Supporting Information

New insights into the HIFU-triggered release from polymeric micelles

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Experimental Section

Analytical methods

Spectrophotometry

The measurements of the amount of release are based on the decrease of nile red (NR) fluorescence upon the release of NR from the core of the micelle into the aqueous solution. The fluorescence of NR depends strongly on the polarity of its environment. Releasing the NR from the hydrophobic core of the micelles into the buffer solution causes a large increase in polarity, which leads to a substantial red shift of the emission spectrum as well as significant decrease of the quantum yield and fluorescence lifetime (figure 4A+B).

After HIFU exposure, the micellar dispersions were collected and the fluorescence emission spectrum (570 – 700 nm) was recorded on a Varian Cary Eclipse fluorescence spectrophotometer with an excitation wavelength of 550 nm. To calculate the percentage of released NR the following equation was used:

$$\text{NR released (\%)} = [(F_0 - F_{HIFU}) / (F_0 - F_{aa})] \times 100\%$$

where F_0 is the fluorescence emission peak intensity at ~600 nm recorded before ultrasound. F_{HIFU} is the fluorescence emission peak intensity at ~600 nm recorded after exposing the NR-loaded micelles to HIFU. F_{aa} is the fluorescence emission peak intensity at ~600 nm of NR (0.5 µg/mL) in ammonium acetate buffer in the absence of polymer.

Dynamic Light Scattering

The average size, polydispersity index (PDI) and the scatter intensity (proportional to number of micelles per volume unit) of the NR-loaded micelles were determined by dynamic light scattering (DLS). The DLS experiments were performed with a Malvern CGS-3 multi-angle goniometer (Malvern Ltd., Malvern, UK) equipped with a JDS Uniphase 22 mW HeNe 632-nm laser, an optical fiber-based detector and a digital LV/LSE-5003 correlator. Autocorrelation functions were analyzed by the cumulants method (fitting a single exponential to the correlation function to obtain the mean size and the PDI) and the CONTIN routine (fitting a multiple exponential to the correlation function to obtain the distribution of particle sizes). The measurements were performed in triplicate at 25 °C and at a 90° angle.

Gel Permeation Chromatography

The molecular weight of the polymers was measured using gel permeation chromatography (GPC) before and after HIFU exposure. In short, two serial Plgel 3 μm MIXED-D columns (Polymer Laboratories) were used with a Waters System (Waters Associates Inc., Milford, MA) with a differential refractometer model 410. DMF containing 10 mM LiCl was used as the eluent at a flow rate of 0.7 mL/min at 40 °C. The samples were dissolved overnight at a concentration of 5 mg/mL in the eluent and filtered through a 0.45 μm filter prior to analysis. The molecular weights were calibrated with poly(ethylene glycol) standards.

^{1}H NMR spectroscopy

^{1}H NMR spectra of the polymers were recorded with a Gemini 300MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA) before and after HIFU exposure. NCL micelles (polymer concentration of 10 mg/mL) in phosphate buffer pH 7.4 were freeze dried. The obtained samples were dissolved in DMSO d6.

^{1}H-NMR (DMSO, d6): 7.5 (b, CO–NH–CH_2), 5.5 (b, CH–OH (HPMAmLac$_2$), 5.3 (b, CH–OH (HPMAmLac$_1$), 5.0 (b, CO–CH (CH_3)–O), 4.1 (b, CO–CH–(CH_3)–OH), 3.6 (b, PEG methylene protons, O–CH_2–CH_2), 3.4 (b, NH–CH_2–CH_2), 1.4, (b, CO–CH–CH_3), 1.3 (b, HO–CH–CH_3), 1.0–0.6 (pHPMAmLac$_n$ main chain protons).

The percentage of pHPMAmLac$_2$ in the block copolymer was determined based on the ^{1}H-NMR spectra: L2/(L1+L2) x100%; 'L1' and 'L2' are assigned as the protons peaks of the hydroxyl group of pHPMAmLac$_1$ and pHPMAmLac$_2$, respectively.

The percentage of –OH groups in the polymer derivatized with methacrylate groups was determined with ^{1}H NMR in DMSO-d6 as follows: $(((m + n)/2)/((m + n)/2) + L1 + L2) \times 100\%$ in which 'm' and 'n' correspond with the two protons of the double bond of methacrylate groups attached either to pHPMAm-Lac$_1$ or pHPMAm-Lac$_2$ chains. 'L1' is the proton of the free alcohol functionality of pHPMAm-Lac$_1$, 'L2′ is the proton of the free alcohol functionality of pHPMAm-Lac$_2$, and '(m + n)/2′ the number of derivatized pHPMAm-Lac$_1$ and pHPMAm-Lac$_2$ chains.

The number average molecular weight (M_n) before HIFU exposure of the block copolymer was determined by ^{1}H NMR from the ratio of the integral of the peak at 3.6 ppm (PEG methylene protons) to the integral of the peak at 4.1 ppm (methine proton (CO-CH (CH_3)-OH). Furthermore, the possible hydrolysis of the lactic acid side groups due to HIFU exposure was

studied using ^{1}H NMR by comparing the integral of the peak at 5.5 (CH–OH (HPMAmLac$_2$) before and after HIFU exposure.

FIGURES

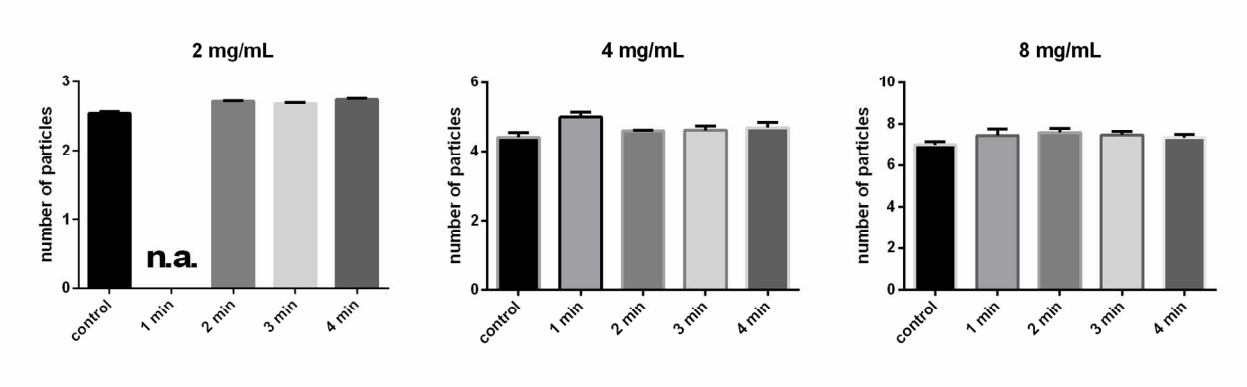

Figure S1. Number of particles as function of exposure time and polymer concentration at pH 5. The number of NCL micelles was measured before (control) and after CW HIFU (20 W) with different exposure times (1, 2, 3 and 4 min) using DLS.

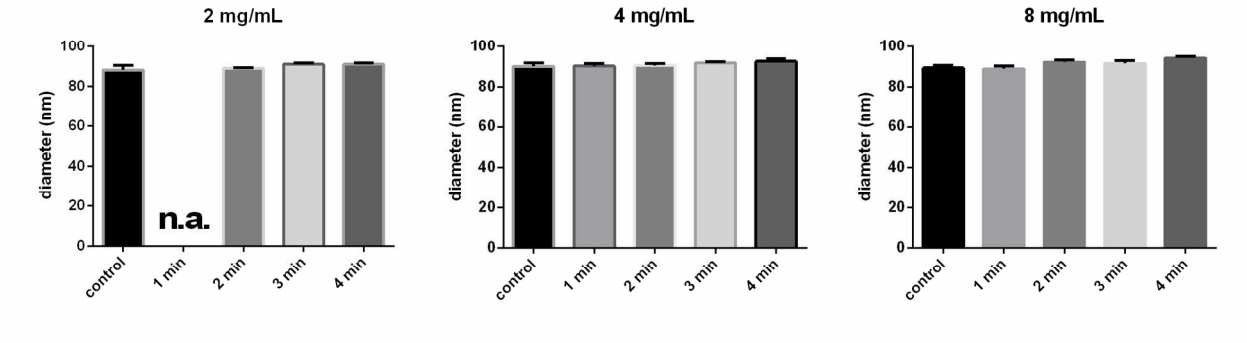

Figure S2. Size of particles as function of exposure time and polymer concentration at pH 5. The size of NCL micelles was measured before (control) and after CW HIFU (20 W) with different exposure times using (1, 2, 3 and 4 min) DLS.

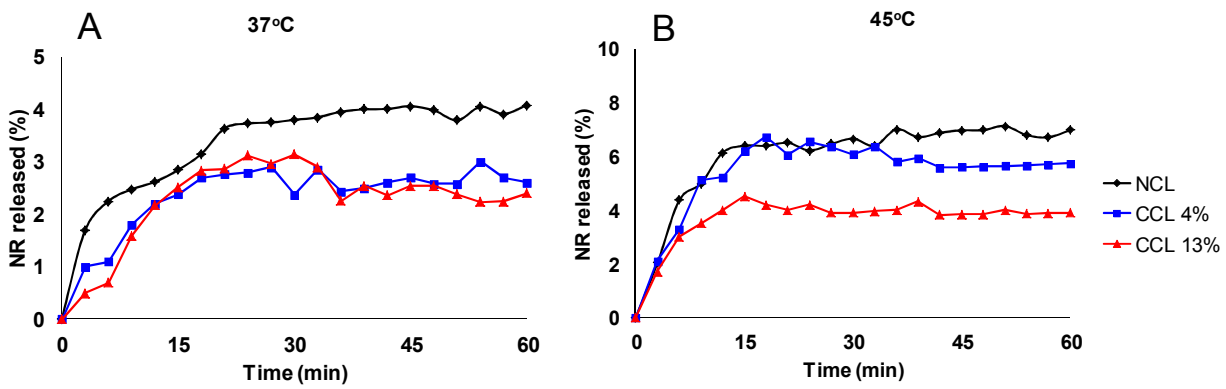

Figure S3. Release of NR from NCL, CCL 4% methacrylation and CCL 13% methacrylation micelles at two different temperatures: 37° C (A) and 45° C (B).

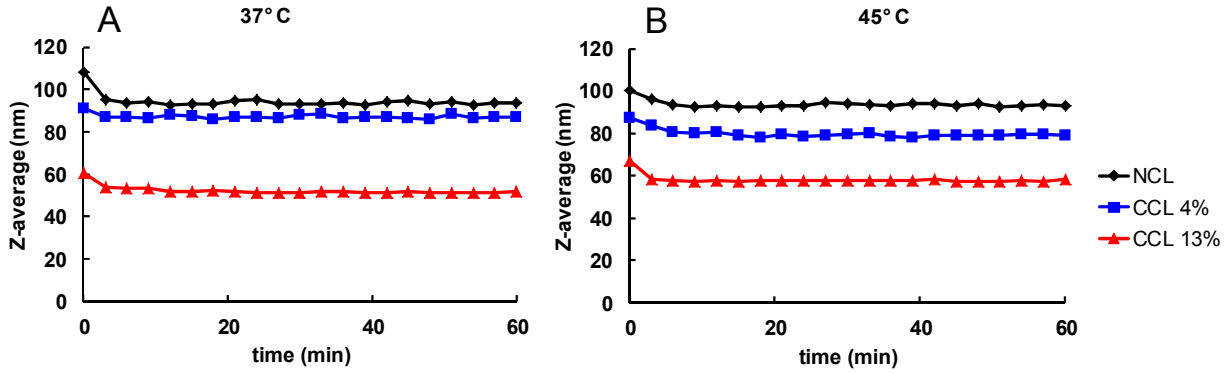

Figure S4. Stability of NR-loaded NCL and CCL micelles determined by DLS at pH 7.4 incubated over time at 37° C (A) and 45° C (B). Figure shows Z-average (in nm) of different micellar formulations as function of time at 2 different temperatures.

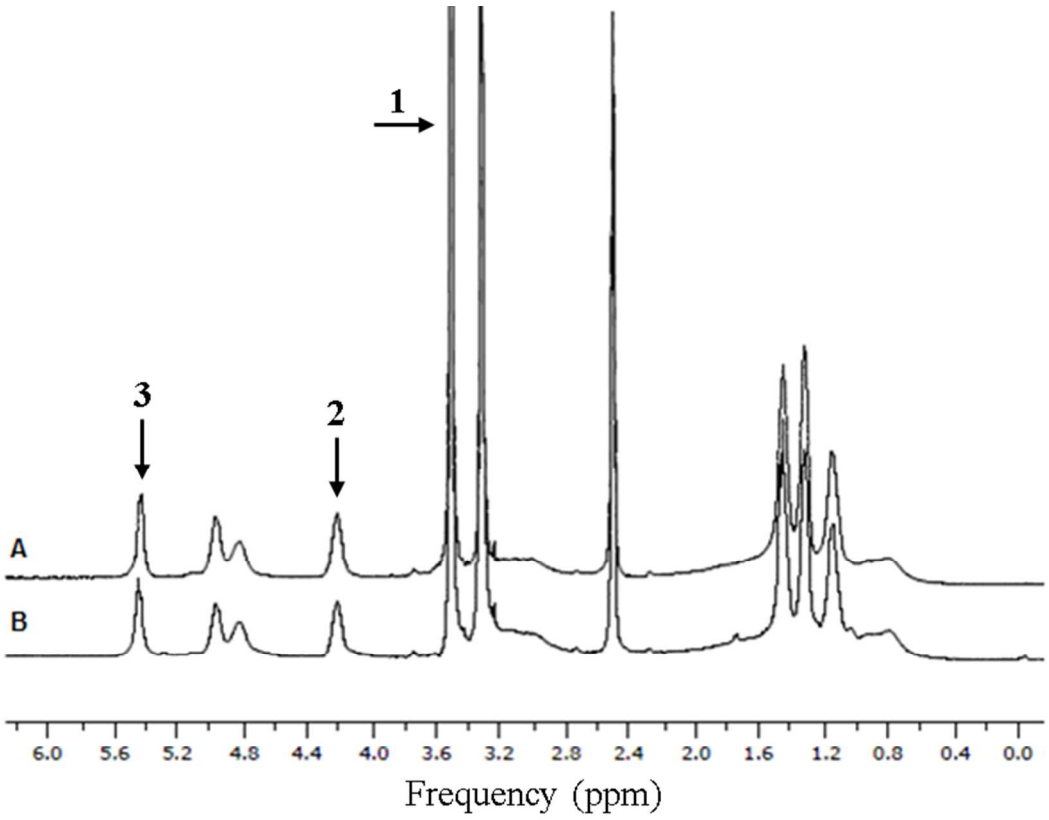

Figure S5. ^{1}H-NMR spectra of mPEG-b-p(HPMAm-Lac$_2$) before (A) and after CW HIFU for 4 minutes at 20 W (B). Arrow 1 indicates the PEG methylene proton resonance peak, arrow 2 indicates the methine proton (CO-CH(CH$_3$)-OH) resonance peak.and arrow 3 indicates the (CH–OH (HPMAmLac$_2$)) resonance peak.

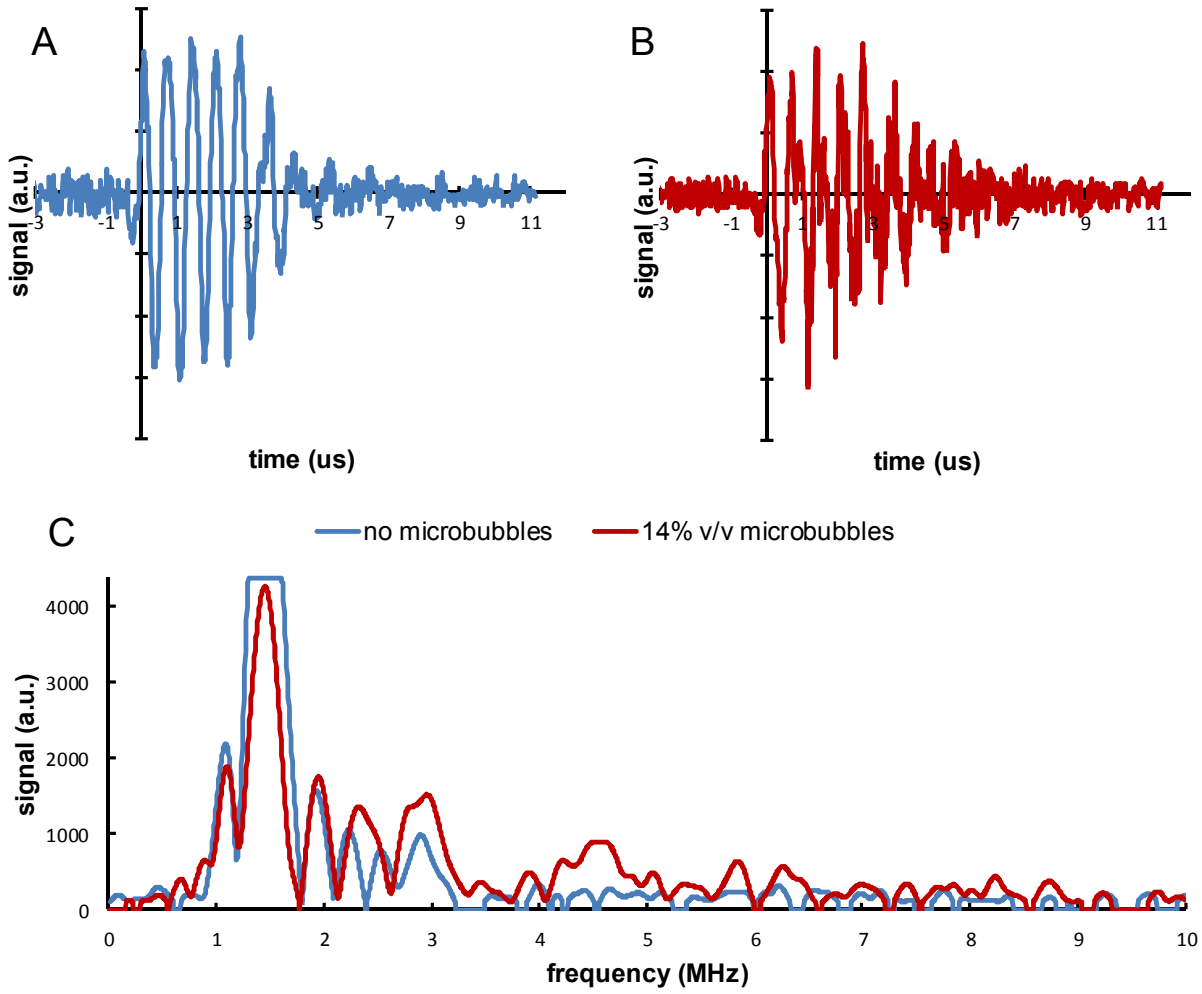

Figure S6. Cavitation detection with and without microbubbles present. The hydrophone signal as function of time shows a disturbance of the measured ultrasound field when cavitating microbubbles are present (B). This is not the case when no microbubbles are present (A). In the Fourier spectrum (0 – 10 MHz range) this leads to ultraharmonics at 3 and 4.5 MHz and increased broadband noise (C).