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Preparation and Characterization of Aligned and Random Nanofibrous Nanocomposite Scaffolds of Poly (Vinyl Alcohol), Poly (ε-Caprolactone) and Nanohydroxyapatite

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Abstract:
Nanofibrous scaffolds produced by electrospinning have attracted much attention, recently. Aligned and random nanofibrous scaffolds of poly (vinyl alcohol) (PVA), poly (ε-caprolactone) (PCL) and nanohydroxyapatite (nHA) were fabricated by electrospinning method in this study. The composite nanofibrous scaffolds were subjected to detailed analysis. Morphological investigations revealed that the prepared nanofibers have uniform morphology and the average fiber diameters of aligned and random scaffolds were 135.5 and 290 nm, respectively. The obtained scaffolds have a porous structure with porosity of 88 and 76 % for random and aligned nanofibers, respectively. Furthermore, FTIR analysis demonstrated that there were strong intramolecular interactions between the molecules of PVA/PCL/nHA. On the other hand, mechanical characterizations show that aligning the nanofibers, could significantly improve the rigidity of the resultant biocomposite nanofibrous scaffolds. The results indicate that aligned scaffolds are suitable for tissue engineering applications.
Keywords: *Electrospinnig; Nanofibrous scaffold; poly (vinyl alcohol); Poly (ε-caprolactone).*

1. INTRODUCTION

Electrospinning is a highly versatile method to process polymer solutions or melts, into continuous fibers with diameters ranging from a few micrometers to a few nanometers. In the electrospinning process, a polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field and an electric charge is included on the liquid surface due to this electric field. When the applied electric field reaches a critical value, the repulsive electrical forces overcome the surface tension forces. Eventually, a charged jet of the solution is ejected from the tip of the Taylor cone and an unstable jet occurs in the space between the capillary tip and collector which leads to polymer fiber formation by solvent evaporation [1, 2].

With smaller pores and higher surface area than regular fibers, electrospun fibers have been successfully applied in various fields such as nano-catalysis, tissue engineering scaffolds, filtration, biomedical, pharmaceutical, optical and environmental engineering [3-9]. Several reports have shown that the electrospun scaffolds serve as a better environment for cell attachment and proliferation, since they resemble the extracellular

matrix (ECM) [10-13]. This special architecture can affect the tissue specific cell morphology, function and mechanical properties. Mimicking the nanoscale structure of ECM is an effective strategy to design and develop tissue-engineered scaffolds [14]. Electrospinning is a well known technique to produce nanofibers and has been used by many researchers to make nanofibrous matrix for tissue engineering applications [15-18]. For nonwovens produced by electrospinning, the fiber arrangement is of great importance. The orientation of nanofibers along a preferred direction is of interest for structural reinforcement with nanofibers or for tissue engineering to give the cells a preferred growth direction. Most native ECMs found in tissues, however, have defined orientation architecture. Therefore, a well-defined architecture is believed to be required to precisely mimic the native ECM. In-vitro studies have demonstrated the influence of alignment on the osteogenic and neurogenic differentiation of stem cells cultured on nanofibrous scaffolds [19,20].

Aligned fibers can, for example, be obtained by the use of rapidly rotating cylindrical collectors, which either serves as counter electrode or are combined with an electrode [21-23]. Combination of synthetic polymers and a bioceramic can take advantages of the mechanical properties, degradation stability and cell affinities of the individual components. Thus, incorporation of nanohydroxyapatite (nHA), into a nanofibrous matrix not only mimics the natural bone structure but also can enhance the mechanical properties and biological response of the scaffolds [24]. The objective of this study was to prepare and characterize aligned and random nanofibrous scaffolds of nHA, poly (ε-caprolactone) (PCL) and Poly (vinyl alcohol) (PVA) witch can be used in bone tissue engineering.

2. MATERIALS AND METHODS

2.1. Materilas

PVA with molecular weight of 72 kD and 98% degree of hydrolysis was obtained from Merck and used without further purification. PCL with molecular weight of 80 kD and nHA (≤200 nm) were obtained from Sigma-Aldrich. N,N-dimethylformamide (DMF) and chloroform were purchased from Merck.

2.2. Methods

2.2.1. PVA solution preparation

To obtain electrospinnable solution, PVA (10 w/w %) was dissolved in de-ionized water and vigorously stirred with a magnetic stir bar at 80 º C for 5 h, then cooled to room temperature and stirred for 3 h at room temperature to ensure homogeneity.

2.2.2. PCL/nHA solution preparation

A mixed solvent of chloroform/ DMF (85/15 v/v) was exploited to obtain a spinnable PCL/nHA dispersion. HA nanoparticles (10.0 w/w %) were dispersed in this solvent to form a suspension. Then PCL pellets were added to the suspension and homogenized by ultrasonic vibrator.

2.2.3. Nanofibrous scaffolds preparation

A double-spinnert electrospinning machine (ANSTCO-N/VI, Asian Nanostructures Technology Co., Iran) was used for the preparation of composite nanofibrous scaffolds (Figure 1). The solutions were fed into 5 mL standard syringes attached to a 21-gauge blunted needle using a syringe pump with a rate of 0.5 and 0.3 mL/hr for PCL/nHA and PVA solution, respectively. A steel grounded collector was used to collect the electrospun nanofibers in a distance of 15 cm from the needle. Aligned nanofibers were formed using a rotating disk setup

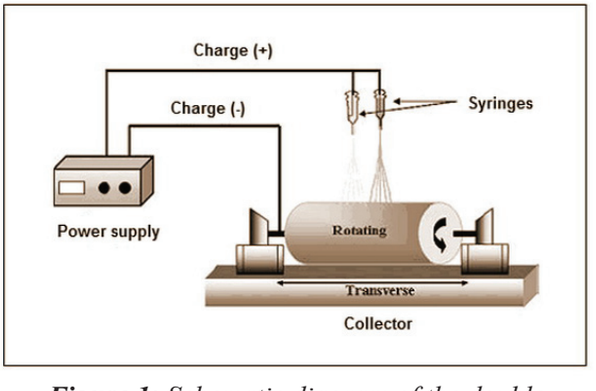

Figure 1: *Schematic diagram of the double-spinneret electrospinning set-up.*

at 3000 rpm with the same parameters to obtain well-aligned nanofibers.

2.2.4. Surface morphology

The morphology of electrospun scaffolds was characterized by scanning electron microscopy (SEM; Vega II XMU instrument Tescan, Czech Republic). Specimens were sputter-coated with gold for 20s and imaged with a back-scattering detector. Fiber diameters of scaffolds were calculated from their respective SEM images using image analysis software (Image J, NIH).

2.2.5. Mechanical testing

Mechanical properties of aligned and random nanofibrous scaffolds were determined by a universal testing machine (Galdabini, Italy) at ambient temperature using a 10-N load cell under a cross-head speed of 50 mm/min. All samples were prepared in the form of rectangular shape with dimensions of 60×10 mm^2 from the electrospun fibrous mats. At least, five samples were tested for each type of scaffolds.

2.2.6. Porosity

The apparent density and porosity characteristics of the electrospun scaffolds were calculated using Eqs. (1) and (2), respectively [25].

$$\text{Apparent density of scaffold } (g/cm^3) = \frac{\text{Mass of scaffold (g)}}{\text{Area of scaffold } (cm^2) \times \text{Thickness of scaffold (cm)}} \quad (1)$$

$$\text{Porosity of scaffold } (\%) = \left(1 - \frac{\text{apparent density of scaffold } (g/cm^3)}{\text{bulk density of scaffold } (g/cm^3)}\right) \times 100 \quad (2)$$

The thickness of aligned and random scaffolds was measured by micrometer. The bulk density of PCL/PVA nanofibrous membrane is known to be 1.24 g cm^{-2} [26].

2.2.7. ATR-FTIR spectroscopy

Chemical analysis of electrospun PCL, PVA, PCL/nHA, PVA/nHA and PCL/nHA/PVA nanofibrous scaffolds was performed by ATR-FTIR spectroscopy. ATR-FTIR spectra of scaffolds were obtained on an Equinox 55 spectrometer (Bruker optics, Germany).

3. RESULTS AND DISCUSSION

3.1. Morphology of nanofibrous scaffolds

SEM micrographs of electrospun nanofibrous scaffolds revealed porous, nanoscaled, fibrous structures (Figure 2). Randomely oriented PCL/nHA/PVA nanofibrous scaffold was obtained as uniform and bead free nanofibers with mean diameter of 290 nm. Aligned PCL/nHA/PVA nanofibers had an average diameter of 135.5 nm, comparatively lower than the random fibers. Use of high speed rotating disk setup for the production of aligned nanofibers imparted a smooth surface morphology with highly aligned and fine fibers. The majority of fibers were within 15º of the orientation axis, and only a few showed significant angular deviation. This deviation in alignment is expected and is due to the rapid deposition of fibers on the grounded collector, which offers insufficient time to completely release the charge resulting in repulsion of fibers depositing and being deposited [27, 28].

3.2. Porosity

The porosity of the scaffolds was calculated by Eq. (2). It was observed that random fibers had a porosity of 88%, whereas the porosity of aligned fibers was 76%. The different orientation of the aligned and random fibers leads to different shape of pores between the fibers. Jose et.al [29] reported similar results in the electrospinning of PLGA/HA nanofibrous nanocomposite scaffolds. The porosity of random scaffolds was 77%, whereas aligned scaffolds had 72% porosity. As shown in Figure 2, smaller and narrower pores are commonly seen on aligned nanofibrous scaffold, but bigger and rounder pores are shown on random ones. By aligning the fibers, aligned nanofiberous scaffolds exhibited a denser structure and a lower porosity than scaffolds made from random nanofibers.

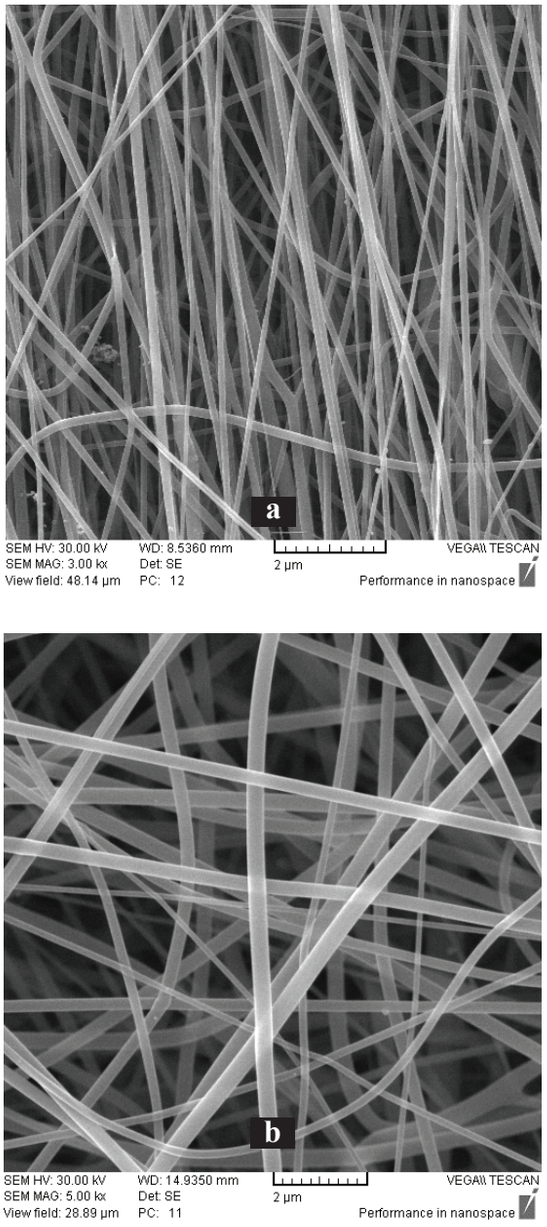

Figure 2: *SEM images of nanofibrous scaffolds (a) random, (b) aligned.*

3.3. Mechanical properties

The mechanical properties of biocomposite nanofibrous scaffolds were evaluated by tensile testing. The stress-strain curves of the samples are shown in Figure 3. As shown in this figure, the tensile strength of random PVA/PCL/nHA

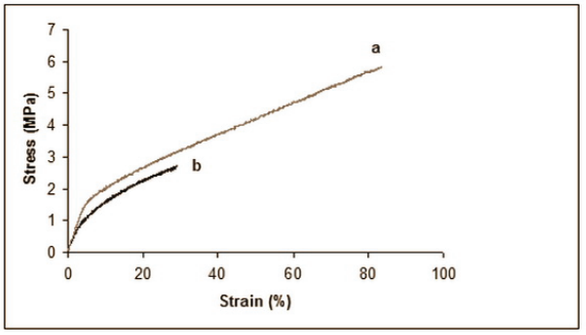

Figure 3: *stress-strain curves for electrospun nanocomposite scaffolds (a) aligned, (b) random.*

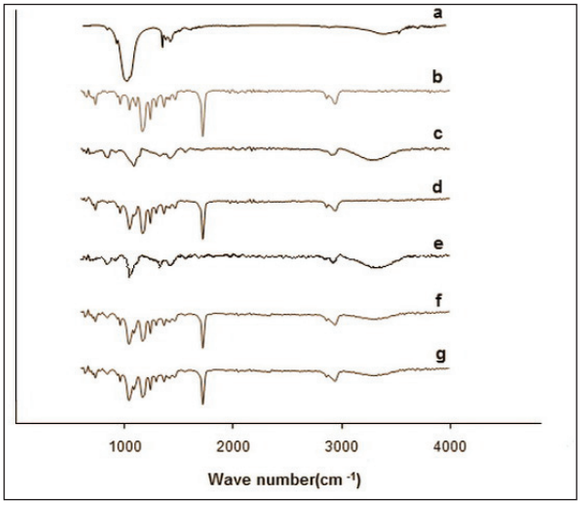

Figure 4: *FTIR spectra for (a) nHA, (b) PCL, (c) PVA, (d) PCL/ nHA, (e) PVA/ nHA, (f) PCL/ nHA/ PVA (random), (g) PCL/ nHA/ PVA (aligned).*

nanofibers was 2.66 MPa and the elastic modulus was 5.22 MPa. By alignment of nanofibers, the tensile strength increased to 5.82 MPa with an elastic modulus of 12.2 MPa. Yin et.al [30] found that the alignment of PLLA nanofibers significantly increased the mechanical properties of prepared nanofiberous scaffolds. The tensile strength of aligned scaffolds was 10-fold that of the random groups (1.88 ± 0.05 vs. 0.17±0.06 Mpa), and the modulus of the aligned scaffolds was 36 times than that of the random group (22.67±5.63 vs. 0.63±0.56 MPa). The increment of elastic modulus and tensile strength is attributed to the highly porous structure of the random nanofibrous

scaffolds. Moreover, during tensile loading, only the fibers oriented along the loading direction experience the stretching force, while the fibers that are oriented perpendicular to the loading direction do not experience any force.

3.4. Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopic analysis was done to characterize functional groups in the fibers in order to confirm the presence of different phase in the scaffold and to discern any possible chemical modification or interaction between phases. Figure 4 shows FTIR spectra for PCL, nHA, PCL/HA, PVA, PVA/nHA and PCL/nHA/PVA scaffolds. Typical infrared bands for PCL-related stretching modes are observed for the PCL and PCL/HA scaffolds. These include 2923 cm^{-1} (asymmetric CH_2 stretching), 2857 cm^{-1} (symmetric CH_2 stretching), 1720 cm^{-1} (carbonyl stretching), 1293 cm^{-1} (C-O and C-C stretching in the crystalline phase) and 1240 cm^{-1} (asymmetric COC stretching). Characteristic PO_4^{-3} absorption bands attributed to HA nanoparticles are seen for each of nHA, PCL/nHA, PVA/nHA and PCL/nHA/PVA scaffolds. These PO_4^{-3} bands are seen at 569 cm^{-1} and 1031 cm^{-1}. PCL/nHA/PVA nanofibers also showed a characteristic broad absorbance at 3265 cm^{-1} (–OH stretching), at 2917 cm^{-1} (symmetric – CH^{2-}), and at 1422 and 1088 cm^{-1} for C–O group of PVA.

4. CONCLUSION

In this study, aligned and random biocomposites of PVA/PCL/nHA nanofibrous scaffolds were prepared via electrospinning techniques. The results reported in this study, demonstrated that the mean fiber diameter and porosity of aligned nanofibers were lower than those of random nanofibers. Moreover, it was found that the mechanical properties of aligned nanofibrous scaffolds were higher than those of randomly oriented scaffolds. The results indicate that aligned nanocomposite scaffolds of PCL/nHA/PVA are suitable candidates for tissue engineering applications.

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