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Mathematical Modeling and Experimental Evaluation for the predication of single nanofiber modulus

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ABSTRACT

Electrospun nanofiber matrices are widely used as scaffolds for the regeneration of different tissues due to similarities with fibrous components of the extracellular matrix. These scaffolds could act as a substrate for inducing mechanical stimuli to cells. The main mechanical stimuli factor in nanofiber scaffolds for determining the cell behaviors is stiffness of single nanofibers. This paper especially highlights the finding that the young's modulus of single nanofibers can be obtained from aligned nanofibers matrix. It is assume that, the modulus of single nanofibers are equal to modulus of completely aligned nanofibers. However, due to difficulty of producing completely aligned nanofibers, the obtained modulus of single nanofiber wouldn't have significant value. Therefore, we propose a new mathematical model to predict the stiffness of single nanofibers from non-perfectly aligned nanofibers matrix.

1. Introduction

Electrospinning provides an efficient and scalable method towards producing bioengineered nano fiberous scaffolds with appropriate structure and excellent mechanical properties for tissue engineering (Wang et al., 2013; Rajzer et al., 2017; Xue et al., 2017; Wang et al., 2017; Wright et al., 2017).

Poly (ε-caprolactone) (PCL) is well-known synthetic polymer, used extensively in biomedical and pharmaceutical applications. In this respect, Nanofiber scaffolds fabricated using polymer by electrospinning would be good candidates for tissue engineering (Baker et al., 2016; Song et al., 2015; Yao et al., 2017; Ren et al., 2017; Salehi et al., 2017; Bansal et al., 2017).

Scaffolds for tissue engineering are designed to mimic the extracellular matrix (ECM) properties of the target tissue to guide tissue regeneration (Sathy et al., 2017). When cells placed in contact with the scaffold, the mechanical interactions between cells and the matrix determine cell fate. In other words, cell behaviors including migration, proliferation, and differentiation are effected by scaffold stiffness due to cell response to mechanical signals (Kennedy et al., 2016; Fernandez-Yague et al., 2015; Higuchi et al., 2013; Lv et al., 2015).

In electrospun scaffolds, however, most of the studies use the bulk mechanical properties of nanofibers matrix as the indicators of stiffness (Song et al., 2015; Liao et al., 2012; Holmes et al., 2016; Tutak et al., 2013). Bulk stiffness of these matrices do not act as a driver of cell behavior at the cell-sensing scale. The stiffness of single nanofibers (SFs) can be sensed only by mechanoreceptors on the cells. Indeed, the effective stiffness of single nanofibers would be the best quantified by the SF Young's modulus (Baker et al., 2016; Plotnikov et al., 2012; Doyle and Yamada, 2016; Reilly and Engler, 2010; Nam et al., 2011).

In order to evaluating the SF modulus, several mechanical testing methods were used. Atomic force microscope (AFM) based mechanical testing method (Yang et al., 2016), as well as commercial nano-tensile tester system (Nano Bionix System, by MTS, USA, acquired by Agilent lately, known as Nano UTM)(Pai et al., 2011; Lim et al., 2008) had been widely used in previous papers, however, these techniques were faced with various challenges.

Three most popular approaches in AFM are included AFM-based tensile testing (Hang et al., 2011; Barber et al., 2015), three-point deformation testing (Stachewicz et al., 2012; Carlisle et al., 2010) and nanoindentation (Gibson, 2014; Janković et al., 2013). In AFM based tensile testing, often necessitates to use combination of SEM–AFM system. SEM imaging needs pretreatment for most of nanofiber samples to provide electrical conductivity, in which mechanical properties of samples may alter by this treatment (Neugirg et al., 2016). The major challenges of AFM-based three-point deformation tests are, difficulty in

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sensing of nano force by AFM cantilevers, anchoring the nanofibers properly on microridges to prevent slippage of nanofibers (Baker et al., 2016) and applying the nanoforce in proper position (Neugirg et al., 2016).

Another approach for measuring the modulus of single nanofiber is nano tensile tester. However, this technique also faced with various challenges including, difficulty in the separation of single nanofibers from the main matrix and transportation to the nano tensile tester and limitation of this technique in testing of nanofibers with a diameter below 1 μm (Tan et al., 2005).

Furthermore, in all above techniques only a single nanofiber among all existed nanofibers in a scaffold were tested. Several papers show that the variation in diameter of nanofibers in the same scaffold and also fineness variation along the length of each fiber could effect on the amount of SF young's modulus (Yang et al., 2016; Stachewicz et al., 2012; Shin et al., 2006). Hence, measuring the modulus of one single nanofiber couldn’t represent the average modulus of single nanofibers in whole scaffold.

In view of challenges for testing one single nanofibers by above methods, it would be appropriate to develop a correlation between the modulus of an individual single nanofiber and that of a nanofibers matrix. Therefore, predicting the modulus of single nanofiber from the modulus of nanofibers matrix could be the best representative of average stiffness of single nanofibers with different diameters in the matrix. In this study, the hypothesis is, the modulus of single nanofiber is equal to modulus of completely aligned nanofibers. Since, the electrospinning of completely aligned nanofibers are difficult and always some misaligned fibers were existed in the matrix, we propose a new mathematical model to predict the SF modulus from aligned nanofibers matrix. Such a finding will have significant impact on the predicting average modulus of single nanofibers with different diameters in a scaffold with lower variation than pervious methods.

2. Experiments and modeling

2.1. Materials

Poly (ε-Caprolactone) PCL ($M_w = 80,000$) was purchased from Sigma Aldrich, Chloroform and methanol from Merck.

2.2. Electrospinning of aligned PCL nanofibers

The polymer solutions (11% W/V) were prepared by dissolving PCL in chloroform/methanol (3/1). The nanofibers were then electrospun by the feed rate of 1.0 ml/h and the voltage of 15 kV. The distance between needle tip and collector was 17 cm. In addition, to produce properly aligned nanofibers, two methods were used (Fig. 1).

2.2.1. Rotary drum

In this method, a drum with high speed rotation (400–3600 rpm) was used for collecting the aligned nanofibers. By increasing the rpm of rotating collector, alignment of nanofibers is increased.

2.2.2. Two parallel electrode

In the second method, fibers are collected perpendicular to the parallel electrode. During the traveling of polymer jet toward the collector, part of the descending jet is attached to one of the electrodes. The remaining portion of the jet will be pulled towards the opposite electrode. These processes are frequently repeated and a matrix of aligned fibers is eventually formed across the electrodes.

2.3. Characterization

For observing the structure of electrospun nanofiberous matrix, the samples were sputtered by gold (BALZERS SCD 004, Germany), and then observed by Scanning Electron Microscopy, SEM, (Seron Technologies AIS2100, Korea). Mechanical tests of the nanofibers were performed by using an Instron 5566 universal testing machine at a crosshead speed of 1 mm/min with a load cell of 100 μN.

2.4. Uniaxial tensile testing

2.4.1. Evaluation of aligned nanofiber modulus

The Young's modulus of all nanofiber samples were evaluated by tensile tester. Every series of samples were measured ten times. For calculating the Young's modulus two methods were used:

1. Density based method (DBM): The surface area of the neat aligned nanofiberous matrix (without consideration of voids between the nanofibers) is determined by:

$$A = W/L_0\rho_t \tag{1}$$

where W and L_0 represent the weight and length of the nanofibers sample, respectively, and ρ_t is the density of polymer (PCL).

In the following, the young modulus is obtained by Eq. (2):

$$E = \frac{F/A}{\Delta L/L_0} \tag{2}$$

where F is the force exerted on the nanofibers under tension, A is the cross-sectional area of nanofiber through which the force is applied, ΔL define the length change of nanofibers and L_0 is the original length of the nanofibers.

2. Counting based method (CBM): Prior to the tensile test, the total surface area of the nanofibers in the matrix were calculated by Eq. (3):

$$A_t = N_t.\ \overline{A}_s \tag{3}$$

where $\overline{A}_s$ is the average surface area of the nanofibers in a matrix obtained from SEM images, N_t is the total number of fibers existed in the matrix and A_t is the total surface area of nanofibers. The total number of nanofibers between the electrodes for each sample was determined directly using an optical microscopy. This number was multiplied by the ratio of 40, which is obtained by the division of total width of sample (20 mm) to the width of micrographs (500 μm). Then the young modulus of matrix is calculated by Eq. (2).

2.4.2. Sample preparation for tensile testing

In this study, a new technique has been developed to prepare samples for direct tensile testing. Each of the mentioned electrospun

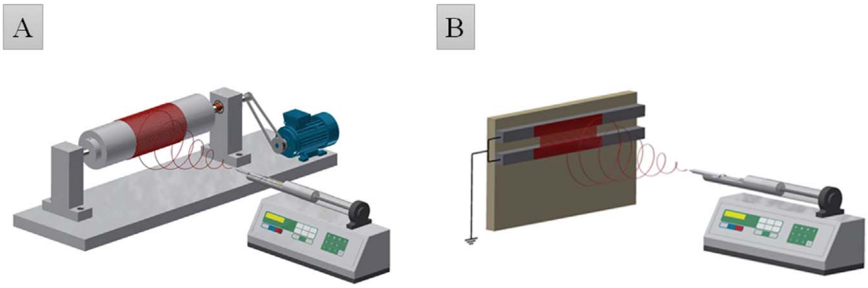

Fig. 1. (A) Rotary drum (B) Two parallel electrode.