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Mechanical properties of electrospun fibrinogen structures

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Abstract

Fibrin and fibrinogen have a well-established track record in tissue engineering due to their innate ability to induce improved cellular interaction and subsequent scaffold remodeling compared to synthetic scaffolds. Use of fibrinogen as a primary scaffold component, however, has been limited by traditional processing techniques that render scaffolds with insufficient mechanical properties. The goal of this study was to demonstrate, based on mechanical properties, that electrospun fibrinogen overcomes these limitations and can be successful as a tissue engineering scaffold or wound dressing. Electrospun fibrinogen scaffolds were characterized for fiber diameter and pore area and subsequently tested for uniaxial mechanical properties while dry and hydrated. In addition, uniaxial mechanical testing was conducted on scaffolds treated to regulate scaffold degradation in serum-containing media by supplementing the media with aprotinin or cross-linking the scaffolds with glutaraldehyde vapor. A linear relationship between electrospinning solution concentration and measured fiber diameter was seen; fiber diameters ranged from 120 to 610 nm over electrospinning concentrations of 80 to 140 mg/ml fibrinogen, respectively. Pore areas ranged from 1.3 μm^2 to 13 μm^2 over the same fibrinogen concentrations. Aprotinin in the culture media inhibited scaffold degradation in a predictable fashion, but glutaraldehyde vapor fixation produced less reliable results as evidenced by mechanical property testing. In conclusion, the mechanical characteristics of electrospun fibrinogen strongly support its potential use as a tissue engineering scaffold or wound dressing.
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Keywords: Fibrinogen; Electrospinning; Tissue engineering; Scaffold

1. Introduction

Fibrinogen is a naturally occurring plasma protein (340 kDa, globular) that functions as a major element in the coagulation cascade, contributing to clot formation and wound healing [1,2]. Fibrinogen has also been defined as fibrin molecules coupled to charged peptides [3]. The fibrinogen molecule is composed of six chains—two Aα, two Bβ, and two γ chains—that are linked by 29 disulfide bonds [4–6]. This is often denoted by $(A\alpha B\beta\gamma)_2$ since the molecule is composed of two identical halves (a dimer of $A\alpha B\beta\gamma$). When fibrinogen is exposed to thrombin, two peptides are cleaved to produce fibrin monomers. These monomers, in the presence of Ca^{2+} and factor XIII, lead to the assembly of stable fibrous clots (insoluble gels) and/or other fibrous structures. These stable structures function as nature's provisional matrix, on which tissues rebuild and repair themselves, making this type of structure an attractive tissue engineering scaffold [7–9].

Fibrinogen-based scaffolds have previously been developed in the form of fibrin gels [7,8,10–14] and wet extrusion

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fibronectin–fibrinogen cables [15,16]. These studies demonstrated that fibrinogen-based scaffolds are easily degradable and non-immunogenic [7,8] and promote increased cell migration [16]. The practical use of fibrin gels, however, has been limited by lack of structural integrity. Use of wet extrusion has been limited by large resulting fiber size; the 200–250 μm diameter ranges in which they are produced are several orders of magnitude larger than the native protein fibers in the extracellular matrix, which typically have diameters of 50–300 nm.

A preliminary study demonstrated that the process of electrospinning could be utilized to produce fibrous, non-woven fibrinogen structures composed of fiber diameters as low as 80 nm [17]. These 80 nm fibers also possessed the ultrastructure (22.5 nm banding) found in native, polymerized fibrinogen fibers. In addition, this study showed that fiber diameter was directly proportional to electrospinning solution concentration, as is typically seen for other natural and synthetic polymers [17].

Electrospinning is accomplished by inducing a large electric potential (typically 15–30 kV direct current) in a polymer solution (or melt) that is separated by some distance from an oppositely charged target to create a static electric field [18]. As electric field potential increases, the electrostatic forces in the solution overcome the surface tension of the solution and a thin liquid jet composed of entangled polymer chains is ejected from the polymer reservoir. This jet then travels through space towards the oppositely charged target. Instabilities within the charged jet define its orientation in space (condition previously described as whipping motion of the fiber [19]). As the liquid jet travels through space, the solvent evaporates forming a fiber that deposits as a dry, fibrous structure, and, eventually, a non-woven mat collects on the target. Over the last several years, both natural and synthetic polymers have been used in electrospun form for tissue engineering applications [17,18,20–32].

While the feasibility of electrospinning non-woven fibrinogen scaffolds of sub-micron diameter fibers has previously been demonstrated, the material properties important to assess performance have not yet been evaluated. The corollary is that additional steps would be required to use fibrinogen in these applications since it can be rapidly degraded by the enzymatic activity of thrombin and other generic serum proteases present in any serum-containing media or a wound site. Thus, the overriding hypothesis for this study is that fibrinogen can be electrospun into a non-woven structure (mat) that displays the biomimicking geometry of a native provisional matrix and subsequently be treated to maintain the necessary mechanical integrity for use as a tissue engineering scaffold or wound dressing. For this study, various concentrations of fibrinogen were electrospun to form non-woven, fibrous structures, which were characterized in terms of fiber diameter and approximate pore area followed by uniaxial mechanical testing of dry, hydrated and treated (to prevent serum degradation) samples.

2. Methods

2.1. Electrospinning

For this study, fibrinogen (Bovine soluble fraction 1, Sigma Aldrich Chemical Co.) was dissolved in nine parts 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma Aldrich Chemical Co.) and 1 part 10× minimal essential medium (MEM) at concentrations of 80, 90, 100, 110, 120, 130, and 140 mg/ml. These concentrations were determined by a preliminary study to be the full range of solution concentrations (inherent viscosities) capable of producing electrospun fibers under ambient conditions. Electrospinning was accomplished by loading the solutions into a 5.0-ml syringe, which was placed in a KD Scientific syringe pump (Model 100) for metered dispensing at 1.8 ml/h. The positive output lead of a high voltage power supply (Spellman CZE1000R; Spellman High Voltage Electronics Corp.), set to 22 kV, was attached to a blunt 18 gauge needle on the syringe as depicted in Fig. 1. A grounded target (2.5 cm wide ×10 cm long ×0.3 cm thick; 303 polished stainless steel) was placed 10 cm from the needle tip and rotated at 500 revolutions per minute and translated at 1.5 cm/s over a 7.5 cm travel distance to evenly coat the mandrel and create a mat of uniform thickness without imparting a large degree of alignment to the deposited fibers.

2.2. Scaffold characterization

Following electrospinning, representative samples were taken from each mat for characterization of fiber diameter and pore area. The samples were dry after the electrospinning process and required only sputter coating with gold (Electron Microscope Sciences Model 550) for scanning electron microscopy (SEM) (JEOL JSM-820 JE Electron Microscope) evaluation from 1000× to 4000× magnification. The SEM micrographs (Polaroids) were digitized with a flat bed scanner (Hewlett–Packard Scanjet 6200C) and then analyzed by ImageTool 3.0 (Shareware provided by University of Texas Health Science Center in San Antonio) to determine the average fiber diameters (average and

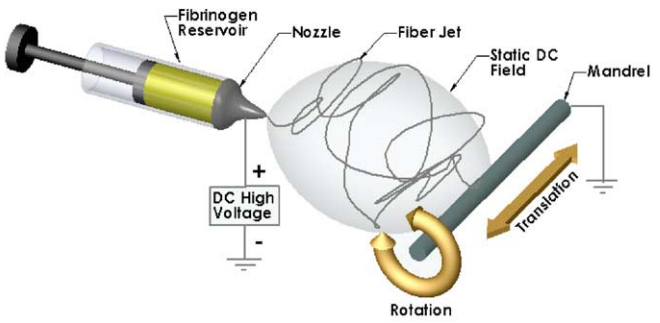
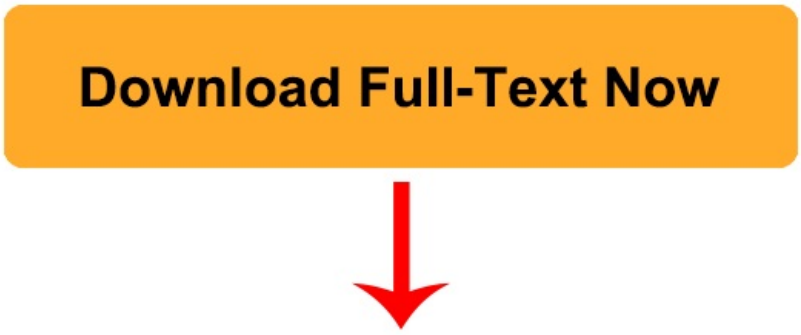


Fig. 1. A diagrammatic representation of the electrospinning process. A syringe is used as the reservoir and a blunt 18 gauge needle is used as the nozzle; a high voltage power supply charges the solution to 15–30 kV.

ID	Title	Pages
2644	Mechanical properties of electrospun fibrinogen structures	10



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