

In Situ Fabrication of Fiber Reinforced Three-Dimensional Hydrogel Tissue Engineering Scaffolds

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SUPPLEMENTAL INFORMATION

Additional Experimental Details

Composite Fabrication. PCL was purchased from The Perstorp Group as CAPA 6800. Poly(L-lactic acid) (PLLA) was purchased from NatureWorks as Ingeo Biopolymer 2003D. PEO was purchased from Dow as two independent powders, POLYOX WSR-N:10 and POLYOX WSR-N:80, and melt blended at 140 °C using a 70/30 (WSR-N:80/WSR-N:10) weight ratio in a co-rotating twin screw extruder (Thermo Scientific TSE 24 MC) with a screw length/diameter ratio of 40:1 after drying under vacuum at 40 °C for 48 hours. Melt blending of PEO provided a rheological viscosity match with both PCL and PLLA for multilayer coextrusion at a temperature of 190 °C. (**Figure S1**) Viscosity was determined on a Galaxy I Model D7054 Melt Flow Indexer (Kayeness Inc.) at a shear rate of 10 sec^{-1} to simulate extrusion conditions.

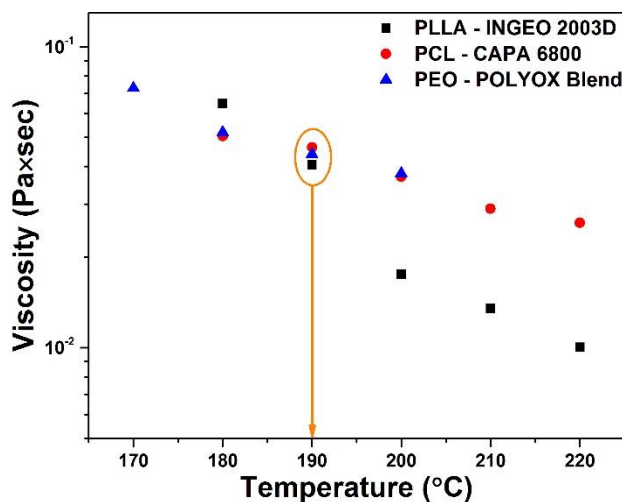


Figure S1. Melt flow index (MFI) of PCL, PEO, and PLLA highlighting the viscosity match for coextrusion at 190 °C for fabrication of both PCL/PEO and PLLA/PEO composites.

Prior to coextrusion, all three materials were dried at 40 °C for 48 hours to remove moisture. The details of fiber/matrix multilayer coextrusion have been described elsewhere.^{Invalid source specified.} All composites were fabricated with 64 total horizontal layers, containing 32 layers consisting of 512 alternating PEO/fiber domains each surrounded by a continuous PEO layer. The melt pump rates of each extruder were systematically manipulated to vary the fiber content of each of the 8 composites, but the total melt pump rate for all three extruders was kept constant at 30 rotations

per minute (RPM). Composites were coextruded through a tape die (12.7 mm width $\times$ 0.5 mm thickness) onto a steel chill roll held at 50 °C and rotating at 50 RPM. The calculated fiber phase content (W_c) in each coextruded composite was determined via **Equation S1** based on relative feed rate, where V_{fiber} and ρ_{fiber} correspond to the melt pump rate and density of the fibrous phase and V_{PEO} and ρ_{PEO} correspond to the melt pump rate and density of PEO. This approximation was verified experimentally by dissolving PCL/PEO and PLLA/PEO composites in $CDCl_3$ and analyzing using 1H nuclear magnetic resonance (NMR, Varian Inova 600 MHz) spectroscopy (**Figure S2**). To visualize fiber distribution throughout the hydrogel volume, a fluorescent dye (Sunset Yellow) was included at a 3% loading in the PCL phase in a separate experimental run under similar coextrusion parameters with relative melt pump rates of 10:20 (PCL:PEO). Although dye may elute from the fiber phase, recent work by Mofidfar and coworkers (ref) has demonstrated significantly reduced elution from these melt processed fibers due to differences in crystalline structures formed from the melt vs. solution processed fibers.¹

$$W_c = \frac{\frac{V_{fiber}\rho_{fiber}}{V_{PEO}\rho_{PEO}}}{\frac{V_{fiber}\rho_{fiber}}{V_{PEO}\rho_{PEO}} + 1} \times 100\% \quad (\text{Equation S1})$$

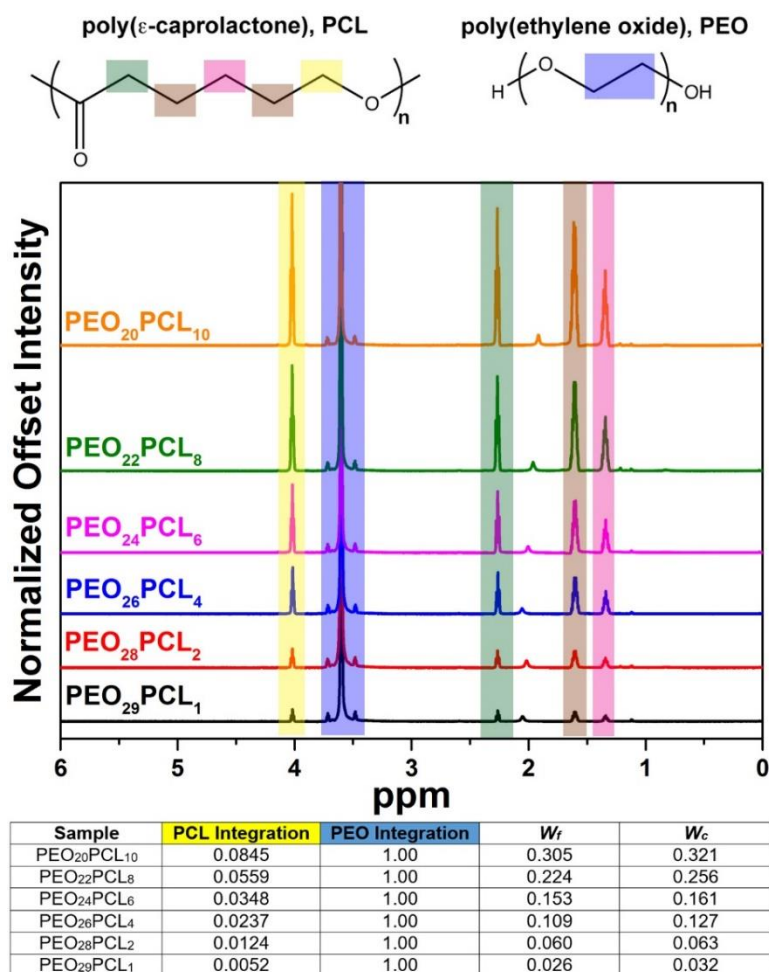


Figure S2. Comparison between weight fractions of PCL in the coextruded PCL/PEO composites at various feed rates calculated using relative PCL/PEO melt pump rates and analytically determined via ^1H NMR.

Wide-angle X-ray Scattering. Wide-angle X-ray scattering (WAXS) was carried out on a Rigaku S-MAX 3000 two-dimensional (2D) small/wide angle X-ray scattering (SAXS/WAXS) instrument with a sealed tube MicroMax-002+ source emitting with a Cu $K\alpha$ X-rays with a wavelength (λ) of 0.1542 nm. Monochromatic X-rays were collimated through three pinhole slits to yield a final spot size of 0.7 mm on each specimen. The sample-to-detector distance was calibrated with silver behenate (AgBe) standard with a (001) scattering vector (q) at a position of 1.076 nm^{-1} to yield a final sample to collector distance of 147.2 mm. Each specimen was exposed to X-ray radiation for 2 hours to allow adequate data collection. The transmission factor of each specimen was measured using a photo-diode placed behind the specimen and used for subtraction

of the background incoherent inelastic X-ray scattering in POLAR software (Precision Works NY, Inc.).

Chain orientation within the fibrous phase was calculated from the background corrected WAXS profiles using Hermans orientation factor (f_H). Azimuthal distributions ($\langle \cos^2 \phi \rangle_{hk0}$) between $0 - 90^\circ$ ($0 - \frac{\pi}{2}$ rad) were obtained for two ($hk0$) reflections ((110) and (200) reflections) of both PCL and PLLA using intensity (I) as a function of azimuthal angle (ϕ) (**Equation S2**). The relationship between the two ($hk0$) azimuthal distributions of PCL and the crystallite c-axis ($\langle \cos^2 \phi \rangle_c$) has been derived already (**Equation S3**). The relationship between the two ($hk0$) azimuthal distributions of PLLA and the crystallite c-axis is determined in subsequent sections of this manuscript (**Equation S4**). These azimuthal distributions were then related to f_H using **Equation S5** with values ranging between -0.5 and 1.0 indicating various degrees of crystal orientation. Values of f_H of -0.5 indicate crystallites with the c-axis oriented perpendicular to the fiber axis and 1.0 indicates crystallites with the c-axis oriented parallel to the fiber axis; a value of 0.0 indicates a random crystallite alignment.

$$\langle \cos^2 \phi \rangle_{hk0} = \frac{\int_0^{\frac{\pi}{2}} I(\phi_{hk0}) \sin \phi_{hk0} \cos^2 \phi_{hk0} d\phi_{hk0}}{\int_0^{\frac{\pi}{2}} I(\phi_{hk0}) \sin \phi_{hk0} d\phi_{hk0}} \quad (\text{Equation S2})$$

$$\langle \cos^2 \phi \rangle_c = 1 - 1.441 \langle \cos^2 \phi \rangle_{110} - 0.559 \langle \cos^2 \phi \rangle_{200} \quad (\text{Equation S3})$$

$$\langle \cos^2 \phi \rangle_c = 1 - 1.309 \langle \cos^2 \phi \rangle_{110} - 0.691 \langle \cos^2 \phi \rangle_{200} \quad (\text{Equation S4})$$

$$f_H = \frac{(3 \langle \cos^2 \phi \rangle_c - 1)}{2} \quad (\text{Equation S5})$$

Tensile Analysis. Both drawn PCL and undrawn PLLA fibers were isolated from coextruded composites using methanol (MeOH, Sigma Aldrich) and tap water (Cleveland, OH). Drawn PCL fibers were isolated from drawn PCL/PEO composites using two six-hour solvation steps in a 70:30 (MeOH:H₂O) solution followed by 0.8 min cm⁻² high pressure delamination and

hydroentanglement using H₂O pressurized to 3.5 MPa. PLLA fibers were isolated from coextruded PLLA/PEO composites again using two six-hour solvation steps in a 70:30 (MeOH:H₂O) solution followed by 0.8 min cm⁻² high pressure delamination and hydroentanglement using H₂O pressurized to 9.0 MPa. PLLA fiber mat purity was analyzed via ¹H NMR in CDCl₃. The void fraction of each fiber mat was obtained by measuring the length, width, and thickness of each sample to determine an occupied volume and weighing the mass of that volume to calculate an apparent density. The apparent density was compared to the density of a solid PCL or PLLA film to obtain a void fraction for each tensile sample (5 specimens for each sample). Individual fiber mats, either drawn PCL or undrawn PLLA, were analyzed under uniaxial tensile deformation along the fiber axis on a Zwick/Roell tensile testing instrument equipped with 100 N load cell (minimum 5 samples per fiber type). Testing was carried out at room temperature at a rate of 100% strain min⁻¹ with a standard 10 mm gauge length. After accounting for the void fraction of each sample, the tensile modulus (E_T) of each fibrous sample was determined using linear regression of the linear elastic deformation region (strain < 1%).

Pore Structure Analysis. Hydrogels containing PCL and PLLA fibers were freeze-fractured using liquid nitrogen (LN₂), and cut with a sharp razor to ensure a smooth surface for imaging. The pore structure of fiber reinforced hydrogels was imaged after freeze drying (-80 °C) to remove water before freeze fracturing to provide a smooth surface. Specimens were mounted on carbon tape and sputter coated with 10 nm of gold at a 90° angle to provide adequate conductivity for imaging via scanning electron microscopy (SEM, JEOL-JSM-6510LV).

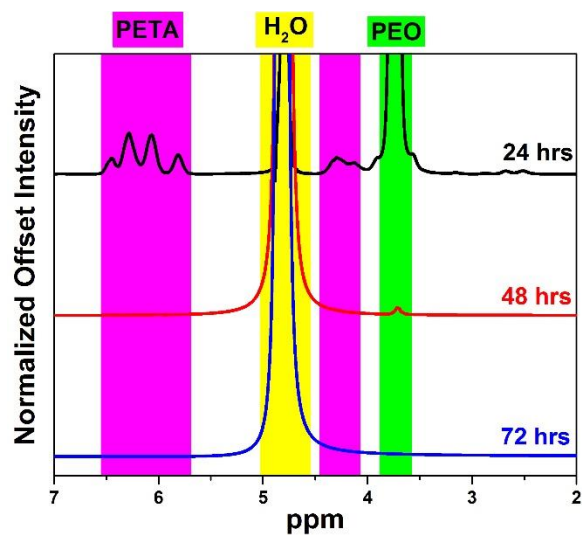


Figure S3. ^1H NMR of PEO hydrogel extractions using H_2O after 24, 48, and 72 hours.

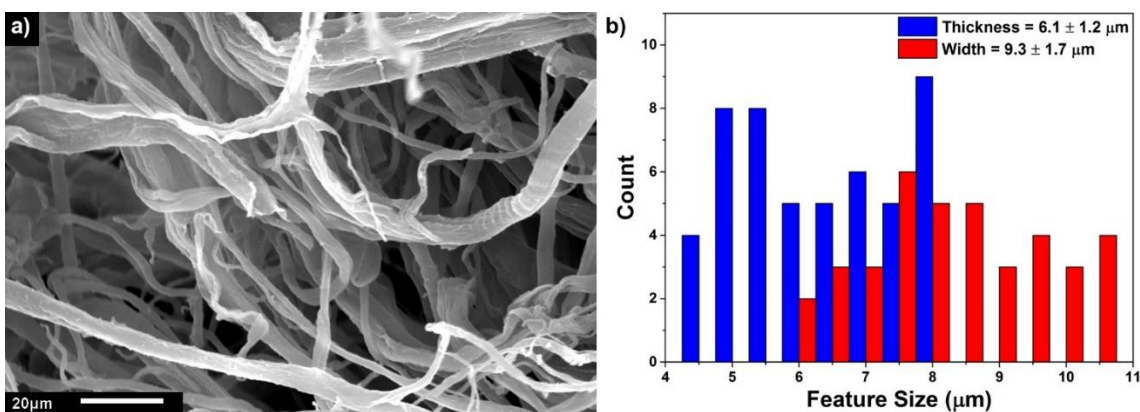


Figure S4. a) SEM micrograph; b) lateral fiber size distribution of PCL fibers isolated for coextruded PCL/PEO composites using highest relative PCL:PEO feed rate.

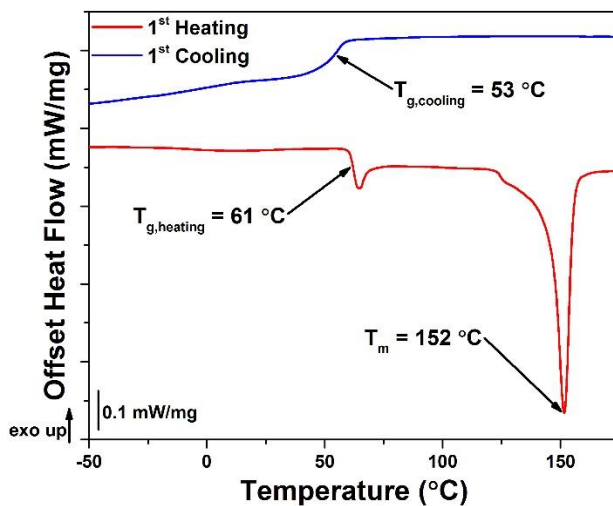


Figure S5. Differential scanning calorimetry (DSC) 1st heating and cooling heat flow of isolated coextruded PLLA fibers detailing glass transition temperature (T_g) and melting temperature (T_m).

Crosslinking optimization. To select appropriate reaction conditions, a range of PEO:PETA concentrations and UV exposure times were explored using a control coextruded PEO tape containing no fibrous phase at a constant UV intensity of $33 \pm 2 \text{ mW/cm}^2$. Initially, UV exposure time was controlled while the PEO:PETA concentration was varied (**Figure S6a-b**). At PEO:PETA ratios below 40:60 (by weight), negligible water uptake was observed, suggesting that there were insufficient PEO binding sites for appropriate swelling. Above 70:30 (by weight) PEO:PETA, dissolution of the composites during swelling occurred, which suggested insufficient crosslink density. This optimization study highlighted the role of PEO:PETA concentration, suggesting that any PEO concentration between 40-70 weight percent (wt%) can be utilized for hydrogel formation. This concentration range is much higher than reported for hydrogels fabricated using end-functionalized low molecular weight PEG, which are due compatible with multilayer coextrusion due to their extremely low viscosity. A 60:40 PEO:PETA was chosen for examination of the impact of UV exposure time (20 – 120 minutes) due to the balance of crosslink density (gel fraction) and water uptake (swelling ratio) (**Figure S6c-d**). As expected, the gel fraction increased and water uptake decreased with increasing reaction time due to the higher crosslink density. To balance adequate crosslinking with appropriate water uptake, 30 minutes was chosen as the optimal UV exposure time for 60:40 PEO:PETA mixture. These conditions were utilized for *in situ* crosslinking of the coextruded matrix fiber composites.

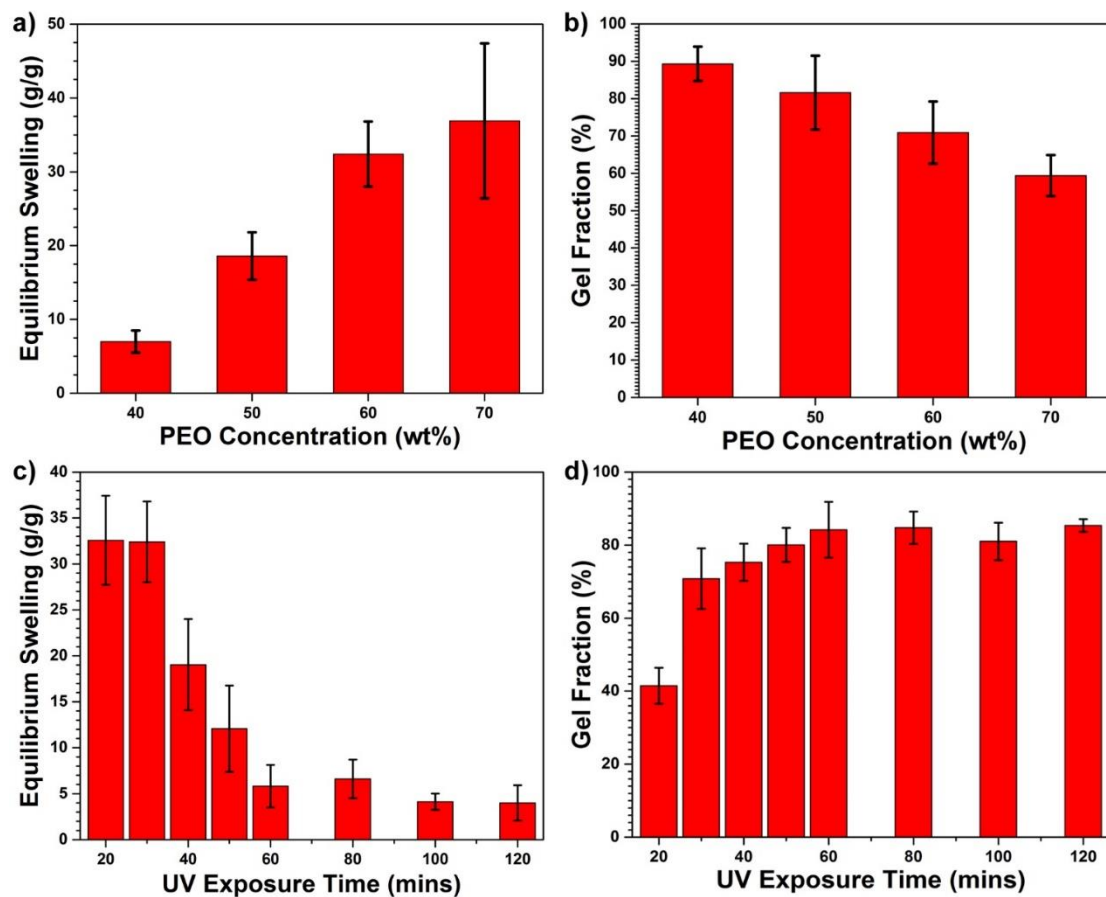


Figure S6. a) Equilibrium swelling ratio and b) gel fraction of PEO/PETA crosslinking as a function of reactant concentration (30 min crosslinking time); c) equilibrium swelling ratio and d) gel fraction of PEO/PETA crosslinking as a function of UV exposure time (60/40 PEO/PETA weight ratio).

Swelling Ratio Calculations. The coextruded fiber weight percent (wt%, W_f) was determined for each PCL/PEO coextruded composite via ^1H NMR (**Figure S2**). Coextruded composites were taken as 2 g samples, and W_f was used for each sample to determine the mass of PEO (M_{PEO}) and mass of fiber (M_F). Crosslinker PETA (M_{PETA}) was added to the dissolved composite mixture in a 60:40 (PEO:PETA) weight ratio for each sample. The fiber weight fraction (A_I) of the xerogel constituents was then determined and related to W_f (**Table S1**).

Table S1. Conversion between W_f and A_1 .							
W_f (%)	Composite Mass (g)	PEO Mass (g)	Fiber Mass (g)	PETA Mass (g)	Total Mass (g)	Swellable Mass (g)	A_1
0.0	2.00	2.00	0.00	1.33	3.33	3.33	0.00
2.6	2.00	1.95	0.05	1.30	3.30	3.25	0.015
6.0	2.00	1.88	0.12	1.25	3.25	3.13	0.037
10.9	2.00	1.78	0.22	1.19	3.19	2.97	0.069
15.3	2.00	1.69	0.31	1.13	3.13	2.82	0.099
22.4	2.00	1.55	0.45	1.03	3.03	2.58	0.149
30.5	2.00	1.39	0.61	0.93	2.93	2.32	0.208

$$A_1 = \frac{\text{Fiber Mass}}{\text{Total Mass}}$$

$$A_1 = \frac{M_f}{M_f + M_{PEO} + M_{PETA}}$$

$$W_f = \frac{M_f}{M_f + M_{PEO}}$$

$$M_f + M_{PEO} = \frac{M_f}{W_f}$$

$$M_{PEO} = \frac{M_f}{W_f} - M_f = M_f \left(\frac{1}{W_f} - 1 \right)$$

$$0.4M_{PEO} = 0.6M_{PETA}$$

$$M_{PETA} = \frac{2M_{PEO}}{3} = \frac{2}{3} \left(M_f \left(\frac{1}{W_f} - 1 \right) \right)$$

$$A_1 = \frac{M_f}{M_f + M_f \left(\frac{1}{W_f} - 1 \right) + \frac{2}{3} \left(M_f \left(\frac{1}{W_f} - 1 \right) \right)}$$

$$A_1 = \frac{1}{1 + \left(\frac{1}{W_f} - 1 \right) + \frac{2}{3} \left(\left(\frac{1}{W_f} - 1 \right) \right)}$$

$$A_1 = \frac{1}{1 + \frac{1}{W_f} - 1 + \frac{2}{3W_f} - \frac{2}{3}}$$

$$A_1 = \frac{1}{\frac{5}{3W_f} - \frac{2}{3}} = \frac{1}{\frac{1}{3} \left(\frac{5}{W_f} - 2 \right)}$$

$$A_1 = \frac{3}{\left(\frac{5}{W_f} - 2 \right)}$$

The equilibrium swelling ratio (S_R) and gel fraction (G_f) were determined based on the water uptake of the PEO/PETA crosslinked matrix. Each sample was weighed individually to determine the initial mass (m_1), the mass after 72 hours of swelling in distilled H₂O (m_2), and the final weight after drying under vacuum for 24 hours (m_3).

$$S_R = \frac{[(m_2 - A_1 m_1) - (m_3 - A_1 m_1)]}{m_3 - A_1 m_1}$$

$$S_R = \frac{m_2 - m_3}{m_3 - A_1 m_1}$$

$$G_f = \frac{m_3 - A_1 m_1}{m_1 - A_1 m_1}$$

$$G_f = \frac{m_3 - A_1 m_1}{m_1(1 - A_1)}$$

The fiber volume fraction (v_f) of the PEO hydrogels derived from coextruded composites was then calculated using the mass ratio and density (ρ_i) as well as water uptake of each individual sample with varying PCL/PEO composition (**Table S2**) and various fiber types (**Table S3**).

$$v_f = \frac{\frac{A_1 m_1}{\rho_f}}{\frac{A_1 m_1}{\rho_f} + \frac{0.6 m_1 (1 - A_1)}{\rho_{PEO}} + \frac{0.4 m_1 (1 - A_1)}{\rho_{PETA}} + \frac{m_2 - m_3}{\rho_{H_2O}}}$$

$$S_R(m_3 - A_1 m_1) = m_2 - m_3$$

$$G_f(m_1(1 - A_1)) = m_3 - A_1 m_1$$

$$m_2 - m_3 = S_R(G_f(m_1(1 - A_1)))$$

$$v_f = \frac{\frac{A_1 m_1}{\rho_f}}{\frac{A_1 m_1}{\rho_f} + \frac{0.6 m_1 (1 - A_1)}{\rho_{PEO}} + \frac{0.4 m_1 (1 - A_1)}{\rho_{PETA}} + \frac{S_R(G_f(m_1(1 - A_1)))}{\rho_{H_2O}}}$$

$$v_f = \frac{\frac{A_1}{\rho_f}}{\frac{A_1}{\rho_f} + \frac{0.6(1 - A_1)}{\rho_{PEO}} + \frac{0.4(1 - A_1)}{\rho_{PETA}} + \frac{S_R G_f(1 - A_1)}{\rho_{H_2O}}}$$

Table S2. Determination of S_R , X_D , and v_f for PEO hydrogels derived from PCL/PEO composites of varying PCL composition.				
W_f	A_1	S_R (g H ₂ O/g Gel)	G_f	v_f (%)
0.000	0.000	32.4 ± 4.4	0.71 ± 0.08	0.00
0.026	0.015	22.7 ± 2.7	0.68 ± 0.08	0.1 ± 0.02
0.060	0.037	9.0 ± 2.3	0.69 ± 0.06	0.4 ± 0.1
0.109	0.069	6.8 ± 1.8	0.73 ± 0.07	1.2 ± 0.3
0.153	0.099	4.7 ± 1.3	0.66 ± 0.07	2.4 ± 0.5
0.224	0.149	3.0 ± 1.7	0.75 ± 0.08	4.7 ± 0.8
0.305	0.208	2.6 ± 1.6	0.74 ± 0.06	7.8 ± 1.5

Table S3. Determination of S_R , G_f and v_f for PEO hydrogels derived from PCL/PEO composites of varying PCL composition.					
<i>Fiber Type</i>	W_f	A_1	S_R (g H ₂ O/g Gel)	G_f	V_f (%)
None	0.000	0.000	32.4 ± 4.4	0.71 ± 0.08	0.0
Undrawn PCL	0.305	0.208	2.6 ± 1.6	0.74 ± 0.06	7.8 ± 1.5
Drawn PCL	0.305	0.208	2.9 ± 1.1	0.72 ± 0.04	7.2 ± 1.3
Undrawn PLLA	0.344	0.239	3.0 ± 0.7	0.69 ± 0.05	7.5 ± 1.6

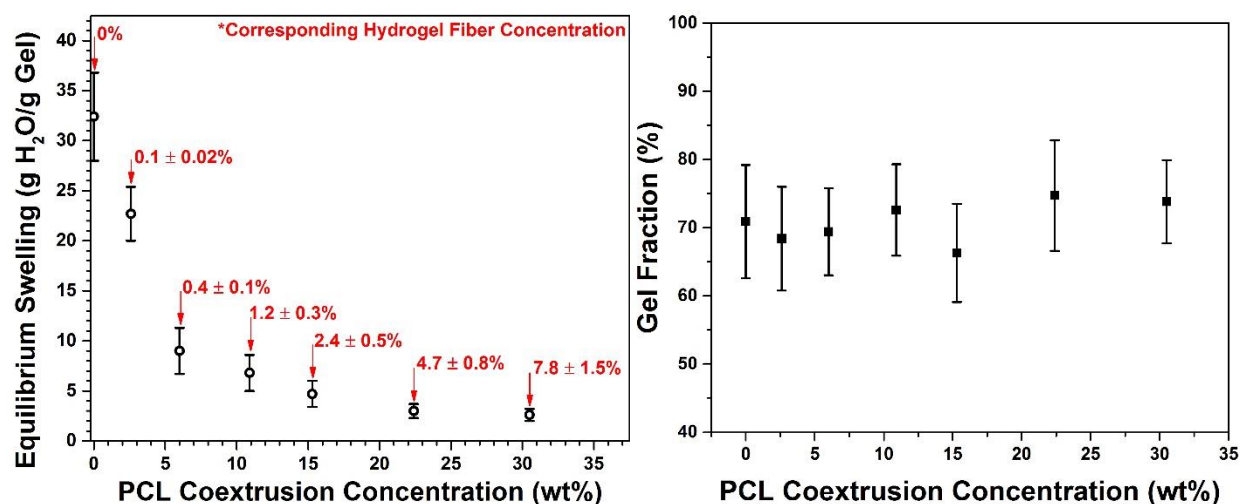


Figure S7. Equilibrium swelling and gel fractions of PCL fiber-reinforced PEO hydrogels at varying fiber content, showing an average crosslink density of $\sim 70\%$.

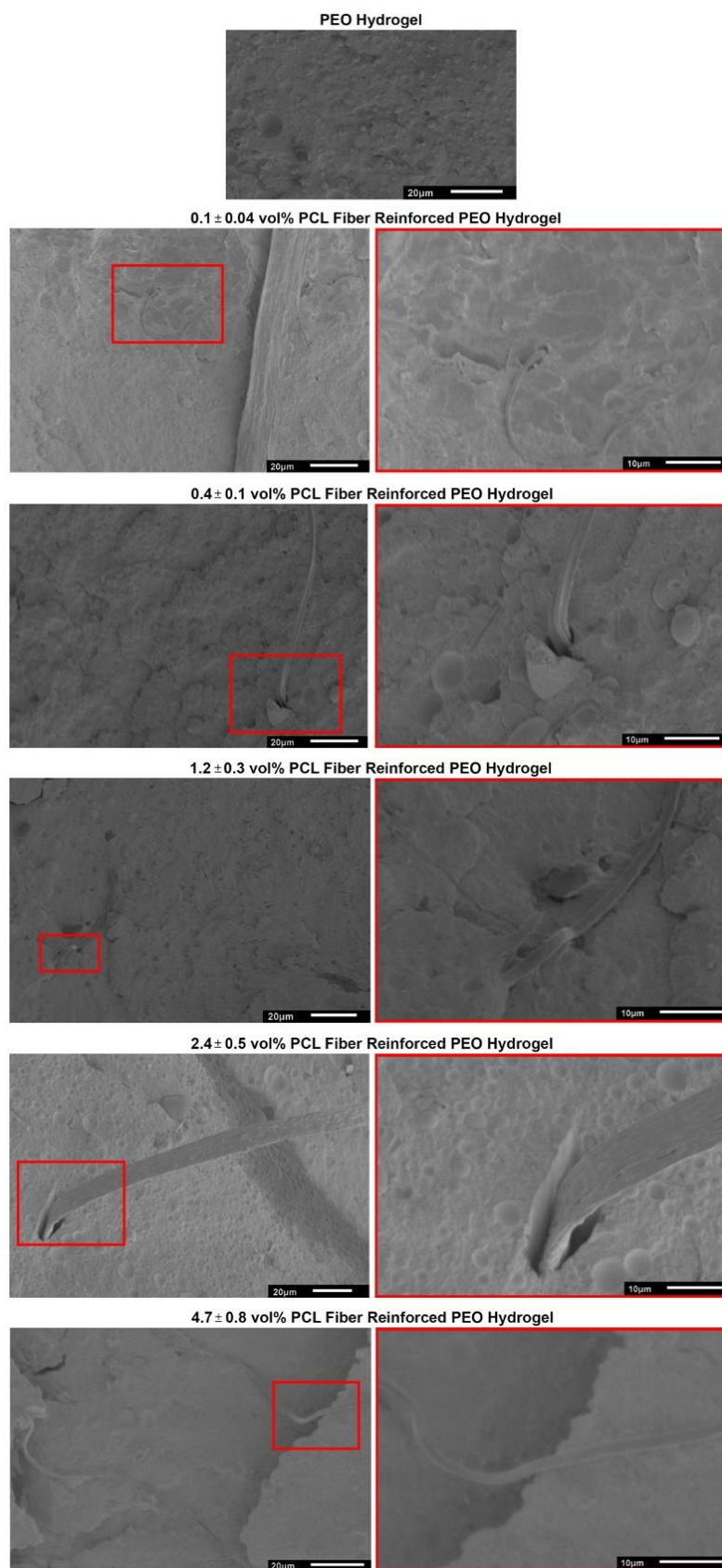


Figure S8. Cross-sectional SEM of fiber reinforced PEO hydrogels of varying PCL fiber content.

Stability determination. The stability of each hydrogel sample was determined via cyclic compression testing over 20 loading/unloading cycles (**Figure S9a**) by measuring the stress (σ) response to compressive strain (ε). Internal friction (F_I) was determined for each loading/unloading cycle and plotted as a function of cycle number (**Figure S9b**).

$$F_I = \int_0^{25} \sigma(\varepsilon)_{loading} \delta\varepsilon - \int_{25}^0 \sigma(\varepsilon)_{unloading} \delta\varepsilon$$

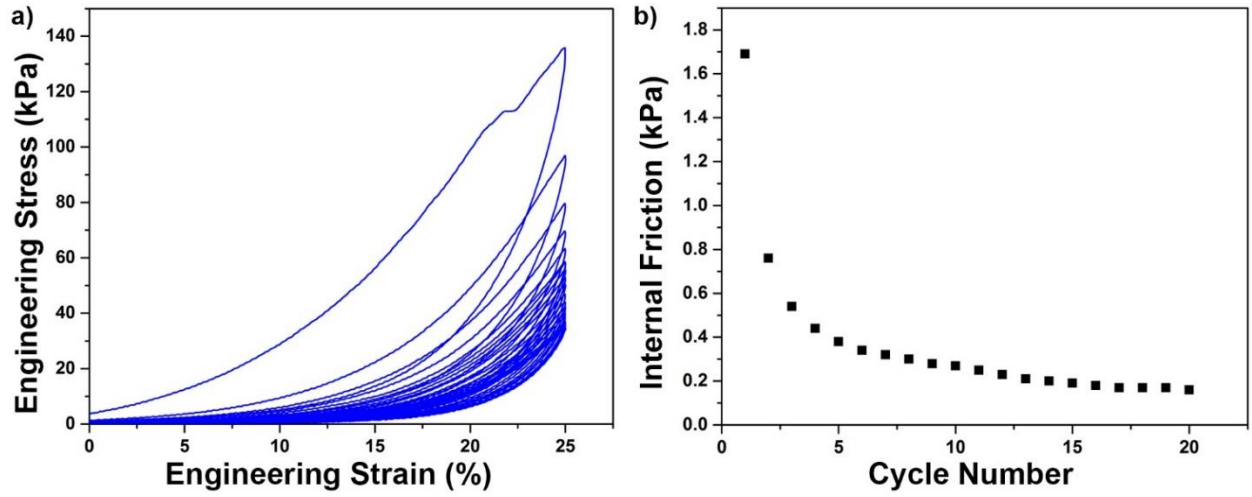


Figure S9. a) Cyclic loading/unloading response to compressive strain; b) F_I plotted as a function of cycle number.

The process was repeated for minimum of 5 samples ($n=5$) (**Figure 10a**), and F_I was averaged for each compressive cycle (**Figure S10b**).

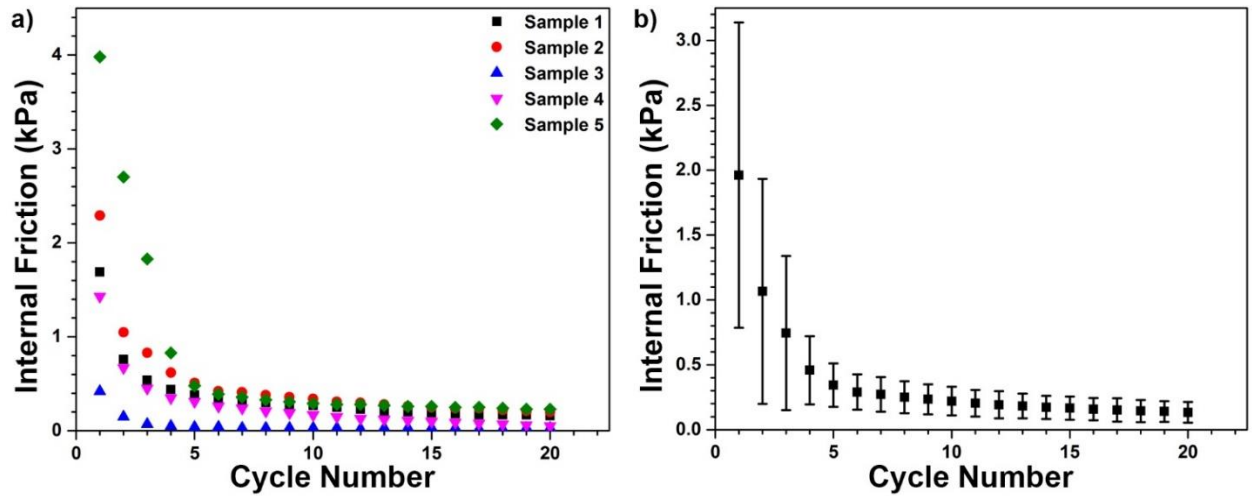


Figure S10. a) F_I plotted as a function of cycle number for 5 independent PEO hydrogel samples containing similar PCL fiber loading; b) averaged F_I values for the 5 independent samples.

However, due to the large variation in the initial F_I values, it was more useful to determine a normalized internal friction (e.g. stability) (**Figure S11a**), which can then be averaged for each compressive cycle (**Figure S11b**). Each value of F_I was divided by the F_I value of the 1st compressive loading cycle for that respective sample to obtain the normalized F_I plots. This normalization approach allowed comparison of the relative stability between samples of varying fiber loading and type (**Figure S12**).

$$Stability = \frac{F_{I,i}}{F_{I,1}} = \frac{\left(\int_0^{25} \sigma(\varepsilon)_{loading} \delta\varepsilon - \int_{25}^0 \sigma(\varepsilon)_{unloading} \delta\varepsilon \right)_i}{\left(\int_0^{25} \sigma(\varepsilon)_{loading} \delta\varepsilon - \int_{25}^0 \sigma(\varepsilon)_{unloading} \delta\varepsilon \right)_1}$$

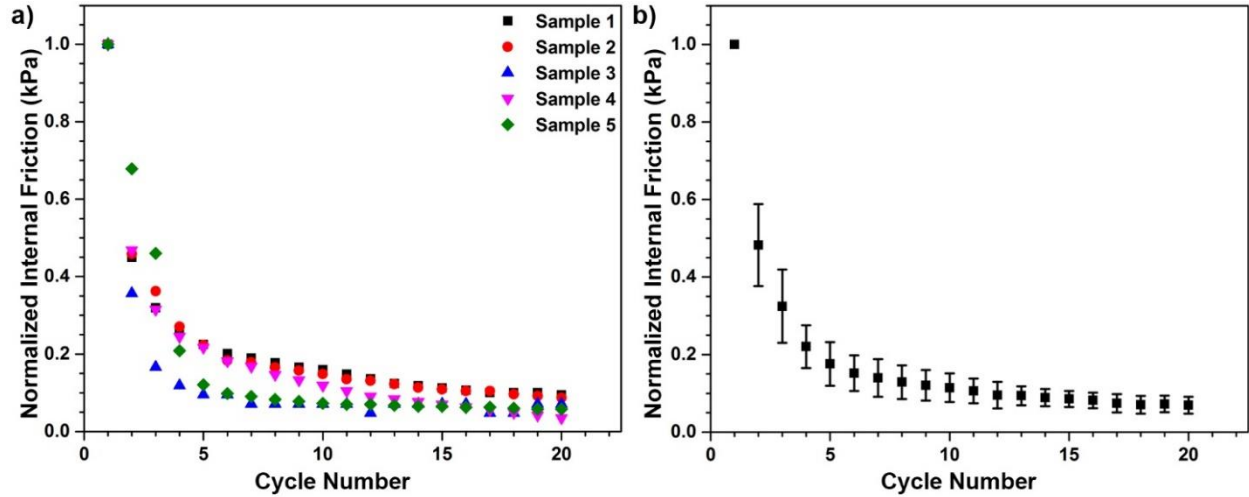


Figure S11. a) Normalized F_I values for each of the 5 independent PEO hydrogel samples; b) averaged normalized F_I values (stability) for the PEO hydrogel.

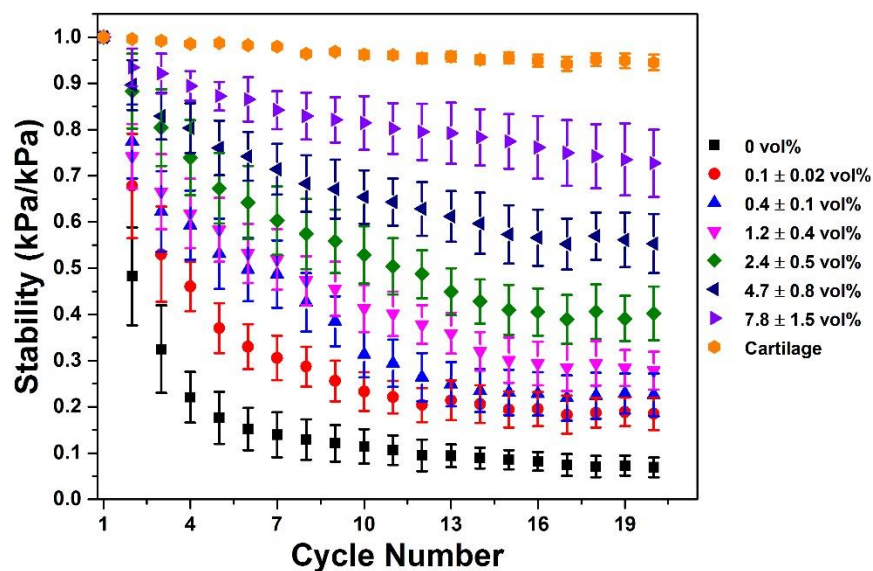


Figure S12. Stability as a function of cycle number for PEO hydrogels of varying PCL fiber content and porcine articular cartilage.

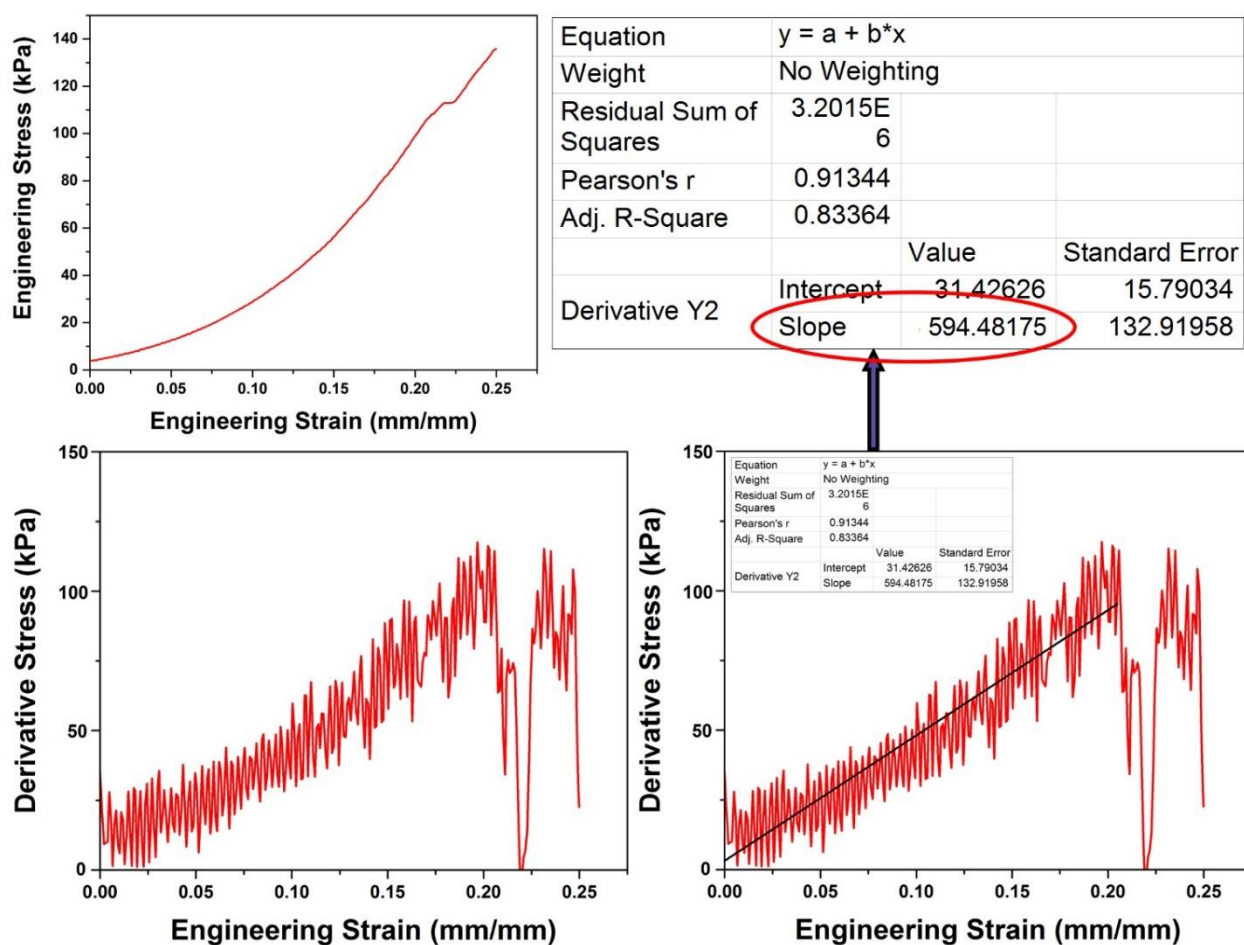


Figure S13. Sample calculation to determine the stiffness of a single hydrogel sample with a stiffness of 594 kPa using the slope of the linear derivative of the compressive loading curve.

Hydrogel modeling. Initially, a simple volume additive model was fit to the experimentally determined stiffness values for PEO hydrogels of varying fiber loading and type (**Table S4**).

Table S4. Experimentally determined stiffness (K_c) and Stability values for PEO hydrogels with varying fiber loading and porcine articular cartilage.		
v_f (%)	K_c (MPa)	<i>Stability</i> (%)
0	0.69 ± 0.04	6.8 ± 2.1
0.1 ± 0.02	0.66 ± 0.12	18.5 ± 3.5
0.4 ± 0.1	0.72 ± 0.11	22.7 ± 4.6
1.2 ± 0.3	0.81 ± 0.09	27.8 ± 4.1
2.4 ± 0.5	0.99 ± 0.14	40.2 ± 5.8
4.7 ± 0.8	1.42 ± 0.16	55.3 ± 6.4
7.8 ± 1.5	1.94 ± 0.21	72.7 ± 7.3
Porcine Articular Cartilage	1.61 ± 0.17	94.5 ± 1.7

Compressive stiffness (K_f) of each fiber type (undrawn PCL, drawn PCL, and undrawn PLLA) fiber mats was determined (**Figure S14a**) along with the stiffness of the PEO hydrogel matrix (K_m) and used to determine the expected stiffness ($K_{c,volume}$) for comparison with the experimentally obtained stiffness (K_c).

$$K_{c,volume} = v_f K_f + (1 - v_f) K_m$$

The volume additive model did not adequately predict the reinforcing effect of the fabricated fiber-reinforced hydrogels, which suggested a more complex phenomena (**Figure S14b,c**). A tensile reinforcement approach based on the Halpin-Tsai model was applied to characterize the mechanics of these fiber-reinforced hydrogels. Values for the tensile modulus of each fiber phase as well the PEO hydrogel matrix were determined (**Figure S15**).

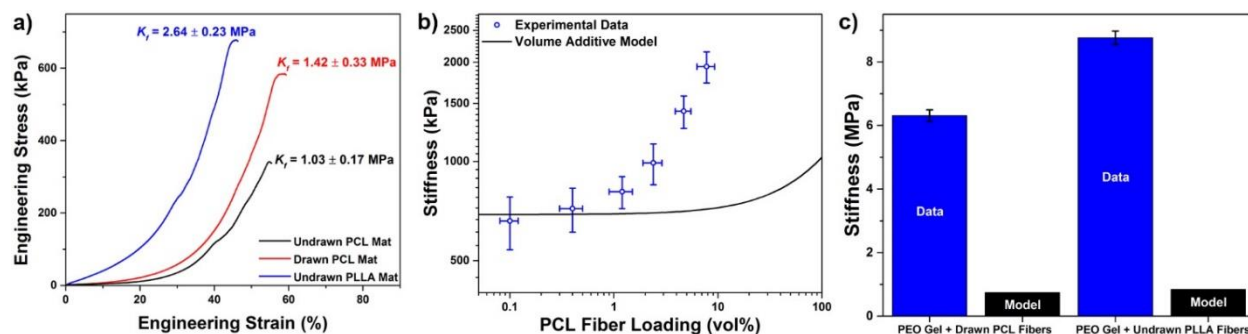


Figure S14. a) Representative compressive loading profiles from undrawn PCL, drawn PCL, and undrawn PLLA coextruded fiber mats; b) volume-additive model using the stiffness of PCL fibers (K_f) and PEO hydrogel matrix (K_m) along with stiffness data obtained at various PCL fiber loadings; c) volume-additive model and stiffness data of fiber-reinforced PEO hydrogels using drawn PCL and undrawn PLLA fibers.

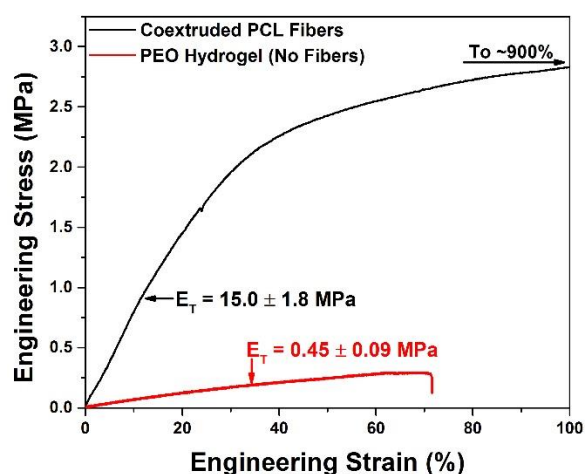


Figure S15. Tensile stress-strain response to uniaxial deformation of coextruded PCL fibers and control PEO hydrogel to determine elastic tensile moduli (E_T) for the Halpin-Tsai model.

Effective Aspect Ratio. SEM imaging revealed that individual fibers were bent and twisted within the hydrogel volume, suggesting an effective aspect (A_{eff}) ratio much lower than the theoretically calculated ideal aspect ratio (**Figure S16**).

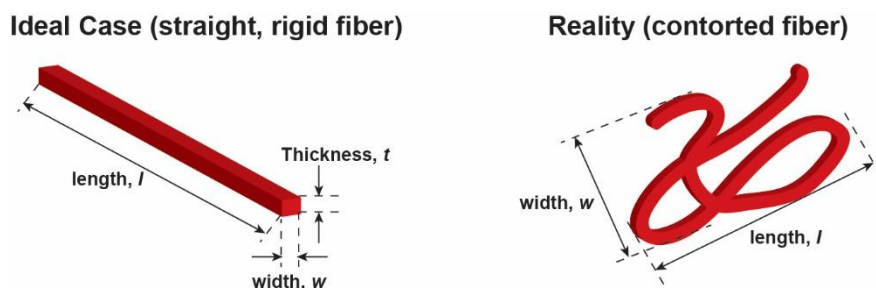


Figure S16. Pictorial representation of an ideal straight, rigid fiber element and a contorted fiber element with decreased effective aspect ratio.

To obtain a relative idea of fiber curvature, the theoretical aspect ratio (A_{theor}) was calculated based on the nominal dimensions of the individual fibers upon exiting the extruder. The highest A_{theor} and lowest A_{theor} were calculated based on nominal fiber width (w) and thickness (t) and a composite cut length (l) of 5 mm (**Table S5**).

$$A_{theor} = \frac{l}{\frac{w + t}{2}}$$

Table S5. Range of theoretical fiber aspect ratios.			
Relative Feed Rates	Thickness, t (μm)	Width, w (μm)	A_{theor}
PCL ₁ PEO ₂₉	1.6 ± 0.4	2.6 ± 0.6	2,380
PCL ₁₀ PEO ₂₀	6.1 ± 1.2	9.3 ± 1.7	650

As shown in **Table S5**, the theoretical aspect ratios of coextruded PCL fibers ranged from 650-2,380. However, it was possible to calculate an A_{eff} using a mechanical percolation model with stiffness data obtained in accordance with the depictions of **Figures S8, S16**, where the critical percolation threshold (v_c) is related to A_{eff} . The percolation exponent (b) and v_c can both be determined by fitting the percolation model to the experimentally determined stiffness data using a custom curve fitting program. The percolation exponent was determined to be 1.712, while v_c was determined to be 0.74% with an R^2 value of 0.942, indicating a very good fit of the percolation model to the experimental data (**Figure S17**). An A_{eff} of 95 was determined, which was below the theoretical aspect ratio range and suggested a high degree of fiber contortion in the undrawn PCL fiber reinforced PEO hydrogels derived from coextruded composites.

$$\begin{aligned}
 P(F) &= 0 & \text{for } v_f < v_c \\
 P(F) &= \left(\frac{v_f - v_c}{1 - v_c} \right)^b & \text{for } v_f > v_c \\
 K_c &= \frac{K_m K_f (1 - 2P(F) + P(F)v_f) + K_f^2 P(F)(1 - v_f)}{K_f (1 - v_f) + K_m (v_f - P(F))} \\
 A_{eff} &= \frac{0.7}{v_c}
 \end{aligned}$$

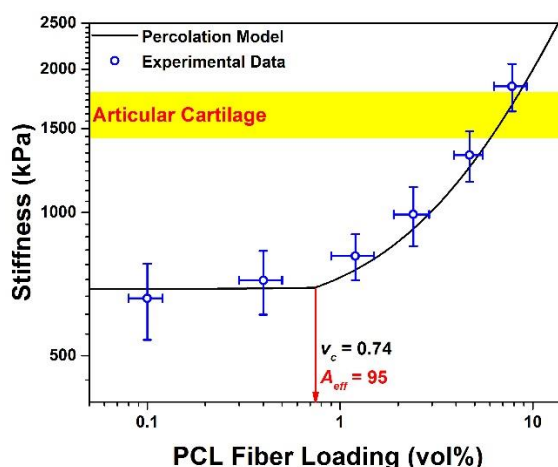


Figure S17. Determination of effective aspect ratio by fitting experimentally obtained data to the percolation model.

Fiber Drawing. Previously, uniaxial drawing of solvated PCL/PEO composites to a draw ratio of 11 ($DR = 11$) resulted in a 30-fold increase in PCL fiber tensile modulus after complete PEO removal. With this framework, the coextruded composites containing PCL fibrous domains surrounded by PEO matrix were uniaxially drawn prior to the *in situ* hydrogel fabrication procedure. Due to the brittle nature of PEO, only composites containing the highest elastic PCL loading (~ 30.5 wt%) could be uniaxially drawn without immediate fracture. At this PCL content, these extruded composites could be extended to $DR = 6$ reliably before failure (**Figure S18a**). After uniaxial drawing, both PEO and PCL chains are oriented as revealed by two-dimensional wide angle X-ray scattering (2D WAXS) (**Figure S18b,c**). However, since PEO was solvated and dissolved in subsequent steps and any chain orientation would be lost, it was not meaningful to determine an orientation parameter for the PEO matrix. Chain orientation within PCL fibers was quantified using the Hermans orientation factor (f_H). Values of f_H range between -0.5 for crystallites with the c-axis oriented perpendicular to the fiber axis to 1.0 for crystallites with the c-axis oriented parallel to the fiber axis; a value of 0.0 indicates a random crystallite alignment. The value $f_H = 0.83$ for PCL indicated a high degree of preferential chain orientation along the fiber axis. Removal of the PEO matrix from the drawn composites produced a PCL fiber mat with lateral

fiber distributions of $4.1 \pm 0.5 \mu\text{m}$ (width) and $3.6 \pm 0.3 \mu\text{m}$ (thickness) (**Figure S19**), which represents a 56% decrease in lateral dimensions from the undrawn PCL fibers. The tensile modulus of the drawn PCL fibers increased by $\sim 820\%$ to $138 \pm 27 \text{ MPa}$ (**Figure 20**), which was anticipated to have a significant impact on hydrogel stiffness.

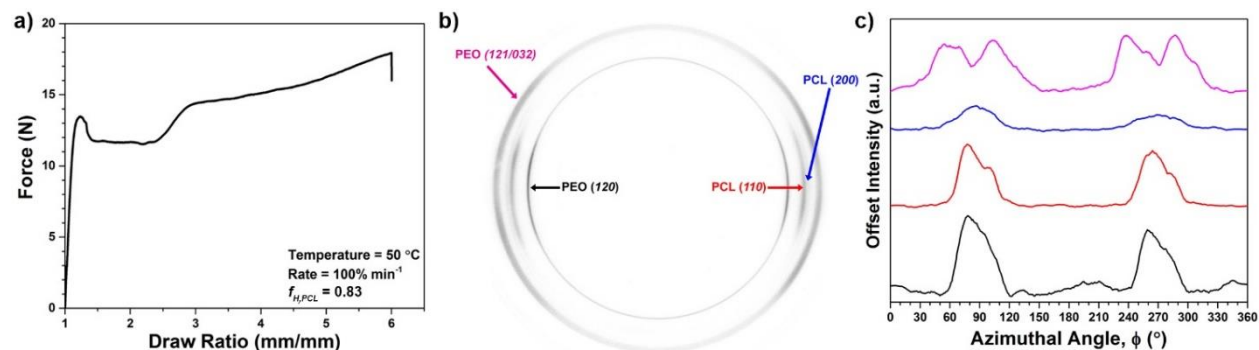


Figure S18. Uniaxial drawing of PCL/PEO coextruded composites.

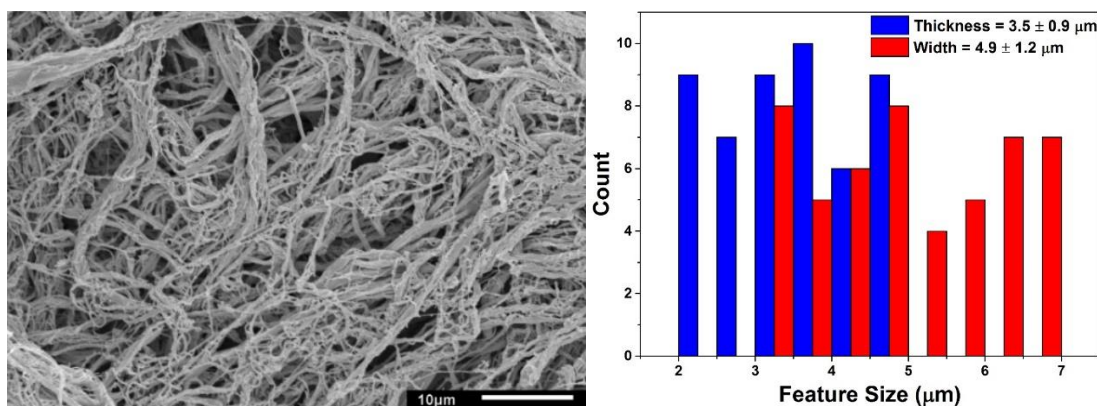


Figure S19. SEM lateral fiber size distribution of PCL fibers drawn to DR = 6.

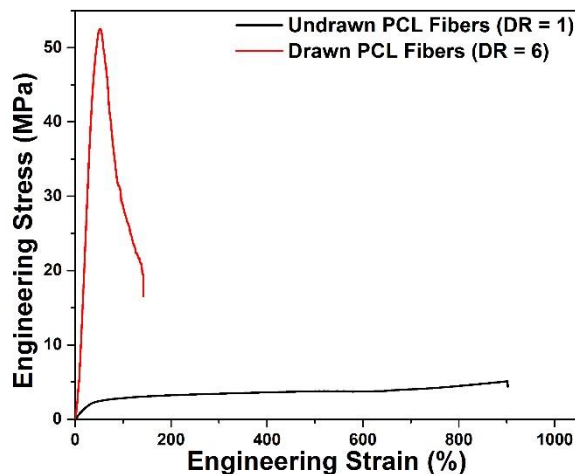


Figure S20. Tensile response of undrawn (DR = 1) and drawn (DR = 6) PCL fibers.

The void fraction of each drawn PCL fiber mat sample for tensile testing was determined by obtaining the apparent mat density and comparing to a continuous PCL film (1.145 g/cm³) (Table S6).

Table S6. Void fraction determination of drawn PCL fiber mats for tensile testing.						
	Length (mm)	Width (mm)	Thickness (mm)	Mass (mg)	Apparent Density (g/cm ³)	Void Fraction
Sample 1	18.54	6.74	0.458	38.9	0.679	0.41
Sample 2	16.37	7.32	0.437	37.3	0.713	0.38
Sample 3	18.43	6.23	0.467	35.8	0.667	0.42
Sample 4	17.27	6.09	0.492	37.5	0.725	0.37
Sample 5	16.98	7.13	0.428	36.4	0.702	0.39

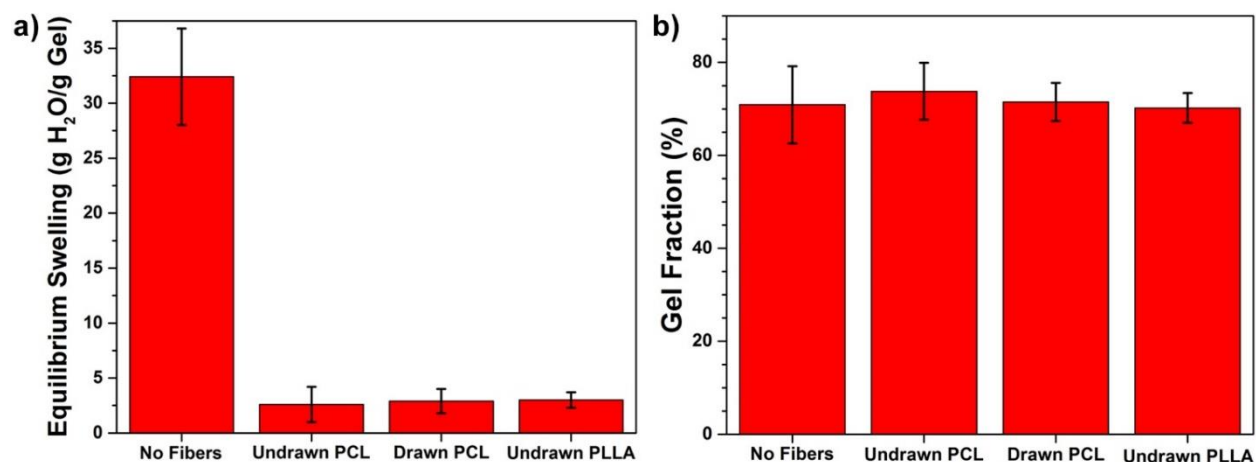


Figure S21. a) Equilibrium swelling ratio and b) gel fraction of control PEO hydrogel and fiber reinforced hydrogels containing undrawn PCL fibers, drawn PCL fibers, and undrawn PLLA fibers.

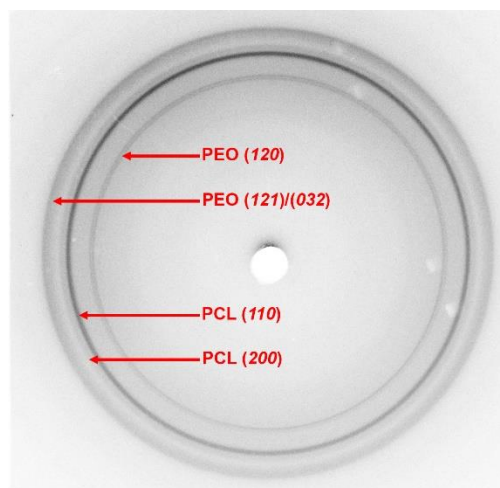


Figure S22. 2D WAXS profile of PCL fiber-reinforced PEO hydrogel displaying isotropic reflections of PEO and PCL, which indicated a random distribution of PCL fibers throughout the hydrogel volume.

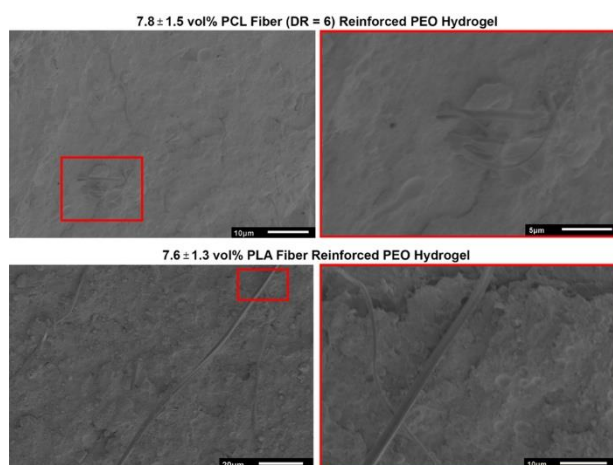


Figure S23. SEM micrographs of PEO hydrogels derived from drawn PCL/PEO composites and undrawn PLLA/PEO composites.

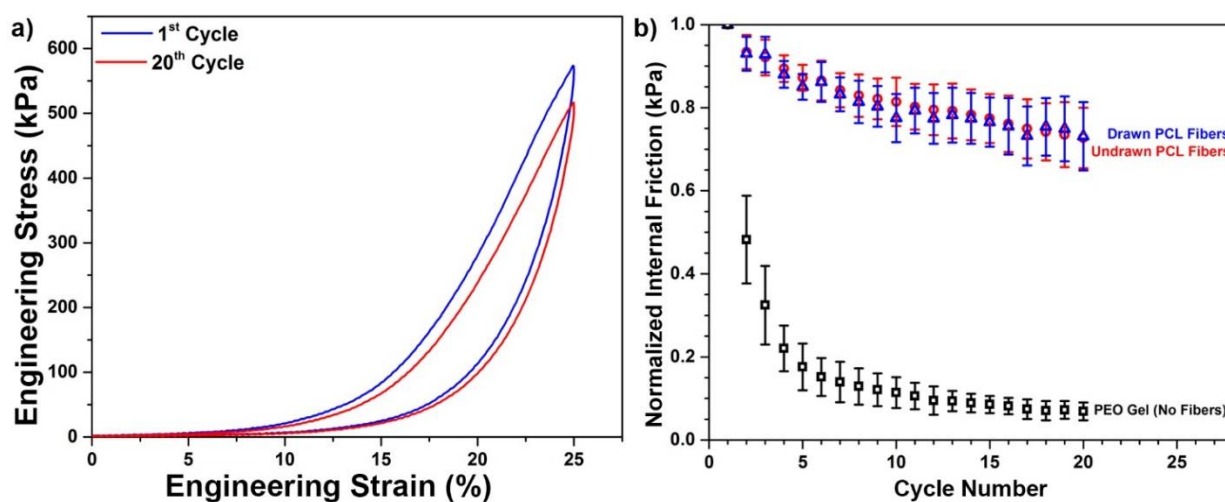


Figure S24. a) Compressive hysteresis loop for PEO hydrogels derived from drawn PCL/PEO composites; b) stability of hydrogels plotted as a function of cycle number.

PLLA washing efficiency. The integral area of “peak a” (**yellow, Figure S25c**) in the ^1H NMR is set to 1.00 to determine the ratio of the area included in “peak c” (**brown, Figure S25c**) and “peak a”. Peak *c* represents three protons in each PLLA repeat unit, and peak *a* represents four protons in each PEO repeat unit. This integral ratio can be utilized to determine the relative mole fraction of each repeat unit present.

$$\left(\frac{\frac{a \text{ mol PEO}}{4}}{\frac{c \text{ mol PLLA}}{3}} \right) = \frac{3a}{4c} \frac{\text{mol PEO}}{\text{mol PLLA}}$$

After determining the molar ratio of PEO to PLLA, we converted the molar ratio to a weight ratio using the molar mass of a single PEO repeat unit (44.05 g mol⁻¹) and a single PLLA repeat unit (72.06 g mol⁻¹).

$$\left(\frac{3a}{4c} \frac{\text{mol PEO}}{\text{mol PLLA}} \right) \left(\frac{1 \text{ mol PLLA}}{72.06 \text{ g PLLA}} \right) \left(\frac{44.05 \text{ g PEO}}{1 \text{ mol PEO}} \right) = 0.458 \left(\frac{a}{c} \right) \frac{\text{g PEO}}{\text{g PLLA}}$$

To perform the integrations, the area for “*peak a*” was set to 1.00, simplifying this relationship to include only one variable (*c*).

$$0.458 \left(\frac{a}{c} \right) \frac{\text{g PEO}}{\text{g PLLA}} = 0.458 \left(\frac{1}{c} \right) \frac{\text{g PEO}}{\text{g PLLA}} = \left(\frac{0.458}{c} \right) \frac{\text{g PEO}}{\text{g PLLA}}$$

We assumed a 1 g PLLA basis, and then found the corresponding weight of PEO that would result from the given peak integration ratio.

$$\left(\frac{0.458}{c} \frac{\text{g PEO}}{\text{g PLLA}} \right) (1 \text{ g PLLA}) = \frac{0.458}{c} \text{ g PEO}$$

The weight percent (W_f) of PLLA in the composite was then determined using the 1 g PLLA value. This 1 g PLLA basis was divided by the total weight determined through the integration ratio from ^1H NMR.

$$W_f = \frac{1 \text{ g PLLA}}{1 \text{ g PLLA} + \frac{0.458}{c} \text{ g PLLA}} = \frac{1 \text{ g PLLA}}{\left(1 + \frac{0.458}{c}\right) \text{ g Total}}$$

This equation simplifies to:

$$W_f = \frac{1}{1 + \frac{0.458}{c}}$$

$$W_f = \frac{1}{\frac{c + 0.458}{c}}$$

$$W_f = \frac{c}{c + 0.458}$$

The peak integrations, along with the corresponding PLLA wt%, are given in **Table S7**.

Table S7. PLA weight fraction in coextruded PLLA/PEO composites and isolated PLLA fiber mats.				
	Peak “a”	Peak “b”	Peak “c”	PLLA wt%
Coextruded Composite	1.00	0.09	0.24	34.4
Washed PLA Fiber Mat	1.00	16.67	48.21	99.1

The void fraction of each PLLA fiber mat sample for tensile testing was determined by determining the apparent mat density and comparing to a continuous PLLA film (1.21 g/cm³) (**Table S8**).

Table S8. Determination of void fraction of coextruded PLLA fiber mats for tensile testing to determine tensile modulus.						
	Length (mm)	Width (mm)	Thickness (mm)	Mass (mg)	Apparent Density (g/cm³)	Void Fraction
Sample 1	13.05	4.87	0.344	9.8	0.448	0.63
Sample 2	12.17	5.63	0.129	3.3	0.375	0.69
Sample 3	14.92	5.03	0.286	10.9	0.508	0.58
Sample 4	14.74	5.74	0.294	9.6	0.387	0.68
Sample 5	13.74	5.28	0.195	6.7	0.472	0.61

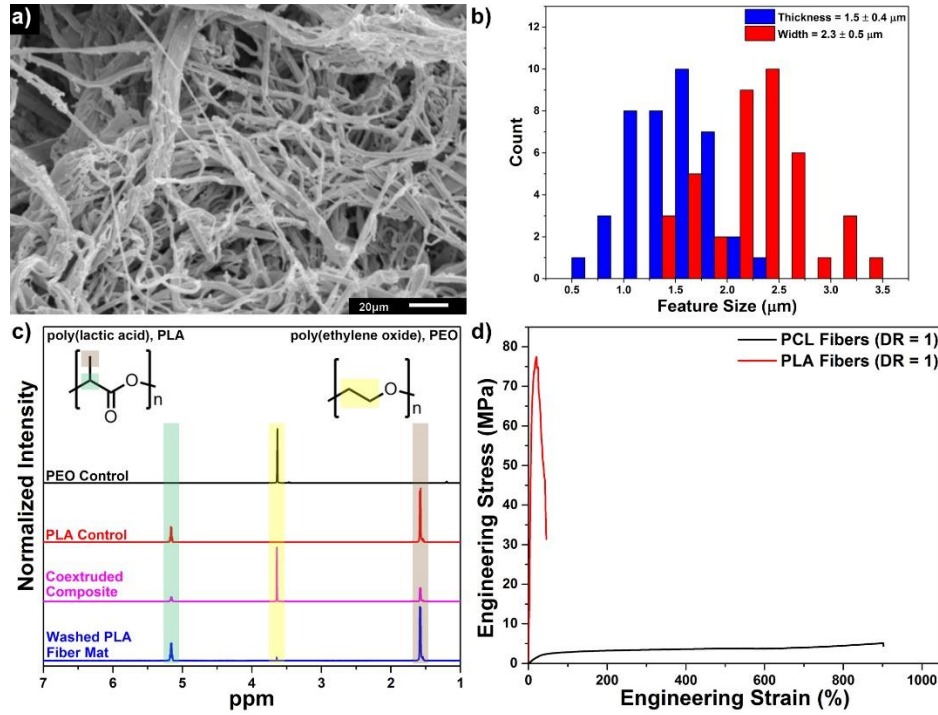


Figure S25. a) SEM micrograph of isolated coextruded PLLA fibers; b) lateral fiber size distribution of isolated coextruded PLLA fibers; c) ^1H NMR spectra of PEO, PLLA, coextruded PLLA/PEO composites and an isolated PLLA fiber mat; d) tensile stress-strain response of undrawn PCL and PLLA fiber mats.

PLLA chain orientation. PLLA chain orientation within coextruded PLLA/PEO composites and isolated PLLA fiber mats was determined using the orthorhombic unit cell dimensions ($a=1.05$ nm, $b=0.61$ nm, $c=2.88$ nm, $\alpha=\beta=\gamma=90^\circ$) of PLLA² according to the original method of Wilchinsky et al.^{3,4}

$$\langle \cos^2 \phi \rangle_c = 1 - \frac{[(1 - 2 \sin^2 p_2) \langle \cos^2 \phi \rangle_1] - [(1 - 2 \sin^2 p_1) \langle \cos^2 \phi \rangle_2]}{\sin^2 p_1 - \sin^2 p_2}$$

$$\langle \cos^2 \phi \rangle_c = 1 - \frac{[(1 - 2 \sin^2 p_{200}) \langle \cos^2 \phi \rangle_{110}] - [(1 - 2 \sin^2 p_{110}) \langle \cos^2 \phi \rangle_{200}]}{\sin^2 p_{110} - \sin^2 p_{200}}$$

(110) Crystallographic Plane

$$p_{110} = 90^\circ - \tan^{-1} \left(\frac{(1)(1.05 \text{ nm})}{(1)(0.61 \text{ nm})} \right)$$

$$p_{110} = 90^\circ - \tan^{-1}(1.798)$$

$$p_{110} = 90^\circ - 60.9^\circ = 29.1^\circ$$

(200) Crystallographic Plane

$$p_{200} = 90^\circ - \tan^{-1} \left(\frac{(0)(1.05 \text{ nm})}{(2)(0.61 \text{ nm})} \right)$$

$$p_{200} = 90^\circ - \tan^{-1}(0)$$

$$p_{200} = 90^\circ - 0^\circ = 90^\circ$$

$$\langle \cos^2 \phi \rangle_c = 1 - \frac{[(1 - 2 \sin^2(90^\circ))\langle \cos^2 \phi \rangle_{110}] - [(1 - 2 \sin^2(29.1^\circ))\langle \cos^2 \phi \rangle_{200}]}{\sin^2(29.1^\circ) - \sin^2(90^\circ)}$$

$$\langle \cos^2 \phi \rangle_c = 1 - \frac{[(1 - 2(1))\langle \cos^2 \phi \rangle_{110}] - [(1 - 2(0.236))\langle \cos^2 \phi \rangle_{200}]}{0.236 - 1}$$

$$\langle \cos^2 \phi \rangle_c = 1 - \frac{[(-1)\langle \cos^2 \phi \rangle_{110}] - [(0.528)\langle \cos^2 \phi \rangle_{200}]}{-0.764}$$

$$\langle \cos^2 \phi \rangle_c = 1 - \left(\frac{-1}{-1} \right) \frac{[\langle \cos^2 \phi \rangle_{110} + 0.528\langle \cos^2 \phi \rangle_{200}]}{0.764}$$

$$\langle \cos^2 \phi \rangle_c = 1 - \frac{[\langle \cos^2 \phi \rangle_{110} + 0.528\langle \cos^2 \phi \rangle_{200}]}{0.764}$$

$$\langle \cos^2 \phi \rangle_c = 1 - 1.309\langle \cos^2 \phi \rangle_{110} - 0.691\langle \cos^2 \phi \rangle_{200}$$

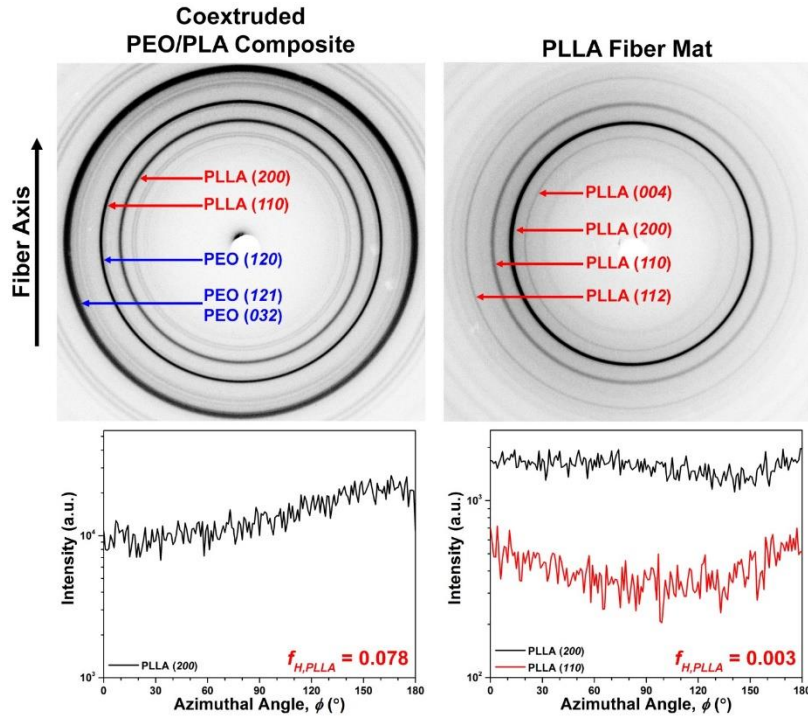


Figure S26. 2D WAXS profiles of PLLA/PEO coextruded composite and PLLA fiber mat.

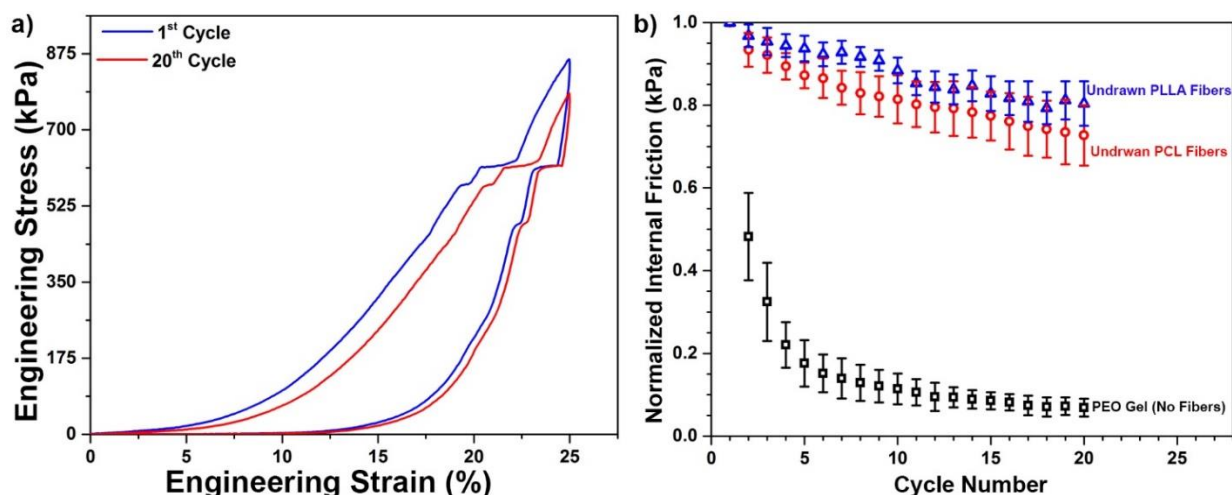


Figure S27. a) Compressive hysteresis loops for PLLA fiber reinforced PEO hydrogel; b) stability plotted as a function of cycle number.

Interestingly, hydrogels derived from undrawn PLLA/PEO composites possessed a very well-defined plateau stress, consistently observed at approximately 650 kPa. This phenomenon did not appear in the control PEO hydrogel, any of the hydrogels derived from PCL/PEO composites, or the isolated PLLA fiber mat. As the stability between loading cycles was quite similar, this plateau stress was likely not the result of permanent mechanical damage to the hydrogel. Similar behavior has been observed in other material systems and has been attributed to the collapse (during compressive loading) and reopening (during compressive unloading) of lamellar type pores in the material.^{5,6} It was suspected that the pore morphology varied between hydrogels reinforced with PCL and PLLA fibers based on the observed mechanical differences (**Figures 3, S24, and S27**). Swollen PEO hydrogels reinforced with the highest PCL fiber content (7.8 ± 1.5 vol%) and PLLA fibers (7.5 ± 1.6 vol%) were freeze-dried to preserve the swollen pore structure and evaluated via cross-sectional SEM. Hydrogels reinforced with PCL fibers possessed a spherical pore morphology with average pore diameter of 13.9 ± 4.5 μm (**Figure S28a**). Conversely, hydrogels reinforced with PLLA fibers possessed a lamellar pore structure with an average pore width and length of 9.2 ± 3.0 μm and 53.1 ± 28.7 μm , respectively (**Figure S28b**).

The difference in pore structure was attributed to the nature of the reinforcing fiber elements and their impact on the elastic swelling characteristics of the individual hydrogels (i.e. elastic PCL vs. rigid PLLA).

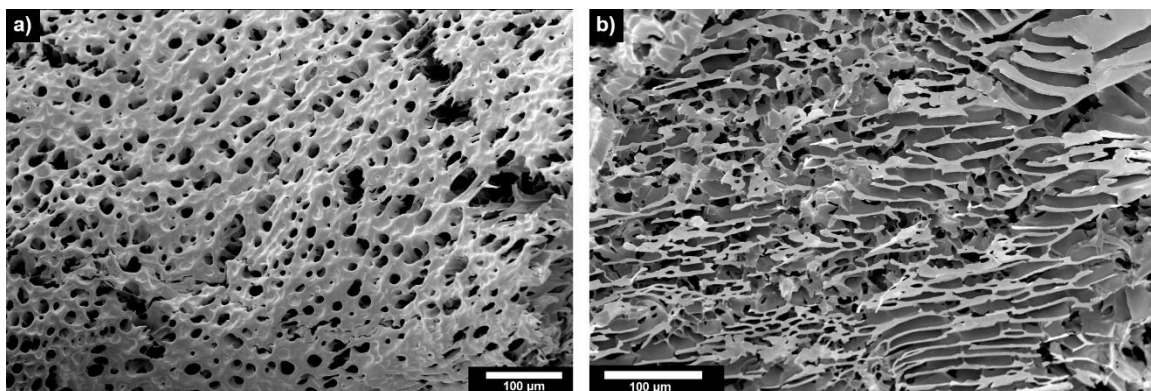


Figure S28. Pore structure of PEO hydrogels reinforced with a) PCL and b) PLLA fibers.

Table S9. Hydrogel stiffness, stability, and storage modulus determined for PEO hydrogels reinforced with undrawn PCL, drawn PCL, and undrawn PLLA fibers.					
Fiber Type	v_f (%)	K_c – Experimental (MPa)	K_c – Model (MPa)	Stability (%)	G' (MPa)
None	0	0.69 ± 0.04	0.69	6.8 ± 2.1	2.5 ± 0.2
PCL (DR = 1)	7.8 ± 1.5	1.94 ± 0.21	1.76	72.7 ± 7.3	3.9 ± 0.3
PCL (DR = 6)	7.2 ± 1.3	6.31 ± 0.18	11.4	73.1 ± 8.2	12.3 ± 1.2
PLLA (DR = 1)	7.5 ± 1.6	8.76 ± 0.21	13.3	80.4 ± 5.4	27.4 ± 1.4

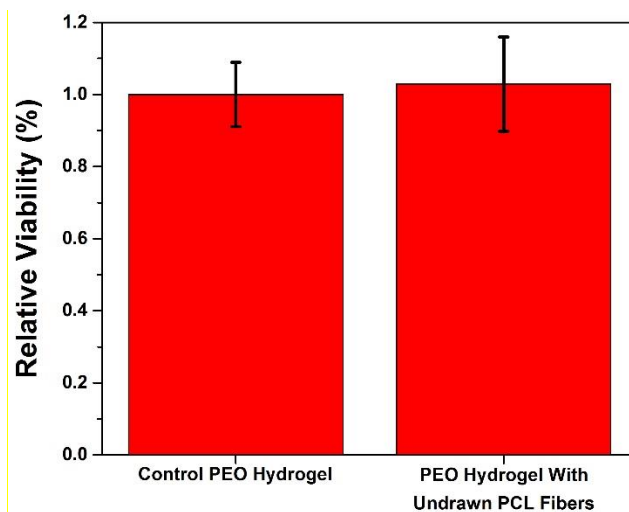


Figure S29. NIH3T3 cell viability for the control PEO hydrogel (normalized to 1.0) and PEO hydrogel containing PCL fibers.

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