SUPPLEMENTARY INFORMATION

Reactive Electrospinning of Degradable Poly(oligoethylene glycol methacrylate)-Based Nanofibrous Hydrogel Networks

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Materials and Methods

Materials: Poly (ethylene oxide) (PEO, M_w = 600,000 g/mol) was purchased from Sigma-Aldrich. Oligo(ethylene glycol) methyl ether methacrylate (OEGMA, M_n = 475 g/mol, Sigma Aldrich, 95%) was purified by passing through a column of basic aluminum oxide (Sigma Aldrich, type CG-20) to remove the methyl ether hydroquinone (MEHQ) and butylated hydroxytoluene (BHT) inhibitors. Acrylic acid (AA, Sigma Aldrich, 99%), 2,2-azobisisobutryic acid dimethyl ester (AIBMe, Wako Chemicals, 98.5%), dioxane (Caledon Labs, 99%), adipic acid dihydrazyde (ADH, Alfa Aesar, 98%), N'-ethyl-N-(3-dimethylaminopropyl)-carbodiimide (EDC, Carbosynth, Compton CA, commercial grade), aminoacetaldehyde dimethyl acetal (Sigma Aldrich, 99%), methacryloyl chloride (Sigma Aldrich, purum), rhodamine 123 (Sigma Aldrich, 85%), and fluorescein isothiocyanate (FITC, Sigma Aldrich, 90%) were all used as received. For all experiments, Milli-Q grade distilled deionized water (DIW) was used. N-(2,2-dimethoxyethyl)methacrylamide (DMEMAm) was synthesized as previously reported (see N. M. B. Smeets, E. Bakaic, M. Patenaude and T. Hoare, Chem. Commun., 2014, 50, 3306–3309).

Synthesis of hydrazide-functionalized POEGMA (POH): AIBMe (37 mg, 0.16 mmol), OEGMA (4.0 g, 8.4 mmol), AA (0.25 g, 3.5 mmol) and dioxane (20 mL) were added to a 50 mL Schlenk three-neck flask and were purged with nitrogen for 30 minutes. Subsequently the solution was placed in an oil bath at 75 °C for 4 hours under magnetic stirring. After removing the solvent, poly(OEGMA-*co*-AA) polymer was purified by dialysis (6+ hours for 6 cycles) and lyophilized. The purified copolymer was dissolved in 100 mL DIW, after which ADH (2.65 g, 15.2 mmol) and EDC (1.18 g, 7.6 mmol) were added to the polymer solution. The pH of the solution was adjusted to pH = 4.75 using 0.1 M HCl continually over the reaction until the pH stabilized (~ 4 hours). The solution was stirred overnight and then purified by dialysis (6+ hours for 6 cycles). The final polymer was obtained by lyophilization and was stored as a 15 w/w% solution in DIW at 4 °C.

Synthesis of aldehyde-functionalized POEGMA (POA): AIBMe (50 mg, 0.22 mmol), OEGMA (4.0 g, 8.4 mmol), DMEMAm (0.60 g, 3.5 mmol) and dioxane (20 mL) were added to a 50 mL Schlenk three-neck flask and purged with nitrogen for 30 minutes. Subsequently the solution was placed in an oil bath at 75 °C for 4 hours under magnetic stirring. After removing the solvent, poly(OEGMA-*co*-DMEMAm) was dissolved in 100 mL of 0.25 M HCl. The solution was stirred for 24 hours and then purified by dialysis (6+ hours for 6 cycles). The final product was obtained by lyophilization and was stored as a 15 w/w% solution in DIW at 4 °C.

Characterization of reactive precursor polymers: The molecular weight of the precursor polymers was analyzed using gel permeation chromatography (GPC) (Waters, Mississauga, ON). Aqueous GPC was performed with a system consisting of a Waters 515 HPLC pump, Waters 717 plus Autosampler and three columns (Waters Ultrahydrogel-120, -250, -500; 7.8 × 300 mm; 6 µm particles). Samples run in an aqueous buffer consisting of 0.5 M sodium nitrate and 25 mM 2-(cyclohexylamino)ethanesulfonic acid maintained at pH 10.0 at 30 °C. The degree of hydrazide functionalization in POH was determined by conductometric base-into-acid titration (Mantech), comparing the number of –COOH groups in the polymer before and after functionalization. The degree of aldehyde functionalization in POA was determined by ^{1}H-NMR (600 MHz, Bruker), taking the ratio of the aldehyde proton signal at 9.52 ppm to the methyl proton signal at 0.81 ppm. The resulting properties of the POH and POA pre-polymers are summarized in Table S1 below.

Table S1. Molecular weight and degree of functionalization of precursor polymers used for nanofibrous hydrogel preparation

Polymer	Functional Group [-]	Theoretical Functional Monomer [mol%]	Actual Functional Monomer [mol%]	M_n [kDa]	PDI [-]	Average # of Functional Groups/Chain
POH	$NHNH_2$	30.0	28.9	33.4	3.3	18
POA	CHO	30.0	27.8	22.8	3.0	13

Synthesis of fluorescein isothiocyanate (FITC)-labeled POH: FITC-labeled POH was prepared by reacting FITC (5 mg) with POH solution (1 g, 15 w/w% in DIW) under magnetic stirring for 12 hours at room temperature. A total of 2 mol% of the available hydrazide groups were targeted for labelling with FITC. The solution was dialyzed (6+ hours for 6 cycles) and lyophilized. FITC-labeled POH was stored at 15 w/w% in DIW at 4°C. All reaction vessels and storage containers were covered by aluminum foil to prevent photobleaching.

Synthesis of rhodamine 123-labeled POA: Rhodamine 123-labeled POA was prepared by reacting rhodamine 123 (5 mg) with POA solution (1g, 15 w/w% in DIW) under magnetic

stirring for 24 hours. Sodium cyanoborohydride (8.25 mg, 10 mol eq. to rhodamine 123) was then added to the solution to generate a stable conjugate via reductive amination, after which the solution was stirred for another 48 hours. The resulting polymer solution was dialyzed (6+ hours for 6 cycles) and lyophilized to dryness. The rhodamine 123-labeled POA was stored at 15w/w% in in DIW at 4 °C. All reaction vessels and storage containers were covered by aluminum foil to prevent photobleaching.

Preparation of electrospun nanofibrous hydrogel: PEO (2.5 w/w%) and either POH or POA (7.5 w/w%) were co-dissolved in DIW, with the resulting PEO/POH and PEO/POA solutions subsequently loaded into separate barrels of a double barrel syringe equipped with a static mixer followed by a blunt-tip 18 G needle. A voltage of 8.5 kV was applied to the tip of the blunt needle. Aluminum foil and aluminum disk were used as collectors for electrospinning, the former for collecting films and the latter for collecting thicker scaffolds. The distance between the needle and the collector was maintained at 10 cm. All electrospun scaffolds were prepared at room temperature.

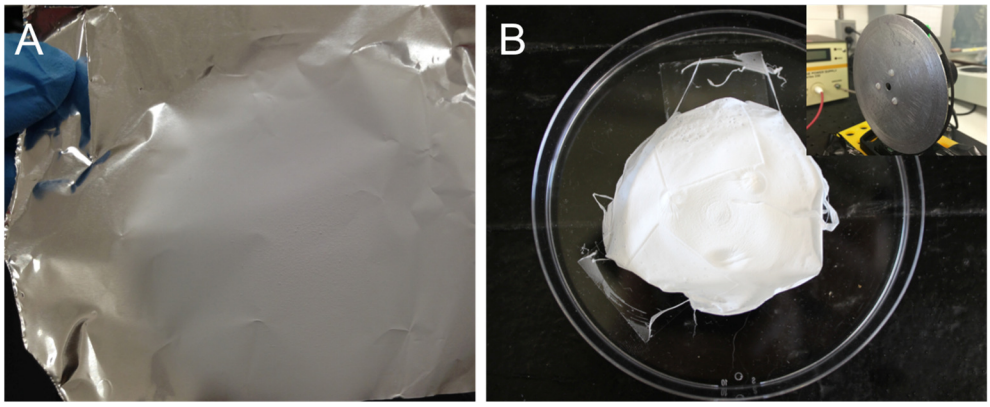

Figure S1. Electrospinning nanofiber mat on (A) aluminum foil and (B) rotating aluminum disk.

Differential scanning calorimetry (DSC) analysis: DSC experiments were conducted using Auto Q20 (TA Instruments) to assess the efficacy of the swelling step for removing the PEO electrospinning aid. First, all samples were heated to 150 °C at a rate of 10 °C/min to eliminate any thermal history. Second, all samples were cooled down to 20 °C at a rate of 5 °C/min and subsequently heated back to 150 °C at a rate of 2 °C/min. Figure S2 shows that melting point endotherms were observed with maximum absolute values of 66 °C and 63 °C for PEO and POH/POA+PEO electrospun nanofiber thermograms respectively due to the crystallization of the high molecular weight PEO; in contrast, a POH/POA bulk hydrogel (prepared without PEO and lyophilized to dryness to match the dry state of the nanofibers) exhibits no transition whatsoever. After the POH/POA+PEO electrospun scaffold was immersed in DIW overnight and then lyophilized, the endotherm disappeared, suggesting that the PEO electrospinning aid could effectively be removed by swelling the scaffold. Note that the degradation results as well as SEM imaging of the scaffolds following the 24 hour time over which DSC indicated complete

removal of the PEO fraction (Figure S3) confirm the scaffold remained intact during this time, again confirming that POH/POA gelation is occurring in this electrospun system.

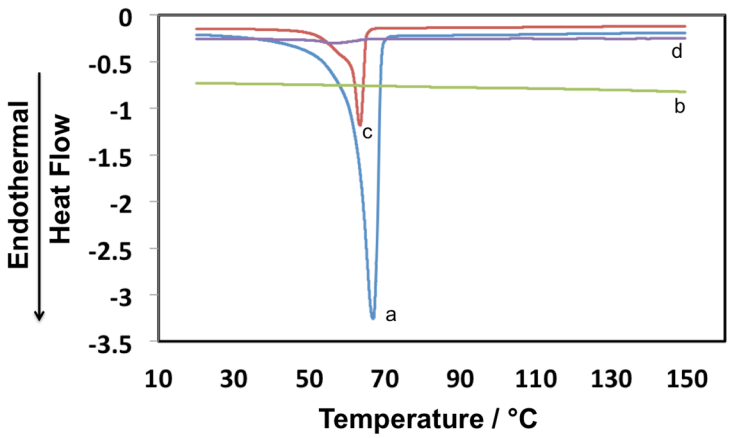

Figure S2. DSC thermograms of a) PEO-only electrospun nanofibers (blue), b) POH/POA bulk hydrogel after lyophilization (green), c) POH/POA+PEO electrospun nanofibers prior to soaking (red), and d) POH/POA+PEO electrospun nanofibers after immersion in DIW overnight (purple)

Microscopy: Optical microscopy of the nanofibrous hydrogels was conducted using a Nikon Eclipse LV100N POL epifluorescence microscope (Nikon Instruments, Mississauga, Ontario, 10× objective lens) that enables direct imaging of the hydrogel when swollen in 10 mM phosphate buffered saline. Nanostructural analysis of the nanofibrous hydrogels in the dry state was conducted using scanning electron microscopy (SEM, Tecan Vega II LSU instrument) using an operating voltage of 10 kV. Samples were imaged directly following electrospinning or lyophilized immediately after swelling in water and sputter-coated with a ~30 nm gold layer prior to imaging to prevent charging. Co-localization of hydrazide and aldehyde-functionalized POEGMA within the hydrogel fibers was confirmed by preparing scaffolds using POH-fluorescein and POH-rhodamine (at the same concentrations outlined above) and observing the resulting scaffolds using confocal laser scanning microscopy (Zeiss LSM 510). Excitation wavelengths of 488 nm (fluorescein) and 543 nm (rhodamine) were used for imaging.

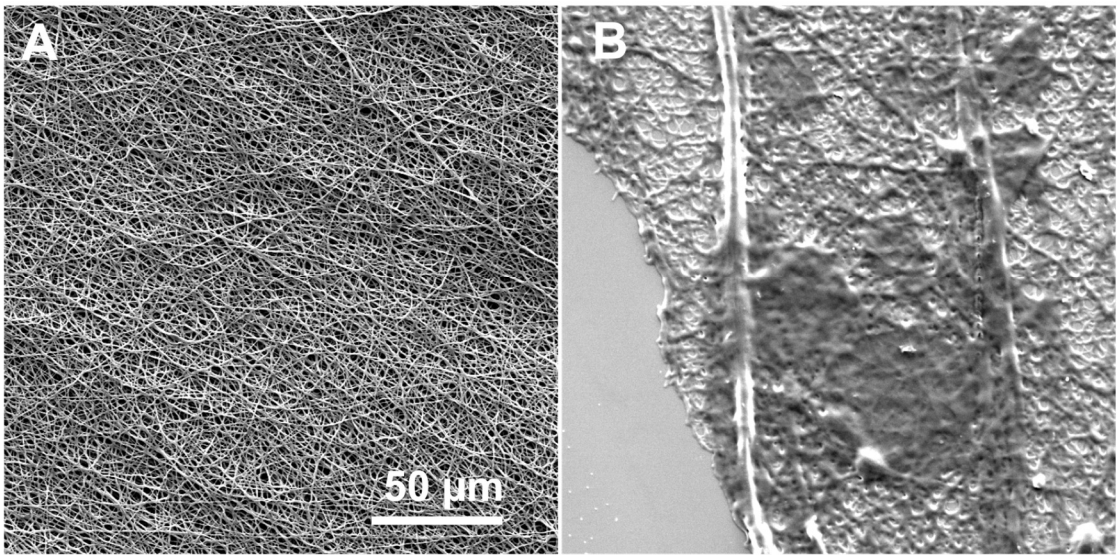

Figure S3. SEM images of POH/POA+PEO electrospun fibers before and after soaking (24 h) in DIW water.

Swelling kinetics: Swelling kinetics were measured at 37 °C in 10 mM PBS at pH 7.4. Electrospun scaffolds were placed into cell culture inserts that were subsequently placed inside a 24-well plate and submerged with 4 mL PBS. At predetermined time intervals, the inserts were removed, excess PBS was wicked off the surface of the hydrogels using a Kimwipe, and the inserts were weighed. The water content was calculated based on this gravimetric data using the equation

$$Swelling\ ratio\ \% = \frac{W_{swollen} - W_{initial}}{W_{initial}} \times 100\%$$

$$Water\ content\ \% = \frac{W_{swollen} - W_{dry}}{W_{swollen}} \times 100\%$$

Error bars represent the standard deviation of the replicate measurements (n=4). Videos of the short-term swelling of PEO-only scaffolds as well as POH/POA+PEO scaffolds are also available as supplementary information for viewing.

As a control, a bulk hydrogel prepared with the same overall composition (2.5 wt% PEO, 7.5 wt% of each POEGMA precursor polymer) was prepared via simple injection of the precursors into a silicone mold with comparable thickness to the electrospun sample tested (1.7 ± 0.2 mm height for the bulk gels compared to 0.4 ± 0.1 mm height for the electrospun samples). Note that precisely matching these dimensions is technically difficult using the molding process; however, all samples were kept thin such that the small differences in height would have minimal impact on changing any swelling kinetics

measured (at least in comparison to the differences in swelling kinetics measured). The bulk hydrogel sample was dried overnight at room temperature (to match the electrospinning sample treatment), after which its swelling was tested using the same gravimetric method outlined above for the nanofibrous hydrogels.

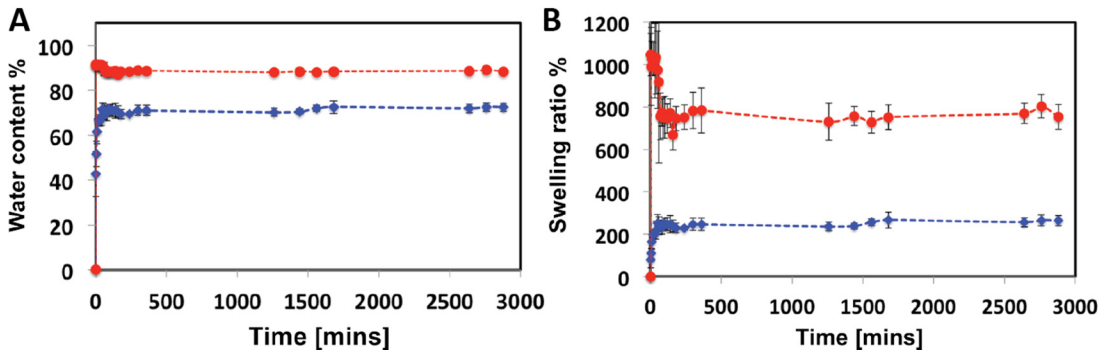

Figure S4. Swelling kinetics of electrospun hydrogel relative to a bulk hydrogel with the same composition and similar dimensions: (A) water content as a function of time; (B) swelling ratio as a function of time.

Degradation kinetics: Degradation kinetics were determined using the same gravimetric method described above for the swelling kinetics measurements but substituting the PBS for 1 M HCl at 37 °C; the acid-catalyzed conditions were selected to provide an effective and rapid assay to compare the degradation of the nanofibrous scaffolds with a corresponding bulk hydrogel prepared as described above for the swelling measurements. Degradation was also tracked in 10 mM PBS as the buffer by continuing to observe the swelling kinetics samples until no residual gel was present in the cell culture insert. In a parallel experiment, samples were collected at different times, immediately lyophilized, and imaged using SEM as per the previously described protocol to track the morphology of the nanofibrous network during degradation. Error bars represent the standard deviation of the replicate measurements (n=4).

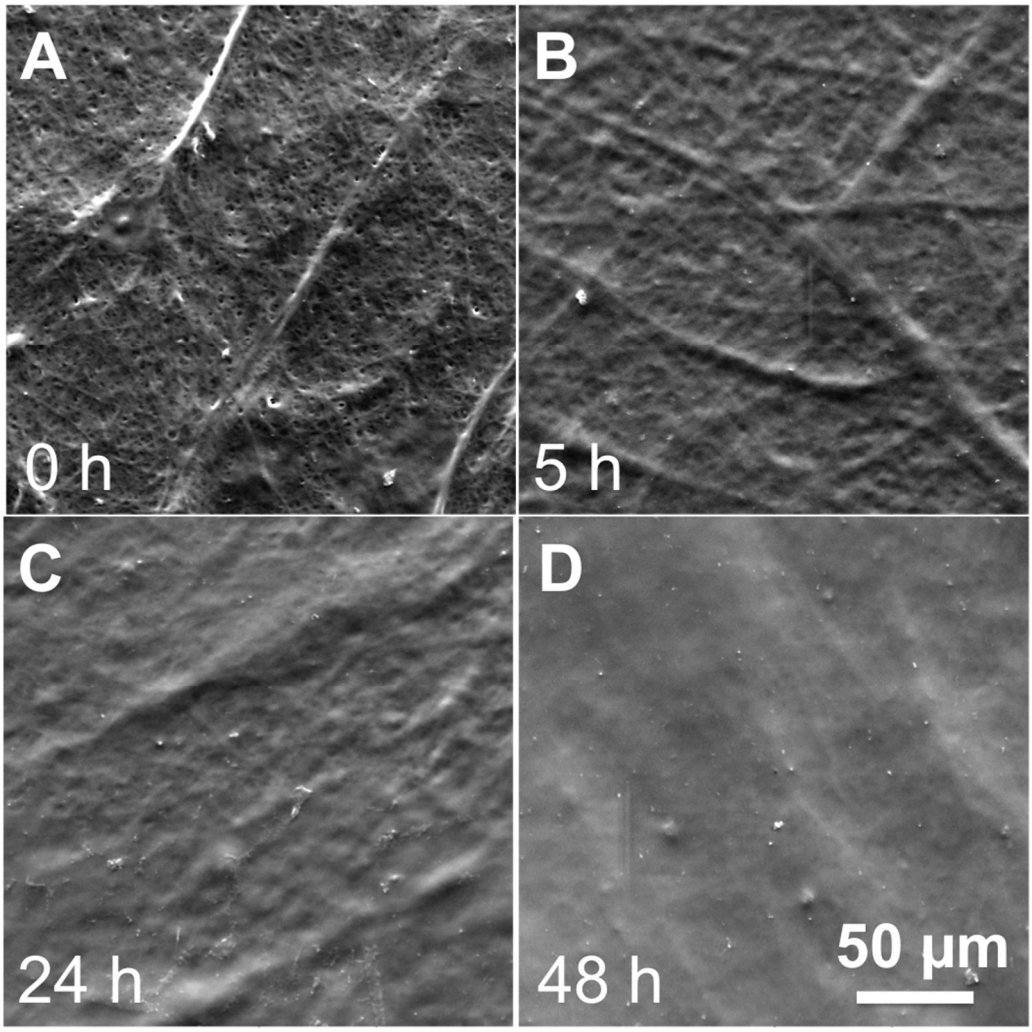

Figure S5. SEM images tracking degradation of nanofibers in 1M HCl at 37 °C after (A) 0 h; (B) 5 h; (C) 24 h; (D) 48 h

Mechanical properties: The tensile properties of dry electrospun scaffolds were tested using a MicroSquisher (CellScale Biomaterials Testing, Waterloo Canada). Samples were mounted via puncture with five pins spaced 0.7 cm apart, then pulled to 10% (swollen samples) or 20% (dry samples) elongation and subsequently allowed to relax. At least 80 cycles were tested, with loading and recovery durations of 20 seconds used for each cycle. Compressive mechanical properties of the swollen nanofibrous hydrogels were subsequently measured directly in 10 mM PBS buffer at room temperature, also using a MicroSquisher. Cantilevers were fabricated using a 559 µm gauge cantilever and a square, 3 mm stainless steel plate. A displacement of 50% compression was used per cycle, with 40 cycles tested and loading and recovery durations of 20 seconds per cycle applied. Videos showing each micromechanical test performed are uploaded for viewing as supplementary information.

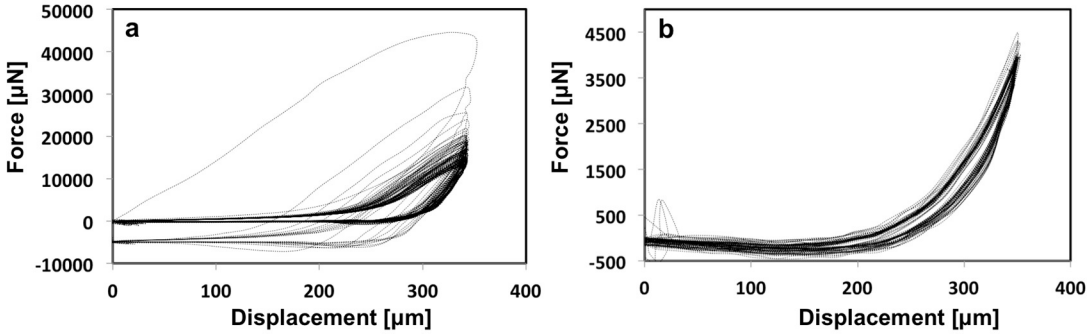

Figure S6. Mechanical properties of POH/POA+PEO nanofibers: (a) tensile cycling of dry nanofiber mat (80 cycles, 20% elongation/cycle); (b) compressive cycling of swollen nanofiber network (PBS, 40 cycles, 50% compression/cycle).

Tensile mechanical properties of swollen nanofibrous hydrogels were assessed in 10 mM PBS (pH=7.4) at room temperature (MicroSquisher). Scaffolds fractured after a few cycles with elongation of 20%, but could be cycled for at least 325 cycles at elongation of 10% (Table S2 and Figure S7).

Table S2. Tensile modulus at different % elongation; the thickness of nanofibrous hydrogels was 0.4 ± 0.1 mm (n=5)

Tensile (%)	Modulus (kPa)
10%	0.26 ± 0.05
20%	1.45 ± 0.80

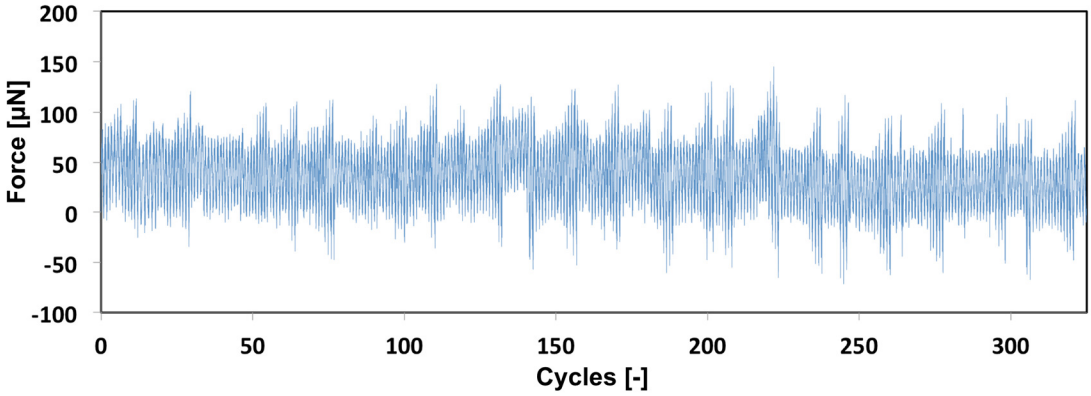

Figure S7. Mechanical properties of POH/POA+PEO nanofibers: tensile cycling of swollen nanofiber mat in 10 mM PBS (325 cycles, 10% elongation/cycle).

Table S3. Compressive testing at different % compression values for both the POH/POA+PEO nanofibrous hydrogels and a bulk hydrogel with the same overall composition as POH/POA+PEO nanofibrous hydrogels. The thickness of bulk hydrogel and nanofibrous hydrogels were 1.7 ± 0.2 mm and 0.4 ± 0.1 mm, respectively. (n=3)

Compression (%)	Compressive Modulus (kPa)	
	Fibrous hydrogel	Bulk hydrogel
20%	0.9 ± 0.3	3.8 ± 1.6
30%	1.7 ± 0.6	4.1 ± 1.8
40%	1.8 ± 0.6	4.6 ± 1.0
50%	2.1 ± 0.1	4.8 ± 1.2

Enzyme Activity: β-galactosidase (β-gal, Grade VI, M_w = 464 kDa, Sigma Aldrich) and alkaline phosphatase (AP, from calf intestine, Roche) was encapsulated in POH/POA+PEO nanofibers directly during the electrospinning process by dissolving the enzyme in the POH precursor solution at concentrations of 2.65U/mL and 2.5U/mL respectively. The resulting POH/POA+PEO scaffolds were then swollen in 10 mM PBS buffer (pH=7.4) at 37 °C. The released protein concentration was measured using a Bradford protein assay (595 nm, Tecan M 1000 plate reader), while the relative enzyme activity was then assessed by adding the chlorophenol red β-galactopyranoside (CPRG) substrate for β-gal and the 3,3',5,5'-tetramethylbenzidine substrate for AP (without removing the scaffolds) and tracking their conversions at 550 nm and 405 nm, respectively, 30 minutes (β-gal) and 5 minutes (AP) after substrate addition (Tecan M 1000 plate reader). The relative (%) enzyme activity was calculated based on a calibration curve constructed from a fresh solution of the enzyme prepared at the same overall concentration in the same PBS buffer.

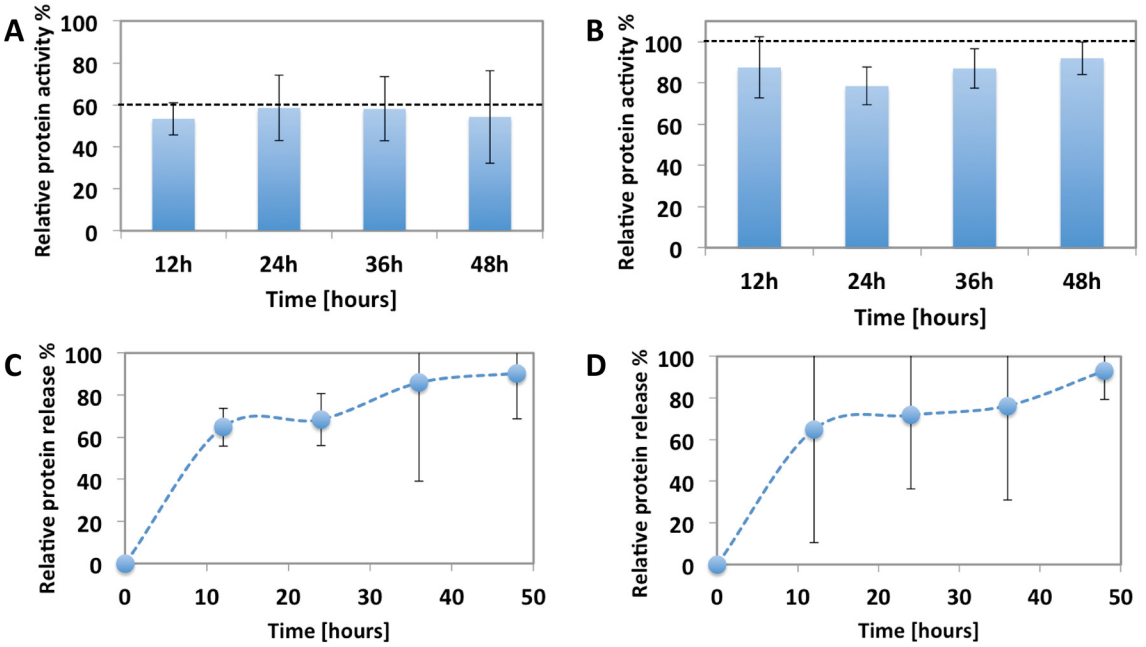

Figure S8. Encapsulation of β-gal and AP in electrospun nanofibrous hydrogel: (A,B) percentage activity of (A) β-gal and (B) AP in electrospun samples relative to the same concentration of enzyme freshly dissolved in the PBS release medium; (C,D) percentage of (C) β-gal and (D) AP release at each time point relative to the total loaded enzyme concentration. (n=4)

AP retains at least 80% of its fresh solution activity while β-gal retains ~60% of its activity in fresh solution following electrospinning after at least 48 hours of incubation in PBS (Fig. S8A-B), demonstrating that the electrospinning method is sufficiently mild to maintain the majority of enzyme activity even after drying during electrospinning and in the presence of hydrazone cross-linking chemistry. Note that electrospinning the same enzymes into a hollow polycaprolactone nanofiber results in only ~30% enzyme activity for AP (ref. 39 in main manuscript), suggesting the inertness of this reactive electrospinning process to biomolecules. In addition, the electrospun fibers release the majority of the encapsulated enzyme within the first few hours of soaking and facilitate nearly 100% release after 2 days, further confirming that the enzymes are not grafted or otherwise bound to the fibers and thus more likely to retain their activity.