

Electric Supplementary Information

Fabrication of ultrathin nanocellulose shell on tough microparticle via emulsion-templated colloidal assembly: towards versatile carrier materials

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Supplementary Experimental methods, Figs. S1 to S6, and Table S1

Experimental methods

Materials. Softwood bleached kraft pulp was kindly provided by Nippon Paper Industries Co., Ltd. in never-dried state. TEMPO, a 2 M sodium hypochlorite solution, sodium bromide, divinylbenzene, 2,2'-azobis(2,4-dimethylvaleronitrile) (ADVN), methylene blue (MB), acridine orange hydrochloride, carboxymethyl cellulose (CMC, DS = 0.52), ranitidine hydrochloride, and other chemicals were obtained from Wako Chemical Co., Ltd., and used as received. Microfibrillated cellulose (MFC, CELISH PC110S, Daicel Chemical Industries, Ltd) and microcrystalline cellulose (MCC, Avicel PH 101, Fluka) were purchased and used without further purification.

Preparation of CNF aqueous dispersion. CNF was prepared from softwood bleached kraft pulp by TEMPO-mediated oxidation, according to a previous report.³⁰ The softwood bleached kraft pulp (1 g) was oxidized with TEMPO (0.1 mmol), sodium bromide (1 mmol), and sodium hypochlorite (10 mmol) in water (100 mL) at pH 10. The oxidized pulp was washed with distilled water by filtration, and CNFs were dispersed in water using a double-cylinder-type homogenizer (Physoctron, Microtec Niton, Japan) at 7,500 rpm for 2 min and an ultrasonic homogenizer (VP-300N, TAITEC) for 5 min. The unfibrillated fraction (< 5%) was then removed by centrifugation at 10,000g for 30 min. The carboxylate content of the CNF was determined to be 1.7 mmol g⁻¹ by electric conductivity titration. 1.0% w/w CNF dispersion was prepared using an evaporator at 40 °C under reduced pressure.

Preparation of CNF-stabilized microparticle. ADVN (0.01–1.0 mmol) was added to divinylbenzene (1 mL) and gently stirred into an ice bath until complete dissolution. Then, the divinylbenzene containing ADVN (1 mL) was added to the CNF aqueous dispersion (9 mL), and CNF-stabilized Pickering emulsion was formed by ultrasonication (60 W, 20 KHz) in an ice bath. The aqueous emulsion was placed in a water bath at 70 °C and gently stirred at 100 rpm for 12 h, to polymerize the encapsulated divinylbenzene. The reaction product was collected by centrifugation, and the sediment was thoroughly washed with

methanol by filtration with a 0.1- μm pore size PTFE membrane (ADVANTEC, Japan). The microparticles were solvent exchanged to water, and redispersed in water by ultrasonication for 1 min. The weight recovery ratios of the microparticles were as high as $\sim 80\%$, with more than 0.1 mmol/mL of ADVN addition. Therefore, an ADVN concentration of 0.1 mmol/mL to DVB was used in this study.

MB adsorption experiment. Adsorption isotherms were determined by the batch equilibrium method. The adsorption experiments were performed at 23 °C with microparticle (10 mg mL⁻¹). The initial concentrations of MB were varied from 0.1 to 20 mg L⁻¹, and the pH was adjusted to be 2.5 or 7.0 using phosphate buffer (0.01 M in the mixture). Here, we focused on the ionic bonds between the CNFs and MB, which is why this pH range was chosen. After reaching adsorption equilibrium (1 week), the concentration of MB in the solution was determined with a UV-vis spectrometer (V-670, JASCO), by monitoring the absorbance of MB ($\lambda = 665$ nm). The amount of adsorbed MB at equilibrium, q_{eq} (mg g⁻¹), was calculated as follows:

$$q_{\text{eq}} = V(C_0 - C_{\text{eq}})/W, \quad (1)$$

where C_0 and C_{eq} (mg L⁻¹) are the concentrations of MB at initial and equilibrium, respectively; and V (L) and W (g) are the volume of the solution and weight of the microparticle, respectively.

Repeated adsorption/desorption experiments using MB were conducted by a flow method. A 40-mg portion of CNF-shelled microparticles was trapped in a 0.20 μm PTFE membrane filter. A 4-mL portion of MB solution (5 mg L⁻¹) was passed through the filter at a constant flow rate of 100 mL min⁻¹. The amount of MB adsorbed onto the microparticles was determined by measuring the concentration of the filtered MB using a UV-vis spectrometer (cycle 1). The microparticles in the membrane filter were washed with 0.01 M phosphate buffer (pH 2.5 or 7.0), and the filtered solution was collected in a 100 mL measuring flask. The amount of MB removed from the microparticle surfaces was calculated based on the concentration of the filtered solution (cycle 2). The microparticles were thoroughly washed with 0.1 M

phosphate buffer (pH 2.5), to completely remove MB still adsorbed on the surfaces, and then washed with 0.01 M phosphate buffer and water to neutralize the carboxyl groups on the microparticle surfaces. Subsequent cycles were performed in the same way as for cycle 1 and 2, up to cycle 10.

Drug release experiment. Ranitidine and mitomycin C were used as model drugs. Aqueous solutions of the drugs were added to the microparticle dispersion, to adsorb the drugs onto the microparticle surfaces. The initial concentrations of the drugs and microparticles were 15 and 20 mg mL⁻¹, respectively. To remove excess drug, the mixtures were washed with distilled water (10 times dilution × 4 times). The pH of the ranitidine-adsorbed microparticle dispersion was set to be 1.0 with the use of 1 M HCl, 3.0 and 7.0 with the use of phosphate buffers, and 5.0 with the use of an acetate buffer. The pH of the mitomycin C-adsorbed microparticle dispersion was set to be 2.5 and 7.0 with the use of phosphate buffers and 4.5 with the use of acetate buffer. The concentration of the buffers was adjusted to be 0.1 M. The release behaviours of the drugs from the microparticle surfaces under different pH conditions were monitored at 37 °C. The samples were collected and the amounts of released ranitidine were determined with a UV-vis spectrometer by measuring the supernatant concentration after centrifugation.

Analyses. The emulsion size distribution was determined with a laser diffraction particle size analyser ($\lambda = 405$ nm; SALD-7500nano, Shimadzu). The emulsion was dispersed in water and analysed with a flow cell unit. A field-emission scanning electron microscope (SEM) (S-4800, Hitachi) imaging was performed at 1 kV. The samples were coated with osmium by an osmium coater (Neo Osmium Coater, Meiwafoysis) at 5 mA for 5 s. The microparticle dispersion was diluted with water down to 0.01% w/v, and the size distribution and surface charge of the microparticles was analysed with a particle size and ζ -potential analyser (Delsa Max™ PRO, Beckman Coulter) at 25 °C. The mechanical properties of the CNF-shelled microparticles were measured on a microcompression testing machine (MCT-510, Shimadzu) equipped with a flat indenter ($\phi = 20$ μ m) at a load speed of 0.45 mN s⁻¹. The adsorption of acridine orange onto

the microparticles was observed by confocal laser scanning microscopy (FV3000, Olympus). Solid-state ^{13}C cross-polarization/magic-angle spinning (CP/MAS) nuclear magnetic resonance (NMR) analysis was performed on an NMR spectrometer (Bruker Avance III 400, Bruker). The spectra were obtained with a 1.0 ms contact time and 3.0 s repetition time. The samples were loaded into a 4 mm zirconia rotor and spun at 10 000 Hz during the measurements. Thermal analysis was performed by simultaneous thermogravimetric-differential thermal analysis (TG-DTA, TG-DTA2000SE, Netzsch) at a heating rate of $5\text{ }^{\circ}\text{C min}^{-1}$ in a N_2 atmosphere.

Data availability. The data that support the plots within this paper and other findings of this study are available from the corresponding authors upon reasonable request.

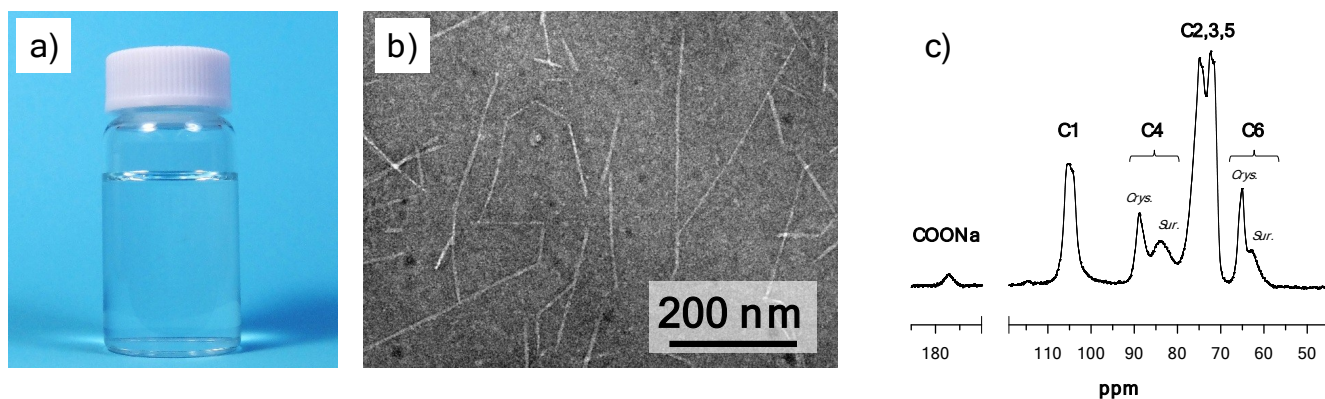


Fig. S1. Photograph of 1.0 % w/w CNF dispersion in water (a), transmission electron microscopy image (b), and solid-state ^{13}C CP/MAS NMR spectra of the CNF (c).

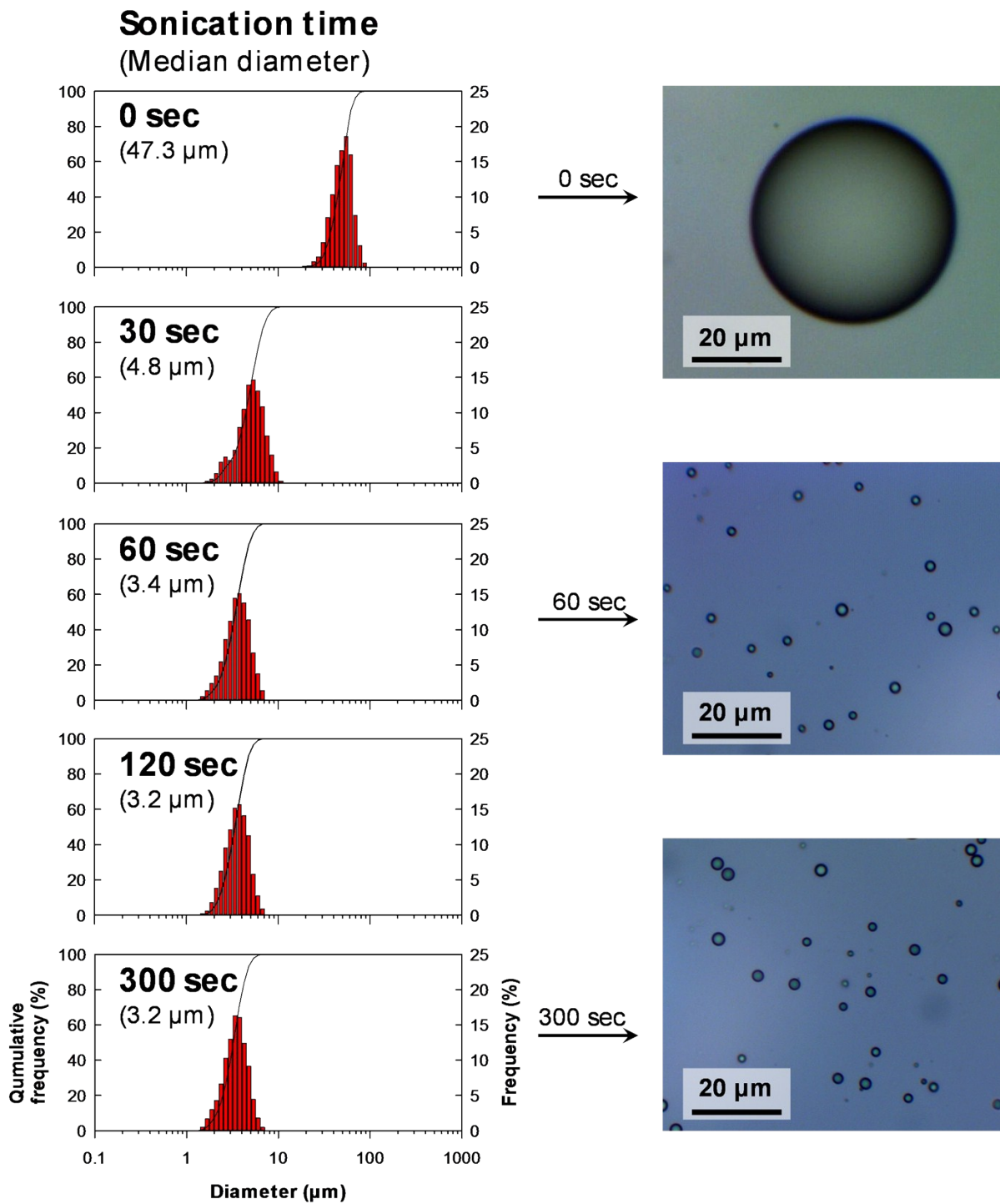


Fig. S2. Size distribution determined by laser diffraction analysis and optical microscopy images of CNF/divinylbenzene Pickering emulsion after different sonication time.

Table S1. Carboxy content and width of nanocelluloses (TEMPO-CNF, MFC, and MCC), and carboxymethyl cellulose used in this study.

	Carboxyl content (mmol/g)	Width (nm)
TEMPO-CNF	1.6	3
MFC ¹	0.05	10-100
MCC ²	0.0	5-10
CMC ³	2.54	-

¹Microfibrillated cellulose, ²Microcrystalline cellulose,

³Carboxymethyl cellulose.

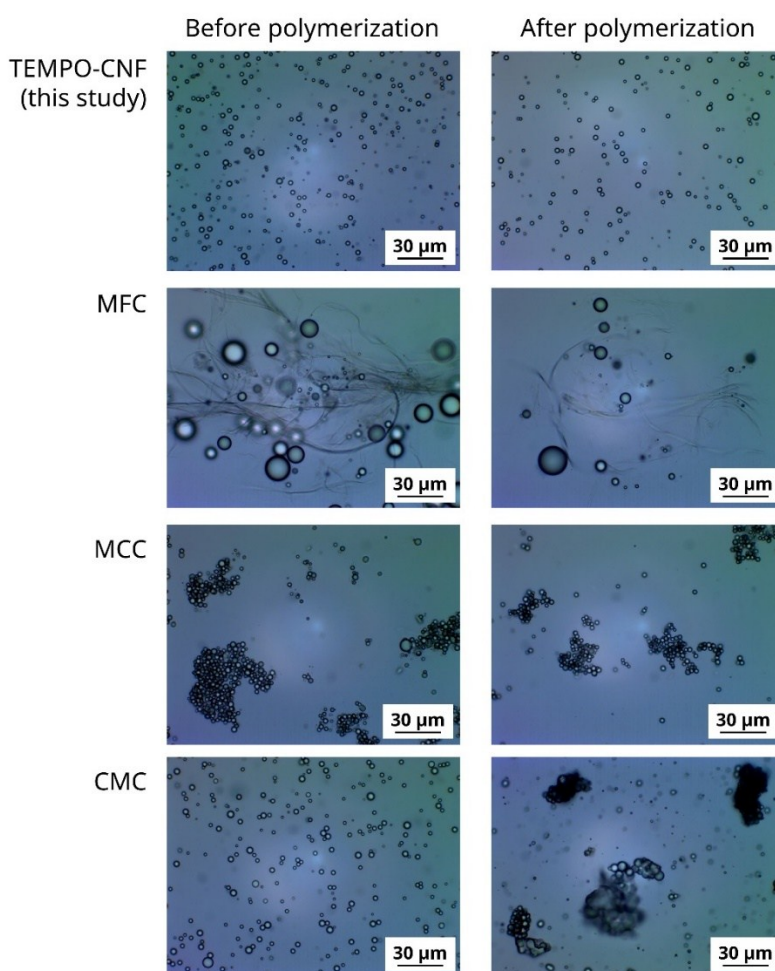


Fig. S3. Optical micrographs of microparticles, using TEMPO-CNF, microfibrillated cellulose (MFC), microcrystalline cellulose (MCC), and carboxymethyl cellulose (CMC) as stabilizers.

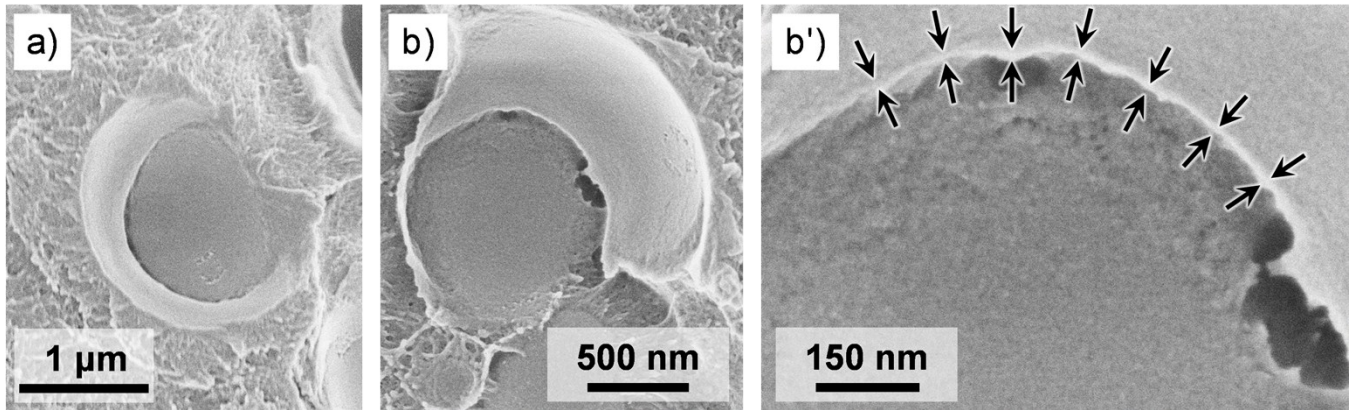
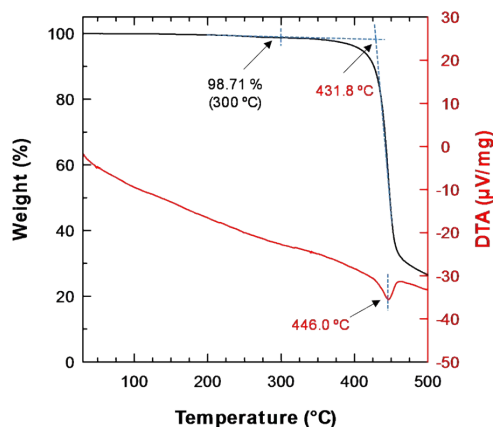
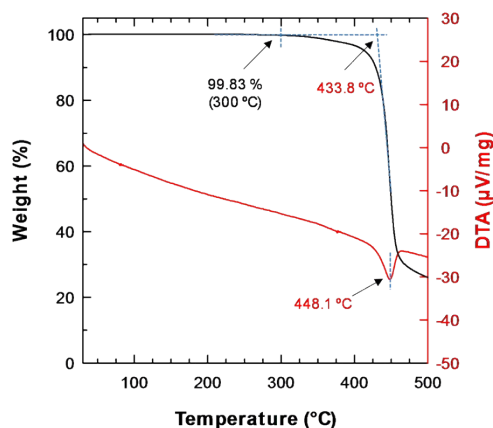


Fig. S4. SEM images of CNF-shelled microparticles: fractured surfaces of the microparticles embedded in acrylic resin (a, b and b').

a) CNF-shelled
microparticle



b) PDVB



c) CNF

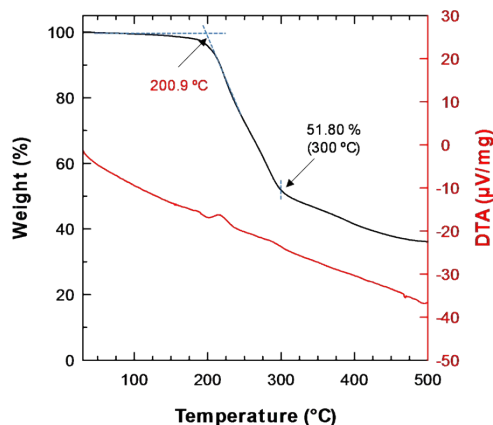


Fig. S5. TG-DTA curves of CNF-shelled microparticle (a), PDVB (b), and CNF (c). Weight content of the CNF in the microparticles was estimated to be ~2.5 wt% (1.6 vol%, assuming that the densities of PDVB and CNF are 1.0 and 1.6 g cm⁻³), as calculated based on weight loss at 300 °C from thermogravimetry-differential thermal analysis.

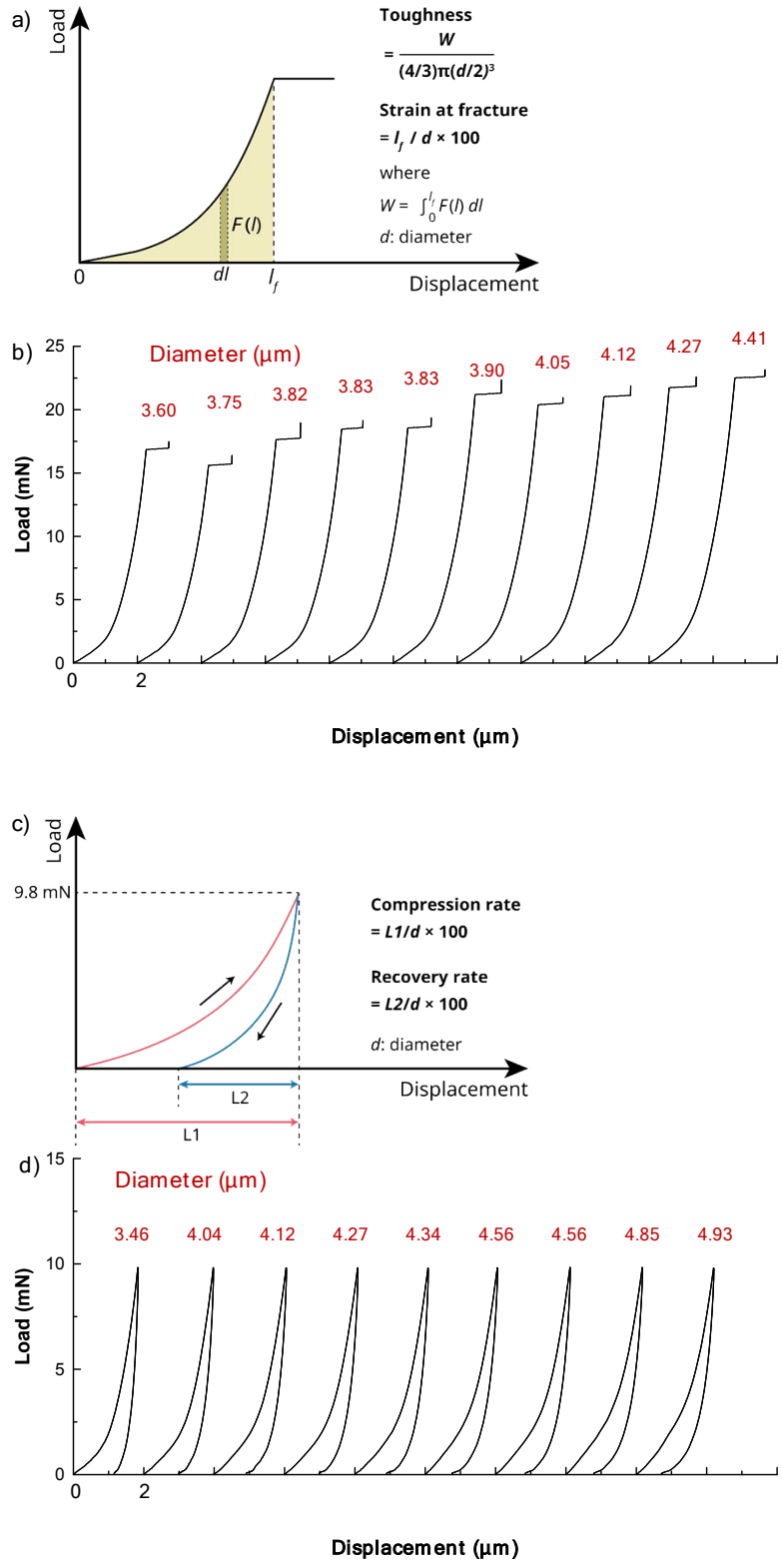


Fig. S6. Load–displacement curves (a, b) and load–unload curves (c, d), shown in order of increasing diameter.