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Regulation of material properties in electrospun scaffolds: Role of cross-linking and fiber tertiary structure

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Abstract

We cross-linked scaffolds of electrospun collagen to varying degrees with glutaraldehyde using an ethanol-based solvent system and subsequently defined how the percentage of cross-linking impacts bulk and microscale material properties and fiber structure. At hydration, electrospun fibers underwent coiling; the extent of coiling was proportional to the percentage of cross-linking introduced into the samples and was largely suppressed as cross-linking approached saturation. These data suggest that electrospun collagen fibers are not deposited in a minimal energy state; fiber coiling may reflect a molecular reorganization. This result has functional/structural implications for protein-based electrospun scaffolds. Changes in fiber topology that develop during post-electrospinning processing may alter monomer organization, mask or unmask receptor binding sites, and/or change the biological properties of these nanomaterials. Hydrated scaffolds were mounted into a custom stretching device installed on a microscope stage and photographed after incremental changes in strain. Changes in fiber alignment were measured using the two-dimensional fast Fourier transform method. Fibers in all scaffolds underwent alignment in response to strain; however, the rate and extent of alignment that could be achieved varied as a function of cross-linking. We propose four distinct modes of scaffold response to strain: fiber uncoiling, fiber reorientation, fiber elongation and interfiber sliding. We conclude that bulk material properties and local microscale architecture must be simultaneously considered to optimize the performance of electrospun scaffolds.

Keywords: Electrospinning; Electrospun collagen; Mechanical testing; 2D FFT; Anisotropy; Fiber alignment; Scaffold design

1. Introduction

Electrospinning can be used to process native, synthetic or blended polymers into fibrous mats that show considerable promise as tissue engineering scaffolds [1,2]. Both scaffold and fiber-level properties can be adjusted at several stages in the electrospinning process. Fiber composition and diameter can be tailored by varying the polymer identity and starting concentration. During electrospinning, fiber alignment (anisotropy) can be selectively increased or decreased by adjusting the mandrel's tangential velocity [3–5]. Once completed, the material properties of a protein-based scaffold can be further modified through various cross-linking protocols [6]. Each of these variables can be manipulated individually or in concert to provide superb control over the chemical, structural and material properties of a tissue engineering scaffold. The functional properties of this unique class of biomaterials can be manipulated by incorporating various growth factors [7], pharmaceuticals [2,8] or cells [9] into a scaffold at the time of fabrication. The control afforded over the electrospinning process theoretically makes it possible to use this fabrication technique to produce complex, multicellular organs [1,10].

We believe that two basic criteria must be satisfied in order to extend the method of electrospinning from the bench top into clinical practice. First, the macroscale mate-

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rial properties and architectural features of the electrospun scaffold must be consistent and mimic the profile of the native tissue of interest. Second, the local microscale environment must provide physiologically relevant binding sites to anchor cells to the surrounding scaffold. It is through these considerations that the resident cells of most organ systems receive the physical and mechanical signals necessary to express the appropriate phenotypic profile [11,12]. The macroscale architecture and material properties of an electrospun scaffold can be manipulated by regulating fiber composition, fiber diameter, fiber cross-linking and the relative degree of anisotropy introduced into a scaffold during the fabrication process. In turn, the physical cues provided by these macroscopic features can be used to modulate cell phenotype. For example, physical cues provided by the introduction of anisotropy into an electrospun scaffold promote fibroblasts [3] and neuronal cells [13] to align in parallel with the primary axis of fiber alignment. These results suggest that it will be possible to use anisotropy to produce constructs composed of highly aligned cells, a cytoarchitectural feature that is fundamental to the structure and function of skeletal muscle, blood vessels and many other hollow organs. Architectural features and scaffold composition also interact to regulate the extent to which a given construct can support the infiltration and migration of local cell populations [14].

In this study, we examined how the extent of cross-linking that is introduced into an electrospun collagen scaffold impacts the structure and response of this type of construct to externally applied mechanical loads. At modest degrees of cross-linking, we report that the fibers of these scaffolds undergo extensive coiling when hydrated. This coiling was largely suppressed as the degree of cross-linking was increased. Overall, we believe that strain is distributed throughout these scaffolds by at least four distinct modes. The systematic application of strain induces fiber straightening (uncoiling), fiber reorientation (fiber realignment along the axis of strain), fiber elongation (changes in fiber length) and interfiber sliding (fibers moving with respect to one another). Our results suggest that fiber topology plays a critical role in determining the stress–strain relationships observed in collagen electrospun scaffolds.

2. Materials and methods

2.1. Collagen isolation

Type I collagen was acid-extracted from calfskin corium (animals <6 months old; Lampire Biologics, Pipersville, PA). Corium was defrosted at 4 °C, diced into 10–20 cm^3 squares and placed into ice-cold distilled water at a concentration of 25–50 g of solid per liter of solution. Glacial acetic acid was added to the suspension to bring the molarity to 0.5 M, the solution was stirred and allowed to incubate for 12 h at 4 °C. The ice-cold suspension was then homogenized in a commercial grade blender (Waring) in 10–15 s bursts to limit heating. After an additional 48 h of incubation at 4 °C under gentle stirring the resulting collagen-rich solution was centrifuged for 12 h at 14,000*g* (4 °C). Pellets were discarded, and the supernatant was dialyzed against 20 vol of 18 MΩ-cm water. Water was changed 3–4 times while maintaining the dialysis at 4 °C. The extract was then frozen to −70 °C and lyophilized until dry. A representative 10% sodium dodecyl sulfate (SDS) gel of fractions isolated by this method and used in our electrospinning experiments is illustrated in Fig. 1.

2.2. Electrospinning

Reagents were purchased from Sigma–Aldrich (St. Louis, MO) unless noted. Collagen was solubilized in 2,2,2-triflouroethanol (TFE) at 55 mg ml^{-1} for 1 h on a clinical rotator [15]. Solutions were loaded into 10 ml syringes capped with a blunt tipped needle. The positive lead of a constant voltage power supply (Spellman CZE1000R; Spellman High Voltage Electronics Corporation) was attached by an alligator clip to the needle; electrospinning was conducted at 22 kV. The collagen solution was delivered to the needle at 8–12 ml h^{-1} using a Harvard profusion pump. A grounded, rectangular, steel mandrel (70 mm × 10 mm × 5 mm) was placed 20 cm away, and served as the target. The mandrel was set to rotate at less than 200 rpm to ensure the production of scaffolds composed of random elements [4]. Starting conditions were adjusted to produce scaffolds composed of fibers that exhibited an average cross-sectional diameter of 1 μm.

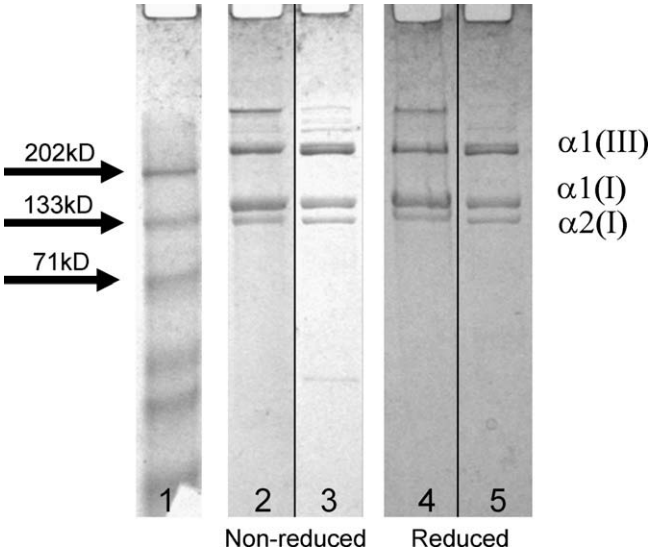

Fig. 1. Collagen analysis by SDS gel electrophoresis. Composite image of two separate gels with samples run under non-reducing (lanes 2 and 3) and reducing conditions (lanes 4 and 5). Lane 1 = mol. Wt. standards; lane 2 = cell prime type I collagen standard; lane 3 = type I collagen isolate from calfskin corium; lane 4 = cell prime type I collagen standard; lane 5 = type I collagen isolate from calfskin corium.