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Research Article

Enhancing Biocompatibility without Compromising Material Properties: An Optimised NaOH Treatment for Electrospun Polycaprolactone Fibres

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This research presents the first optimised protocol for submersion of electrospun polycaprolactone (PCL) fibres in sodium hydroxide (NaOH) to improve surface hydrophilicity, and hence biocompatibility, without compromising material properties. The study comprised two aims: (1) identify the leading NaOH concentration (0, 0.1, 1, and 10 M) and submersion time (0, 1, 4, and 24 h) to improve hydrophilicity with minimal impact on tensile properties and (2) once identified, undertake material characterisation and *in vitro* testing for validation. 1 M 4 h (NaOH concentration: 1 M, submersion time: 4 h) improved hydrophilicity (aligned fibres at 0 M NaOH and 0 h submersion time reduced from $97 \pm 3^{\circ}$ to $6 \pm 2^{\circ}$; and random fibres at 0 M 0 h reduced from $105 \pm 4^{\circ}$ to $15 \pm 7^{\circ}$) with minimal impact on tensile strength (9% and 6% loss aligned and random, respectively). 1 M 4 h-treated scaffolds demonstrated no significant change in material properties, yet notably improved protein adsorption and attachment, viability and elongation of 3T3 fibroblasts 4 h postseeding. Thus, 1 M 4 h is optimal for successful wet chemical treatment of electrospun PCL and presents a simple and economical method to easily enhance biocompatibility without compromising scaffold integrity.

1. Introduction

Electrospinning is a popular technique used within the biomaterials and tissue engineering community, as the produced fibres create scaffolds with controlled architectures that mimic the extracellular matrix of many different tissues [1, 2]. Fibre scaffolds can be produced from a range of suitable materials, including natural and synthetic, and selection often depends on the function of the scaffold: whether it needs to be sufficiently load-bearing and/or is a temporary structure and over what period of time. In the majority of biomaterial applications, the scaffold must be receptive to the adhesion and proliferation of cells [3].

Electrospinning natural polymers, such as collagen and elastin, are highly attractive as they are bioactive and similar to the basic structural components of the tissue matrix, and thus inherently supportive of cell adhesion, viability, and proliferation. However, variation between batches and their relative expense means alternative synthetic polymers, which are consistently mass-produced and cheap, are frequently chosen [4]. FDA-approved synthetic polymers include polyesters (poly(lactic) acid, poly(glycolic) acid, and poly(ε-caprolactone)), which are inert, biodegradable materials. In particular, poly(ε-caprolactone) (PCL) has found extensive use in tissue engineering and drug delivery applications [5]. However, PCL shows a low apparent surface hydrophilicity, particularly in fibre form, which ultimately decreases the ability of cells to adhere to its material surface [6]. Hydrophilicity can be improved by submerging PCL fibres in culture medium prior to cell seeding, as proteins are able to adsorb onto the fibre surface, and thus provide artificial binding sites for cells to adhere to; though it is worth noting that this attachment is via weak Van der Waals forces and cells can be easily removed from the fibre surface [7]. In order to modify the hydrophilicity of PCL and strengthen the adhesion between material and cell, treatments to alter the material's surface chemistry are required

which introduce and present functional groups that are attractive for cell binding [8, 9].

To improve the surface hydrophilicity and hence cell adhesion, there are four main modification methods for the treatment of electrospun PCL fibres: (1) coelectrospinning with active materials, (2) plasma treatment, (3) surface graft polymerisation, and (4) wet chemical method. An excellent review of these modification methods is available from Yoo et al. [10].

Methods (2) to (4) involve treatment of prefabricated fibrous structures; whereas, coelectrospinning and blending with active agents allows nanoparticles and functional segments of polymer to be directly presented on the fibre surface, which increases surface free energy and hence decreases water contact angle [10–13]. A study by Li et al. demonstrated that the inclusion of hydroxyapatite with PCL during electrospinning yielded scaffolds supportive of both osteoblast and fibroblast growth and improved mechanical properties compared to PCL fibres alone [14].

Plasma modification using different feed gases, such as air, oxygen, ammonia, and carbon dioxide, have all successfully altered the surface chemical composition of electrospun fibres by generating carboxylic acid groups, hydroxyl groups, and amine groups on the fibrous surface leading to their improved biocompatibility [15–18]. Martins et al. investigated the effects of oxygen and argon plasma treatment of electrospun PCL fibres and found that changes in surface roughness and hydrophilicity affected the response of three different cell lines [19]. However, whilst used by a number of researchers, modification of fibrous meshes is not totally effective as the plasma is unable to penetrate deep between the fibres, particularly if the scaffold is relatively dense [10].

Surface graft polymerisation, instigated by free radicals released from either a plasma or UV radiation source, has been performed on a number of materials for biomaterial applications because it not only improves the hydrophilicity, but also provides multifunctionalities on the surface to increase cell attachment and proliferation [20–22]. Ma et al. modified the surface of electrospun PCL fibres initially by air plasma treatment before immersing in a gelatin solution, which physically adsorbed the gelatin to the PCL surface [15]. Culturing with endothelial cells demonstrated significantly improved attachment and growth over the fibres and maintained expression of key markers typical for this cell type.

Wet chemical methods are able to modify the fibres throughout the scaffold as they involve complete immersion in an acidic or basic solution. When immersed in a sodium hydroxide (NaOH) solution, for example, the ester linkages along the polymer backbone break by random chemical scission, which leads to hydroxyl and carboxylic acid groups being exposed on the polymer surface [10, 23]. Hydrophilicity is subsequently improved, as these groups are freely available to hydrogen bond with water molecules because of their slightly electronegative effects and less steric hindrance [23]. However, the concentration of hydrolysing agents and submersion time in aqueous solution can significantly affect the scaffold's material properties, such as mechanical integrity, and it is necessary to determine the ideal concentration and time for this type of technique [24]. Review of the literature yielded several papers where electrospun PCL fibres were treated with NaOH; however, all three utilised different concentrations and submersion times. The first study was conducted in early 2007 by Chen et al., where a concentration of 5 M and 3-hour submersion time was investigated and found to improve cell attachment and spreading across the fibrous surface [25]. Later that year, Park et al. investigated 1 N NaOH for 1 hour and found that the PCL fibres strongly supported the attachment and growth of osteoblasts compared to nontreated fibres [26]. A third study by Ghasemi-Mobarakeh et al. used NaOH treatment at 1.25 M at several different times (1, 4, and 12 hours) and whilst this treatment improved hydrophilicity of the fibres, the main focus was to further bind matrigel to the scaffold and not to determine an optimal protocol [27].

There remains a need for a definitive NaOH submersion protocol to be established and shared with the scientific community. As such, the objective of this research was to optimise the concentration of NaOH and the submersion time to improve the hydrophilicity without compromising the material properties, such as mechanical integrity, of the fibrous scaffold. The study was split into two aims: firstly, to investigate the effect of NaOH concentration (0, 0.1, 1, and 10 M) and time (0, 1, 4, and 24 h) in terms of hydrophilicity and tensile properties for both aligned and randomly orientated electrospun PCL fibres. Secondly, after identifying a concentration and submersion time, which yielded a significant improvement in hydrophilicity without compromising the mechanical integrity of the scaffold, further material characterisation and initial cell response to this treatment protocol was investigated.

2. Materials and Methods

2.1. Fabrication of Poly(ε-Caprolactone) (PCL) Fibre Scaffolds. A 10%w/v solution of PCL (Mn 80,000 g/mol; Sigma) dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma) was electrospun using the following parameters: applied voltage—11 kV, collector voltage—3 kV, Distance from needle to collector—20 cm, and flow-rate through a 21G needle—1 ml/hr. Fibre mats (~100 μm thick) were collected on a rotating mandrel ($\emptyset = 12\,cm$): 50 RPM for random orientation and 600 RPM for aligned.

2.2. Scaffold Surface Treatment with Sodium Hydroxide (NaOH). Initially, four incubation time points (0, 1, 4, and 24 h) and four different concentrations of NaOH (Sigma) (0, 0.1, 1, and 10 M) were examined, where 0 hours equated to approximately 5 seconds. Electrospun PCL fibres were fully submerged in 10 ml NaOH solution and placed on an orbital shaker (70 RPM) at room temperature for the desired treatment time. Following this, the treated PCL fibres were washed three times in deionised (DI) water and soaked in DI water overnight. After this time, the DI water was removed, and the treated fibres were placed into an ultralow freezer (-85°C) for 20 min. Finally, fibres were vacuum-dried at -40°C for 1.5 h.

2.3. Experiment 1: Optimising NaOH Treatment Time and Concentration

2.3.1. Water Contact Angle (WCA). Using a Krüss DSA100 goniometer in static sessile drop mode, a 10 μl deionised water droplet was deposited from the end of an 8-gauge needle onto the surface of the electrospun PCL fibre scaffold and optically captured. Drop Shape Analysis Software using circle fitting measured and calculated the contact angle ($n = 6$ per group).

2.3.2. Tensile Test. Scaffolds were cut to 30 mm × 5 mm and fixed with tape to cardboard windows (internal dimensions: 20 mm × 10 mm and external dimensions: 35 mm × 15 mm), leaving an overall test length of 20 mm. Cardboard windows were gripped within tensile testing clamps and positioned within a tabletop uniaxial testing machine (Instron 3344) with 10 N load cell. The sides to the cardboard window were cut immediately prior to commencing the tensile test to failure at 5 mm/min crosshead speed ($n = 5$ per group).

2.3.3. Mass Loss. Changes in mass were detected using a microbalance. Scaffolds were cut to 30 mm × 5 mm and weighed before and after NaOH treatment (dried under vacuum overnight at room temperature) for each group ($n = 5$) and compared to 0 M 0 h.

2.4. Experiment 2: Material Characterisation and Biological Response to Optimised NaOH Protocol

2.4.1. Gel Permeation Chromatography (GPC). As described by Bosworth and Downes [28], GPC (Applied Chromatography Systems Ltd.) was used to determine the molecular weight distributions of random and aligned PCL fibres (NaOH-treated and untreated; $n = 9$ per group). Samples were individually dissolved in distilled tetrahydrofuran (THF; Fisher Scientific) at a concentration of 0.2% *w*/*v*. 100 μl of solution was injected into the GPC, which had been previously calibrated with polystyrene (PS) standards (Pressure chemical standards) in THF with known molecular weights ranging from 600 – 7.7×10^6 g mol^{-1}. Distilled THF with flow rate 1 ml min^{-1} was used as the mobile phase. The column (Phenomenex) was composed of Phenogel 5 μm, with pore sizes 500 Å, 5×10^4 Å, and 5×10^6 Å. The average molecular mass distributions were determined using PSS WinGPC software.

2.4.2. Differential Scanning Calorimetry (DSC). Thermal analysis was performed using DSC Q100 (TA Instruments, version 9.9) as previously described in Bosworth et al. [29]. Scaffolds ($n = 5$ per group) were sealed in aluminium pans and exposed to a single heat cycle from 0°C to 100°C with 10°C/min heating rate. Nitrogen gas flow was 50 ml/min. Universal Analysis 2000.v.4.2E software (TA Instruments) was used to quantify the melting temperature (T_m) and enthalpy of fusion (ΔH_m). Material crystallinity was determined by comparing the ΔH_m with that for 100% crystalline PCL (135.44 J g^{-1}) as measured by Hartman et al. [30].

2.4.3. Scanning Electron Microscope (SEM). To reduce charging, scaffolds (10 × 10 mm) were gold sputter-coated for 2 min. Scaffold morphology ($n = 2$ per group) was assessed by SEM (Phenom Pro) operating at 5 KeV with 10 mm working distance. Fibremetric software (Phenom Pro) was used to measure fibre diameter ($n = 80$).

2.4.4. Cell Culture and Scaffold Preparation. 3T3 fibroblast cells were cultured within T75 flasks with 12 ml of Dulbecco's Modified Eagle's Medium (high glucose) (DMEM-HG; Sigma) supplemented with 5 ml antibiotic and 50 ml fetal bovine serum (15% *v*/*v*) at 37°C with 5% humidified CO_2. Scaffolds were cut into 20 mm^2 squares and mounted within 24-well plate CellCrowns (Scaffdex Ltd.). They were sterilised overnight in 70% *v*/*v* ethanol and then washed several times in sterile Phosphate-Buffered Saline (PBS; Sigma). Scaffolds were transferred into sterile, low-binding 24-well plates (Corning) and presoaked in culture media prior to cell seeding.

(2.4.4.1) NanoOrange Assay. Adsorption of proteins to the scaffold surface was determined by using a NanoOrange Protein Quantitation Kit (Life Technologies). 1 ml of 50 μg/ml Bovine Serum Albumin (BSA; Sigma) in PBS was added to each scaffold ($n = 6$ per group) and incubated for 90 min at 37°C and 5% humidified CO_2. After this time, 200 μl of solution was removed from each sample and placed within a sterile microfuge tube (Eppendorf), which contained 200 μl of NanoOrange reagent working solution. Microfuge tubes were heated to 92°C for 10 minutes and then cooled to room temperature (>20 min). 100 μl of solution was transferred from each microfuge tube to a 96-well plate and analysed using a fluorescent plate reader (excitation wavelength: 485 nm, emission: 590 nm). Test samples were compared to BSA as a positive control.

(2.4.4.2) Live/Dead Assay. Cell viability on electrospun PCL fibres was determined by live/dead assay. Firstly, 50,000 3T3 cells were seeded onto each PCL scaffold with 1 ml media and incubated for 4 hours. After this time, the live/-dead assay solution (Life Technologies) was prepared; 10 μl ethidium homodimer (EthD-1), 4 μl calcein-AM, and 10 ml PBS were mixed together. 500 μl mixed dye solution was added to each scaffold and then incubated for 20 minutes at 37°C and 5% humidified CO_2. Scaffolds ($n = 2$ per group) were imaged using a confocal microscope (Leica CTR6500) at 10x magnification, and Z-stack images were grouped to provide a single image.

(2.4.4.3) Scanning Electron Microscopy—Cell Morphology. SEM was used to image cell morphology relative to the fibrous scaffold for each group ($n = 2$). Scaffolds seeded with 50,000 3T3 cells were fixed after 4 hours in culture. As outlined in [31], scaffolds were washed in PBS and fixed in 1.5% *v*/*v* glutaraldehyde (TAAB Laboratories) in PBS for 30 min at 4°C. They were subsequently dehydrated through increasing concentrations of ethanol (50–100% *v*/*v*), followed by chemical drying in hexamethyldisilazane

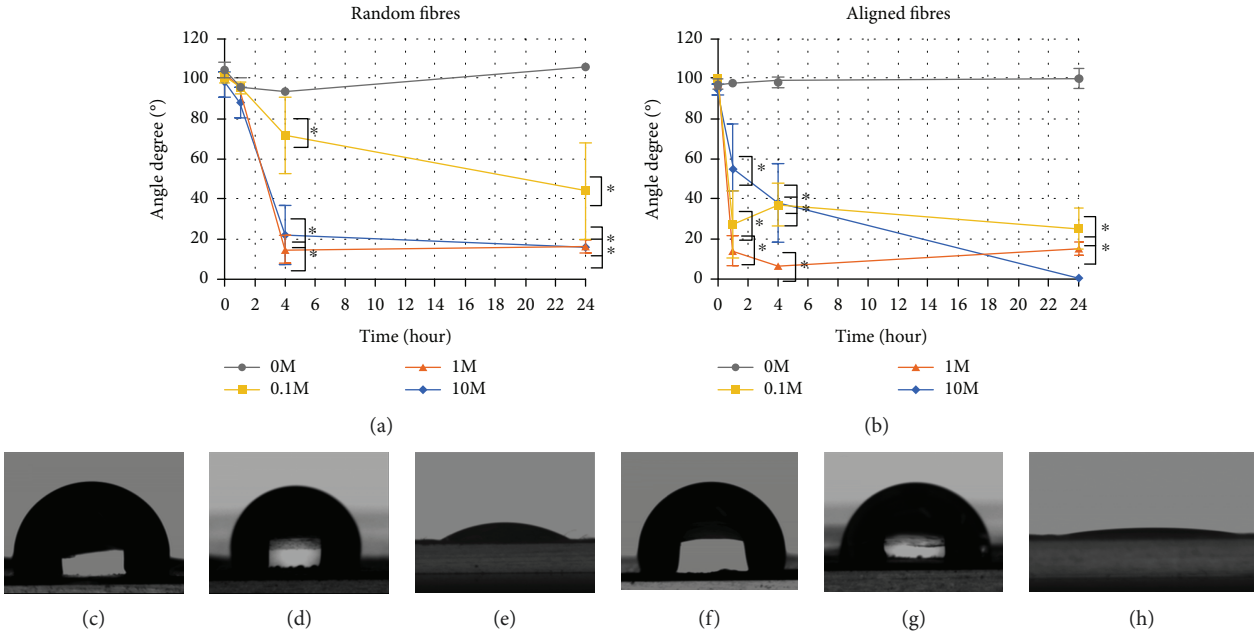

Figure 1: Water contact angle data demonstrating effects of NaOH treatment on scaffold hydrophilicity for electrospun PCL fibres with random (a) and aligned (b) orientations. (c–h) are representative images of single water droplets on the scaffold surface: (c) random, untreated; (d) random, 0 M 0 h; (e) random, 1 M 4 h; (f) aligned, untreated; (g) aligned, 0 M 0 h; and (h) aligned, 1 M 4 h. Data are shown as mean ± st. deviation with two-way ANOVA and Tukey's multiple comparisons test ($^*p < 0.05$; $n = 4$).

(Sigma). Samples were gold sputter-coated for 2 min and visualised by SEM (Phenom Pro) operating at 5 KeV.

2.5. Statistical Analysis. Graphpad Prism 7 was used to compare NaOH-treated PCL scaffold data to untreated PCL controls. Datasets were checked for normality and all except fibre diameter were normally distributed. As such, data is presented as mean ± standard deviation. WCA, mass loss, and tensile strength were analysed using a two-way ANOVA with Tukey's posttests and 95% confidence level. Protein adsorption was analysed using one-way ANOVA with Tukey's posttests and 95% confidence level.

3. Results

3.1. Objective 1: Determine the Ideal Concentration and Submersion Time of Electrospun PCL Fibres in NaOH. Random and aligned PCL fibres were exposed to 16 different test conditions and characterised in terms of water contact angle, tensile strength, and mass loss.

3.1.1. Water Contact Angle. Noticeable differences in water contact angle (WCA) were observed for both groups (Figure 1). Irrespective of orientation, fibre hydrophilicity was improved following the submersion in NaOH for 1 hour or more. Aligned fibres demonstrated significant reductions in contact angle for all concentrations (≥1 h) compared to fibre mats briefly submerged in 0 M NaOH (deionised water) for 0 h (~5secs). The highest concentration and submersion time could not be analysed as the scaffolds disintegrated. For aligned fibres at 1 M concentration, WCA reduced from 96.9° ± 2.5° to 6.3° ± 1.6° after 4 hours; and ten-fold weaker concentrations demonstrated WCAs of 26.8° ± 16.8° (1 h), 37.0° ± 10.6° (4 h), and 24.8° ± 10.2° (24 h). Random fibre mats equally demonstrated improvements in scaffold hydrophilicity, but these were less pronounced with significant changes mostly occurring after four hours exposure to NaOH. Submersion times of 4 hours and more resulted in significant reductions in WCA for all concentrations except 0 M. Lowest WCA (16.0°) was obtained for random fibres exposed to 10 M 24 h.

3.1.2. Tensile Strength. NaOH-treated fibres were subjected to tensile loading to failure (Figure 2). 0 M 0 h demonstrated a clear difference in strength depending on the initial fibre orientation: 3.6 MPa ± 0.7 MPa (aligned) and 2.1 MPa ± 0.1 MPa (random). Following treatment with each test group, the greatest reduction in tensile strength was observed for aligned fibres at 1 M 24 h (2.2 MPa ± 0.3 MPa; 40% decrease) and random fibres, 0.1 M 24 h with 74% reduction in strength compared to 0 M 0 h. Scaffold stiffness remained consistent for random fibres regardless of applied NaOH treatment group, except for 10 M 0 h as these disintegrated when mounting to the cardboard test windows. A similar trend was observed for aligned fibres, where stiffness was relatively stable for all groups except for 10M 24h, which resulted in a total loss of 64% compared to 0 M 0 h scaffolds.

3.1.3. Mass Loss. As a consequence of NaOH-induced scission, the scaffolds were measured for changes in mass pre- and posttreatment. Figure 3 demonstrates an overall loss in mass following surface treatment, which was particularly noted after 24 h submersion with 52% and 32% loss for aligned and random, respectively. A general trend showed

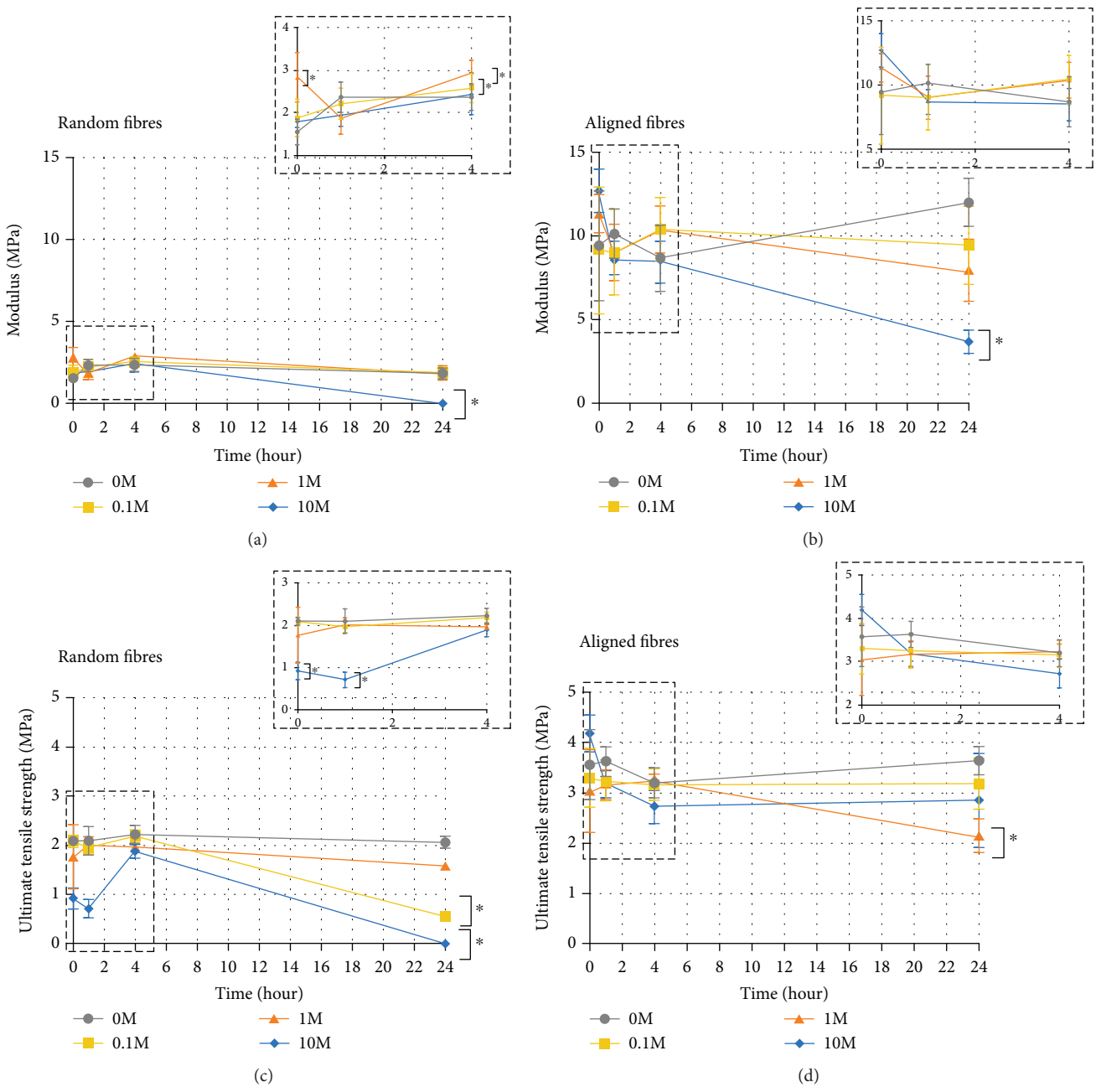

Figure 2: Tensile testing data demonstrating effects of NaOH treatment on modulus and ultimate tensile strength for electrospun PCL fibres with random (a, c) and aligned (b, d) orientations. Data are shown as mean ± st. deviation with two-way ANOVA and Tukey's multiple comparisons test ($^*p < 0.05$; $n = 5$).

mass loss increased with increasing NaOH concentration and greatest reduction was observed for aligned fibres.

3.2. Objective 2: Determine the Effect of 1 M 4 h on Material and In Vitro Properties for Electrospun PCL Fibres. The data obtained in the first objective indicated a NaOH surface treatment of 1 M 4 h to be optimal for both aligned and random electrospun PCL fibres. Consequently, the second objective of the study was to further characterise the material properties and cell response to 1 M 4 h NaOH-treated fibres.

3.2.1. Molecular Mass. The molecular mass of the treated scaffolds was measured by Gel Permeation Chromatography (GPC) and compared to untreated fibres 0 M 0 h (Table 1). Comparison between these two groups for both random and aligned fibre orientations revealed a reduction in molecular mass for treated fibres of <5%.

3.2.2. Thermal Properties and Percentage Crystallinity. Differential Scanning Calorimetry was used to assess the thermal properties (melting temperature and Enthalpy of Fusion)

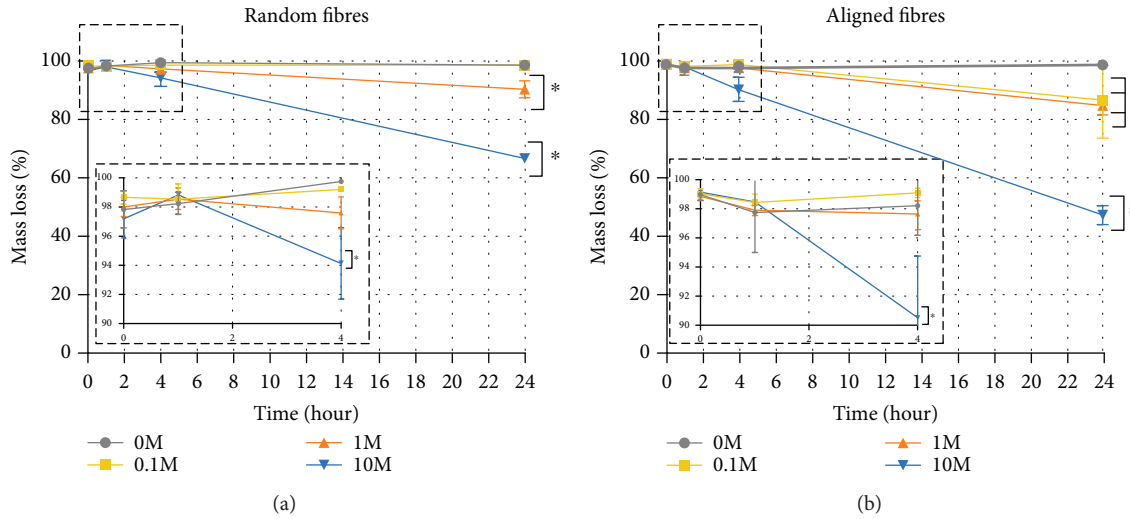

Figure 3: Effects of NaOH treatment on scaffold percentage mass loss for electrospun PCL fibres with random (a) and aligned (b) orientations. Data are shown as mean ± st. deviation with two-way ANOVA and Tukey's multiple comparisons test ($^*p < 0.05$; $n = 4$).

Table 1: Molecular weight data for treated (1 M 4 h) and untreated (0 M 0 h) electrospun nanofibres.

	Random nanofibres		Aligned nanofibres	
	0 M 0 h	1 M 4 h	0 M 0 h	1 M 4 h
M_n (g/mol)	$11.1 \pm 0.6 \times 10^4$	$10.5 \pm 0.3 \times 10^4$	$10.6 \pm 0.3 \times 10^4$	$10.2 \pm 0.3 \times 10^4$
M_w (g/mol)	$16.7 \pm 0.6 \times 10^4$	$16.6 \pm 0.1 \times 10^4$	$16.9 \pm 0.3 \times 10^4$	$16.6 \pm 0.1 \times 10^4$

Where M_n represents the number average molecular weight and M_w the weight average molecular weight. Data are presented as mean ± st. deviation with one-way ANOVA and Tukey's posttests ($n = 9$).

Table 2: Thermal properties and crystallinity data for treated (1 M 4 h) and untreated (0 M 0 h) electrospun nanofibres.

	Random nanofibres		Aligned nanofibres	
	0 M 0 h	1 M 4 h	0 M 0 h	1 M 4 h
T_m (°C)	59.04 ± 0.69	59.06 ± 0.48	58.88 ± 0.47	58.93 ± 0.34
ΔH_m (J/g)	58.74 ± 2.03	60.87 ± 2.10	53.08 ± 10.53	60.15 ± 1.75
χ_c (%)	43.19 ± 1.49	44.75 ± 1.55	39.03 ± 7.75	44.23 ± 1.29

T_m represents the melting point, ΔH_m the enthalpy of fusion, and χ_c the crystallinity. Data are presented as mean ± st. deviation with one-way ANOVA and Tukey's posttests ($n = 5$).

and changes in percentage crystallinity (Table 2). The data demonstrated no significant difference in these parameters across all test groups. Crystallinity remained at ~45% for random and ~44% for aligned fibres following treatment with 1 M 4 h NaOH.

3.2.3. Scaffold Morphology. SEM was used to assess changes in scaffold topography, particularly fibre diameter and morphology (Figure 4). SEM images demonstrated no morphological changes to the fibre surface or fibre diameter following contact with 1 M NaOH for 4 hours. Random and aligned networks were retained and fibre surfaces remained smooth with no visible topographical features. Fibre diameter distributions remained comparable between test groups indicating no substantial change in fibre size.

3.2.4. Protein Adsorption. NanoOrange was used to determine the quantity of protein capable of adsorbing onto the scaffold surface following NaOH treatment (Figure 5). There was a significant increase in the level of adsorbed protein on both random (41%) and aligned (24%) scaffolds subjected to 1 M 4 h compared to untreated counterparts.

3.2.5. Cell Viability and Morphology. The attachment, viability, and morphology of 3T3 fibroblasts were determined by live/dead assay and SEM 4 hours after cell seeding (Figure 6). Irrespective of fibre orientation, a greater number of viable (green) cells were detected on NaOH-treated scaffolds compared to untreated. SEM images similarly presented this observation between test groups and further demonstrated changes in cell morphology, where cells appeared spread and elongated across the underlying fibre

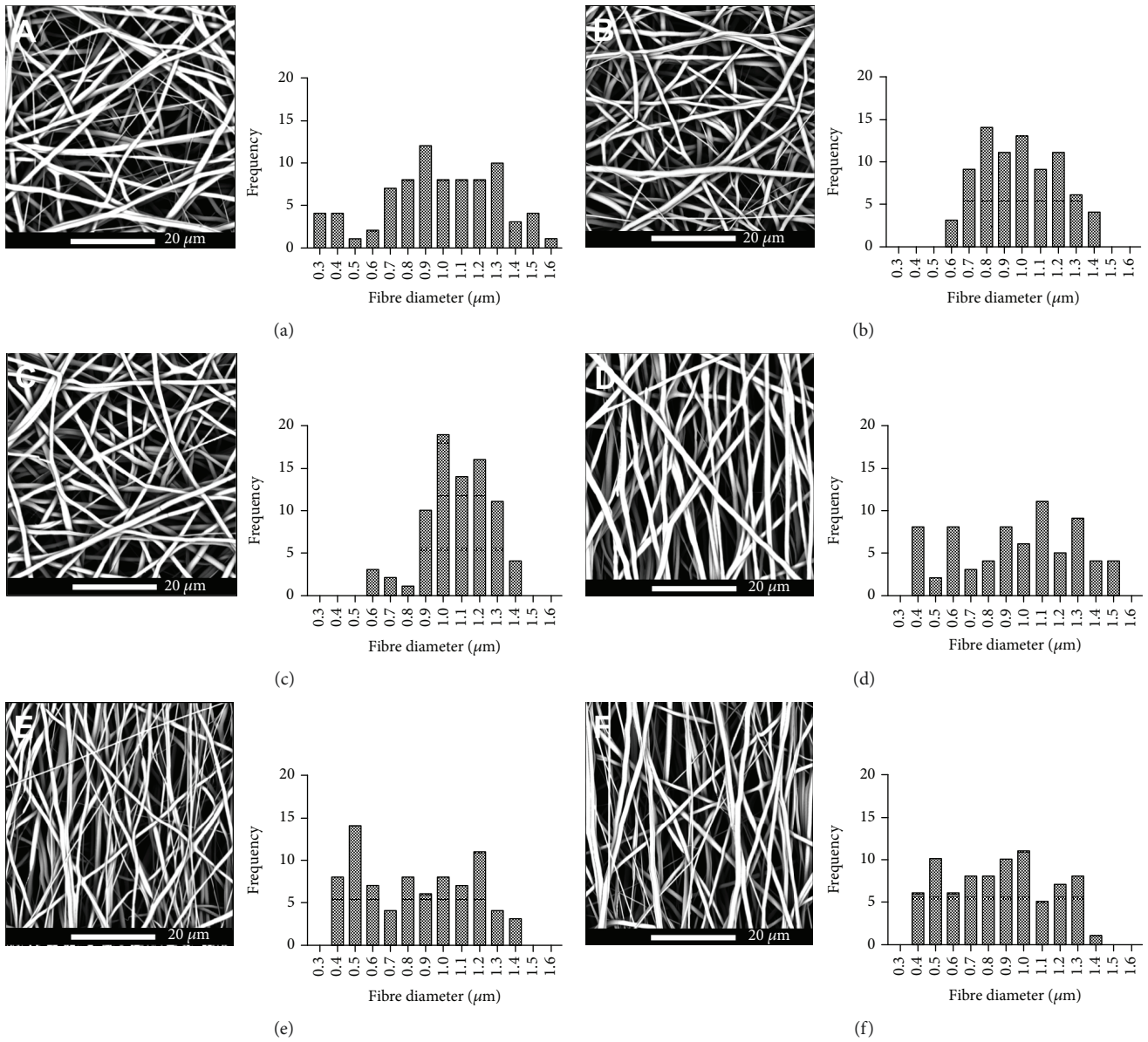

Figure 4: Scanning electron microscopy images demonstrating effects of NaOH treatment on scaffold morphology and fibre diameter for electrospun PCL fibres (magnification ×5,000): (a) random, untreated; (b) random, 0 M 0 h; (c) random, 1 M 4 h; (d) aligned, untreated; (e) aligned, 0 M 0 h; and (f) aligned, 1 M 4 h. Fibre diameters presented as frequency distributions ($n = 80$).

topography for treated fibres, yet possessed a rounded morphology on the untreated.

4. Discussion

This study is aimed at determining the optimal concentration and submersion time of electrospun PCL fibres in NaOH, making it available as a surface treatment protocol for other users of electrospinning, particularly with PCL, for biomaterial and tissue engineering applications. NaOH surface treatment alters the surface chemistry of PCL (and other polymers) to create and expose hydrophilic functional groups. The first objective of this study was to determine the effect of NaOH concentration and submersion time on surface hydrophilicity and tensile properties.

4.1. Determine the Ideal Concentration and Submersion Time of Electrospun PCL Fibres in NaOH. Two-dimensional (2D) sheets of PCL fibres were separated by orientation to give groups of random and aligned fibres that were subsequently exposed to 16 different test conditions. Measuring the contact angle of deionised water droplets on the scaffold surface enabled the hydrophilicity following treatment to be assessed. Figure 1 highlights distinct changes in WCA and ultimately hydrophilicity for both random and aligned fibres following a minimum treatment time of 1 hour. This demonstrates the power of NaOH to quickly break up the ester bonds within the polymer chains and expose hydrophilic functional groups. Between the two orientations, aligned fibres exhibited the most distinct change in WCA for all NaOH concentrations, which may be due to their different

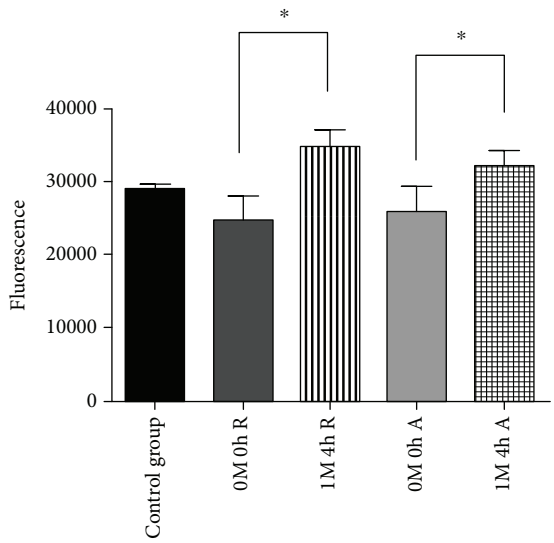

Figure 5: Quantification of protein deposition on NaOH-treated (1 M 4 h) and untreated (0 M 0 h) electrospun PCL fibres with random (R) and aligned (A) orientations. Data are shown as mean ± st. deviation with one-way ANOVA and Tukey's multiple comparisons test ($^*p < 0.05$; $n = 9$).

architectures, where fibres aligning preferentially adjacent to each other presents an overall larger exposed surface area, whereas fibres within a random network are protected by more fibres crossing over each other indiscriminately creating more fibre-fibre contact points [32]. This could further explain the fragility and fragmentation of aligned fibres following 10 M 24 h treatment compared to random fibres subjected to the same parameters.

Whilst a significant improvement in fibre hydrophilicity has been achieved, which demonstrates the success of this wet treatment method, it is important to investigate the effects on other pertinent material properties, such as mechanical strength. Electrospun PCL fibres are currently being researched as scaffolds to aid tendon repair and regeneration [30, 32], as such the tensile properties of these devices are crucial to their success in situ as tendons repeatedly withstand high tensile forces. As expected, aligned fibres were able to withstand considerably higher tensile strengths compared to random and this is in agreement with other published studies [30, 33]. Tensile properties remained relatively consistent between groups: the greatest loss in strength and stiffness was detected after submersion times of 24 hours for random (0.1 M and 10 M) and aligned (1 M and 10 M). This would suggest that the initial molecular mass of PCL was sufficient to withstand random scission of the ester bonds and still be able to provide mechanical support to the overall structure. Furthermore, the arbitrary arrangement and entanglement of molecular chains within the individual fibres may also continue to provide strength when pulled along their length [31].

In addition to assessing the hydrophilicity and tensile properties of these 16 test groups, the change in bulk mass of the scaffold pre- and posttreatment was also determined (Figure 3). For random fibres, the greatest mass loss was measured after 24 hours for 1 M and 10 M; this was also observed for aligned fibres with the addition of 0.1 M. Overall greatest mass loss occurred for aligned scaffolds, which again may be attributed to an increased area of scaffold that is exposed compared to random fibre networks that possess a number of contact points between fibres [32]. For both scaffold orientations, minor mass loss occurred at 1 and 4 hours for all concentrations except 10 M 4 h, suggesting that short treatment times and low molarities of NaOH were sufficient to limit mass loss and damage to the scaffold whilst notably improving the hydrophilicity.

In order to demonstrate a significant improvement in material hydrophilicity whilst retaining sufficient tensile properties and scaffold integrity, the data indicated a NaOH surface treatment of 1 M 4 h to be optimal for both aligned and random electrospun PCL fibres. Consequently, the second objective of this study was to further characterise the material properties and cell response to random and aligned fibrous scaffolds treated with 1 M NaOH for 4 hours.

4.2. Determine the Effect of 1 M 4 h on Material and In Vitro Properties for Electrospun PCL Fibres. GPC determined the molecular mass of 1 M 4 h-treated random and aligned fibre scaffolds and compared these to 0 M 0 h. As shown in Table 1, the effect of this treatment time had minimal impact on the molecular weight (Mn and Mw) with only a slight decrease detected overall, which suggests this concentration and submersion time has elicited a degree of polymeric chain scission but without overly compromising the molecular structure within the PCL fibres. Assessment of thermal properties, particularly changes in crystallinity, demonstrated no significant differences across all test groups (Table 2). PCL is a semicrystalline polymer, and this property contributes to the material's strength; no significant difference, as a consequence of wet chemical treatment, was detected in the polymer's crystallinity, which is further reflected in the tensile testing data. Furthermore, the data obtained in this study is similar to other published data for electrospun PCL fibre crystallinity values [31, 34]. SEM assessed the effect of wet chemical treatment on scaffold topography, in terms of fibre diameter and morphology (Figure 4). The fibre surface remained smooth with no distinct change in diameter following 4-hour submersion in 1 M NaOH, which is in line with the limited loss in scaffold mass previously observed in Figure 3. Characterisation of 1 M 4 h-treated aligned and random scaffolds demonstrated no significant effect on the material properties, with preservation of tensile properties, molecular weight, crystallinity, and scaffold morphology and integrity. Yet scaffold hydrophilicity was markedly improved, and thus the biocompatibility was subsequently assessed *in vitro*.

The purpose of the study was to improve the hydrophilicity, and thus biocompatibility of the electrospun fibres by use of a wet chemical method. Water contact angle testing in objective 1 of this study demonstrated significant improvement in fibre hydrophilicity following submersion in NaOH, which was most improved when fibres were subjected to treatment at 1 M 4 h. Assessment of enhanced

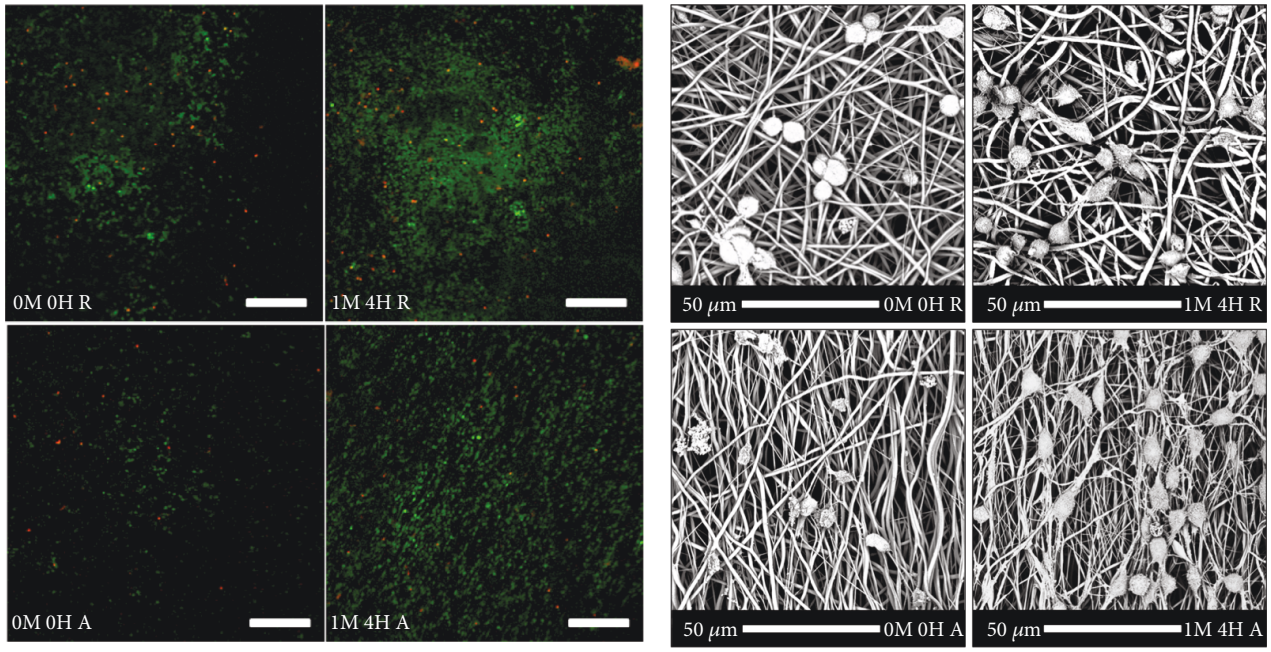

Figure 6: (a) Representative fluorescent images demonstrating effects of NaOH scaffold treatment on 3T3 fibroblast cell viability via live/dead assay for random (R) fibres (0 M 0 h and 1 M 4 h) and aligned (a) fibres (0 M 0 h and 1 M 4 h). Scale bar = 100 μm, magnification ×10. (b) Representative scanning electron microscopy images demonstrating effects of NaOH scaffold treatment on 3T3 fibroblast morphology for random (R) fibres (0 M 0 h and 1 M 4 h) and aligned (a) fibres (0 M 0 h and 1 M 4 h). Scale bar = 50 μm; magnification ×2,500.

biocompatibility was further determined by protein adsorption assay and short-term culture of 3T3 fibroblasts. NanoOrange, which is sensitive to 10 ng/ml, was used to determine the adsorption of protein on the surface of 1 M 4 h-treated scaffolds with comparison to untreated (0 M 0 h). As shown in Figure 5, there was a significant increase in the level of adsorbed protein on the random and aligned scaffolds that had been subjected to NaOH treatment. This would suggest that the treatment protocol devised in the first part of this study is more than sufficient for encouraging deposition of protein and in turn cell adhesion. Viability of 3T3 fibroblasts on NaOH-treated and untreated scaffolds was assessed by live/dead after 4 hours (Figure 6(a)). Comparison between treated and untreated (0 M 0 h) scaffolds highlighted a qualitative difference between groups, with those submersed in 1 M NaOH for 4 hours presenting a greater number of viable (green) cells compared to untreated fibres. Representative SEM images also indicated a greater number of cells on NaOH-treated scaffolds (Figure 6(b)). Furthermore, cells displayed different morphologies between the test groups after 4 hours, appearing to extend over the fibre surface (following contact guidance cues of the underlying fibres) for 1 M 4 h scaffolds, whilst possessing predominantly rounded morphologies on untreated counterparts. This is indicative of cell preference for the treated scaffold and their ability to adhere to the free chemical groups on the fibre surface, which is in agreement with a previous study by Chen et al., where 3T3 fibroblasts were found to have well-spread morphologies on NaOH-treated fibres compared to rounded cells on nontreated 12 hours after seeding [25].

5. Conclusions

Collectively, the data presented has demonstrated the main objective of the research—establishment of an optimised protocol for the improvement in biocompatibility and retention of key material properties for electrospun PCL random and aligned fibres. Investigating several different concentrations of NaOH (0, 0.1, 1, and 10 M) and submersion times (0, 1, 4, and 24 h) demonstrated electrospun PCL were ideally treated at a concentration of 1 M and submersion time of 4 hours, irrespective of fibre orientation. This protocol yielded significant improvements in water contact angle, protein adsorption, and initial cell attachment without compromising the fibre scaffold material properties, including tensile strength and topography. As such, submersion for 4 hours in 1 M NaOH provides an optimal surface treatment for electrospun PCL fibres.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest.

Acknowledgments

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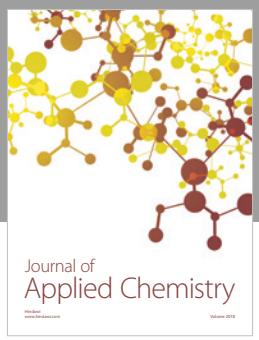
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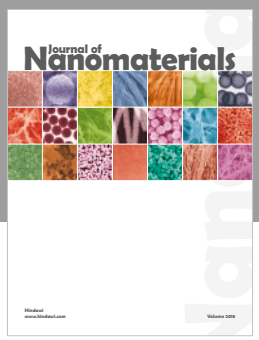
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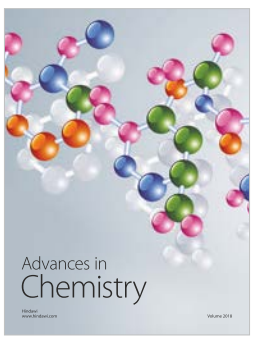
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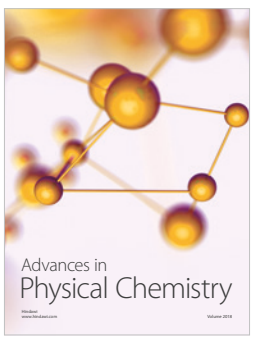
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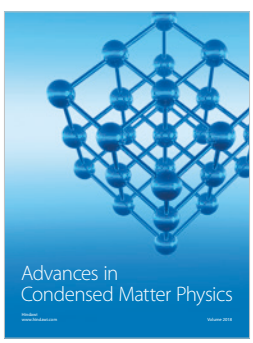
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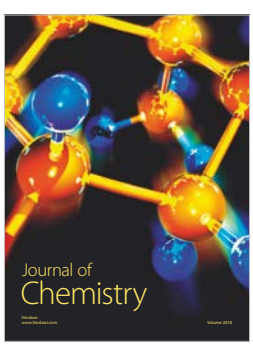
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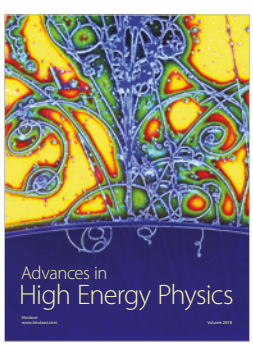
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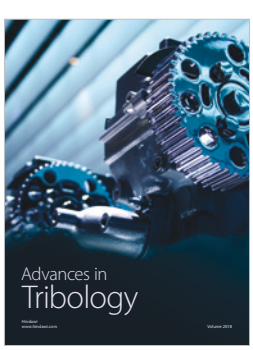
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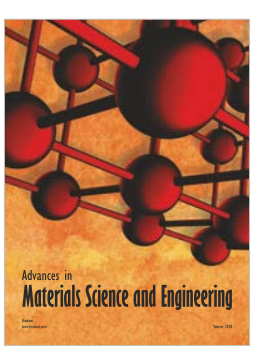
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