Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Downregulation of MIM protein inhibits cellular endocytosis process of magnetic nanoparticles in macrophages

Peng Zhao,ab Meng Cao,ab Lina Song,ab Hao Wu,ab Ke Hu,ab Bo Chen,ab Qiwei Wangab and Ning Gu *ab

a State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, P.R. China. E-mail: guning@seu.edu.cn

b Collaborative Innovation Center of Suzhou Nano Science and Technology, Suzhou 215123, PR China

S1. Effects of MNPs on the expression of MIM

To exclude the potential interference of MNPs to the experiment as to confirm the differences between the control and test groups are caused by the expression of MIM, we tested the influence of MNPs on the expression of MIM in normal RAW 264.7 cells, to avoid the interference of MNPs to the experiment. And the results indicated that incubation with Fe_2O_3@DMSA did not influence the MIM expression in macrophage RAW 264.7.

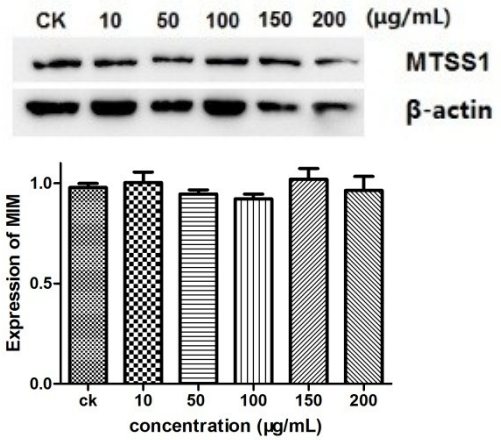

Fig. S1 Effects of Fe_2O_3@DMSA on the expression of MTSS1. Result no statistical significance was evident between MNP treated and untreated group at concentration of 10-200 μg/mL.

S2. Assay of particle uptake efficiency

Calcein fluorescence quenching assay

Calcein is an interesting iron ligand whose fluorescence is quenched upon binding to iron. As a consequence, it has been studied as a sensor for iron and is regarded as a useful tool for monitoring cytosolic iron and assessing the dynamics of intracellular iron in living cells. Cells were seeded in 96-well plates(black) at a density of 1×10^4 cells/well and cultured for 24 hours, then incubated with 1 μM calcein/AM for 20 minutes. After incubation, washed the cells 3 times with the PBS, and replaced with fresh medium containing MNPs in concentration of 100 μg/mL, and monitor the fluorescence quenching in a 5 minutes gap, with the exposure time from 0 to 60 minutes. The fluorescence was monitored at an excitation of 490 nm and an emission of 515 nm via a microplate reader(infinite M200, Tecan, Switzerland).

Calcein indicated MNPs uptake

Cells were exposed to Fe_2O_3@DMSA (100 μg/mL) after incubated with Calcein/AM for 20 minutes, and the fluorescence quenching of the cells were monitored in a 5 minutes gap, recorded in Fig. S2. In both normal RAW 264.7 cells and RAW 264.7^{MIM-} cells, with the extension of incubated time, the degree of fluorescence quenching increased. And with the increase of exposure time, the difference of fluorescence quenching efficiency became visible between the two cell lines, especially after 30 minutes exposure. The fluorescence intensity decreased rapidly, indicating the extracellular iron was transported into the cells. That is to say, the uptake of MNPs was faster in normal RAW 264.7 cells than RAW 264.7^{MIM-} cells, and the degree of fluorescence quenching was due to the amount of intracellular iron internalization.

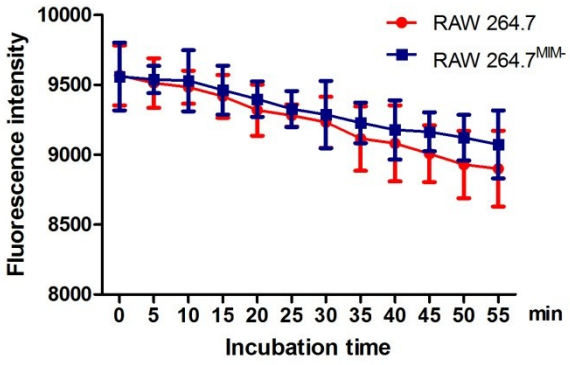

Fig.S2 calcein indicated MNPs uptake by cells. MNPs internalized into cells were determined by the quenching of calcein fluorescence. Compared to transfected cells RAW 264.7^{MIM-}, RAW 264.7 cells showed more rapid and steady fluorescence quenching when exposure with 100 μg/mL concentration Fe_2O_3@DMSA.