Supporting Information

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Novel self-healing nanocomposite hydrogels based on antifouling poly(carboxybetaine) with superior mechanical properties

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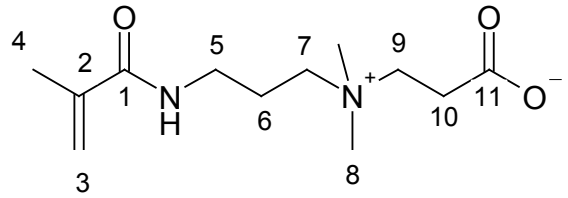
Synthesis of (3-methacryloylamino-propyl)-(2-carboxy-ethyl)-dimethylammonium (carboxybetaine methacrylamide, CBMAA-3) monomer

The carboxybetaine methacrylamide (CBMAA-3) monomer was synthesised as reported earlier[1] (see Scheme 1). Briefly, DMAPM (17 g, 100 mmol) was dissolved in 100 mL of dry THF and cooled to 0 ^{o}C. Subsequently, β-propiolactone (9 g, 125 mmol) was dissolved in 30 mL of dry THF for each monomer and added dropwise under nitrogen for 3 h. The reaction was allowed to proceed for 24 h at 4 ^{o}C. The white precipitate was filtered-off and subsequently washed with dry THF and ether. The product was dried under high vacuum. The structure of the monomer was confirmed by ^{1}H- and ^{13}C-NMR.

Characterisation: ^{1}H- and ^{13}C-NMR



^{1}H NMR (400 MHz, D2O): δ = 1.907 (s, 3H, H^{4}), 1.912-2.09 (m, 2H, H^{6}), 2.64 (t, 2H, H^{10}, $J_{9,10}$ =7.7Hz), 3.06 (s, 6H, H^{8}), 3.29-3.37(m, 4H, H^{5}, H^{7}), 3.53 (t, 2H, H^{9}, $J_{9,10}$ = 7.7 Hz) 5.45-5.46 (m, 1H, H^{3trans}), 5.69-5.70 (m, 1H, H^{3cis}).

^{13}C NMR (100 MHz, D$_2$O): δ = 17.68 (C^{4}),22.35 (C^{6}), 30.82 (C^{10}), 36.31 (C^{5}), 50.62 (C^{8}), 61.30 (C^{7}), 62.05 (C^{9}), 121.44 (C^{3}), 138.92(C^{2}), 172.16 (C^{1}), 176.66 (C^{11}).

Protein Fouling test

In our previous work, we investigated the resistance to non-specific protein adsorption achieved on poly(HEMA) hydrogels when increasing amounts of zwitterionic comonomers were added.[2] All the hydrogels containing zwitterionic comonomers showed an unprecedented reduction of fouling from full blood plasma. In the present work we explore a new type of hydrogels synthesised by copolymerisation of HEMA and CBMAA-3 and physically cross-linked by Laponite XLG. In order to confirm that the material presented herein also possesses antifouling properties, we evaluated the non-specific protein adsorption from a 10 % foetal bovine serum (FBS) solution by UV-Vis spectroscopy.

Hydrogel rods of 2.5 cm length and 0.5 cm diameter were swollen in PBS at 25ºC to equilibrium and then were immersed in 4 mL of 10% FBS. The samples were gently shaken in 10% FBS during 1 hour at room temperature and then removed. UV spectra of FBS before (blank) and after incubating the hydrogel (both diluted 1:4 in PBS) were recorded using a Hitachi U-1900 spectrophotometer in the region λ = 600-200 nm using a 1 cm cuvette (Figure S1).

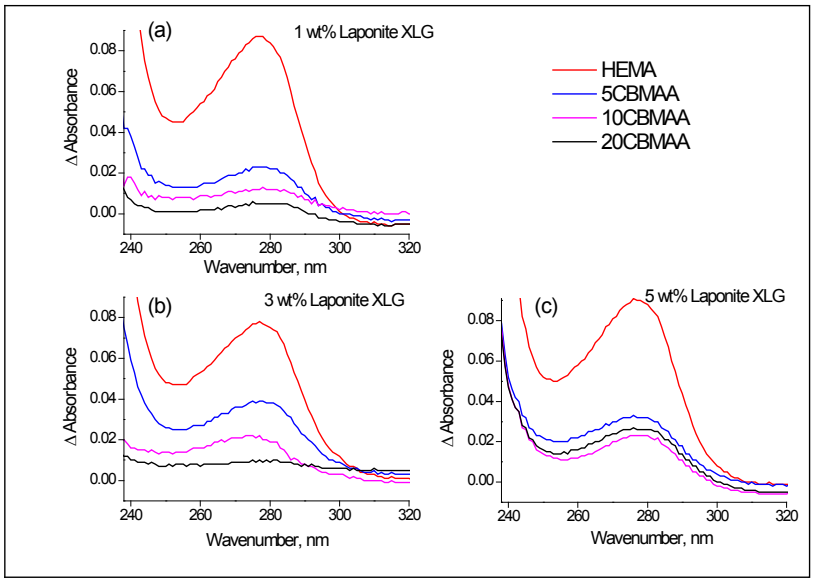


Fig. S1 UV-Vis absorbance spectra of FBS (after subtraction of the blank) after incubating hydrogels with 1% (a), 3% (b) and 5% (c) Laponite XLG.

Protein fouling was estimated from the difference in intensity of the protein absorbance[3] peak at λ = 277 nm by subtracting the spectra of solution contacted with the hydrogels from the blank spectrum. The protein fouling was expressed as the relative amount of adsorbed protein (%) taking the hydrogel with HEMA1LN as the reference. For the determination of the influence of Laponite XLG on the fouling of hydrogels, the fouling on a chemically cross-linked poly(HEMA) hydrogel, without Laponite XLG, was also measured (HEMA0LN). For the purpose of this comparison, HEMA was polymerised in the presence of 80 wt% of water and 0.5 wt% of ethylene glycol dimethacrylate, initiated by ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED).

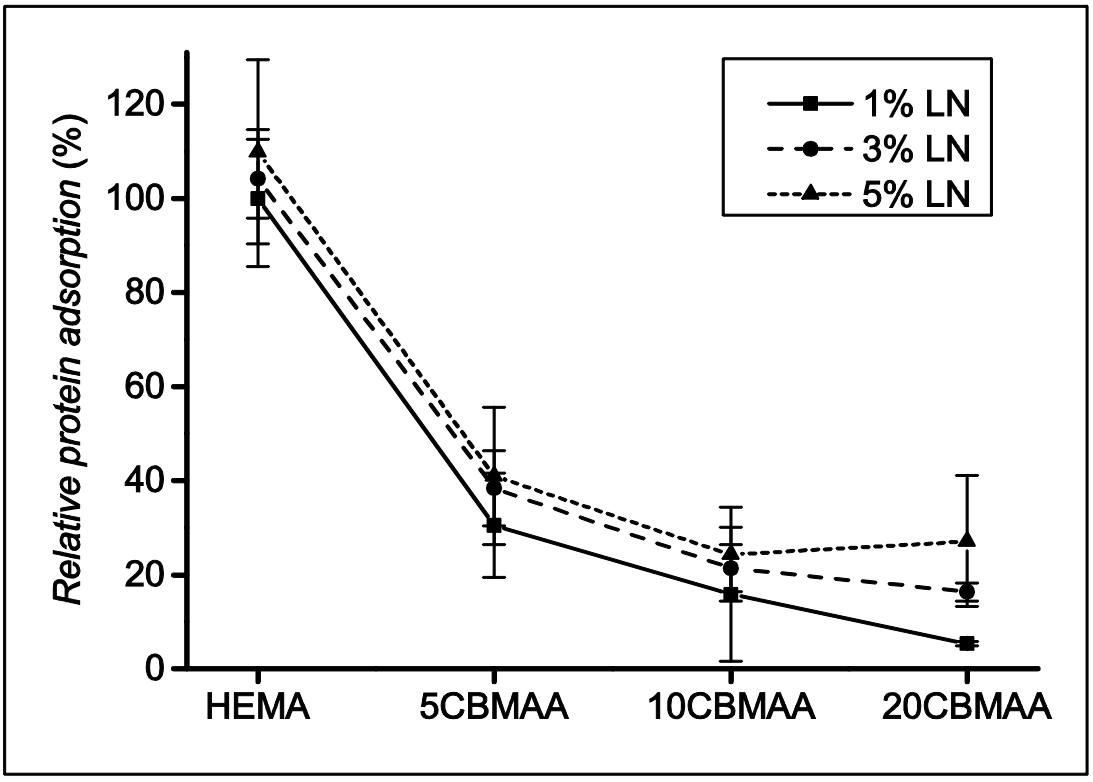


Fig. S2 Non-specific protein adsorption on hydrogels from 10 % FBS, measured by UV-Vis Spectroscopy.

The fouling for HEMA0LN amounted to about 60 % of the fouling observed on the physically cross-linked poly(HEMA) with 1% Laponite XLG and without zwitterionic comonomer (HEMA1LN). Further increases in the amount of Laponite XLG (HEMA3LN and HEMA5LN) caused only a minor increase in the fouling (Fig. S2). However, with the

addition of only 5 mol% of CBMAA-3 comonomer to the polymerisation feed, a dramatic reductions in the fouling (60 to 70 %) are achieved regardless the concentration of Laponite. A subsequent increase in the ratio of CBMAA-3 to 10 mol% further reduced the fouling by 85 %, 80 % and 75 % (Fig. S2) for hydrogels with 1, 3 and 5 wt% of Laponite XLG respectively. Remarkably, for hydrogels containing 20 mol% of CBMAA-3 and 1 wt% of nanoparticles (20CBMAA1LN), a suppression of more than 95 % of the fouling is attained. These results confirm the antifouling characteristics of the newly presented materials, with a trend similar to what has been previously reported.[2]

References

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2. N. Y. Kostina, C. Rodriguez-Emmenegger, M. Houska, E. Brynda and J. Michálek, *Biomacromolecules*, 2012, **13**, 4164-4170.
3. E. Brynda, J. Drobník, J. Vacík and J. Kálal, *Journal of Biomedical Materials Research*, 1978, **12**, 55-65.