Electronic Supplementary Information (ESI)

Doubly crosslinked hydrogels prepared from vinyl-functionalised pH-responsive hollow particle dispersions

Robert Bird[a], Somjit Tungchaiwattana[a], Tony J. Freemont[b] and Brian R. Saunders[a,]*

[a]*Biomaterials Research Group, School of Materials, The University of Manchester,*

Grosvenor Street, Manchester, M1 7HS, UK.

[b]*Tissue Injury and Repair Group, School of Medicine, Stopford Building,*

The University of Manchester, Oxford Road, Manchester, M13 9PT, UK.

Experimental Details

MATERIALS:

Methylmethacrylate (MMA), methacrylic acid (MAA) and *t*-butylmethacrylate (*t*-BMA) were purchased from Aldrich and were used as received. Poly(vinyl pyrrolidone) (PVP) was also purchased from Aldrich (average molecular weight = 40,000 g/mol). FITC-dextran (average molar mass of 4000 g/mol) was purchased from Aldrich and used as received. Poly(MMA-*co*-*t*-BMA) was prepared by conventional free-radical copolymerisation. The copolymer was hydrolysed to give poly(MMA-*co*-MAA). Details concerning these procedures have been given elsewhere[1].

Preparation of pH-responsive hollow poly(MMA-co-MAA) particles

Poly(MMA-*co*-MAA) (1 g) was dissolved in 22 ml of a mixed CH_2Cl_2 (84 vol.%) / methanol (16 vol.%) solvent. This was then added into 60 ml of water containing 4 wt.% PVP and sheared at 10,000 rpm (whilst cooled to 0 ^{o}C), using a Silverson LR4 high speed mixer. The emulsification continued for 30 seconds after addition of the polymer solution. The emulsion was then allowed to stir gently overnight to remove CH_2Cl_2. The product was purified by repeated centrifugation and redispersion in water in order to remove excess PVA. It was then filtered through a 50 μm filter.

Preparation of pH-responsive vinyl-functionalised hollow poly(MMA-co-MAA)/AEM particles

100 g of 1.5 wt% poly(MMA-*co*-MAA) particles maintained at pH of 6.4 using 0.4 M phosphate buffer solution was prepared. To this *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC, 1.4 g, 7.3 mmol) and *N*-hydroxysuccinimide (NHS, 0.9 g, 7.8 mmol) were added. The dispersion was stirred for 15 min at room temperature before adding purified 2-aminoethyl methacrylate hydrochloride (AEM, 0.3 g, 1.8 mmol). The reaction was allowed to proceed for approximately 18 h at room temperature before purification by repeated centrifugation and redispersion in water. The use of pH = 6. 4 ensured that AEM remained chemically stable[2].

Preparation of singly crosslinked poly(MMA-co-MAA)/AEM particles

Crosslinking was performed by heating 1 wt% of poly(MMA-*co*-MAA)/AEM dispersion to 70 °C in the presence of 0.25 wt% ammonium persulfate (APS, 0.011 M) for 8 h. After being allowed to cool the dispersion was purified by repeated centrifugation and redispersion in water.

Preparation of singly crosslinked poly(MMA-co-MAA)/AEM(2) particles

The initial EDC coupling process used to prepare poly(MMA-*co*-MAA)/AEM particles was repeated using the same reagents and proportions as described above. This gave vinyl-functionalised pH-responsive hollow particles.

Preparation of doubly crosslinked poly(MMA-co-MAA)/AEM(2) hydrogels

0.6 g of a 5 wt.% dispersion of poly(MMA-*co*-MAA)/AEM(2) particles was prepared containing, 0.2 wt% Irgacure 2959 and sufficient NaOH (1M) solution to achieve a pH of 8.1. The resulting gel was placed in a 20 mm diameter dish (1 mm depth) and covered using a 0.06 mm thick sheet of polypropylene. The sample was UV irradiated for 2 h at 254 nm using a UV Crosslinker (Ultra-violet Products LtD) with a calculated flux of 7.4 $mWcm^{-2}$.

***PHYSICAL MEASUREMENTS*:**

GPC. GPC was conducted using a Shodex R101 Refractive index detector and was run using THF at room temperature. The columns contained Phenomenex Phenogel 5um beads with 500, 10^4 and 10^6 angstrom pore sizes. The flow rate was 1mL / min.

NMR ^{1}H NMR spectroscopy was conducted using D_2O and a Bruker 300 instrument.

Potentiometric titration was conducted using a Mettler Toledo DL15 Titrator. Titration was performed on 40 ml of a 1 wt.% uncrosslinked dispersion using 0.1M NaOH.

Optical microscopy was conducted with an Olympus BX41 microscope and white transmitted light.

SEM was performed using a Philips XL30 FEG SEM instrument. Dispersions were deposited on SEM stubs by evaporation at room temperature. Gels were freeze-fractured using liquid nitrogen.

TEM measurements were performed using a Philips CEM 200 operating at 200 keV after being deposited onto Holey carbon film TEM grid (Agar Scientific Ltd.).

Fluorescence miscroscopy was conducted using a Nikon Eclipse 50i microscope. The sample was illuminated using a mercury lamp filtered using a DAPI (blue) filter which allows transmission of light at 475 nm.

Rheology measurements were performed at 25 ^{o}C using a TA AR-G2 rheometer using a 250 μm gap with a 20 mm diameter steel plate at 1% strain. The dispersions and gels investigated were allowed to stand for 30 min prior to measurement.

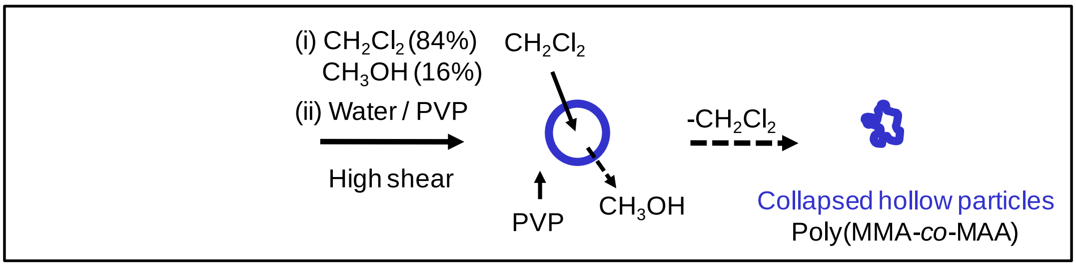
Measurement of Swelling Ratio

A drop of 0.1 wt% dispersions in water was placed between a microscope slide and cover slip. A drop of 0.1 M buffer solution of specific pH was added at the edge of the slip and allowed to diffuse between the slide and slip. Particles were tracked using a digital video camera as the buffer solution diffused through the dispersion and the images analysed to determine average diameters and volume swelling ratios. At least 25 particles were tracked in this way for each data point. The volume swelling ratio is the ratio of the particle volume at a given pH to that for the non-swollen particles. The pH corresponding to non-swollen poly(MMA-*co*-MAA)/AEM particles was 7.0. Swelling experiments for the DX hydrogels were performed using 0.1 M buffer solutions. The swelling ratio was determined gravimetrically.

FITC-dextran permeation experiments

A single drop of a 0.1 wt.% FITC dextran (4,000) was placed on top of a small gel sample. This was left for 30s before the excess was gently removed (touched dry). The gel was then stored in a water-saturated atmosphere for different periods of time before a thin slices were removed and imaged using fluorescence microscopy (See Fig. S7).

SUPPLEMENTARY FIGURES



Scheme S1. Preparation of pH-responsive hollow poly(MMA-*co*-MAA) particles.

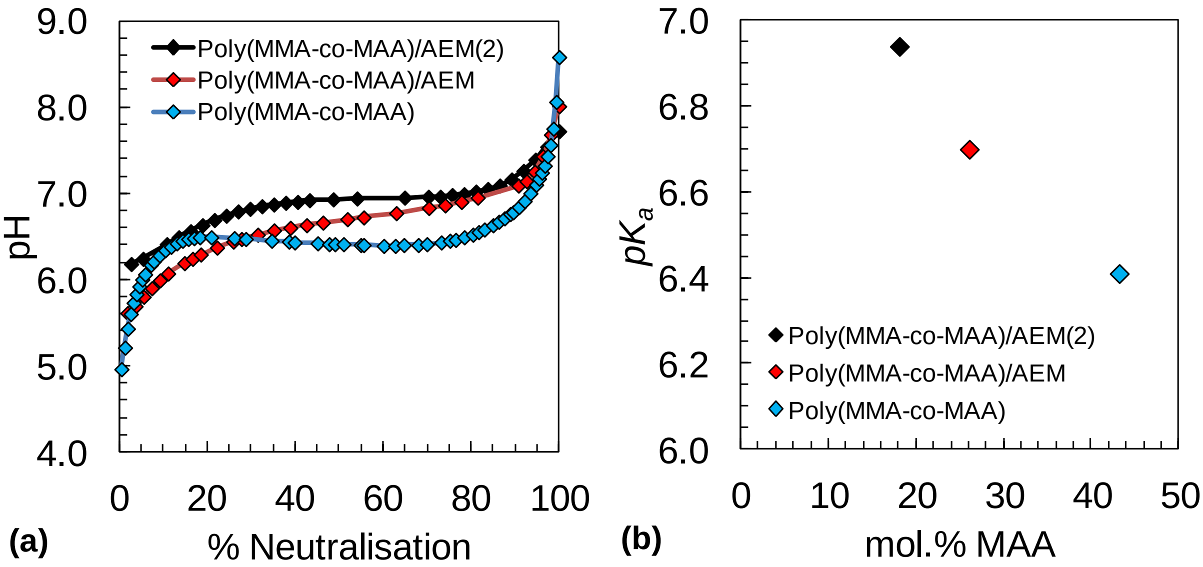


Fig. S1 (a) Titration data and (b) variation of apparent pK_a with mol.% MAA for hollow particles. The identities are shown.

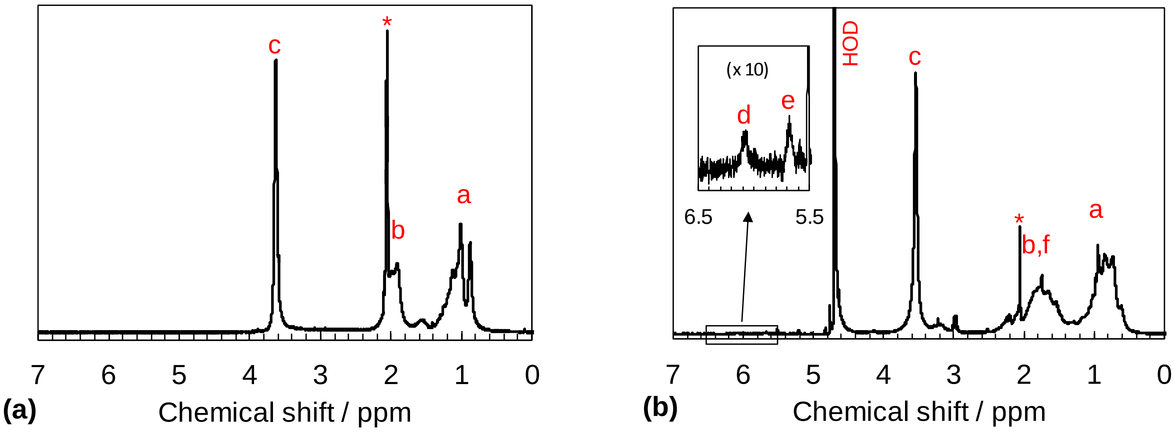


Fig. S2 ^{1}H NMR spectra for (a) poly(MMA-*co*-MAA) measured in acetone-d_6 and (b) non-crosslinked poly(MMA-co-MAA)/AEM in D_2O. The pH was adjusted with a small amount of NaOH to promote dissolution. Solvent peaks are indicated with an asterisk.

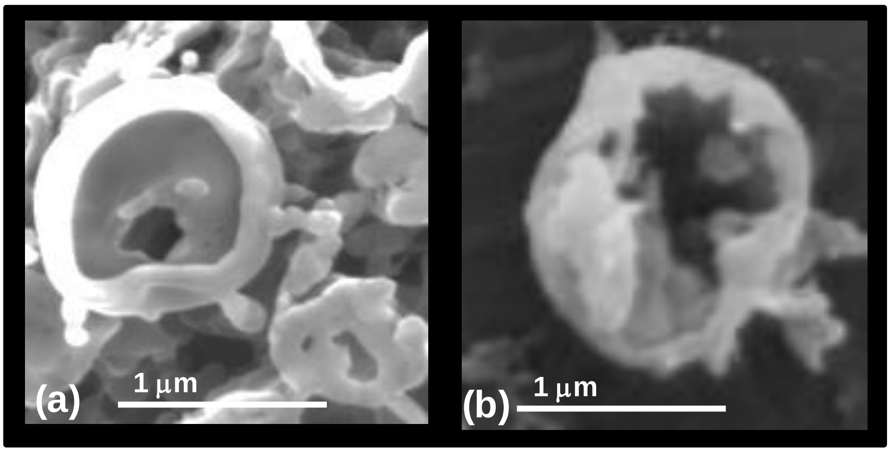


Fig. S3. SEM images of fragmented hollow SX poly(MMA-co-MAA)/AEM particles deposited at pH = 7.7(a) or 8.1 (b).

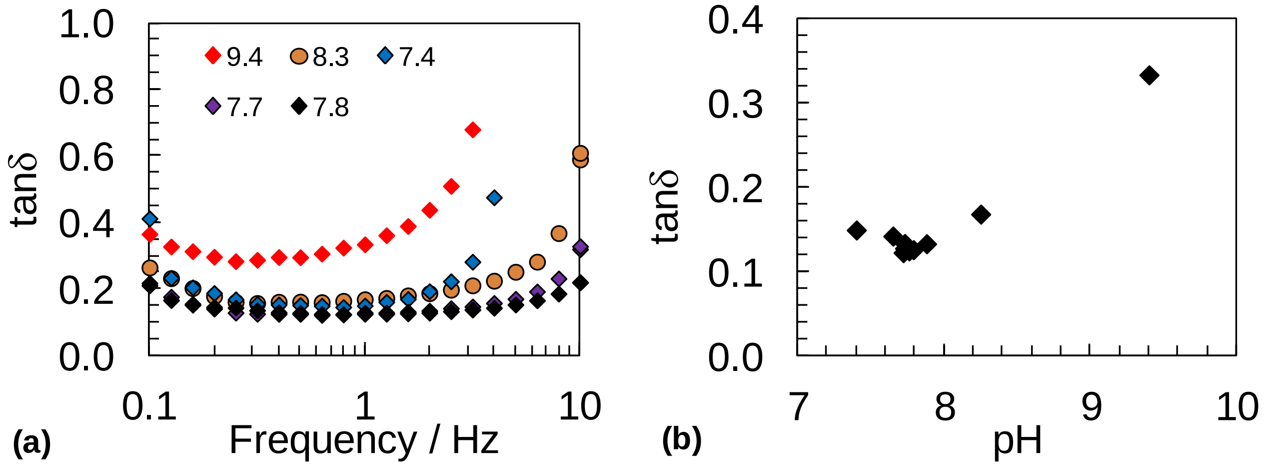


Fig. S4 (a) Frequency sweep data (b) variation of tanδ with pH for SX poly(MMA-*co*-MAA)/AEM physical gels. These data were measured at a strain of 1% and frequency of 1 Hz. All gels had a particle concentration of 5 wt.%

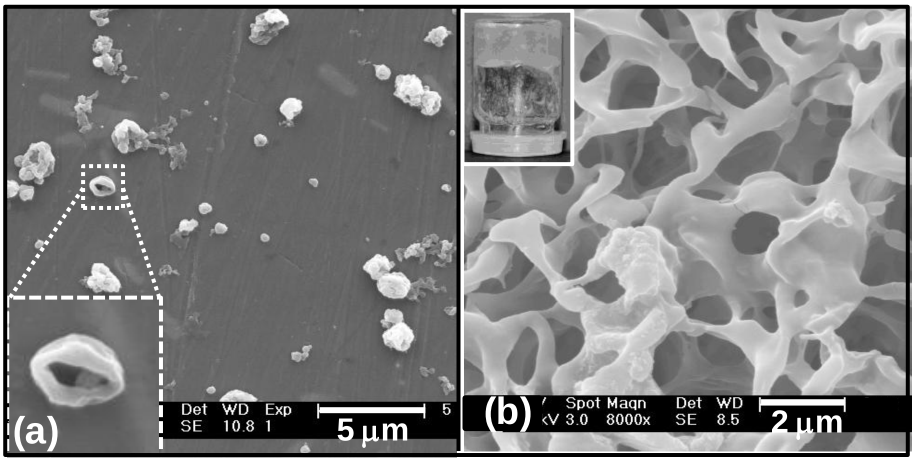


Fig. S5.(a) Shows an SEM image of collapsed SX poly(MMA-*co*-MAA)/AEM(2) particles deposited from a dispersion with a pH of ca. 6. (b) shows an SEM image for a freeze-dried physical gel (gel shown in inset), obtained at a pH of ca. 8.1.

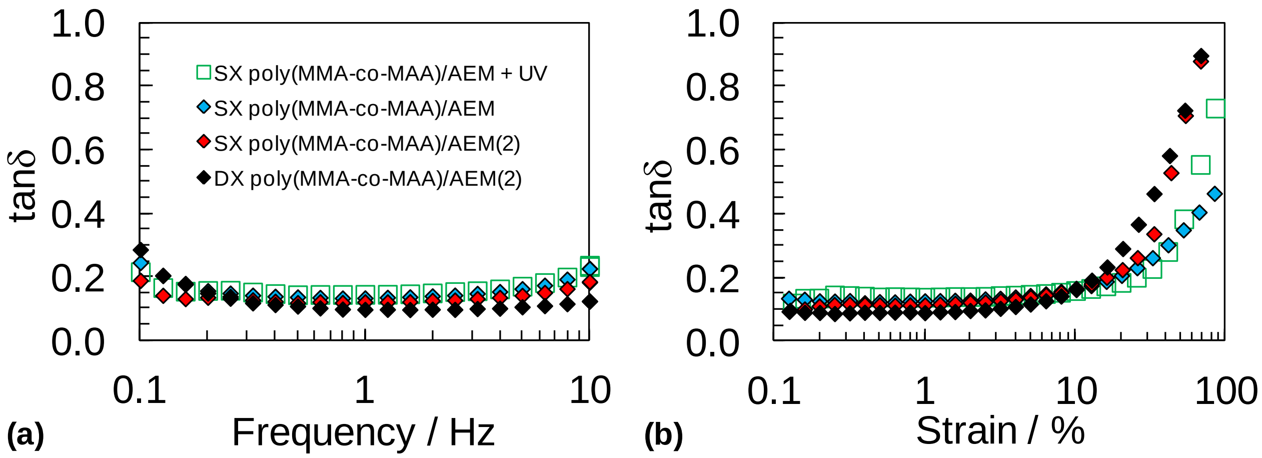


Fig. S6. Variation of tanδ with (a) frequency and (b) strain for DX and SX hydrogels. The particle concentration used was 5 wt% and the pH was about 8.0.

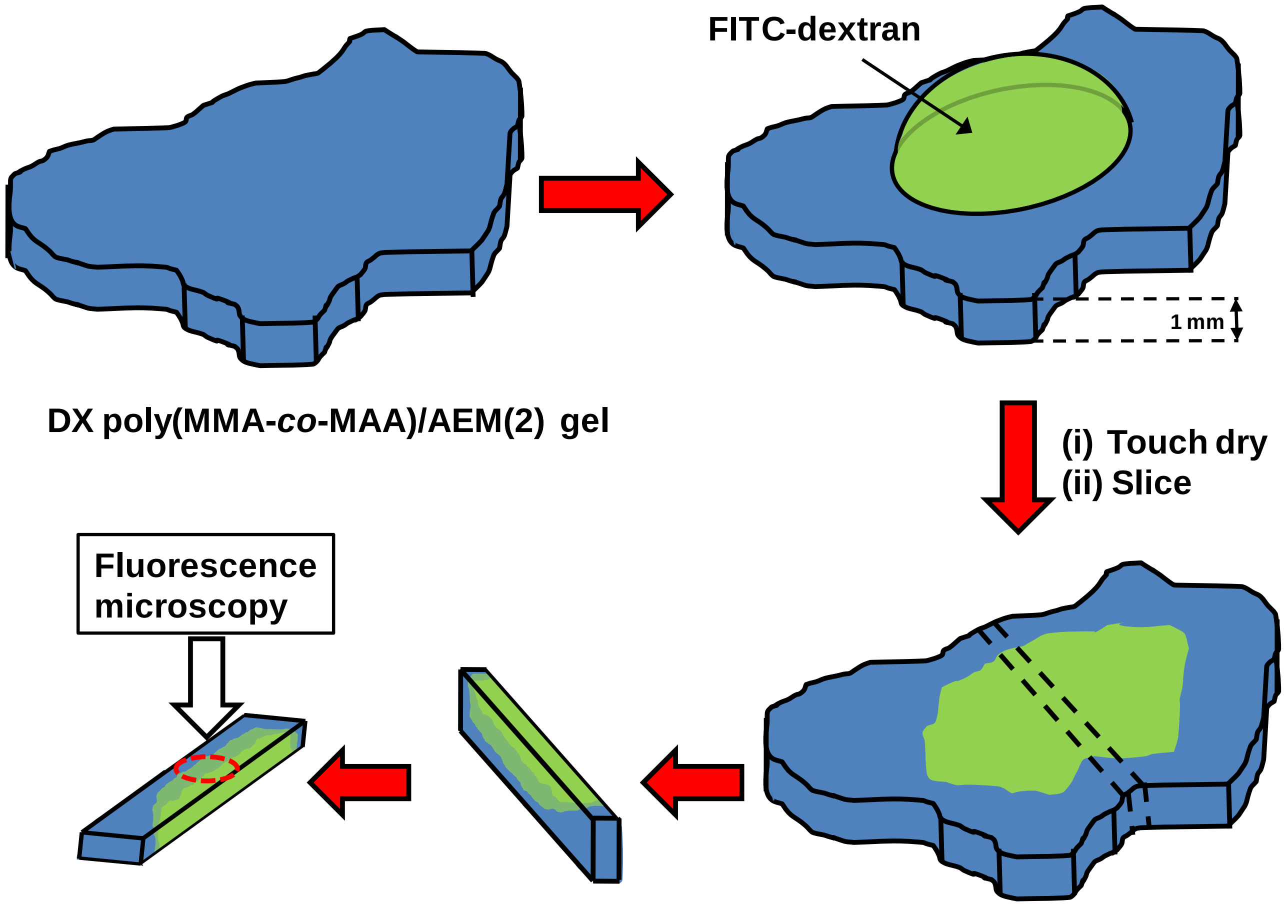


Fig. S7. Depiction of experimental procedure used to probe FITC-dextran permeation through the DX poly(MMA-*co*-MAA)/AEM(2) hydrogel.

References

1. R. Bird, T. J. Freemont, and B. R. Saunders. *Chem. Commun.* 2011, **47**, 1443.
2. K. L. Thompson, E. S. Read, and S. P. Armes. *Polym. Deg. Stab.* 2008, **93**, 1460.