Supporting Information for

Development of dual-component protein microparticles in all-aqueous system for biomedical application

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1. Formation of PAH~BSA microparticles

Bovine Serum Albumin (BSA) and Poly (allylamine hydrochloride) (PAH, M_w 17,500) were dissolved in the dextran phase and the PEG phase respectively at the same concentrations (5, 10, 15 and 20mg/ml). For instance, water with 10*wt*% dextran and 10mg/ml BSA were electrosprayed into an immiscible aqueous phase containing water with 8*wt*% PEG and 10mg/ml PAH. After the droplet phase was electrosprayed into the continuous phase, dextran-in-PEG emulsion droplets were formed. Moreover, the droplets solidified and became filled with complexed polyelectrolytes, due to the complexation of the two oppositely charged components of PAH and BSA induced by the corresponding electrostatic attraction.

2. Determination of the partitioning coefficient of polyanion and polycation in the dextran-rich and PEG-rich phase.

The partitioning coefficient is a ratio of the concentration of a compound in the two immiscible phases of a mixture of two solvents.[1, 2] BSA was used as an example to demonstrate how the partitioning coefficients were obtained experimentally. Initially, a 20*wt*% dextran solution and a 16*wt*% PEG solution were mixed at a 1:1 volume ratio and left sitting until the resultant solution mixture separated into two distinct immiscible phases, with the PEG-rich phase above the dextran-rich phase. Afterwards, equal volumes of the dextran-rich phase and the PEG-rich phase were extracted. Different known amounts of BSA were dissolved in the extracted PEG solutions. Subsequently, the absorbance of the solutions at 280nm was measured using a UV-vis spectrophotometer with the pure extracted PEG-rich solution as a blank. The resultant calibration curve of absorbance as a function of the BSA concentration can be used to calculate the concentration of BSA in the PEG-rich phase, as shown by the plot in Figure S1.

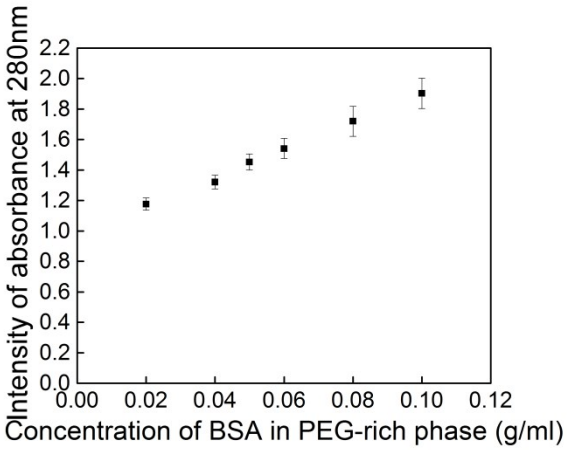

Figure S1. Calibration curve of the absorbance as a function of the BSA concentration in the PEG-rich phase.

The regression equation was A= 9.235$C_{BSA\text{-}PEG}$+0.980, where A was the absorbance value; $C_{BSA\text{-}PEG}$ was the concentration of BSA in the PEG-rich phase. Correlation coefficient R= 0.997, range of concentration= 0.02-0.1g/ml.

A calibration curve of absorbance values as a function of the BSA concentration in the dextran-rich phase was also obtained using the same procedure with the pure extracted dextran-rich solution as blank and used in subsequent measurements to calculate the concentration of BSA in the dextran-rich phase, as shown by the plot in Figure S2.

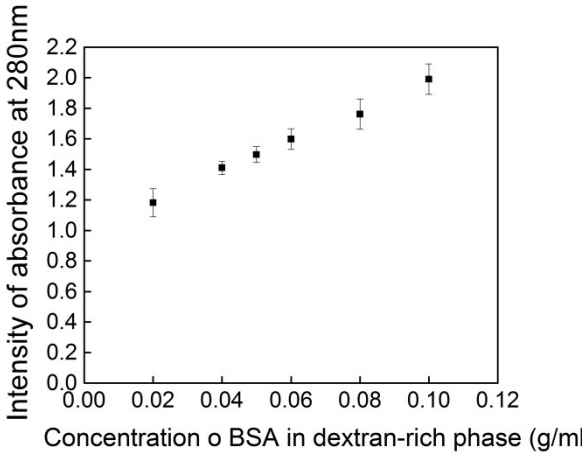

Figure S2. Calibration curve of absorbance as a function of the BSA concentrations in the dextran-rich phase.

The regression equation was $A= 9.835C_{BSA\text{-}dextran}+0.999$, where $C_{BSA\text{-}dextran}$ was the concentration of BSA in the dextran-rich phase. Correlation coefficient R= 0.997, range of concentration= 0.02-0.1g/ml.

Afterward, the concentrations of BSA partitioned into the dextran-rich, and PEG-rich phases can be calculated using the previously obtained calibration curves. Briefly, 0.1g BSA was dissolved in a 2ml mixture of 20wt% dextran solution and 16wt% PEG solution at a 1:1 volume ratio before the mixture was left sitting to obtain a fully phase-separated mixture, with the PEG-rich top phase and the dextran-rich bottom phase both containing equilibrium concentrations of BSA. Afterward, equal volumes of the dextran-rich phase and the PEG-rich phase containing BSA were extracted, and the absorbance values of the two solutions at 280nm were measured using a UV-vis spectrometer as the test samples. Then, the concentrations of BSA partitioned in the dextran-rich, and PEG-rich phases were calculated using the previously obtained calibration curves. Based on an absorbance value of 1.333 at 280nm in the dextran-rich solution, the concentration of BSA in the dextran-rich phase was calculated using the calibration curve as (1.471-0.999)/9.835= 0.048g/ml. Then, the partitioning coefficient of BSA in the dextran-rich phase was determined to be 0.048/0.05= 0.69. Similarly, with an absorbance value of 0.998 for the tested PEG-rich solution, the corresponding concentration of BSA in the PEG-rich phase was calculated using the calibration curve as (0.998-0.980)/9.235= 0.0155g/ml. Then, the partitioning coefficient of BSA in the PEG-rich phase was determined to be 0.002/0.05= 0.04. The same protocols were used to obtain the partitioning coefficients of PAH, Hb, and IgG.

3. Force-deformation curve and elastic modulus for rat red blood cells by AFM

Elastic moduli of natural RBCs from rats were also measured following the elastic moduli measurement by AFM in the Experimental Section. The value was found to be 14kPa, as shown in Figure S3, which was consistent with the values reported in the literature.[3]

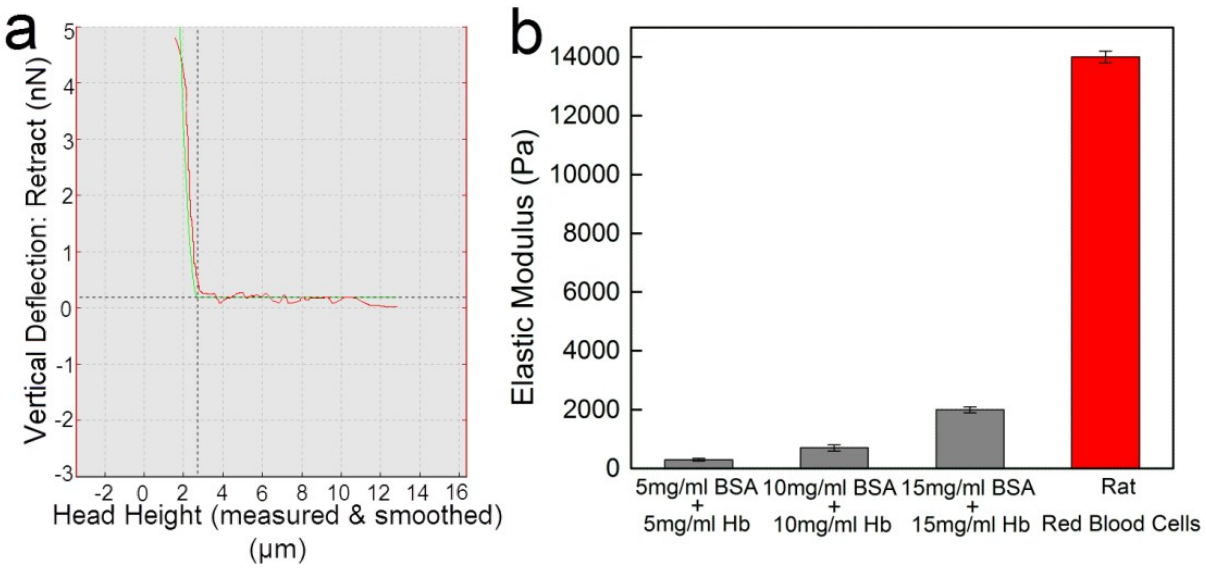

Figure S3. (a) Force-deformation curves for the rat RBCs. (b) Elastic modulus of rat RBCs compared with Hb~BSA particles with different Hb and BSA concentrations.

4. The height and cryo-SEM observation of Hb~BSA microparticles

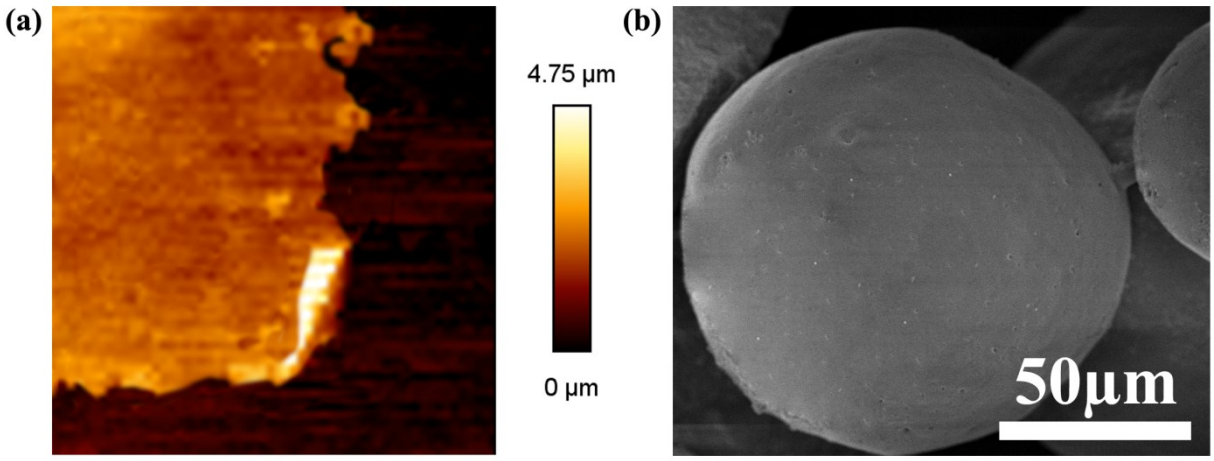

Figure S4. (a) The height of Hb~BSA microparticles estimated by analysis of AFM images of the microparticles. (b) Cryo-SEM images of Hb~BSA microparticles after passing through the capillary device of 80µm.

5. Patterns of polydimethylsiloxane (PDMS) microfluidic blood capillary device

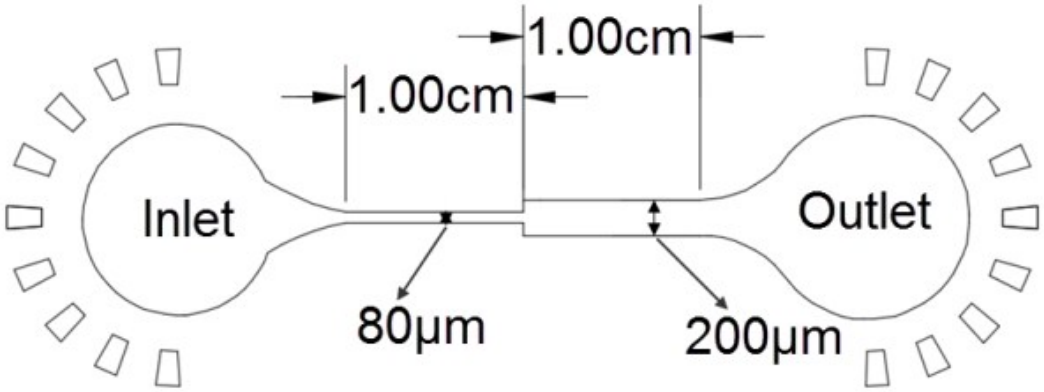

Figure S5. Schematic diagram of the microfluidic blood capillary device mimicking the narrow blood vessels for characterizing the flexibility of hybrid protein hydrogel particles.

6. Zeta potential of DCP microparticles

Samples	PAH~BSA	Hb~BSA	Hb~lgG
Zeta potentials in deionized water (mV)	-0.27 ± 0.15	-0.83 ± 0.22	+0.14 ± 0.03

References:

[1] A. Leo, C. Hansch, D. Elkins, *Chemcal. Rev.* **1971**, *71*, 525.

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[3] Y. Fung, *Biomechanics: Mechanical Properties of Living Tissues by Yuan-Cheng Fung*, *Springer, New York, N. Y.* **1993**.