ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Sequence-Specific Crosslinking of Electrospun, Elastin-Like Protein Preserves Bioactivity and Native-Like Mechanics

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Nanofibrous elastin-like protein engineered as a tunable mimic of the extracellular matrix

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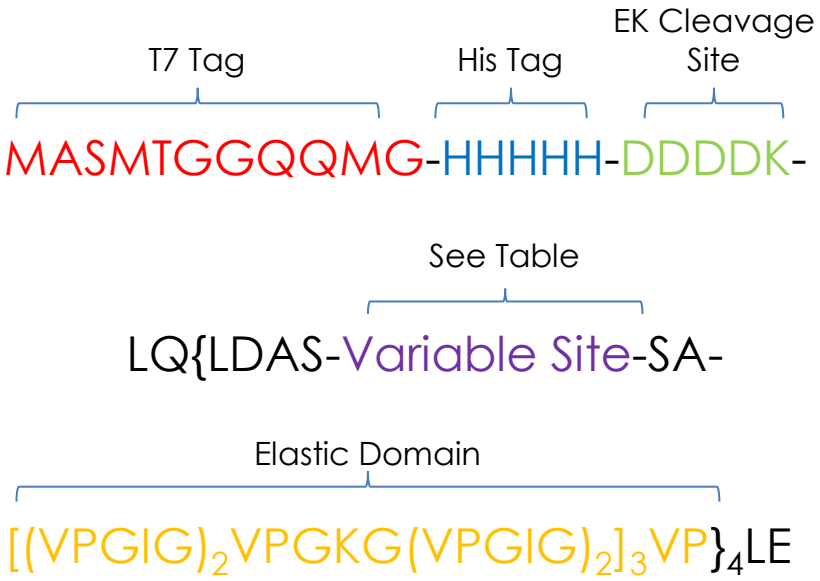

Protein Name	Variable Domain
ELP-RGD	TVYAVTG**RGD**SPASSAA
ELP-RDG	TVYAVTG**RDG**SPASSAA

Figure S1. Amino acid sequence of the bioactive family of ELPs.

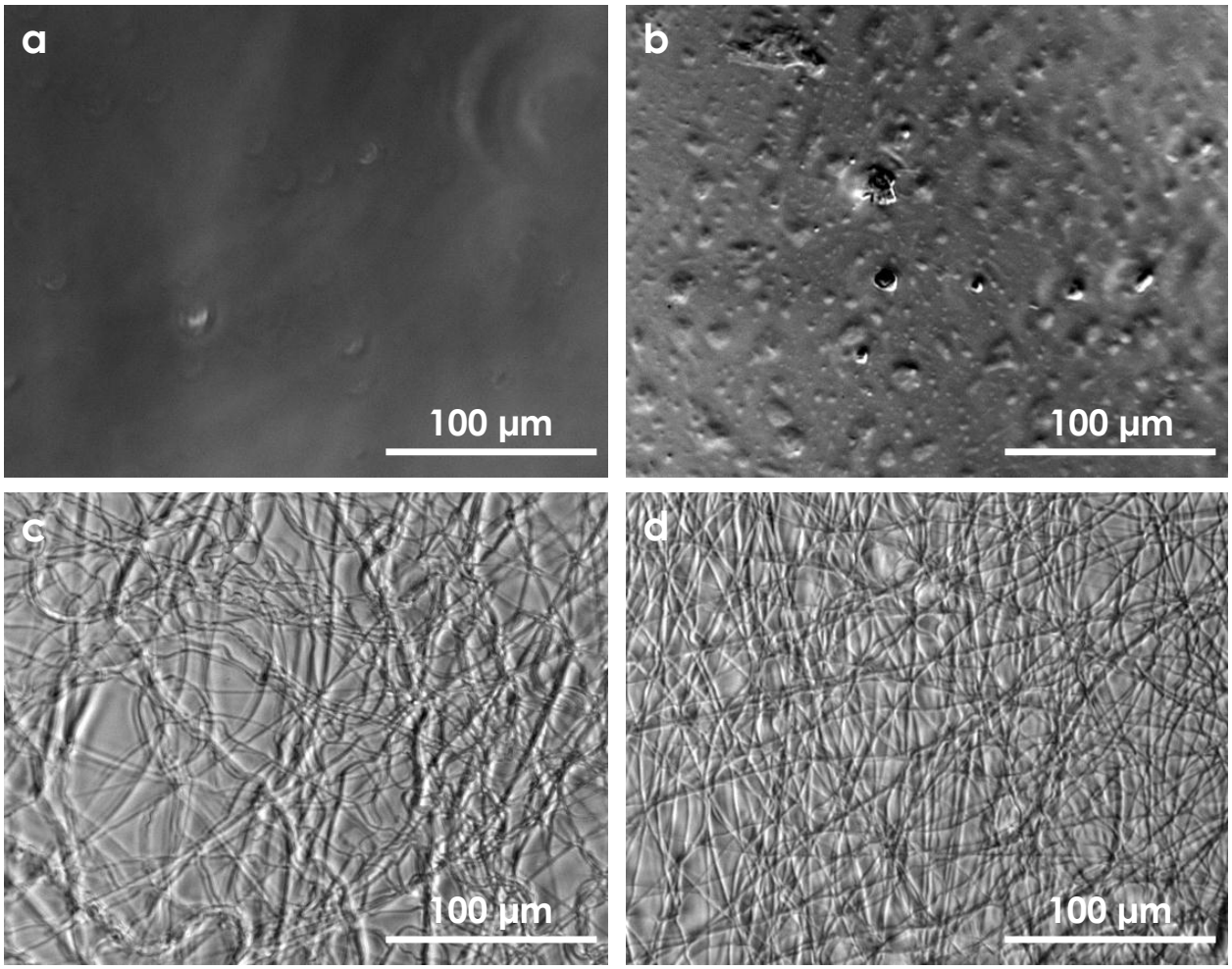

Figure S2. Phase contrast micrographs of electrospun ELP hydrated after various steps in the crosslinking protocol. Electrospun ELP is incubated in physiological buffer after a) no crosslinking, b) initial hydration with a minimal volume of high NaCl buffer, but no glutaraldehyde, c) glutaraldehyde vapor exposure only, and d) glutaraldehyde vapor exposure followed by hydration with a minimal volume of high NaCl buffer without additional glutaraldehyde.

Supporting Information Experimental Section

Scanning electron microscopy: Samples were coated with gold (Hummer V Sputter Coater) and analyzed at an acceleration voltage of 5 kV (Hitachi S-3400N VP-SEM).

Quantification of LCST: Turbidity was read at 300 nm (Molecular Devices SpectraMax Plus^{384} Spectrophotometer). The temperature was increased at a rate of 0.3 °C min^{-1} with an equilibration time of 30 s min^{-1}.

Bulk tensile testing: Hydrated fabrics were cut into rectangular strips, mounted with a gap of 4 mm, and stretched at room temperature until rupture (Instron 5848).

Culture of rMSCs: Samples were rinsed three times with sterile PBS, transferred to a 24-well plate, seeded with 30,000 rMSCs (p38) in 500 □L medium (Dulbecco's minimal essential medium supplemented with, unless otherwise noted, 20% fetal bovine serum, 100 U mL^{-1} penicillin, and 0.1 mg mL^{-1} streptomycin), and incubated at 37°C, 5% CO_2 for 24 h. For metabolic assays, rMSCs were maintained in serum-free medium for 24 h and incubated with 0.5 mg mL^{-1} sodium resazurin for 4 h before quantifying fluorescence (excitation 560 nm, emission 590 nm).

Protein expression and purification: Sequences were cloned into pET15b (Novagen) plasmids using traditional recombinant techniques and expressed in Escherichia coli, BL21(DE3) (induced with 1mM b-isopropyl thiogalactoside at OD600 ~0.6, 37 °C, expressed for 6 h). The wet cell pellet was resuspended in TEN Buffer (1 g/mL, 1 mM PMSF), sonicated, and agitated overnight at 4 °C. The pH was adjusted to ~9 with 4 N NaOH, incubated at 4 °C for 1 h, and centrifuged at 4 °C (1 h, 17,700 g). NaCl was added to the supernatant to a final concentration of 1 M. This solution was agitated overnight at 4 °C, incubated at 40 °C shaking for 3 hrs, and centrifuged at 40 °C (1 h, 3,584 g). The pellet was resuspended in water (0.1 g/mL), agitated overnight at 4 °C. Warm (40 °C, 1 M NaCl) and cold (4 °C, pH ~9) purifications were then repeated twice. To de-salt before lyophilization, solutions were dialyzed (10,000 MWCO) and agitated stirred (200 rpm) three times for over 4 h eacf in 4 °C, deionized water. After lyophilization, typical protein yields are 25–50 mg L^{-1}.

Confocal fluorescent microscopy: Samples were fixed (4% formaldehyde in PBS, 20 min), blocked (10% normal goat serum, 0.3% Triton X-100 in PBS solution, 3 h, and stained (cell nuclei with DAPI and actin cytoskeleton with tetramethylrodamine-conjugated phalloidin (Invitrogen), overnight at 4 °C). Samples were washed twice with PBS after each step. A laser scanning confocal inverted fluorescence microscope (Leica DMI 4000B) was used to acquire images.

CARS microscopy: The set-up for CARS microscopy is described in detail elsewhere [Enejder, A., Brackmann, C. & Svedberg, F. Coherent Anti-Stokes Raman Scattering Microscopy of

Cellular Lipid Storage. IEEE J. Sel. Top. Quantum Electron. 16, 506-515 (2010)]. Briefly, the system consists of a Nd:Vanadate pump laser (Picotrain, HighQ Lasers GmbH, Hohenems, Austria) and a ring-cavity optical parametric oscillator (OPO; Emerald ring cavity OPO, Angewandte Physik & Elektronik GmbH, Berlin, Germany). The 1064 nm beam from the pump laser is directly aligned into the microscope and overlaid with the output of the OPO. The beams are directed into the microscope (Nikon Eclipse TE2000-E, Nikon) through a mirror scanning unit and focused on the sample by an objective (Nikon Plan Fluor 40x/1.30 oil). The OPO was tuned to 817 nm to probe the 2845 cm^{-1} vibration and to 811 nm to probe the 2930 cm^{-1} vibration in the sample. CARS signals were detected in forward direction using a single-photon counting photomultiplier tube (PMT; PMC-100-1, Hamamatsu, Japan) at 632 nm and 656 nm, respectively. Samples were imaged in unlabeled, wet condition. Both cells and ELP-RGD scaffold generate a CARS signal at 2845 cm^{-1}, whereas the scaffold (ELP-RGD) only displays a distinct resonance at 2930 cm^{-1}. With imaging software (ImageJ) the cells were identified by subtracting the 2930 cm^{-1} from the 2845 cm^{-1} image.