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奈米鐵合金在細胞內外的磁感作用

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Evaluating ultrasmall superparamagnetic iron oxide (USPIO) by macrophage cell line Raw 264.7

Abstract

There are different kinds of ultrasmall superparamagnetic iron oxide (USPIO) used currently in the application of cell trafficking. Although the relaxivity of these iron oxide particles are well documented, the amount of cellular uptake and signal in MR system differs. The size of the iron oxide particles and the charge of the molecule accounts for only part of the character. Thus an efficient way to measure the uptake of the iron oxide particles is demanding. We demonstrated that it is an efficient way to evaluate the intracellular amount of the iron oxide particles by using mouse macrophage (Raw 264.7) as a cellular uptake system. Three different iron oxide particles were incubated with the Raw 264.7 for different time period. The uptake of these iron oxide particles was measured by flow cytometry, and microscopy. The amount of particle uptake measured from flow cytometry correlates well with the signal change determined by MR. Besides, the release of nitric oxide by Raw 264.7 is a indicator of inflammatory regulation. We tested USPIO about the release of the NO which revealed the USPIO has no influence on the regulation of inflammation. We conclude that measuring the uptake of iron oxides particles by using mice macrophage is an efficient way to evaluate the character of these iron oxide particles.

Mouse macrophage cell line Raw 264.7 is a good *in vitro* model for studying USPIO, because the USPIO was uptake *in vivo* by reticuloendothelial system which resembles Raw 264.7.

We tested three different kinds of iron oxide nanoparticles, USPIO201, USPIO202 (TANbead, Taiwan), and Resovist ® (Schering, Germany). USPIO 201 and USPIO 202 have Fe3O4 core with different coating. The size of the nanoparticle is ranged from 5~8 nm. USPIO-201 is positively charged while USPIO-202 is negatively charged. Resovsit ® is used widely as the magnetic resonance (MR) contrast medium especially for detecting hepatic tumors.

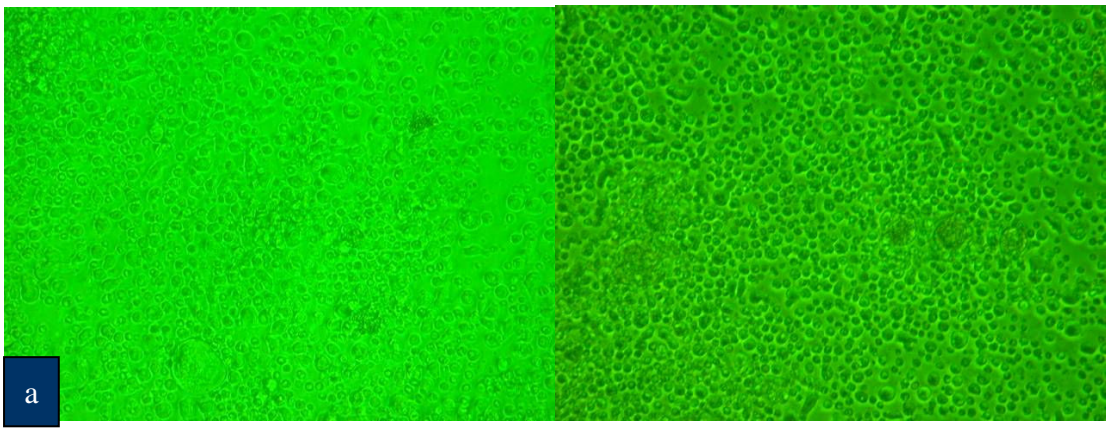


Fig 1a fig 1b

Fig.1 a. Microscope of mice macrophage cell line Raw 264.7 at low power field. b. After incubation with Resovist® (Schering, Germany) for 24 hours, the granularity of the Raw264.7 increased dramatically.

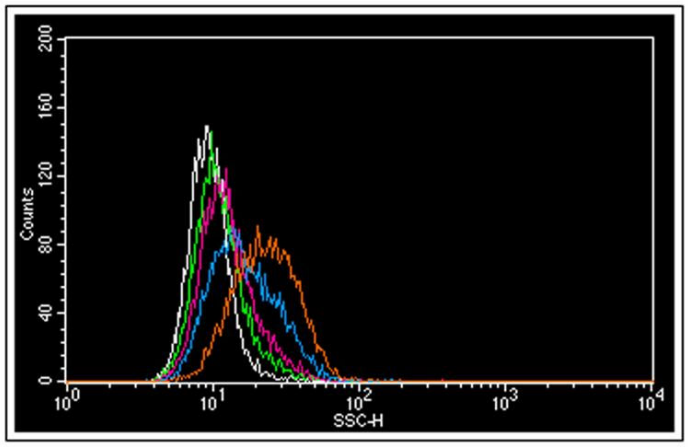
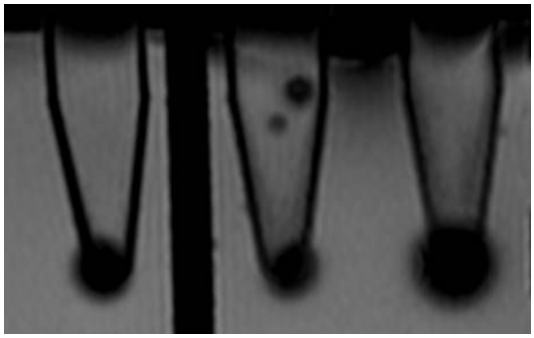


Fig.2

Flow cytometry of the Raw 264.7 cells incubated with Resolvist® at different concentration and incubation time revealed the granularity of the Raw 264.7 increased as the concentration or incubation time increased. The finding suggest that the uptake of USPIO increased at more concentrated condition or increased incubation time (White: no Resovist; green: 2.5umole/ml for 4 hours; pink: 2.5umol/ml for 24 hours; blue: 10umol/ml for 4 hours; orange: 10umol/ml for 24 hours)



3a

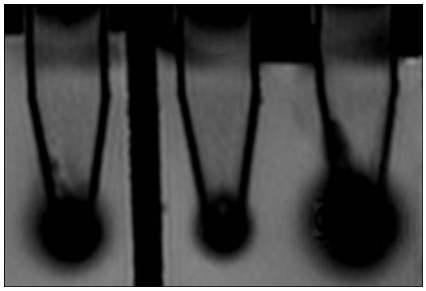


fig 3b

Fig.3

MR image of Raw 264.7 cells after uptake of USPIO-201, USPIO-202 and Resovist respectively. The cell amount is 1×10^5 (a) and 1×10^6 (b). The amount of iron oxide uptake differs in these three different USPIO and the signal intensity change differs.

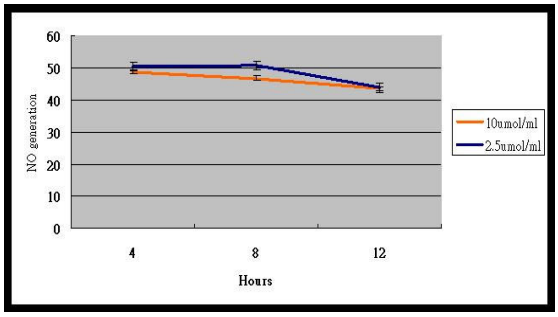


Fig. 4

RAW 264.7 cells were plated at 2×105 cells/well in 96-well plate and then incubated with or without LPS (1 μg/ml) in the presence of various concentrations of Resolvist for 24 h. The nitrite accumulation in the supernatant was assessed by Griess reaction. Each 50 μl of culture supernatant was mixed with an equal volume of Griess reagent [0.1% *N*-(1-naphthyl)-ethylenediamine, 1% sulfanilamide in 5% orthophosphoric acid] and incubated at room temperature for 5 min. The absorbance at 540 nm was measured in an automated microplate reader, and a series of known concentrations of sodium nitrite was used as a standard.

Conclusion

As the technique of cell trafficking and molecular imaging methods in MR develops, more iron oxides nanoparticles emerged for the clinical and research usage. While testing these compound for its character *in vivo* is more complicated, we proposed

the method of using Raw 264.7 cell line as an efficient and simple way to measure these new USPIO *in vitro*.

References

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