

Electronic Supplementary Information

Zwitterionic cryogels for sustained release of proteins

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Materials and Methods

Materials. Bovine serum albumin (BSA) was purchased from Fisher Scientific (Pittsburgh, PA). [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA, 99.5%) was purchased from Chem-Impex Int'l Inc (Wood Dale, IL), and glycerol dimethacrylate (GDMA, $\geq 90\%$) was purchased from Tokyo Chemical Industry (TCI) (Tokyo, Japan). N,N'-Methylenebis(acrylamide) and myoglobin (from equine heart) were purchased from Sigma Aldrich (St. Louis, MO). 2-Hydroxyethyl methacrylate (HEMA, 97.0%), ammonium persulfate (APS, $\geq 98.0\%$), and N,N,N,N-tetramethylethylenediamine (TEMED, $\geq 99.0\%$) were purchased from Acros Organics (Morris Plains, NJ). Phosphate buffered saline (PBS) with calcium and magnesium was purchased from HyClone (Logan, UT). All chemicals were used as received.

Gel preparation. Gels were prepared using SBMA or HEMA monomers, GDMA or MBA as crosslinker, and APS/TEMED as initiator. For instance, BSA-loaded GDMA-crosslinked SBMA hydrogels were prepared by dissolving 50 mg of SBMA, 7 mg of GDMA and 10 mg BSA in 0.45 mL water. Polymerization was initiated using 50 μL of APS solution (13.6 mg/mL in water) and 0.85 μL of TEMED. Then, the reaction mixtures were poured into plastic syringes (3 mL, inner diameter 0.5 cm) and polymerized at room temperature or $-20\text{ }^{\circ}\text{C}$ for 24 h to prepare RTgels or cryogels, respectively. Cryogels were thawed at room temperature. To remove unreacted monomers and other ingredients, the hydrogel was washed three times with PBS (5 mL) for at least 2h each for the first and second washings, and overnight for the last washing. To determine BSA loading efficiency, the released BSA amount in the washing solutions were measured via UV-Vis spectrophotometer at 280 nm (Genesys 10S UV-Vis spectrophotometer, Thermo Scientific). Myoglobin-loaded poly(SBMA) cryogels were prepared using 2.6 mg myoglobin, other parameters were the same as the BSA-loaded cryogel synthesis conditions.

Protein release experiments. To determine the protein release profiles from the gels, BSA was used as a model protein. BSA-loaded gels were placed into a 20 mL glass bottle filled with 5 mL of PBS (pH 7.4) or MES buffered saline (pH 5.5) and incubated at 37 °C. At predetermined time points, release solutions were collected and replaced with 5 mL of fresh PBS. To determine the released BSA amounts, absorption at 280 nm was measured by using an UV-Vis spectrophotometer and released amounts were calculated using a calibration curve.

Swelling tests. In order to determine the swelling behavior of the hydrogels, wet hydrogels were dried in an incubator at 37 °C, and then weighed to determine the mass of dried samples ($m_{\text{dry gel}}$). Then, they were soaked in distilled water and allowed to swell. The hydrogels were taken at selected time intervals. A kimwipe was used to remove the excess water from the hydrogel surface, and then they were weighed ($m_{\text{wet gel}}$). The swelling ratio of the hydrogel was calculated according to the following equation:

$$\text{Swelling ratio} = (m_{\text{wet gel}} - m_{\text{dry gel}}) / m_{\text{dry gel}}$$

Characterization. The morphology of a cross section of the dried samples was visualized by scanning electron microscopy (SEM). Hydrogels were freeze-dried to protect the pore structures. After gold sputter coating, SEM images of the hydrogels were taken on a FEI Quanta 600i Environmental Scanning Electron Microscope under high vacuum conditions.

Supporting Figures

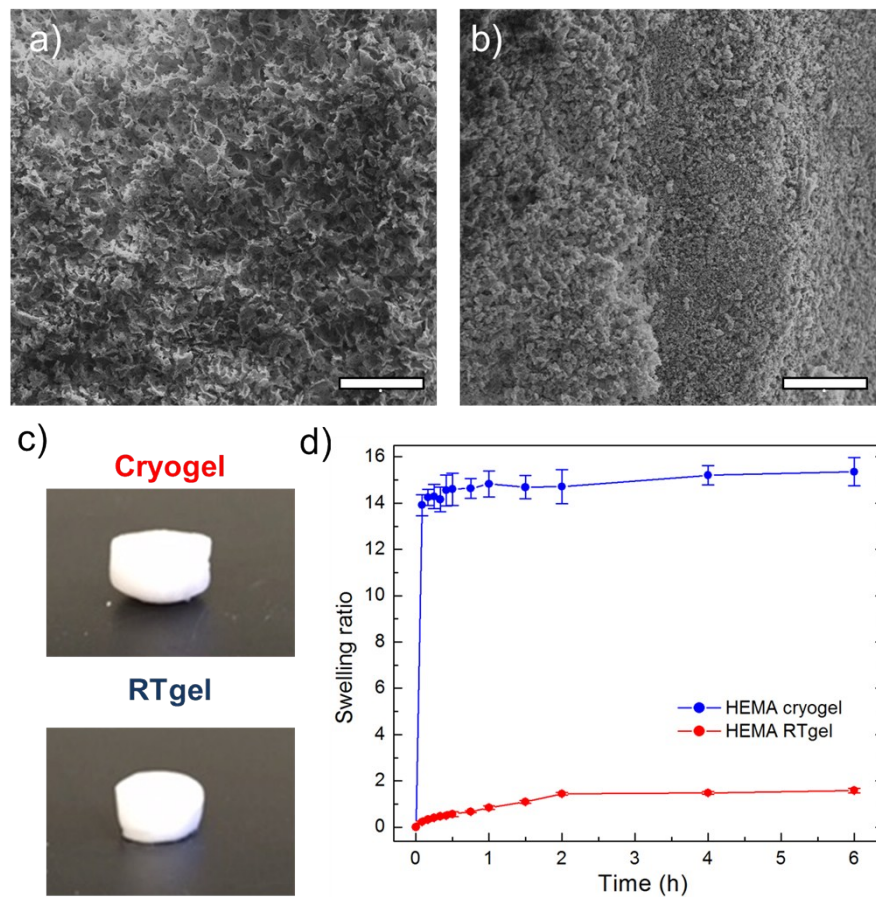


Fig. S1 Characterization of the poly(HEMA) hydrogels. SEM images of (a) poly(HEMA) cryogel and (b) poly(HEMA) RTgel. Scale bars are 250 μm . (c) Photographs of a poly(HEMA) cryogel and RTgel. (d) Swelling of the dried poly(HEMA) hydrogels in water over time.

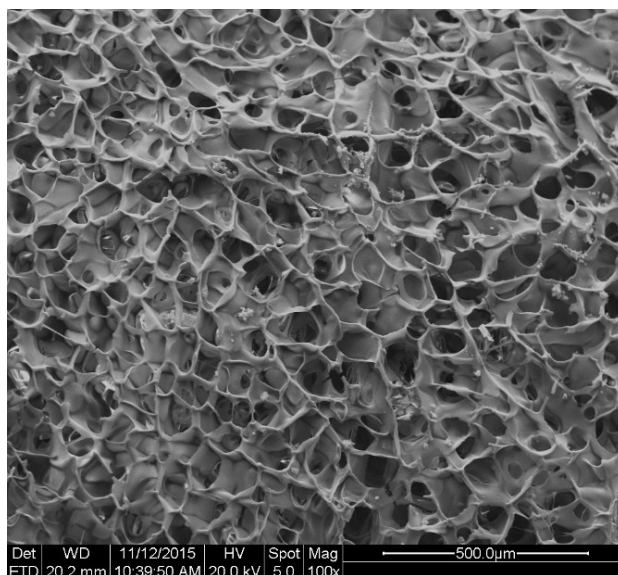


Fig. S2 Scanning electron photomicrograph of a BSA-loaded (20 mg/mL) poly(SBMA) cryogel. Scale bar is 500 μm .

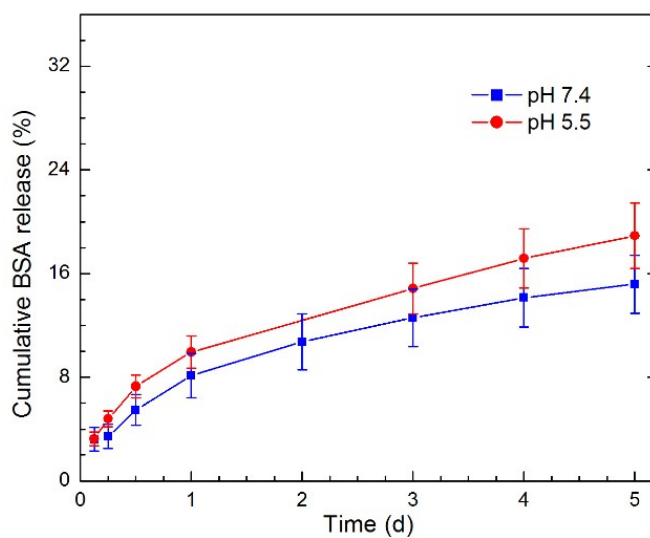


Fig. S3 BSA release from poly(SBMA) cryogels at acidic (pH 5.5) and neutral (pH 7.4) conditions. The gels were prepared using 100 mg/mL SBMA, 14 mg/mL GDMA and 20 mg/mL BSA.

Table S1. Synthesis conditions of different poly(SBMA) and poly(HEMA) gels.

Sample	SBMA (mg/mL)	HEMA (mg/mL)	GDMA (mg/mL)	MBA (mg/mL)	BSA (mg/mL)	Polymerization conditions (temperature and time)
SBMA cryogel 1	100	-	14	-	20	-20 °C, 24 h
SBMA cryogel 2	100	-	-	9.4	20	-20 °C, 24 h
SBMA RTgel	100	-	14	-	20	RT, 24 h
HEMA cryogel	-	47	-	-	20	-20 °C, 24 h
HEMA RTgel	-	47	-	-	20	RT, 24 h