

Thermoresponsive, stretchable, biodegradable and biocompatible poly(glycerol sebacate)-based polyurethane hydrogels

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Supporting Information

Determination of the Effective Crosslink Density of PEUs

The polymer volume fraction in the swollen state (ϕ_2) and the polymer volume fraction in the gel in the relaxed state (ϕ_0) were calculated by Equation S1 and S2,^{1,2}

$$\phi_0 = \frac{V_{dry}}{V_{relaxed}} \quad (S1)$$

$$\phi_2 = \frac{V_{dry}}{V_{swollen}} \quad (S2)$$

where V_{dry} , $V_{relaxed}$ and $V_{swollen}$ are the PEU polymer volume fractions in dry, relaxed and swollen state, respectively. The polymer volume fractions of relaxed, swollen and dried PEU polymers (n = 5) were calculated by Equation S3,^{1,2}

$$V_x = \frac{m_{air} - m_{heptane}}{\rho_{heptane}} \quad (S3)$$

where the subscript x refers to the dry, or relaxed or swollen state, respectively. m_{air} is the mass of the corresponding PEU polymer in air at dry, relaxed (after the crosslinking process) or swollen (after 24 h saturation in PBS solution at 37 °C) state, $m_{heptane}$ is the mass of the corresponding PEU polymer in n-heptane at dry, relaxed or swollen state, and $\rho_{heptane}$ is the density of n-heptane (0.7 g ml⁻¹).

Proof-of-concept Preparation of PEU Microspheres

PEU microspheres were prepared by an oil-in-oil solvent evaporation method.³ Briefly, the PEU/solvent mixture (40 mL of the final reacted solution, as described in Section 2.3) was dispersed in pre-heated mineral oil (100 mL at 55 °C) in the presence of Span 80 surfactant (2

mL) and stirred with an overhead stirrer at 400 rpm for 5 h, allowing further crosslinking and solvent evaporation. The microspheres were collected by filtration and washed five times with hexane to remove the mineral oil. Cleaned microspheres were kept in ethanol and stored at 5 °C in a fridge until further use. SEM analysis (Camscan S2) was performed on dry and gold coated (Emscope SC500) microspheres at 5 kV. The sizes of the dry microspheres were evaluated by using ImageJ software (n = 170). Only fully defined microspheres were considered for geometrical measurements.

Proof-of-concept Preparation of PEU scaffolds

PEU-1450 scaffolds were prepared based on our previously established method.^{4,5} Briefly, the PEU/solvent mixture (40 mL of the final reacted solution, as described in Section 2.3) was cast into a non-sticky Teflon-coated metal baking tray (cylindrical cavities; diameter: 60 mm; purchased from a local store) and placed in a freeze-dryer (FreeZone Triad Freeze Dry System, Labconco Co., USA) for lyophilisation. The solutions were cooled during the freezing stage to -30 °C and held at the temperature for 5 h, allowing the solutions to freeze completely. During the primary drying stage the solutions were heated to -5 °C (heating rate of 1 °C min⁻¹) and sublimated for 10 h under vacuum. In the secondary drying stage, the temperature was raised to 20 °C (heating rate of 5 °C min⁻¹) and kept for 5 h. SEM analysis (Camscan S2) was performed on dry and gold coated (Emscope SC500) scaffold at 5 kV. The pore sizes of the dry scaffold microstructures were calculated by using ImageJ software (n = 315). Only fully defined pores were considered for geometrical measurements.

Supporting Figures

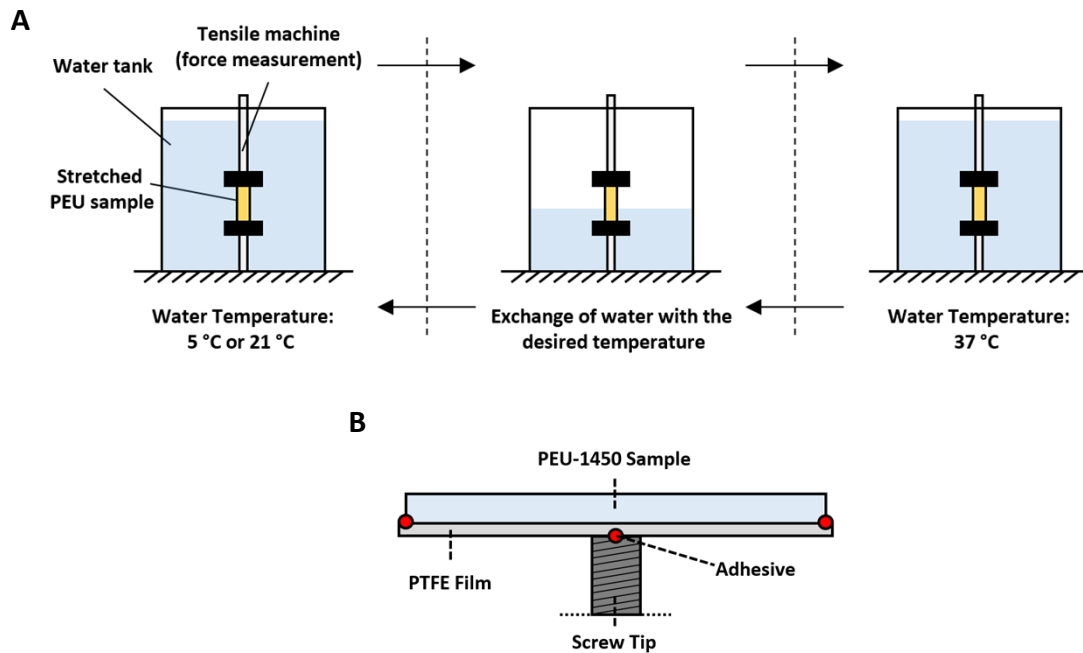


Figure S1. (A) Schematic illustration of the water-temperature activated force generation measurements. The water temperature was alternated from 5 °C to 37 °C, or from 21 °C to 37 °C, with an interval of 10 min. The change of water temperature was achieved rapidly by exchanging the same volume of water with the desired temperature. (B) Schematic illustration of the cantilever stripe test setup.

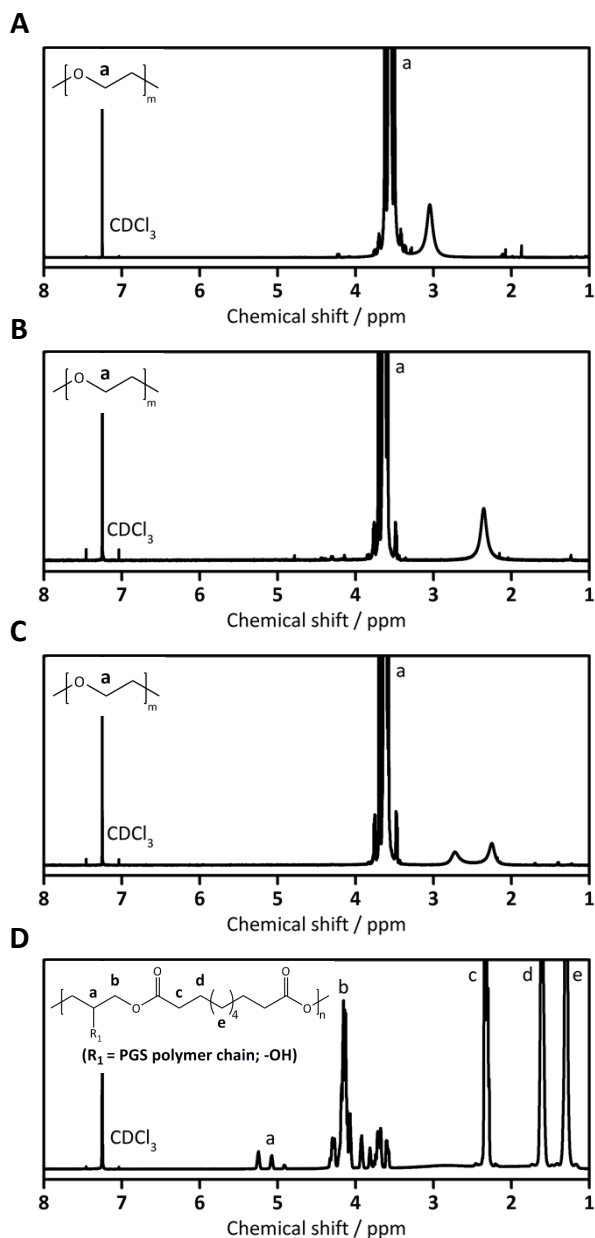


Figure S2. ^1H NMR spectra of (A) PEG-400, (B) PEG-1000, (C) PEG-1450 and (D) pre-PGS. (A-C) Methylene protons of PEG were assigned at 3.6 ppm (position “a”). (D) Methylene protons of glycerol were assigned at 4.05-4.35 ppm and 4.92-5.24 ppm (position “a” and “b”), while sebacic acid related methylene protons were assigned at 1.29, 1.61 and 2.32 ppm (position “c”, “d” and “e”).

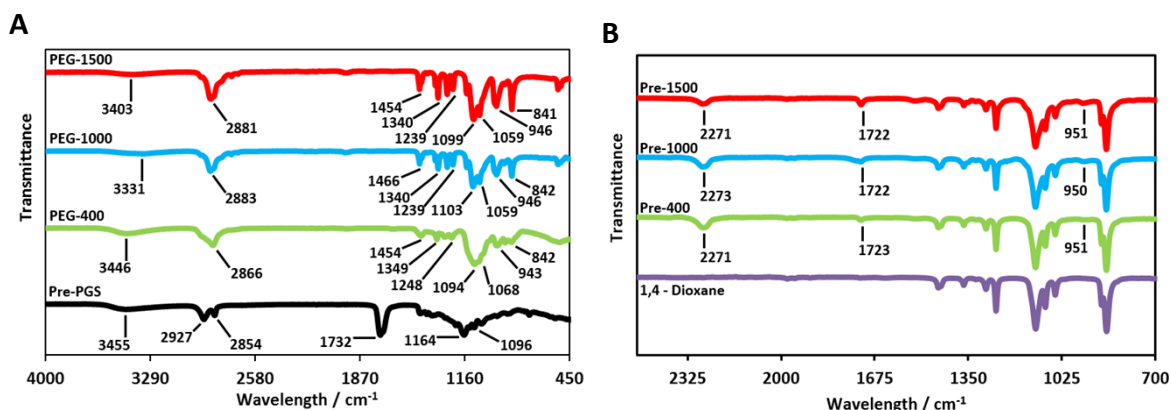


Figure S3. (A) ATR-FTIR spectra of pre-PGS, PEG-400, PEG-1000 and PEG-1450. Briefly, pre-PGS was characterized by the stretching vibration of -OH at 3455 cm^{-1} , C-H at 2927 cm^{-1} and 2854 cm^{-1} , C=O and C-O at 1732 cm^{-1} and 1164 cm^{-1} , C-O at 1096 cm^{-1} and 1048 cm^{-1} .^{6,7} The PEGs were distinguished by the stretching vibration of -OH and C-H at 3400 cm^{-1} and 2880 cm^{-1} , the bending vibration of C-H at 1454 cm^{-1} and 1340 cm^{-1} , O-H at 1240 cm^{-1} , the stretching vibration of C-O and C-O-C at around $1150\text{--}1050\text{ cm}^{-1}$ and of C-C at 943 cm^{-1} and 842 cm^{-1} .^{8,9} (B) FTIR spectra of dissolved NCO-terminated PEG pre-polymer derivatives (pre-400, pre-1000 and pre-1450) in 1,4-dioxane. The ATR-FTIR spectra were shifted vertically for clarity.

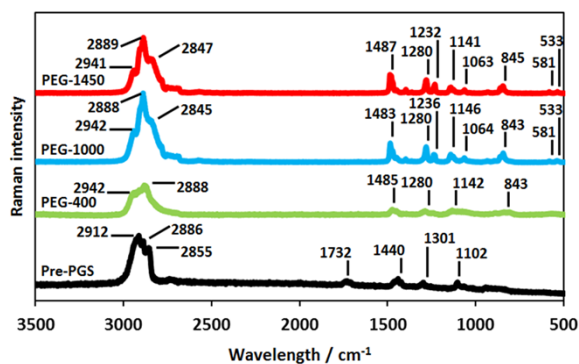


Figure S4. (A) Raman spectra of pre-PGS, PEG-400, PEG-1000 and PEG-1450. The Raman spectra were shifted vertically for clarity. Briefly, pre-PGS related bands are distinguished by the stretching vibration of C-O at 1102 cm⁻¹, the twisting and bending vibrations of CH₂ at 1298 cm⁻¹ and 1440 cm⁻¹, the stretching vibration of C=O at 1732 cm⁻¹ and CH₂ in the range of 2700-3000 cm⁻¹.^{6,10} PEG associated bands are characterized by the bending vibrations of C-C-O at 533 cm⁻¹ and 581 cm⁻¹, the rocking vibration of CH₂ at 843 cm⁻¹, the stretching vibrations of C-O at 1064 cm⁻¹ and 1146 cm⁻¹, the twisting vibrations of CH₂ at 1236 cm⁻¹ and 1286 cm⁻¹, the bending vibration of CH₂-CH₂ at 1444 cm⁻¹ and 1484 cm⁻¹, and the strong bending and stretching vibration of CH₂ in the range of 2700-3000 cm⁻¹.^{11,12}

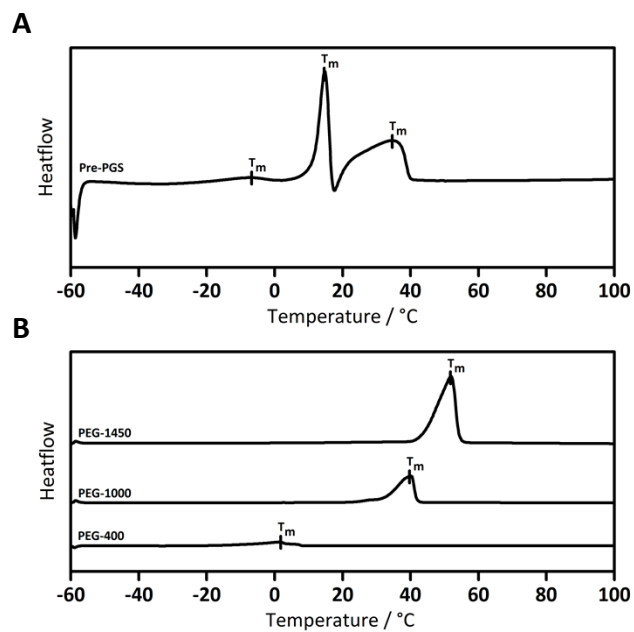


Figure S5. (A) DSC curve of pre-PGS, characterized with three distinct T_m of -7.6 °C, 14.4 °C and 33.4 °C. (B) DSC curves of PEG-400, PEG-1000 and PEG-1450 characterized with T_m 's of 2.1 °C, 40.1 °C and 50.6 °C, respectively. DSC curves were shifted vertically for clarity.

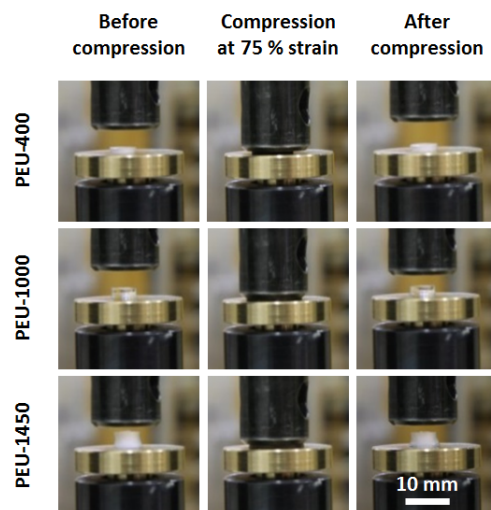


Figure S6. Images of the compressive behavior of PEU hydrogels after 75% strain compression, presenting no damage and fully recovery after load is released.

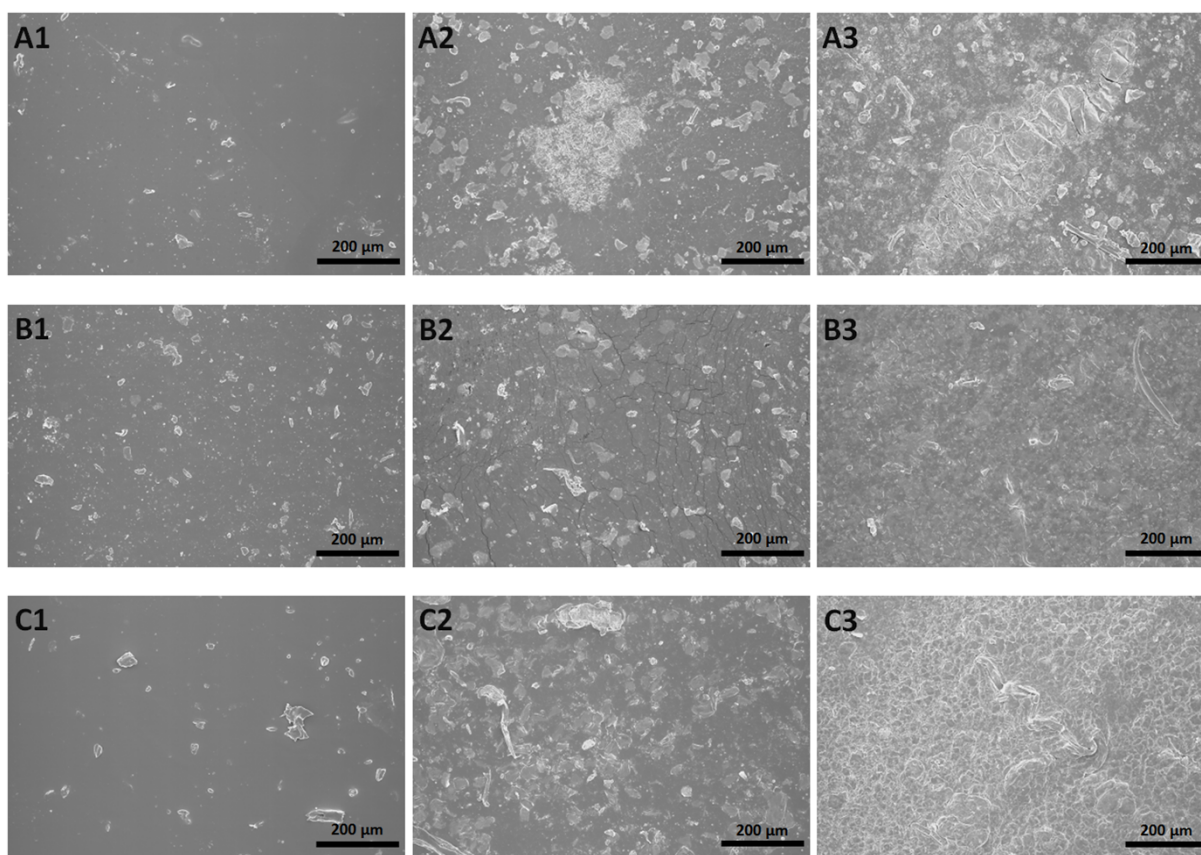


Figure S7. SEM micrographs of vacuum-dried (A1-3) PEU-400, (B1-3) PEU-1000 and (C1-3) PEU-1450 film surfaces: (A1, B1, C1) untreated, (A2, B2, C2) in enzyme-free PBS solution and (A3, B3, C3) in enzyme-including PBS solution after 31 days at 37 °C. The results illustrate the enhanced degradation in the presence of the enzyme.

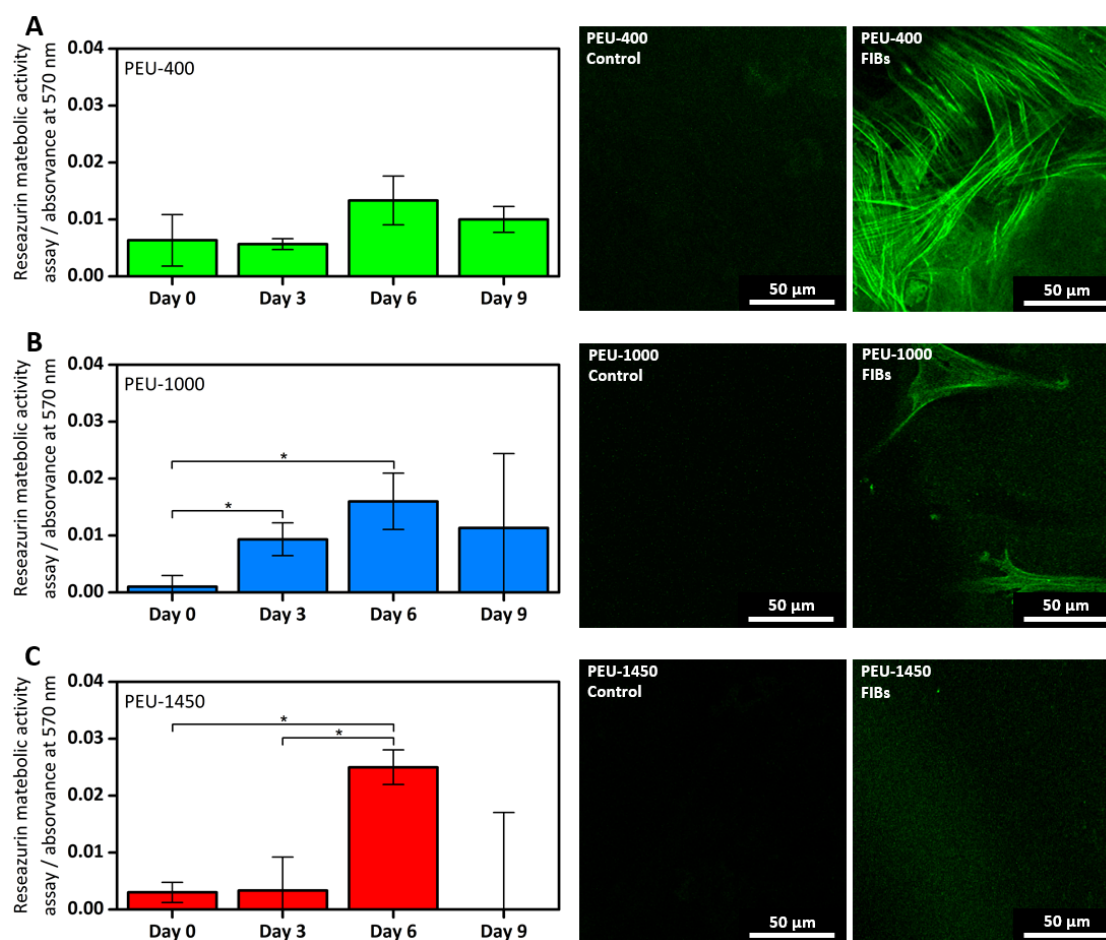


Figure S8. Metabolic activity assay of FIBs cultured on (A) PEU-400, (B) PEU-1000 and (C) PEU-1450 after subtracting the data for the cell-free PEU control sample, along with representative confocal fluorescence micrographs following 9 days of *in vitro* cultivation. FIBs showed relatively constant metabolic activities on the PEU-400 hydrogels, and presented on the PEU-1000 and PEU-1450 hydrogels a significant increase of metabolic activity from day 0 to day 6. The representative confocal fluorescence micrographs present highly confluent cell populations on the PEU-400 and PEU-1000 hydrogel surfaces, while the PEU-1450 hydrogels presented relatively poor cell populations on the surfaces. It is assumed that the seeded cells detached from the PEU-1450 hydrogels and proliferated in part on the well plate surfaces, due to the hydrogels thermosensitive properties and its relatively higher mass swelling ratio alterations under temperature changes. The data are represented as mean \pm standard deviation ($n = 3$; $* = p < 0.05$, two sample *t*-test).

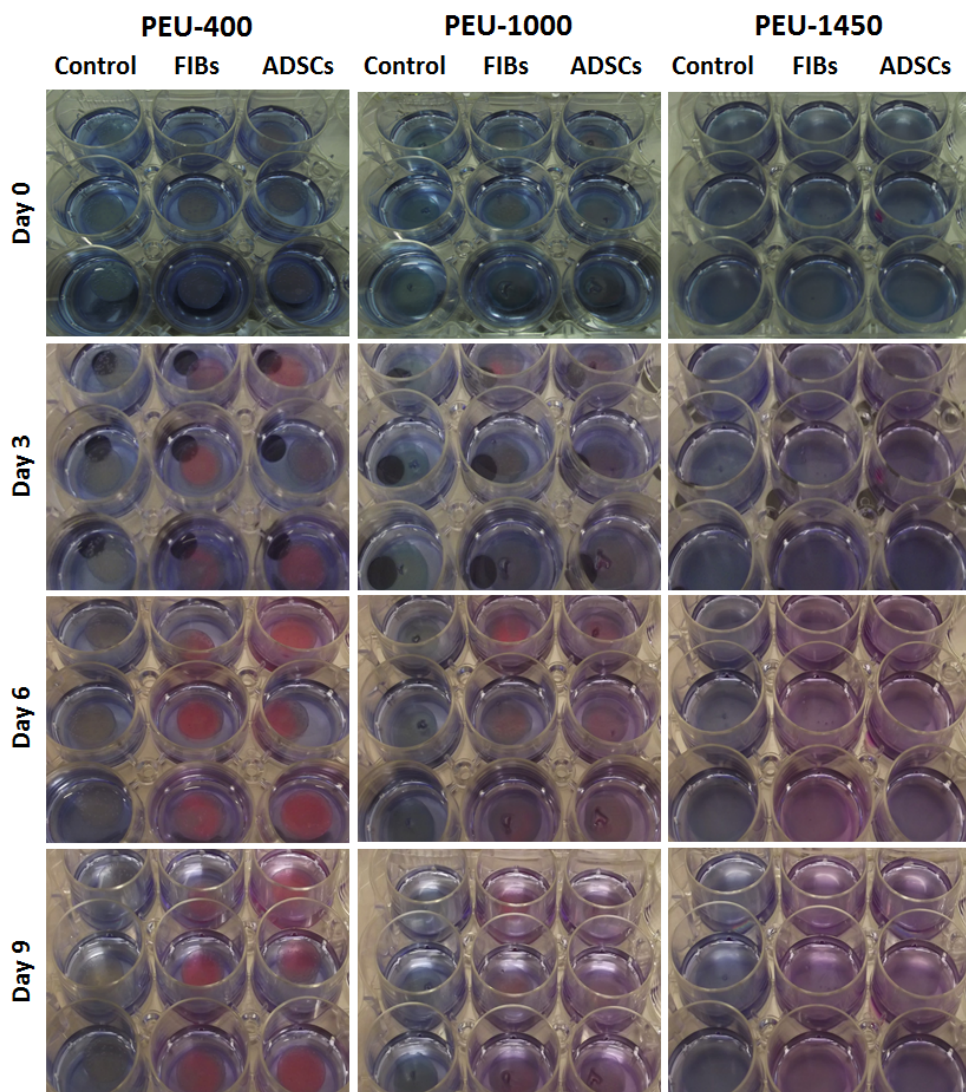


Figure S9. Images of performed metabolic activity assay tests (at days 0, 3, 6 and 9), illustrating the resazurin color change for FIBs and ADSCs cultured PEUs in comparison to cell-free PEU control specimens. Briefly, the assay is based on the mechanism that blue resazurin can only be reduced to pink resorufin by proliferating cells. Thus, the production of pink resorufin correlates confidently with cell viability and proliferation.

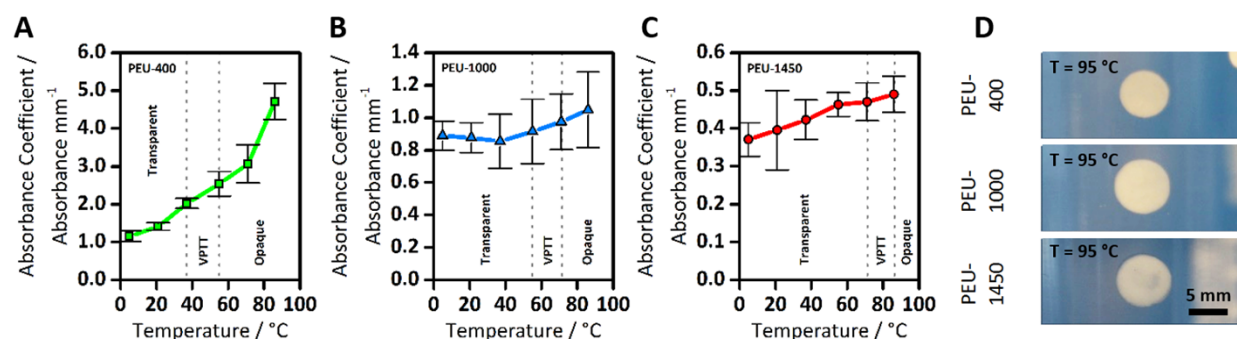


Figure S10. Estimation of the volume phase transition temperature (VPTT) of (A) PEU-400, (B) PEU-1000 and (C) PEU-1450 films by the cloud point method and defined in a temperature range at the onset of cloudiness by optical conformation.¹³ Briefly, the absorbance coefficient (absorbance/sample thickness) at 600 nm was measured as a function of the PBS solution temperature (monitored at 5, 21, 37, 55, 71 and 86 °C) with a UV-Vis spectrometer (Perkin Elmer Lambda 900). Pre-hydrated samples ($n = 5$; 24 h saturation in PBS solution at 21 °C) were submerged for 2 h in the temperature monitored PBS solution, and the absorbance of the individual swollen specimens and the sample thickness were measured. No sharp VPTT transitions were observed in the figure; PEU-400, PEU-1000 and PEU-1450 specimens presented a VPTT within the range of 37-55 °C, 55-71 °C and 71-86 °C, respectively. The VPTT can be defined as the critical temperature below which the hydrogel swells (hydrophilic characteristics) and above which the hydrogel contracts (hydrophobic characteristics).¹⁴ The results imply that the VPTT is adjustable and that the incorporation of higher MWt PEG increased the VPTT, due to its enhanced hydrophilicity.¹³ (D) Swollen PEUs were transparent at low medium temperatures (see Figure 5E in the manuscript) but changed to opaque at higher temperatures, which is associated with temperature-dependent phase separation of the hydrogels from the aqueous solution.

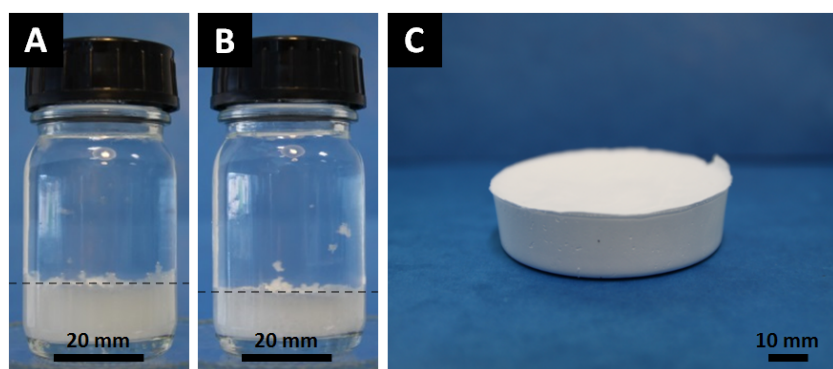


Figure S11. Images of the swelling/deswelling behaviour of PEU-1000 microspheres (See Figure 10A) after 24 h immersion in PBS solution at (A) 5 °C and (B) 37 °C. The microsphere specimens presented at a low temperature a higher degree of swelling which shrank at a higher temperatures, as indicated from the level of the microsphere specimens in the bottle. (C) PEU-1450 scaffold specimen with interconnected porous structure (See Figure 10B), fabricated via freeze-drying.

References

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