Microstructure-dependent mechanical properties of electrospun core–shell scaffolds at multi-scale levels

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Article history:
Received 13 August 2015
Received in revised form 19 December 2015
Accepted 22 December 2015
Available online 1 January 2016

Keywords:
Core–shell electrospinning
Electrospun fiber mechanics
Electrospun scaffold mechanics

Abstract

Mechanical factors among many physiochemical properties of scaffolds for stem cell-based tissue engineering significantly affect tissue morphogenesis by controlling stem cell behaviors including proliferation and phenotype-specific differentiation. Core–shell electrospinning provides a unique opportunity to control mechanical properties of scaffolds independent of surface chemistry, rendering a greater freedom to tailor design for specific applications. In this study, we synthesized electrospun core–shell scaffolds having different core composition and/or core-to-shell dimensional ratios. Two independent biocompatible polymer systems, polyetherketone (PEKK) and gelatin as the core materials while maintaining the shell polymer with polycaprolactone (PCL), were utilized. The mechanics of such scaffolds was analyzed at the microscale and macroscales to determine the potential implications it may hold for cell-material and tissue-material interactions. The mechanical properties of individual core-shell fibers were controlled by core–shell composition and structure. The individual fiber modulus correlated with the increase in percent core size ranging from 0.55 ± 0.10 GPa to 1.74 ± 0.22 GPa and 0.48 ± 0.12 GPa to 1.53 ± 0.12 GPa for the PEKK–PCL and gelatin-PCL fibers, respectively. More importantly, it was demonstrated that mechanical properties of the scaffolds at the macroscale were dominantly determined by porosity under compression. The increase of scaffold porosity from 70.2% ± 1.0% to 93.2% ± 0.5% by increasing the core size in the PEKK–PCL scaffold resulted in the decrease of the compressive elastic modulus from 227.67 ± 20.39 kPa to 14.55 ± 1.43 kPa while a greater changes in the porosity of gelatin-PCL scaffold from 54.5% ± 4.2% to 89.6% ± 0.4% resulted in the compressive elastic modulus change from 484.01 ± 30.18 kPa to 17.57 ± 1.40 kPa. On the other hand, the biphasic behaviors under tensile mechanical loading result in a range from a minimum of 5.42 ± 1.05 MPa to a maximum of 12.00 ± 1.96 MPa for the PEKK–PCL scaffolds, and 10.19 ± 4.49 MPa to 22.60 ± 2.44 MPa for the gelatin-PCL scaffolds. These results suggest a feasible approach for precisely controlling the local and global mechanical characteristics, in addition to independent control over surface chemistry, to achieve a desired tissue morphogenesis using the core–shell electrospinning.

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1. Introduction

Tissue engineering approaches provide a viable method for the replacement or regeneration of damaged/diseased tissues. Tissue scaffolds need to be specifically designed for target tissues in order to modulate cells to elicit appropriate phenotypic behaviors and subsequent tissue morphogenesis (Fisher and Mauck, 2013; Langer and Vacanti, 1993). When designing and engineering such tissue replacements, it is critical to address various requirements to meet the biochemical and physical traits of the native tissues. Thus, an in-depth understanding of the interaction between the cells and the physiochemical properties of the scaffolds is essential for enhancing efficacy of the implanted tissue for successful therapeutic tissue regeneration (Khademhosseini et al., 2006; Leach, 2006; Stevens and George, 2005; Xu and Simon, 2005). This fundamental understanding is especially important when stem cells are considered as a cell source for the regeneration of tissues. The behaviors (i.e., proliferation and differentiation) of stem cells such as induced pluripotent stem cells (iPSC), adipose-derived stem cells (ASC), and mesenchymal stem cells (MSC), have shown to be controlled by their microenvironments or ‘cell niche’ (Engler et al., 2006; Park et al., 2011; Pek et al., 2010). In addition to soluble factors, physical environmental factors significantly affect stem cell behaviors. Specifically, mechanical properties of scaffolds (e.g., stiffness) have shown to direct stem cell differentiation independent from material surface chemistry (Huang et al., 2005; Nam et al., 2011). In addition, mechanically different scaffolds elicit different compliances to extrinsic mechanical forces in dynamic culture environments, which subject residing cells to different stress/strain environments to modulate stem cell behavior (Nerurkar et al., 2011). Therefore, scaffolds with well-designed and precisely controlled mechanical properties are essential to enhance tissue morphogenesis of stem cell-based engineered tissue.

The mechanical characteristics of the scaffold influence the differentiation of progenitor cells towards desired phenotypic tissue possibly via modulating the attachment and spreading of the cells that regulate cell morphology. Several studies have shown that control of stem cell morphology via substrate stiffness determines subsequent stem cell differentiation, suggesting the mechanical properties as an important design criterion for scaffolds (Binulal et al., 2010; Engler et al., 2006; Maldonado et al., 2015; Park et al., 2011; Pek et al., 2010). In addition to the ‘intrinsic’ mechanical properties of scaffolds, their performance under extrinsic mechanical stimuli, i.e., forces generated by bodily movement in vivo or ‘mechanical training’ of engineered tissues in vitro, is just as influential for directing stem cell differentiation. For example, dynamic compressive or tensile loading to stem cell/scaffold constructs that mimics the native mechanical environments has shown to induce phenotype-specific differentiation of stem cells, e.g. compressive and tensile forces for chondrogenesis and tendonogenesis, respectively (Bosworth et al., 2014; Lee et al., 2010; Mauck et al., 2000; Moroni et al., 2006). Such in-vivo-like extrinsic mechanical stimulation to the cells within scaffolds is likely determined by the bulk or macroscale mechanical properties of the scaffolds. Not only the mechanical loading direct stem cell differentiation, but it is also essential for maintaining tissue-specific phenotypes of the differentiated cells to support tissue maturation (Bosworth et al., 2014; Gurjarpadhye et al., 2015; Seliktar et al., 2000). Therefore, tissue engineers must simultaneously take into account the macroscale mechanical characteristics of scaffolds for controlled tissue morphogenesis as well as the microscale level properties that control cellular behaviors through modulating the localized cell-material interactions.

With this in mind, electrospinning presents a cost-effective and versatile technique for fabricating nano- micro-structures possessing a high surface area-to-volume ratio, suitable for tissue engineering scaffolds. It also provides precise control over the physical and biological properties via control over various electrospinning parameters including solution properties (natural or synthetic polymer/solvent concentration, viscosity, conductivity) and processing parameters (solution flow rate, applied electric field, collection distance) (Li et al., 2002). The fibrous scaffolds that resemble the morphology of native extracellular matrix (ECM) have shown to be capable of addressing a vast array of potential bioengineering applications (Blackstone et al., 2014; Bosworth et al., 2014; Moroni et al., 2006; Nerurkar et al., 2011; Schenkel-Layland et al., 2009). Recently, we have shown that the stiffness of electrospun scaffolds modulates self-renewal of iPSCs (Maldonado et al., 2015) and differentiation of MSCs (Nam et al., 2011). In addition, we have also shown that electrospun scaffolds provide an appropriate platform to actuate scaffold-seeded cells with physiological mechanical stimulation, resulting in enhanced tissue morphogenesis (Nam et al., 2009).

In this regard, core–shell (also referred to as coaxial or core–sheath) electrospinning further allows for tight control over the physiochemical properties of the scaffolds. The method utilizes concentric needles to encapsulate one material within another in the form of monolithic fibrous structure. Advantages of the core–shell structure include the capability of embedding growth factors or drugs (Jiang et al., 2005; Jiang et al., 2006), or more relevantly in this study, the modulation of the intrinsic mechanical properties while maintaining the homogenous surface chemistry of the shell (Blackstone et al., 2014; Sun et al., 2003). Utilizing an identical shell material with varied core size/material composition enables decoupling of the mechanical factors from the surface chemistry of the scaffolds. This maintains a chemically uniform exterior for cell interaction while providing a means to subject the cells to independently controlled mechanical environments (Zhang et al., 2004, 2005). More importantly, individual electrospun fiber mechanics can be controlled independently from the macroscale scaffold mechanics, presenting further engineering control over the physiochemical properties for various in vitro and in vivo applications.

The objective of this study is to understand the relationship between the microscale (i.e. individual fibers) and macroscale (i.e. bulk fibrous scaffolds) mechanical properties of electrospun core–shell scaffolds. The influence of polymer materials and dimensions of the core–shell structures on the mechanical characteristics of these scaffolds were evaluated. Ultimately, we demonstrate a methodology to control the intrinsic individual fiber mechanics independent of the bulk
scaffold properties, which is useful to develop scaffolds having tissue-specific mechanical properties required for tissue engineering applications.

2. Materials and methods

2.1. Scaffold fabrication

All reagents and materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Core–shell composite scaffolds of poly(etherketoneketone) (PEKK, core)-poly(e-caprolactone) (PCL, shell) or gelatin (core)-PCL (shell) were synthesized by coaxial electrospinning as described previously (Nam et al., 2011). Briefly, the shell polymer solution was prepared by dissolving 11 wt% of PCL (Mw=80,000) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Oakwood Products, Inc., West Columbia, SC). For the core polymer solutions, gelatin (type A, from porcine skin in powder form) or PEKK (OXPEKK-IG100, Oxford Performance Materials, South Windsor, CT) were dissolved in HFIP at a concentration of 10 wt%. A custom dual concentric nozzle 16G (OD: 1.65 mm, ID: 1.26 mm) – 22G (OD: 0.70 mm, ID: 0.41 mm) was utilized to synthesize the shell electrospun fibers (Fig. 1A). Programmable syringe pumps (NE-1010, New Era Pump Systems Inc., Farmingdale, NY) were used in tandem to independently feed the core and shell polymeric solutions (Fig. 1B). An electrical field ranging from 500–600 kV/m was applied to polymer solutions using a high voltage D.C. power supply (Model: FC60R2, Glassman High Voltage, Inc., High Bridge, NJ) to achieve a stable Taylor Cone. Non-woven electrospun fibrous mats were collected on a grounded 3" x 3" aluminum plate. A total of ten different conditions were utilized to synthesize scaffolds with the two previously mentioned core (PEKK or gelatin)-shell (PCL) compositions having different core sizes; each electrospun core–shell fiber composition was fabricated by reciprocally varying the ratio between the core flow rate and the shell flow rate while maintaining the overall flow rate at 11 mL/hr at the following core–shell (C:S) ratios: 1:10, 2:9, 3:8, 4:7, and 5:6. These conditions have a percent core flow rates defined as 9%, 18%, 27%, 36%, and 45%, respectively.

2.2. Morphological characterization of electrospun fibers

The morphology of the electrospun core–shell fibers was characterized using a scanning electron microscope (SEM, Phillips/FEI XL30–FEG, Hitachi, Japan). The average fiber diameter was determined from the measurements of at least 50 fibers per condition using ImageJ software. To observe the cross-section of the core–shell fibers for core-to-shell dimensional measurements, cryogenic fracturing was performed by submerging the fiber mat into liquid nitrogen. The cryo-fractured cross-sections were then characterized by SEM to assess the core and overall fiber diameters. Additionally, samples were submerged in phosphate-buffered solution (PBS) for one month to determine the stability of the scaffolds in an aqueous solution. SEM images were taken for the 9% and 45% core flow rate fibers both before and after PBS incubation.

A liquid displacement method was used to determine the pore volume of the bulk scaffolds as described elsewhere (Guan et al., 2005). Briefly, a 6 mm diameter biopsy punch (Integra Miltex, York, PA) was used to make cylindrical samples from the as-spun fiber mats. Thicknesses of punched cylinders were measured with digital calipers with a ±0.01 mm precision. Each sample was weighed out, submerged into ethanol, and placed under vacuum to allow complete ethanol penetration into the scaffolds. Residual ethanol on the surface of the cylinders was removed, then reweighed to obtain the mass and subsequent volume of ethanol contained within the interconnected porous network of fibers (n = 6).

2.3. Mechanical characterization of electrospun fibers/scaffolds

Individual core–shell electrospun fibers were collected on square copper grids with a 100 μm gap distance (Supplementary Fig. 1). The mechanical properties of the individual fibers were then tested by three point bending using an MFP-3D atomic force microscopy (AFM) system (Asylum Research, Santa Barbara, CA) (Cere and Timoshenko, 1999; Ugural, 1991). A silicon tetrahedral cantilever (AC240TS, Olympus, Japan) with a nominal spring constant of approximately at 2 N/m was used to deflect the mid-point of the fibers to a pre-determined trigger force of 20 nN. The indentation/retraction speed was 2 μm/s. The force-displacement curves were utilized to determine the elastic modulus of the core–shell fibers. All conditions were tested with at least 7 independent samples (n = 7).

To measure the macroscale compressive properties of the scaffolds, a custom compression device was utilized as previously described (Supplementary Fig. 2) (Nam et al., 2009). Briefly, a static impermeable platen with the dimensions of 9.5 mm in diameter was attached to a 1 kg load cell (Model 11, Honeywell Sensing and Control, Columbus, OH).
submicron linear translational stage (AVL-125, Aerotech, Pittsburgh, PA) was used to incrementally deliver a compressive strain along the fiber deposition direction (Fig. 1C). A tare load of 0.02 N was used to ensure that scaffolds were in contact with the impermeable platen. The samples were then compressed at 5% strain increments of the scaffold thickness where the load was held for 90 s until achieving a total of 20% applied strain per sample (Korhonen et al., 2002). The modulus was then calculated from a linear range of the stress-strain curve. All conditions were tested with at least 6 samples per condition (n=6).

In addition, tensile dog bones of the electrospun fiber mats were cut to have the dimensions of an overall length of 40 mm, and a sample gauge width of 20 mm, where the length of the grip section was 10 mm. The gauge length for the reduced section was 15 mm, and the width was 4 mm. The filet radius shoulders were cut using a 6 mm biopsy punch to create a 3 mm radii to ensure a smooth transition from the grip section to the reduced section. Tensile testing parallel to the direction of the fiber deposition layer (Fig. 1D) was carried out using a load frame (model 1322, Instron, Norwood, MA) equipped with a 1 kg load cell. A cross-head speed of 5 mm/min was utilized until complete sample failure. All conditions were tested with at least 5 samples per condition (n=5).

2.4. Theories of mathematical relationship between mechanical properties and morphology

The mathematical relationship between the microscale/macroscopic mechanical properties and morphology of the fibrous structures was investigated using mechanical analysis. First, the relationship between the Young’s modulus of individual core-shell fibers and its geometry was explained by the rule of mixtures in Eq. (1)

\[ E_f = f E_c + (1-f) E_s \]  

(1)

\[ f = \frac{V_c}{V_o} = \frac{r_c^2 - r_s^2}{r_s^2} \cdot \frac{1}{r_c^2 - r_s^2} = \left( \frac{r_s}{r_c} \right)^2 \]  

(2)

where \( V_o, V_c, r_o, r_c \) and \( f \) represent volume of the core, volume of the shell, radius of the core, radius of the shell, and the length of the fiber, respectively. Here, a geometric parameter \( f \) was introduced to represent the cross-sectional volumetric fraction of the core, which was experimentally determined. Young’s moduli of materials that comprises the core or shell are 0.345 GPa, 3.45 GPa, and 2.5 GPa for PCL, PEKK, and gelatin, respectively (Eshraghi and Das, 2010; Fukae and Midorikawa, 2008; Midorikawa et al., 2012).

Secondly, the relationship between the bulk compressive modulus and scaffold porosity was determined by Eq. (3) (Gibson and Ashby, 1997; Zein et al., 2002):

\[ E = C(100-P)^n \]  

(3)

where \( C \) is a material-specific constant, \( n \) is a structural constant, and \( P \) is the porosity of the electrospun fiber mat. For materials which possess a relatively high porosity (\( P > 50\% \)), a structural constant of \( n=2 \) can be used. Experimentally determined porosities were compared to respective compressive moduli to derive a constant, \( C \), for each combination of materials used in this study.

Finally, the relationship between the bulk tensile modulus and the mass density was determined by Eq. (4) (Gibson and Ashby, 1997; Sonnenschein, 2003):

\[ \left( \frac{E}{E_0} \right) = \left( \frac{\rho}{\rho_0} \right)^2 \]  

(4)

where the ratio between apparent Young’s modulus of porous scaffolds (\( E \)) and Young’s modulus of the individual solid fiber (\( E_0 \)) is proportional to the ratio of the square of the relative mass density of the scaffold (\( \rho \)) to the mass density of the individual solid fiber (\( \rho_0 \)).

The Pearson’s \( r \) correlation coefficient is reported to compare these model prediction to the experimentally determined results for the individual fiber, compressive and tensile scaffold moduli for both the PEKK–PCL and gelatin–PCL polymer systems (Bland and Altman, 1994).

2.5. Statistical analysis

All experiments presented were conducted with at least 5 samples (n=5), and data is represented as mean ± standard deviation. Each set of data was analyzed using SPSS (v.19.0) to determine significance by one-way ANOVA. All the data sets presented rejected the null-hypothesis with \( p=0.000 \), confirming their statistical differences among the conditions within the data sets. To correlate electrospun fiber/scaffold morphologies to mechanical properties, Pearson’s correlation analysis was conducted (Fisher, 1934).

3. Results

Various core (C)-shell (S) dimensional ratios of coaxial electrospun fibers were synthesized to systematically investigate how composition and morphology affect the mechanical properties of electrospun scaffolds at different length scales. Two different solution systems, PEKK–PCL and gelatin–PCL, were utilized to produce electrospun fibers. Distinctive C-S dimensional ratios were achieved by systematically modulating feeding flow rate of the core and shell components. The overall microstructures of these fibers showed a similar cylindrical morphology with an average diameter of 5.16 ± 0.43 μm and 4.58 ± 0.35 μm in diameter (Fig. 2A–E and K–O) for the PEKK–PCL and gelatin-PCL systems, respectively. The cross-sectional examination revealed that the core diameter increases with the greater percent core flow rate for both the PEKK–PCL (Fig. 2F–J) and gelatin-PCL (Fig. 2P–T) systems. The percent core flow rates of 9%, 18%, 27%, 36%, and 45% resulted in varying percent core sizes for the different polymer systems used, and are listed in Table 1 along with their respective core and fiber diameters. The results demonstrate that approximately 100% increase in core diameter can be achieved while maintaining relatively the same overall fiber diameter for both systems (Fig. 3A and B). Porosity measurements showed that the variation in the C-S ratios resulted in significant changes in the scaffold porosity, ranging from 70.2 ± 1.0% at the 9% core flow rate condition to 93.2 ± 0.5% for the 45% core flow rate condition for the PEKK–PCL scaffolds,
and from 54.5 ± 4.2% for 9% core flow rate to 89.6 ± 0.4% for 45% core flow rate for gelatin-PCL fibers (Fig. 3C and D). In general, the scaffold porosity increases with the increased volume ratio of the core material to the total fiber volume. Additionally, the 9% and 45% core flow rate scaffolds were subjected to PBS incubation for one month to test the stability of the fibrous structure. The scaffolds did not wettability issues (Supplementary Fig. 3). As shown in Fig. 4 there were no noticeable changes in the structural conformation of the scaffolds. This is evident by comparing the fibers before PBS incubation for the scaffolds that were synthesized by both the 9% core flow rate (Fig. 4A, E, I and M) and 45% core flow rate (Fig. 4C, G, K and O) to after the incubation (Fig. 4B, F, J, N, D, H, L and P) for both polymer systems.

The elastic moduli of individual PEKK–PCL and gelatin-PCL fibers (shown in Fig. 5C and D) were determined from the force–displacement curves by a three point bending test using AFM (Fig. 5A and B). As expected, the elastic modulus of individual core–shell fiber was positively related to the percent core flow rate. The elastic modulus of the PEKK–PCL core–shell fibers...
ranged from 0.55 ± 0.96 GPa for the 16% core size, up to 1.74 ± 0.22 GPa for the 59% core size. Likewise, the gelatin-PCL fibers exhibit values ranging from 0.48 ± 0.12 GPa to 1.53 ± 0.12 GPa, for the 14% and 59% core size, respectively. The experimental measurements agreed well with the prediction by Eq. (1) having a correlation coefficient of 0.995 for PEKK–PCL and 0.934 for gelatin-PCL and resulting significance of \( p = 0.000 \) and 0.002, respectively. In comparison, solid PCL fibers having a similar fiber diameter to the overall fiber diameter of these core–shell fibers exhibit a modulus of 0.32 ± 0.04 GPa (data not shown), similar to the value reported in literature (Eshraghi and Das, 2010). These results demonstrate that core–shell electrospinning can achieve more than 5-fold increase in individual fiber elastic modulus via modulating core dimension while maintaining the overall fiber dimension and the same surface chemistry (i.e., shell material).

To determine the relationship between morphological characteristics and macroscale mechanical properties of core–shell fibers/scaffolds, three dimensional scaffolds were subjected to unconflned compressive loads (Fig. 6). PEKK–PCL (Fig. 6A) and gelatin-PCL (Fig. 6B) scaffolds were subjected to the compressive strains from 5% to 20% applied strain to determine compressive equilibrium modulus (Korhonen et al., 2002). In general, the compressive modulus of core–shell scaffolds decreased as the percent core size increased. The compressive moduli of the PEKK–PCL composite fibers ranged from 14.6 ± 1.4 kPa at 59% core sized fibers to 227.7 ± 20.4 kPa for the 16% core sized fibers (Fig. 6C). Similarly, the gelatin-PCL fiber mats ranged from 17.6 ± 1.4 kPa at the 59% core size, to a high of 484.0 ± 30.2 kPa at the 14% core sized fibers (Fig. 6D). Interestingly, the experimentally determined compressive moduli were not proportionally related to the elastic modulus of individual fibers predicted by Eq.(1) (Fig. 6C and D). Rather, there was a clear relationship between the compressive modulus and the porosity and it well agrees with the mathematical predictions by Eq. (3) (dotted lines in Fig. 6E and F), with correlation coefficients of 0.997 and 0.996 for PEKK–PCL and gelatin-PCL scaffolds, respectively. These correlation coefficients corresponded to significance \( p \) values of 0.000 for the PEKK–PCL and 0.000 for the gelatin-PCL scaffolds. Overall, compressive mechanical properties of electrospin core–shell scaffolds were correlated highly with the macroscale porosity of the electrospun fiber mats rather than the elastic modulus of individual fibers.

The stress–strain curves for PEKK–PCL and gelatin-PCL scaffolds having various percent core sizes under tensile loading are presented in Fig. 6A and B. As compared to gelatin-PCL scaffolds, PEKK–PCL scaffolds exhibited a ductile behavior, in which the failure of the samples occurred beyond 100% strain. Interestingly, there was a biphasic relationship between the core–shell ratio and

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**Fig. 3** – Morphological characterization of electrospun core–shell fiber/scaffold with various core/shell dimensional ratios at micro- and macroscales for (A and C) PEKK–PCL and (B and D) gelatin-PCL systems. (A and B) The overall fiber diameter and core diameter were measured with respect to the change of % core flow rate that was used to control the core/shell dimensional ratios. (C and D) At a macroscale, the porosity of the scaffolds composed of the core–shell fibers with various core-to-shell dimensional ratios was compared to % core flow rate. Standard deviation of samples is smaller than markers in some conditions.
the tensile modulus (Fig. 7C and D). Both the PEKK-PCL and gelatin-PCL scaffolds reached the maximum tensile moduli at approximately 45% and 32% core sized fibers, respectively. Greater than these thresholds in percent core size led to a decrease in the tensile moduli. Below the thresholds, i.e., the 45% and 32% core sized fibers for the PEKK-PCL and gelatin-PCL, respectively, the change of the tensile moduli is proportionally related to the individual fiber modulus predicted by Eq. (1) (dotted lines in Fig. 7C and D). Beyond these core sizes, however, porosity appears to be the dominant factor that determines the tensile modulus. The tensile modulus decreased from 12.0 ± 2.0 MPa at the 45% core sized fibers to 5.4 ± 1.1 MPa for the 59% core sized fibers for the PEKK-PCL scaffolds. Similarly, the maximum tensile modulus of the gelatin-PCL scaffolds was 22.6 ± 2.4 MPa at the 32% core sized fibers while it decreased to 16.8 ± 3.6 MPa when the core size increased to 59%. Remarkably, the tensile modulus of the scaffolds above the scaffold porosities of 45% (PEKK-PCL) and 32% (gelatin-PCL) was well predicted by Eq. (3), with the correlation coefficients of 0.878 and 0.995, and significance of \( p = 0.050 \) and 0.000, for PEKK-PCL and gelatin-PCL scaffolds, respectively (Fig. 7E and F).

4. Discussion

When designing an electrospun scaffold for tissue engineering applications, it is important to consider the physical properties that will ultimately contribute towards the desired use or projected outcome of the material. Considering the fact that both intrinsic (e.g., stiffness) and extrinsic (e.g., compliance to external forces) mechanical properties of the scaffolds influence stem cell fate, it is especially vital to take into account the scaffold characteristics delineating mechanics at the micro- and macroscales. In this study, we have demonstrated that the mechanical properties of individual core–shell electrospun fibers can be modulated independent of the properties of the bulk scaffolds composed of such fibers. More specifically, we showed that the mechanical properties of individual core–shell fibers can be modulated by controlling the dimensional ratio of respective core-to-shell components. In addition, we described how such a structural control at the microscale impacts the mechanics at both microscale and macroscale. Several other studies have successfully tested the mechanical characteristics of individual electrospun fibers (Croisier et al., 2012) and/or selective bulk scaffold traits (D’Amore et al., 2014; Drexler and Powell, 2011; Wong et al., 2008). However, to our best knowledge, this study is the first to systematically investigate the mechanical properties of individual core–shell fibers and their influence on bulk scaffold mechanics under both compressive and tensile loadings.

The mechanical properties of the two core–shell model systems investigated in this study, PEKK-PCL and gelatin-PCL, are robustly dictated by the volume fractions of core and
shell components as predicted by a simple rule of mixtures. Interestingly, the compressive moduli (along the direction normal to the longitudinal axis of individual fibers) of the bulk scaffolds do not seem to strongly depend on the elastic moduli of individual fibers (Fig. 8A). Instead, the bulk compressive mechanics has a strong dependency on the scaffold porosity. As the scaffolds are compressed, the packing density increases and they undergo a densification by reducing pore volume between the fibers (Fig. 8B). Therefore, the compressive modulus decreases to the second order of the relative fiber density, agreeing well with previous studies that reported a power-law relating porosity to the compressive stiffness as in Eq. (3) (Woodfield et al., 2004; Zein et al., 2002).

Unlike mechanical behaviors under compressive loading, which was predominantly modulated by bulk porosity regardless of component materials, tensile moduli of the scaffolds (along the longitudinal direction of individual fibers) are apparently influenced by both individual fiber mechanics and bulk scaffold porosity. Both PEKK–PCL and gelatin-PCL core–shell fiber scaffolds exhibited an increase in the tensile modulus when the percent core size increased. However, the tensile modulus exhibits a biphasic profile when the porosity reached a certain threshold (which is approximately 85% porosity for both polymer systems). When the electrospun scaffold is subjected to a tensile loading in the parallel direction of the fibers, the fibers initially align longitudinally to the applied force while increasing their packing density (Fig. 8C) (Johnson et al., 2007; Johnson et al., 2009). We speculate that the balance between intrinsic material properties of individual core–shell fibers and bulk scaffold properties (i.e., porosity or relative fiber density) determines tensile modulus. It implies that porosity is a critical factor determining core–shell scaffold mechanics, in addition to individual fiber modulus which was commonly regarded as a deterministic factor for electrospun scaffold mechanics under tensile loading. Indeed, Soliman et al. demonstrated the tensile mechanics (ultimate stress, ultimate strain, and Young's Modulus) of electrospun fibers to be dependent on the fiber packing density (Soliman et al., 2011).

Together, these results clearly demonstrate the feasibility of core–shell electrospinning to decouple scaffold surface chemistry from mechanical factors, and enable modification of mechanical cellular environments at the microscale without negatively impacting mechanical properties of scaffolds against applied forces at the macroscale. The cell-material interface can be tailored to achieve desirable biochemical microenvironments via modifications in shell materials regardless of the core polymer used in electrospun core–shell
fibers. This has the ability to directly regulate cellular behaviors from attachment, self-renewal to differentiation of stem cells (Nerurkar et al., 2011; Schenke-Layland et al., 2009). In addition, such surface chemistry modification can be achieved independent of mechanical properties of the scaffolds by utilizing core materials with different mechanical properties. For example, the cell-material interface of both PEKK–PCL and gelatin-PCL scaffolds can be modified to accommodate MSC attachment. A comparable individual fiber modulus of the two is expected to induce a similar cellular behavior under a static condition. However, vastly different mechanical behaviors of the scaffolds under compressive loading will likely induce different degree of MSC differentiation, e.g., towards more chondrogenic phenotype in the softer PEKK–PCL scaffolds as compared to more osteogenic differentiation in the stiffer gelatin-PCL scaffolds.

More importantly, all of the mechanical testing results presented in this study reveal a comprehensive outlook on both the microscale (i.e. individual fibers) and macroscale (i.e. bulk scaffolds) for the mechanical aspects of electrospun core–shell fibrous scaffolds (Supplementary Tables 1 and 2). Although the increase in core content in these fibers increases the individual fiber modulus, this does not always affect the bulk material properties, especially under

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**Fig. 6** – Compressive mechanical characterization of the scaffolds composed of (A, C and E) PEKK–PCL and (B, D and F) gelatin-PCL core–shell fibers. (A and B) Representative compressive stress-strain curves of scaffolds with various % core sizes. Insets show the curves for the core size greater than 26 and 16% for PEKK–PCL and gelatin-PCL, respectively to reveal the differences in mechanical responses. (C and D) Compressive modulus of scaffolds with different % core sizes shows a lack of proportional relationship to the individual fiber modulus predicted by Eq. (1) (MP1, dashed line). (E and F) The relationship between compressive modulus and porosity was compared to a mathematical prediction by using Eq. (3) (MP3, dashed line).
compression. Similarly, the overall scaffold porosity dominates over the individual fiber properties to determine tensile modulus when the porosity is above a certain threshold. Therefore, our results suggest that core–shell electrospinning can provide a means to simultaneously tailor mechanical properties of scaffolds at both micro- and macroscale to achieve the desired and controlled cell niche at the cell and tissue levels.

5. Conclusions

We demonstrated that the mechanical properties of core–shell electrospun fibers can be modulated by controlling the composition and the dimension of core, decoupled from the cell-interfacing surface (shell) chemistry. More importantly, we showed that mechanical properties of such fibers/scaffolds at the micro- and macroscale can be independently regulated by modulating micro- (core size) and macro-structure (scaffold porosity). Considering significant influence of various physiochemical cell niche on cellular behavior and subsequent tissue morphogenesis, the ability to independently tailor surface chemistry, micro- and macro-mechanical properties substantially increases the freedom of scaffold design. By modulating the respective core–shell dimensional ratios we have not only shown that the mechanics of the scaffolds can be regulated, but these fibers hold an additional promise for use in controlled drug release.

Fig. 7 – Tensile mechanical characterization of scaffolds composed of (A, C and E) PEKK–PCL and (B, D and F) gelatin-PCL core–shell fibers. (A and B) Representative tensile stress–strain curves of scaffolds with various % core sizes. (C and D) Tensile modulus of scaffolds with different % core sizes shows a proportional relationship to the individual fiber modulus predicted by Eq. (1) (MP1, dashed line) for smaller % core sizes. (E and F) The relationship between tensile modulus and porosity was compared to a mathematical prediction by using Eq. (4) (MP4, dashed line).
applications for tissue engineering purposes. Our insights in the relationship between the mechanical-morphology dependency at the micro- and macroscale are, therefore, expected to provide a better understanding to scaffold synthesis and aid for developing artificial tissues in biomedical engineering.

Disclosure

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

This work was supported by UCR Initial Complement Funding.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jmbbm.2015.12.034.

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