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# Magnetic microgels for drug targeting applications: Physical–chemical properties and cytotoxicity evaluation

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ABSTRACT

Magnetoresponsive microgels with high saturation magnetization values have been obtained by a strategy based on the miniemulsion method using high colloidal stability organic carrier ferrofluid as primary material. Hydrophobic nanoparticles $Fe_3O_4$/oleic acid are densely packed into well-defined spherical nanoparticle clusters coated with polymers with sizes in the range 40–350 nm. Physical–chemical characteristics of magnetic microgels were investigated by TEM, SAXS, XPS and VSM measurements with the focus on the structure–properties relationship. The impact of magnetic microgels loaded with anticancer drug mitoxantrone (MTO) on the non-adherent human T cell leukemia line Jurkat was investigated in multiparameter flow cytometry. We showed that both MTO and microgel-loaded MTO penetrate into cells and both induce apoptosis and later secondary necrosis in a time- and dose dependent manner. In contrast, microgels without MTO are not cytotoxic in the corresponding concentrations. Our results show that MTO-loaded microgels are promising structures for application in magnetic drug targeting.



## 1. Introduction

Magnetic nanoparticle systems are receiving continuously increasing interest in the biomedical field for diagnosis and treatment [1–4] due to their multiple functionalities as MRI contrast agents, magnetic hyperthermia treatments and magnetically guided drug delivery [5–7]. Superparamagnetic nanoparticles embedded in a polymer matrix [8], in particular in microgels, are highly promising magnetic carriers which offer several benefits concerning encapsulation of therapeutics, multivalency for bioconjugation, as well as mechanical and chemical stability in the specific bio-environment. The high magnetic moment of the functionalized carriers is among the most important requirements for successful applications in biomedicine, in particular for magnetic targeting [9]. Magnetic nanoparticle clusters in a polymer shell, summing up the magnetic moments of individual nanoparticles, were obtained by in situ coprecipitation of magnetic nanoparticles in microgels as microreactors [10,11] and also by strongly polar solvent induced destabilization of a ferrofluid [12]. The controlled clusterization of magnetic nanoparticles, followed by encapsulation of the densely packed magnetic core in a polymer shell proved to be a facile and reproducible procedure when using the well-established ferrofluid technology and oil-in-water miniemulsion procedure [13–15].

In this paper we present the oil-in-water miniemulsion based clustering process of surface coated magnetic nanoparticles, followed by entrapping the close packed magnetic core into the functional polymer shell. The efficient control of the evaporation induced self-assembly of magnetic nanoparticles, while keeping the superparamagnetic behavior of the resulted clusters was possible by starting with individually dispersed surfactant coated MNPs in a volatile organic solvent, i.e. from a highly stable ferrofluid. The structure, size characteristics and chemical composition were determined by transmission electron microscopy (TEM), Small Angle X-ray Scattering (SAXS) and X-ray Photoelectron Spectroscopy (XPS) respectively, and the magnetic properties were investigated by vibrating sample magnetometer (VSM). The anticancer drug loading and multiparameter flow cytometry analyses finally reveal the promising characteristics of the obtained magnetic microgel particles for drug targeting applications.

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## 2. Experimental section

### 2.1. Synthesis of magnetic microgels

The magnetic microgels were obtained using a two steps synthesis procedure [16]: (i) the preparation of magnetic nanoparticle clusters (NPC) by oil-in-water miniemulsion technique [15,17]; (ii) the coating of NPC with cross-linked polymer shells such as poly(N-isopropylacrylamide) (M-pNIPA) or poly(N-isopropylacrylamide)-polyacrylic acid (M-pNIPA-pAAc). The first step involved the emulsification of toluene based ferrofluid, containing $Fe_3O_4$ nanoparticles coated with a hydrophobic monolayer of oleic acid, in aqueous solution with sodium dodecyl sulfate (SDS) as surfactant. Any excess (free) oleic acid was eliminated from the initial ferrofluid. The mixture was treated ultrasonically to obtain small stable droplets of ferrofluid. An UP400S Compact Ultrasonic processor (100 W, 24 kHz; Hielscher Ultrasonics GmbH) with PC control, sonotrode made of titanium was used to obtain the magnetic miniemulsion. The as prepared magnetic miniemulsion was heated to 100 °C to remove the toluene and then was carefully washed several times with methanol-water mixture, magnetically separated and redispersed in water.

In the second step, the NPC coated with SDS were coated either with one polymer shell pNIPA or with two shells pNIPA-pAAc using layer by layer free radical polymerization. In a typical synthesis procedure, the aqueous solution containing NPC, the monomer (NIPA or AAc) and the cross-linker N,N-bisacrylamide (BIS) was stirred for 10 min, after that the oxidant ammonium persulfate (APS) was added to start the polymerization. The reaction mixture was kept under argon atmosphere at temperature 70 °C and vigorous stirring. The as prepared magnetic microgel was precipitated using excess of acetone, washing several times to remove the unreacted products and redispersed in water.

### 2.2. Characterization methods

Transmission electron microscopy was carried out on a JEOL 1010 microscope to investigate the morphology of NPC and magnetic microgels. Structural investigations of NPC and microgels were performed by SAXS measurements using synchrotron radiation at the P12 BioSAXS facility with high brilliance X-ray beam (PETRA III storage ring – EMBL/DESY Hamburg). The beamline is optimized for solution scattering experiments and allows the investigations of water based suspensions of magnetic microgels. X-ray Photoelectron Spectroscopy was used to determine the surface chemical composition of NPC and microgels. XPS spectra were collected on an XPS spectrometer SPECS equipped with a dual-anode X-ray source Al/Mg, a PHOIBOS 150 2D CCD hemispherical energy analyzer, a multi-channeltron detector with vacuum maintained at $1 \times 10^{-9}$ Torr using AlKα X-ray source (1486.6 eV) operated at 200 W. The particle suspension was dried on the indium foil to allow the XPS measurements. XPS data analysis and curve fitting was performed using CasaXPS software with a Gaussian–Lorentzian product function and a non-linear Shirley background substraction. The static magnetization of the samples was measured by means of vibrating sample magnetometry at room temperature using an ADE Technologies VSM 880 magnetometer.

### 2.3. Cytotoxicity experiments

UV-B sterilized microgels were incubated with mitoxantrone (MTO) for 96 h. The effective loading of nanoparticles with MTO was calculated from the measurements of unbound MTO in the supernatant by an established HPLC method [18].

For assessment of toxicity we employed the non-adherent human T cell leukemia cell line Jurkat (DSMZ ACC 282). Cell culture was performed at 37 °C and 5% $CO_2$ in RPMI 1640 medium supplemented with 10% Fetal Calf Serum (FCS), 1% glutamine, 1% penicillin-streptomycin (all from Invitrogen Life Technologies, Karlsruhe, Germany), and 1% HEPES (10 mM, pH 7.2) (Merck KGaA, Darmstadt, Germany). For the experiments, the cells were adjusted to a density of $2 \times 10^5$ cells/ml in cell culture media. 1 ml of the cell suspensions were seeded into 48 well plates (Greiner Bioone, Frickenhausen, Germany) and incubated with unloaded and MTO loaded microgels. Untreated cells and cells treated with soluble MTO served as controls. The experiments were performed in triplicates. After 24 h and 48 h incubation 50 μl aliquots of the cells were stained for 30 min at 4 °C with 250 μl of a mixture of 20 μg/ml Propidium iodide (PI, Sigma-Aldrich, Taufkirchen, Germany), 10 nM DiIC1(5), 1 μg/ml Hoechst 33342 (Invitrogen Life Technologies, Karlsruhe, Germany) and 0.5 μg/ml FITC-labelled Annexin A5 (kindly provided by Internal Medicine 3, University Hospital Erlangen, Germany) in Ringer's solution (Baxter Healthcare, Zurich, Switzerland) as previously reported by Munoz et al. [19].

Finally, the cells were analyzed employing a Gallios flow cytometer (Beckman Coulter, Fullerton, CA, USA). Excitation for FITC and PI was at 488 nm, the FITC fluorescence was recorded on FL1 sensor (525/38 nm BP), the PI fluorescence on FL3 sensor (620/30 nm BP), the DiIC1(5) fluorescence was excited at 638 nm and recorded on FL6 sensor (675/20 nm BP), and the Hoechst 33342 fluorescence was excited at 405 nm and recorded on FL9 sensor (430/40 nm BP). The MTO fluorescence was excited at 638 nm and recorded by the FL-7 sensor (725/20 nm BP). Electronic compensation was used to eliminate bleed through fluorescence. Data analysis was performed with Kaluza software (Beckman Coulter, Fullerton, CA, USA).

## 3. Results and discussion

### 3.1. Morphology and structure of magnetic microgels

Well defined near spherical NPC have been obtained by oil-in-water miniemulsion method. Hydrophobic nanoparticles, $Fe_3O_4$ coated with oleic acid from the ferrofluid are densely packed into spherical clusters stabilized with SDS, as shown in Fig. 1. The aggregation observed in Fig. 1 is caused by the preparation of the sample for TEM investigation. The analysis of the TEM images enabled the determination of the diameters distributions of the nanoparticle clusters. As shown in the inset in Fig. 1, NPC have sizes in the range of

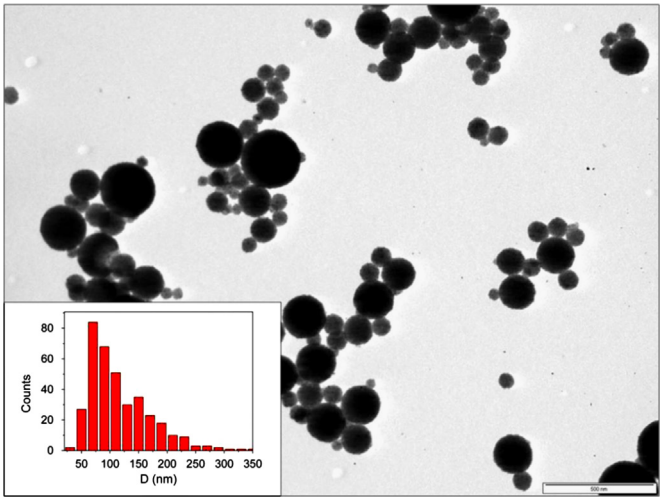


**Fig. 1.** TEM image of magnetic nanoparticle clusters stabilized with SDS (the bar is 500 nm). Inset: the diameters distribution.