Published in final edited form as:

J Mater Sci Mater Med. 2012 November ; 23(11): 2679–2695. doi:10.1007/s10856-012-4739-7.

Seamless, axially aligned, fiber tubes, meshes, microbundles and gradient biomaterial constructs

Rod R. Jose,

Department of Biomedical Engineering, Science and Technology Center, Tufts University, 4 Colby Street, Medford, MA 02155, USA

Roberto Elia,

Department of Biomedical Engineering, Science and Technology Center, Tufts University, 4 Colby Street, Medford, MA 02155, USA

Matthew A. Firpo,

Department of Surgery, School of Medicine, The University of Utah, 30 N 1930 E, Salt Lake City, UT 84132, USA

David L. Kaplan, and

Department of Biomedical Engineering, Science and Technology Center, Tufts University, 4 Colby Street, Medford, MA 02155, USA

Robert A. Peattie

Department of Biomedical Engineering, Science and Technology Center, Tufts University, 4 Colby Street, Medford, MA 02155, USA

Abstract

A new electrospinning apparatus was developed to generate nanofibrous materials with improved organizational control. The system functions by oscillating the deposition signal (ODS) of multiple collectors, allowing significantly improved nanofiber control by manipulating the electric field which drives the electrospinning process. Other electrospinning techniques designed to impart deposited fiber organizational control, such as rotating mandrels or parallel collector systems, do not generate seamless constructs with high quality alignment in sizes large enough for medical devices. In contrast, the ODS collection system produces deposited fiber networks with highly pure alignment in a variety of forms and sizes, including flat ($8 \times 8 \text{ cm}^2$), tubular (1.3 cm diameter), or rope-like microbundle (45 μm diameter) samples. Additionally, the mechanism of our technique allows for scale-up beyond these dimensions. The ODS collection system produced 81.6 % of fibers aligned within 5° of the axial direction, nearly a four-fold improvement over the rotating mandrel technique. The meshes produced from the 9 % (w/v) fibroin/PEO blend demonstrated significant mechanical anisotropy due to the fiber alignment. In 37 °C PBS, aligned samples produced an ultimate tensile strength of $16.47 \pm 1.18 \text{ MPa}$, a Young's modulus of 37.33 MPa, and a yield strength of $7.79 \pm 1.13 \text{ MPa}$. The material was 300 % stiffer when extended in the direction of fiber alignment and required 20 times the amount of force to be deformed, compared to aligned meshes extended perpendicular to the fiber direction. The ODS technique could be applied to any electrospinnable polymer to overcome the more limited uniformity and induced mechanical strain of rotating mandrel techniques, and greatly surpasses the limited length of standard parallel collector techniques.

1 Introduction

Electrospinning is a cost-effective nanofiber production technique gaining popularity due to its ease of use, broad polymer compatibility, and receptivity to system modifications. The basic electrospinning technique involves the generation of a strong electric field between a polymer solution passing through a metallic capillary tip or spinneret set to high voltage and a grounded collection plate [1–3]. When the voltage reaches a critical value, solution charge overcomes the surface tension of the deformed drop of the polymer solution, producing a jet at the spinneret tip that travels towards the collector plate [3–5]. Along its path, the jet undergoes a series of electrically induced bending instabilities as solution fractions of varying charge repel and attract each other. This results in extensive stretching of the jet through a violent whipping mechanism known as the instability region [2, 5, 6], which transforms the dissolved molecules within the jet into thousands of stiffened nanoscale fibers. Residual solvent evaporates from the surface of the fibers as they descend towards the collector, reducing the fiber diameter and compacting the fibers. A dense network of dry nanofibers is ultimately deposited on the collector.

Fiber deposition patterns at the system collector are determined by the shape, size, and position of the system collector [5–7], which controls the size and curvature or diameter of the instability region. Through appropriate design of the collection system, electrospinning configurations can be modified to tune the mechanical properties, porosity and degradability by tissue-resident enzymes of the electrospun fibers [7–13]. Specific properties can be selected to optimize deposited fiber networks for particular applications including planar meshes, tubes and three-dimensional scaffolds [13–18]. Electrospun materials have been used as wound dressings, tissue scaffolds, nerve guides, vascular grafts, drug delivery vehicles and affinity membranes [11, 13, 15, 19–23]. Each of these applications has benefited from the development and implementation of novel hardware modifications imparting new capabilities not previously available on common commercial electrospinning systems.

Consistent control of fiber organization throughout the mesh is important not only for the reliable production of desired mechanical properties, but also because when cells are seeded onto electrospun meshes, the nanofibrous topography has been shown to have a strong influence on cellular organization and integration, which is critical to proper scaffold function [24–26]. In addition, it may be desirable that as the material degrades or cells infiltrate deeper into the scaffold, a consistent nanofiber topography be presented, to maintain cell viability. Accordingly, methods for controlling the alignment of deposited nanofibers are crucial for many clinically important applications of electrospun biomaterials.

Unfortunately, current methods for controlling alignment have significant disadvantages. Fiber alignment can be improved simply through the use of multiple collectors in parallel, but the resulting product size is limited [27, 28]. At present, the most popular collection method for directing fiber alignment is the rotating mandrel or wheel, a process in which fibers deposit onto the surface of a rapidly rotating mandrel collector, aligning in the direction of mandrel rotation [29–31]. However, although mandrel systems are capable of producing much larger aligned fiber samples than parallel collector techniques, the alignment quality is inferior. Mechanical forces exerted by the rotating mandrel on the fibers as they deposit introduce inconsistent areas of strain to each fiber, degrading fiber diameters and causing fiber breaks, fatigue, and plastic deformation.

Parallel collector and rotating mandrel techniques have several additional drawbacks that are common to both. Neither is capable of producing a controlled gradient in random-to-aligned fiber orientations blended into the direction of fiber alignment. Such a capability could

address the difficulty of merging two interconnected tissue types, such as in muscle–tendon or ligament–bone junctions. In addition, current methods are incapable of electrospinning seamless, true axially aligned nanofibrous tubes, which are important for providing topological cues capable of guiding endothelial cell morphology and neural outgrowth in appropriate orientations [13, 25, 30, 32–35]. As a result, there remains a need for hardware configurations that can produce high purity, axially aligned meshes, seamless tubes and scaffolds with fiber orientation and density gradients in the direction of alignment.

To overcome these limitations, a novel, highly reliable but very low cost collection system has been developed that effectively drives the electrospinning process by manipulating the instability region. This oscillating ground deposition system (ODS) operates by continuously switching an electrical ground back and forth between two collector plates. High purity deposited fiber alignment is achieved by the resulting switching of the instability region electric field. The system can be used with any polymers capable of properly responding to standard electrospinning hardware, although optimal operating parameters such as the switching period and solution physico-chemical properties will be specific to the particular polymer. Below, the system operation is described, and several high purity nanofibrous electrospun materials with morphologies and fiber orientations not previously electrospinnable are presented. These materials can enable clinically applicable constructs not currently available to the biomedical community.

2 Materials and methods

2.1 Materials

Cocoons of the silkworm *Bombyx mori* were supplied by Tajima Shoji Co. (Yokohama, Japan). Sodium carbonate, lithium bromide, methanol, polyethylene oxide (PEO) and polyethylene glycol (PEG) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Slide-a-Lyzer dialysis cassettes were purchased from Pierce, Inc. (Rockford, IL, USA).

2.2 Preparation of electrospinning solutions

The capabilities of the ODS system were tested and optimized using silk fibroin and blended fibroin/PEO solutions, which were prepared following published procedures [36]. In brief, *B. mori* silk cocoons were boiled in 0.02 M aqueous Na_2CO_3 for 40 min to extract the sericin component and isolate the silk fibroin protein. Isolated silk fibroin was then washed three times for 20 min in deionized water and allowed to dry for 48 h at room temperature. Dried silk was dissolved in 9.3 M LiBr at 60 °C for 3 h, and the resulting 20 % (w/v) solution was dialyzed against water using a Slide-a-Lyzer dialysis cassette (molecular weight cutoff 3500) for 2 days to remove salts. The ensuing concentration of aqueous silk fibroin ranged from 5 to 7 % (w/v), which was calculated by weighing the remaining solid after drying. This solution was then concentrated by dialyzing against 20 % (w/v) PEG for varying times to produce 5.78–25.34 % (w/v) silk fibroin aqueous solutions.

All silk fibroin solutions were stored at 4 °C until use. The same solutions were also used to produce silk fibroin films for comparison. Before spinning, each solution below the concentration of 20 % (w/v) was blended with 5 % (w/v) PEO in the ratio of four parts aqueous silk fibroin solution to one part PEO solution, to improve electro-spinnability and flexibility of the fibers.

2.3 Electrospinning hardware and process

To allow control of the environmental conditions in which electrospinning was carried out, which is crucial for successful fiber production, the ODS was housed in a closed cabinet divided into three shared environment sections by two shelves (Fig. 1a). The electrospinning

cabinet and shelves were composed of melamine laminate coated particle board. This material was chosen for its heat-resistant, non-conductive, wipe-clean properties. Additionally, the material provides insulating properties when working with solvents requiring temperature control. Fiber deposition on the cabinet surface during electrospinning was negligible. The top compartment housed the polymer solution to be spun and a syringe pump (Orion, model M362, Thermo Fisher Scientific Inc., Barrington, IL, USA) that delivered the solution to a spinneret. It also held a dual zone digital thermometer and humidity meter with external probe (# 63-1032, Radioshack CO, Fortworth, TX, USA) and a 500 W heating element with thermostat (#36415K26, McMaster-Carr, Inc., Atlanta, GA, US) that preheated and maintained the polymer solution temperature. These components rested on two $8 \times 15 \times 1 \text{ cm}^3$ stone tiles that increased the thermal inertia of the compartment.

The middle compartment served as a dedicated electrospinning zone. Collectors were mounted to its floor. The floor position and orientation could be adjusted to increase or decrease electrospinning area as desired for particular applications. A square hole measuring $45 \times 45 \text{ cm}^2$ was cut from the floor to permit air circulation between the middle and bottom compartments. It was covered by a ceramic plate resting on small standoffs that provided further thermal inertia to help keep the cabinet environment uniform. Ambient temperature and humidity in the middle compartment were controlled by a hot plate (#31745K15, McMaster-Carr, Inc., Atlanta, GA, US) and aluminum heat sink located in the bottom compartment. Beakers of deionized water were placed on the hot plate to increase humidity when needed.

A blunt tip 16-gauge 304 stainless steel dispensing needle (#6710A23, McMaster-Carr, Inc., Atlanta, GA, USA) protruded through the upper shelf into the middle cabinet and served as the system spinneret. The luer lock fitted dispensing needle measured 50 mm long, with a 1.2 mm ID, and 1.7 mm OD. A $2.5 \times 7.5 \text{ cm}^2$ diameter aluminum focal plate mounted to the spinneret helped guide the spinning fibers toward the collectors. A variable output, high voltage transformer (model HV350, Information Unlimited Inc., Amherst, NH, US) driven by a 12 V, 12 amp power supply (Pyramid Inc., Brooklyn, NY, USA) supplied current to the spinneret and focal plate.

During the electrospinning process, the fibroin or fibroin/PEO solution was delivered through the spinneret needle at a flow rate of $40 \mu\text{L}/\text{min}$. A voltage of 10 kV at a current of $1 \mu\text{A}$ was applied to the needle, focal plate and the electrospinning solution.

Several different styles of collectors were used to fabricate meshes with different geometries and fiber patterns. Standard meshes consisting of randomly oriented fibers were produced using a single, solid, stationary, round, 8 cm diameter $\times$ 0.5 cm thick aluminum plate as collector (Fig. 2a). To electrospin these meshes, the plate was permanently connected to electrical ground. Fibers deposited on the collector surface.

In contrast to standard randomly oriented fiber meshes, meshes composed of aligned fibers were produced using a mechanically driven switch and a series of collectors designed specifically for the ODS (Fig. 2b–d). The novel mechanism of the ODS, which caused fibers to be deposited between the collectors with uniform axial alignment, was the use of a mechanical switch functioning at a programmable frequency to continuously alternate the location of ground between two independent collectors. This switch consisted of a single pole, double throw, manual rocker switch (#7030K53, McMaster-Carr, Inc., Atlanta, GA, USA), driven by a 115 VAC 20 rpm gearmotor (#6142K55, McMaster-Carr, Inc., Atlanta, GA, USA) through a camshaft (Fig. 1b). Normally the switch was operated at 0.33 Hz, but its toggling frequency could be varied between 0.1 and 1 Hz by varying the motor speed. If

desired, toggling frequency can be further varied by substituting with motors producing different rotation speeds.

To fabricate planar meshes with uniform, axial fiber alignment, two electrically independent, 8 cm width $\times$ 0.3 cm thick, flat, zinc-plated steel plates served as collectors (Fig. 2b). The plates were mounted on 25 cm high ceramic stands fastened to adjustable PVC legs that allowed angle and spacing adjustments. They were positioned 23 cm below the droplet and 8 to 23 cm from each other in different trials. Fiber alignment was controlled by connecting each collector to a separate electrically independent output of the switch. During electrospinning, first one collector was provided with electrical ground for a half period, normally 1.5 s, while the second collector was disconnected. Then for the next half period, ground was switched to the second collector while the first collector was disconnected. After a full period, it was switched back to the first collector. This oscillation of ground was maintained continuously until the electrospinning process was completed. Fibers deposited between the collectors.

Planar meshes with axial fiber density gradients were produced with the same arrangement as uniform, axially aligned fibers, but using lower solution concentrations of 8.5 % (w/v) fibroin/PEO to induce a higher rate of fiber breaking and recoiling.

To fabricate seamless electrospun tubes with walls consisting of uniform, axially aligned fibers, the flat collector plates were replaced with aluminum rotating cylindrical collectors measuring 1.3 cm outer diameter and 1 cm inner diameter (Fig. 2c). Two separately grounded, short, hollow cylindrical collectors positioned 13 cm from each other were placed 18 cm from the droplet. An automated collection system was designed that allowed these collectors to rotate around their own axis as fibers were deposited (Fig. 2d). In this mechanism, collector rotation was driven by a separate, compact 12 VDC, 1.3 rpm gear motor (#6409K12, McMaster-Carr, Inc., Atlanta, GA, USA), through 2.5 cm OD acetal timing belt drive pulleys (#57105K13, McMaster-Carr, Inc., Atlanta, GA, USA) and 1 cm neoprene timing belts (#6484K23, McMaster-Carr, Inc., Atlanta, GA, USA). Again, electrical ground was alternated between the two collectors. Axially aligned fibers spanning the gap between the collectors were deposited as the collectors rotated, creating seamless tubes with consistent and even nanofiber coverage. The tube diameter was determined by the collector dimensions, while the overall tube length was determined by the distance between the collector pair.

In the microbundle electrospinning process, a 9 % (w/v) fibroin/PEO blended solution was used with the rotating collectors, as in the tube electrospinning process. After the desired quantity of aligned fibers had been deposited in the form of a tube, rotation of the two collectors was stopped. Then rotation was initiated at 1.3 rpm for one collector in the pair while the other was held fixed. This process was continued until the fibers were wound into rope-like microbundle of acceptable length.

For comparison purposes, tubular aligned samples were also produced using a rotating mandrel collector at 3,000 RPM, at a charging voltage of 9.5 kV at a current of 1 μ A, from 9 % (w/v) fibroin/PEO at a flow rate of 40 μ L/min.

2.4 Fiber post-processing

In some trials, deposited fiber meshes were used as spun without further processing. In other trials, it was desired to treat the completed meshes with methanol, to induce β -sheet formation, increase the mesh crystallinity and thereby improve mesh mechanical properties, resiliency, and water insolubility. In those trials, following published protocols [13], samples

were submerged in 99.9 % (w/v) methanol for 5 min after removal from the collectors. They were then laid on parafilm and allowed to air dry.

2.5 Fibroin film fabrication

Aqueous fibroin solution prepared as described above was used to cast films, for comparison of mechanical properties with the meshes. Films were fabricated as previously described [37], blending aqueous fibroin with 99 % (w/v) glycerol to produce a 25 % (w/v) solution. The resulting solution was poured onto polystyrene plates and allowed to dry in ambient conditions. Films were 120 μm thick, and were cut to shape using the stencil described in Sect. 2.8. After cutting, samples were submerged in 99.9 % (w/v) methanol for 5 min to increase the film crystallinity. They were then laid on parafilm and allowed to air dry.

2.6 Morphology

Deposited fiber ultrastructural patterns were evaluated by scanning electron microscopy (SUPRA 55VP ultra high resolution FE-SEM, Carl Zeiss Inc., Thornwood, NY, USA). Finished samples were mounted on aluminum pegs, then sputter coated with gold nanoparticles for 1 min. Fiber features were imaged at high magnification at an accelerating voltage of 2 kV.

2.7 Mesh characterization

To calculate fiber alignment, five SEM images with a fiber density of at least 100 fibers per frame were selected from each of the following categories: as-spun standard random fibers, methanol treated standard random fibers, as-spun aligned fibers produced using the ODS collectors, methanol treated aligned fibers produced using the ODS collectors and as-spun aligned fibers produced using the rotating mandrel. The angles of each fiber were measured by overlaying straight lines on the images using ImageJ software (National Institutes of Health, Bethesda, MD, USA). To compensate for the bending of individual fibers, the overlaid line was broken into segments until the measured fiber stayed within one fiber diameter of the line at all bends. The angle of each resulting segment was then measured and recorded. Measurements from each image were placed into 10° bins ranging from -90 to 90° , and the resulting fiber distribution $d(\theta)$ was fit by least squares analysis to a Gaussian form

$$d(\theta) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(\theta-\mu)^2}{2\sigma^2}} \quad (1)$$

where θ is the fiber angle, μ is the mean angle and σ^2 is the distribution variance.

Using ImageJ software, the threshold of each image was set to exclude void space. The ratio of excluded pixels to total pixels per image was then used to estimate void area fraction and sample porosity.

To determine the sample overall size, dried meshes were laid on parafilm and their length and width measured with calipers. Separately, a stylus profilometer (Dektak 6 M, Veeco Inc., Plainview, NY, USA) was used to measure sample thickness and roughness. For that purpose, the sections were mounted on glass slides and fixed with a drop of 99 % (w/v) methanol. The profilometer stylus had a radius of 12.5 μm and force was set at 3 mg.

Roughness (R_a) was expressed as $R_a = 1/n \sum_{i=1}^n |y_i|$ where y_i is the difference in height between any measured point and the calculated mean sample height.

2.8 Mechanical testing

Tensile testing of random and aligned fiber meshes and films was performed with a uniaxial mechanical tester (model 3366, Instron Inc., Norwood, MA, USA), with a length between clamps of 35 ± 3 mm. Samples were excised using a stencil in a shape such that the cross sectional area of the specimen smoothly reduced to 12 mm in the center. Shapes of aligned samples were cut in the direction of alignment as well as perpendicular to the direction of alignment. Three samples per group were tested in both simulated physiological conditions in PBS at 37 °C, and in ambient air at 22 °C. Samples were pre-cycled for three 30 s intervals at a rate of 1 mm/min, then pulled at a rate of 1 mm/min until failure.

2.9 Electrostatic simulation

As part of the ODS development, to understand the physics governing its electrospinning process, static electric fields in the apparatus were simulated using the commercial software COMSOL MultiPhysics (AltaSim Technologies, Inc., Worthington, OH, USA). Three separate calculations were performed to investigate spatial distributions of electric field and electric potential. These included a standard parallel collection system at close range with both collectors permanently connected to a single ground, standard parallel collectors spaced 20 cm apart, and the ODS with electrically independent collectors spaced 20 cm apart to demonstrate the effect of grounding only one collector.

Electric fields in the electrospinning compartment are governed by

$$\nabla^2\Phi=0 \quad (1)$$

and

$$E=-\nabla\Phi \quad (2)$$

where $\Phi(x, y, z)$ is the scalar electric potential at any point in the compartment and $E(x, y, z)$ is the electric field at that point. Boundary conditions were taken as

$$\Phi=10 \text{ kV} \quad \text{at the spinneret surface} \quad (3)$$

$$\Phi=0 \text{ kV} \quad \text{at the collector surface} \quad (4)$$

The system of Eqs. (1–4) was solved using the Electrostatics module within COMSOL. For the simulations, two $8 \times 2.5 \times 0.3 \text{ cm}^3$ aluminum collectors mounted on 16 cm concrete stands, a 6 cm long 16 gauge aluminum spinneret, and a 2.5 cm thick aluminum focal plate with a 7.5 cm diameter were positioned inside a $76 \times 61 \times 45 \text{ cm}^3$ block of air to represent the interior of the cabinet during electrospinning. Simulated components were drawn full-scale, taking the default material properties for aluminum, concrete, and air. The spinneret to collector distance was 23 cm with a 10 cm offset.

Results were plotted using the Gradient Contour function within COMSOL to illustrate the electric potential range, from 10 kV at the spinneret to ground at the collectors. To emphasize the midrange of electric potential (4.0–4.5 kV) and highlight the differences in electric fields produced by the different collection systems, 500 contour lines were also displayed. Simultaneously, the Arrow Volume 3D plot group was used to illustrate the direction and magnitude of the electric field, which drives the electrospinning process.

3 Results

To date, imperfect deposited fiber alignment and the associated strain fields have limited many potential applications of aligned electrospun fiber meshes for new scaffolds for biomaterials and regenerative medicine. Here we describe electrospun materials with near uniform alignment and no residual stress, prepared by a novel technique in which a single electrical ground is continuously switched from one collector to another and back at a constant frequency. Using the ODS, constructs with walls consisting of uniformly aligned fibers were fabricated in a variety of forms, including planar meshes, tubes and wound bundles. The physico-chemical properties of these meshes were evaluated in vitro.

3.1 Aligned material morphology

SEM images showed the ODS was able to couple high quality alignment with the large material size capabilities of common commercial collection systems. Representative images of samples fabricated from a fibroin/PEO blended solution showed specific characteristic ultrastructures (Fig. 3, taken with a collector distance of 24 cm and 22 C, 44–46 % relative humidity ambient conditions). Planar meshes spun with random fiber alignment (Fig. 3a–c), which were manufactured for comparison purposes as a control, showed a consistent, homogeneous, dull appearance at low or no magnification (Fig. 3a). At high magnification, these meshes consisted of a uniform distribution of randomly oriented nanofibers of diameters in the range of 200–250 nm (Fig. 3b, c). Untreated fibers were relatively widely spaced with fewer cross-bridges (Fig. 3b), while methanol treatment produced more densely packed fibers with tighter spacing and more cross-bridges (Fig. 3c).

In contrast, planar, axially aligned meshes (Fig. 3d–f) showed a characteristic reflective sheen at low or no magnification due to the fiber alignment (Fig. 3d). In samples not exposed to methanol, nanofibers were densely packed with very uniform alignment and limited cross-bridging (Fig. 3e). Methanol treatment induced crystallization of the silk fibroin component and increased fiber density of the mesh while maintaining the uniformity of alignment. However, it also facilitated interfiber associations which led to the spacing of some fibers, producing the appearance of fiber bundles separated by gaps spanned by small groups of fibers (Fig. 3f).

Seamless tubes composed of axially aligned nanofibers (Fig. 3g–i) also showed the reflective sheen at low magnification (Fig. 3g). At high magnification, under as-spun conditions the axial alignment of the fibers making up the tube wall was clear on both the inner and outer surfaces of the wall (Fig. 3h, i).

The distribution of fiber alignment was quantified by analysis of SEM imaging (Fig. 4). All data were well fit by a Gaussian distribution. Values for μ and the standard deviation, σ , in each case are given in Table 1. As expected, the ODS technique produced significantly improved alignment compared to either randomly spun fibers or aligned, mandrel-spun fibers. Random fibers deposited and not methanol-treated showed no preferred orientation with respect to any reference fiber (Fig. 4a). Fibers were found in all possible orientations, with no predominant direction. Only 7.2 % of fibers were oriented within $\pm 5^\circ$ of a reference direction. In contrast, the rotating mandrel technique produced 22.1 % of non-methanol-treated fibers aligned within 5° (Fig. 4b).

Most importantly, collection with the ODS system resulted in 81.6 % of deposited, non-methanol-treated fibers aligning within 5° of reference (Fig. 4c). This was an 11-fold improvement over random deposition, and nearly quadruple the performance of the mandrel system. When comparing the alignment within 10° , samples produced with the rotating mandrel technique had 45.9 % of fibers aligned within 10° of reference, which was slightly

more than double the 19.7 % of fibers found aligned in random meshes. In contrast, the ODS collection system more than doubled the performance of the rotating mandrel, while exhibiting a fraction of the variance, with 92.5 % of its deposited fibers aligning within 10° (Table 1).

Moreover, these trends were preserved after methanol treatment (data not shown). After methanol treatment, the alignment of fibers in random meshes was reduced to 3 % within 5°, and 11 % of fibers within 10°, of reference. However, methanol-treated, ODS-deposited meshes maintained alignment of 56 % of fibers within 5°, and 70 % of fibers within 10°. Although fiber alignment within 5° was reduced by 32 % after methanol treatment compared to prior to treatment, alignment was still 87 % greater than non-treated samples produced with the mandrel. Fiber alignment within 10° in meshes produced with the ODS system was reduced only by 20.4 % after methanol treatment and was still 33 % greater than non-treated samples produced with the mandrel. Methanol treatment more than doubled the variance of ODS meshes, but variance still remained a fraction of that produced with the rotating mandrel.

3.2 Geometric properties

In general, ODS-fabricated, axially aligned samples were found to be capable of being handled and prepared at thinner thicknesses than other meshes. Of six axially aligned samples, the thinnest sample measurement was $2.80 \mu\text{m}$ and the mean thickness was $5.26 \pm 2.26 \mu\text{m}$. The six randomly aligned samples electrospun directly onto a collector had a mean thickness of $18.18 \pm 3.18 \mu\text{m}$. The randomly oriented fiber meshes also exhibited a significantly rougher surface than axially aligned samples, with mean roughness, R_a , of $1.58 \pm 1.43 \mu\text{m}$, compared to axially or perpendicularly aligned fiber meshes, which exhibited mean roughness of 0.57 ± 0.27 and $0.45 \pm 0.13 \mu\text{m}$, respectively.

Randomly oriented, non-methanol-treated meshes had the greatest void fraction of all samples, $29.15 \pm 16.75 \%$. ODS-fabricated, aligned meshes exhibited the second largest void area fraction, at $11 \pm 4.59 \%$. Compared to the standard random meshes, the aligned meshes exhibited a 62 % lower void area, due to tighter interfiber packing upon deposition. Interestingly, however, methanol treatment was associated with significant fiber rearrangement. Methanol treatment of randomly oriented meshes resulted in a general contraction of the fibrous mesh and a subsequent reduction of void area by 81, to $5.55 \pm 3 \%$. In contrast, the void area of aligned fiber meshes reduced only by 45 % after methanol-treatment, to 6.03 ± 2.41 , 8.6 % more void area than was preserved by randomly oriented meshes.

3.3 Mechanical properties

As would be expected for highly aligned fibrous materials, the mechanical properties of planar meshes fabricated with the ODS system were highly anisotropic. Stress-strain behavior of aligned, methanol-treated meshes formed from fibroin/PEO were measured both at 37 °C in PBS to simulate physiologic conditions and at 22 °C in ambient air (Fig. 5; Table 2). In PBS, when loaded in their axial direction, aligned samples produced an ultimate tensile strength (UTS) of $16.47 \pm 1.18 \text{ MPa}$, a Young's modulus of $37.33 \pm 1.33 \text{ MPa}$ and a yield strength of $7.79 \pm 1.13 \text{ MPa}$ (Fig. 5a). They had 10 times the UTS of aligned samples loaded in their transverse direction ($1.58 \pm 0.28 \text{ MPa}$). They also were four times stiffer than when extended transversely ($9.78 \pm 0.7 \text{ MPa}$), and had more than 20 times the yield strength ($0.40 \pm 0.2 \text{ MPa}$).

UTS of ODS samples aligned in the direction of strain was not significantly different in dry and wet conditions when compared using an unpaired *t* test with $n = 3$ ($P < 0.05$). However,

when extended in ambient air compared to in 37 °C PBS, the ODS meshes stiffened significantly, producing Young's moduli of 774 ± 141 MPa axially and 459 ± 25.8 MPa transversely. In addition, dry samples were nearly five times stronger than wet samples when extended transversely to the direction of fiber alignment, producing an UTS of 7.68 ± 0.84 MPa, presumably because shielding of loose hydrophobic and electrostatic interactions in aqueous solutions allows are more rapid dissociation of the fibers under tensile stress.

3.4 Novel material configurations

In addition to planar meshes and tubes, several other material forms were fabricated with the ODS that either cannot be fabricated at all by other electrospinning techniques or can only be fabricated with poor quality.

3.4.1 Aligned fiber gradient meshes—Planar meshes with a smooth transition of fiber organization in the direction of fiber alignment (Fig. 6). To produce these meshes, deposited aligned fibers spanning the collector gap but lacking the structural integrity to support their suspension were allowed to break and recoil towards each collector. The result was a mesh with a symmetric gradient composed of dense but less aligned regions near the collectors (shown at low magnification in Fig. 6a, b, and at high magnification in Fig. 6d) that transitioned continuously into a less dense but well aligned region in the mesh center (also shown at low magnification in Fig. 6a, b, and at high magnification in Fig. 6c). In these meshes, aligned fibers integrated with the more random end regions (Fig. 6b), provided support and redistributed stress, end to end, through interactions with the randomized fibers. The widths and densities of the center and end regions could be controlled by varying the polymer solution concentration, thereby altering the individual fiber strength and breaking rate during electrospinning (Fig. 7). Lowering the polymer solution concentration below a threshold resulted in all fibers breaking (Fig. 7a, taken at 7.8 % (w/v). However, increasing to the concentration to 8.6 % (w/v) fibroin/PEO produced fiber alignment gradients (Fig. 7b), while further increases produced very consistent and dense aligned fiber meshes [Fig. 7c, 9.4 % (w/v)].

3.4.2 Axially aligned seamless tubes—Seamless tubes (Fig. 3) produced using rotating cylindrical collectors (Fig. 2d). The tube diameter was determined by the collector dimensions, while overall tube length was determined by the distance between the collector pair. SEM images of tube cross-sections showed the fibers in both the luminal and exterior surfaces to maintain axial alignment (Fig. 3g, j).

3.4.3 Axially aligned microbundles—Rope-like microbundles fabricated by winding deposited fibers around each other through asynchronous rotation of the collectors (Fig. 8a). Stopping the rotation of one collector while the opposite collector continued to rotate produced enough tension to reduce the fibers to a tightly bound bundle. SEM images show that the fibers maintained alignment as they overlapped in layers leading to the point of microbundle origin (Fig. 8b, white braces). White arrows in Fig. 8b represent the orientation of fiber alignment. The fiber density and bundle thickness of the final wound bundle was controlled by varying the thickness of the fiber layer prior to winding. The winding process was allowed to continue until a desired bundle length was achieved.

SEM images comparing morphology of sections of the same microbundle before and after methanol treatment (Fig. 8c, d) show the microbundle to be highly compacted with significantly reduced void space after methanol treatment. The microbundle diameter constricted 50 %, to 45 μm diameter, after methanol induced crystallization, near the range of natural silk fibers.

4 Discussion

A variety of biopolymers were electrospun successfully using the ODS, including gelatin and a combined hyaluronic acid-gelatin solution (data not shown). However, results are presented here only for constructs fabricated from silk fibroin. Of the many naturally occurring polymers that can be successfully electrospun, silk fibroin of the larval silkworm *Bombyx mori* is a popular option for biomedical applications due to its strength, low immunogenicity, and processability. Many specific techniques have been developed to facilitate the preparation of fibroin solutions for electrospinning, and conditions for electrospinning silk from aqueous solutions have been optimized [13, 36, 38–40]. As a result, the environmental safety conditions associated with electrospinning using organic solvents can be avoided. Electrospun fibroin silk is robust, non-degrading, highly biocompatible and has been used in a wide number of in vitro and in vivo applications [38–44]. It is therefore particularly suited for demonstrating the ODS capabilities without the complex conditions surrounding electrospinning other biopolymers.

4.1 Mechanism of alignment

COMSOL simulations (Fig. 9) gave significant insight into the mechanism by which aligned fibers deposit in the ODS. For comparison purposes, as a control, the case of two parallel collectors with both collectors permanently grounded (Fig. 9a, b) was analyzed before investigating the ODS configuration. Parallel collector systems with all collectors grounded have been used for many years by other authors to produce small samples of aligned electrospun material [26, 27]. However, it has not been possible to fabricate larger samples with them. The simulations suggest that when both collectors are grounded simultaneously, the length of electrospinnable samples is limited by dispersion of the necessary electric field as the collectors are moved apart. When parallel collectors are spaced only 4 cm apart (Fig. 9a), the electric field between them has sufficient magnitude to cause the electrospinning instability region to take a shape with a single base spanning both. As a result, the instability zone gives rise to aligned fiber deposition.

In contrast, as the grounded collectors are positioned increasingly further apart, the field at the center of the collectors weakens significantly. At a spacing of 20 cm (Fig. 9b), the electric field intensity needed to successfully drive the collection of fibers between the collectors does not develop, because the fixed potential difference between the spinneret surface and the collector surfaces is spread over a larger distance. The instability region then either centers its base on one preferred collector at a time, or develops fibers to a different stage at each collector at the time of deposition, thereby preventing production of aligned fibers.

It is possible to address the deficiency in magnitude of the electric field between the collectors by adding negative applied voltage directly to the collectors. However, this approach can produce significant fiber breaking, due to increased tension forces at the collectors and collisions with subsequent fibers resulting from the intense electrical attraction [9]. Better results can be achieved by increasing the dimensions of the collector plates, but the collector size required may not be practical [9].

In contrast to standard parallel collection, in the ODS configuration, with two collectors present but only one grounded, an entirely different electric potential distribution develops (Fig. 9c, which also shows 20 cm collector spacing). With the ODS arrangement, a highly focused region with a very high potential gradient forms at the active collector surface, driving fiber deposition onto that collector. However, when activation is switched to the second collector, the electrodynamics of the system inverts and the body of electrospinning fibers is shifted toward it. Reducing the number of collectors activated at any given time

results in tight focus of the electric field on the active collector (Fig. 9c). Fiber deposition between the collectors with very pure alignment can then be achieved without altering the voltages at the spinneret and polymer reservoir, which maintains polymer jet stability.

In addition, the ODS collection system has been designed to intensify aligned fiber deposition by inducing additional lateral bending instability on the fiber cloud. In turn, the intensity of the induced lateral instability enhances the expansion of the fiber whipping mechanism in a controlled direction. As the bending instability drives the whipping mechanism to leave the deactivated first collector and shift to the activated second collector, fibers are extended in one controlled direction and deposited, bridging the two collectors.

While fibers are traveling toward the active ODS collector, inconsistent charges, stretching, and subsequent solvent evaporation transform portions of them to a stage of completion which leaves them very stiff [4–6, 45]. After activation switches to the other collector, free segments of those fibers are driven toward the new collector. The strength of that response is determined by the amount of remnant charge, solvent, and viscosity of these fiber portions.

Because this instability is generated intermittently between two parallel collectors rather than radiating from a single collector, the resulting nanofiber extensions are generated in a parallel, aligned manner even before coming in contact with a collector surface. This active collection process imparts the capability to produce aligned fiber materials with a much greater length than can be produced with a standard parallel collection system.

4.2 Alignment quality

Nanofiber meshes fabricated with the ODS demonstrated far more consistency and uniformity of alignment at all scales than meshes previously reported by other authors that were nominally described as “aligned” [24, 46, 47]. Quantitative analysis of fiber alignment showed ODS deposition to produce meshes with over 80 % of the fibers aligned within $\pm 5^\circ$ of the reference direction (Fig. 4). In comparison, fiber alignment within meshes fabricated by other techniques was significantly less uniform (Fig. 10). At very high magnification, non-ODS-fabricated silk meshes produced using a rotating mandrel collector appeared to provide recognizable alignment (Fig. 10a). However, that alignment was achieved only at very fine scale, or only in select regions of the mesh. When viewed at lower magnification, to show larger areas, it was apparent that alignment was not maintained throughout these meshes (Fig. 10b). The alignment of meshes reported by other authors using rotating mandrel collectors also demonstrates similar inconsistency [24, 46]. In contrast, ODS-fabricated meshes were aligned with very high purity at all scales (Fig. 10c, d). Moreover, Fig. 10c, d are representative of all the meshes fabricated with the ODS. Once electrospinning conditions had been optimized, alignment quality of similar purity and consistency was produced in all trials, regardless of the overall mesh geometry.

Standard electrospinning and rotating mandrel systems deposit fibers directly onto supportive collectors. In contrast, in ODS-fabricated meshes, high purity of alignment is further reinforced by elimination of the supportive collector, which is known to be a mechanism for increasing fiber alignment in parallel collector systems [9, 48, 49]. Because the ODS mesh is composed of gap-spanning fibers collected between the grounded plates, rather than directly onto them, it contains less broken, weak, and misaligned fiber fragments.

Further, deposited nanofibers preserved their alignment even after chemical treatment with methanol to induce β -sheet transition, by bundling with neighboring nanofibers (Fig. 3d). This also appears to be a method of maintaining transverse integrity, as fibers originating in one bundle cross over to another to give rise to a fine-scale lattice. As fiber bundles compacted, small crevices opened between them. Those crevices also maintained alignment

with the direction of the fiber bundles. In applications related to meshes serving as cell scaffolds, such crevices may provide pores that serve to enhance cell-influencing topography. In addition, it may be possible to design meshes in which larger pores serve to isolate individual cells from one another, preventing cell–cell interactions and hindering cellular processes when that is desirable.

4.3 Mechanical properties

Because of the collector style and the collection method, aligned fiber meshes fabricated with the ODS can be thinner than corresponding meshes with randomly oriented fibers. In addition, the ODS does not induce residual mechanical strains in the deposited fibers. The resulting meshes therefore have more extensibility and much less residual stress and strain than meshes collected using rotating mandrels, in which stresses are induced as fibers are deposited on a rotating surface.

Furthermore, mats with randomly ordered fibers are normally electrospun directly onto flat collectors or foil-wrapped surfaces. Removal of these mats from the collector surface can damage the fibers, particularly if residual solvent is present and creates surface tension forces that increase the strength of adhesion of the mat to the collector. In contrast, in the ODS technique, fibers are deposited between collectors, with no backing support. After spinning, even very thin aligned samples can be excised from between the collectors with no need for removal from a surface and therefore no risk of fiber damage associated with the removal process.

The UTS of aligned, methanol-treated ODS meshes in the fiber direction, 16–17 MPa, is comparable to the UTS of silk films reported by other authors, which are in the range of 8–16 MPa [50, 51]. However, ODS meshes present a much higher surface area than do films, due to the nonwoven individual fiber network. This network allows for delocalization of stresses by fiber shifting and rearrangements that make meshes more crack tolerant compared to films [8].

In addition, ODS-synthesized meshes showed significantly greater strain at failure than do randomly oriented meshes and films. It seems likely that such higher strain is possible because fibers deposited between the collectors of multiple-collector systems retain remnant charges distributed throughout the fiber body at the time of deposition because they do not come into direct contact with the counter electrode collection plates. These charges cause interfiber repulsions, producing a bulb-like haze of repelling fibers around the main mesh until the charges dissipate [4, 45]. Fibers may dissipate charge by interacting with the atmosphere or may recoil at both ends toward each collector, thereby shortening themselves. Depending on the rate of fiber shortening due to recoiling, the fiber may still exhibit some curvature and remain longer than the gap distance at time of contact with newer fiber depositions. Because electrospinning is a continuous process, newer fibers will deposit directly onto the previous depositions before they have completely dissipated charge [4], which flattens the bulb-like curvature of older bowing fibers into the mesh. By compressing the bulb, the mesh may become a collection of aligned fibers which span the collector gap with very slight repeating curvatures.

As would be expected because of the uniformity of the fiber alignment, ODS meshes showed much greater strength in their axial direction than in the transverse direction, in both dry and hydrated conditions (Fig. 5). In ODS meshes, strength in the transverse direction primarily reflects the ability of each fiber to adhere to neighboring fiber bundles. In comparison, other techniques for fabricating aligned meshes leave large numbers of randomly oriented fibers, which can then provide additional support and contribute to transverse strength [52]. ODS meshes lack those fibers, and accordingly have substantially

decreased transverse strength. In some applications, such mechanical anisotropy may represent a limitation of the ODS technique. However, that issue could be addressed by layering sheets of aligned fibers at alternating angles to improve mechanical properties in all directions.

4.4 Novel materials

One of the largest challenges facing potential tendon grafts is the difficulty of merging two interconnected tissue types, such as muscle–tendon or tendon–bone junctions [22, 28, 53]. This is important for both cell orientation and for the reliable mechanical properties necessary for controlled muscle, tendon, or ligament function. Transitions with step changes in tissue stiffness of strength localize stress and create sites with greater potential to be reinjured. However, fabricating implant materials with gradual transitions in stiffness and strength matching native conditions is dependent on controlling the organization of deposited fibers. Stationary and rotating mandrel systems are not capable of producing meshes with controlled transition gradients along the direction of fiber alignment.

In contrast, the ODS has the ability to electrospin such smooth transitional meshes (Fig. 6). Deposited aligned fibers that span the collector gap but lack the structural integrity to support themselves break and recoil towards each collector, producing denser, less aligned portions at each end of the scaffold (Fig. 6a). As fibers are deposited, the continuous process of compacting of recoiling fibers creates a symmetric gradient in the mesh organization (Fig. 6a). The mesh ends become densely packed, and the dense end regions can support the central, less dense region while also redistributing transmitted stresses. Their presence suggests the possibility of fabricating meshes with controlled, continuously varying fiber density and mechanical properties suitable for applications in which material property gradients are desirable.

It is a significant challenge to produce nanofibrous biodegradable vessels and tubes that possess both a seamless tubular structure and axial fiber alignment. Axially aligned tubes reported in the current literature have either been produced using techniques incorporating a seam, or seamlessly but only heretofore with random or circumferentially oriented fibers [13, 20, 30–32, 34]. The automated tubular collectors developed in this work were able to produce the first generation of seamless, electro-spun, axially aligned nanofibrous tubular meshes, which may prove promising as tubular grafts and scaffolds (Fig. 3). True seamless tubes are not possible with rotating mandrel collection systems, which can only produce randomly or circumferentially aligned nanofibrous tubes.

The automated tubular collectors can also be used to enable the production of wound microbundles (Fig. 8). The microbundle is highly compacted, and void space has been significantly reduced after methanol treatment. However, while only 2–4 fibroin fibers would fit in this cross sectional area, these novel bundles contain 80,000 fibroin fibers within the same area.

5 Conclusions

A novel, reliable, low cost system for the deposition of aligned electrospun nanofibers with very high purity has been demonstrated. The ODS is able in principle to deposit aligned fibers from any biopolymer solution that can be electrospun. Meshes with over 80 % of fibers within 5° of the axial direction were easily achieved, a nearly four-fold improvement in alignment over fibers deposited by a traditional rotating mandrel technique. At the same time, mechanical complications associated with the rotating mandrel were bypassed. Meshes can be synthesized by the ODS in a variety of formats, including flat planar meshes, seamless axially aligned tubes, aligned microbundles and gradient orientation meshes. As

might be expected given their alignment purity, meshes demonstrated highly anisotropic mechanical properties. Under simulated physiological conditions, meshes produced a UTS of 16.47 ± 1.18 MPa in their alignment direction, an order of magnitude larger than when loaded transversely, and were 300 % stiffer when extended in the direction of fiber alignment than when extended transversely. However, although distinctly anisotropic, these mechanical properties were of the same order of magnitude as meshes fabricated by other techniques. The ODS technique overcomes the limited uniformity and induced mechanical strain associated with mechanical wheel techniques, and greatly surpasses the limited length of standard parallel collector techniques. Compared to the standard parallel collector techniques, the ODS system adds additional influence over electrically induced bending instability, which can be varied in both intensity and frequency. This allows manipulation of fiber deposition over greater distances. The effective distance limit of our technique has not yet been achieved, which suggests great potential for future scale-up.

Acknowledgments

Supported by National Science Foundation award CBET-0932456, and partially by NIH awards EY020856 and EB002520, and AFOSR award FA9550-10-1-0172. We thank Dr. Robert White for contributing microscale profilometry measurements, and Dr. Ethan Golden for his assistance in design and fabrication.

References

- Shenoy S, Bates W, Frisch H, Wnek G. Role of chain entanglements on fiber formation during electrospinning of polymer solutions: good solvent, non-specific polymer? polymer interaction limit. *Polymer*. 2005; 46:3372–84.
- Deitzel JM, Kleinmeyer JD, Hirvonen JK, Tan NC. Controlled deposition of electrospun poly(ethylene oxide) fibers. *Polymer*. 2001; 42:8163–70.
- Angammana C, Jayaram SH. The effects of electric field on the multijet electrospinning process and fiber morphology. *Ind Appl IEEE*. 2011; 47:1028–35.
- Schreuder-Gibson H, Gibson P, Tsai P, Gupta P, Wilkes G. Cooperative charging effects of fibers from electrospinning of electrically dissimilar polymers. *Int Nonwovens J*. 2005; 13:39–45.
- Reneker DH, Yarin AL, Fong H, Koombhongse S. Bending instability of electrically charged liquid jets of polymer solutions in electrospinning. *J Appl Phys*. 2000; 87:4531.
- Yarin AL, Koombhongse S, Reneker DH. Bending instability in electrospinning of nanofibers. *J Appl Phys*. 2001; 89:3018.
- Gaumer J, Prasad A, Lee D, Lannutti J. Structure–function relationships and source-to-ground distance in electrospun polycaprolactone. *Acta Biomater*. 2009; 5:1552–61. [PubMed: 19233754]
- Stachewicz U, Peker I, Tu W, Barber AH. Stress delocalization in crack tolerant electrospun nanofiber networks. *ACS Appl Mater Interfaces*. 2011; 3:1991–6. [PubMed: 21545103]
- Beachley V, Wen X. Effect of electrospinning parameters on the nanofiber diameter and length. *Mater Sci Eng C*. 2009; 29:663–8.
- Saraf A, Lozier G, Haesslein A, Kasper FK, Raphael RM, Baggett LS, Mikos AG. Fabrication of nonwoven coaxial fiber meshes by electrospinning. *Tissue Eng C*. 2009; 15:333–44.
- Thakur RA, Florek CA, Kohn J, Michniak BB. Electrospun nanofibrous polymeric scaffold with targeted drug release profiles for potential application as wound dressing. *Int J Pharm*. 2008; 364:87–93. [PubMed: 18771719]
- Barnes CP, Pemble CW, Brand DD, Simpson DG, Bowlin GL. Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol. *Tissue Eng*. 2007; 13:1593–605. [PubMed: 17523878]
- Zhang X, Wang X, Keshav V, Wang X, Johanas JT, Leisk GG, Kaplan DL. Dynamic culture conditions to generate silk-based tissue-engineered vascular grafts. *Biomaterials*. 2009; 30:3213–23. [PubMed: 19232717]

14. Sell SA, Francis MP, Garg K, McClure MJ, Simpson DG, Bowlin GL. Cross-linking methods of electrospun fibrinogen scaffolds for tissue engineering applications. *Biomed. Mater.* 2008; 3:045001. [PubMed: 18824779]
15. Yoshimatsu K, Ye L, Lindberg J, Chronakis IS. Selective molecular adsorption using electrospun nanofiber affinity membranes. *Biosens Bioelectron.* 2008; 23:1208–15. [PubMed: 18226521]
16. Cai Z-X, Mo X-M, Zhang K-H, Fan L-P, Yin A-L, He C-L, Wang H-S. Fabrication of chitosan/silk fibroin composite nanofibers for wound-dressing applications. *Int J Mol Sci.* 2010; 11:3529–39. [PubMed: 20957110]
17. Kempf M, Miyamura Y, Liu P-Y, Chen AC-H, Nakamura H, Shimizu H, Tabata Y, Kimble RM, McMillan JR. A denatured collagen microfiber scaffold seeded with human fibroblasts and keratinocytes for skin grafting. *Biomaterials.* 2011; 32:4782–92. [PubMed: 21477857]
18. Lee H, Yeo M, Ahn S, Kang D-O, Jang CH, Lee H, Park G-M, Kim GH. Designed hybrid scaffolds consisting of polycaprolactone microstrands and electrospun collagen-nanofibers for bone tissue regeneration. *J Biomed Mater Res B.* 2011; 97:263–70.
19. Ahmad Z, Stride E, Edirisinghe M. Novel preparation of transdermal drug-delivery patches and functional wound healing materials. *J Drug Target.* 2009; 17:724–9. [PubMed: 19845489]
20. Yao L, O'Brien N, Windebank A, Pandit A. Orienting neurite growth in electrospun fibrous neural conduits. *J Biomed Mater Res B.* 2009; 90:483–91.
21. Weber N, Lee Y-S, Shanmugasundaram S, Jaffe M, Arinzeh TL. Characterization and in vitro cytocompatibility of piezoelectric electrospun scaffolds. *Acta Biomater.* 2010; 6:3550–6. [PubMed: 20371302]
22. Ladd MR, Lee SJ, Stitzel JD, Atala A, Yoo JJ. Co-electrospun dual scaffolding system with potential for muscle–tendon junction tissue engineering. *Biomaterials.* 2011; 32:1549–59. [PubMed: 21093046]
23. Ma Z, Chen F, Zhu Y-J, Cui T, Liu X-Y. Amorphous calcium phosphate/poly(d, l-lactic acid) composite nanofibers: electrospinning preparation and biomineralization. *J Colloid Interface Sci.* 2011; 359:371–9. [PubMed: 21536302]
24. Murugan R, Ramakrishna S. Design strategies of tissue engineering scaffolds with controlled fiber orientation. *Tissue Eng.* 2007; 13:1845–66. [PubMed: 17518727]
25. Aviss KJ, Gough JE, Downes S. Aligned electrospun polymer fibres for skeletal muscle regeneration. *Eur Cells Mater.* 2010; 19:193–204.
26. Xie J, MacEwan MR, Ray WZ, Liu W, Siewe DY, Xia Y. Radially aligned, electrospun nanofibers as dural substitutes for wound closure and tissue regeneration applications. *ACS Nano.* 2010; 4:5027–36. [PubMed: 20695478]
27. Xie J, Li X, Xia Y. Putting electrospun nanofibers to work for biomedical research. *Macromol Rapid Commun.* 2008; 29:1775–92. [PubMed: 20011452]
28. Xie J, Li X, Lipner J, Manning CN, Schwartz AG, Thomopoulos S, Xia Y. “Aligned-to-random” nanofiber scaffolds for mimicking the structure of the tendon-to-bone insertion site. *Nanoscale.* 2010; 2:923–6. [PubMed: 20648290]
29. Wolfe PS, Madurantakam P, Garg K, Sell S, Beckman MJ, Bowlin GL. Evaluation of thrombogenic potential of electrospun bioresorbable vascular graft materials: acute monocyte tissue factor expression. *J Biomed Mater Res A.* 2010; 92:1321–8. [PubMed: 19353561]
30. McClure MJ, Sell S, Simpson DG, Walpoth BH, Bowlin GL. A three-layered electrospun matrix to mimic native arterial architecture using polycaprolactone, elastin, and collagen: a preliminary study. *Acta Biomater.* 2010; 6:2422–33. [PubMed: 20060934]
31. Wu H, Fan J, Chu C-C, Wu J. Electrospinning of small diameter 3-D nanofibrous tubular scaffolds with controllable nanofiber orientations for vascular grafts. *J Mater Sci Mater Med.* 2010; 21:3207–15. [PubMed: 20890639]
32. Drilling S, Gaumer J, Lannutti J. Fabrication of burst pressure competent vascular grafts via electrospinning: effects of microstructure. *J Biomed Mater Res A.* 2009; 88:923–34. [PubMed: 18384169]
33. Liu X, Chen J, Gilmore KJ, Higgins MJ, Liu Y, Wallace GG. Guidance of neurite outgrowth on aligned electrospun polypyrrole/poly(styrene-beta-isobutylene-beta-styrene) fiber platforms. *J Biomed Mater Res A.* 2010; 94:1004–11. [PubMed: 20694967]

34. Griffin J, Delgado-Rivera R, Meiners S, Urich KE. Salicylic acid-derived poly(anhydride-ester) electrospun fibers designed for regenerating the peripheral nervous system. *J Biomed Mater Res A*. 2011; 97:230–42. [PubMed: 21442724]
35. Ahmad Z, Nangrejo M, Edirisinghe M, Stride E, Colombo P, Zhang HB. Engineering a material for biomedical applications with electric field assisted processing. *Appl Phys A*. 2009; 97:31–7.
36. Lovett M, Cannizzaro C, Daheron L, Messmer B. Silk fibroin microtubes for blood vessel engineering. *Biomaterials*. 2007; 28:5271–9. [PubMed: 17727944]
37. Lu S, Wang X, Lu Q, Zhang X, Kluge Ja, Uppal N, Omenetto F, Kaplan DL. Insoluble and flexible silk films containing glycerol. *Biomacromolecules*. 2010; 11:143–50. [PubMed: 19919091]
38. Jin H-J, Fridrikh SV, Rutledge GC, Kaplan DL. Electrospinning *Bombyx mori* silk with poly(ethylene oxide). *Biomacromolecules*. 2002; 3:1233–9. [PubMed: 12425660]
39. Zhang X, Reagan MR, Kaplan DL. Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv Drug Deliv Rev*. 2009; 61:988–1006. [PubMed: 19643154]
40. Jin H. Human bone marrow stromal cell responses on electrospun silk fibroin mats. *Biomaterials*. 2004; 25:1039–47. [PubMed: 14615169]
41. Wang H, Zhang Y, Shao H, Hu X. Electrospun ultra-fine silk fibroin fibers from aqueous solutions. *J Mater Sci*. 2005; 40:5359–63.
42. Wang S, Zhang Y, Wang H, Yin G, Dong Z. Fabrication and properties of the electrospun polylactide/silk fibroin-gelatin composite tubular scaffold. *Biomacromolecules*. 2009; 10:2240–4. [PubMed: 19722559]
43. Wei K, Li Y, Kim K-O, Nakagawa Y, Kim B-S, Abe K, Chen G-Q, Kim I-S. Fabrication of nano-hydroxyapatite on electrospun silk fibroin nanofiber and their effects in osteoblastic behavior. *J Biomed Mater Res A*. 2011; 97:272–80. [PubMed: 21442728]
44. Min B-M, Lee G, Kim SH, Nam YS, Lee TS, Park WH. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials*. 2004; 25:1289–97. [PubMed: 14643603]
45. Kumar A, Wei M, Barry C, Chen J, Mead J. Controlling fiber repulsion in multijet electrospinning for higher throughput. *Macromol Mater Eng*. 2010; 295:701–8.
46. Timnak A, Gharebaghi FY, Shariati RP, Bahrami SH, Javadian S, Emami SH, Shokrgozar MA. Fabrication of nano-structured electrospun collagen scaffold intended for nerve tissue engineering. *J Mater Sci Mater Med*. 2011; 22:1555–67. [PubMed: 21526410]
47. Nandakumar A, Fernandes H, de Boer J, Moroni L, Habibovic P, van Blitterswijk Ca. Fabrication of bioactive composite scaffolds by electrospinning for bone regeneration. *Macromol Biosci*. 2010; 10:1365–73. [PubMed: 20799255]
48. Ramakrishna, S. An introduction to electrospinning and nanofibers. World Scientific Pub Co Inc.; Singapore: 2005.
49. Vaquette C, Cooper-White J. Increasing electrospun scaffold pore size with tailored collectors for improved cell penetration. *Acta Biomater*. 2011; 7:2544–57. [PubMed: 21371575]
50. Kundu J, Dewan M, Ghoshal S, Kundu SC. Mulberry non-engineered silk gland protein vis-à-vis silk cocoon protein engineered by silkworms as biomaterial matrices. *J Mater Sci Mater Med*. 2008; 19:2679–89. [PubMed: 18283532]
51. Rajkhowa R, Levin B, Redmond SL, Li LH, Wang L, Kanwar JR, Atlas MD, Wang X. Structure and properties of biomedical films prepared from aqueous and acidic silk fibroin solutions. *J Biomed Mater Res A*. 2011; 97A:37–45.
52. Tong H-W, Wang M, Lu WW. Electrospinning and evaluation of PHBV-based tissue engineering scaffolds with different fibre diameters, surface topography and compositions. *J Biomater Sci Polym Ed*. 2011; 23:779–806.
53. Li X, Xie J, Lipner J, Yuan X, Thomopoulos S, Xia Y. Nanofiber scaffolds with gradations in mineral content for mimicking the tendon-to-bone insertion site. *Nano Lett*. 2009; 9:2763–8. [PubMed: 19537737]

Fig. 1. Oscillating deposition signal (ODS) collection system. **a** Schematic diagram illustrating the electrospinning cabinet and hardware. **b** Schematic diagram illustrating the switch design. In the tests reported here, the switch operated at 0.33 Hz

Fig. 2. Schematic diagrams of the standard and ODS collectors. **a** Standard single, flat collector used to collect nanofibers in a randomized orientation. **b** Dual, flat ODS collectors used to produce planar aligned nanofiber samples. **c** Tubular ODS collectors used to produce hollow seamless aligned nanofiber tubular or microbundle samples. **d** Schematic diagram of the automated tubular collection system

Fig. 3. SEM images of standard, random and ODS, aligned, planar meshes and tubes, produced from 9 % (w/v) fibroin/PEO blended solution, with an applied voltage of 10 kV at a current of 1 μ A, a collector distance of 24 cm, a flow rate of 40 μ L/min, and ambient conditions of 22 $^{\circ}$ C and 44–46 RH %. *Box insert in the upper left corner of the images indicates scale.* **a** Macroscopic, low magnification image of a standard randomly oriented mesh. **b** High magnification image of random mesh as spun. **c** High magnification image of a standard, random mesh after methanol-induced crystallization. **d** Macroscopic, low magnification image of an ODS-fabricated planar aligned mesh. **e** High magnification image of an as-spun ODS aligned mesh. **f** High magnification image of an aligned mesh after methanol-induced crystallization. **g** Low magnification image showing the end of an ODS-fabricated seamless aligned tube. A section of the inner surface of the tube wall highlighted by a *dashed box* is shown in high magnification in the insert. **h** Even lower magnification macroscopic image of the same tube. *Highlighted boxes* indicate the regions shown in (g) and (i). **i** Image demonstrating the axial fiber alignment of the exterior surface

Fig. 4.

Measurements of the distribution of fiber alignment for as-spun samples. Bars represent measured data, and the associated curves are the best fit Gaussian form. **a** Random deposition on a standard collector. **b** Ostensibly aligned deposition on a rotating mandrel. **c** Aligned deposition on the ODS collection system. The ODS collection system produced significantly improved results, nearly quadrupling the performance of the rotating mandrel, with less variance, by aligning 81.6 % of fibers within $\pm 5^\circ$ of the intended fiber direction

Fig. 5. Stress–strain behavior of methanol-treated, ODS-fabricated aligned, planar meshes, demonstrating the anisotropy of mechanical properties due to the high fiber alignment. Elongation rate of 1 mm/ min, mean of $n = 3$ samples. **a** Testing in PBS at 37 °C with comparison to standard randomly oriented meshes and fibroin films. **b** Testing in ambient air at 22 °C

Fig. 6. SEM images showing **a** comparison of the excising parameters for premium aligned (*white dotted box*) or orientation gradient (*black dotted box*) scaffolds at early stage of production. **b** Finished excised gradient mesh after increasing crystallization with methanol, **c** high magnification, demonstrating the highly aligned fibers found within the self-cleaning area of the mesh, **d** high magnification, demonstrating the increased inclusion of randomly oriented fibers with closer proximity to each collector end

Fig. 7. SEM images showing the effect of varying the polymer solution concentration. **a** 7.8 % (w/v) fibroin/PEO blend. **b** 8.6 % (w/v) fibroin/PEO blend. **c** 9.4 % (w/v) fibroin/PEO blend

Fig. 8.

Photo and SEM images of microbundle production. **a** Micro-bundles produced from fibroin/PEO solution. *Dotted lines indicate the regions shown in detail in (b) and (c).* **b** Microbundle origin, 150× magnification. *White arrows indicate fiber alignment leading to the microbundle origin (white braces).* **c, d** Morphology of sections of the same microbundle before (**c**) and after (**d**) inducing crystallization with methanol

Fig. 9. COMSOL simulations of the electric field and electric potential produced with dual flat plate collectors. The direction and length of the *black arrows* represent the direction and magnitude of the electric field which drives the electrospinning process. *Color gradient* illustrates the electric potential. **a** Standard parallel collection with collectors positioned 4 cm apart. **b** Standard parallel collection with collectors positioned 20 cm apart. **c** ODS collection with collectors spaced 20 cm apart (Color figure online)

Fig. 10.

SEM images comparing the alignment of fibers deposited on a rotating mandrel with fibers deposited using the ODS. **a** High magnification image of silk fibers produced with the rotating mandrel at 3,000 RPM. **b** Lower magnification image of the sample shown in **(a)**. *Dashed lines* indicate the region shown in **(a)**. Note the poor alignment and mesh inconsistency shown at low magnification. **c** High magnification image of silk fibers produced with the ODS. **d** Lower magnification image of the sample shown in **(c)**. *Dashed lines* indicate the region shown in **(c)**. Note the high purity of alignment at all magnifications

Table 1

Summary of the distributions of fiber angles of electrospun fibers produced using the ODS or rotating mandrel collection to achieve aligned fiber deposition, and fibers produced using a single stationary collector which results in randomly oriented fiber deposition

	ODS	Mandrel	Standard random
Frequency distribution			
% Fibers aligned within 5°	81.604	22.093	7.229
% Fibers aligned within 10°	92.453	45.930	19.679
Gaussian distribution			
Best-fit values			
Amplitude	81.62	17.07	6.613
SD	4.294	16.79	89.05
95 % confidence intervals			
Amplitude	80.34–82.90	12.65–21.49	5.745–7.481
SD	4.162–4.426	11.77–21.81	55.73–122.4

Table 2

Summary of mechanical measurements, all units in MPa

Type	UTS	Failure strain (%)	Young's moduli	Yield strength
Wet				
Transverse	1.58 ± 0.28	29 ± 14	9.78 ± 0.70	0.40 ± 0.20
Axially	16.47 ± 1.18	143 ± 16	37.33 ± 1.33	7.79 ± 1.13
Random	2.86 ± 0.18	115 ± 42	28.96 ± 0.53	3.21 ± 0.84
Fibroin film	2.79 ± 0.14	54 ± 18	19.55 ± 1.52	1.10 ± 0.19
Dry				
Transverse	7.68 ± 0.84	2.5 ± 0.8	459 ± 25.8	4.22 ± 0.17
Axially	17.55 ± 1.87	4.0 ± 1.9	774 ± 141	13.23 ± 2.52

Transverse properties of uniaxially aligned meshes loaded perpendicular to the fiber direction; *Axially* properties of uniaxially aligned meshes loaded in the fiber direction; *Random* properties of meshes with randomly deposited fibers; *Fibroin film* properties of films