Electron Paramagnetic Resonance as a new sensitive tool to assess the iron content in cells and tissues for MRI cell labeling studies

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Introduction

The ability to track the migration of metastatic cells when leaving the seeding tumor should become an invaluable tool to understand this phenomenon and propose new therapeutic anti-cancer strategies. Magnetic cell labeling is a promising technique to detect metastatic cancer cells with magnetic resonance imaging. Usually, cells are incubated with iron oxides (T_2 contrast agent) in order to uptake the particles before being injected in vivo. In addition to MRI protocols, there is generally a need for complementary techniques able to confirm and quantify the cells that have migrated inside a host tissue. We propose here to implement Electron Paramagnetic Resonance (EPR) as a very sensitive method to quantify iron oxide concentration (in cells and tissues). Iron oxide particles exhibit an EPR spectrum, which directly reflects the number of iron oxide particles in a sample. EPR spectroscopy has already been proposed as a method of quantifying the accumulation of iron oxide inside tissues (1). In order to compare EPR with existing methods (Perl's Prussian blue reaction, and fluorimetry), we labeled tumor cells (Melanoma B16F10-luc, fibrosarcoma KHT-luc) and fibroblasts (3T3) with fluorescent iron oxide particles, and defined the limit of detection of the different techniques.

Material and methods

Cell labelling: Melanoma B16F10, fibrosarcoma KHT and fibroblast 3T3 were grown in full medium. One day before the experiment, particles of iron oxide (Molday Ion Rhodamine B, MIRB supplied by Biopal) were incubated (50µg Fe/ml) overnight.
Incorporation of MIRB in cells: Cells were rinsed three times with PBS, trypsinized, and counted. For EPR measurements, the cells were resuspended in pure water with 20% Dextran in order to be analyzed by EPR. Cells were analyzed by cytochemistry: cell membranes were imaged by wga-fluorescein, cell nucleus was imaged with Hoechst 33342, and the MIRB particles were coupled to rhodamine B. Cells supensions were analyzed in hematocrit tubes (75 µl) using a Bruker EMX EPR (9 GHz) spectrometer with the typical parameters: 30 Gauss modulation amplitude, 10.11 mW power, 3251 G center field, field 4000 G sweep width. For colorimetric analysis, cells were resuspended in 200µL HCl 10M and digested for 24h. The solution was then mixed with 400µL of 5% potassium ferrocyanide solution. The OD700 was measured 15min after addition of the ferrocyanide solution. Fluorescence quantification of the MIRB accumulated inside the cells was done using a λ_{abs}= 485 nm and a λ_{em} = 620 nm. Limit of detection was defined as the standard deviation of the calibration curves multiplied by three.

Results and discussion

MIRB iron oxide particles accumulated intracellularly with no remaining particles in the plasma membrane (Fig 1). A typical EPR spectrum of MIRB particles is shown on Fig.2. EPR was able to give an objective and accurate information about labeling efficiency (requiring a very limited amount of sample). Iron oxide quantification studies with EPR show that all the cells uptake from 10 to 20 pg Fe/cell. The fluorescence was not usable to quantify the iron oxide content in cells due to the auto-fluorescence of the samples. With the present setup, the limit of detection of MIRB particles was 104 ng/ml (7.8 ng detected) using EPR and 860 ng/ml (172 ng detected) using Perl's reaction.

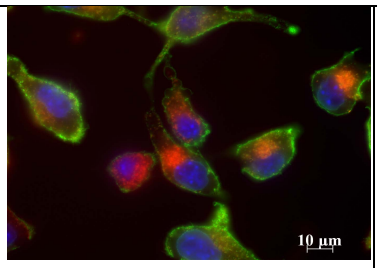

Fig1: B16F10 labeled with MIRB (in red), membranes in green and nucleus in blue.

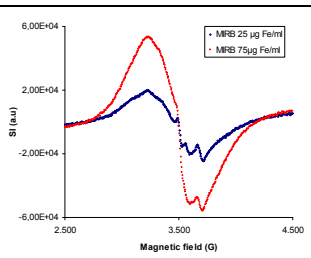

Fig2: EPR spectrum of MIRB.

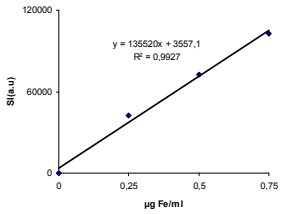

Fig3: Calibration curve
EPR signal intensity as a function of iron content

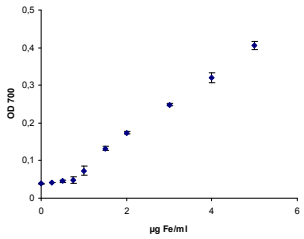

Fig4: Calibration curve
Optical density as a function of iron content

Conclusions

EPR is a fast, easy, and highly sensitive method to quantify iron oxide content after magnetic labelling. Moreover, EPR allows ex vivo Fe quantification, a good way to confirm and quantify MRI results after cell tracking experiments.

References

1. Radermacher et al, Invest Radiol.2009, Jul;44(7):398-404.