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Preparation and Characterization of Biocompatible Electrospun Nanofiber Scaffolds

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Abstract

Nanoscale fibers were prepared for the fabrication of scaffolds by using a strong electrostatic field on the polymer solution. Electrospinning is widely applied for production of drug delivery, tissue engineering, and regenerative medicine systems as well as biosensors and enzyme immobilization. Nanofibers, thanks to their high surface area to volume ratio, can also mimic the extracellular matrix, thus it has been recognized as a suitable technique for the fast fabrication of scaffolds. This article demonstrates the fabrication of several nanofibrous scaffolds from biopolymers such as polycaprolactone, poly(lactic acid), poly(lactide-co-glycolide), poly(lactide-co-caprolactone) and poly(hydroxybutyrate-co-hydroxy valerate). The characterization and comparison of the scaffolds were achieved based on the morphology and surface characteristic of the nanofibers. The samples showed hydrophobic characteristic, thus a plasma surface treatment was applied successfully to increase hydrophilicity and the effect of the treatment was evaluated based on the wettability and the change in elemental composition of the surface based on X-ray photoelectron spectroscopy.

Keywords

electrospinning, nanomaterials, scaffolds, plasma treatment

1 Introduction

Nanotechnology and nano-scale science have been a major part of research in the past few decades. The increasing need for discovering something new on a nano-scale level encouraged scientists to develop nanofibers with controllable pore structure [1, 2] for several fields of application such as filtration [3-5], military protective clothing [6-8], nanosensors [9, 10], wound dressings [11-13], drug delivery systems [14-17], enzyme immobilization [18, 19] and scaffolds for tissue engineering [20, 21].

Nanofibers can be manufactured with several methods like drawing [22, 23], template synthesis [24, 25], self-assembly [26], phase separation [27, 28] and electrospinning [29-32]. The last one is the most commonly used technique as it has been bursting into the industry in the past few years. It is a quite simple, versatile and reliable method to produce nanofibers from synthetic and natural polymers with diameters in the submicron scale [33]. Electrospinning involves the application of a high electric field to generate nanofibers from a charged polymer solution or melt. Electrospinning parameters can be changed to affect the properties of the nanofibers [14]. These parameters can be the concentration of the polymer solution, polymer molecular weight, conductivity of the polymer solution, voltage, flow rate of the solution, solvent and the distance between the tip and the collector. Additives to the solution can play a major role in controlling the properties of the solution, such as electrical conductivity, dielectric constant, surface tension, and viscosity [34].

It has been determined that nanofibrous structures can create excellent artificial scaffolds for cell cultivation by mimicking the fibrous structure of the extracellular matrix (ECM) as the size of the fibers can be similar to the structure of the ECM. Owing to this advantageous feature, scaffolds have the ability to be seeded with cells and

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engrafted into the human body to stimulate regeneration and regrowth of tissues [31]. These nanofibrous scaffolds have a huge impact on cell migration, embedding, proliferation, and differentiation. The nanofibrous scaffolds can make it possible to grow tissues such as skin [35, 36], muscle [37, 38], nerves [39-41], veins [42-44], bone [45, 46], cartilage [47, 48] and ligament [49, 50].

Increased academic interest have shown recently in exploiting the electrospinning technique to produce nanofibers from biocompatible polymers such as polycaprolactone (PCL) [51], poly(lactic acid) (PLA) [52, 53], poly(-lactide-co-glycolide) (PLGA) [54, 55], polyurethanes [56], silk fibroin [57], collagen [58, 59], hyaluronic acid [60], cellulose [61] and their blends [62, 63].

In the present work, fabrication and optimization of nanofibrous scaffolds from PCL, PLA, PLGA, poly(-lactide-co-caprolactone) (PLC) and poly(hydroxybutyrate-co-valerate) (PHB / HV) biocompatible polymers were achieved using electrospinning method. The scaffold prepared from these biocompatible polymers is a possible choice for the cultivation of cells for different tissue engineering application. Although these polymers are biocompatible, the hydrophobicity of the scaffolds must be reduced in order to promote cell adhesion and migration. Plasma treatment was applied to eliminate this adverse feature. Characterization and comparison of the scaffolds were accomplished based on morphology and surface characteristic.

2 Experimental

2.1 Materials

The polymers used in the experiment were the following: polycaprolactone (PCL, Perstorp, Capa™ 6800, M_w = 80,000). poly(lactic acid) (PLA, Purasorb® PL 24, M_w = 339,000), poly(lactide-co-glycolide) (PLGA, Purasorb® PLG 8523 (85 % PLA), M_w = 362,000) and poly(lactide-co-caprolactone) (PLC, Purasorb® 8516 (85 % PLA), M_w = 221,000) was purchased from Corbion (Netherlands). Polyhydroxybutyrate (PHB, M_w = 973,000) and poly(hydroxybutyrate-co-hydroxy valerate) (PHB / HV, type L®, 87 % PHB, M_w = 600,000) were obtained from Biomer (Germany). Chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and N,N-dimethylformamide (DMF) was obtained from Merck Millipore (USA).

2.2 Electrospinning process

To perform electrospinning, an infusion pump (Aitecs SEP-10S Plus syringe pump, Lithuania) was used and high voltage provided by a direct current power supplier (NT-35 High Voltage DC Supply MA2000, Hungary) was applied on an electrostatic spinneret (with an inner diameter of 0.5 mm). Nanofibers were collected on conventional aluminum foil immobilized to the grounded collector plate. The high voltage, the collector distance, and the dosing speed were optimized for each solution. All of the experiments were carried out at room temperature.

2.3 Scanning electron microscopy (SEM)

The morphology of the nanofibers and the surface of the scaffolds were examined by a JEOL JSM-6380LA scanning electron microscope within the settings of 15 kV acceleration voltage and 10-15 mm sample distance from the microscope in high vacuum. The samples were placed on a copper tool and coated with gold in argon atmosphere in order to avoid electrostatic charging. The experiments must have been performed quickly to prevent the nanofibers from melting.

2.4 Surface treatment by plasma

Since the nanofibers are electrostatically charged and quite hydrophobic, surface treatment by plasma is inevitable to increase the hydrophilicity of the scaffolds. The nanofibrous structures were treated with a Femto V1 (Diener Electronic Plasma Surface Technology, Ebhausen, Germany) apparatus at 30 W in high vacuum. The duration of the plasma treatment was optimized using PCL scaffolds.

2.5 Water contact angle measurement

The measurement of the contact angle has been performed using the sessile drop technique. After applying a drop of distilled water (20 μL) on the scaffold, a photo was taken of the sample carefully, positioning the contact of the drop in line with the camera's lenses. The contact angles were measured digitally and noted for further use.

2.6 X-ray photoelectron spectroscopy (XPS)

XPS tests were performed using a Kratos XSAM 800 (Manchester, UK). The samples were induced by Mg Kα radiation at 1258.6 eV energy level. The analizator was working in fixed analyzer transmission (FAT) mode with the setting of the transmission window at 40 eV energy level. Small resolution spectra were registered with the step size of 0.5 eV and high-resolution spectra with 0.1 eV. Qualitative evaluation of spectra was carried out with Kratos Vision 2 and the quantitative evaluation with XPS MultiQuant computer program.

3 Results and discussion

3.1 Preparation of nanofibrous scaffolds

Nanofibrous scaffolds were prepared by electrospinning technique for the purpose of cell cultivation and tissue engineering. In the experiments, the following polymers were used and compared: PCL, PLA, PLGA, PLC and PHB / HV (Fig. 1).

The structure of the nanofibrous system and the diameter of the fibers have a huge impact on the *in vitro* and *in vivo* availability of the scaffolds, so the optimization of the electrospinning technology was necessary.

Since the concentrations of the polymer solution have great influence on fiber diameter during electrospinning, several solutions of different concentrations were prepared from PCL, PLA, PHB / HV, PLC and PLGA polymers. The optimal concentrations for each polymer are summarized in Table 1.

The polymers were dissolved in the mixture of halogenated organic solvents and dimethyl-formamide (DMF). DMF has high dielectric constant and is widely used as an addition to form a mixture solvent system to obtain ultrathin fibers with uniform distribution [34].

The electrospinning parameters such as collector distance (30-35 cm), flow rate (7-9 mL/h), and voltage (25 kV) had been optimized for each polymer in order to eliminate the beads and other surficial defects on the scaffolds. While optimizing the parameters, the aluminum foil was applied on the collector and later when using the optimal settings, inert foils were used. Poly(ethyleneterephthalate) (PET), polypropylene (PP) and PLA foil were tested and the PP foil was chosen for further investigation based on the good process handling. The electrospinning of biocompatible polymers on an inert carrier makes it possible to test the potential applicability of scaffolds in an easy to use plate system.

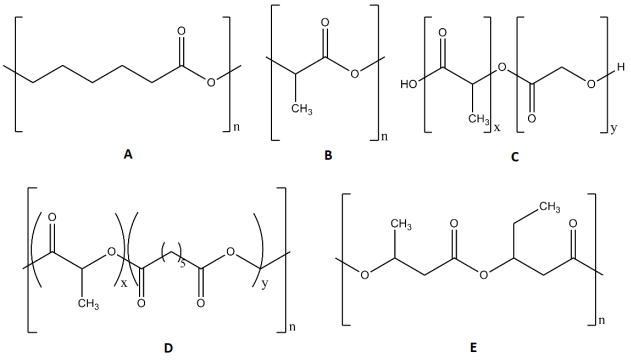

Fig. 1 Structure of the applied polymers: PCL (A), PLA (B), PLGA (C), PLC (D), PHB / HV (E).

Table 1 Optimized solution concentrations of different polymers.

Polymer	Concentration (w/V)%	Solvent
PCL	12.0	dichlormethane:DMF 1:1
PLA	5.0	chloroform:DMF 6:1
PHB / HV	12.0	chloroform:DMF 4:1
PLC	10.0	chloroform:DMF 6:1
PLGA	6.0	chloroform:DMF 6:1

3.2 Morphology

Optical microscope and scanning electron microscope (SEM) were used to analyze the structure of the nanofibrous scaffolds and the morphology of the fibers prepared using the optimal solution compositions (Fig. 2, Table 2).

SEM image of the polymers showed that the fibers had random orientation and the diameter of the fibers was submicron sized, thus the electrospinning proved to be satisfactory. Fibers in a submicron range are promising for cell cultivation since this range of fiber diameters is suitable for the biomimetics of the extracellular matrix. The density of the fibers was similar; however, the PHB / HV scaffold consisted of a thick layer of fiber.

3.3 Surface characteristic

The scaffolds were hydrophobic, thus low-pressure air plasma surface treatment was carried out to increase the wettability of the samples [64, 65]. The optimization was achieved to make the samples hydrophilic for the use of cell cultivation. The wettability of the nanofibrous scaffolds was quantified by contact angle measurements to evaluate the effect of plasma treatment (Table 3). The samples had high contact angle and hydrophobic characteristic before the plasma treatment. However, the PHB / HV scaffold had better wettability, because the contact angle is affected not only by the surface chemistry but also by the surface roughness [66, 67]. Based on the SEM images the PHB / HV scaffolds had different fiber density and surface roughness (Fig. 2).

Table 2 Average fiber diameters average and standard deviation (SD) for the scaffolds

Polymer	Polymer concentration (%)	Average fiber diameter (nm)	SD (%)
PCL	12.0	655	22 %
PLA	5.0	763	15 %
PLGA	6.0	643	23 %
PLC	10.0	481	20 %
PHB / HV	12.0	769	20 %

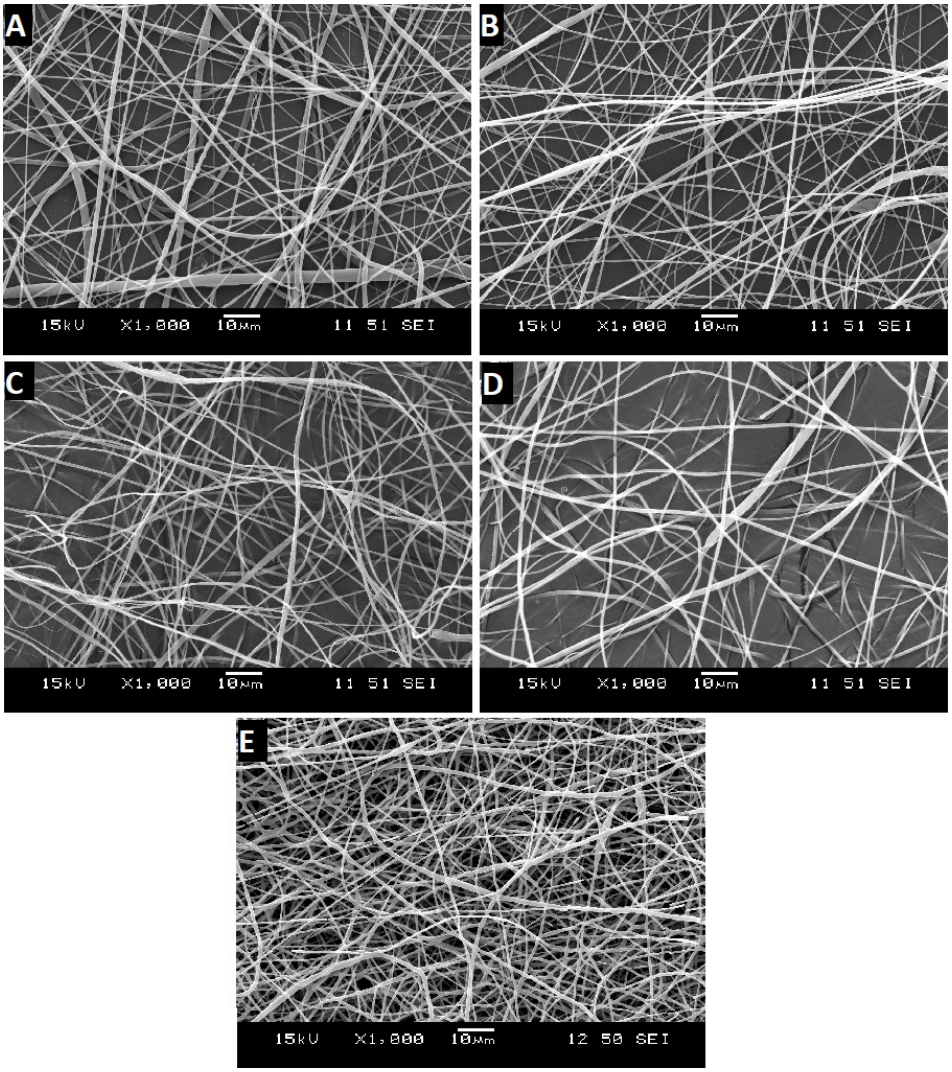

Fig. 2 SEM images of nanofibrous scaffolds: PCL (12.0 %) (A), PLA 5.0 % (B), PLGA 6.0 % (C), PLC 10.0 % (D), PHB / HV 12.0 % (E)

Table 3 Contact angle of distilled water on the scaffold surface before the plasma treatment, right after and 5 days later

Polymer	Before treatment	After treatment	5 days later
PCL (10 s)	113°	46°	46°
PCL (1 min)		21°	37°
PLA (1 min)	103°	34°	59°
PLC (1 min)	113°	56°	94°
PLGA (1 min)	116°	46°	54°
PHB / HV (1 min)	85°	59°	65°

The plasma treatment was successful to decrease the contact angles and to increase the wettability of the scaffolds. By increasing the duration of plasma treatment of the PCL scaffold, the contact angle decreased. The 5 days storage resulted in the increase of the contact angles of the 1 minute plasma treated samples. On the contrary, the contact angle of the 10 seconds plasma treated sample remained the same during the storage. Regarding the stability of wettability, the shorter plasma treatment would be more advantageous, since it polarized mainly the surface of the scaffold and the polymer molecules remained less mobile. However, the 1 minute plasma treatment resulted better wettability after the plasma treatment and after 5 days storage as well. The other polymer scaffolds were, therefore, treated for 1 minute. The surface treatment had a different influence on the electrospun materials since the hydrophobic features of the polymers are different (Table 3).

To understand the effect of plasma treatment, the chemical composition of the surface of the PCL scaffold was examined using X-ray photoelectron spectroscopy on the untreated and the 1 min plasma treated sample (the day after surface treatment). The atomic composition of the

surface was determined in order to gain information about the differences between the scaffold before and after the modification (Table 4). The surface composition of the scaffold proved to be different from the theoretical value calculated for pure PCL. This can be ascribed to that, the polar, hydrophilic groups face inwards in the fibers as a consequence of the drying process. During the electrospinning the polar solvent first evaporates from the surface of the polymer jet, resulting to direct the groups on the polymer chain toward the remaining solvent and other polymer molecules inside the fibers. The lack of hydrophilic groups on the surface could explain the low oxygen ratio and the large contact angle of the samples.

The binding energy of the atoms was determined and similar energy profiles were detected during the measurement, the intensities of the oxygen atoms were corresponding. As far as the carbon atoms are concerned, the equivalent binding energies were identified on the spectra (Fig. 3).

In Fig. 3 the carbon and oxygen atoms are numbered on the structure of PCL polymer to make the evaluation easier. The C5 label means the carbon atom right next to the oxygen atom with an ether bond.

As it can be seen in the spectra, the surface treatment had an enormous impact on the scaffold. It has been observed that the untreated samples comprised more hydrocarbon-related carbon atoms than the theoretical ratio. Carbonyl oxygens (O1) found to be in majority compared to ethereal ones in both scaffolds. Although the overall ratio of oxygen atoms increased, their types did not change. The ratio of carbonyl carbon atoms increased, whilst the top layer of hydrocarbons – probably an air-condensed hydrophobic layer – vanished completely after the plasma treatment. The molecule became more polarized and consequently hydrophilic due to the removal of the inwards oriented (due to drying) surface molecules. After the plasma treatment, the polymer scaffolds were wettable because of the presence of newly generated oxygen-containing groups on the surface.

Table 4 Percent composition of C and O atoms in the PCL scaffolds: MM-PCL = untreated PCL scaffold, MM-PCL-P1 = plasma treated PCL scaffold

Sample name	Chemical composition		O/C atomic ratio (%)
	Oxigen (%)	Carbon (%)	
PCL theoretical	25.0	75.0	33.3
MM-PCL	18.6	81.4	22.9
MM-PCL-P1	21.5	78.5	27.4

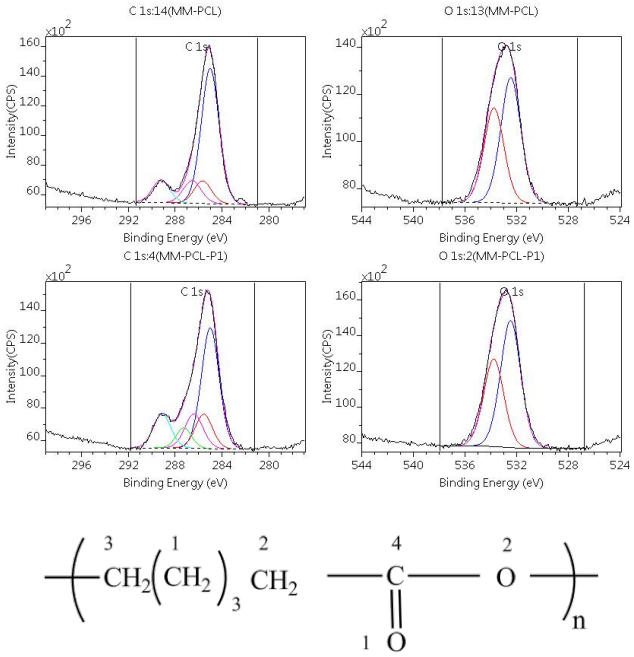

Fig. 3 XPS spectra according to the carbon and oxygen atoms: MM-PCL = untreated PCL scaffold, MM-PCL-P1 = plasma treated PCL scaffold and the structural formula of PCL with the atoms labeled

Table 5 Atomic percent composition according to all the atoms in the polymer: MM-PCL = untreated PCL scaffold, MM-PCL-P1 = plasma treated PCL scaffold

Sample name	Atomic percent (%)						
	O1	O2	C1	C2	C3	C4	C5
Theoretical	12.5	12.5	37.5	12.5	12.5	12.5	-
MM-PCL	10.6	8.0	54.2	8.9	9.3	8.9	-
MM-PCL-P1	12.8	8.8	39.4	11.0	11.4	10.9	5.7

4 Conclusion

Nanofibrous scaffolds were prepared from biodegradable polymers and copolymers by applying the electrospinning technique. The polymer concentrations, the solution compositions, and the electrospinning parameters were optimized to achieve adequate fibers in a submicron range. The electrospun fibers of PCL, PLA, PHB / HV, PLC, and PLGA polymer solutions were deposited on an inert PP carrier and was placed in a cell cultivation plate to obtain an easy to use the system for the comparison of the applicability of scaffolds. The prepared scaffolds were characterized based on morphology, wettability, and surface chemical composition. The PCL scaffolds comprised more hydrocarbon-related carbon atoms than the theoretical ratio and the samples were quite hydrophobic. The drying induced orientation of the polar groups on the polymer chain presumably the reason for the deviation from the theoretical value. The surface treatment

of the scaffolds was necessary to increase the hydrophilicity of the scaffolds. Based on the optimization a 1-minute plasma treatment was applied on the samples. The total ratio of oxygen atoms had increased on the surface of the plasma treated sample. The use of plasma treatment was successful to increase the hydrophilicity because of the removal of the oriented surface molecules and the newly generated oxygen-containing groups on the surface. The molecules on the surface became more polarized and as a consequence, the scaffold had good wettability.

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