Supplemental material for: Thermoresponsive and biocompatible poly(N-isopropylacrylamide)-cellulose nanocrystals hydrogel for cell growth

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S-1. Methodology - Osmotic dehydration

Hydrogels were prepared at the laboratory scale using the osmotic dehydration principle, as illustrated in Figure S-1.

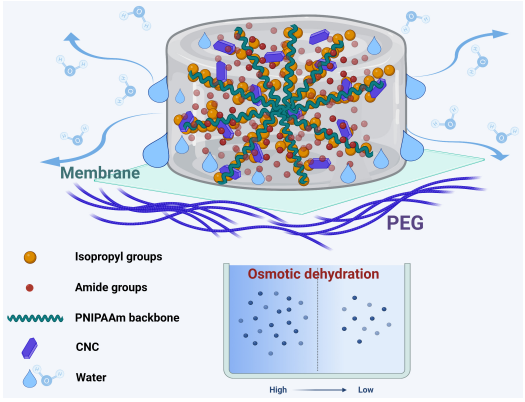

Figure S-1: Schematic of crosslinking of PNIPAAm-cellulose nanocrystals to form hydrogel using osmotic dehydration at room temperature.

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S-2. Methodology - Biocompatibility studies

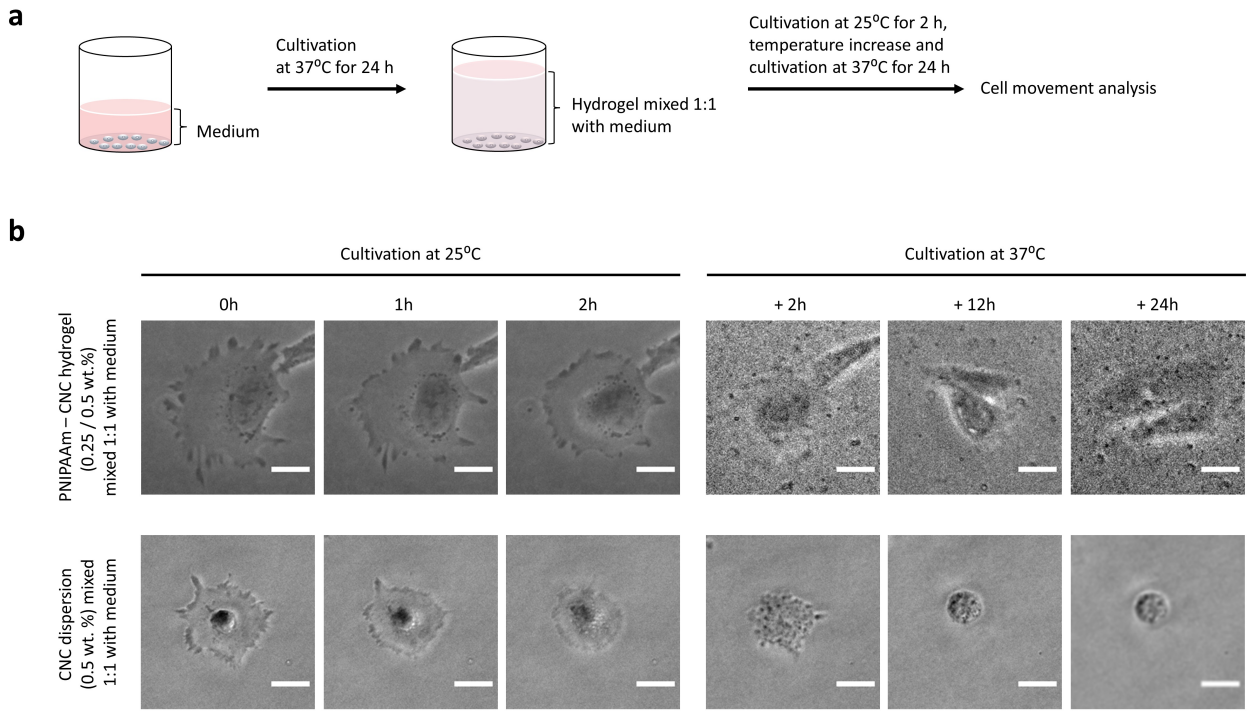


Figure S-2: This Figure is related to Figure 7 in the main manuscript. Biocompatibility of the hydrogel and temperature-mediated actuation of the cells using different setups. (a) Schematic of the alternative biocompatibility assay setup. Hydrogel was mixed 1:1 with cell culture medium, and the mixture was applied on top of cells. Samples were incubated at 25°C and 5 % CO_2 for 2 h and time-lapse imaged every 10 min. Then the temperature was increased to 37°C and the cells were incubated for 24 h while time-lapse imaging was continued at every 10 min. The images were taken with 20x objective by EVOS FL auto microscope. Snaphots of single cells below PNIPAAm-CNC hydrogel mixed 1:1 with medium or CNC solution mixed 1:1 with medium at different time points are shown.

S-3. Interaction between PNIPAAm and CNCs

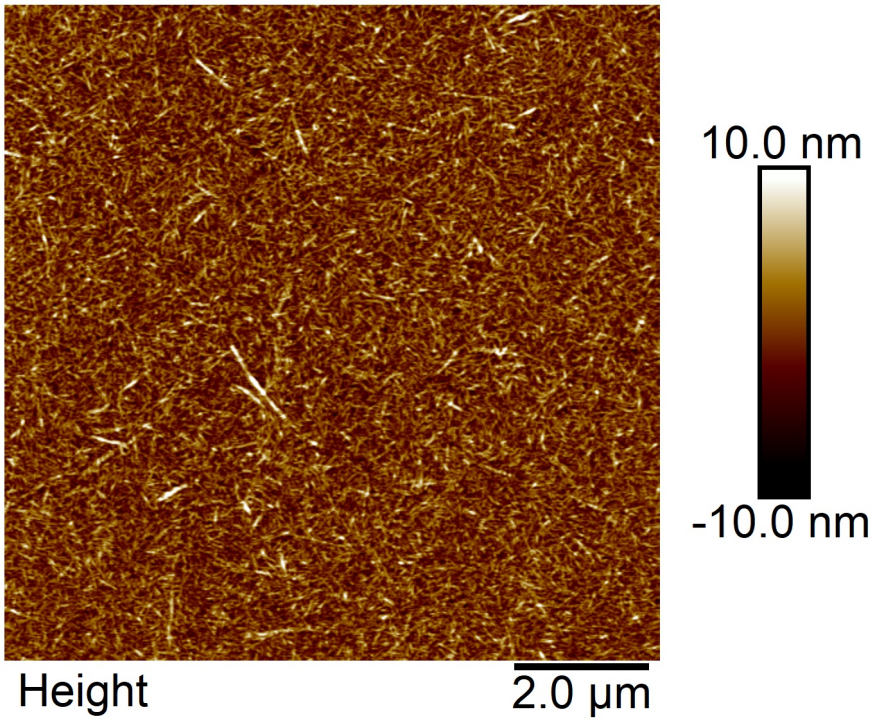


Figure S-3: AFM image of carboxylated CNCs from dissolving pulp. The RMS (root-mean-square) roughness of the film is 2.17 nm.

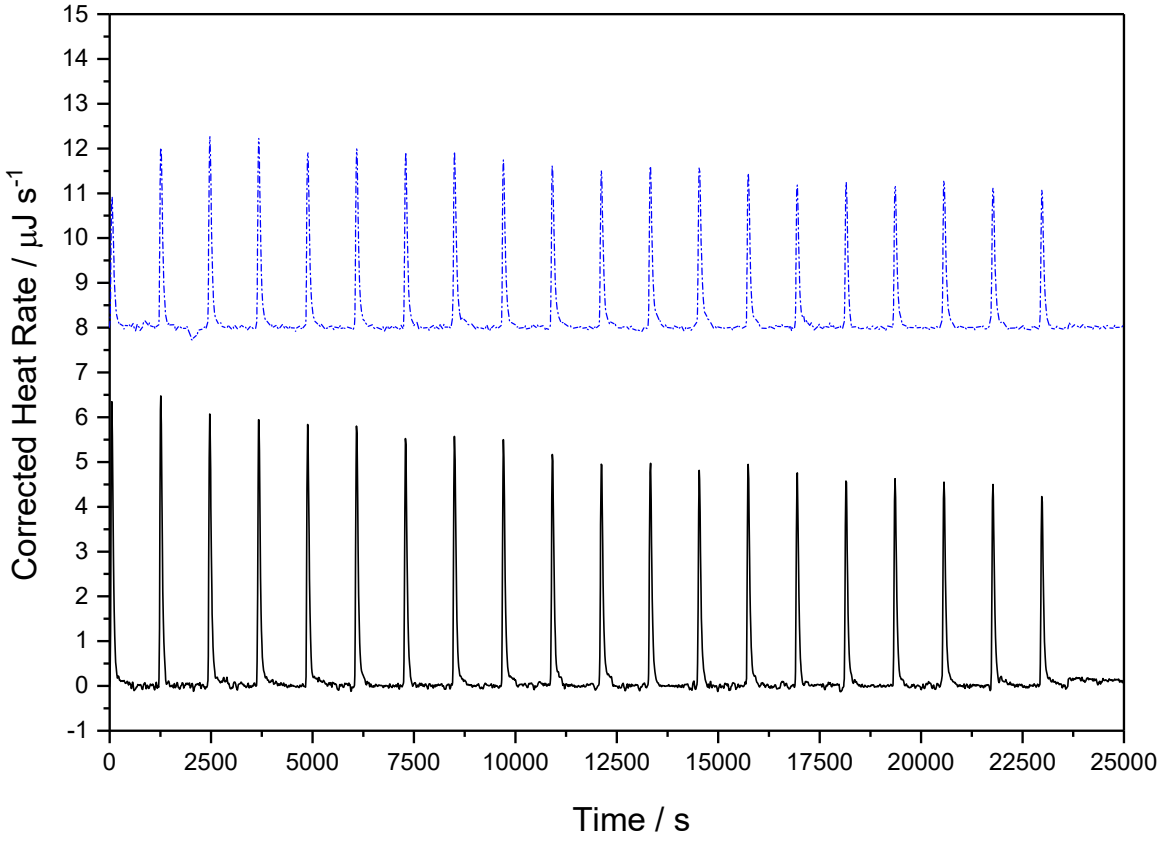
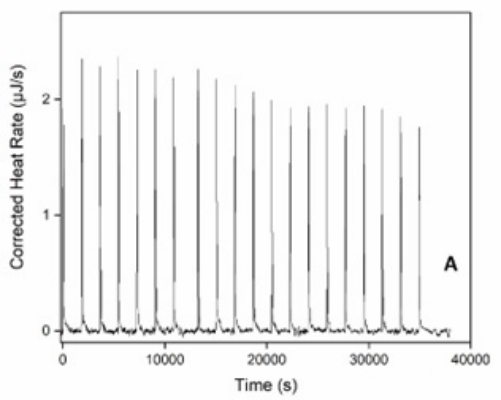
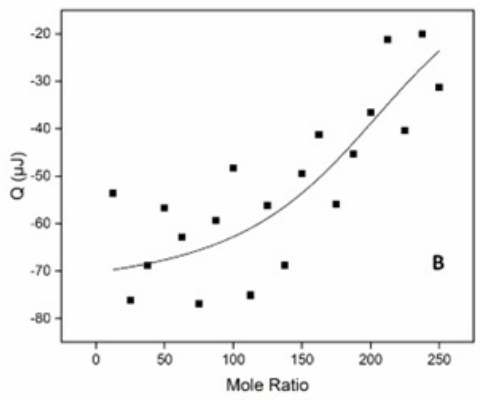


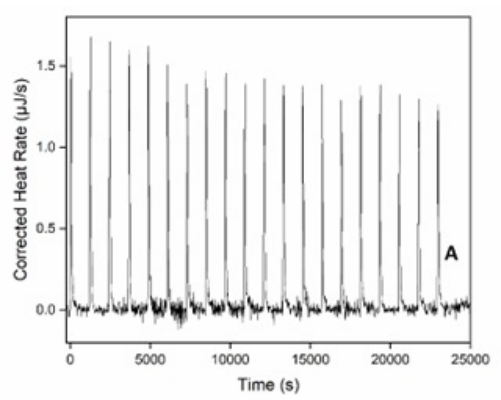
Figure S-4: Calorimetric trace (solid line) with the dilution of 3.8 wt. % PNIPAAm to water (dash dotted line).



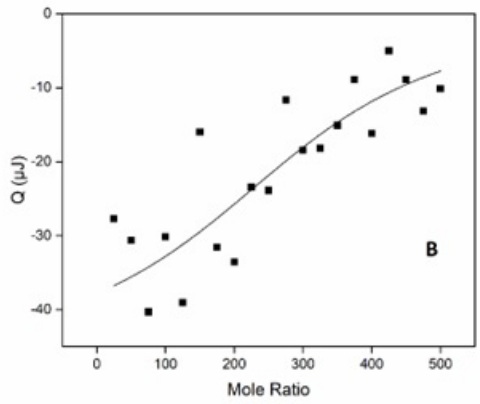
(a) 2 wt. % PNIPAAm; 0.5 wt. % CNC



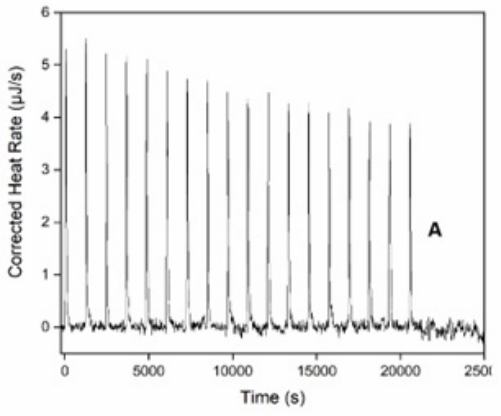
(b) 2 wt. % PNIPAAm; 0.5 wt. % CNC



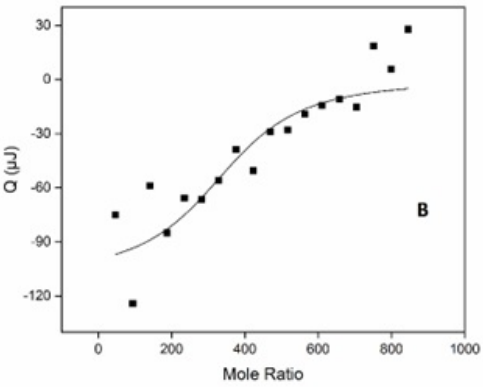
(c) 2 wt. % PNIPAAm; 0.25 wt. % CNC



(d) 2 wt. % PNIPAAm; 0.25 wt. % CNC



(e) 3.8 wt. % PNIPAAm; 0.25 wt. % CNC



(f) 3.8 wt. % PNIPAAm; 0.25 wt. % CNC

Figure S-5: (A) Isothermal titration calorimetric trace (ITC) to study the thermodynamic characteristics of the interaction between CNC and PNIPAAm; (B) Integral plot obtained performing subsequent injections of 10 μL 2-3.8 wt. % PNIPAAm to 800 μL 0.25-0.5 wt. % CNC, and fit according to the Levenberg-Marquardt algorithm. Chemical structures of the compounds of interest and values obtained by ITC for the adsorption of PNIPAAm on CNCs.

Table S-1: DSC endotherms of 0.25-4 wt.% PNIPAAm - 0.5, 3.5, 6 % CNC hydrogels.

CNCs, wt.%	PNIPAAm, wt.%	endo at °C	J g^{-1} at ≈ 30°C	endo at °C	J g^{-1} at ≈ -10°C
0.5	0.25	32.1	0.13		
	0.5	31.6	0.09	-10.2	7.4
	1	31.2	0.18	-9.5	9.6
	2	30.8	0.31	-9.8	13
	4	32	1	-10.4	1
3.5	0.25				
	0.5	32.3	0.93	-10.3	1.2
	1	32.2	0.35	-10.3	4.2
	2	30.8	0.44	-10.2	8.3
	4	32.5	0.9	-10.3	0.4
6	0.25			-9.3	8.7
	0.5	30.7	0.09	-10.2	7.7
	1	30.5	0.22	-10	7.8
	2	32	0.32	-9.7	4.8
	4	31.3	0.74	-9.8	5

S-4. Rheology

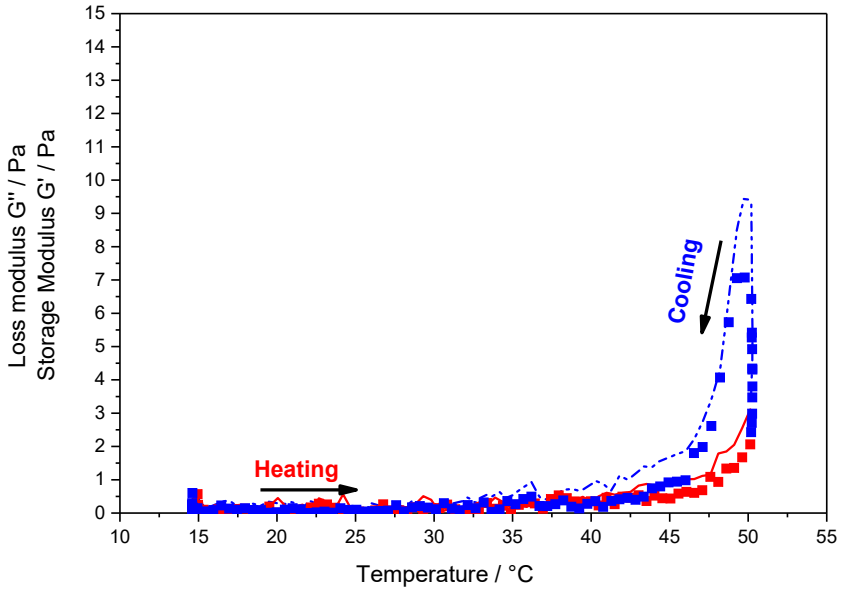


Figure S-6: Storage and loss modulus of pure 0.25 wt. % PNIPAAm.

S-5. Supplementary videos

Movies S1-S6 contain original time-lapse data of cell cultivation at 25°C and 37°C for medium control 1 and 2, PNIPAAm (0.25 wt. %) - CNC (0.5 wt. %) hydrogel, PNIPAAm (0.25 wt. %) - CNC (0.5 wt. %) hydrogel mixed 1:1 with medium, CNC (0.5 wt. %) dispersion, CNC (0.5 wt. %) dispersion mixed 1:1 with medium. Movies S7-S14 contain original original time-lapse data of cell cultivation either at 25°C, or at 37°C, where the movement of individual cells have been tracked.

Supplementary Movie S1: Cell cultivation at 25C_37C_Medium control 1
Supplementary Movie S2: Cell cultivation at 25C_37C_Medium control 2
Supplementary Movie S3: Cell cultivation at 25C_37C_PNIPAAM_CNC hydrogel
Supplementary Movie S4: Cell cultivation at 25C_37C_PNIPAAM_CNC hydrogel mixed with medium
Supplementary Movie S5: Cell cultivation at 25C_37C_CNC dispersion
Supplementary Movie S6: Cell cultivation at 25C_37C_CNC dispersion mixed with medium
Supplementary Movie S7: Cell movement analysis at 25C_Medium control 1
Supplementary Movie S8: Cell movement analysis at 37C_Medium control 1
Supplementary Movie S9: Cell movement analysis at 25C_Medium control 2
Supplementary Movie S10: Cell movement analysis at 37C_Medium control 2
Supplementary Movie S11: Cell movement analysis at 25C_CNC_PNIPAM hydrogel
Supplementary Movie S12: Cell movement analysis at 37C_CNC_PNIPAM hydrogel
Supplementary Movie S13: Cell movement analysis at 25C_CNC_PNIPAM hydrogel mixed with medium
Supplementary Movie S14: Cell movement analysis at 37C_CNC_PNIPAM hydrogel mixed with medium