Published in final edited form as:

Mater Sci Eng C Mater Biol Appl. 2012 March 1; 32(2): 215–221. doi:10.1016/j.msec.2011.10.021.

The Mechanical Properties of Dry, Electrospun Fibrinogen Fibers

Stephen Baker^a, Justin Sigley^a, Christine R. Carlisle^a, Joel Stitzel^b, Joel Berry^{a,c}, Keith Bonin^a, and Martin Guthold^{a,*}

^aDepartment of Physics, Wake Forest University, Winston-Salem, North Carolina 27109

^bDepartment of Biomedical Engineering, Wake Forest University Health Sciences, Winston-Salem, NC, 27157

Abstract

Due to their low immunogenicity, biodegradability and native cell-binding domains, fibrinogen fibers may be good candidates for tissue engineering scaffolds, drug delivery vehicles and other medical devices. We used a combined atomic force microscope (AFM)/optical microscope technique to study the mechanical properties of individual, electrospun fibrinogen fibers in dry, ambient conditions. The AFM was used to stretch individual fibers suspended over 13.5 μm wide grooves in a transparent substrate. The optical microscope, located below the sample, was used to monitor the stretching process. Electrospun fibrinogen fibers (diameter, 30–200 nm) can stretch to 74 % beyond their original length before rupturing at a stress of 2.1 GPa. They can stretch elastically up to 15 % beyond their original length. Using incremental stress-strain curves the viscoelastic behavior of these fibers was determined. The total stretch modulus was 4.2 GPa while the relaxed elastic modulus was 3.7 GPa. When held at constant strain, fibrinogen fibers display stress relaxation with a fast and slow relaxation time of 1.2 s and 11 s.

In comparison to native and electrospun collagen fibers, dry electrospun fibrinogen fibers are significantly more extensible and elastic. In comparison to wet electrospun fibrinogen fibers, dry fibers are about 1000 times stiffer.

Key terms

Electrospinning; nanofibers; stress-strain curves; atomic force microscope (AFM); fluorescence microscope

Introduction

With a concentration of a few g/L, fibrinogen is among the most abundant proteins in blood plasma; it is also non-immunogenic and relatively easy to purify. Its major physiological role is to form a meshwork of nanoscopic fibrin fibers – the major structural component of a

© 2011 Elsevier B.V. All rights reserved.

*Corresponding Author: Martin Guthold, Ph.D., Department of Physics, 7507 Reynolda Station, Wake Forest University, Winston-Salem, North Carolina 27109, Phone: 336-758-4977, Fax: 336-758-6142, gutholdm@wfu.edu.

^cPermanent address: Joel L. Berry, Ph.D. Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, Alabama 35233

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

hemostatic blood clot - in the event of vascular injury. Fibrinogen also plays a role in platelet adhesion and wound healing [1]. These physiological and biological properties of fibrinogen may make fibrinogen fibers a good candidate for use in biomedical devices, such as tissue engineering scaffolds, drug delivery vehicles, cell substrates, wound pads, sutures, and others [2]. Some of these devices are used in wet (aqueous) conditions; others may be used in dry and/or wet conditions. Most of these devices, such as drug delivery vehicles, wound pads and sutures, may transition from a dry (manufacture, storage) to a wet environment (application). It is, thus, important to investigate their properties in dry (ambient) and wet conditions.

The performance of biomedical devices does not only depend on the physiological and biological properties of their components, but also on the *mechanical* properties of their components. Clearly, the device components need to endow the device with sufficient mechanical integrity and it may be important to maintain this integrity in dry and wet conditions. In other applications, such as drug delivery vehicles, wound pads, or sutures it may be beneficial to have a strong, stable material in dry conditions, that then changes to a softer, pliable, biocompatible, digestible material in the body. The mechanical properties of devices can have a strong influence on the biological function of the device. For example, the differentiation of cells depends on the mechanical properties of the substrate; stem cells grown on hard, medium hard and soft substrates differentiated into bone, muscle and nerve cells, respectively [3–5].

The performance of a device can also depend on the device topography at the nanoscopic and microscopic level. For example, cells grow better on substrates mimicking the dimensions and porosity of the extracellular matrix, rather than on flat, featureless substrates [2].

Thus, biomedical devices often need to fulfill the following requirements: They need to mimic the fibrous, porous topography of the extracellular matrix at the microscopic level; they need to have specific mechanical properties at the microscopic, and macroscopic level; and they need to be fashioned from biocompatible materials. Additionally, they should be stable under storage, and it may be beneficial if they change properties in the body under wet conditions.

Electrospinning technology offers the potential to control material, structural, and mechanical properties of biocompatible scaffolds and devices. In electrospinning, an electric field created by a high-voltage source causes a jet of polymers in a volatile solvent to elongate into ever thinner fibers, speeding evaporation so that nanometer diameters can be achieved. The fibers are drawn to a lower voltage surface or collection plate [6, 7]. Electrospinning has been used to fabricate biomaterials for bone, ligament, blood vessel, peripheral nerve, skin, cartilage, muscle, heart, and heart valve [8–15].

Electrospun fibers, and fibers in the extracellular matrix, have a diameter on the order of a hundred nanometers. Until recently, it has been difficult to determine the mechanical properties of these nanoscopic fibers, since a suitable methodology was missing. We have developed a combined atomic force microscope (AFM)/optical microscope technique to determine the mechanical properties of individual nanoscopic fibers in buffer or ambient conditions [16–18]. Using standard cantilevers, this technique can measure forces in the 10^{-2} to 10^4 nN range and should, thus be applicable to many native biological fibers, electrospun fibers, or other nanoscopic fibers [19]. Here, this technique was used to determine the mechanical properties of single, dry electrospun fibrinogen fibers. These results complement our experiments on electrospun fibrinogen fibers in wet (aqueous) conditions [17]. We report extensibility, elasticity, stiffness and relaxation behavior. We

found that electrospun fibrinogen fibers are easy to make, stable (in dry and wet environments), and they are more extensible and elastic than electrospun collagen fibers [20, 21], and may, thus, become the fiber of choice for some biomedical devices.

Methods and Materials

1.1 Substrate preparation

Preparation of the striated substrate is based on soft lithography and micromoulding in capillaries [22]. Briefly, a PDMS (polydimethylsiloxane) stamp was prepared by pouring dimethylsiloxane plus catalyst (Sylgard, Dow Corning Corp, Midland, MI) onto an SU-8-silicon master grid (gift from Prof. Superfine, University of North Carolina, Chapel Hill) in a Petri dish. The polymer was cured at 70°C for 1 h. The PDMS stamp was removed from the master and pressed into a 10 µl drop of Norland Optical Adhesive-81 (NOA-81, Norland Products, Cranbury, NJ) on top of a 60 mm × 24 mm, #1.5 microscope cover slide (Thomas Scientific, Swedesboro, NJ). The NOA-81 was cured for 70 s with UV light (365 nm setting, UVP 3UV transilluminator, Upland, CA) and the stamp was removed. The substrate pattern had 6.5 µm wide ridges separated by 13.5 µm wide and 6 µm deep channels.

1.2 Formation of electrospun fibrinogen fibers

Fibrinogen fibers were electrospun based on the procedures developed by Wnek et al [23]. A solution of 100 mg/ml lyophilized bovine fibrinogen, (Sigma-Aldrich Chemical Co.), 9 parts 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma Aldrich), and 1 part minimum essential medium (MEM, 10× MEM, Gibco, Invitrogen cell culture) was prepared and placed in a 1 ml, 4 mm diameter syringe (Becton-Dickinson, Franklin Lakes, New Jersey). The syringe was equipped with a 20 gauge blunt needle (Howard Electronic Instruments, Kansas) attached to Teflon tubing (Small Parts Inc.). The Teflon tubing connected to a 3 mm piece of 20 gauge hypodermic tubing (Small Parts Inc.). The syringe was placed in a syringe pump (PHD 2000 Infusion Syringe Pump, Harvard Apparatus, Holliston, Massachusetts), the hypodermic tubing was maintained at a voltage of 22 kV (Spellman High Voltage Electronics Corporation) and the solution was dispensed at a rate of 2 ml/hr toward a grounded substrate a distance of 16 cm away. Fibers were spun for 5–10 s onto each substrate which consisted of a striated cover slide taped to the front of a grounded copper plate. Fiber preparation was done in a large plexiglass box with access to open air (ambient conditions and air pressure), at room temperature (23°C). As is typical in electrospun fibers, the solvent evaporates as the fibers form and get stretch out in the electric field, resulting in dry fibers on the substrate. The fiber sample was removed from the electrospinning apparatus and was stored in a small plastic box in ambient conditions until further use. We did not determine the residual hydration level of the fibers that might occur due to the ambient humidity in the laboratory. However, since all fibers were treated and stored the same way, and since our results were reproducible across several measurements, we assume that the residual water content was very low and consistent across fibers. A schematic of the setup, and an SEM image of electrospun fibers on the striated substrate are shown in Figure 1.

1.3 Combined microscopy and manipulation

Fibrinogen fiber manipulations and force acquisitions were performed using a combined atomic force and inverted optical microscopic technique [16, 17, 24]. The AFM (Topometrix Explorer, Veeco Instruments, Woodbury, NY) rests on a custom-made stage on top of an inverted microscope (Zeiss Axiovert 200, Göttingen, Germany) (Figure 2A). The fibrinogen sample is sandwiched between the AFM and optical microscope. The stage is designed to allow for independent movement of the objective, AFM cantilever and electrospun

fibrinogen sample. Illumination for the sample is provided by the camera light inside the AFM.

The AFM cantilever tip (NSC35/AIBS force constant 14 N/m, MikroMasch, Wilsonville, OR) was placed in the center of a groove next to a fiber for manipulation. The tip, controlled by a nanoManipulator (3rd Tech, Chapel Hill, NC), was then used to laterally stretch the fiber at a rate between 306 and 395 nm/s. Images and movies of the manipulation were collected by a Hamamatsu EM-CCD C9100 Camera (Hamamatsu Photonics KK, Japan) and IPlab software (Scanalytics, Fairfax, VA) (Figure 2C). Meanwhile, the tip travel distance, s , elapsed time and left-right photodiode signal, I_l , were recorded by the nanoManipulator software.

1.4 Stress and strain calculation

Stress and strain values were calculated as previously reported [16, 17]. The left-right photodiode signal, I_l , was recorded during a manipulation to determine the lateral force via, $F_l = K_C \cdot I_l$. The lateral force spring constant K_C , was determined from cantilever beam

mechanics, $K_C = \frac{Ewt^3}{6l^2(h+t/2)} \cdot S_n$, where E is the Young's modulus of silicon (1.69×10^{11} N/m²) w , t , and l are the cantilever width, thickness and length respectively, S_n is the normal sensor response of the cantilever, and h is the height of the tip. The length and width of the cantilever and the height of the tip were determined using the optical microscope, and the thickness was calculated using the resonance frequency of the cantilever,

$$f = 0.276 \cdot \sqrt{\frac{Ewt^3}{\rho(\pi \cdot h^3 \cdot l^3 + 2.832 \cdot w \cdot t \cdot l^4)}}, \text{ where } \rho = 2330 \text{ kg/m}^3 \text{ is the density of silicon.}$$

The stress was determined by dividing the force applied to the fiber by the cross-sectional area of the fiber, A . The cross-sectional area, $A = \pi \cdot (D/2)^2$, was determined by AFM imaging the fiber on top of the ridge (adjacent to the groove where it was manipulated). Imaging was done in noncontact mode and the diameter, D , was extracted from the

topography data; assuming a circular cross-section. Stress, $\sigma = \frac{F_{\text{fiber}}}{A}$, was calculated assuming a constant fiber radius (engineering stress) and using the trigonometric relationship between the lateral force measured by the AFM tip, F_l , and the force applied to

the fiber F_{fiber} : $F_{\text{fiber}} = \frac{F_l}{2 \sin \beta}$, where $\beta = \arctan \frac{s}{L_{\text{initial}}}$ (see Figure 2B). The strain of the fiber was determined using the initial length of the fiber, recorded by the optical images, and the distance of the tip travel, s , recorded by the nanoManipulator. The engineering strain,

$\epsilon_{\text{Eng}} = \frac{L' - L_{\text{initial}}}{L_{\text{initial}}}$, where L' is the length of the stretched fiber and L_{initial} is the initial length of the fiber (Figure 2B) and the true strain, $\epsilon_{\text{True}} = \ln(1 + \epsilon_{\text{Eng}})$, were calculated. We choose to use true strain over engineering strain because we believe that it is a more accurate representation of the actual mechanical properties of these fibers at high strains.

Results

Fibrinogen fibers were electrospun from a 100 mg/ml fibrinogen solution, onto a striated substrate for mechanical testing. The striated substrate had 13.5 μm wide and 6 μm deep grooves and 6.5 μm wide ridges. We then viewed the fibers under the optical microscope, and selected single fibers for manipulation that spanned the ridges, as shown in Figure 2B and 2C. As the fibers were pulled, we observed the manipulation with the optical microscope to ensure that each fiber stayed attached to the ridges. We have found that

fibrinogen fibers are very sticky. They attach non-specifically and very strongly to the cured optical adhesive substrate, without any further treatment. Slippage may occasionally occur at the interface between the fibers and the substrate; however, these slippage events can be easily detected in the AFM force data traces and/or in the optical microscopy images and movies. We are familiar with data that do show slippage, since other, non-biological fibers, such as electrospun PCL fibers, often do show slippage (data not shown). Data in which the fiber detached from either ridge were not included in the analysis. The average diameter of the manipulated fibers, as determined by AFM, was 95 nm, with a range from 30 nm to 200 nm.

2.1 Fiber extensibility

We first determined the maximum extensibility, $\epsilon_{\max}$, of electrospun fibrinogen fibers, i.e. the strain at which the fiber ruptures, by stretching the fibers parallel to the ridges until they broke (Figure 2C). The fibers were pulled at a rate between 306 and 395 nm/s. As the fibers were stretched the force required to stretch the fibers increased (Figure 3). In the figure the force increases until the fiber reaches a strain of 57%, at which point the fiber ruptures and the force drops to zero. The average maximum extensibility, $\epsilon_{\max}$, was found to be 74 ± 22 % before rupturing at a stress of 2.1 ± 1.5 GPa (all values presented as average $\pm$ standard deviation). Figure 3C, shows a histogram of the maximum extensibility. The change in slope of the graph at ~ 35 % strain (Figure 3A, B) indicates that the modulus of electrospun fibrinogen fibers decreases with increasing strain; this phenomenon is known as strain softening. All manipulated fibers showed strain softening and the average change in slope, taken as the initial slope divided by the slope after softening, was 2.9 ± 1.1 . This change in slope occurred at ~ 20 % strain.

2.2 Fiber viscoelastic properties

Next, the elastic limit of electrospun fibrinogen fibers was determined. The elastic limit, as described here, is the maximum strain after which the fiber is still able return to its original length once the applied force is removed. We first pulled the fiber to a small strain and then retracted the AFM tip to allow the fiber to return to its initial position. If permanent deformation did not occur the fiber was pulled to a greater strain and returned again. This process was repeated, increasing the strain at each step, until permanent deformations could be seen in the fiber. After visual inspection of permanent fiber deformation, the stress-strain curves were also used to verify the strain at which the fiber deformed (Figure 4). In Figure 4A, the first three pulls, blue, green, and red, respectively, do not reach the elastic limit and the fiber returns to its original position and shape. This can be seen from the plot because the stress returns to zero as the true strain reaches zero. The black curve, however, shows the elastic limit for this fiber has been exceeded, since the stress goes to zero (no tension) before the strain reaches zero. That is, in this curve, the fiber had some slack due to permanently induced deformations. Figure 4B shows a histogram of the elastic limit; the average elastic limit is $\epsilon_{\text{elastic}} = 15 \pm 4$ %.

We also determined the total and elastic modulus for dry electrospun fibrinogen fibers. Figure 5 shows an example of an incremental stress as a function of true strain curve for dry electrospun fibrinogen fibers. Figure 5A shows a plot of the true strain as a function of time. For this example, the fiber was pulled to a strain of $\epsilon = 67$ % and held for 21 seconds. It was then pulled to a strain of $\epsilon = 80$ % and held for 31 seconds. Lastly it was pulled to a strain of $\epsilon = 102$ % and held for 34 seconds. The fiber ruptured after further pulling. Figure 5C, D shows a plot of stress as a function of time for the same fiber manipulation. The plots together show that as the fiber is held at constant strain the stress decays, but it does not decay to zero stress. This is indicative of viscoelastic behavior.

Figure 5B shows the stress as a function of true strain for the same fiber manipulation. The red curve is the raw data, the black solid line is the total modulus determined by the slope between adjacent stress peaks, and the black dashed line is the elastic component of the total modulus determined by fitting an exponential curve to the stress decay. The mean value for total modulus of dry electrospun fibrinogen fibers was 4.2 ± 3.4 GPa and the elastic modulus was 3.7 ± 3.1 GPa.

We did not observe any trend in mechanical properties related to fiber diameter, indicating that electrospun fibrinogen fibers may have a relatively homogeneous crosssection. For *natural fibrin* fibers, we did observe a diameter-dependence of the Young's modulus – the Young's modulus decreased with increasing diameter [18, 25] – which indicates that natural fibrin fibers are denser in the center than at the periphery of the crosssection.

The incremental stress versus time curves can also be used to determine the stress relaxation times. Figure 5D shows a typical relaxation curve. The relaxation times are determined by fitting a double exponential curve, $y = a \exp(-t/\tau_1) + b \exp(-t/\tau_2) + c$, to the relaxation curve. For dry electrospun fibrinogen fibers the fast relaxation time was $\tau_1 = 1.2 \pm 0.4$ s and the slow relaxation time was $\tau_2 = 11 \pm 5$ s. A single exponential fit can only fit either the slow or fast relaxation and therefore did not fit the data at either the beginning or the end of the relaxation curve.

Discussion

The physiological function fibrinogen is to form a mesh of fibrin fibers - the major structural component of hemostatic blood clots – in the event of vascular injury. The fibrin network also provides a scaffold for binding of tissue cells such as monocytes, fibroblasts, and endothelial cells while fibrin degradation products attract monocytes and neutrophils [26]. The biocompatibility of fibrinogen and its role in tissue repair and proliferation make fibrin(ogen) fibers a natural choice for medical devices, including tissue engineering, drug delivery, or wound healing applications. Moreover, fibrinogen can be easily electrospun into nanoscopic fibers. Electrospinning is done in ambient conditions, and thus, the fibers in the initial device are dry. The device may then be further processed (e.g. loaded with drugs, fashioned into a new shape, etc) and stored until use. Subsequent use will then often involve exposing the device to the aqueous environment of the body. It is, thus, important to determine the properties of the fibers comprising medical devices in dry and wet conditions.

We determined the mechanical properties of individual, dry, electrospun fibrinogen fibers. The fibers had an average diameter of 95 nm, the same order of magnitude as natural fibrin fibers and fibers of the extracellular matrix. Electrospun fibrinogen fibers are relatively stiff and remarkably extensible and elastic, when compared to other natural, electrospun, or even synthetic fibers of similar stiffness (Table 1 and [27]). For example, dry electrospun collagen fibers ($e_{\max} = 33\%$; $e_{\text{elastic}} = 1\text{--}2\%$) [20], native collagen fibers ($e_{\max} = 12\text{--}68\%$; $e_{\text{elastic}} = 3\text{--}8\%$), the fibers in the spokes of spider webs (*Araneus* MA silk, ($e_{\max} = 27\%$) or even nylon ($e_{\max} = 15\text{--}50\%$; $e_{\text{elastic}} = 8\%$) are less extensible.

Natural, *wet* uncrosslinked fibrin fibers have a modulus of 4 MPa [18], and they have been shown to be extraordinarily extensible ($e_{\max} = 226\%$ for uncrosslinked fibers) [16]. Wet, electrospun fibrinogen fibers retain some of the mechanical properties of the native fibrin fibers as they have a similar modulus of 17.5 MPa and large extensibility ($e_{\max} = 130\%$) [17]. Thus, upon drying, electrospun fibrinogen fibers become nearly a 1000 times stiffer, but only somewhat less extensible. Wet and dry fibrinogen fibers may be useful in applications that require extensible and elastic nanofibers. Thus, these fibers may be able to fill a unique niche in medical or material science applications.

We would like to propose a molecular mechanism why electrospun fibrinogen fibers become so much stiffer upon drying. The 1000-fold increase in stiffness suggests that the removal of water stiffens or fixes molecular elements that are soft and flexible in the presence of water. Very recent experiments suggest that a key element providing fibrin fibers with its low modulus, elasticity and extensibility is the long, flexible, mostly unfolded alpha-C region [28, 29]. This region may also play a significant role in influencing the mechanical properties of electrospun fibrinogen fibers. We would like to propose that water is critical to keep this region solvated and unfolded, and that removal of water may severely reduce the mobility and extensibility of this region. Thus, the mechanical properties of fibrin and fibrinogen fibers, are dominated by the soft, extensible alpha-C region in the presence of water; in the absence of water this region becomes stiffer (or is fixed), and other elements, for the example the alpha-helical coiled coils may play a more dominant role.

Fibrinogen is a highly water-soluble molecule. It occurs at micromolar concentrations in blood; yet, it does not polymerize until thrombin activates it by removing fibrinopeptides A and B to expose binding sites A and B. Given this high water solubility, it is surprising that electrospun fibrinogen fibers are stable in aqueous buffers, even without any activation or crosslinking, as was observed in our previous study [17]. In the following paragraph we would like to discuss this surprising stability of electrospun fibrinogen fibers. There are some lines of evidence indicating that fibrinogen largely maintains its native structure under electrospinning conditions. For example, the CD spectra of fibrinogen in aqueous buffer and in electrospinning solvent are similar; and the mechanical properties of native fibrin fibers and electrospun fibrinogen fibers are similar. The A:a interactions, which direct the half-staggered assembly of fibrin into proto-fibrils, are missing in the electrospun fibrinogen fibers, raising the question ‘what holds the electrospun fibrinogen fibers together in aqueous buffers?’. There must be additional or strengthened interactions in electrospun fibrinogen fibers. We speculate that the interactions between the alpha C-region, which exist in fibrin fibers, might also be present in electrospun fibers. In soluble fibrinogen, this region is folded back and tucked into the central domain. Electrospinning conditions may untuck these regions allowing them to interact with each other. This could be tested by spinning fibers from a mutant fibrinogen that don’t have this region. Electrospun collagen fibers, a different protein without such a flexible region, are not stable in aqueous buffer (without crosslinking). Additional interactions between the fibrinogen molecules in the fibers may be provided by the salt and amino acids contained in the minimum essential medium (MEM) of the spinning buffer. In fact, it is difficult to spin fibrinogen fibers without MEM.

Previous researchers have reported on the use of electrospun fibrinogen as a candidate for tissue scaffolds. McManus et al. electrospun fibrous mats of fibrinogen and evaluated their bulk mechanical behavior under dry and wet conditions [30]. They found that wet conditions increased extensibility but modulus and peak stress were significantly reduced. In a later study, it was found that human bladder smooth muscle cells readily migrated into electrospun mats of fibrinogen and initiated remodeling through collagen production [31].

From a biomechanical viewpoint, electrospun fibrinogen fibers offer composition and elasticity features that favor cell attachment and the potential for remodeling. The RGD amino acid sequence (Arg-Gly-Asp) is the universal cell recognition motif and can be found at positions A α 95-97 and A α 572-574 on the fibrinogen molecule, while RGD independent integrin binding sites occur on the fibrinogen γ -chain and RGD independent cell interactions have been reported [32]. Many cell types such as endothelial cells, smooth muscle cells, keratinocytes, fibroblasts, and leukocytes and growth factors such as fibroblasts growth factor-2 (FGF-2) and vascular endothelial cell growth factor (VEGF) have been shown to bind to fibrin(ogen) [32–34]. Cellular adhesion and proliferation along with the mechanical properties of the fibers are likely to be important components of

engineered load-bearing structures like blood vessels and bone where cell attachment and subsequent mechanotransduction are essential to tissue remodeling. Native tissues, like arteries for example, exhibit a highly elastic material behavior at lower physiologic pressures (elastin dominates) and a stiffening behavior at higher pressures (collagen dominates) [35]. Fibrinogen could well serve as the elastic component for engineered arteries. In the case of engineered valves for pediatric patients, the degradability of fibrinogen may be important so that the valve can remodel as the patient grows.

Single fiber data, as presented in this paper, will inform the design of electrospun medical devices and tissue scaffolds, and will, thus, ultimately allow intelligent and guided design of scaffolds for medical devices.

Highlights

- Fabricated dry, electrospun, fibrinogen fibers; average diameter, $D_{\text{avg.}} = 95$ nm.
- Determined mechanical properties with combined atomic force/optical microscope.
- Fibers are very extensible ($\epsilon_{\text{max}} = 74\%$) and elastic ($\epsilon_{\text{elastic}} = 15\%$).
- Fibers rupture strength: 2.1 GPa
- Fiber total modulus, $E_{\text{tot.}} = 4.2$ GPa; elastic modulus, $E_{\text{el.}} = 3.7$ GPa.
- Fiber stress relaxation times: $\tau_1 = 1.2$ s and $\tau_2 = 11$ s.

Acknowledgments

This research was supported by the NIH, R41 CA103120 (MG); R21EB006539 (JS & JB); NSF, CMMI-0646627 (MG); and the American Heart Association, 081503E (CRC). We thank the NIH research resource P41 EB002025 for general support.

References

1. Hantgan, RR.; Lord, ST. Fibrinogen Structure and Physiology. In: Colman, RW.; Marder, VJ.; Clowes, AW.; George, JN.; Goldhaber, SZ., editors. Hemostasis and Thrombosis. Basic Principles and Clinical Practice. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 285-316.
2. Ayres CE, Jha BS, Sell SA, Bowlin GL, Simpson DG. Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol. 2010; 2:20–34. [PubMed: 20049828]
3. Chowdhury F, Na S, Li D, Poh YC, Tanaka TS, Wang F, Wang N. Nat. Mater. 2010; 9:82–88. [PubMed: 19838182]
4. Engler AJ, Sen S, Sweeney HL, Discher DE. Cell. 2006; 126:677–689. [PubMed: 16923388]
5. Reilly GC, Engler AJ. J. Biomech. 2010; 43:55–62. [PubMed: 19800626]
6. Huang Z-M, Zhang YZ, Kotaki M, Ramakrishna S. Composites Science and Technology. 2003; 63:2223–2253.
7. Reneker DH, Chun I. Nanotechnology. 1996; 7:216–223.
8. Badami AS, Kreke MR, Thompson MS, Riffle JS, Goldstein AS. Biomaterials. 2006; 27:596–606. [PubMed: 16023716]
9. Foley JD, Grunwald EW, Nealey PF, Murphy CJ. Biomaterials. 2005; 26:3639–3644. [PubMed: 15621254]
10. Kumbar SG, Nukavarapu SP, James R, Nair LS, Laurencin CT. Biomaterials. 2008; 29:4100–4107. [PubMed: 18639927]
11. Lee CH, Shin HJ, Cho IH, Kang YM, Kim IA, Park KD, Shin JW. Biomaterials. 2005; 26:1261–1270. [PubMed: 15475056]

12. Li WJ, Tuli R, Okafor C, Derfoul A, Danielson KG, Hall DJ, Tuan RS. *Biomaterials*. 2005; 26:599–609. [PubMed: 15282138]
13. Liao I, Liu JB, Bursac N, Leong KW. *Cellular and Molecular Bioengineering*. 2008; 1:133–145. [PubMed: 19774099]
14. Tillman BW, Yazdani SK, Lee SJ, Geary RL, Atala A, Yoo JJ. *Biomaterials*. 2009; 30:583–588. [PubMed: 18990437]
15. Vesely, I. *Cardiovascular Tissue Engineering*. Lee, RT., editor. Los Angeles: The Saban Research Institute; 2005. p. 743-755.
16. Liu W, Jawerth LM, Sparks EA, Falvo MR, Hantgan RR, Superfine R, Lord ST, Guthold M. *Science*. 2006; 313:634. [PubMed: 16888133]
17. Carlisle CR, Coulais C, Namboothiry M, Carroll DL, Hantgan RR, Guthold M. *Biomaterials*. 2009; 30:1205–1213. [PubMed: 19058845]
18. Liu W, Carlisle CR, Sparks EA, Guthold M. *J. Thromb. Haemost.* 2010; 8:1030–1036. [PubMed: 20088938]
19. Zhao HP, Feng XQ, Gao HJ. *Applied Physics Letters*. 2007; 90
20. Carlisle CR, Coulais C, Guthold M. *Acta Biomaterialia*. 2010; 6:2997–3003. [PubMed: 20197123]
21. Yang L, Fitié CFC, van der Werf KO, Bennink ML, Dijkstra PJ, Feijen J. *Biomaterials*. 2008; 29:955–962. [PubMed: 18082253]
22. Xia YN, Whitesides GM. *Angewandte Chemie-International Edition*. 1998; 37:551–575.
23. Wnek GE, Carr ME, Simpson DG, Bowlin GL. *Nano Letters*. 2003; 3:213–216.
24. Peng L, Stephens BJ, Bonin K, Cubicciotti R, Guthold M. *Microscopy Research and Technique*. 2007; 70:372–381. [PubMed: 17262788]
25. Guthold M, Liu W, Stephens B, Lord ST, Hantgan RR, Erie DA, Taylor RM, Superfine R. *Biophysical Journal*. 2004; 87:4226–4236. [PubMed: 15465869]
26. Clark, RAF. *Fibrin and wound healing*. Nieuwenhuizen, W.; Mosesson, MW.; DeMaat, MPM., editors. *Fibrinogen*: 2001. p. 355-367.
27. Guthold M, Liu W, Sparks EA, Jawerth LM, Peng L, Falvo M, Superfine R, Hantgan RR, Lord ST. *Cell Biochemistry and Biophysics*. 2007; 49:165–181. [PubMed: 17952642]
28. Houser JR, Hudson NE, Ping L, O'Brien ET Iii, Superfine R, Lord ST, Falvo MR. *Biophysical Journal*. 2010; 99:3038–3047. [PubMed: 21044602]
29. Carlisle CR, Ariens RAS, de Willige SU, Standeven KF, Guthold M. *Biophysical Journal*. 2011 (under revision).
30. McManus MC, Boland ED, Koo HP, Barnes CP, Pawlowski KJ, Wnek GE, Simpson DG, Bowlin GL. *Acta Biomaterialia*. 2006; 2:19–28. [PubMed: 16701855]
31. McManus M, Boland E, Sell S, Bowen W, Koo H, Simpson D, Bowlin G. *Biomedical Materials*. 2007; 2:257–262. [PubMed: 18458483]
32. Laurens N, Koolwijk P, De Maat MPM. *Journal of Thrombosis and Haemostasis*. 2006; 4:932–939. [PubMed: 16689737]
33. Sahni A, Baker CA, Sporn LA, Francis CW. *Thrombosis and Haemostasis*. 2000; 83:736–741. [PubMed: 10823272]
34. Sahni A, Francis CW. *Blood*. 2000; 96:3772–3778. [PubMed: 11090059]
35. Fung, WC. *Biomechanics: Mechanical Properties of Living Tissues*. 2 ed.. New York: Springer; 1993.
36. Silver FH, Christiansen D, Snowhill PB, Chen Y, Landis WJ. *Biomacromolecules*. 2000; 1:180–185. [PubMed: 11710098]
37. Shadwick RE. *Journal of Applied Physiology*. 1990; 68:1033–1040. [PubMed: 2341331]
38. Sasaki N, Odajima S. *J. Biomech.* 1996; 29:655–658. [PubMed: 8707794]

Figure 1. Electrosprinning

(A) Schematic of the Electrosprinning apparatus. The syringe pump is used to regulate the flow of the solution through the blunt needle tip. The tip is held at 22 kV and the pump rate is 2 ml/hr. Fibers are spun onto a glass slide prepared with ridges and attached to a grounded copper plate. (B) SEM image of the electrospun fibrinogen fibers suspended over the grooves of a striated substrate. Scale bar is 7.00 μm .

Figure 2. Fibrinogen Fiber Manipulation

(A) Schematic of fibrinogen fiber manipulation. The fiber is suspended over the grooves of a striated substrate. The AFM tip, located above the sample, pulls on the fiber while the optical microscope, located below the sample, acquires images and movies of the manipulation. Figure adapted from [17]. (B) Top view schematic of fiber manipulation. L_{initial} is the initial length of the fiber, L' is the length of the stretched fiber and s is the distance the tip has traveled. L' can be found trigonometrically from L_{initial} and s , and the strain can be calculated from these quantities (see text). Image adapted from [17]. (C) Optical microscopy movie frames of a fiber being stretched and broken. The fiber is on the left in the first photograph and the cantilever (large, dark object) and AFM tip (marked by an asterisk) are visible on the right. The broken fiber is to the right of the cantilever in the fourth image. Scale bar is $14\ \mu\text{m}$.

Figure 3. Extensibility

(A) Lateral force vs. true strain for $\epsilon_{\max}$. The fiber is continuously stretched until it breaks. Strain softening is visible at 34% strain, as the slope of the curve decreases. The extensibility or breaking strain of this fiber is 57%. Lateral Force is shown in units of nA to understand the general shape of the curve. (B) Lateral force is shown in nN. True strain was plotted starting at 10% strain because this is where true strain and engineering strain differ,

and because the error in $F_{\text{fiber}} = \frac{F_r}{2 \sin \beta}$ can become very large for small β (small strains). (C)

Maximum extensibility for dry fibrinogen fibers (n=47). The average maximum extensibility was 74 ± 22 %.

Figure 4. Elastic Limit

(A) Stress vs. true strain for the elastic limit ($\epsilon_{\text{elastic}}$) of dry fibrinogen fibers. The fiber was pulled four times with the first pull being shown in blue. Since the stress and strain return to zero at the same point when the fiber is allowed to relax, $\epsilon_{\text{elastic}}$ has not been reached. Pulls 2 (green) and 3 (red) show the same kind of relaxation. Pull 4 (black) shows that the elastic limit has been reached because the return stress reaches zero before the strain reaches zero.

(B) Elastic limit distribution for dry fibrinogen fibers ($n = 47$). The average elastic limit was $15 \pm 4\%$.

Figure 5. Incremental Stress-Strain Curves and Relaxation

(A) True strain versus time for a fiber that was pulled incrementally. The fiber was stretched to a given length and held at a constant strain while allowing the fiber to relax. The plateaus on the graph indicate where the AFM tip was stopped and the fiber was held at constant strain during the manipulation. (B) Stress versus true strain for dry electrospun fibrinogen fibers that were pulled incrementally. The red curve is the raw data for the fiber, the black solid line is the total modulus determined by the stress before relaxation, and the black dashed line is the elastic modulus component of the total modulus determined by fitting an exponential curve to the relaxation. (C) Stress versus time for a fiber that was

pulled incrementally. The stress was shown to decay exponentially during the time when the fiber is held at a constant strain, showing viscoelastic behavior for dry electrospun fibrinogen fibers. **(D)** Relaxation curve for dry electrospun fibrinogen fiber. The curve was fit with a double exponential function which produced two relaxation times. The fast relaxation time for this fiber was $\tau_1 = 1.5$ s and the slow relaxation time was $\tau_2 = 20$ s.

Table 1

A comparison of the available, mechanical properties of electrospun fibrinogen, native fibrin, electrospun collagen and native collagen fibers. $\epsilon_{\max}$, maximum extensibility; $\epsilon_{\text{elastic}}$, elastic limit; E_0 , relaxed (elastic) Young's modulus; E_{∞} , (total) Young's modulus; τ_1 , fast relaxation time, τ_2 , slow relaxation time; h, strain hardening factor (ratio of total Young's modulus at high strain to total Young's modulus at low strain). A number $h > 1$ indicates strain hardening, a number $h < 1$ indicates strain softening.

FiberType	$\epsilon_{\max}^a$	$\epsilon_{\text{elastic}}^a$	E_0 (MPa)	E_{∞} (MPa)	τ_1 (s)	τ_2 (s)	h	Ref
Dry, electrospun fibrinogen fibers	110% ^a	16% ^a	4200	3700	1.2	11	0.3	<i>This paper</i>
Wet, electrospun fibrinogen fibers	130%	-	17.5	7.2	3.0	55	~0.5	[17]
Wet, crosslinked fibrin fibers	147%	50–75%	8.0	4.0	2.1	49	1.9	[18]
Wet, uncrosslinked, fibrin fibers	226%	60–120%	3.9	2.0	2.9	54	3.21	[18]
Dry, electrospun collagen fibers	33%	<2%	-	10,000 – 200 ^c	-	-	<0.1 ^d	[20, 21]
Wet, crosslinked electrospun collagen fibers ^b	-	-	-	200 – 70 ^c	-	-	-	[21]
Native tendon collagen fibers	12	-	-	7,500 – 160	-	-	-	[36–38]

^aFor better comparison, engineering strain, $\epsilon_{\text{Eng}} = \frac{L^* - L_{\text{initial}}}{L_{\text{initial}}}$, is reported in the table for all fibers. In the text of this paper we report true strain $\epsilon_{\text{True}} = \ln(1 + \epsilon_{\text{Eng}})$. For dry, electrospun fibrinogen fibers, $\epsilon_{\max}$, true = 74% $\epsilon_{\text{elastic}}$, true = 15% (as in manuscript text), and $\epsilon_{\max}$, eng. = 110%, $\epsilon_{\max}$, eng. = 16% (as in table).

^bUncrosslinked, electrospun collagen fibers are not stable in aqueous buffers.

^cThe modulus of electrospun collagen fibers strongly depends on the radius, with thinner fibers having a larger modulus; fiber diameter ranged from about 200 nm to 800 nm [20] and 150 nm to 500 nm [21].

^dElectrospun collagen fibers show extreme strain softening and h may be significantly smaller than 0.1 [36], turkey tendon; [37], pig digital tendon; [38], bovine Achilles tendon.