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Magnet-induced behavior of iron carbide (Fe7C3@C) nanoparticles in the cytoplasm of living cells

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The uptake of superparamagnetic Fe7C3@C nanoparticles into living cells and their behavior once inside the cell was investigated. The cells used were shown to absorb the nanoparticle aggregates over the first 30 minutes. After absorption, these aggregates moved towards the center of the cell and accumulated near the cell nucleus. No toxic effects on cell physiology were observed. In a magnetic field, the particles aligned in the cells along magnetic lines and shifted to the magnet's side. During long-term cultivation, Fe7C3@C nanoparticle aggregates were ultimately discarded via exocytosis.

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

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1. Introduction

A new type of superparamagnetic nanoparticles (NPs), with chemical formula Fe7 C3@C was recently obtained by us at high pressure and temperature and studied via physico-chemical and biological methods [1,2]. Prolonged biological exposure demonstrated that Fe7C3@C NPs display highly efficient cellular uptake and did not affect cytophysiological parameters for *in vitro* cultured pig kidney epithelial (PK) cells.

2. Experimental section

For live imaging, pig kidney cells were placed on glass-bottomed Petri dishes (LabTek, USA) at a density of 10^5 cells/ml and incubated with Fe7C3@C NPs for 24 h. Cell observation was performed in an environmental chamber kept at 37 °C and under 5 % 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 [1]. Illumination conditions (ND filters, lamp voltage, exposure time) were set to minimize phototoxicity.

For further 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 h with subsequent post-fixation in 1 % OsO_4 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.

During long-term cultivation, cells discarded Fe7C3@C aggregates on the surface of the plasma membrane via 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 with a uniform distribution (Fig. 1).

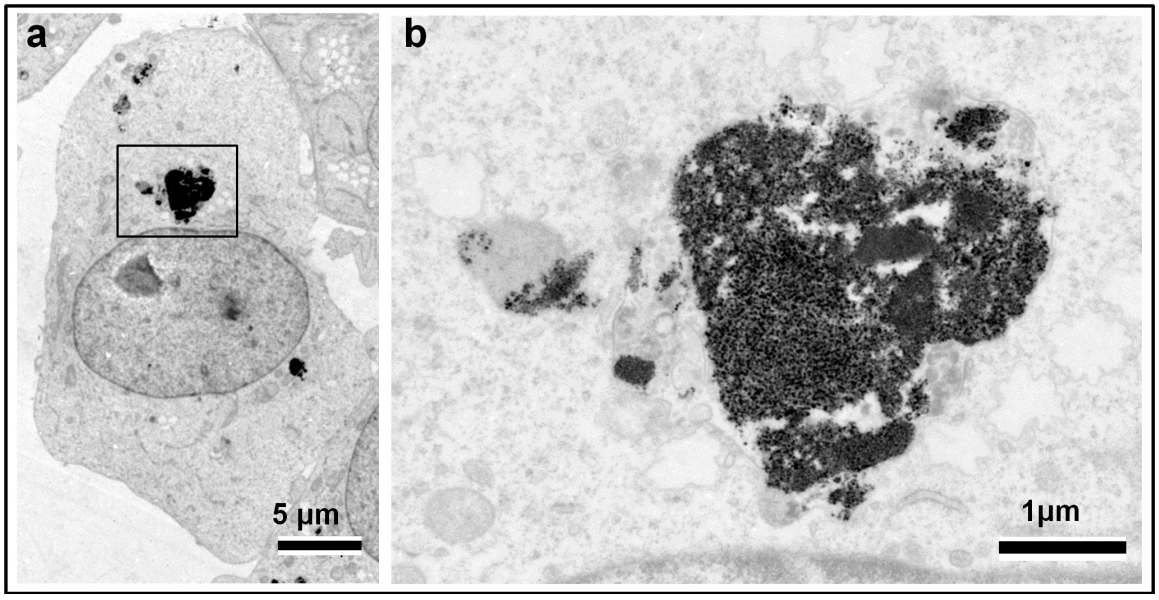

FIG. 1. Transmission electron microscopy photos of the cell with Fe7C3@C nanoparticles aggregates. a) Whole cell image on small magnification; b) High magnification of selected region. Scale bar: a – 5 μm, b – 1 μm

In a magnetic field, uneven distribution of Fe7C3@C aggregates was observed - it was 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.

4. Conclusions

Our experiments demonstrated active endocytosis-mediated uptake of Fe7C3@C nanoparticles and the absence of any significant effects of it to cell behavior.

The magnetic properties of the Fe7C3@C NPs are sufficient for successful manipulation at the intracellular level.

The non-toxic, biologically-compatible paramagnetic Fe7C3@C carbide NPs can be used as efficient vectors for the targeted delivery of biologically-active compounds both intracellularly and throughout the entire organism.

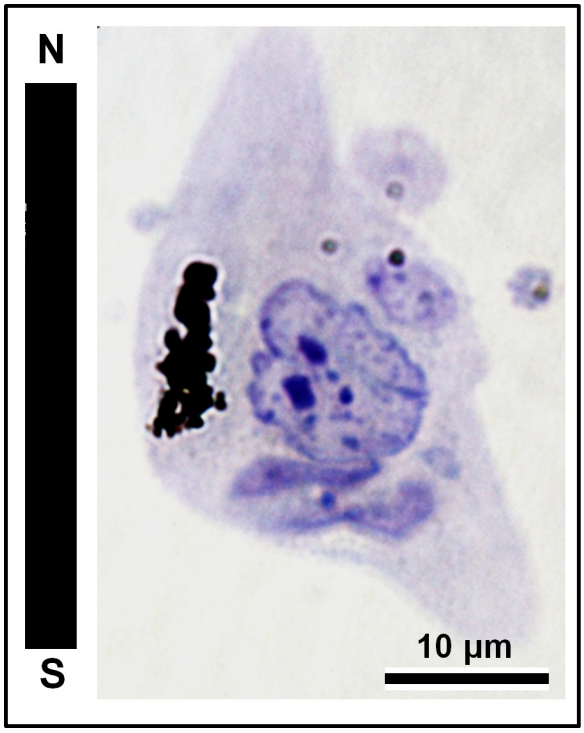

FIG. 2. Light microscopy photo of the cell with Fe7C3@C nanoparticles in magnetic field. Nanoparticles aggregate oriented along magnetic field lines and shifted to the magnet side. Scale bar 10 μm

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