Fracture behavior of multilayer fibrous scaffolds featuring microstructural gradients

W Khoo, SM Chung, Shing Chee Lim, Cheng Yee Low, Jenna M. Shapiro, Ching Theng Koh

Faculty of Mechanical and Manufacturing Engineering, University of Tun Hussein Onn Malaysia, Johor, Malaysia
Faculty of Engineering, University of Bristol, Bristol, United Kingdom

ABSTRACT

Multilayer nanofibrous scaffolds that mimic the microstructure gradient of connective tissues have shown promising results in tissue regeneration, but the effect of these gradients on the mechanical performance of fiber meshes is poorly understood. In this study, trilayer nanofibrous gelatin scaffolds with gradually increasing fiber diameters (227–679 nm) and pore sizes (1.14–4.93 μm²) were fabricated using a sequential electrospinning process, producing a fiber density gradient over the scaffold thickness. The mechanical properties of the fibrous scaffolds were evaluated using uniaxial tensile and fracture tests. Deformation of microscopic crack tip openings was simulated using finite element analysis. Results from uniaxial and fracture tensile tests showed that the mechanical properties of fibrous scaffolds were governed by network architectures. The microstructure gradient yielded corresponding changes of material properties over the scaffold thickness. Simulation results showed different stress distribution and energy dissipation at each layer of the graded scaffolds. Finite element analysis also revealed that a combination of network density and alignment gradients can improve fracture toughness of graded scaffolds. This study provides guidelines and methodologies for designing tailored gradient fibrous scaffolds to more closely mimic the structural and mechanical properties of native interfacial tissues.

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1. Introduction

Multilayered fibrous structures have a variety of functions in multiple applications, ranging from filtration, to textiles, to tissue engineering. In filtration, multilayer structures affect pressure drop and dust holding efficiency [1, 2]. Multilayer fibers in textiles can act as an acoustic absorption material to reduce noise [3]. Further, multilayer fibrous materials are used as tissue engineering scaffolds to provide suitable microenvironments for cells to support the repair and regeneration of injured tissues.

Microstructural and compositional gradients have been found in several interface tissues, such as ligament-to-bone [4], tendon-to-bone [5] and cartilage-to-bone [6]. These gradients play important roles in transmitting loads from soft to hard tissues [7–9]. Variations in extracellular matrix composition and microstructural architecture were also found in connective tissues, including cartilage [10]. In cartilage, this variation leads to directional stiffness and fracture behavior of graded scaffolds were observed. Computational finite element analysis of the microscopic fracture at the crack tip of three dimensional graded fibrous scaffolds was performed. Microstructural architecture variations, including gradients in network density and fiber alignment, were also simulated to provide future design guidelines for functionally graded scaffolds.

2. Materials and methods

2.1. Sample preparation

2.1.1. Sample preparation

A 25 wt% solution of cold water skin gelatin with a molecular weight of 60 kDa (Sigma Aldrich, USA) in a mixture of 90 wt% glacial acetic acid (Merck, Germany) and 10 wt% water was prepared. The gelatin solution was stirred overnight at room temperature before loading into a 50 mL plastic syringe (Terumo, UK) in our bespoke electrospinning apparatus. The solution was pumped through a blunt 23 gauge needle (Terumo, UK) at a constant feed rate by a syringe pump (KD Scientific, USA). A voltage was supplied between the needle and a grounded metal collector using a high voltage power supply (Glassman, UK) to produce nanofibers.

A sequential electrospinning process was used to produce functionally graded scaffolds consisting of distinct trilayer microstructures. For each layer, electrospinning process parameters of voltage, feed pump rate, working distance, and deposition time were varied. In the first step, operating parameters were set to voltage \( V = 9 \, \text{kV} \), pump rate \( f = 0.15 \, \text{ml/h} \) and distance \( d = 20 \, \text{cm} \). After 30 h of electrospinning, the machine parameters were then adjusted to \( V = 15 \, \text{kV} \), \( f = 0.3 \, \text{ml/h} \) and \( d = 20 \, \text{cm} \) for the second step. After 6 h of the electrospinning process, the machine parameters were finally adjusted to \( V = 15 \, \text{kV} \), \( f = 0.45 \, \text{ml/h} \) and \( d = 15 \, \text{cm} \) for the third step. The process was completed after 45 min of electrospinning.

In order to compare mechanical properties of the individual layers, three homogeneous scaffolds were also fabricated. Homogeneous scaffolds with densely packed fibers (DH) were fabricated using the parameters of the first step of the sequential process. Homogeneous scaffolds with moderately packed fibers (MH) were fabricated using the second step process parameters. Homogeneous scaffolds with sparsely packed fibers (SH) were fabricated using the third step parameters for 2 h, rather than 45 min. Eight to twelve samples were prepared from each type of scaffold for tensile and fracture tests. The thickness for each sample was measured three times using digital calipers (Facom, France). The thicknesses of graded, DH, MH and SH scaffolds were 0.56 ± 0.13, 0.16 ± 0.04, 0.34 ± 0.05 and 0.33 ± 0.06 mm, respectively.

2.1.2. Morphology characterization and quantification

Cross-sectional and top-view morphologies of the graded scaffolds were observed via scanning electron microscopy (SEM, Hitachi, USA). To observe the morphology of each layer, samples were prepared by cutting squares of approximately 10 mm × 10 mm from the scaffold after each step of the electrospinning process. To observe the cross-sectional morphology, following the completion
of the sequential electrospinning process, the graded scaffold was cut across its thickness using a scalpel and visualized using SEM.

All samples were gold coated before SEM observation at an accelerating voltage of 10 kV. The network morphology, including fiber diameters, pore sizes and fiber orientation of each layer, were quantified using image analysis software ImageJ (NIH, Bethesda, MD, USA) [35]. For pore size measurement, the SEM images were first converted to black and white. The area of the void space between fibers was measured as pore size, using the measuring tool in ImageJ. The average fiber diameters and pore sizes were determined by measuring and averaging the diameters of ten randomly chosen fibers and pore sizes respectively from one SEM image for each type of scaffold.

2.2. Computational analysis

2.2.1. Finite element modeling

Meshes were generated using MATLAB (Version 2017, MathWorks, Natick, MA, USA). Finite element analysis was performed with Abaqus (Version 2017, SIMULIA, Providence, RI, USA). Models were constructed by depositing fibers in separate x-y planes in a three-dimensional circular unit model. These fibers were placed at random locations, with a slope \( m \) following the user-defined angles \( \Theta \) (Fig. 1). The defined angle \( \Theta \) represents the alignment of the fibers and generates random lines inclined from negative to positive slopes (Eq. (2)).

\[
\tan(-\Theta) < m < \tan(\Theta)
\]

The models had the first layer \((i = 1)\) placed at \( z_1 = 0 \). Consecutive layers were placed in a separate plane at \( z_i = z_i - \Delta z \), with offset distance \( \Delta z \) from the previous layer. The offset distance was determined from the sum of fiber radii of both adjacent layers.

Random fibers were crosslinked by assigning the same nodes at the intersection points. Two types of crosslinking modeled in this work include intralayer crosslinking, where 15% of the intersection points on each layer were randomly crosslinked, and interlayer crosslinking, where 15% of the intersection points of fibers located on two adjacent layers were randomly crosslinked. Fiber density, \( \rho_f \) was defined as the sum of fiber length per unit area, and was calculated using MATLAB.

Both density and alignment gradient models were studied in this paper, involving the construction of three types of graded networks: density graded (DG), alignment graded (AG), and density and alignment graded (DAG) networks. Homogeneous multilayer networks were also modeled. The DG networks have density gradually reduced over the network thickness. The density gradient was quantified as the percentage fiber density change between fibrous networks located on two consecutive layers. The AG networks had fiber orientation gradually changed over the network thickness. The DAG networks had both density and alignment changed over the thickness.

Fibrous network models were constructed with two levels of fiber density (Table 1). The number of fiber \( N \) was assigned to randomly generate fibers in a circular unit of fibrous network. The value of fiber density \( \rho_f \) was then determined from the networks in MATLAB. The first level of fiber density ranged from 6.6 to 6.8 \( \mu m \) while the second level ranged from 67 to 68 \( \mu m \). The DG networks were constructed with gradients ranging from 10% to 58%. Alignment angles were assigned from 15° to 90° at each layer in AG and DAG networks. All case studies were repeated three times to account for the stochastic nature of the fibrous networks.

Meshes generated from MATLAB were imported into the finite element software, Abaqus. Fibers were modeled with Timoshenko fibrous microstructure around the fibrous microstructure located on two consecutive layers. The AG networks in a circular unit model. These fibers were placed at random locations, with a slope \( m \) following the user-defined angles \( \Theta \) (Fig. 1). The defined angle \( \Theta \) represents the alignment of the fibers and generates random lines inclined from negative to positive slopes (Eq. (2)).

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Meshes generated from MATLAB were imported into the finite element software, Abaqus. Fibers were modeled with Timoshenko beam (B31) and were analyzed using nonlinear finite element analysis, which considers large strain and rotation. All fibers were defined with diameter \( r \) of 300 nm, Young’s modulus, \( E \), of 100 MPa and fracture strength, \( \sigma_f \) of 30 ± 4 MPa.

2.2.2. Fracture analysis

Detailed deformation of the fibrous microstructure around the crack tip was modeled by constructing fibrous networks in a circular unit (radii ranging from 5 to 75 \( \mu m \)) with a notch length corresponding to the unit radius. All nodes located on the perimeter of the circle were assigned with displacements \( u_1 \) and \( u_2 \) (Eqs. (3) and (4)), which are associated with the macroscopic crack tip field for homogeneous and isotropic materials. The applied displacements were calculated from the classical equations of linear elastic fracture mechanics [38]:

\[
\begin{align*}
\frac{1}{\pi} \left[ \frac{\Delta K_1}{\sigma_f} \right]^2 &= \frac{1}{2} \left[ 1 + \frac{2\sin^2\Theta}{2} \cos^2\frac{\Theta}{2} \right] \\
\frac{1}{\pi} \left[ \frac{\Delta K_2}{\sigma_f} \right]^2 &= \frac{1}{2} \left[ 1 + \frac{2\cos^2\Theta}{2} \sin^2\frac{\Theta}{2} \right]
\end{align*}
\]
4.93 ± 4 which result in changes in microstructural architecture over the distinct layers produced by the sequential electrospinning process.

3.1. Morphology of homogeneous and graded electrospun scaffolds

3. Results

3.1. Morphology of homogeneous and graded electrospun scaffolds

Fig. 1. Schematic illustration of (a) experimentally produced graded electrospun scaffolds and (b) finite element model for density graded fibrous networks.

The network parameters of homogeneous (H), density graded (DG), alignment graded (AG) and density and alignment graded (DAG) networks.

<table>
<thead>
<tr>
<th>Network name</th>
<th>Fiber density, ( p_f (\text{1st layer})(\mu\text{m}^{-1}) )</th>
<th>Density gradient, ( G(%) )</th>
<th>Fiber alignment, ( \Theta(°) )</th>
<th>Model size, ( r(\mu\text{m}) )</th>
<th>Fiber number, ( N_{\text{1st layer}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>6.8 ± 0.1</td>
<td>0</td>
<td>90</td>
<td>75</td>
<td>1170</td>
</tr>
<tr>
<td>H2</td>
<td>68.5 ± 0.9</td>
<td>0</td>
<td>90</td>
<td>5</td>
<td>800</td>
</tr>
<tr>
<td>DG1</td>
<td>6.7 ± 0.1</td>
<td>10 ± 2</td>
<td>90</td>
<td>75</td>
<td>1170</td>
</tr>
<tr>
<td>DG2</td>
<td>68.0 ± 0.9</td>
<td>20 ± 3</td>
<td>90</td>
<td>5</td>
<td>800</td>
</tr>
<tr>
<td>AG</td>
<td>67.6 ± 0.9</td>
<td>49 ± 1</td>
<td>90</td>
<td>5</td>
<td>800</td>
</tr>
<tr>
<td>DAG</td>
<td>68.0 ± 0.5</td>
<td>13 ± 2</td>
<td>15, 52.5, 90</td>
<td>5</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>68.3 ± 0.2</td>
<td>29 ± 2</td>
<td>15, 52.5, 90</td>
<td>5</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>68.0 ± 0.5</td>
<td>50 ± 1</td>
<td>15, 52.5, 90</td>
<td>10</td>
<td>1600</td>
</tr>
</tbody>
</table>

Table 1

The origin was assigned at the notch root in polar coordinates \((r, \theta)\). \( K_I \) is the stress intensity factor for mode I. The shear modulus, \( G \), was assigned to be 4 MPa, with a Poisson’s ratio, \( v \), of 0.3, where \( \varepsilon = (3 - v)/(1 + v) \). The fracture points presented in this paper refer to the crack opening when the first fiber element exceeds its fracture strength.

3.2. Mechanical testing on homogeneous and graded electrospun scaffolds

The analysis of the mechanical performance of homogeneous and graded scaffolds is shown in Fig. 5. Graded scaffolds exhibited the largest tensile strength with a mean of 2.80 ± 0.41 MPa, which was significantly different to that of the DH scaffolds. The tensile strength of the SH scaffold was the lowest, with a mean strength was 0.17 ± 0.18 MPa. The elastic modulus of the SH (13.30 ± 3.02 MPa) and MH scaffolds (14.46 ± 3.15 MPa) were not significantly different. As with tensile strength, the fracture toughness of the graded scaffold (\( G_c = 1.16 ± 0.23 \text{kJ/m}^2 \)) was comparable to the DH scaffold (\( G_c = 1.50 ± 0.20 \text{kJ/m}^2 \)). SH scaffolds had the least fracture toughness of 0.17 ± 0.03 kJ/m².
Fig. 2. Microstructural architecture of graded scaffolds. (a) Cross section of graded scaffold. (b) High magnification of the cross section, showing the constituent layers of the graded scaffold, enclosed by rectangular boxes in (a).

Fig. 3. Morphology and fiber orientation of (a) graded and (b) homogeneous scaffolds.

Fig. 4. Quantification of (a) fiber diameter and (b) pore size for homogeneous and each layer in the graded scaffolds. The groups marked with same Roman numerals (I, II, III and IV) are not significantly different ($p > 0.05$).
Fig. 6 shows the failure mechanism of graded scaffolds, which was visualized using SEM. The crack of the top layer started to propagate while the notch in the middle and bottom layers blunted at strain $\varepsilon_1$. As the scaffold was further stretched to strain $\varepsilon_2$, the crack of middle and bottom layers also started to propagate across the scaffold. At strain $\varepsilon_3$, the crack of all three layers propagated across the scaffold.

### 3.3. Design of graded fibrous networks using FEA

Fig. 7 shows the fracture of DG and DAG networks corresponding to various gradients. The network fracture is described by stress intensity factor $K$, which corresponds to the $K$-dominant strain field assigned at the boundaries when the first fiber element exceeds its tensile strength. The DG networks have consistent fracture points corresponding to an increase of network density gradient. The stress intensity factor of both H1 and DG1 networks with low fiber density of $6.6-7.8 \, \mu m^{-1}$ increased to more than twice that of the H1 and DG1 networks with $67.6-68.5 \, \mu m^{-1}$ density. In addition to a network density gradient, fiber alignment gradients were incorporated in AG and DAG networks. The AG network had similar fracture behavior to the homogeneous networks with equivalent fiber densities. The DAG networks showed a larger stress intensity as compared to DG networks with similar fiber densities.

The deformation and stress distribution at the crack tip were examined during fracture of homogeneous and graded networks (Fig. 8). The fracture of DG networks occurred at the crack tip of the densest network, which had the largest stress concentration. The fracture point of AG networks, which had similar network density for all layers, was located at the layer comprised of randomly oriented fibers. The fibers in AG networks aligned parallel to the notch dissipate energy by rearranging and realigning fibers perpendicular to the crack tip during crack tip opening. In contrast to DG networks, the fracture of DAG networks occurred at the layer with
sparse fiber density and randomly oriented fibers. In order to encourage more fiber rearrangement and realignment to dissipate energy, aligned fibers were associated with the densest layer of the DAG scaffolds.

4. Discussion

In this paper, we present both experimental and computational methods for producing functionally graded multilayer nanofibrous scaffolds and evaluating their mechanical properties and fracture behaviors. Gelatin was chosen as the scaffold material due to its biodegradability, biocompatibility and ready availability at low cost. Moreover, in vitro studies have shown that gelatin promotes cell proliferation [39], adhesion, and infiltration [40].

Trilayer gelatin nanofibrous scaffolds featuring fiber density gradients across their thicknesses were fabricated using a sequential electrospinning method. Shifts from dense to sparse fiber packing and fiber diameter were achieved by altering the electrospinning process parameters. These process parameters included solution feed rate (0.15 ml/h to 0.45 ml/h), needle-to-plate distance (15 cm to 20 cm) and applied voltage (9 kV to 15 kV). However, the chemical composition of the polymer solution (e.g., molecular weight and concentration) was maintained throughout the fabrication process to avoid inconsistencies in fiber properties across layers associated with compositional changes [16,24].

Besides fiber diameter and packing density, pore size is another critical parameter in tissue engineering; modulating pore size is important for encouraging cell growth and migration or alternatively, limiting cell motility [41]. Further, pore size is always related to porosity, which contributes to scaffold permeability, enabling transport of nutrients, oxygen and waste [16,42]. Scaffolds constructed with pore size gradient architectures were shown to promote anisotropic cell distribution within the scaffold, similarly to native tissues such as cartilage [43]. Our results also demonstrated a pore size gradient within the scaffolds; we believe such architecture will impact in vitro cellular response. However, further studies are needed to investigate the potential of these graded scaffolds in guiding zonal cell and matrix composition.

Homogeneous scaffolds with comparable composition and morphology to corresponding layers of the graded scaffolds were produced and mechanically tested. Uniaxial tensile and fracture tests on homogeneous scaffolds with sparse, moderate and dense networks demonstrate statistically significantly distinctive mechanical properties, including tensile strength and fracture toughness (Fig. 5). The graded scaffolds, constructed of three layers with similarly sparse, moderate and dense networks, were likely to have the local mechanical properties increased over the scaffold thickness, resulting in the observation of layer-by-layer fracture (Fig. 6).
The finite element analysis on the microscopic deformation near the crack tip also shows that fibers at each layer rearranged and realigned differently during crack tip opening according to the network density.

Functionally graded fibrous scaffolds aim to replace sharp interfaces with gradual changes at the interface to prevent discontinuous material failure. Finite element analysis revealed that fracture of functionally graded networks can be controlled by tailoring the gradients of both network density and fiber alignment. Networks with fiber density gradients (DG) exhibit failure starting at the densest layer and show similar fracture toughness to homogeneous scaffolds. DAG networks featuring microstructural gradients in both density and fiber orientation over their thickness had improved fracture toughness as compared to DG networks. The DAG networks, which have aligned fibers at the densest network layer, postponed failure by rearranging and realigning the orientation of fibers from parallel to perpendicular to the notch to dissipate energy. The fiber rearrangement and realignment distributes stress instead of confining it within a small area [44]. This mechanism improves the fracture toughness of multilayer graded fibrous networks.

The current study has outlined experimental and computational methodologies for generating biomimetic functionally graded materials to recapitulate interface and connective tissues. In terms of mechanical properties, networks featuring both fiber density and alignment gradients are promising scaffold designs for these native tissues. One limitation in this work is that gelatin scaffolds require further crosslinking prior to use in biomedical applications, in order to prevent dissolution in aqueous media at physiological temperates [45]. The degree of crosslinking and hydration of the construct will influence the ultimate scaffold mechanical properties [46], requiring further study of these factors on fracture behavior. In the future, we also plan to experimentally fabricate DAG networks based upon the computational models and compare their observed mechanical properties and fracture behavior to the simulations. We will also assess biocompatibility of the graded electrospun scaffolds, and observe how tailoring mechanical properties of the graded electrospun scaffolds affects cellular viability, ECM production and cell-scaffold interactions.

5. Conclusion

A trilayer scaffold with a microstructural architecture gradient has been successfully produced using a sequential electrospinning technique. The scaffold displayed a gradual increase in fiber diameter and pore size corresponding with a decrease in fiber packing densities from the bottom to top layer. Mechanical properties of both the functionally graded scaffolds and homogeneous scaffolds with fiber morphologies corresponding to that of each of the three layers of the graded scaffold were measured using uniaxial tensile and fracture tests. Mechanical properties of the homogeneous scaffolds, including elastic modulus, tensile strength, and fracture toughness, varied according to microstructural differences. This indicated that the mechanical properties of functionally graded electrospun scaffolds could be tailored by controlling the microstructure of the individual layers. This resulted in a gradual increase in modulus over the thickness of the graded scaffold, which led to the layer-by-layer crack propagation during fracture analysis. Finite element analysis of density-graded scaffolds indicated that fibers rearrange and deform differently at each network layer resulting in inhomogeneous strain distribution over the network thickness. Simulations of scaffolds combining gradients in both fiber density and alignment had larger crack tip openings before fracture, resulting in improved toughness. This work presents experimental methods of fabrication and computational methods of modeling the mechanical and fracture behavior of multilayer scaffolds. Consideration of the type and degree of gradient is critical for tailoring mechanical properties of the scaffold for specific applications. The methods presented herein can be used for the rational design of functionally graded multilayered fibrous scaffolds for interface and connective tissue engineering applications.

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