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Electrospun 1,6-diisocyanatohexane-extended poly(1,4-butylene succinate) fiber mats and their potential for use as bone scaffolds

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ABSTRACT

Ultrafine 1,6-diisocyanatohexane-extended poly(1,4-butylene succinate) (PBSu-DCH) fibers were best fabricated by electrospinning from 22% w/v PBSu-DCH solution in 90:10 v/v dichloromethane/trifluoroacetic acid under the electric field of 17 kV/20 cm. The diameters of these fibers were 172 ± 3 nm. Due to their fibrous nature, the obtained PBSu-DCH fiber mats exhibited high values of advancing/receding water contact angles (i.e., 114°/79°) and porosity (69%). Indirect cytotoxicity evaluation of the PBSu-DCH fiber mats based on the viabilities of human osteosarcoma cells (SaOS-2) and mouse fibroblasts (L929) revealed that the fibrous materials did not release any substance in the level that was harmful to the cells. The potential for use of the PBSu-DCH fiber mats as substrates for bone cell culture was further evaluated *in vitro* with SaOS-2 in terms of the ability to support the attachment and to promote the proliferation and the differentiation of the seeded/cultured cells. Comparative studies were made against corresponding solvent-cast PBSu-DCH films. The results indicated that the bone cells grown on the surface of the fiber mats could attach, proliferate and express alkaline phosphatase (ALP), an early osteogenic proliferation marker, better than they did on the surface of the films. The evidence obtained in this work implies the potential for use of the electrospun PBSu-DCH fiber mats as bone scaffolds.

1. Introduction

Electrospinning (e-spinning) is a process capable of fabricating ultrafine fibers with diameters in micrometer down to nanometer range [1]. In this process, a continuous, ultrafine filament is drawn from a polymer liquid (i.e., solution or melt) through a spinneret by electrical forces and deposits randomly on a conductive collector. When a high electrical potential is applied to the polymer liquid, excess or uncompensated charges are accumulated on the surface of a pendant droplet of the polymer liquid at the tip of the spinneret. When the electric field reaches to a point beyond which the electrical forces overcome that of the surface tension, a charged polymer jet is ejected from the conical apex of the pendant droplet and accelerates towards the collector screen. As the jet travels in the air, it thins down by the electrical forces (i.e., Coulombic repulsion of the mutual charges) and, at the same time, the jet finally dries out or cools down, leaving ultrafine fibers in the form of a non-woven fabric on the collector screen [1]. Due to the inherently high surface area to volume/mass ratio, high porosity, and vast possibilities for surface functionalization, some proposed uses of the electrospun (e-spun) fiber mats are as filters [2], composite reinforcements [3,4], carriers for the delivery of drugs [5,6], and scaffolds for cell/tissue culture [7–15].

Tissue engineering is an interdisciplinary technology that combines materials engineering, cellular biology and genetic engineering into developing biological substitutes for defected or damaged tissues [16]. In this approach, a biocompatible scaffold is required to support the adhesion of the seeded cells and to guide and to promote the proliferation and the differentiation of the cells in order to generate new tissues. Ideally, a functional scaffold should mimic the structure and biological functions of native extracellular matrix (ECM) matters [8], so as to provide necessary support and regulate cellular activities [17]. Depending on the size of pores generated by randomly-depositing fibers during the e-spinning process whether it is smaller or larger than that of the cells, e-spun fiber mats could be envisioned as either a two-dimensional (2D) or

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a three-dimensional (3D) substrate for cell/tissue culture. The popularity for the use of e-spun fiber mats as substrates for cell/tissue culture stems from the resemblance of the underlying fibrous structure to the collagen bundles in the natural ECM [8]. Several biocompatible materials have been fabricated into fibers by e-spinning and the resulting e-spun fiber mats have been tested for their potential for use as cell/tissue culture substrates [7–15]. In attempts to be used as bone scaffolds, e-spun fiber mats of polycaprolactone [10–12], poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [14], and silk fibroin [15] have previously been evaluated in our group.

Poly(1,4-butylene succinate) (PBSu) or poly(tetramethylene succinate) is one of the biodegradable and biocompatible aliphatic polyesters that is synthesized from the polycondensation reaction of bis(4-hydroxybutyl)succinate (BHBSu) oligomer, which, in turn, is prepared by the direct esterification of 1,4-butanediol and succinic acid [18]. PBSu has excellent mechanical properties and can be applied to a range of applications, e.g., mulch films, packaging films, bags and 'flushable' hygiene products, via conventional melt-processing techniques. Various aspects of PBSu have been studied and reported in the literature: some of which are structure and morphology [18], bulk crystallization behavior [19], melting behavior [20], effect of degradation products on plant cultivation [21], *in vitro* enzymatic degradation [22] and *in vitro* biocompatibility studies with osteoblasts that had been isolated from the calvaria of neonatal Sprague–Dawley rats [23]. Preparation of PBSu in an e-spun ultrafine fibrous form has been reported to be successful with either chloroform or dichloromethane as the solvent [24–26]. To improve the electrospinnability of the PBSu solutions in these solvents, another liquid, such as 2-chloroethanol, 1-chloro-2-propanol, 3-chloro-1-propanol [24] or ethanol [26], can be added into the solutions to improve some of their properties, particularly to decrease the evaporation rate of the resulting solutions.

As demonstrated in the work of Li et al. [23] that PBSu in the form of melt-pressed films could support the proliferation of the native rat osteoblasts in a similar level to tissue-culture polystyrene plate (TCPS; positive control) and that the cells that had been cultured on the PBSu films for 14 d showed a greater alkaline phosphatase (ALP) activity than those cultured on TCPS, it is of our interest to investigate whether PBSu in the form of e-spun fibrous membranes could support the attachment and, at the same time, promote the proliferation of the bone cells in a similar manner. The main objective of the present work, therefore, focuses on the *in vitro* biocompatibility studies of the e-spun PBSu fiber mats using human osteosarcoma cells (SaOS-2) as reference. Comparisons were also made against the cells that had been seeded or cultured on TCPS (positive control) and the corresponding solvent-cast films (internal control).

2. Experimental details

2.1. Materials

Poly(1,4-butylene succinate) extended with 1,6-diisocyanatohexane (PBSu-DCH; pellet form; CAS number: 143606-53-5; batch number: 09717ED; melt index = 10 g/10 min at 190 °C/2.16 kg) was purchased from Sigma-Aldrich (USA). Dichloromethane (DCM, CH_2Cl_2; Fisher Scientific, UK) and trifluoroacetic acid (TFA, CF_3COOH; Fluka, Switzerland) were used to prepare a mixed solvent system for PBSu-DCH. All other chemicals were of analytical reagent grade and used without further purification.

2.2. Electrospinning (e-spinning)

PBSu-DCH solutions were first prepared in a mixed solvent system of DCM and TFA (at 90:10 v/v) under a mechanical stirring at room temperature. Prior to e-spinning, the as-prepared PBSu-DCH solutions were characterized for their shear viscosity and electrical conductivity using a Brookfield DV-III programmable viscometer and an Orion 160 conductivity meter, respectively. In the e-spinning, each of the as-prepared PBSu-DCH solutions was contained in a 5 mL glass syringe, which was connected to a blunt 20-gauge stainless steel hypodermic needle, used as the nozzle. Both the syringe and the needle were tilted about 45° from a horizontal baseline to ascertain the constant presence of a solution droplet at the tip of the needle. A Kd Scientific syringe pump was used to control the feed rate of the solution (at about 1 mL h^{-1}). An aluminum (Al) sheet, wrapped around a home-made rotating cylinder (width and OD of the cylinder ≈ 15 cm; rotational speed ≈ 50 rpm), was used as the collector. A Gamma High Voltage Research D-ES30PN/M692 dc power supply was used to charge the solution, by connecting the emitting (positive) electrode to the nozzle and the grounding one to the collector. In order to find the optimal conditions for the e-spinning of PBSu-DCH, various parameters were varied: they were solution concentration (i.e., 18–24% w/v), applied electrical potential (i.e., 14, 17 and 20 kV) and collection distance (i.e., 15, 20 and 25 cm). The e-spinning was carried out at ambient conditions. The e-spun fiber mats were dried *in vacuo* at room temperature for 12 h to remove as much solvent as possible. Comparatively, PBSu-DCH films were also prepared by solvent-casting of a PBSu-DCH solution in chloroform (i.e., 8% w/v) in Petri dishes and the castings were dried slowly at room temperature. Where applicable, the room temperature was 25 ± 1 °C.

2.3. Characterization

The morphological appearance of the e-spun fiber mats was observed by a JEOL JSM-5200 scanning electron microscope (SEM). The specimens for SEM observation were prepared by cutting Al sheets covered with the e-spun fiber mats and the cut sections were carefully affixed on copper stubs. Each specimen was gold-coated using a JEOL JFC-1100E sputtering device prior to SEM observation. Diameters of the individual fibers in the e-spun fiber mats were measured directly from the SEM images with 10,000× magnification using a SemAphore 4.0 software, with the average value being calculated from at least 100 measurements.

Thermal properties of the fiber mat and the film specimens were investigated using a Mettler-Toledo DSC822^e differential scanning calorimeter (DSC) and a Perkin–Elmer Pyris Diamond thermogravimetric/differential thermal analyzer (TGA). DSC was used to observe the melting behavior of the specimens. Each specimen of about 5 mg, sealed in an Al DSC pan, was heated from 25 to 150 °C at a rate of 10 °C min^{-1}. The apparent degree of crystallinity of PBSu-DCH, either in the form of e-spun fiber mats or solvent-cast films, was assessed from the enthalpy of fusion that was obtained from the heating thermogram. TGA was used to evaluate thermal degradation behavior of both types of specimens. Each specimen of about 5 mg was heated from 30 to 500 °C at a rate of 10 °C min^{-1} in a nitrogen atmosphere.

Mechanical integrity in terms of yield strength, tensile strength, Young's modulus and elongation at break of the fiber mat and the film specimens (rectangular shape, 10 mm × 70 mm) was investigated using a Lloyd LRX universal testing machine (gauge length = 30 mm and crosshead speed = 20 mm min^{-1}). Wettability of the surfaces of the fiber mat and the film specimens was assessed by dynamic water contact angle measurements using a KRÜSS 10-MK2 drop shape analyzer, equipped with a syringe and a flat-tipped needle. Both the advancing and the receding water contact angles were recorded as incremental amount of water was

either added to or withdrawn from the water drops (initial volume = 10 μL).

Lastly, porosity (ε) of the fiber mat specimens was investigated based on the difference between the bulk density of the fiber mat specimens (ρ_{sc}) and that of PBSu-DCH (ρ_{PBSu}) (i.e., about 1.3 g cm^{-3}), according to the following equation:

$$\varepsilon(\%) = \left[1 - \frac{\rho_{sc}}{\rho_{PBSu}}\right] \times 100. \quad (1)$$

The bulk density of the fiber mat specimens was measured by a Sartorius YDK01 density measurement kit. For comparison, the bulk density of the film specimens was also measured.

2.4. Cell culture and cell seeding

Two types of cells were used in this study: 1) mouse fibroblasts (L929) and 2) human osteosarcoma cells (SaOS-2). The cells were cultured as monolayer in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA), supplemented by 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotic and antimycotic formulation [containing penicillin G sodium, streptomycin sulfate, amphotericin B (Invitrogen, USA)]. The medium was replaced once in every 2 d and the cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Each specimen was cut into circular discs (about 15 mm in diameter) and the discs were individually placed in wells of a 24-well tissue-culture polystyrene plate (TCPS; Biokom Systems, Poland). The specimens were sterilized in 70% ethanol for 30 min and then washed with autoclaved de-ionized water. The specimens were later immersed in DMEM overnight. For cell seeding, SaOS-2 from the cultures were trypsinized [0.25% trypsin containing 1 mM EDTA (Invitrogen, USA)], counted by a hemacytometer (Hausser Scientific, USA), and seeded at a density of about 36,000 cells cm^{-2} on the specimens and empty wells of the 24-well TCPS (positive control). Each specimen was pressed with a metal ring (about 12 mm in diameter) to ensure a complete contact between the specimen and the well. The cultures were maintained at 37 °C in an incubator.

2.4.1. Indirect cytotoxicity evaluation

The biocompatibility of the e-spun PBSu-DCH fiber mats and the corresponding solvent-cast films was first assessed by indirect cytotoxicity evaluation, using L929 and SaOS-2 as reference cells, based on a procedure adapted from the ISO10993-5 standard test method. The test was conducted in empty wells of the 24-well TCPS. Extraction media were first prepared by immersing specimens cut from the fiber mat and the film samples in wells of the 24-well TCPS containing a quantity of serum-free medium (SFM; containing the same composition as DMEM, but without FBS) for 24 h. L929 and SaOS-2 were separately cultured in wells of the 24-well TCPS in serum-containing DMEM for 24 h to allow cell attachment. The cells were then starved with SFM for 24 h. After that, the medium was replaced with an extraction medium and the cells were re-incubated for 24 h. The viability of the cells cultured with fresh SFM was used as control. Finally, the viability of the cells cultured with the fresh SFM (i.e., control) and the as-prepared extraction media was determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma-Aldrich, USA) assay.

The MTT assay is based on the reduction of yellow tetrazolium salt to purple formazan crystals by dehydrogenase enzymes secreted from the mitochondria of metabolically active cells. Thus, it is the measure of the mitochondrial activity of the cells, which, for a given type of cells, can be used to represent the number of viable cells. First, each specimen was incubated at 37 °C for 1 h with 250 μL/well of MTT solution at 0.5 mg mL^{-1} without phenol red. After incubation, MTT solution was removed and a buffer solution containing dimethylsulfoxide (DMSO; Carlo Erba, Italy) (900 μL/well) and glycine buffer (pH = 10) (125 μL/well) was subsequently added into the wells to dissolve the formazan crystals. The solutions, after 10 min of rotary agitation, were then transferred into a cuvette and placed in a thermospectronic Genesis 10 UV–visible spectrophotometer, from which the absorbance at 570 nm, representing the viability of the cells, was measured.

2.4.2. Cell attachment and cell proliferation

For the attachment study, SaOS-2 were allowed to attach on the fiber mat and the film specimens and empty wells of the 24-well TCPS for 1, 4 and 16 h, respectively. At each specified seeding time point, the viability of the attached cells was quantified by the MTT assay. Each specimen after cell seeding was rinsed with phosphate buffered saline solution (PBS; Sigma-Aldrich, USA) to remove unattached cells prior to the quantification for the cell viability. Since no studies related to the expression of attachment proteins nor the strength of the attached cells were carried out, this evaluation only served as the qualitative measure of the cell attachment. For the proliferation study, the cells were first allowed to attach on the specimens for 24 h. The proliferation of the cells on the specimens was determined on days 1, 2 and 3 after cell culturing. After the attachment period of 16 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) and the viability of the cells was then quantified by the MTT assay.

2.4.3. Morphological observation of seeded/cultured cells

After the removal of the culture medium, the cell-seeded or the cell-cultured specimens were rinsed with PBS twice and the cells were then fixed with 3% glutaraldehyde solution [diluted from 50% glutaraldehyde solution (Electron Microscopy Science, USA) with PBS] at 500 μL/well. After 30 min, they were rinsed again with and kept in PBS at 4 °C. After the cell fixation, the specimens were dehydrated in ethanol solutions of varying concentration (i.e., 30, 50, 70 and 90%, respectively) and in pure ethanol for about 2 min each. The specimens were then dried in 100% hexamethyldisilazane (HMDS; Sigma, USA) for 5 min and finally dried in air after the removal of HMDS. Finally, the specimens were mounted on copper stubs, coated with gold, and observed by SEM.

2.4.4. Alkaline phosphatase (ALP) activity of cultured cells

SaOS-2 were cultured on the e-spun PBSu-DCH fiber mat and the corresponding solvent-cast film specimens for 3, 5 and 10 d to observe the production of alkaline phosphatase (ALP). The specimens were rinsed with PBS after the removal of the culture medium. Alkaline lysis buffer (10 mM Tris–HCl, 2 mM $MgCl_2$, 0.1% Triton-X100, pH 10) (100 μL/well) was added and the specimens were scrapped and then frozen at −20 °C for at least 30 min prior to the next step. An aqueous solution of 2 mg mL^{-1} *p*-nitrophenyl phosphate (PNPP; Zymed Laboratories, USA) mixed with 0.1 M amino propanol (10 μL/well) in 2 mM $MgCl_2$ (100 μL/well) having a pH of 10.5 was prepared and added into the specimens. The specimens were incubated at 37 °C for 2 min. The reaction was stopped by 50 mM NaOH at 0.9 mL/well and the extracted solution was transferred to a cuvette and placed in the UV–visible spectrophotometer, from which the absorbance at 410 nm was measured. The amount of ALP was then calculated against a standard curve. To determine the ALP activity, the amount of ALP had to be normalized by the amount of the total proteins. In the protein assay, the specimens were treated in the same manner as previously described up to the point where the specimens were frozen. Then, bicinchoninic acid (BCA; Pierce Biotechnology, USA) solution

was added into the specimens. The specimens were incubated at 37 °C for 2 min. The absorbance of the medium solution was then measured at 562 nm by the UV–visible spectrophotometer and the amount of the total proteins was calculated against a standard curve.

3. Results and discussion

The present contribution can be divided into two parts. The first part focuses on the e-spinning of PBSu-DCH to find the optimal condition for the fabrication of PBSu-DCH fibers with a suitable morphology (i.e., smooth fibers without the presence of beads), by carefully investigating the effects of solution concentration, applied electrical potential and collection distance on the morphology of the obtained fibers. The second part then focuses on the characterization of the e-spun PBSu-DCH fiber mats which had been fabricated with the optimized condition for their thermal, mechanical, physical and *in vitro* biological characteristics. Comparisons were made against the corresponding solvent-cast films.

3.1. E-spinning of PBSu-DCH fiber mats

The effect of the concentration of PBSu-DCH solutions on the morphology of the e-spun fibers was first investigated. Prior to the e-spinning, the PBSu-DCH solutions in 90:10 v/v DCM/TFA were characterized for their shear viscosity and electrical conductivity (see Table 1). A monotonous increase in both of the property values with an increase in the solution concentration was evident. The observed increase in the shear viscosity of the solutions was due obviously to the increase in the number of chain entanglements when the solution concentration increased. On the other hand, the observed increase in the electrical conductivity of the solutions could be a result of the increase in the ionic species associated with the acidolytic degradation of PBSu-DCH when it is in contact with the strong acid TFA ($pK_a = 0.59$) in the mixed solvent system. The hydrolysis could occur at a urethane linkage [i.e., a product between an isocyanate group of 1,6-diisocyanatohexane (or hexamethylene diisocyanate, HDI) and a hydroxyl group of 1,4-butanediol residue], an amide linkage (i.e., a product between an isocyanate group of HDI and a carboxylic group of succinic acid residue) or an ester linkage of a PBSu-DCH chain.

Though not completely related to the hypothesized acidolytic degradation of PBSu-DCH with TFA, TFA has been used to cleave *tert*-butyloxycarbonyl group (tBOC) used to protect the amino group of a carboxyterminal amino acid during polypeptide or protein synthesis [27,28]. Wang et al. [29] suggested that TFA could be used to trigger the degradation of polycaprolactone (PCL) segments through acid-catalyzed hydrolysis, while Cohen et al. [30] reported that TFA selectively cleaved bonds between polyester repeating units in polyesters and polyurethanes. Notwithstanding, Kenwright et al. [31] showed that a direct interaction of poly(ethylene terephthalate) (PET) with TFA only resulted in the modification of the hydroxyl end groups of PET, without a significant degradation of the main chains.

Table 1
Shear viscosity and electrical conductivity of PBSu-DCH solutions ($n = 3$).

Solution concentration (% w/v)	Shear viscosity (mPa s)	Electrical conductivity ($S\,cm^{-1}$)
18	132 ± 5	0.59 ± 0.02
20	151 ± 6	0.69 ± 0.01
22	166 ± 4	0.75 ± 0.01
24	176 ± 5	0.80 ± 0.01

E-spinning of these PBSu-DCH solutions into ultrafine fibers was first carried out under an electrical potential of 17 kV that was applied over a collection distance of 20 cm. Fig. 1 shows representative SEM images of the obtained fibers. At concentrations lower than 18% w/v (results not shown), discrete beads were the dominant products. At such low solution concentrations, surface tension of the solutions was the dominant factor, which was responsible for the capillary instability of the charged jet [1]. At 18% w/v, a combination of smooth and beaded fibers was evident (see Fig. 1a). At this concentration, the increased chain entanglements allowed the jet to be more efficient in stabilizing the jet against the formation of the beads [1]. Increasing the solution concentration to 20 and 22% w/v resulted only in the formation of smooth fibers (see Fig. 1b,c). At 24% w/v, fibers with elongated beads, in addition to the smooth fibers, reappeared (see Fig. 1d). The formation of the fibers with elongated beads could be a result of a combination of factors, e.g., the relatively high shear viscosity and the rather fast evaporation of the solvent, that caused a droplet of the polymer solution at the tip of the nozzle to gel up rather rapidly and, upon being ejected from the nozzle, did not have enough time to become fully stretched. As for the size of the obtained fibers, the increase in the solution concentration, hence the shear viscosity, was responsible for the observed increase in the diameters of the obtained fibers [32], e.g., from 159 ± 10 nm at 18% w/v to 248 ± 8 nm at 24% w/v.

The effects of the applied electrical potential and the collection distance on the morphology of the e-spun fibers were further investigated with the 22% w/v PBSu-DCH solution in 90:10 v/v DCM/TFA and the results are shown in Table 2. At a given collection distance, an increase in the applied electrical potential used to charge the polymer solution generally resulted in the observed decrease in both the number and the size of the beads. The increase in the applied electrical potential was responsible for the increase in the electrical forces imposed on a droplet of the polymer solution at the tip of the nozzle as well as the subsequent, ejected jet. During e-spinning, the accumulation of the droplet of the polymer solution at the tip of the nozzle may not be constant. Over time, the size of the droplet grew, despite a constant ejection of the jet from the apex of the droplet cone. The ejection of the overgrown droplet could present either as a discrete droplet or as a part of the jet, which for the latter, under the action of the electrical forces, would become elongated. As the applied electrical potential increased, the size of the ejected droplets on average (over a period of time) would decrease, due to the increase in the electrical forces acting on them (viz. the ejection of droplets occurred as soon as the electrical forces overcame both the viscoelastic force and the surface tension).

At a given applied electrical potential, a combination of smooth fibers and discrete and/or elongated droplets was observed at the collection distances of 15 and 25 cm. Interestingly, at the collection distance of 20 cm, smooth fibers were the dominant feature, regardless of the applied electrical potentials investigated. At the shortest collection distance investigated (i.e., 15 cm), the electrical forces acting on a droplet of the polymer solution at the tip of the nozzle and the ejected jet were the greatest, thus the likelihood for the observed formation of either discrete or elongated beads. At the longest collection distance investigated (i.e., 25 cm), on the other hand, the lowest electrical forces exerting on both the polymer droplet and the ejected jet, in combination with the long path distance and the relatively high volatility of the solvent used, should be the main contributors for the presence of the elongated beads observed, as electrical forces were not great enough to fully elongate the ejected droplets prior to their solidification. With regards to the size of the obtained fibers, an increase in either the applied electrical potential or the collection distance generally decreased the fiber diameters, which could be attributed to the

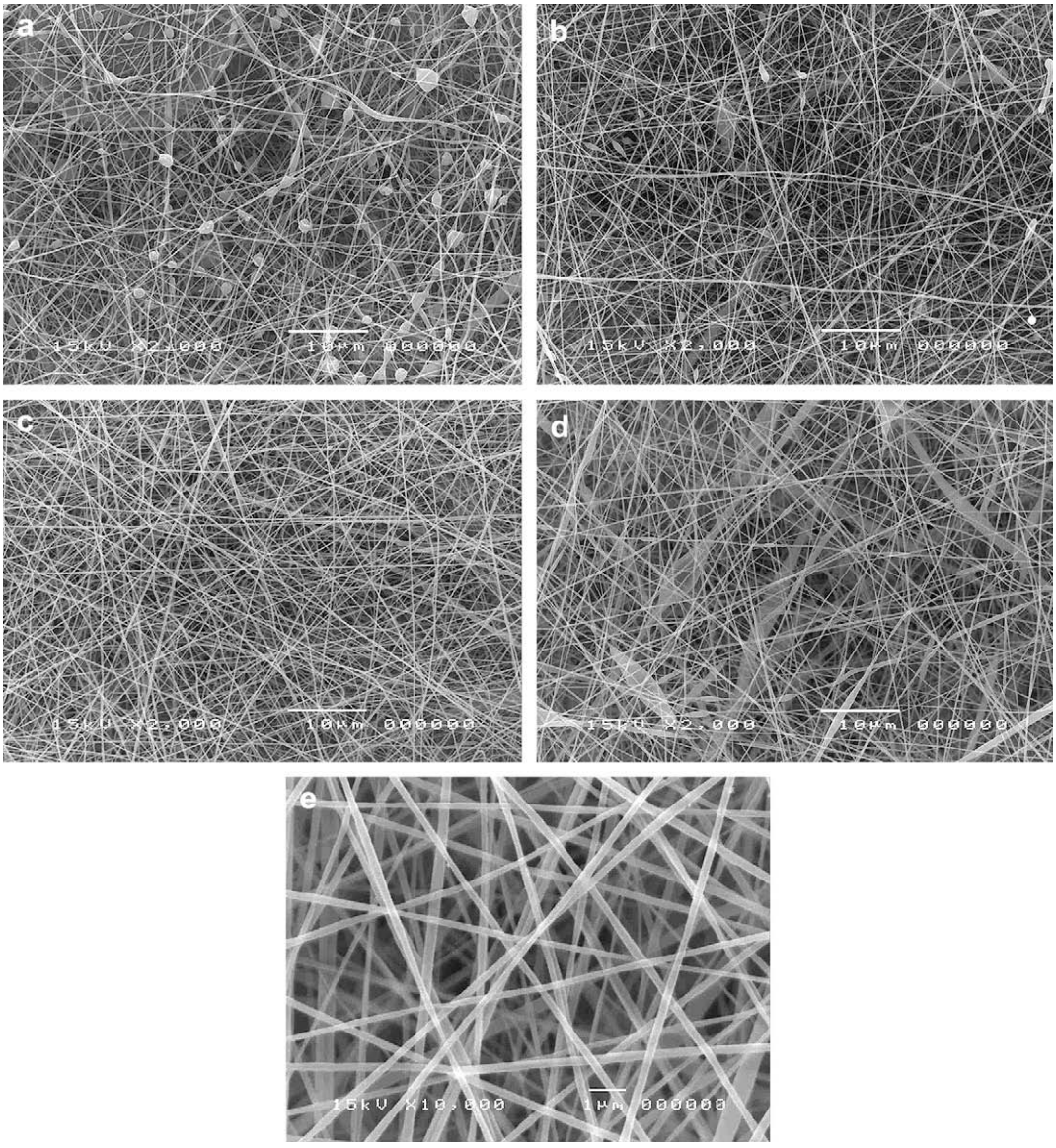


Fig. 1. Representative SEM images (scale bar = 10 μm and magnification = 2000×) illustrating the effect of solution concentration on morphology of PBSu-DCH fibers that had been electrospun from PBSu-DCH solutions in 90:10 v/v dichloromethane/trifluoroacetic acid at various concentrations: (a) 18, (b) 20, (c) 22 and (d) 24% w/v, under an applied electrical potential of 17 kV and a collection distance of 20 cm. Panel (e) represents a SEM image of (c) at a higher magnification (i.e., scale bar = 1 μm and magnification = 10,000×).

increase in the electrical forces acting on the jet and the increase in the path distance of the jet, respectively [32].

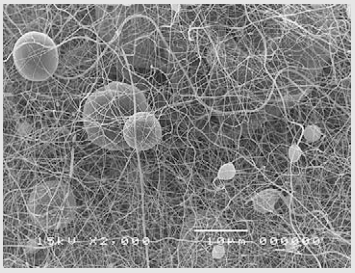
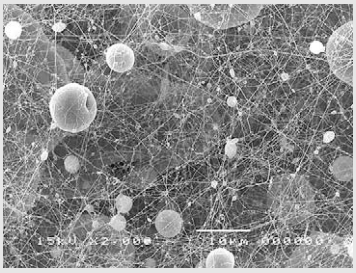
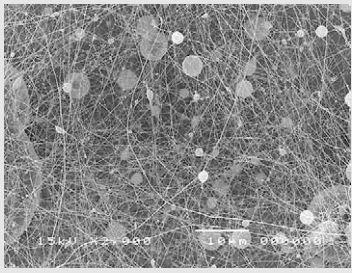
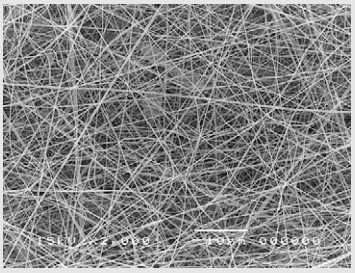
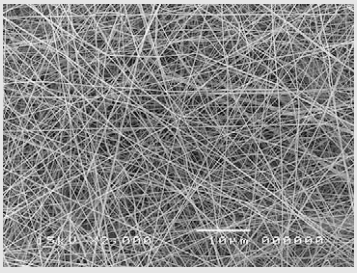
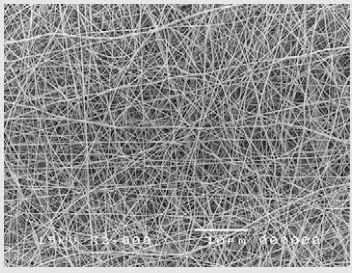
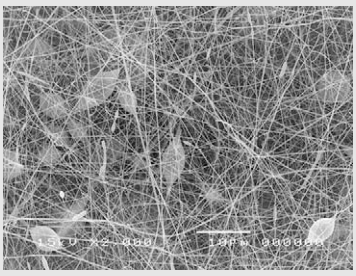
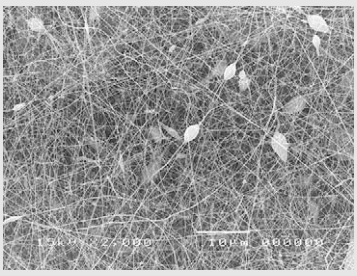
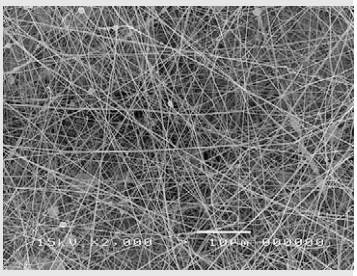
Of all the spinning conditions investigated, it is apparent that the e-spinning of 22% w/v PBSu-DCH solution in DCM/TFA under the applied electrical potential of 17 kV and the collection distance of 20 cm produced exclusively the smooth fibers (see Fig. 1e). The diameters of these fibers were 172 ± 3 nm. Jeong et al. [24] reported that the average diameters of the e-spun fibers from PBSu solutions in various solvent systems (i.e., chloroform/2-chloroethanol, dichloromethane/2-chloroethanol and chloroform/3-chloro-1-propanol) ranged between 125 and 315 nm. In addition, Liu et al. [25] reported that, for the e-spun fibers from PBSu solutions in chloroform, the diameters of the obtained fibers ranged between 200 nm and 1 μm.

3.2. Thermal properties of PBSu-DCH fiber mats and films

Thermal properties of the e-spun PBSu-DCH fiber mat and the corresponding solvent-cast film specimens as well as those of the as-received pellets were characterized by DSC and TGA. In DSC, each specimen was heated from 25 to 150 °C at a rate of 10 °C min^{-1} to observe the melting behavior of the original crystalline state of the specimen. In TGA, each specimen was heated from 30 to 500 °C at a rate of 10 °C min^{-1} in an inert atmosphere to observe its thermal degradation behavior. Table 3 summarizes the melting temperatures (T_m; as obtained from HEAT1), the enthalpies of fusion (ΔH_f; as obtained from HEAT1) as well as the corresponding apparent degree of crystallinity values, and the thermal degradation temperatures (T_d; as obtained from TGA) of the studied materials.

The melting temperatures (T_m) of the fiber mat, the film, and the as-received pellet specimens of PBSu-DCH were 113.4, 111.2 and 114.1 °C, respectively. Miyata and Masuko [19] reported a T_m value of about 110 °C for quenched PBSu films, while Jeong et al. [24] and Liu et al. [25] found that the T_m values for their e-spun PBSu fiber mats were about 114 and 108 °C, respectively. Clearly, our values agreed well with these previous reports. The values associated with the enthalpy of fusion of these specimens were 73.9, 96.9 and 85.1 J g^{-1}, respectively, which corresponded to the apparent degree

Table 2
Representative SEM images (scale bar = 10 μm and magnification = 2000×) illustrating the effects of applied electrical potential and collection distance on morphology of PBSu-DCH fibers that had been electrospun from 22% w/v PBSu-DCH solution in 90:10 v/v dichloromethane/trifluoroacetic acid.

Collection distance (cm)	Applied electrical potential (kV)		
	14	17	20
15			
20			
25			

of crystallinity values of 37.0, 48.4 and 42.6%, respectively. Jeong et al. [24] reported similar values of the apparent degree of crystallinity of the e-spun PBSu fiber mats to be about 37– 42%, while Liu et al. [25] reported a slightly greater value of about 45%. The lower crystallinity value that was observed for the e-spun fiber mats in comparison with those of the films and the as-received pellets should be attributed to the rapid solidification of the charged jet upon its acceleration to the collector. This can be understood as the rapid solidification hinders the development of crystallinity, a result of the rapid decrease in the chain mobility [24]. The latter may have an additional, confinement effect from the one dimensional character of the fibers, as the growth of the crystalline entity in the radial direction of the fibers is somewhat restrained. Lastly, PBSu-DCH, whether in the form of the e-spun fiber mats, the solvent-cast films, or the as-received pellets, exhibited similar thermal degradation temperatures (T_d) of about 380 °C.

Table 3
Some thermal characteristics of electrospun PBSu-DCH fiber mats and corresponding solvent-cast films as well as those of as-received PBSu-DCH pellets ($n = 3$).

Thermal property	Electrospun fiber mats	Solvent -cast films	As-received pellets
Melting temperature, T_m (°C)	113.6	111.2	114.1
Enthalpy of fusion, ΔH_f (J g^{-1})	73.9	96.9	85.1
Apparent degree of crystallinity (%)[a]	37.0	48.4	42.6
Degradation temperature, T_d (°C)	381.5	379.1	380.5

[a] Based on the equilibrium enthalpy of fusion value of 200 J g^{-1} [19].

3.3. Mechanical and physical properties of PBSu-DCH fiber mats and films

The e-spun PBSu-DCH fiber mats and the corresponding solvent-cast films were further characterized for certain mechanical and physical properties (see Table 4). For these investigations, the thickness of both the fiber mats and the films was about 120 ± 10 μm. For the current set-up of the e-spinning apparatus, it required about 10 h to obtain the fiber mats with the specified thickness. The yield strength, the tensile strength and the Young's modulus of the fiber mats were 10.1 ± 1.0, 1.17 ± 0.06 and 43.4 ± 8.4 MPa, respectively. Much greater values were observed for the corresponding films (i.e., 24.4 ± 0.7, 2.93 ± 0.08 and 617 ± 40 MPa, respectively). The results indicated that the fiber mats were much softer than the film counterparts, which is also reflected from the fact that the elongation at break of the fiber mats was greater than that of the film counterparts (i.e., 122 ± 13 versus 47.0 ± 11.0%). Li et al. [23] reported much greater values (i.e., tensile strength = 33 MPa, Young's modulus = 0.5 GPa and elongation at

Table 4
Some mechanical and physical characteristics of electrospun PBSu-DCH fiber mats and corresponding solvent-cast films.

	Electrospun fiber mats	Solvent-cast films
Mechanical property ($n = 10$)		
Yield strength (MPa)	10.1 ± 1.0	24.4 ± 0.7
Specific yield strength (MPa cm^3 g^{-1})	24.9	17.2
Tensile strength (MPa)	1.17 ± 0.06	2.93 ± 0.08
Specific tensile strength (MPa cm^3 g^{-1})	2.88	2.06
Young's modulus (MPa)	43.4 ± 8.4	617 ± 40
Specific Young's modulus (MPa cm^3 g^{-1})	107	435
Elongation at break (%)	122 ± 13	47.0 ± 11.0
Physical property ($n = 5$)		
Advancing water contact angle (°)	114 ± 3	70 ± 3
Receding water contact angle (°)	79 ± 3	49 ± 2
Hysteresis (°)	35	21
Bulk density (g cm^{-3})	0.406 ± 0.070	1.420 ± 0.080
Porosity (%)	68.7	–

break = 126%) for the melt-pressed PBSu films (1.8 mm think @ 0.5 mm min^{-1} crosshead speed).

Both the fiber mat and the film specimens were also characterized for the wettability of their surfaces in terms of the advancing/receding water contact angles. Evidently, both water contact angle values of the e-spun fiber mats were greater than those of the film counterparts (i.e., 114°/79° versus 70°/49°), the results indicating the more hydrophobicity or the less hydrophilicity of the surface of the fiber mats in comparison with that of the films. The observed hydrophobicity of the fiber mats in comparison with that of the films should be a result of the surface roughness that introduced multiple contacting points on the water surface such that the interface between the water droplet and the fiber mat surface was not exactly solid/liquid [33]. Li et al. [23] reported a value of 59° (sessile drop method) for the 1.8-mm thick melt-pressed PBSu films. Due to the porous nature of the e-spun PBSu-DCH fiber mats, porosity is definitely an important quantity and it can be estimated from the difference between the bulk density of the fiber mats and the density of PBSu-DCH (i.e., about 1.3 g cm^{-3}). With the measured bulk density of the e-spun fiber mats of 0.406 g cm^{-3}, their porosity was estimated to be around 69%. Zhang et al. [26] reported a value of the bulk density of the e-spun PBSu fiber mats (0.25 mm in thickness) to be 0.282 g cm^{-3}, corresponding to the estimated porosity of the fiber mats of about 75%.

From an engineering point of view, comparison of the mechanical properties of different materials can be made by normalizing their property values with their respective density, i.e., the specific property values [34]. In addition to the bulk density of the e-spun fiber mats, that of the PBSu-DCH films was also measured and it was found to be about 1.420 g cm^{-3}. Based on these values, the specific mechanical properties of both the PBSu-DCH fiber mats and the films were calculated (see Table 4). Evidently, the specific yield and the specific tensile strengths of the fiber mats were slightly greater than those of the film counterparts, while the specific Young's modulus of the fiber mats was still lower than that of the films.

3.4. *In vitro biological characterization of PBSu-DCH fiber mats and films*

3.4.1. *Indirect cytotoxicity evaluation*

Even though it was shown that PBSu is biocompatible with the native rat osteoblasts [23], the assessment with regards to the biocompatibility of PBSu-DCH after having been fabricated into fibrous and film forms needs to be carried out since DCM/TFA and chloroform, known toxic substances, were used as the respective solvent systems for the preparation of PBSu-DCH into the e-spun fiber mats and the solvent-cast films. Both human osteosarcoma cells (SaOS-2) and mouse fibroblasts (L929) were used in the assessment. L929 were used here just to comply with the ISO10993-5 standard test method. For both types of cells, about 36,000 cells/well were seeded in empty wells of TCPS.

The viabilities of the cells after having been cultured with an extraction medium, prepared by immersing each of the fiber mat and the film specimens in wells of TCPS containing a measured quantity of SFM for 24 h, are reported in Fig. 2. The viabilities of the cells that had been cultured with the fresh SFM were used as control. Evidently, for each type of the cells, all of the extraction media from both the fiber mat and the film specimens were non-toxic to the cells, as the absorbance values, signifying the viability of the cells, were all greater than that of the control. Interestingly, L929, which had been cultured with either the fresh SFM or the extraction media, exhibited the absorbance values that were greater than did SaOS-2. A similar behavior has also previously been observed and reported [10,14]. This could be a result of the difference in the mitochondrial activities of the two cell types or simply a result of the difference in the attachment and/or the proliferation of the two cell types on TCPS (during the re-incubation period of 24 h). Based on the results obtained, both the PBSu-DCH fiber mats and the films released no substance in the level that was harmful to the cells and they could be further evaluated for their suitability as substrates for bone cell culture.

3.4.2. *Cell attachment, cell proliferation and morphology of seeded/cultured cells*

The ability to support the attachment and to promote the proliferation of cells is a prerequisite property of a functional scaffold. In the present studies, the potential for use of the e-spun PBSu-DCH fiber mats as scaffolds for bone cell culture was assessed using the human osteosarcoma cells (SaOS-2). Comparative studies were also made against the corresponding solvent-cast films and TCPS (i.e., control). Fig. 3 illustrates the absorbance values signifying the viabilities of the cells that had been seeded or cultured on the surfaces of these substrates for various cell seeding or cell culturing times. For the attachment study (see Fig. 3a), SaOS-2 were seeded on the surfaces of these substrates and allowed to attach on

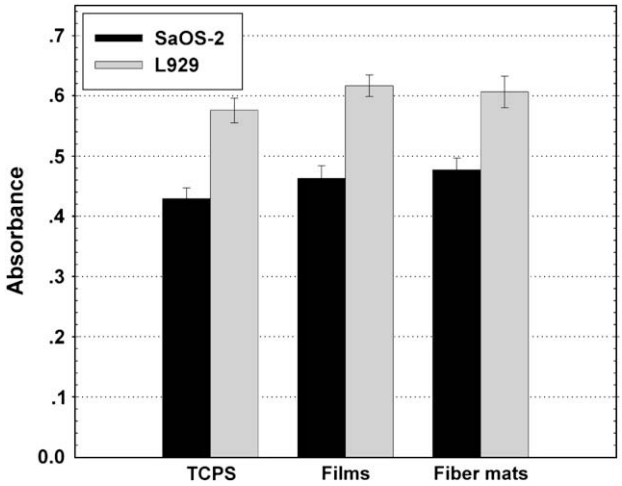


Fig. 2. Indirect cytotoxicity evaluation of electrospun PBSu-DCH fiber mats and corresponding solvent-cast films based on the viability of human osteosarcoma cells (SaOS-2) and mouse fibroblasts (L929) that had been cultured with the extraction media from the specimens. The viability of the cells that had been cultured with fresh serum-free medium was used as control.

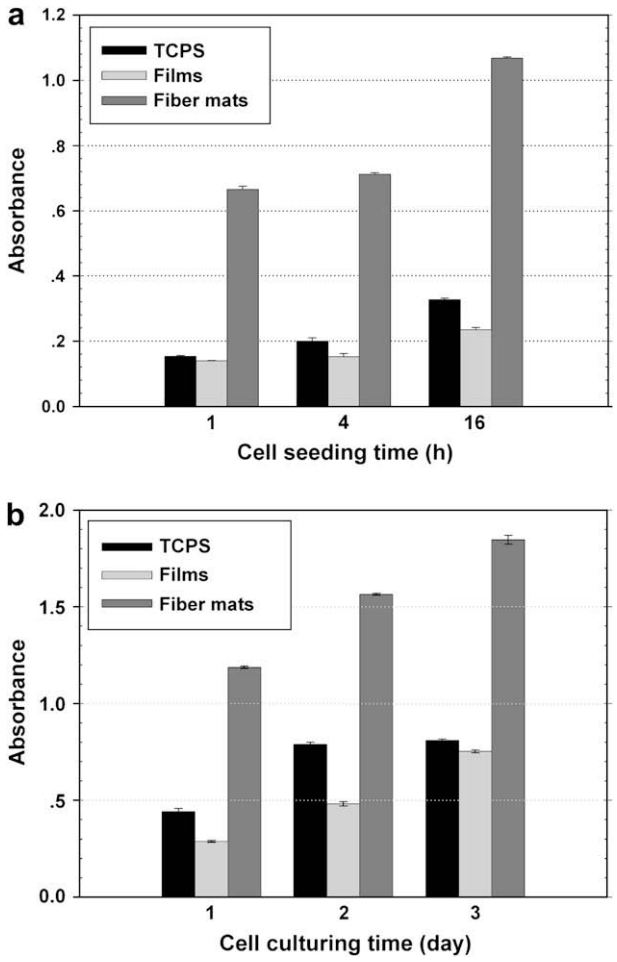


Fig. 3. Viabilities of (a) attached and (b) proliferated SaOS-2 that had been seeded or cultured on electrospun PBSu-DCH fiber mats, corresponding solvent-cast films and tissue-culture polystyrene plate (TCPS; control) as a function of cell seeding or cell culturing time.

the surfaces for 1, 4 and 16 h. For any given substrate, the viability of the attached cells increased monotonically with an increase in the cell seeding time. At any given cell seeding time point, the viability of the cells that attached on the surface of the fiber mats was significantly greater than those on the corresponding films and TCPS, which could be a result of the surface area of the fiber mats that is supposedly greater than those of the films and TCPS.

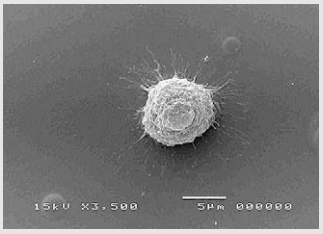
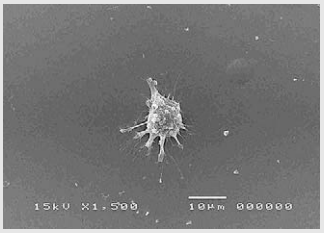
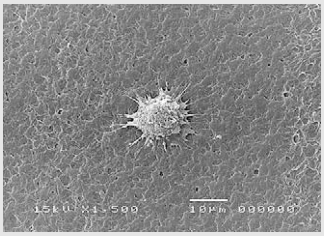
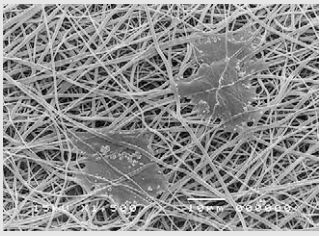
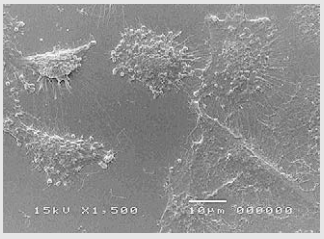
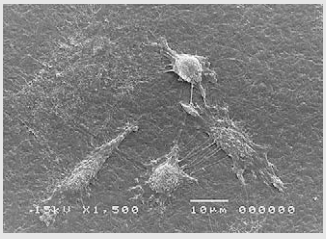
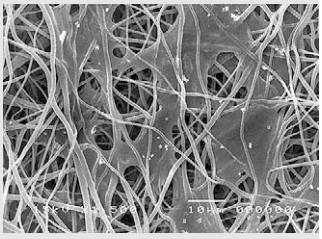
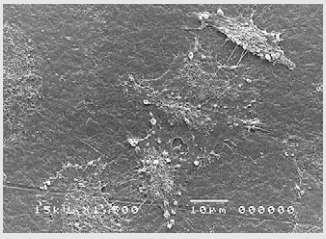
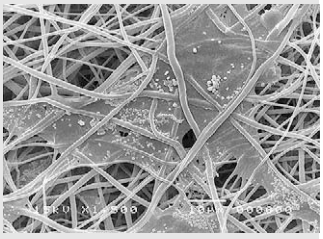
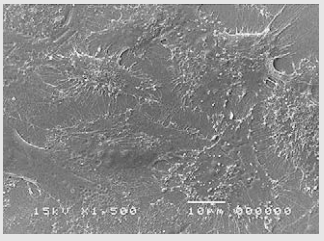
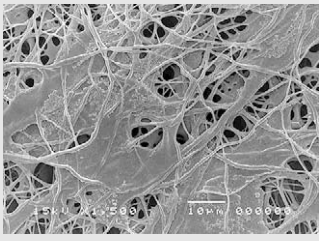
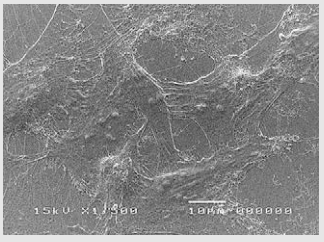
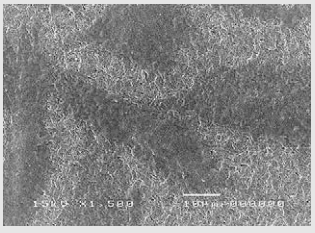
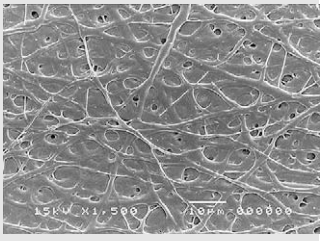
For the proliferation study (see Fig. 3b), the cells, after having been allowed to attach for 16 h, were cultured on the surfaces of the different substrates for 1, 2 and 3 day(s). Interestingly, while the viabilities of the proliferated cells on both the fiber mat and the film substrates increased monotonically with an increase in the cell culturing time, that of the cells on TCPS appeared to reach a constant value after they had been cultured on the surface for 2 days. The saturation in the viability of the cells that had been cultured on TCPS during 2 and 3 days of cell culturing should be due to the cells that might have proliferated until they reached the confluence as the cells fully covered the 2D surface of TCPS as a monolayer. On the other hand, due to the lowest attachment of the cells on their 2D surface, the films showed a steady increase in the viability of the proliferated cells to reach a level that was slightly lower than that on TCPS on day 3. For the e-spun fiber mats however, the viability of the cells that had been cultured on their surface, at any given cell culturing time point, was the greatest. This could be attributed to the roughness of the fiber mat surface that provided greater surface area upon which the cells could proliferate. Similar behavior was observed for SaOS-2 that had been cultured on the surfaces of the fiber mat and the film substrates of other biodegradable polyesters, e.g., poly(caprolactone) (PCL) [11] and poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and their 50/50 w/w blend [14].

SEM observation of the cells that had been seeded or cultured on the surfaces of the e-spun PBSu-DCH fiber mats, the corresponding solvent-cast films and the glass substrates was investigated further to observe the morphology of the cells, the intercellular interaction and the interaction of the cells towards the surfaces. The glass substrates were used as the control surface instead of the TCPS. The seeded and the cultured cells were examined in two magnification levels, i.e., low and high. The purpose of the low-magnification SEM images (i.e., 500×) is to display the number and the morphology of the cells in general over a wide observation area (see Supplementary Data), while that of the high-magnification SEM images (i.e., 1500× or 3500×) is to provide a closer examination on the morphology of the individual cells (see Table 5).

For any given type of the substrates, the number of the cells generally increased with an increase in the cell seeding/culturing times. Despite assuming the round cell shape, the majority of the cells that had been seeded on the glass substrates for 1 and 4 h started to show an evidence of filopodia (i.e., slender cytoplasmic projections extending from the leading edge of the cells), which suggests the probability of the cells to attach onto the surface. The full cytoplasmic expansion of the cells on the glass substrates was realized after they had been seeded on the glass surface for 16 h. Interestingly, the number of the cells that had been cultured on the glass surface for 2 days was not much different from that of the cells that had been cultured for 3 days, a result that is in accord with the observed constancy in the viabilities of the cells that had been cultured on TCPS for 2 and 3 days (see Fig. 3b). On the other hand, the majority of the cells that had been seeded on the surface of the PBSu-DCH films for 1 h were still round with no evidence of any kind of cytoplasmic projections. At 4 h after cell seeding, while some cells still appeared in their round shape, many of the cells showed an evidence of filopodia, with few cells even appeared in their expanded state. At 16 h after cell seeding, while a large number of cells were well expanded, certain cells were still round with an evidence of filopodia that helped their anchorage onto the surface. The full cytoplasmic expansion of the cells on the film surface was observed after 1 day of cell culturing.

Interestingly, almost all of the cells that had been seeded on the PBSu-DCH fiber mats for 1 h were round, with no evidence of filopodia. In the first approximation, the result may have suggested that the attachment of the cells on the fibrous substrate was poor. Careful consideration with regards to the size and the shape of the cells clearly showed that these cells were larger than those observed on the surfaces of both the glass substrates and the PBSu-DCH films and that the cells appeared to be more flat, suggesting that the cells already assumed their expanded state on the surface. In addition, since the size of the cells was much larger than the diameters of the individual fibers, each cells adhered to the fibrous surface on multiple fibers. A similar result was observed with SaOS-2 that had been cultured on the surface of the PCL fiber mats [11], which also featured the individual fibers that were much smaller (i.e., about 950 nm on average) than the size of the cells. The expanded state of the cells was evident at all other cell seeding/culturing times, with the number of cells monotonically increasing to fully cover the surface of the fibrous substrate on day 3 after cell culturing. Interestingly, at time points greater than or equal to 4 h, the seeded or the cultured SaOS-2 seemed to integrated particularly well into the underlying porous structure of the fiber mats. This feature was not observed with the cells that were seeded or

Table 5
Representative high-magnification SEM images (i.e., 3500× or 1500×) illustrating morphology of attached and proliferated SaOS-2 that had been seeded or cultured on electrospun PBSu-DCH fiber mats, corresponding solvent-cast films and glass substrates (i.e., control) as a function of cell seeding or cell culturing time.

Cell seeding/culturing time	Glass substrates	Solvent-cast films	Electrospun fiber mats
1 h			
4 h			
16 h			
1 d			
2 d			
3 d			

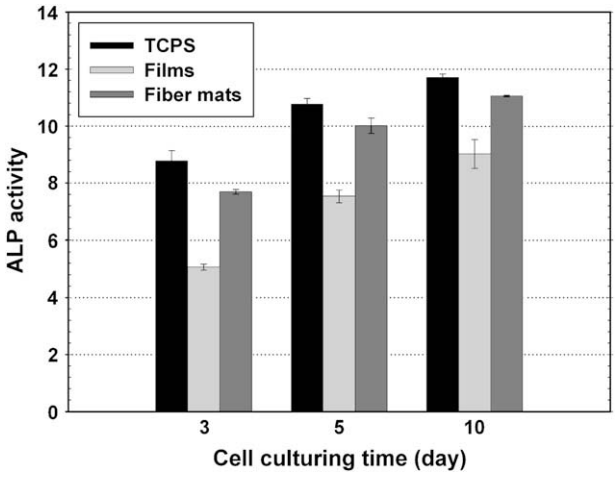


Fig. 4. Alkaline phosphatase (ALP) activity of SaOS-2 that had been cultured on electrospun PBSu-DCH fiber mats, corresponding solvent-cast films and TCPS on days 3, 5 and 10 after cell culturing.

cultured on the PCL fiber mats [11], as the cells appeared to 'float' on the fibrous surface rather than integrated into the underlying porous structure of the fibrous materials.

3.4.3. Alkaline phosphatase (ALP) activity

In addition to the ability to support the attachment and to promote the proliferation of the seeded/cultured cells, a functional scaffold should also be able to promote the differentiation of the cells such that the cells can completely express their phenotypic functions. Here, the ability of SaOS-2 to express alkaline phosphatase (ALP), an enzyme believed to be responsible for the cleavage of the inorganic phosphate for mineralization of osteoblasts [35], was used to evaluate the ability of the e-spun PBSu-DCH fiber mats and the corresponding solvent-cast films in supporting the differentiation of the cultured cells. The results of the analyses are graphically shown in Fig. 4. The ALP activity of the cells that had been cultured on TCPS was used as the control condition.

For any given type of the substrates, the ALP activity was found to monotonically increase with an increase in the cell culturing time. At any given time point, the cells that had been cultured on TCPS expressed the greatest amount of ALP, followed by those on the fiber mats. Since it is a known fact that the expression of ALP occurs immediately after the down-regulation of the proliferation period of the cultured bone cells [12,36], which could be marked by the physical contacts of the proliferated cells. According to the MTT results shown in Fig. 3b and the low-magnification SEM images of the cultured cells shown in Supplementary Data, the cells that had been cultured on TCPS appeared to reach the confluence after 2 days of cell culturing. It is, therefore, logical to postulate that the expression of ALP should begin shortly before or on day 2 after cell culturing. On the other hand, based on the low-magnification SEM images of the cultured cells shown in Supplementary Data, the cells that had been cultured on the fiber mats appeared to fully cover the surface on day 3 after cell culturing, while those that had been cultured on the film counterparts did not yet fully cover the surface. Based on this observation, it can be postulated that the expression of ALP of the cells that had been cultured on the fiber mats should begin on or shortly after day 3 of cell culturing, while that on the film counterparts should do so after day 3. Due to the differences in the onset times for the expression of ALP of the cells that had been cultured on the surfaces of these substrates, the observation of the ALP activities shown in Fig. 4 is well received.

4. Conclusions

In the present contribution, PBSu-DCH was successfully fabricated into ultrafine fibers by e-spinning from PBSu-DCH solutions in 90:10 v/v DCM/TFA. The effects of solution concentration, applied electrical potential and collection distance on the morphology of the obtained fibers were investigated just to find the optimal condition for fabricating e-spun PBSu-DCH fibers that were smooth. Among the various conditions investigated, PBSu-DCH was best spun from 22% w/v solution under the electrical potential of 17 kV that was applied over a collection distance of 20 cm. The average diameter of these fibers was about 170 nm. The obtained fibers were characterized for their thermal, mechanical and physical characteristics. The PBSu-DCH fiber mats exhibited the melting temperature of about 113 °C, the apparent degree of crystallinity of about 37% and the thermal degradation temperature of about 380 °C. The yield strength, the tensile strength and the Young's modulus of the fiber mats were 10.1 ± 1.0, 1.17 ± 0.06 and 43.4 ± 8.4 MPa, respectively. The fibrous nature of the materials was responsible for the relatively high values of advancing/receding water contact angles (i.e., 114°/79°) and porosity (69%). Finally, the potential for use of the e-spun PBSu-DCH fiber mats as substrates for bone cell culture was evaluated *in vitro* using human osteosarcoma cells (SaOS-2) against the corresponding solvent-cast films. The results showed that the fiber mats were superior to the films as the bone cells grown on the fiber mat surface could attach, proliferate and express ALP much better than they did on the film surface.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.polymer.2009.01.042.

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