Supplementary Information

Sulfation of Thiol-Maleimide Crosslinked Hydrogel Modulates Material Properties and Cell Biocompatibility

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Degree of Functionalization by ^{1}H-NMR recorded in D_2O

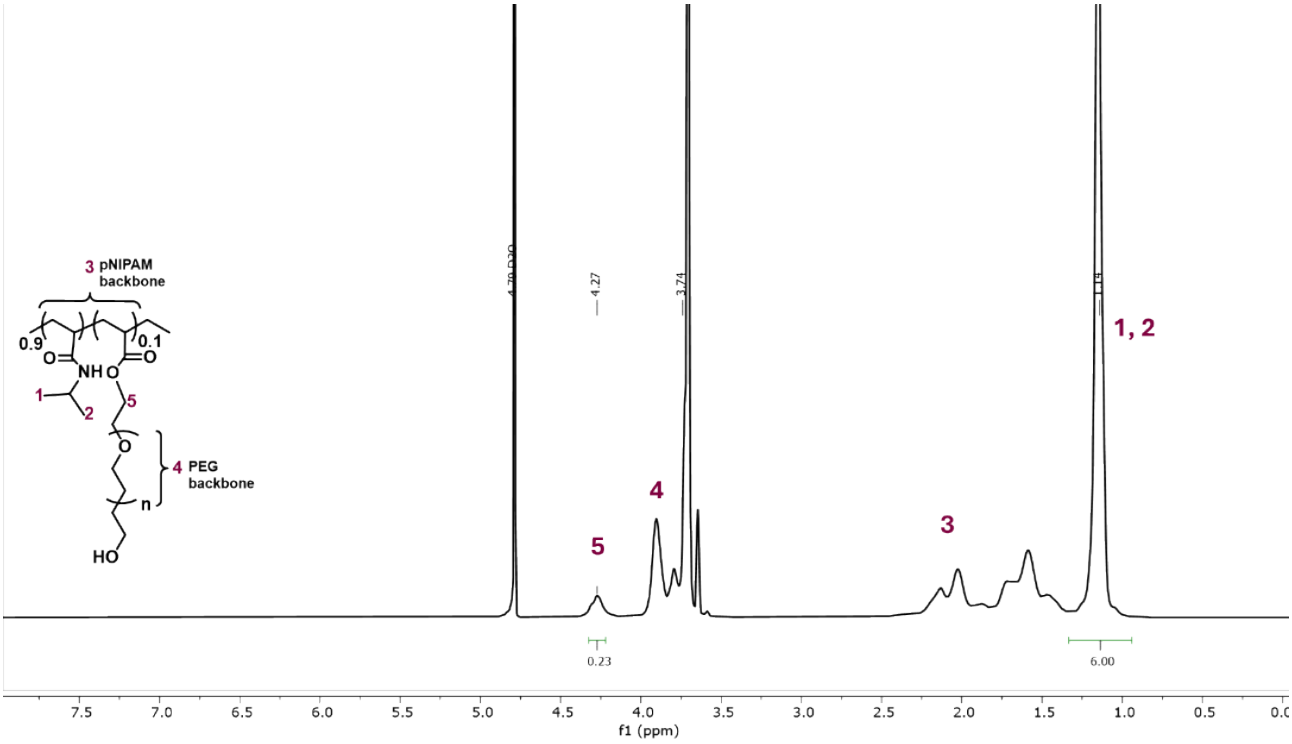

Figure S 1 *The ^{1}H NMR spectrum of Copol-OH$_{(10\%)}$ displays the characteristic proton resonances. The degree of functionalization was determined using Equation 1 with the integration values of signals 1 and 2 relative to signal 5.*

Equation 1*:Degree of Functionalization: Copolymerization*

$$DF\,[\%] = \frac{\left(\frac{peak\ integral}{number\ of\ protons}\right)}{1 + \left(\frac{peak\ integral}{number\ of\ protons}\right)} * 100 = \frac{\frac{0.23}{2}}{1 + \frac{0.23}{2}} * 100 = 10\ \%$$

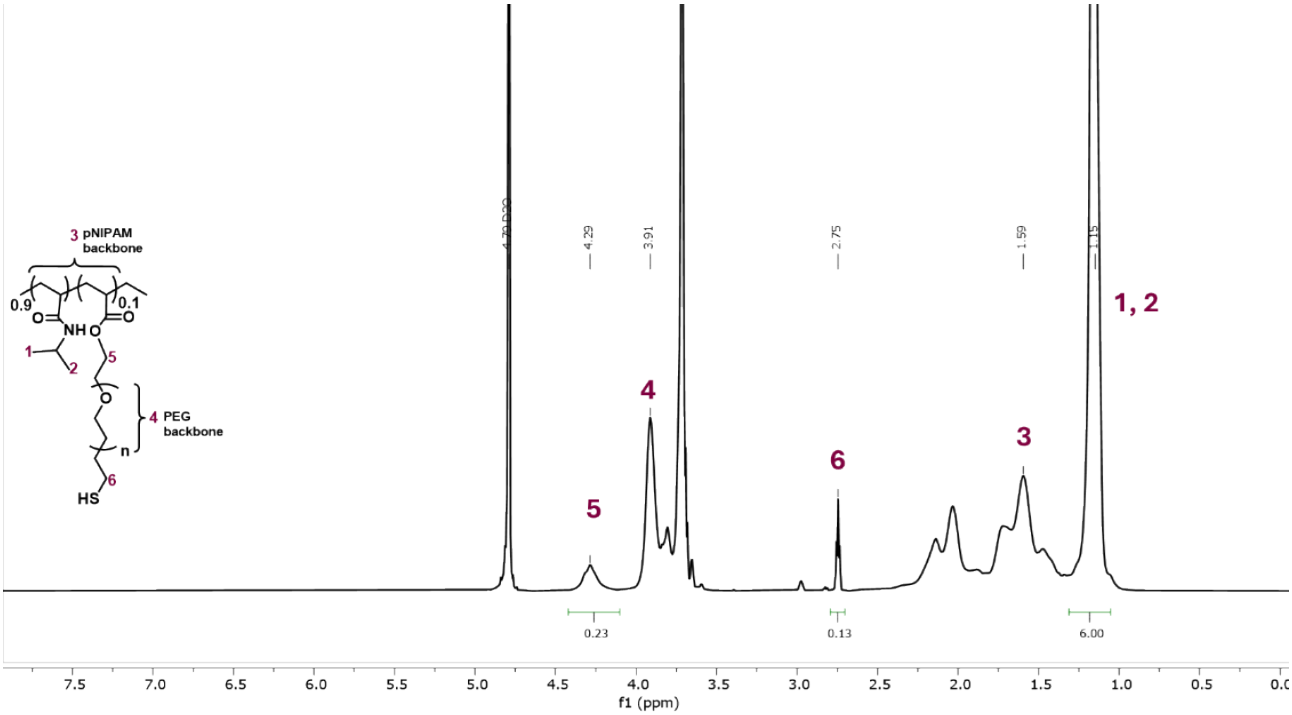

Figure S 2: *The ^{1}H NMR spectrum of Copol-SH$_{(5\%)}$ displays the characteristic proton resonances. The degree of functionalization was determined using Equation 1 with the integration values of signals 1 and 2 relative to signal 5 and signal 6 for the mesylgroup next to the free thiol.*

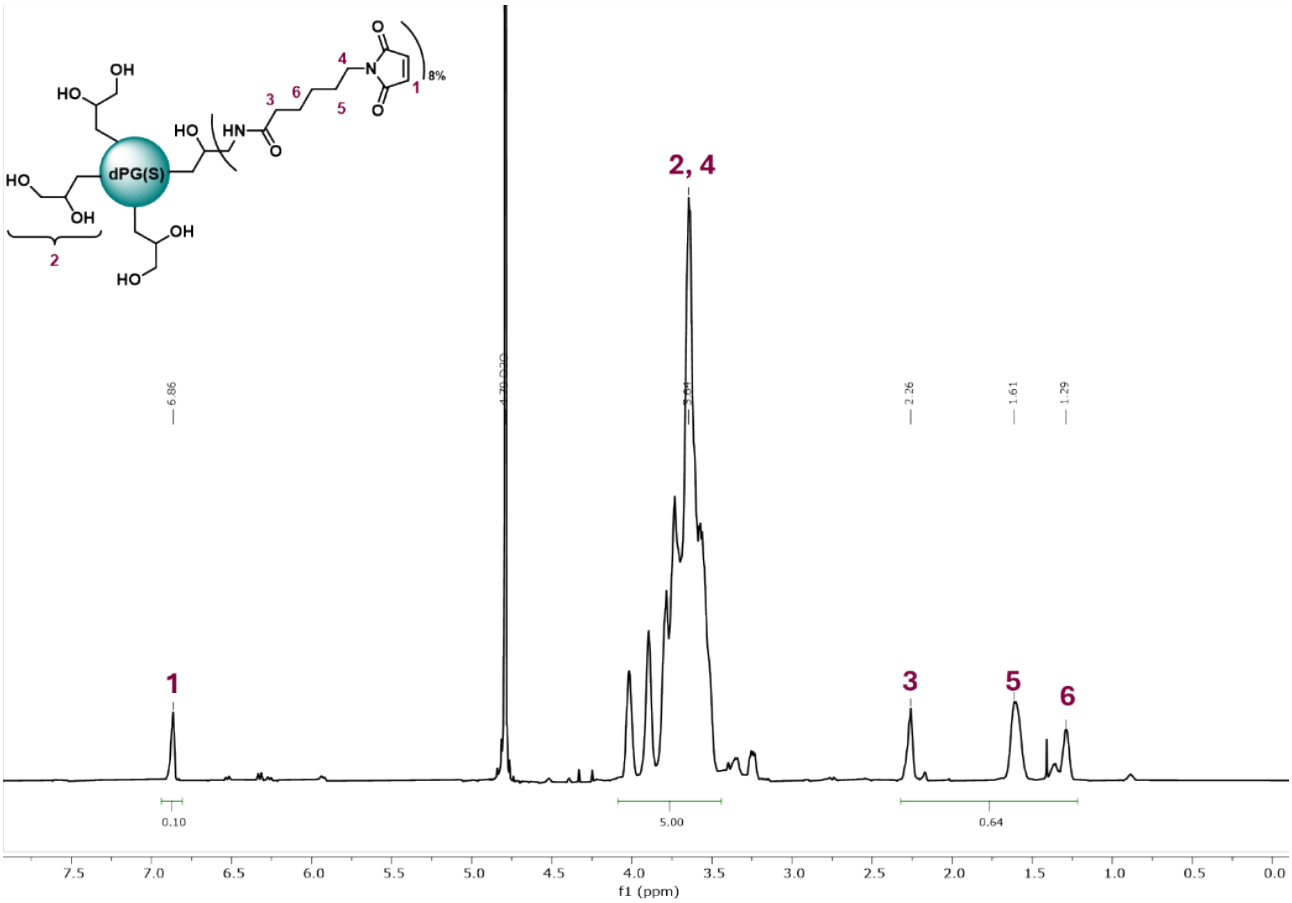

Figure S 3: *The ^{1}H NMR spectrum of dPG(S)-Mal$_{(8\%)}$ displays the characteristic proton resonances. The degree of functionalization was determined using Equation 1 with the integration values of signals 1 relative to signal 2 for the dPG-backbone and maleimide group.*

Degree of Sulfation by Elemental Analysis

Equation 2*: Degree of Sulfation*

$$DS = \frac{M_P + (\#OH \times M_{Sulfate} \times p_S)}{M_S \times \#OH} \times 100$$

Mp = 6500; #OH = 48, ps = 15.5%, Ms=32

dPGS: DS = 50% (elemental analysis (EA): N = 1.84, C = 19.22, S = 15.50, H = 3.20)

Determination of reactive Thiol content by Ellman´s Assay

We quantified the number of free, reactive thiol groups of pNIPAM-co-PEG-SH using the Ellman's assay. Herein, the model disulfide 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) will be reduced by a thiol group quantitatively releasing the anion TNB^{2-}. Monitoring this reduction by UV-Vis-spectroscopy enables the quantification of thiol groups per polymer in reference to a standard molecule. **Copol-SH** was determined to have 5.75 thiol groups per polymer.

The thiol content was also determined by **NMR and GPC** analysis, yielding a value of 7.5 groups per polymer chain. This calculation is based on the integration of methylene protons adjacent to the thiol group; however, oxidized disulfides are difficult to distinguish from this signal. In contrast, the Ellman's assay exclusively quantifies free thiols, providing a direct measure of reactive groups.

Zeta potential of dPG-N_3 and dPGS-N_3

Table 1: Overview table of the surface charge by zeta potential

	ζ (mV)
dPG-N_3	+2.95 ± 1.09
dPGS-N_3	-29.98 ± 2.51

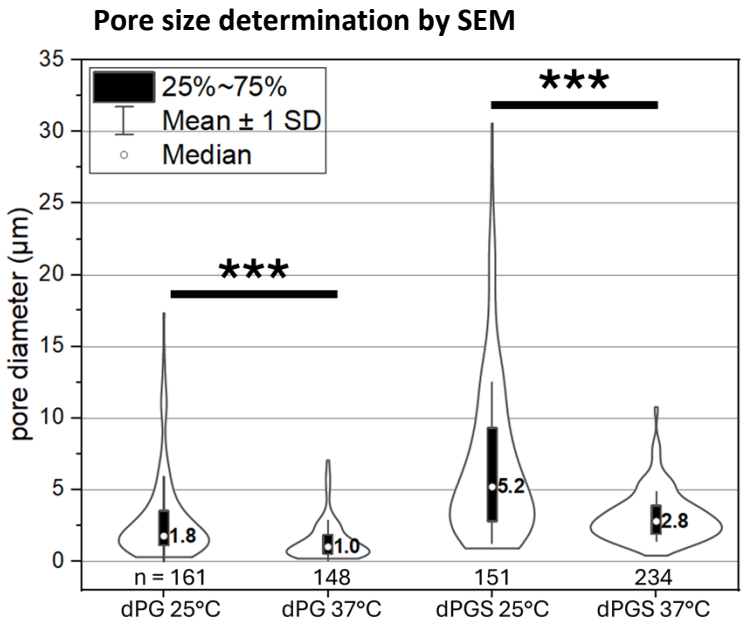

Figure S 4: *Overview of SEM evaluation at 25°C and 37°C. Custom FIJI macro with two image replicates per condition were analysed, and the resulting data were pooled.*

Hydrogel swelling studies

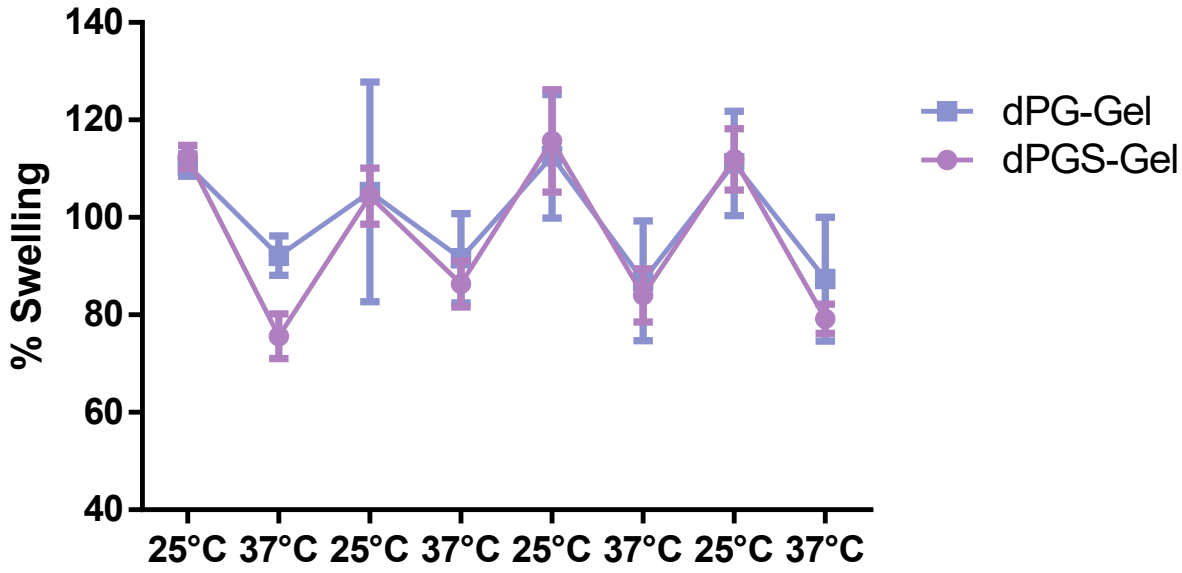

Figure S 5: *Swelling study of dPG and dPGS hydrogels at 25°C and 37°C.*

Hydrogel samples (100 µL each) were prepared, and their initial weights were recorded. Subsequently, 200 µL of PBS was added to each hydrogel, and the samples were incubated 24 h at 25 °C. After incubation, excess surface liquid was carefully removed, and the hydrogels were measured and weighed.

Following this, the hydrogels were incubated at 37 °C for 2 hours, after which the excess liquid was removed, and the samples were weighed again. To assess the reversibility of the swelling behavior, an additional measurement was performed after adding 200 µL of PBS and incubating the hydrogels at 25 °C.

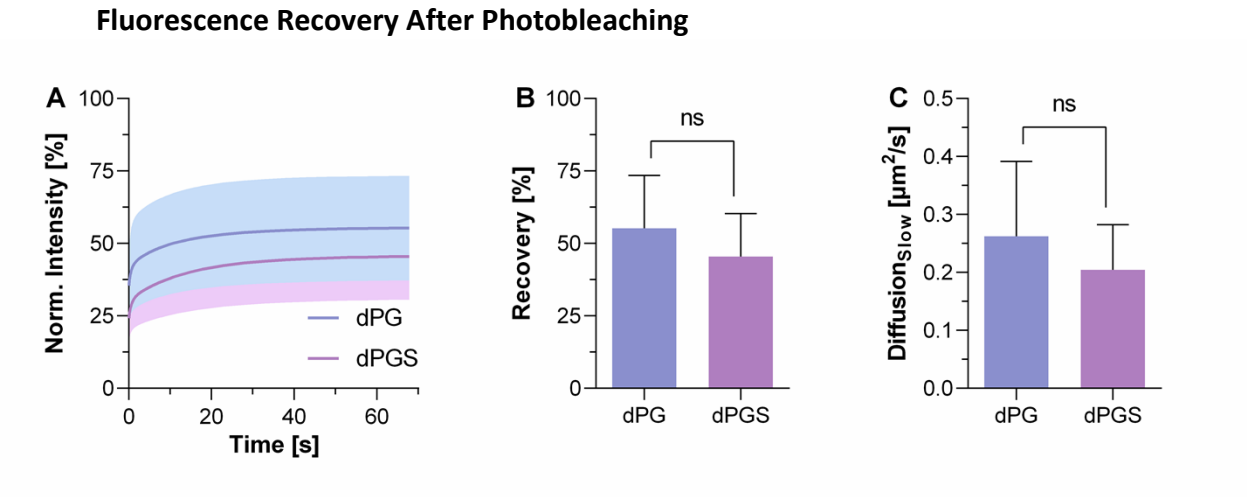

Figure S 6: *FRAP analysis of FITC–Albumin (FITC-HSA) diffusion in dPG and dPGS hydrogels. (A) Normalized fluorescence recovery over time. (B) Quantified mobile fraction of FITC–dextran. (C) Diffusion kinetics. Data shown as mean ± SD*

GAG-like staining (Alcian Blue)

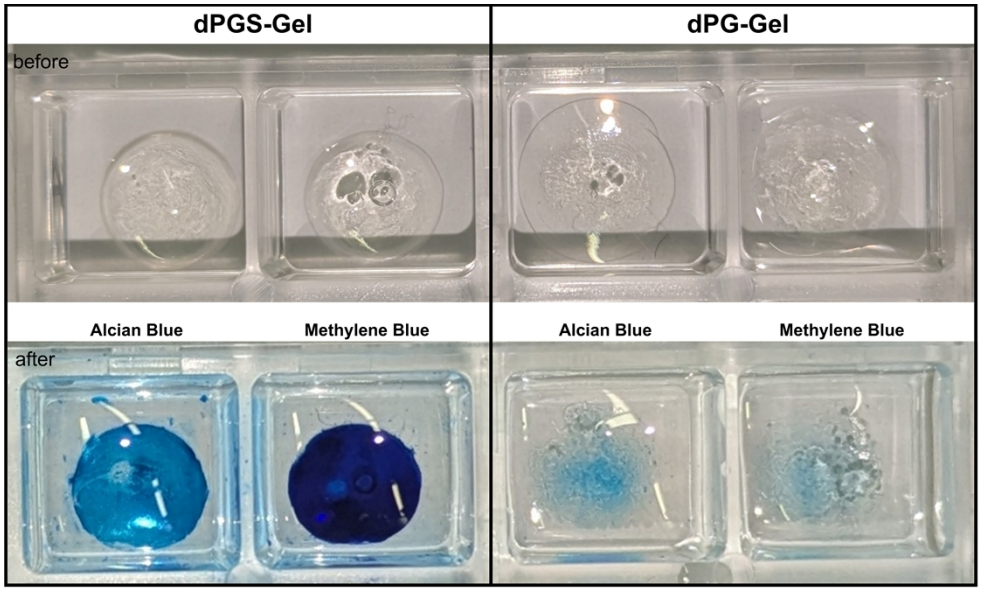

Figure S 7: *Qualitative Alcian blue and methylene blue staining of dPGS- and dPG-based hydrogels.*

dPG- and dPGS-based hydrogels (100 µL, 5 wt% each) were formed and washed three times with PBS (15 min per wash) to remove unbound polymer. The gels were then incubated overnight with 300 µL of an aqueous Alcian blue / methylene blue solution. On the next day, excess dye was removed, the gels were washed with 1% acetic acid and subsequently three times with PBS. As shown in the photograph provided below, the dPGS hydrogels retained a markedly more intense blue colour after the washing steps than the corresponding dPG hydrogels, which appeared only faintly stained. This GAG-like staining thus qualitatively confirms that the sulfated dPGS networks exhibit substantially stronger binding of positively charged dyes than the non-sulfated dPG networks, consistent with enhanced electrostatic interactions between the sulfate groups and cationic species.

Overview of the Cell Viability data

Table 2: *Overview table of cell viability data dPGS-Mal, dPG-Mal, Copol-SH and the corresponding hydrogels with A549, HeLa and MCF7 cells.*

	Cell viability %		
	A549	HeLa	MCF7
dPGS-Mal	101.7 ± 5.0	98.3 ± 11.4	76.9 ± 1.8
dPG-Mal	101.3 ± 5.7	91.6 ± 9.2	96.5 ± 6.0
Copol-SH	108.6 ± 4.9	45.4 ± 7.5	63.0 ± 4.4
dPGS-Gel	93.1 ± 4.2	76.0 ± 3.6	52.6 ± 3.9
dPG-Gel	85.2 ± 2.5	59.5 ± 4.8	96.1 ± 7.1