MAGNET-INDUCED BEHAVIOR OF IRON CARBIDE Fe7C3@C NANOPARTICLES IN THE CYTOPLASM OF LIVING CELLS.

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The intake of superparamagnetic Fe7C3@C nanoparticles with living cells and their behavior into the cell was investigated. It has been shown that cells absorbed aggregates of nanoparticles during first 30 minutes. After absorption these aggregates moved towards the center of the cell and accumulated near the cell nucleus. No toxic effect to the cell physiology was observed. In a magnetic field, the particles align in the cells along the magnetic lines and shifted to the magnet side. During long-term cultivation Fe7C3@C nanoparticles aggregates to be discarded during exocytosis.

Key words: superparamagnetic Fe7C3@C nanoparticles, living cells, electron microscopy, magnetic field.

Introduction
A new type of superparamagnetic nanoparticles (NPs), with chemical formula Fe7C3@C was recently obtained by us at high pressure and high temperature and studied by physico-chemical and biological methods [1, 2]. The longtime biological experiences were demonstrated that Fe7C3@C NPs display high efficiency of cellular uptake and do not affect cytophysiological parameters of in vitro cultured pig kidney epithelia (PK) cells.

2. Experimental section
For live imaging, pig kidney cells were placed on glass-bottomed Petri dishes (LabTek, USA) at density of 10^5 cells/ml and incubated with Fe7C3@C NPs for 24h. Cell observation was
performed in an environmental chamber kept at 37°C and under 5% CO₂. The chamber was mounted on Olympus IX70 inverted microscope equipped with CCD-camera Orca-RT+(Hamamatsu, Japan) and controlled by Micromanager 1.4 software [1]. Illumination conditions (ND filters, lamp voltage, exposure time) were set to minimize phototoxicity.

For further TEM experiments, PK were washed several times with fresh pre-warmed media 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% OsO₄ and embedding in Epon (Sigma, USA). Serial ultrathin sections (70 nm) were prepared with Leica ultramicrotome and observed with JEM 1011 (JEOL, Japan) at 100 kV.

3. Results and discussion

Cells were capable of capturing magnetic nanoparticles (MNP) by upper part of the cell membrane, and from the surface of the cultivation substrate during motion process. Immunofluorescence studies using intracellular endosomal membrane marker showed that MNP aggregates can be located in endosomes or lying free in the cytoplasm.

![Fig 1](image1.png)

**Fig 1.** Transmission electron microscopy photos of the cell with Fe7C3@C nanoparticles aggregates. a) Whole cell image on small magnification; b) High magnification of selected region. Scale bar: a – 5 µm, b – 1µm.
During long-term cultivation, cells discarded Fe7C3@C aggregates on the surface of the plasma membrane during exocytosis. These aggregates were reabsorbed later by the same or adjacent cells. In the absence of a magnetic field Fe7C3@C aggregates localized in the central region of the cells around the nucleus, its distribution was uniform (Fig. 1).

In a magnetic field uneven distribution of Fe7C3@C aggregates was observed - it advantageously arranged on the side of the cell facing the magnet (Fig. 2). Electron microscopy analysis of these cells showed that the aggregates are often located in the cytoplasm of cells along microtubules.

![Fig 2. Light microscopy photo of the cell with Fe7C3@C nanoparticles in magnetic field.](image)

**Nanoparticles aggregate oriented along magnetic field lines and shifted to the magnet side. Scale bar 10µm.**

4. Conclusions

1. Our experiments demonstrated active endocytosis-mediated uptake of Fe7C3@C nanoparticles and absence of any significant effects of it to cell behavior.
2. Magnetic properties of the Fe7C3@C are sufficient for successful manipulation at the intracellular level.
3. Non-toxic, biologically compatible paramagnetic Fe7C3 @ C carbide nanoparticles can be used as efficient vectors for targeted delivery of biologically active compounds both intracellularly and in the whole organism.

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