Polypeptide affined interpenetrating hydrogels with tunable physical and mechanical properties

Farshad Oveissi, Sina Naficy, * Thi Yen Loan Le, David F. Fletcher, Fariba Dehghani

School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, New South Wales, 2006, Australia

Correspondence to: Sina Naficy (E-mail: sina.naficy@sydney.edu.au)

SUPPORTING INFORMATION

Synthesis of Pluronic®F-127 dimethacrylate (FDMA)

To synthesis F-127 dimethacrylate (FDMA), 1.25 g Pluronic® F-127 (MW=12,500 Da) was added to 8.5 ml dichloromethane and 56 μL trimethylamine (MW=101.19 Da). Methylacryloyl chloride (MAC) (MW=104.53 Da) was added to the solution, and the whole batch was stirred at room temperature under nitrogen for three hours. Afterwards, the reaction proceeded for 72 h. The side product of this reaction was TEA.HCl that formed in DCM. Hence, samples were purified by diluting 60 times with Milli-Q water and dialysed with 1000 Da membrane (Spectrumlab, USA). Eventually, the functionalised Pluronic® was freeze-dried at –35 °C for 48 h. The product of this stage, which was a fine white powder, named FDMA hereafter.

The integration of the vinyl protons and the methylene group belonging to the poly(ethylene oxide) segment of F-127 produces FDMA. To confirm such chemical bonds, the dried FDMA was dissolved in D_2O for ^{1}H-NMR measurements. Spectrum was acquired at 298 K using Bruker Avance III 600 MHz NMR spectrometers. The obtained spectrum was processed using TOPSPIN3 (Bruker, Karlsruhe, Germany). The ^{1}H-NMR spectrum confirmed the formation of dimethacrylate end-capped Pluronic® F-127 via incorporating the methacryloyl groups (Figure S1). The chemical shifts at 5.62 and 6.18 ppm (doublet, one proton, peak "a" in Figure S1) correspond to unsaturated bonds of the hydrogen atoms at each tail of FDMA. The spectrum also shows the binding of the methacryloyl moieties, the last methylene group of polyethylene oxide block by the generated ester group, indicated as a signal at 4.3 ppm (triplet, two protons, peak "c" in Figure S1).
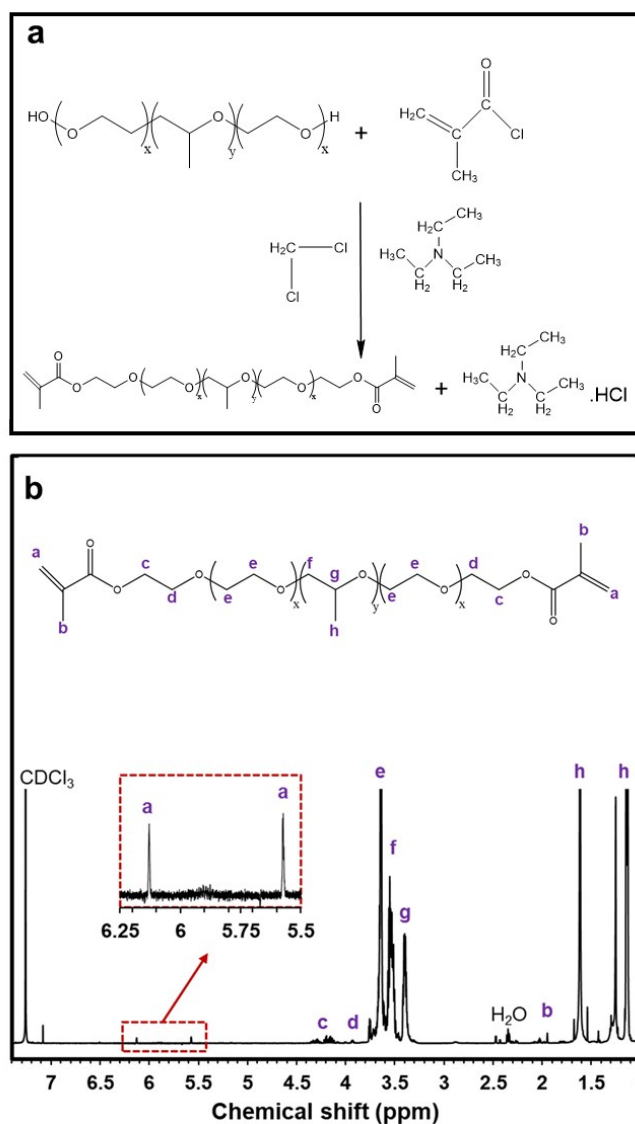
Figure S1. Reaction scheme for synthesis of Pluronic® F127 dimethacrylate (FDMA) (a), and ^{1}H NMR spectrum (b) of FDMA (x = 99 and y = 67).

Chemical bonding of succinimide groups to the network (FTIR analysis)

The fabricated hydrogels with (PPHUN) and without NAS (PPHU) were characterised using FTIR (Nicolet 6700, ThermoFisher Scientific, USA). The FTIR signals were expressed as percentage transmittance (%Transmittance) over a range of 500–4000 wavenumbers (cm^{-1}). The occurrence of the peak at 1700 cm^{-1} in PPHUN spectrum confirmed the chemical bonds of succinimide groups with the network (Figure S2). Similar band was observed and reported as the ester group of NAS in previous studies.[1, 2] The peak at 1080 cm^{-1} referred to stretching of ether groups (-C-O-C-) in HEMA and FDMA.[3] The band between 1500 to 1600 cm^{-1} was due to the stretching of N-H from HPU. The band at 960 cm^{-1} occurred because of stretching

the carbonyl group (C=O).[3] Finally, the obtained spectra demonstrated a peak at 1635 cm^{-1} indicating a stretch in alkenes (C=C) of FDMA and HEMA.[4]
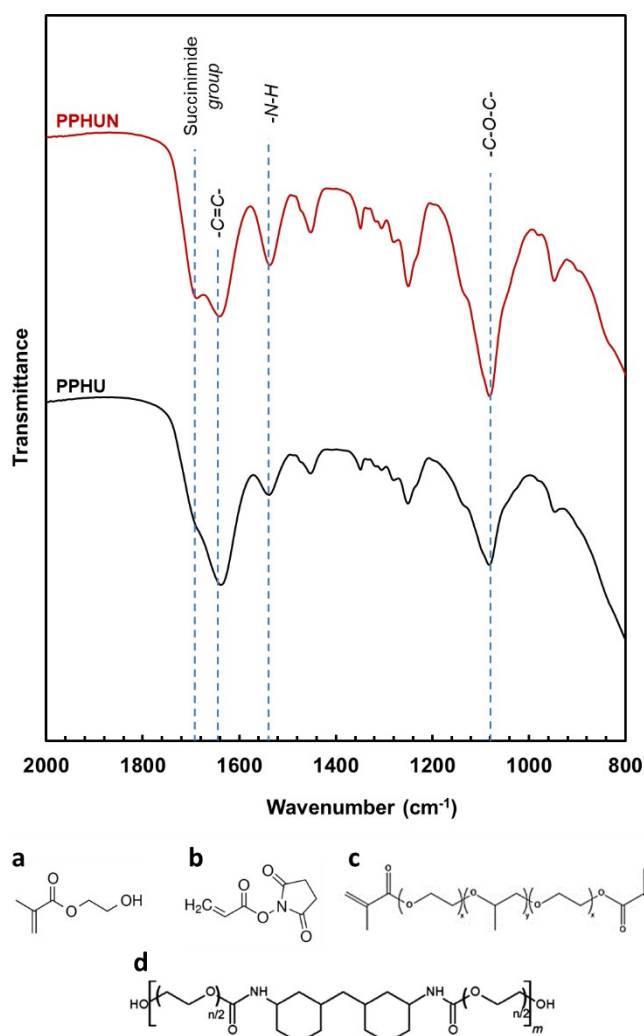
Figure S2. FTIR spectra of the fabricated hydrogel with NAS (PPHUN) and without NAS (PPHU) after synthesis from their building units: HEMA (a), NAS (b), FDMA (c), and HPU(d).

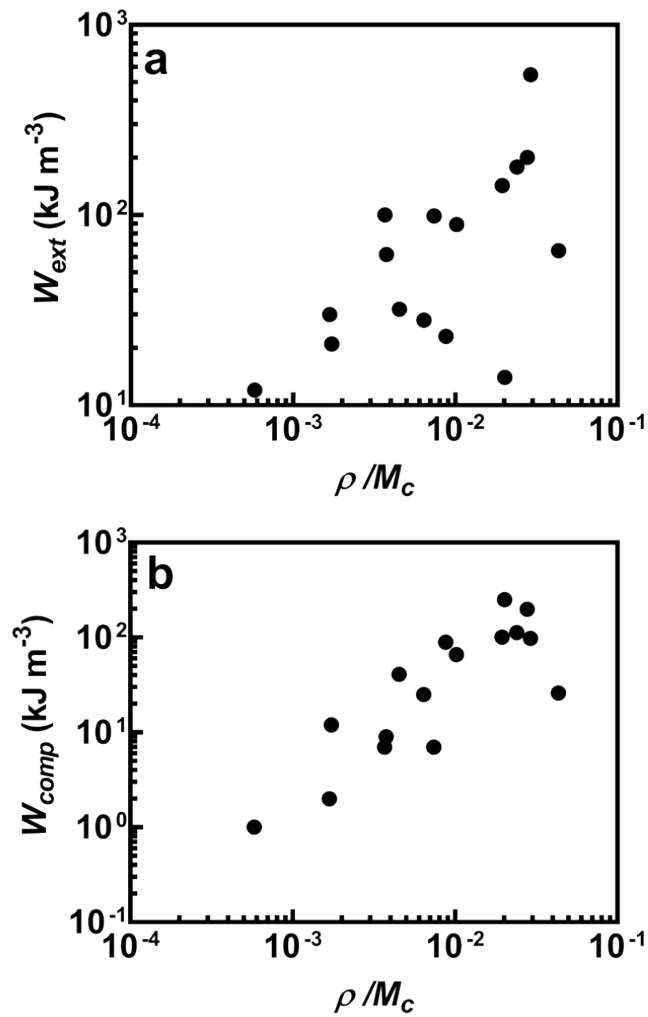**Work of compression and tensile as a function of crosslinkers**

The macroscopic deformation of a network is related to the crosslinkers through the following equation:[5]

$$G_0 = \frac{\rho RT}{M_c}$$

(S1)

where G_0 is the shear modulus of the dry network, ρ is the density of dry network, R is the universal gas constant, T is the temperature, and M_c is the average molecular weight of the chains between two adjacent crosslinking points. The increase in degree of crosslinking result in decrease in the molecular weight between two adjacent crosslink points and subsequent increase in ρ/M_c. It must be noted that the shear modulus, G_0 in Equation S1 is in dry state which is different from the shear modulus in swollen state, G. These two shear moduli are related with each other through the cubic root of swelling ratio:[5]

$$G_0 = G\, q^{\frac{1}{3}}$$

(S2)

For all synthesised PPHUN in this study, the work of extension and compression versus crosslinking component was plotted in Figure S3. As can be seen, by increasing the degree of crosslinking, the network become more entangled and crosslinked therefore, more energy would be required to break the samples. By increasing the ρ/M_c parameter up to two orders of magnitude, the work of extension and compression increased more than two and three orders of magnitude, respectively.

Figure S3. Work of extension (a) and work of compression (b) as a function of crosslinker density

4

References

1.   J. Starling, C. M. da Silva, M. S. Silva Dantas, Â. de Fátima and R. L. Oréfice, *International Journal of Adhesion and Adhesives*, 2011, **31**, 767-774.

2.   J. Guan, Y. Hong, Z. Ma and W. R. Wagner, *Biomacromolecules*, 2008, **9**, 1283-1292.

3.   A. Ramírez-Jiménez, C. Alvarez-Lorenzo, A. Concheiro and E. Bucio, *Radiation Physics and Chemistry*, 2012, **81**, 27-32.

4.   A. Sosnik, D. Cohn, J. S. Román and G. A. Abraham, *Journal of Biomaterials Science, Polymer Edition*, 2003, **14**, 227-239.

5.   G. M. Spinks and S. Naficy, in *Hydrogels*, CRC Press, 2018, pp. 62-81.