Supplementary Information

Nanodesigned magnetic polymer containers for dual stimuli actuated drug controlled release and magnetic hyperthermia mediation

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Experimental Section

Materials: 2,2′-azobisisobutyronitrile (AIBN, 98%) and methacrylic acid (MAA, 99%) were purchased from Acros and used after recrystallization from methanol and purification by vacuum distillation, respectively. Ferrous chloride tetrahydrate (FeCl₂·4H₂O) was purchased from Fluka. N,N′-methylelenbisacrylamide (MBAAm, 96%) and Potassium nitrate (KNO₃, 99%) were obtained from Acros and used as received. N,N′-Bis(acryloyl)cystamine (BAC, 98%) and hexamethylenetetramine (HETM, 99%) were purchased from Alfa Aesar and used without further purification. Poly(ethylene glycol) methyl ether methacrylate (PEGMA) (average Mn=475) and ethyleneglycol (EG) were used as received from Aldrich and Merck, respectively. Glutathione (GSH reduced, ≥ 98%) was received from MP Biomedicals. DNR was provided by Pharmacia &Up John. Acetonitrile (Aldrich) was dried over calcium hydride and purified by distillation before use.

Preparation of hollow P(MAA-co-PEGMA-co-MBAAm-co-BAC) (PMAAS-S) microspheres: Monodisperse PMAA microspheres were synthesized by distillation precipitation polymerization in acetonitrile with AIBN as initiator in the absence of any crosslinker as described in our previous work.¹ In the presence of the previously described PMAA seeds, PMAA@PMAAS-s core-shell microspheres were synthesized, by the second-stage polymerization of MAA, PEGMA and the two crosslinkers, MBAAm and BAC. Briefly, PMAA microspheres (0.30 g), MAA (0.91 g, 10.6 mmol), PEGMA (0.27 g, 0.6 mmol), BAC (0.14 g, 0.54 mmol) and MBAAm (0.08 g, 0.52 mmol) were dispersed in 90 mL of acetonitrile. The total amount of the crosslinkers was 9 mol% corresponding to the monomers of MAA, PEGMA, MBAAm and BAC. After the homogenization of the suspension by ultrasonic treatment for 10 min, AIBN (0.02 g, 0.1 mmol, 1.4 wt% relative to the monomers)
was added. The procedure was the same as above. The PMAA$_{S,S}$ microcontainers were obtained after immersing the core-shell microspheres in ethanol for 24 h at room temperature for selective dissolution of PMAA cores. The final product was dried in vacuum at room temperature till constant weight.

In order to study the redox responsiveness of the microcontainers, three derivatives have been prepared with different molar ratios of the two crosslinkers (PMAA$_{S,S}$-low, PMAA$_{S,S}$-medium, and PMAA$_{S,S}$-high with 0.25, 1.04 and 1.77 mol BAC/mol MBAAm, respectively), while their total amount remained constant.

**Preparation of magnetic PMAA$_{S,S}$@Fe$_3$O$_4$ microcontainers:** PMAA$_{S,S}$ microcontainers (0.10 g), FeCl$_2$·4H$_2$O (0.30 g), KNO$_3$ (0.05 g) and HETM (1.0 g) were mixed in 60 mL EG/water (volume ratio, 1:4). The suspension was heated at 80 °C under N$_2$ for 2 hours. The formed dark brown precipitate was collected by centrifugation and washed three times with distilled water.

**Daunorubicin Loading and Release:** DNR was added to a magnetic microcontainers (PMAA$_{S,S}$@Fe$_3$O$_4$) suspension in phosphate buffer pH 7.4 (final magnetic microcontainers concentration 1 mg/mL). The resulting suspension remained under gentle agitation (220 rpm) for three days at 25 °C. Unloaded DNR was removed after centrifugation. The concentration of DNR was determined by UV absorption spectroscopy, monitoring DNR absorbance at 482 nm. Calculations were based on a standard curve of DNR. The encapsulation efficiency (EE) was calculated according to the following formula: EE (%) = (weight of loaded drug/weight of drug in feed) x 100.

The release behavior of DNR-loaded magnetic microcontainers was investigated under acidic (citrate buffer, pH 5.0) or physiological (phosphate buffer, pH 7.4) pH conditions and in the presence of increasing concentration of GSH (0.001
to 20 mM) at 37 °C. At given time intervals, aliquots were withdrawn and centrifuged. The DNR concentration was determined in the supernatant by measuring the absorbance at 482 nm. Release experiments were repeated twice and results are expressed as the mean value of released DNR (%) ± standard deviation.

**Measurement of heating ability:** Magnetic hyperthermia measurements were performed with a 2.4 kW Easyheat 0224 system (Easyheat®, Ameritherm Inc). A copper coil (diameter 25 mm, 8 loops) was used in order to submit samples to an AMF. Samples were prepared by dispersing various amounts of magnetic microcontainers in Teflon tubes containing 3.0 ml of deionized water. Glass tubes were coated with insulating material and were centered in the coil. Recording of temperature was conducted with an RF-immune fiber optic probe. Temperature was acquired every 1 sec. At the beginning of the measurement the background temperature was recorded for 60 seconds and was subtracted as baseline temperature.

**Characterization:** XRD measurements were made using a Siemens D-500 diffractometer using CuKα radiation (\(\lambda=1.5418\AA\)). An FEI CM 20 microscope equipped with a Gatan GIF 200 energy filter was utilized for TEM studies. The EFTEM images were obtained using the three-window method.² A FEI Quanta Inspect Scanning Electron Microscope with W (Tungsten) filament 25kV was employed for EDX spectroscopy. FT-IR spectra were collected over the range of 4000–380 cm⁻¹ using a Perkin-Elmer Precisely Spectrum 100 Spectrometer. A VSM model 155 with a Bell 640 Gaussmeter, source Danfysik System 8000 (-2 to 2 T) (A/S, Mollehaven 31, DK-4040 Jyllinge, Denmark) was equipped in order to record the magnetic loops. DLS measurements were performed by a Zetasizer Nano™ from Malvern Instruments. In the data presented in this study, each measurement represents the average value of 10 measurements, with 20 s integration time for each
measurement. UV-vis absorption spectra were measured on a Jasco V-650 spectrophotometer.

References


Dynamic Light Scattering (DLS) measurements

Figure S1. Characteristic size distribution of PMAA_{S,S} nanocontainers at pH 7.4.
Fourier Transform Infra-Red spectroscopy (FT-IR)

Figure S2 depicts the FT-IR spectra of the samples during the synthetic process. A PMAA template FT-IR spectrum is presented in curve a. Curve b demonstrates the core-shell nanospheres spectrum with an additional peak at 1540 cm$^{-1}$ referring to the vibration of the amide group of PMBAAm parts. In curve c, the considerable decrease of the peak at 1697 cm$^{-1}$ is due to the successful removal of the PMAA core by dissolution in ethanol. This decrease leads to the appearance of two characteristic peaks at 1635 cm$^{-1}$, corresponding to the stretching vibration of the carbonyl unit in PMBAAm and at 1108 cm$^{-1}$, attributed to the PEGMA ether bond. The characteristic peak at 572 cm$^{-1}$ (curve d) is assigned to the stretching vibration of Fe-O bond in the magnetic microcontainers.

![FT-IR spectra](image)

**Figure S2.** FT-IR spectra of: (a) PMAA template, (b) PMAA@PMAA$_{s,s}$ core-shell microspheres, (c) PMAA$_{s,s}$ microcontainers and (d) PMAA$_{s,s}$@Fe$_3$O$_4$ magnetic microcontainers
**pH-sensitivity of magnetic microcontainers**

DLS measurements were made in order to evaluate the pH-sensitivity of the fabricated magnetic microcontainers throughout the desired pH region and similar behavior with PMAAS-S microcontainers was confirmed. The region below pH 6.0 was excluded because partial detachment of the external magnetic nanoparticles deposited on the polymer shell has been observed at low pH.

![pH-sensitivity of the fabricated magnetic microcontainers measured by DLS.](image)

**Figure S3.** pH-sensitivity of the fabricated magnetic microcontainers measured by DLS.
Raman spectroscopy

Raman spectrum (red line) was collected using a Renishaw InVia Raman microscope using an Ar⁺ laser with 514 nm at room temperature. The band deconvolution was performed by utilizing Wire software (Renishaw). Lorentzian profiles of deconvolution (green lines) and curve fitting (blue line) are shown in Figure S4.

**Figure S4.** Lorentzian profiles of deconvolution (green lines) and curve fitting (blue line) of magnetic microcontainers’ Raman spectrum (red line).
Thermal Gravimetric Analysis (TGA)

The weight fraction of magnetic nanoparticles deposited on the microcontainers’ shell was estimated by TGA, employing a TA Instruments TGA Q50. The weight loss at 600 °C is 59% (w/w). This calculation indicates that the weight fraction of MNPs is approximately 41% (w/w).

Figure S5. Thermal gravimetric analysis of magnetic microcontainers performed in nitrogen atmosphere from room temperature to 600 °C with a heating rate of 10 °C · min⁻¹.
Assessment of dispersion stability

The dispersion stability of the DNR-loaded magnetic microcontainers was assessed by DLS measurements. An amount of loaded microcontainers, identical to that used during the drug release studies, was dispersed in physiological buffer (phosphate buffer, pH 7.4). DLS measurements were made every 10 minutes for 1 hour. The described procedure was repeated 3 times and average results with standard deviation are presented in the following figure.

Figure S6. Dispersion stability of the DNR-loaded magnetic microcontainers
EDX spectroscopy analysis

**Figure S7.** EDX spectroscopy analysis of magnetic microcontainers, PMAAS-S-low, PMAAS-S-medium and PMAAS-S-high, prepared with 0.25, 1.04 and 1.77 mol BAC/mol MBAAm, respectively.