

Supporting Information:

Materials

We used a variety of synthetic and naturally occurring polymers including polyethylene oxide (PEO, $M_v=1,000kD$ Sigma-Aldrich, Milwaukee, WI), gelatin type A from Sigma, poly (lactic acid) (PLA polymer 2002D, NatureWorks®, Minnetonka, MN) with a melt index of 4-8 g/10 min (ASTM D1238) and poly(acrylic acid) (PAA, $M_v=450kD$, Sigma-Aldrich). Chloroform (99.9% HPLC grade), hydrochloric acid, sodium hydroxide, and acetic acid (glacial) were purchased from Sigma-Aldrich (Milwaukee, WI) and dimethylformamide (98.5%) was purchased from VWR (San Dimas, CA). Fluorescent Microspheres (FluoSpheres®, 2% solid suspension, 0.2 μm diameter) was purchased from Molecular Probes, Inc. (Eugene, OR). All reagents were used as received without further purification.

Fabrication

Solution preparation: PEO was dissolved at a concentration of 5 wt% in deionized (18 Ω /cm) water (Millipore, Billerica, MA) at room temperature. Gelatin powder was dissolved at a concentration of 14 wt% in 20 v/v% acetic acid at 30°C. PAA at a concentration of 8 wt% was dissolved in deionized water at room temperature and then neutralized with sodium hydroxide to reach both half and full neutralized states. PLA was dissolved in chloroform at varied concentration of 4-10 wt% at room temperature. To prepare polymer emulsions, gelatin solution was added slowly to 8 wt% PLA in chloroform in the ratio of 1:50 (vol.) and vortexed for 5 min prior to RJS. For microsphere encapsulated samples, 10 μL of microsphere suspension was added under dark conditions to PEO solution and vortexed for 10 min. prior to RJS. The concentration of beads was 5-6 $\times 10^6$ per ml of polymer solution. For tissue engineering studies, PLA was dissolved at concentrations of 8 wt% in chloroform:dimethylformamide (80:20) before fiber fabrication.

Fiber fabrication: The RJS system consisted of a polypropylene reservoir with a diameter of 12.5 mm and height of 25.4 mm (Figure S1). The reservoir had two sidewall orifices with diameter (D) of 340

μm and L: D ratio of 9, where L is the orifice length depicted in Figure 1b. The perforated reservoir was attached to the shaft of a brushless motor (model BND23 from Peromatic GmbH, Switzerland) and rotation speed was controlled by a circuit board. The circuit is equipped with a manual rotation speed control to change the rotation of the motor before or during RJS. The polymer solution was continuously fed to the reservoir via polyethylene tube connected to a 50 ml syringe placed in the cradle of syringe pump (KD Scientific, Holliston, MA). Rotation started immediately after filling the reservoir. The resulting fibers were collected on a stationary round collector. Collected fibers were removed and weighed after certain period of time to evaluate production rate. The production rate was 5-6 g/h which is ~10 times higher than the production rate of standard electrospinning. To study effect of orifice geometry on fiber geometry, another orifice with diameter of 650 μm and L: D ratio of 5 was built.

Preparation of fibrous scaffold for cell culture: Fibrous scaffolds from PLA and gelatin were prepared as described above and were affixed to 25 mm glass coverslips using polydimethylsiloxane adhesive at the edges. After sample mounting, gelatin nanofibers were cross-linked by exposing to vapor of 4 ml glutaraldehyde in a 9 cm x 10 cm x 12 cm sealed container for 12 hours. Following cross-linking, samples were allowed to dry overnight to vaporize any remnant glutaraldehyde, and rinsed with 1X PBS. Samples were then sterilized by soaking in ethanol with exposure to a germicidal lamp in a laminar flow hood for 8 hours. After sterilization, PLA fibers were incubated in 50 $\mu\text{g}/\text{ml}$ fibronectin solution for 24 hours and rinsed with 1X PBS before cell culturing.

Cell culture: Neonatal rat left ventricular cardiomyocytes were isolated from 2-day old neonatal Sprague-Dawley rats as previously reported¹. All procedures were approved by the Harvard Animal Care and Use Committee. Reagents were obtained from Sigma unless otherwise indicated. Ventricles were surgically isolated and homogenized by washing in Hanks balanced salt solution followed by digestion with trypsin and collagenase with agitation overnight at 4°C. Subsequently, cells were re-suspended in M199 culture medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum

(FBS), 10 mM HEPES, 3.5 g/L glucose, 2mM L-glutamine, 2 mg/L vitamin B-12, and 50 U/mL penicillin and seeded onto the nanofiber scaffolds at a density of 350,000 cells/mL. Samples were incubated under standard conditions at 37°C and 5% CO₂. After an additional 48 hours the media was exchanged with maintenance media (M199 media supplemented as above but with 2% FBS) to minimize growth of fibroblasts inevitably present in the primary harvest cardiomyocyte population.

Sample characterization: Viscosity Measurements: Rheological measurements were made on freshly prepared PLA solutions for determining the concentration regimes. PLA solutions ranging from 0.1 to 12 wt% were loaded into the viscometer (Model AR-G2, TA instruments, New Castle, DE) fitted with a cone and plate spindle (model 987864, 40 mm cone diameter, 3°, 59', 56" cone angle and 109 µm gap) and viscosities were measured under steady state shear rate from 0.1-3,000 s⁻¹. All PLA solutions showed Newtonian behavior over low range of shear rates; however, it should be noted that shear thinning occurred at higher shear rates. The zero-shear viscosity (η_0) was determined over the Newtonian region. Figure S2, shows the flow behavior of PLA solutions ranging from 0.1 to 12 wt% at variable shear rates. The critical polymer concentration was calculated based on the zero-shear viscosities over the Newtonian region. The polymer contribution to the η_0 was studied by defining the specific viscosity (η_{sp}) in:

$$\text{Specific viscosity } (\eta_{sp}) = \frac{\eta_0 - \eta_s}{\eta_s} \quad (\text{S1})$$

where η_s is solvent viscosity. The η_{sp} is plotted as a function of concentration in Figure 3a for the PLA solutions. Changes in the slope marked the onset of the semidilute unentangled, semidilute entangled and concentrated regimes². The concentrated regime (c^*) was found to be 6 wt%.

Surface tension measurement: The surface tension of the polymer solution was measured based on Du Nouy ring method with Sigma700 Tensiometer (KSV instruments)³.

Scanning Electron Microscopy: Fiber samples removed from the collector and mounted on sample stubs and coated with Pt/Pd using a sputter coater (Denton Vacuum, Moorestown, NJ) to minimize charging during imaging. The samples were imaged using Zeiss Ultra field-emission scanning electron microscope (Carl Zeiss, Dresden, Germany). Images were acquired and analyzed using image analysis software (Image J, National Institutes of Health, US). A total of 100-300 fibers were analyzed (5 random fields of view per sample) to determine the fiber diameter. The fiber diameter distribution were reported as first, second and third quartile as 25th, 50th and 75th percentile. To observe cardiac cell morphology on fibrous scaffolds by SEM, after 4 days culturing the samples were fixed with 2% of glutaraldehyde/paraformaldehyde for 4 hours and dehydrated with a graded concentration (30-100%) ethanol. Then the samples were dried with a critical point dryer and sputter coated with Pt/Pd for 90s before imaging.

Immunostaining: Cardiomyocytes were fixed 4 days after seeding. Media was removed, cells were rinsed in 37°C PBS, then immediately fixed in a 4% solution of paraformaldehyde with 0.01% Triton X-100 in phosphate-buffered saline at 37°C. During the 15 minute fixation period, cells were equilibrated at room temperature. After fixation, myocytes were rinsed in room temperature PBS and stained. Myocytes were stained by inverting the coverslip on a solution of PBS containing 4', 6'-diamidino-2-phenylindole hydrochloride (DAPI, 30 nM) (Invitrogen, Eugene, OR). The first stain also contained a 1:100 dilution of anti-sarcomeric α -actinin monoclonal antibody (clone EA-53, Sigma, St. Louis, MO) and was incubated for 1 h at RT. Before the secondary stain, coverslips were rinsed in PBS. Secondary stains contained a 1:200 dilution of alexa-fluor 488 goat anti-mouse IgG (H+L) antibody (Invitrogen, Eugene, OR). After incubation, coverslips were rinsed and mounted on glass coverslides until imaged.

Confocal Microscopy: Dispersion of fluorescent beads into the fibers was imaged with Zeiss LSM 5 LIVE Confocal Microscopy (Carl Zeiss, Dresden, Germany). Images were acquired under 40x/1.3 Oil

DIC objective lens with 488 nm wavelength emission. Images of cardiomyocytes on PLA and gelatin fibers were acquired under 40x/1.3 Oil DIC objective lenses with 405 nm and 488 nm wavelength emissions. Images were analyzed and displayed using ImageJ (NIH, Bethesda, MD).

Jet break-up analysis: To elucidate the mechanism of jet break-up and bead formation, the capillary number (Ca) was calculated for all samples based on definition of ratio of Weber number (We) to Reynolds number (Re). For calculating these two dimensionless numbers, jet exit velocity was estimated first in the rotating frame by measuring the difference in liquid height, Δh , and using the following formula:

$$V = \Delta h \cdot (D/2)^2 / R^2 \cdot t \quad (S2)$$

where R is radius of reservoir, D is diameter of the orifice, and t is the duration of experiments. Thereby, the jet exit velocity, U , based on the stationary frame was calculated as:

$$U = \sqrt{V^2 + R^2 \omega^2} \quad (S3)$$

where ω is the rotation speed in $\text{rad}\cdot\text{s}^{-1}$.

Supporting References:

1. Feinberg, A. W.; Feigel, A.; Shevkoplyas, S. S.; Sheehy, S.; Whitesides, G. M.; Parker, K. K. *Science* **2007**, 317, (5843), 1366-1370.
2. Wang, C.; Chien, H. S.; Yan, K. W.; Hung, C. L.; Hung, K. L.; Tsai, S. J.; Jhang, H. J. *Polymer* **2009**, 50, (25), 6100-6110.
3. Grant, J.; Lee, H.; Liu, R. C. W.; Allen, C. *Biomacromolecules* **2008**, 9, (8), 2146-2152.

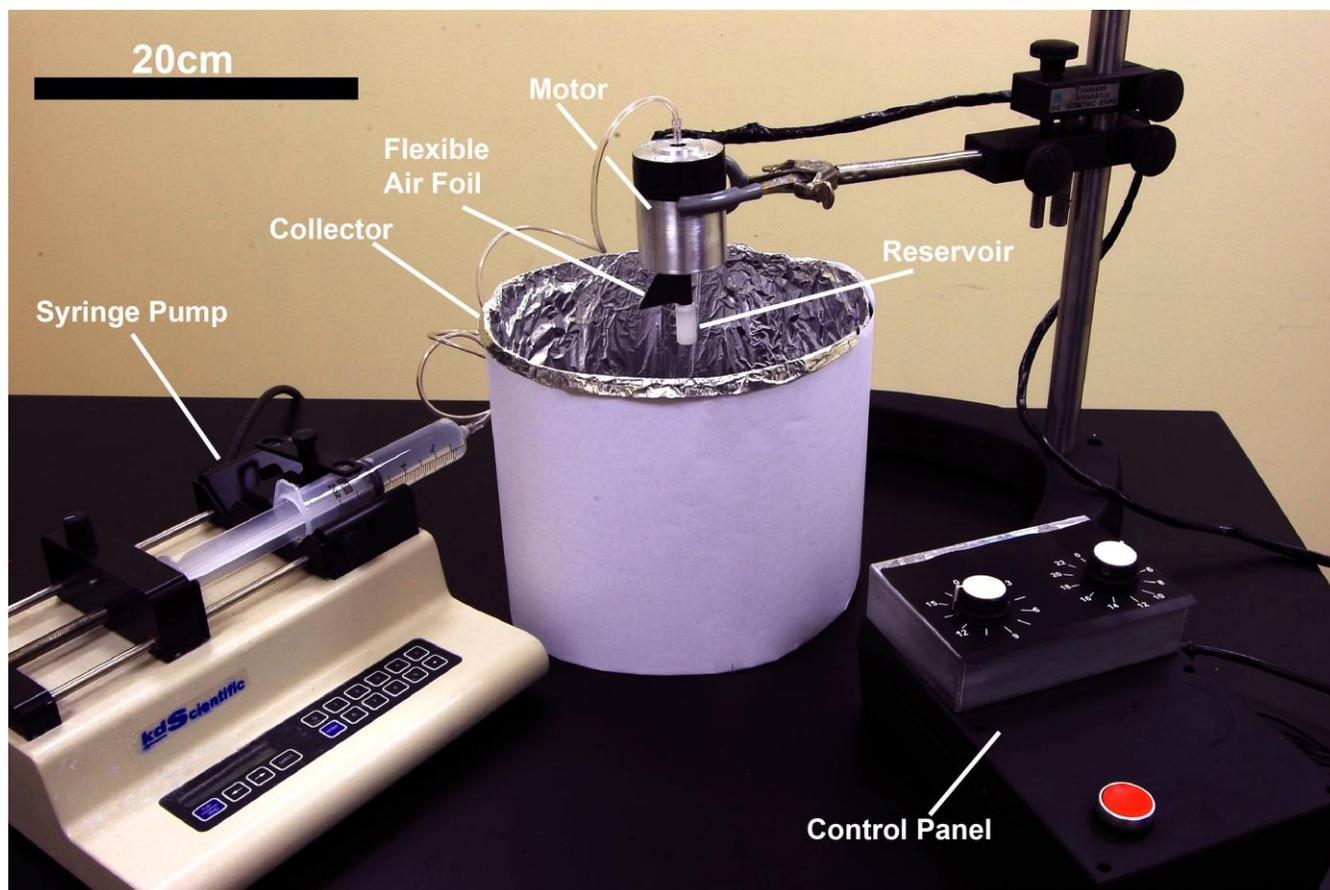


FIGURE S1. Rotary jet-spinning (RJS) system.

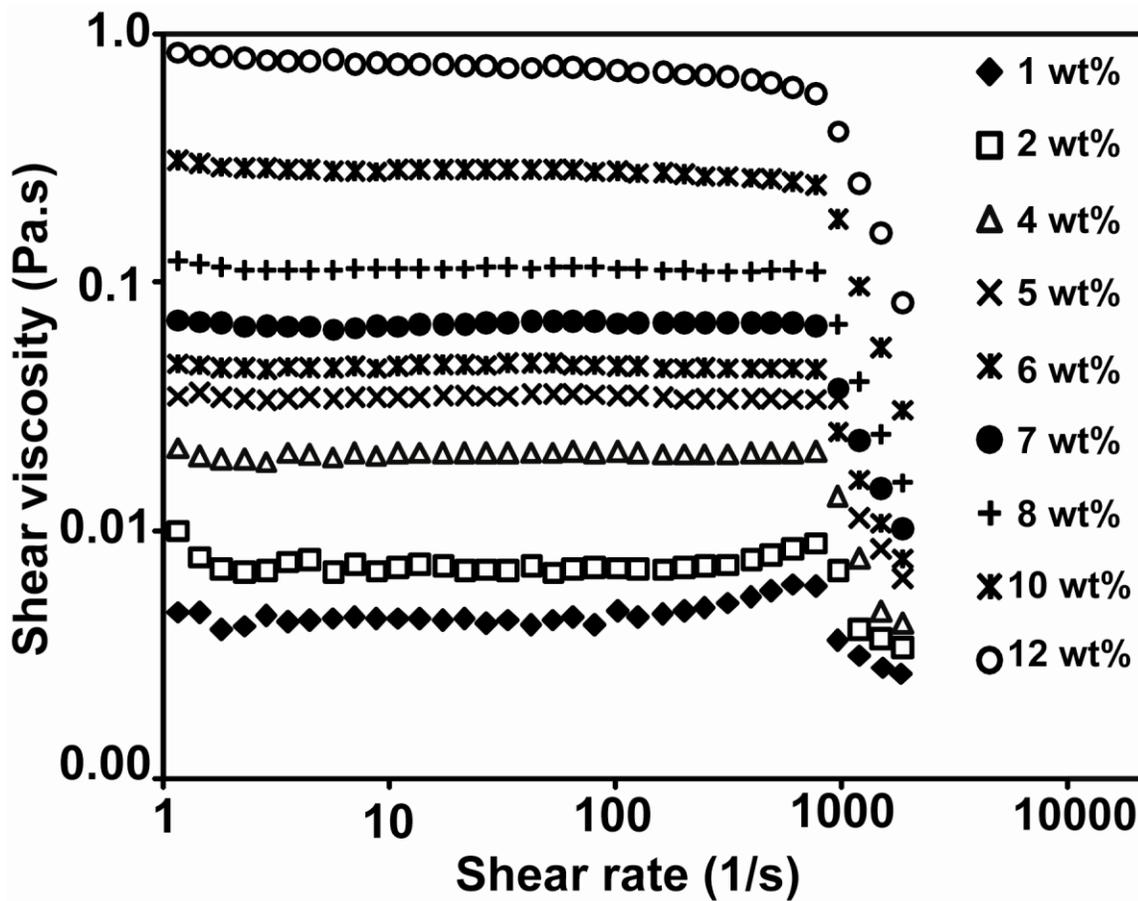


FIGURE S2- Plot of viscosity as a function of shear rate for PLA at different concentrations.