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Nanofibrillar thermoreversible micellar microgels

Diego Velasco[a], Mokit Chau[a], Heloise Therien-Aubin[a], Alexander Kumachev[a], Ethan Tumarkin[a], Zhongfan Jia[d], Gilbert C. Walker[a], Michael Monteiro[d], Eugenia Kumacheva*[a,b,c]

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Materials

A diblock copolymer, poly(*N*-isopropyl acrylamide)-*block*-polystyrene (PDI=1.09, M_n(SEC)=8200), was synthesized by reversible addition-fragmentation chain transfer aqueous-phase emulsion polymerization[1] and stored as a powder. The degrees of polymerization of the poly(*N*-isopropyl acrylamide) and polystyrene blocks were 37 and 36, respectively. Fluorinated oil, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoro-methyl-hexane (3M™ Novec™ Engineered Fluid, HFE-7500, viscosity 0.77 cP) was purchased from 3M (Canada). A fluorinated surfactant, poly (perfluoropropylene)-block-poly (propyleneglycol)-*block*-poly (ethyleneglycol)-*block*-poly (propylene-glycol)-*block*-poly (perfluoropropylene), was prepared as described elsewhere.[2] The surfactant was dissolved to a concentration of 0.5 wt.% in the fluorinated oil. Phosphate buffer saline (PBS, pH=7.4) was purchased from Gibco-BRL (Rockville, MD). SU-8 photoresist was supplied by MicroChem, U.S.A. Sylgard 184 Silicon Elastomer kit was received from Dow Corning Corp. (Midland, MI). The deionized water was obtained from the Millipore Milli-Q water purification system.

Preparation of micellar solutions and gels

To prepare an aqueous solution of long worm-like micelles, a powder of the poly(*N*-isopropyl acrylamide)-*block*-polystyrene copolymer was induced in an Eppendorf plastic tube and a PBS solution was added to reach copolymer concentration of 11 wt. %. The solution was vortexed for 1-2 min and subsequently, equilibrated for 3 hrs.

A solution of short micelles was prepared as described elsewhere.[1] Briefly, 3 mL of an aqueous solution of long micelles, an unpolymerized styrene monomer and sodium dodecyl sulfate were added to an emulsion of 60 µL toluene in 10 mL of water. The system was equilibrated at room temperature for 4 days. The sample was then sonicated at an ultrasound power of 14 Watt, using pulses of 15 sec and the time intervals of 10 sec between the cycles (Vibra-CellTM, Sonics). After sonication, the solution was freeze-dried. An 11 wt. % solution of short micelles was prepared using the same procedure as for the preparation of the solution of long micelles.

To determine gelation temperature, 5_g, of the micellar solution, it was heated from 25 °C to a desired temperature at a heating rate of 0.25 °C /min and maintained at this temperature for 10 min. Gelation of the solution was verified by flipping off the Eppendorf tube. Liquification of the gel upon cooling was carried out in a similar manner.

Measurements of the Young's modulus of the hydrogels

The mechanical properties of the hydrogels were characterized using Atomic Force Microscopy (AFM). The measurements were performed on 40 µm-diameter particles (microgels) using an atomic force microscope (MFP-3D, Asylum Research, Santa Barbara, CA) with a conducting probe module (a modified cantilever holder with a trans-impedance amplifier) and a software for operating mode-control (ORCA kit, Asylum Research, Santa Barbara). The experiments were conducted at (37 ± 0.1) °C in PBS solution using a gold coated silicon-nitride tipless cantilever with a width of 43 µm (Applied NanoStructures, U.S.A). The spring constant of the cantilever was experimentally determined to be 0.09 ± 0.005 N/m by calibrating it using the thermal noise method.[3]

For each microgel composition, a minimum of five microgels were examined by collecting five force-distance curves for each of them. The force indentation curves were fitted to a modified Hertz law equation using IgorProsoftware.[4]

Characterization of hydrogel structure

To examine the structure of hydrogels, we used a critical point drying method reported elsewhere.[5,6] A copolymer powder was introduced in an Eppendorf plastic tube and deionized water (first) and methanol (second) at an optimized water/methanol molar ratio of 5/1 were added to reach a copolymer concentration of 11 wt. %. (Other molar ratios were used to verify that there is no qualitative change in hydrogel structure). After being vortexed for 1-2 min, the solution formed a gel.[7,8] Prior to scanning electron microscopy (SEM) experiments, the hydrogel was subjected to the critical point drying step by placing it into a metal vessel and gradually replacing water/methanol mixture with liquid CO_2. Subsequently, the temperature and the pressure of the system were raised from 23 °C and 14.69 p.s.i. (1 atm) to the CO_2 critical point of 31 °C and 1072 p.s.i. (73 atm). The liquid CO_2 transferred to the vapour state, without changing the morphology of the gel. The dehydrated gels were imaged using a

Hitachi S-3400N scanning electron microscope.

Release of fluorescent microbeads embedded in the hydrogel

Fluorescence microscopy images of ~6 µm-diameter poly(methyl methacrylate) microspheres (CaliBRITE™3, BD Biosciences, Canada) coated with fluorescein isothiocyanate in a micellar solution at 25 °C and in the micellar gel at 37 °C were captured using a Leica Laser Confocal Microscope (gain: 844V, λ_{ex} =480 nm, power 20 % of 30 mW, detection range: 505-540 nm). The microspheres were introduced in the solution of short micelles, a droplet of the solution was placed on a glass slide and heated to 37 °C to form a gel. The gel was liquefied by cooling it to 25 °C.

Fabrication of microfluidic devices

Photolithographic masters were prepared from SU-8 25 photoresist (Micro-Chem) in bas-relief on silicon wafers. The MF devices were fabricated in poly (dimethylsiloxane) (Sylgard 184, Dow Corning) using a standard soft lithography method.[9] Prior to experiments, a MF device was maintained for 48 h in an oven at 140 °C.

Microfluidic emulsification

A micellar solution and a fluorinated oil HFE-7500 (viscosity 0.77 cP) containing 0.5 wt% of the fluorinated surfactant were supplied to the T-junction droplet generator at 25 °C using two independent syringe pumps (Harvard Apparatus 33 Dual Syringe Pump, USA). To generate microgels from the mixture of long and short micelles, the corresponding solutions were mixed prior to the T-junction in a serpentine channel with a length of 250 mm. Following emulsification, the droplets travelled in the downstream channel toward the outlet of the MF device and exited the device through the outlet tubing (HPFA tubing, Upchurch Scientific, U.S.A.).

Dimensions of the microfluidic device

The height of the MF device was 150 µm. The width of the horizontal channel supplying the HFE-7500 oil phase and the width of the orthogonal channel supplying a micellar solution were 150 and 20 µm, respectively.

Characterization of droplets and micellar microgels

The distribution of the diameters of droplets of the micellar solutions and the corresponding microgels was characterized by analysing optical microscopy images of 100 droplets or microgels by using Image Pro 5.0 (media Cybernetics, USA) software.

Variation in gelation temperature of micellar gels in deionized water and PBS solution

We examined the variation in gelation temperature, T_{gel}, of the solutions of long and short micelles and their mixtures in deionized water and PBS solution. Fig. S1 shows the variation in T_{gel} of the individual micellar solutions and their mixtures, plotted as a function of the ratio of weight concentrations, α, of th the long-to-short micelles. All the systems showed qualitatively similar sol-gel transitions, when the temperature of the solution was changed: the values of T_{gel} were in the range from 27 to 30 °C in PBS solution and from 33 to 38 °C in deionized water. A moderate decrease in T_{gel} occurred with an increasing content of the long micelles. Weak hysteresis was observed in the heating-cooling cycles. The hysteresis was caused by the formation of hydrogen bonds between C=O groups and H-N groups of PNIPAm in the gels, which acted as cross-linking points, impairing chain dissociation upon cooling.[10] We note that ~8-10°C reduction in T_{gel} was observed for micellar solutions in PBS solution, in comparison with that in deionized water. The presence of salts such as NaCl, KCl, Na_2HPO_4 and KH_2PO_4 in the PBS solution led to the "salting out" effect for the PNIPAm chains, thereby lowering their dehydration temperature.[11]

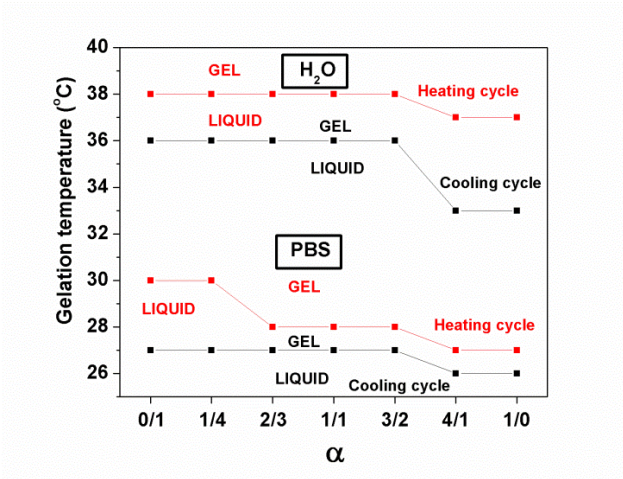

Figure S1. Variation in T_{gel} with the ratio of weight concentrations, α, of long-to-short micelles in PBS solution (pH= 7.4) and in deionized water. The total polymer concentration in the solution was 11 wt%. The data were collected from the temperature ramp experiments by heating (—) and cooling (—) the system at the rate of 0.25 °C /min.

Fig. S1 gives guidance for the preparation and incubation of the micellar hydrogels in deionized water and PBS solution: to achieve gelation, the solutions had to be heated and maintained at the temperature above 27-30 °C and 37-38 °C for PBS and deionized water, respectively, while hydrogel dissociation into micelles was expected upon cooling to 26-27 °C and 33-35 °C respectively.

Variation in the mean diameter of the precursor droplets formed by MF emulsification

Fig. S2 shows the variation in the mean diameter, D, of the droplets formed by individual and mixed micellar solutions, plotted as a function of the volumetric flow rate, Q_o, of the continuous oil phase. The composition of the mixed solutions was varied by changing the ratio of the flow rates of the solutions of long and short micelles from 0/1 to 1/0, while maintaining Q_d at 0.1 mL/h. The diameter of the droplets reduced with increasing

value of Q_o, due to the increasing shear stress imposed on the stream of the micellar solutions. The value of D varied from ~80 to ~115 µm, which was a typical range of microgel diameters used for cell encapsulation,[12] however smaller droplets could be formed by increasing Q_o. For $Q_o \leq 6$ mL/h (when the effects of interfacial tension and viscosity of the droplet phase were significant with respect to the shear stress imposed by the oil phase), the solution of short micelles formed droplets with a smaller diameter.[13] For all the micellar solutions, MF emulsification produced droplets with polydispersity not exceeding 2.6%

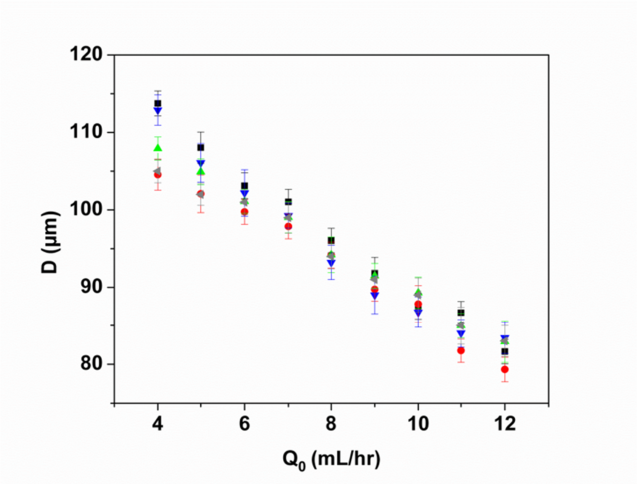

Figure S2. Variation in the mean diameter, D, of precursor droplets formed by MF emulsification of individual solutions of long and short micelles and their mixtures in PBS solution, plotted as a function of the flow rate of the continuous phase, Q_o, for the ratio of flow rates of the solutions of long-to-short micelles of 0/1 (■) , 1/4 (▼), 1/1 (▲), 3/2 (◀) and 1/0 (●). The flow rate of the droplet phase, Q_d, was 0.1 mL/hr. Polymer concentration in the droplet phase was 11 wt.%.

[a] *Department of Chemistry, University of Toronto, 80 Saint George Street, Toronto, Ontario M5S 3H6, Canada*
[b] *Institute of Biomaterials & Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario M5S 3G9, Canada*
[c] *Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario M5S 3E5, Canada*
[d] *Australian Institute for Bioengineering and Nanotechnology. The University of Queensland, Brisbane QLD 4072, Australia*

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