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Tailoring hydrogel mesh structure through custom-designed poly(ether urethane)s for advanced drug delivery systems in biomedical applications

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Chemical characterization of poly(ether urethane)s

To prove their successful synthesis, all the *ad hoc* customized poly(ether urethane)s (PEUs) (i.e., SHE3350, AHE600, AHE1500, AHE6000) were chemically characterized through Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy, Size Exclusion Chromatography (SEC) and Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy according to the methods reported by Pappalardo et al.[1]

Methods

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

ATR-FTIR spectroscopy was performed using a Pelkin Elmer Spectrum 100 instrument equipped with an ATR accessory (UATR KRSS) with a diamond crystal. SHE3350, AHE600, AHE1500 and AHE6000 were analysed at room temperature (RT) in the spectral range 4000-600 cm^{-1} (resolution 4 cm^{-1}, 32 scans). As references, the PEG3350, PEG600, PEG1500 and PEG6000 spectra were also registered according to the same method. All the spectra were analysed using the Perkin Elmer Spectrum Software.

Size Exclusion Chromatography (SEC)

PEUs molecular weight distribution profile was assessed using an Agilent Technologies 1200 Series (California, USA) instrument equipped with a Refractive Index Detector (RID) and two Waters Styragel columns (HR1 and HR4) equilibrated at 55 °C. N,N-dimethylformamide (DMF, HPLC grade, Carlo Erba Reagents, Milano, Italy) added with 0.1% w/v LiBr (Sigma Aldrich, Milano, Italy) was used as mobile phase. For SEC analysis, the PEUs were solubilized at 2 mg/mL concentration in the mobile phase and the resulting solutions were filtered using a 0.45 µm poly(tetrafluoroethylene) (PTFE) syringe filter (LLG International, Meckenheim, Germany). The number-average molecular weight ($\overline{M_n}$), weight-average molecular weight ($\overline{M_w}$), and polydispersity index (D) were calculated using the Agilent ChemStation Software by referring to a calibration curve based on poly(ethylene oxide) (PEO) standards with a peak molecular weight M_p ranging between 982 and 205,500 Da.

Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

^{1}H NMR spectroscopy was performed by using a Bruker Avance NEO spectrometer (Bruker Biospin AG, Fällanden, Switzerland) equipped with an 11.75 T superconducting magnet (500 MHz ^{1}H Larmor frequency) and a Bruker multinuclear SMARTProbe probe. Samples were prepared by solubilizing SHE3350 in deuterium oxide (D_2O, 99.8%, Sigma Aldrich, Milano, Italy), and AHE600, AHE1500 and AHE6000 in deuterated dimethyl sulfoxide (d_6-DMSO, 99.8% D with 0.03% TMS, Sigma Aldrich, Milano, Italy) at 1.3% w/v concentration. As control samples, N-Boc serinol (N-Boc), Boc-protected SHE3350 (Boc-SHE3350) and 4-hydroxybenzaldehyde (BA) were also analysed according to the same protocol.

Results

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

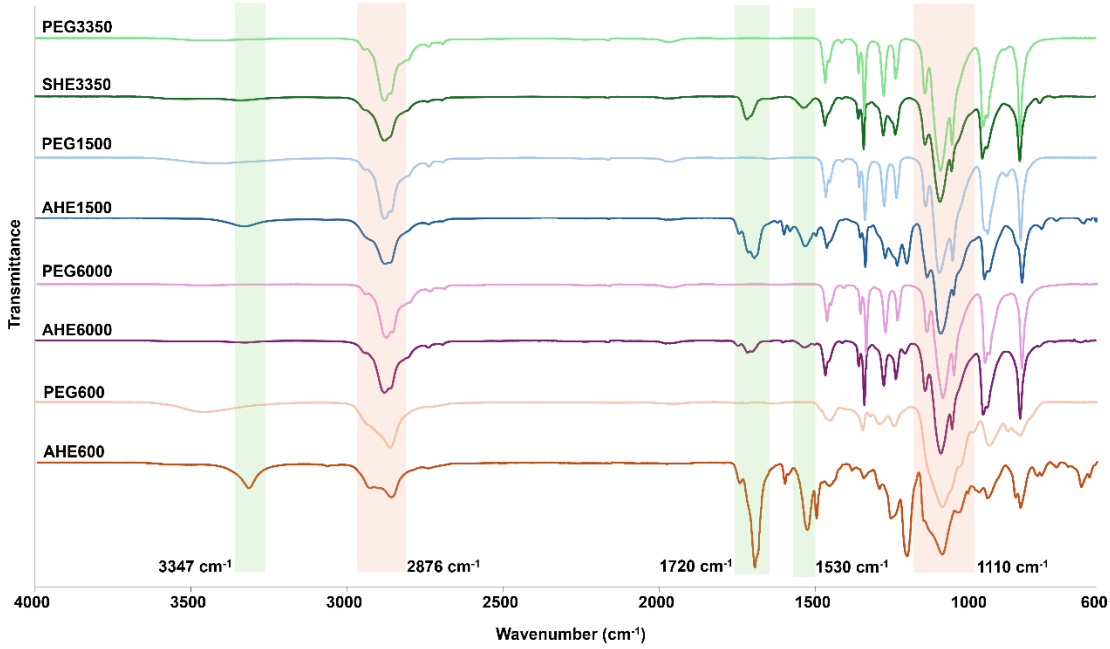

Figure S1. ATR-FTIR spectra of poly(ethylene glycol) with number average molecular weight $\overline{M_n}$ 3350 Da (PEG3350), 1500 Da (PEG1500), 6000 Da (PEG6000), 600 Da (PEG600), and of the corresponding PEUs SHE3350, AHE1500, AHE6000, AHE600. The rectangular shapes highlight the typical signals of poly(ethylene glycol) (light orange) and newly formed urethanes bonds (light green).

Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

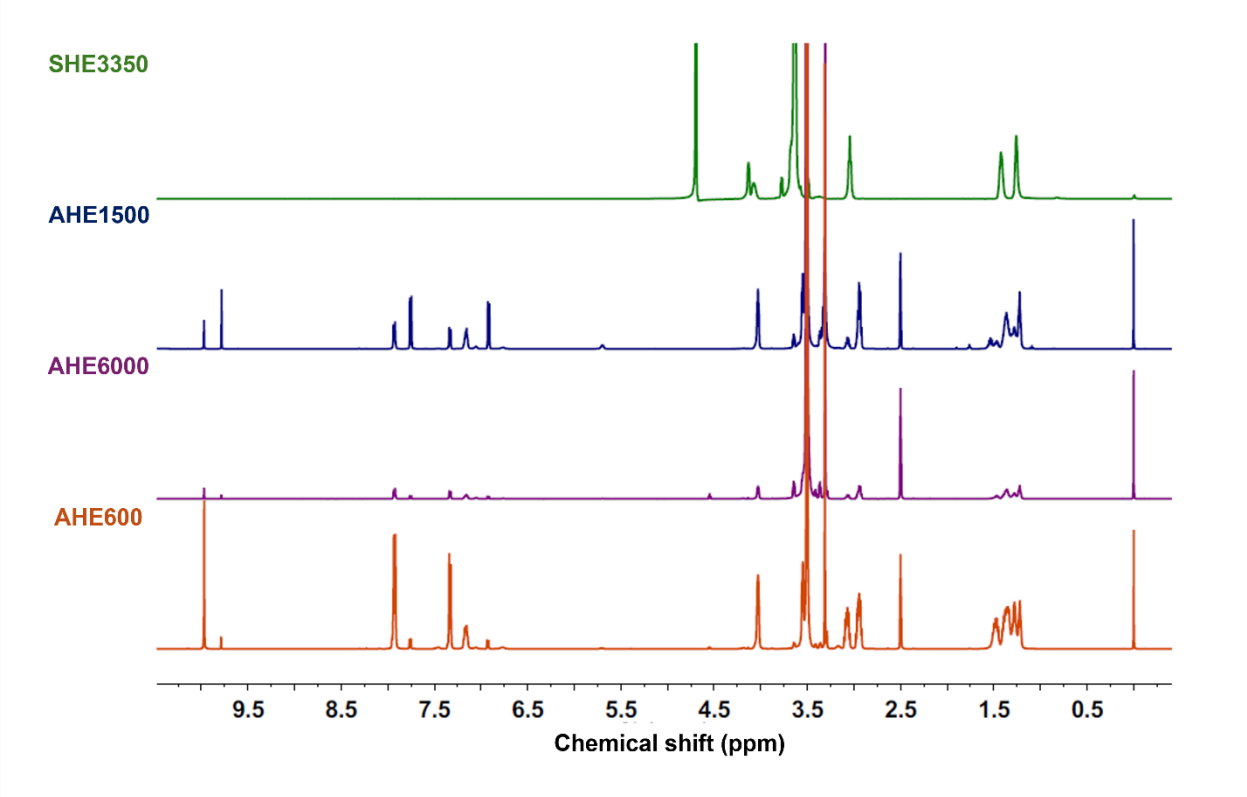

Figure S2. ^{1}H NMR spectra of the synthesized PEUs SHE3350, AHE1500, AHE6000 and AHE600. The complete signals assignment to the corresponding protons for SHE3350 and AHE1500 has already been reported in our previous work.[1] Analogous considerations apply to both AHE6000 and AHE600.

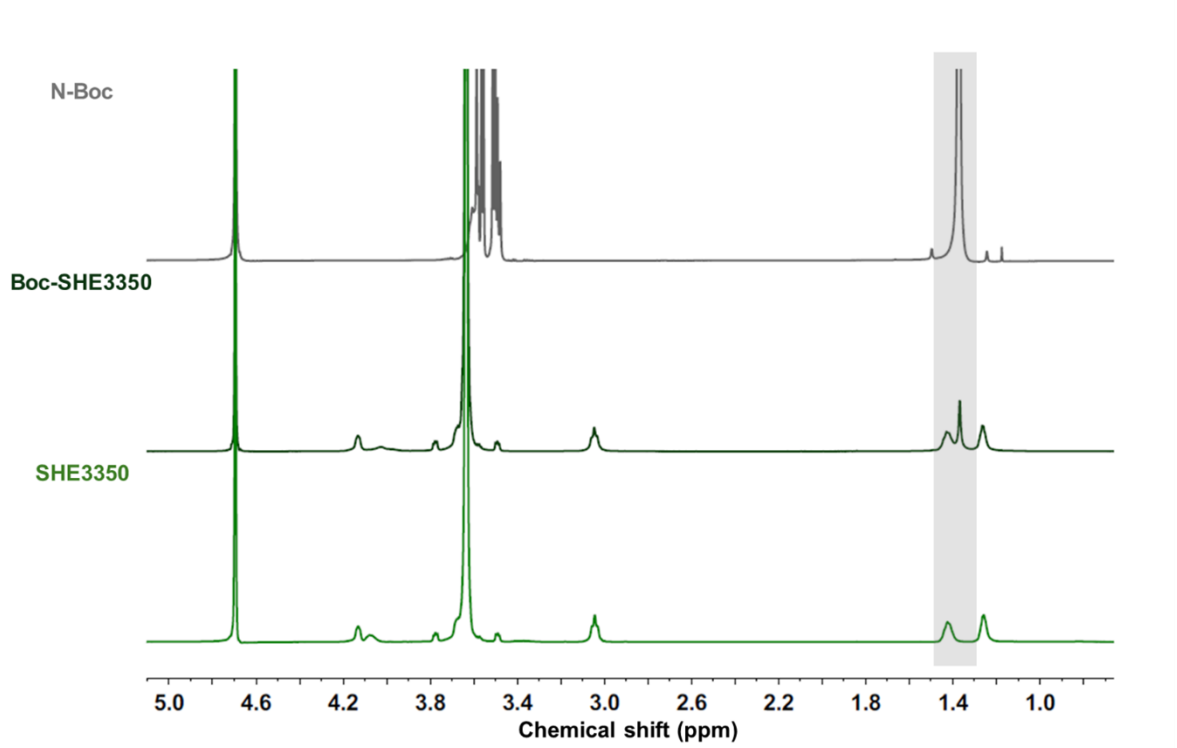

Figure S3. ^{1}H NMR spectra of N-Boc serinol (N-Boc), Boc-protected SHE3350 (Boc-SHE3350) and SHE3350. The rectangular light grey shape highlights the peak at 1.36 ppm ascribed to the hydrogens involved in the Boc group.

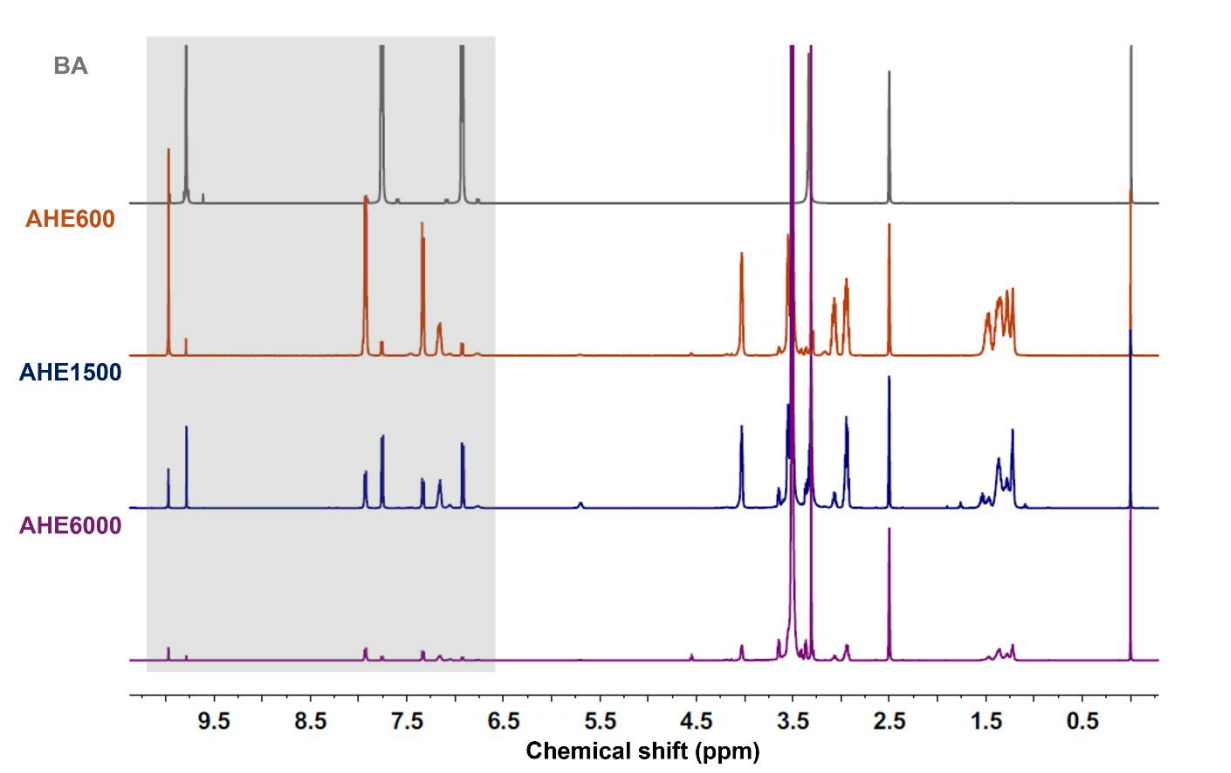

Figure S4. ^{1}H NMR spectra of 4-hydroxybenzaldehyde (BA) and the aldehyde-terminated PEUs AHE600, AHE1500 and AHE6000. The rectangular light grey shape highlights the peaks ascribed to the benzaldehyde protons.

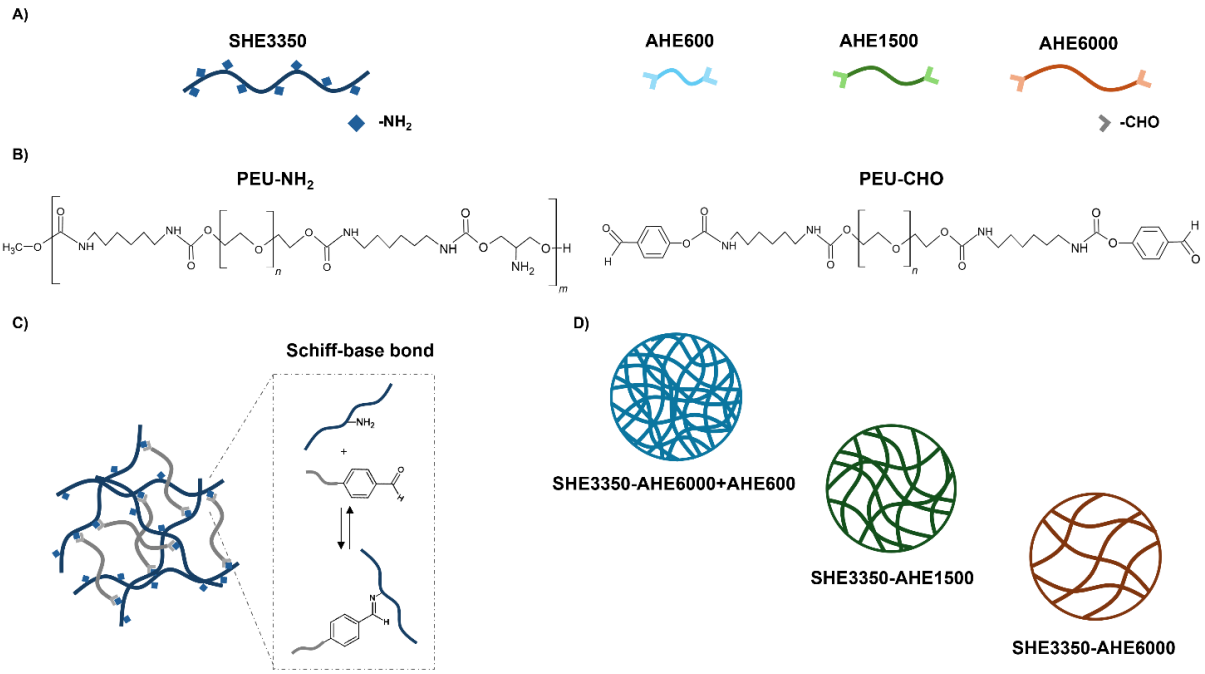

Figure S5. Schematic illustration of **A)** Poly(ether urethane) (PEU) SHE3350 bearing pendant primary amines, and aldehyde-terminated AHE600, AHE1500 and AHE6000; **B)** Chemical structures of PEU-NH_2 and PEU-CHO; **C)** Mechanism of Schiff-base hydrogel formation; **D)** Representation of the three hydrogel formulations differing in polymer composition and structural properties.

Characterization of hydrogels based on Schiff-base crosslinking with tuneable mesh size

Method

Estimation of pore size distribution

The developed Schiff-base hydrogels with tuneable mesh size were thermally characterized by Differential Scanning Calorimetry (DSC) analyses (NEXTA© DSC200, Hitachi, Japan) according to the protocol reported in the paper (Materials and Methods section). From the endothermic peaks of the DSC thermograms, the pore radius distribution curves were obtained according to Manikoth et al. and Raja and Fathima, as follows:[2,3]

- The radius R_p (nm) of the pores present within the hydrogels was expressed as function of the depression of the melting temperature of water confined into the mesopores compared to the bulk water ($\Delta T=T-T_0$, with T_0 being the triple point of water):

$$R_p = -\frac{32.33}{\Delta T} + 0.68$$

- The pore volume distribution dV/dR_p (cm³/(g·nm)) was derived from the following equation:

$$\frac{dV}{dRp} = \frac{\Delta V}{R_i - R_{i+1}}$$

where R_i-R_{i+1} are pore radii corresponding to consecutive points of temperature and ΔV (cm³/g) the pore volume calculated as follows:

$$\Delta V = \frac{H_i}{W_v \times m \times 1000}$$

where H_i (J) are the enthalpies to the corresponding melting temperatures derived from the heat capacity, m (g) is the sample mass, and W_v (J/cm³) is the volumetric latent heat calculated from the specific latent heat of fusion W_s (J/g) and the specific volume of solvent V (cm³/g):

$$W_v = \frac{W_s}{V}$$

$$W_s = -(0.0556(T_i' - T_0)^2 - 11.39(T_i' - T_0) - 332)$$

$$V = 1.000132\left(1 - 0.000091T_i' + (1.035 \times 10^{-5}){T_i'}^2\right)$$

with T_i' being the average temperature interval calculated according to the following equation:

$$T_i' = \frac{T_i + T_{i+1}}{2}$$

Results

Table S1. Thermal transition data (melting temperature T_m and melting enthalpy ΔH_m) of the recorded heating cycle for SHE3350-AHE1500, SHE3350-AHE6000, SHE3350-AHE6000+AHE600.

Sample	T_m (°C)	ΔH_m (J/g)
SHE3350-AHE1500	-17.1 0.2	15.0 0.5
	0.8 0.3	188.6 8.0
SHE3350-AHE6000	-15.2 0.1	19.6 1.0
	0.7 0.1	177.6 0.6
SHE3350-AHE6000+AHE600	-15.2 0.2	18.9 1.1
	0.4 0.2	177.6 5.1

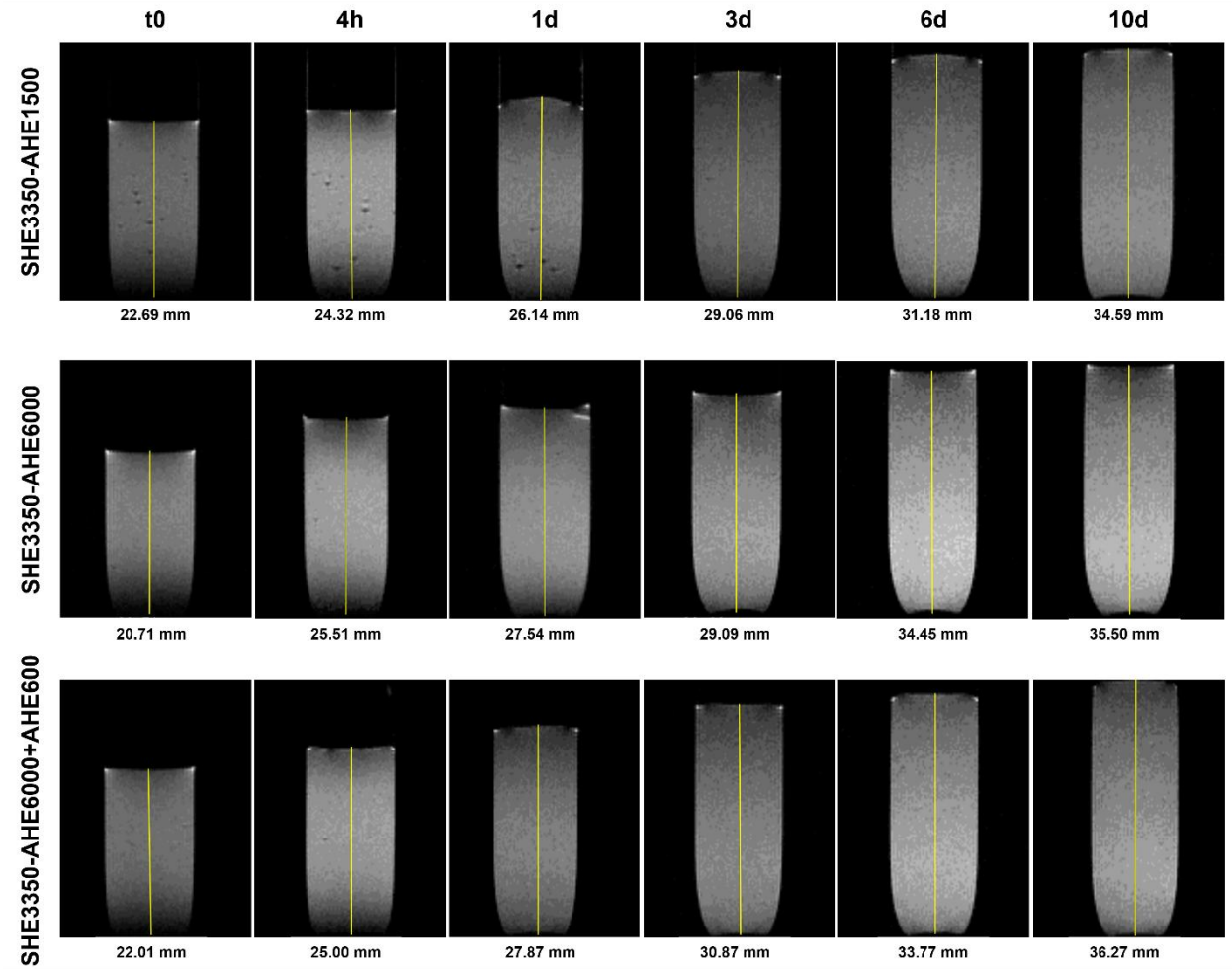

Figure S6. T_1-weighted MRI images of SHE3350-AHE1500, SHE3350-AHE6000 and SHE3350-AHE6000+AHE600 gels incubated for different time intervals (t0, 4h, 1, 3, 6 and 10d) in contact with phosphate-buffered saline (pH 7.4, 37 °C). Yellow straight lines identify the performed measurements of sample size along the longitudinal axis.

References

1 R. Pappalardo, M. Boffito, C. Cassino, V. Caccamo, V. Chiono and G. Ciardelli, *ACS Omega*, 2024, **9**, 45774–45788.

2 R. Manikoth, I. Kanungo, N. N. Fathima and J. R. Rao, *Carbohydr. Polym.*, 2012, **88**, 628–637.

3 I. S. Raja and N. N. Fathima, *Springerplus*, 2014, **3**, 1–10.