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# New superparamagnetic fluorescent Fe@C-$C_5ON_2H_{10}$-Alexa Fluor 647 nanoparticles for biological applications

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The structure and physical properties of superparamagnetic Fe@C nanoparticles (Fe@C NPs) as well as their uptake by living cells and behavior inside the cell were investigated. Magnetic capacity of Fe@C NPs was compared with $Fe_7C_3$@C NPs investigated in our previous work, and showed higher value of magnetic saturation, 75 emu.g$^{-1}$ (75 Am$^2$·kg$^{-1}$), against 54 emu.g$^{-1}$ (54 Am$^2$·kg$^{-1}$) for $Fe_7C_3$@C. The surface of Fe@C NPs was alkylcarboxylated and further aminated for covalent linking to the molecules of fluorochrome Alexa Fluor 647. Fluorescent Fe@C-$C_5ON_2H_{10}$-Alexa Fluor 647 NPs (Fe@C-Alexa NPs) were incubated with HT1080 human fibrosarcoma cells and investigated using fluorescent, confocal laser scanning and transmission electron microscopy. No toxic effect on the cell physiology was observed. In a magnetic field, the NPs became aligned along the magnetic lines inside the cells.

**Keywords:** superparamagnetic fluorescent Fe@C nanoparticles, electron microscopy, magnetic field.

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## 1. Introduction

The search for applications of magnetic nanoparticles (NPs) in biology and medicine has become an area of extensive research. The influence of shape, size, composition, modifications and magnetic properties of NPs in living cells is being actively explored. Superparamagnetic NPs with chemical formula $Fe_7C_3$@C were previously obtained by us at high pressure and temperature and investigated by physico-chemical and biological methods [1–4].

In this work, new superparamagnetic carbon coated iron NPs (Fe@C) with higher magnetic saturation compared with $Fe_7C_3$@C were treated by acids to eliminate uncoated iron NPs, selected by magnet, chemically modified, labeled with fluorescent marker Alexa Fluor 647 and studied for further biological application.

## 2. Experimental section

The pristine NPs of Fe@C formula (carbon coated magnetic iron nanopowder of 25 nm APS purchased from Sigma Aldrich, USA) were suspended in water and treated by high power ultrasound to break up large aggregates. Fe@C NPs were then treated by a mixture of $H_2SO_4$ and HCl acids to eliminate the uncoated iron NPs, and then selected by magnet to dispose of non-magnetic NPs. The carbon onion-like surface of NPs was alkylcarboxylated and further aminated similar to work [5] for covalent linking to the molecules of fluorochrome Alexa Fluor 647. The magnetization curve was obtained out in GREMAN laboratory on a Quantum Design physical property measurement system magnetometer (PPMS).

These fluorescent superparamagnetic NPs were added to HT1080 human fibrosarcoma cells seeded on glass-bottomed Petri dishes (LabTek, USA) at density of $10^5$ cells/ml. NPs concentration in the culture medium was 20 $\mu$g/ml. After 24 h of co-cultivation, a permanent commercial NdFeB magnet (cube with 5 mm edge length, magnetic field of 0.15 T), protected by gold shell, was placed in Petri dish [4]. Cells were observed at such conditions for 16–24 h using time-lapse confocal microscopy. An environmental chamber was kept at 37 °C and

under 5 % of $CO_2$. The chamber was mounted on an Olympus IX70 inverted microscope equipped with CCD-camera Orca-RT+ (Hamamatsu, Japan) and controlled by Micromanager 1.4 software. Illumination conditions (ND filters, lamp voltage, exposure time) were set to minimize photo toxicity. Subsequently, cells were washed several times with fresh pre-warmed medium to remove free particles, fixed in 2.5 % glutaraldehyde in 100 mM phosphate buffer (pH 7.4) for 2 hours with subsequent post-fixation in 1 % $OsO_4$, and finally embedded in Epon (Sigma, USA). Serial ultrathin sections (70 nm) were prepared with Leica ultramicrotome and observed using JEM 1011 transmission electron microscope (JEOL, Japan) at 100 kV.

## 3. Results and discussion

The obtained suspension contained NPs of spherical shape with average size of 25 nm, as specified by the provider and was shown by our HRTEM study. Their surface was coated with multiple layers of onion-like carbon. A magnetization curve (M vs. H) of Fe@C, obtained at room temperature with SQUID instrument, showed the typical superparamagnetic behavior with a high value of magnetic saturation, 75 emu.g$^{-1}$ (75 Am$^2$ kg$^{-1}$), which was higher than that for $Fe_7C_3$@C 54 emu.g$^{-1}$ (54 Am$^2$ kg$^{-1}$ ). It was shown that cells began to absorb the aggregates of NPs during first 30 minutes of co-cultivation. Prolonged biological experiments demonstrated that Fe@C NPs displayed high efficiency of cellular uptake and didn't affect cytophysiological parameters (cell proliferation, spreading, and cell death) of cultured HT1080 human fibrosarcoma cells. In a magnetic field, aggregates of Fe@C NPs became aligned along the magnetic lines inside the cells (Fig. 1a). Fluorescence signal of Alexa Fluor 647 was precisely co-localized with the NPs aggregates visible in phase contrast microscopy (Fig. 1b).

Transmission electron microscopy analysis showed that aggregates of Fe@C NPs were localized directly in the cell cytoplasm without membrane surrounding. We did not detect an ultrastructure defects of cells containing NPs. Inside the cells, NPs retained carbon onion-like layers on their surface, known to be stable in cellular environment [1,2] which protected cells from pure iron influence (Fig. 1c-e).

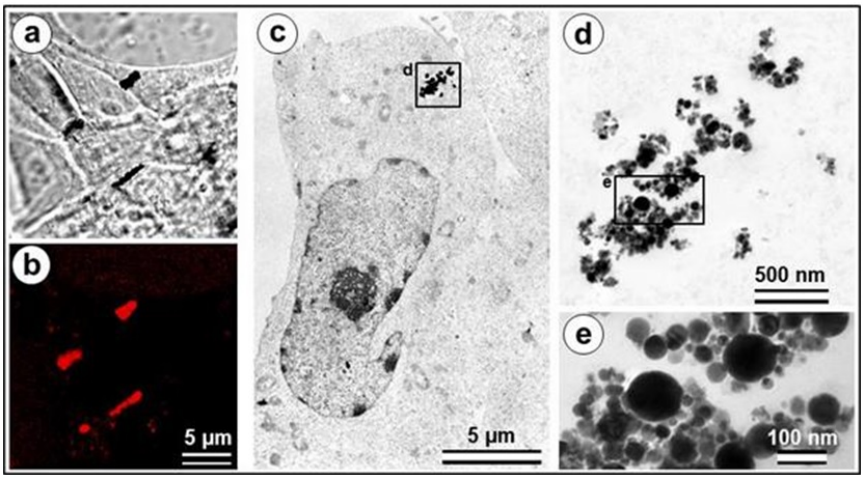


FIG. 1. Representative micrographs of the cells with Fe@C-Alexa NPs inside under magnetic field. Cells were incubated with 20 $\mu$g/ml of NPs for 24 h, then placed in magnetic field for 16 h. NPs aggregates are oriented along the magnetic field lines. a) Phase contract microscopy; b) confocal laser scanning microscopy of the same cells; c) transmission electron microscopy photograph of whole cell with Fe@C-Alexa NPs aggregates inside at low magnification; d) magnified view of Fe@C-Alexa NPs aggregates from the boxed area on panel c; e) high magnification of Fe@C-Alexa NPs in selected region on panel d

## 4. Conclusion

Fe@C-Alexa NPs are internalized by cells and show no significant effect on their cytophysiology.

The Magnetic properties of Fe@C-Alexa NPs are significantly higher than those for $Fe_7C_3$@C NPs and are therefore sufficient for successful manipulation at the intracellular level. The combination of confocal and phase-contrast microscopy shows that the fluorescent signal detected inside the cells corresponds to Fe@C-Alexa NPs.

Electron microscopy reveals that inside the cells, NPs retain carbon onion-like layers on their surface.

Fluorescent labeling of NPs with fluorochrome Alexa Fluor 647 provides an opportunity to reliably identify Fe@C-Alexa NPs inside the cells and in perspective inside the body of experimental animals.

Since the new Fe@C-Alexa NPs exhibit better superparamagnetic properties than previously-used NPs, they penetrate efficiently into the cell, do not cause a physiological harm and do not lose the protecting carbon layers and the fluorescent label, thus providing good opportunity for biological applications.

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