FORCE-INDUCED INTERNALIZATION OF MAGNETIC NANOPARTICLES INTO LIVING CELLS

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Summary We have systematically studied the use of magnetic force to facilitate the cellular delivery of biomolecules carried by superparamagnetic iron oxide nanoparticles (SPIOs). We found that magnet force enhanced SPIO internalization into HeLa cells by 2-10 folds, depending on the magnitude of magnetic force and fluid drag force exerted on individual SPIOs. Magnetic force enhances cellular uptake of SPIOs by increasing the accumulation of SPIOs near cell surface and activating caveolae-mediated endocytosis.

INTRODUCTION

SPIOs are formed of maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4) nanocrystals with a diameter less than 15-20 nm. Cellular/tissue distribution of SPIOs can be controlled with an externally magnetic field, which is intensively explored for magnetically guided delivery of small drug molecules and therapeutic nucleotides, peptides and proteins. Successful delivery requires the development of biocompatible SPIOs that efficiently carry therapeutic substances into target cells and subcellular compartments. However, previous studies showed controversial uptake rates and endocytic pathways of SPIOs, due to lack of control on particle size, magnetization and coating. The variability stresses the need to systematically investigate the effect of magnetic force with SPIOs of uniform core size and surface coating.
To elucidate the mechanisms governing magnetically guided cellular uptake of SPIOs, we systematically studied the uptake of SPIOs with different core size and coating thickness by human cancer cervical cells, HeLa. The quantity and the intracellular distribution of internalized SPIOs were examined with flowcytometry and fluorescence microscopy.

MATERIALS AND METHODS

Nanoparticle synthesis

Six SPIOs with different core size (8, 12 and 15 nm) and surface coating (DSPE-mPEG2000 and DSPE-mPEG5000) were synthesized. To obtain uniform SPIOs, we first synthesized 8 nm magnetite nanocrystals by thermodecomposition of $Fe(acac)_3$ [1]. 12 nm and 15 nm nanocrystals were synthesized by sequential seed-mediated growth from 8 nm nanocrystals. As-synthesized nanocrystals were capped with a layer of oleic acid and oleylamine and dispersed in toluene. To make water-dispersible SPIOs, the nanocrystals were coated with amphiphilic DSPE-mPEG copolymers using dual solvent exchange method [2]. The final core sizes were determined by TEM (Figure 1). In order to examine intracellular distribution of SPIOs with fluorescence microscopy, the SPIOs were labelled with DiI (ex/em=549nm/565nm).

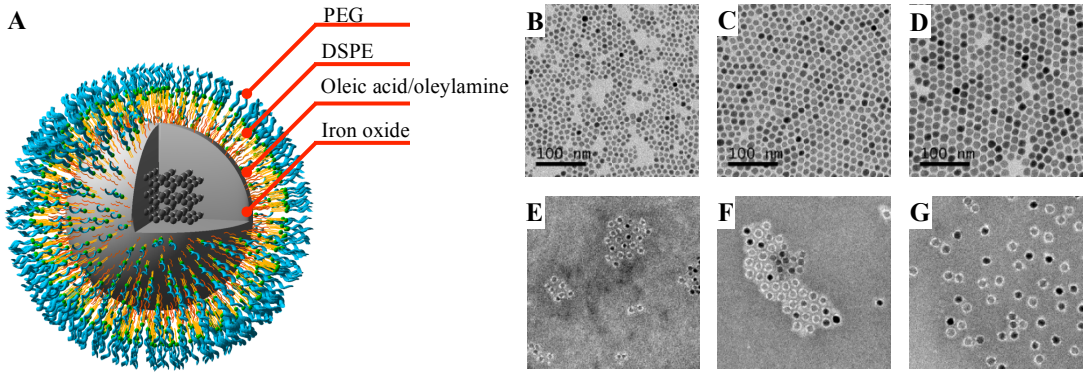

Figure 1: TEM micrographs of SPIOs. (A) Schematic diagram of the structure of SPIOs. (B, C and D) are as synthesized 8, 12 and 15 nm magnetite nanocrystals. (E, F and G) are corresponding nanocrystals coated with DSPE-mPEG2000. In (E, F and G), the nanoparticles were negatively stained with methylamine tungstic acid. The DSPE-mPEG2000 coating was visualized as unstained white layer.

Quantification of cellular uptake of SPIOs with applied magnetic force

HeLa cells were cultured with DMEM supplemented with 10% FBS. After initial passage in tissue culture flasks, all cells were grown in 4-well chamber glass with a bottom thickness of approximately 200 μm. The cells were cultured with medium containing 100 μg Fe/ml SPIOs at 37°C for 1 hour. To apply magnetic force, the chamber glass was placed on a magnetic plate assembled with circular NdFeB permanent magnets (d = 2.5 mm, h = 1.0 mm) (Figure 2). After incubation, the cells were detached with trypsin-EDTA. The fluorescence intensity of internalized SPIO-DiI was measured with a C6 flowcytometer. To examine endocytic pathways, we incubated SPIOs with cells

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at 4°C or 37°C in the presence of filipin (inhibitor of caveolae mediated endocytosis). Endosomes formed by clathrin mediated endocytosis were stained with Cell Light reagent targeting Rab5a.

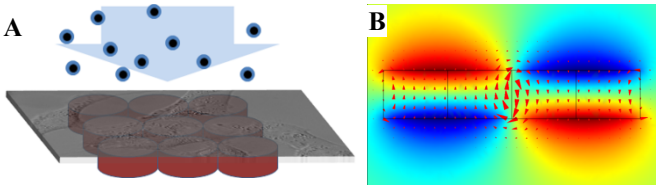

Figure 2: Schematic Diagram of experimental settings. (A) Schematic diagram of experimental setting. The magnetic plate was assembled with small circular magnets. The magnetization was along the axial direction. (B) Computed magnetic field (z-component only).

RESULTS

Our study indicated that the magnet force significantly enhanced cellular uptake of SPIOs (Figure 3). Internalized SPIOs mainly accumulated in the intracellular vesicles in the perinuclear region. The effect of magnetic force increased with the core size as a result of improved core magnetization and consequently larger magnetic force (Figure 4A). The ratios between internalized SPIOs with and without a magnetic field were 6.3, 6.7 and 10.0 for 8 nm, 12 nm and 15 nm cores with DSPE-PEG2000 coating respectively. The ratios decreased as the molecular weight of PEG increased from 2000 Da to 5000 Da, presumably due to both increased fluid drag force and reduced interaction between SPIO and cell membrane. The internalized SPIOs (15 nm core and DSPE-mPEG2000) after one hour incubation on the magnetic plate were approximately 3.1±0.7 pg Fe/cell.

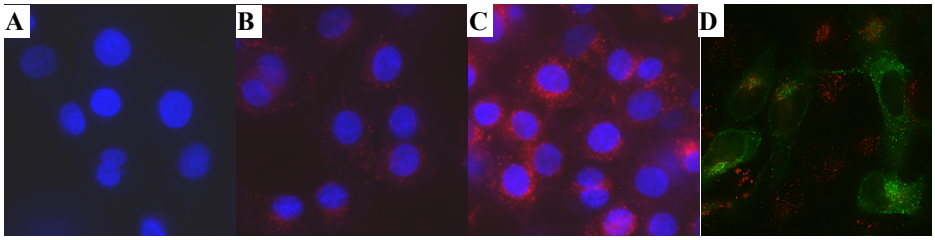

Figure 3: Fluorescence microscopy of cells treated with SPIOs. (A) Untreated cells. (B) Cells incubated with 15 nm SPIO with DSPE-mPEG2000. (C) Cells incubated with the same SPIOs under magnetic field. (D) Cells incubated with the SPIOs under magnetic field. Clathrin coated early endosomes were labelled with Cell Light reagent (green). In (A, B and C), cell nuclei were counterstained with Hoescht 33342 (blue).

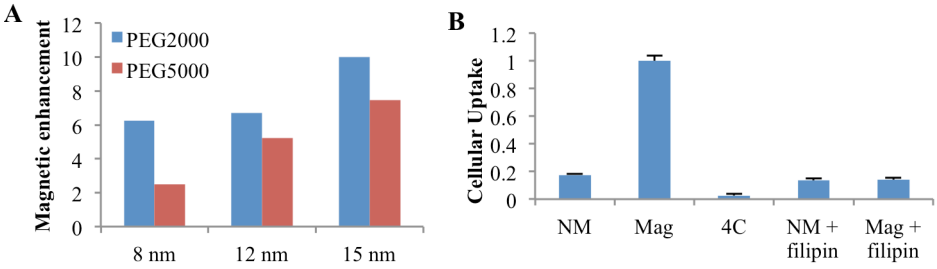

Figure 4: Quantification of cellular uptake of SPIOs driven by applied magnetic force. (A) The ratio of SPIO uptake with and without magnetic treatment. (B) Uptake of SPIO by cells incubated at 4°C or treated with filipin.

Further studies showed that incubating the cells at 4^0C inhibited cellular uptake of SPIOs under the magnetic field, whereas filipin abolished magnetic enhancement but had minimal effect on spontaneous uptake of SPIOs by HeLa cells (Figure 4B). Fluorescence microscopy examination indicated that internalized SPIOs showed poor co-localization with clathrin coated early endosomes (Figure 3D).

CONCLUSIONS

Our results suggest that magnetic forces enhance cellular uptake of SPIOs by (1) increasing accumulation of SPIOs near cell surface and (2) activating caveolae mediated endocytosis. Our on-going studies include exploring SPIO surface modifications that can control intracellular distribution of SPIOs and investigating magnetic control of SPIOs in blood flow.

References

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[2] Tong, S., et al., *Self-assembly of phospholipid-PEG coating on nanoparticles through dual solvent exchange.* Nano Lett, 2011. 11(9): p. 3720-6.

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