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Artificial neural network for modeling the elastic modulus of electrospun polycaprolactone/gelatin scaffolds

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ABSTRACT

Scaffolds for tissue engineering (TE) require the consideration of multiple aspects, including polymeric composition and the structure and mechanical properties of the scaffolds, in order to mimic the native extracellular matrix of the tissue. Electrospun fibers are frequently utilized in TE due to their tunable physical, chemical, and mechanical properties and porosity. The mechanical properties of electrospun scaffolds made from specific polymers are highly dependent on the processing parameters, which can therefore be tuned for particular applications. Fiber diameter and orientation along with polymeric composition are the major factors that determine the elastic modulus of electrospun nano- and microfibers. Here we have developed a neural network model to investigate the simultaneous effects of composition, fiber diameter and fiber orientation of electrospun polycaprolactone/gelatin mats on the elastic modulus of the scaffolds under ambient and simulated physiological conditions. The model generated might assist bioengineers to fabricate electrospun scaffolds with defined fiber diameters, orientations and constituents, thereby replicating the mechanical properties of the native target tissue.

1. Introduction

Providing an alternative to conventional transplants through the development of biomimetic scaffolds is one of the primary objective of tissue engineering (TE) [1]. An ideal TE scaffold, as a simulator of the native extracellular matrix (ECM), should have the physical, biological and mechanical requisites of the target cells. The native ECM is a molecular complex made up of proteins, especially collagen, and polysaccharides, comprising a three-dimensional hierarchical fibrous structure with nanoscale dimensions [2]. Recently nanofibrous scaffolds have received much attention in TE due to their similarity to the fibrillar structure of native ECM, both morphologically and dimensionally [3]. The porous nature of the nanofibers allows cells to migrate into and proliferate in the scaffold, and the transport of nutrients and metabolic waste through the porous nanofibers [4]. Among the various available methods used to fabricate nanofibrous scaffolds, electrospinning remains the most popular technique owing to its simplicity and inexpensive nature, as well as its ability to form nano- and microfibers from a wide range of synthetic and natural polymers. For the fabrication of a bio-scaffold the critical issue for bioengineers is the careful selection of biocompatible polymers with proportional degradability to remodel the tissue concerned.

Polycaprolactone (PCL), a semicrystalline linear hydrophobic polymer, is one of the most commonly used FDA approved polymers that has been electrospun either alone or in combination with other synthetic or natural polymers to fabricate nanofibrous scaffolds for bone, cartilage, nerve, blood vessel, skin, corneal, cardiac, tendon and ligament tissue regeneration. The lack of surface cell recognition sites and poor hydrophilicity of pure PCL scaffolds make them unsuitable for cell adhesion, proliferation, and differentiation and, hence, the blending of PCL with natural polymers such as gelatin and collagen has been used to combine both the mechanical durability of the synthetic component (PCL) and the cell affinity of the natural protein [5].

Although collagen is an adhesion protein present in the native ECM which enables cell attachment and proliferation through

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specific interactions between its RGD domains and integrin receptors in the cell membrane, electrospinning of collagen is an expensive way of making gelatin fibers [6]. Gelatin is a natural biopolymer derived from collagen by controlled hydrolysis and is biocompatible, biodegradable and commercially available at relatively low cost [7].

Electrospun PCL/gelatin scaffolds have been used as substrates for skin, bone, nerve, cardiac, and blood vessel TE by many researchers [5,7–12]. However, the mechanical properties of PCL/gelatin nanofibers require modification so as to mimic the target tissue of regeneration. The mechanical properties of electrospun PCL/gelatin scaffolds is mainly influenced by the ratio of PCL to gelatin [5], but like other electrospun scaffolds it also depends on the orientation [13–15] and diameter of the fibers [14]. A proper understanding of the relationship between these parameters and the macro-mechanical properties of electrospun scaffolds will help bioengineers fabricate optimized scaffolds for any desired purposes primarily by controlling the scaffold composition and architecture.

Artificial neural networks (ANN) are a modeling tool to solve linear and nonlinear multivariate regression problems [16]. The massive interconnected structure makes an ANN an exceptional tool which is translated through input data, while it has the ability to model incomplete data without being affected by data noise. Such techniques have proved more efficient than standard modeling techniques such as the response surface methodology (RSM). Various parameters affect the electrospinning process, such as the solution properties and processing conditions, along with other ambient parameters with known or unknown effects on each other, thus making the electrospinning process a complex procedure, which also highlight the need to employ an ANN model instead of classical statistical tools [17]. RSM is a statistical technique commonly utilized to estimate a quadratic model and cannot be applied to complicated cases such as electrospinning. Collinearity of the parameters influencing the electrospinning procedure is another limitation of using RSM modeling for this application [18].

In recent years ANN have been applied to model the electrospinning process, mostly aimed at predicting the diameter of electrospun polyacrylonitrile [19–21], polyethylene oxide [22], polyurethane [23] and nylon-6,6 [17] nanofibers. At the same time an ANN-based model was demonstrated to predict the water retention capacity of electrospun polyacrylonitrile fibers [24]. Although the capability of neural network to model the mechanical properties of materials is well known, particularly those of textile structures [25–27], no attempt has been made until now to apply an ANN-based model to predict the mechanical properties of electrospun fibers. Therefore the main objective of the present study was to develop an ANN-based model to analyze the nonlinear effect of the above mentioned parameters on the elastic modulus of electrospun PCL/gelatin fibers, which is one of the most widely used scaffolds used in TE. During this study fibers of different compositions, fiber diameters and orientations were fabricated by combining different weight ratios of PCL and gelatin, with varying total solution concentrations and mandrel rotation speeds, and their relation to the elastic modulus was interpreted using an ANN-based model.

2. Experimental and modeling

2.1. Materials

Polycaprolactone ($M_w = 70{,}000–90{,}000$), gelatin type A (300 Bloom) from porcine skin, 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and phosphate-buffered saline (PBS) were all purchased from Sigma–Aldrich (Singapore).

2.2. Scaffold fabrication

Polymer solutions with concentrations of 8%, 11%, 14%, and 17% w/v were prepared by dissolving PCL and gelatin at weight ratios of 30:70, 50:50, 70:30, and 90:10 at room temperature with stirring for a period of 22 h. The rotation speed of the collector (rotating mandrel) was set to 50, 200, 700, or 1200 r.p.m. A set of experiments based on a Taguchi L'16 orthogonal array [28,29] was conducted to study the influence of composition, fiber diameter, and fiber orientation on the elastic modulus of electrospun PCL/gelatin scaffolds by changing variable parameters such as the individual polymer weight ratio of PCL to gelatin, total solution concentration, and mandrel rotation speed.

Other electrospinning parameters were kept approximately constant for all the experiments. The polymer solution was electrospun from a 3 ml syringe attached to a 27 G blunted stainless steel needle at a flow rate of 3–5 ml h^{-1}. A positive voltage (10–12 kV) was applied to the polymer solution and the distance between the syringe tip and the mandrel collector was maintained at a distance of 13 cm, while the mandrel length and diameter were 10 and 14 cm, respectively. For a better comparison of the mechanical properties of the nanofibrous mat membrane with a thickness of 60–80 μm was collected for all scaffolds. The collector was regularly moved to ensure a uniform thickness of the scaffold along the length of the mandrel. The electrospinning parameters to prepare different samples according to the Taguchi method are summarized in Table 1.

2.3. Morphological characterization

Structural characterization of the scaffolds was performed using scanning electron microscopy (FEI-Quanta 200F, The Netherlands) at an accelerating voltage of 10 keV, after sputter coating with gold (JEOL JFC-1600 Auto fine coater, Japan). Imaging was carried out in such a manner that the circumferential direction (the direction of mandrel rotation) of the samples was vertical in the images. Fiber diameter and orientation were determined from scanning electron microscopy (SEM) images using image analysis software (ImageJ, National Institutes of Health, Bethesda, MD), with the fiber diameter being the average value of 100 random points in the respective SEM images.

The fiber orientations were evaluated with a novel ImageJ plug-in, OrientationJ [30,31]. Three images per sample were used to quantify the alignment of the nanofibers. The coherency coefficients ranged from 0 to 1, where values close to 0 and 1 refer to random and aligned fibers, respectively [31]. Therefore, two parameters for orientation evaluation were considered, the dominant fiber direction and the coherency coefficient in the dominant direction to demonstrate the direction and intensity of fiber orientation, respectively. The average coherency coefficient is an index of the alignment intensity (alignment index (AI)). To minimize the dominant alignment direction errors arising during placement of the samples on the SEM plate the alignment directions were categorized in 10° increments and the angles were defined relative to the horizontal axis.

2.4. Uniaxial tensile testing

The electrospun scaffolds were dried in a vacuum oven and used for further experiments. The thickness of the specimens was measured using a Mitutoyo digital micrometer. The elastic modulus of the electrospun scaffolds was determined by uniaxial tensile testing of rectangular specimens (30 mm length × 10 mm width) using an Instron 5943 table top tester, with a 50 N load cell and at an extension rate of 10 mm min^{-1}. An algorithm for Young's modulus determination provided by the Bluehill3 software over a

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