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The mechanical properties of dry, electrospun fibrinogen fibers

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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.

1. 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 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, digestable 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 levels. 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 levels; 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.

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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.

2. Methods and materials

2.1. 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.

2.2. 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 part 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/h 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 stretch 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 Fig. 1.

2.3. 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) (Fig. 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.

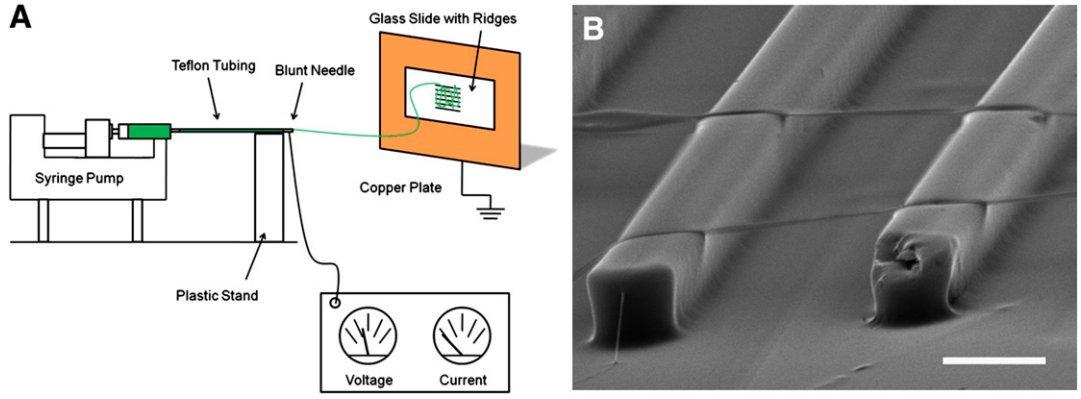

Fig. 1. Electrospinning. (A) Schematic of the electrospinning apparatus. The syringe pump is used to regulate the flow of the solution through the blunt needle tip. The tip is held at 22 kV and the pump rate is 2 ml/h. Fibers are spun onto a glass slide prepared with ridges and attached to a grounded copper plate. (B) SEM image of the electrospun fibrinogen fibers suspended over the grooves of a striated substrate. Scale bar is 7.00 μm.