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In vitro toxicity of magnetic fluids evaluated for macrophage cell lines ☆

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Abstract

In this paper the in vitro toxicity of magnetic fluids is evaluated for macrophage cell lines.
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Keywords: Magnetic fluids; Macrophage cell lines; Toxicity

1. Introduction

Biological applications of superparamagnetic nanoparticles need a previous evaluation of direct in vivo and in vitro cytotoxicity. Considering the growing number of different nanoparticles preparations now available and their great variability both for the core composition or for their external surface, in vitro tests are to be proposed complementary to in vivo tests. Toxicity of nanoparticles appears to be very dependent either on their physical state or their chemical composition.

In vivo toxicity of ionic and citrate-coated nanoparticles has been previously reported to have in vivo cytotoxic effects for macrophage in mice [1].

No such toxicity has been found with five different nanoparticles potentially useful in Magnetic Resonance Imaging [2] or used in vivo for cellular labelling [3].

In the present work, we used mouse (RAW) and human (THP1) cell lines as a cytotoxic model, where THP1 are able to be switched from a monocytic to an active phagocytic state, after differentiation with retinoic acid. This allowed us to distinguish effects due to adsorbtion and pinocytosis from phagocytosis.

2. Materials and methods

Iron oxide nanoparticles: Ferrofluid-dimercaptosuccinic acid (FFDMSA) and Dextran-coated ferrofluid (FFDX) were produced by Mediport Cardiotechnick GmBH (Wiesenweg 10, D12247, Berlin). Dextran sulfate molecular weight was 60 000–100 000, the exact concentration of coated molecules is not known. For FFDMSA, the molar ratio SH/Fe was estimated to be 2%. Magnetization of 40 mT corresponded to a concentration of 3×10^7 particles/ml ($M_s = 5\,\mathrm{mT}$), with a mean size of 9 nm (electronic microscopy). Basic-ion ferrofluid was a kind gift of Prof. Pileni (Jussieu University, Paris). This ferrofluid was maintained at basic pH as ionic particles, and was flocculated near neutrality in the presence of culture medium. Starting concentration of particles (first dilution) was 1.22×10^{15} particles/ml. Cells were cultivated in MEM medium supplemented with 10% fetal calf serum (GIBCO) at

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37°C, 5% $C0_2$. Cell growth was determined by direct counting of viable cells (Trypan blue) using a Mallassez hemocytometer. THP1 were differentiated into active macrophage after retinoic acid treatment (10 μM) for 48 h. For inhibition of cell division, RAW cells were irradiated at 100 Gy (^{60}Co source), culture medium was changed immediately after irradiation. The first dilution of particles was 0.5 ml for 10 ml culture medium. Cells were put in contact with particles for 4 h, and medium was discarded and replaced. Effects of free iron were inhibited by the use of a chelator (1 mg/ml Synsorb B, Fluka). Cells were collected after a 0.1% trypsin treatment and viability was assessed with the Trypan blue exclusion test.

3. Results

The three preparations of superparamagnetic nanoparticles tested show quite different effects on cell viability, dependent on the cell line tested. Toxicity of the FFDMSA preparation was assessed by the growth curve of the RAW cell line (Fig. 1a), this effect was dose

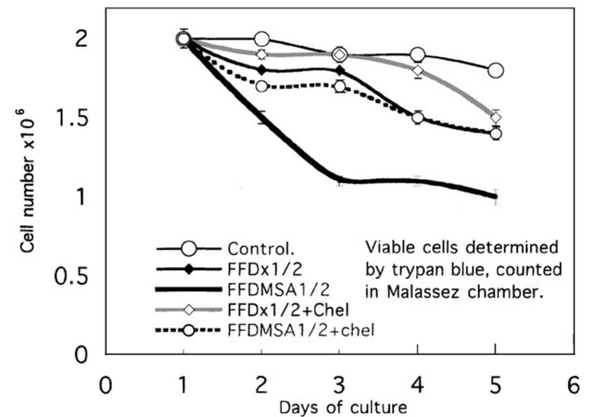
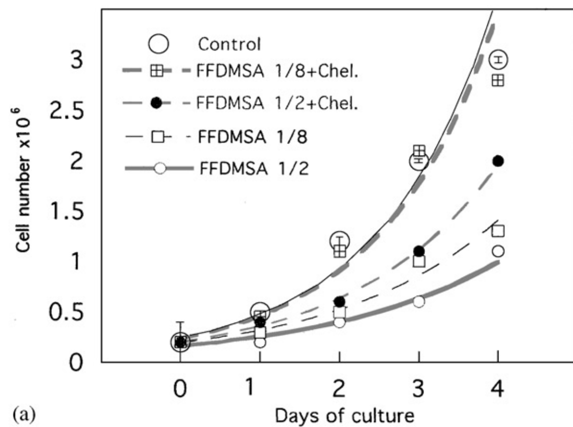


Fig. 2. Viability of irradiated RAW cells exposed to ferrofluids,



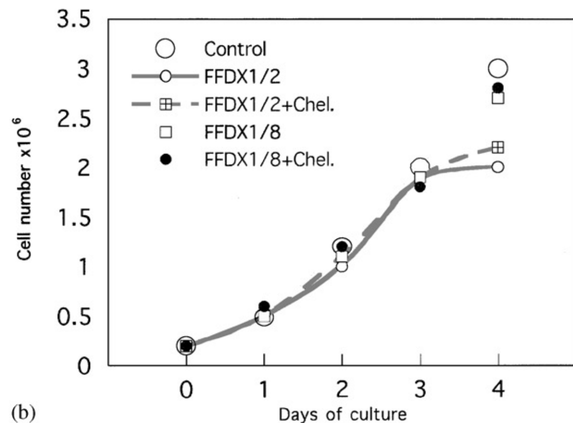
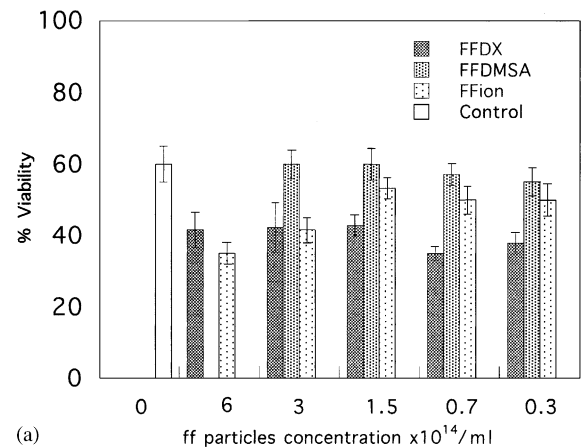


Fig. 1. (a) Cell growth curve of RAW cells: effects of FFDMSA, $\frac{1}{2} = 1.1 \times 10^{15}$ particles/ml, Chel. = iron chelator, SEM = 2% and (b) cell growth curve of RAW cells: effects of FFDX, $\frac{1}{2} = 1.1 \times 10^{15}$ particles/ml, Chel = iron chelator.



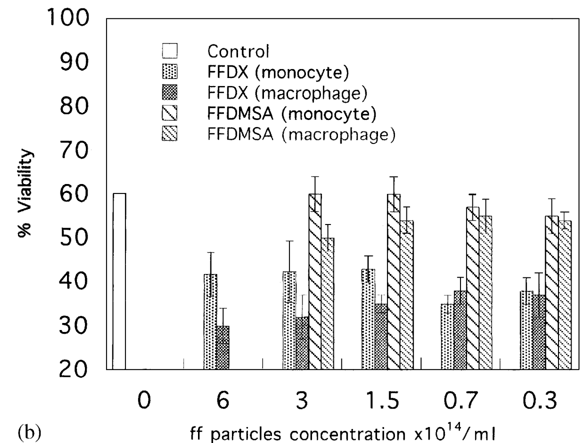


Fig. 3. (a) Viability of monocytic THP1 cells exposed to ferrofluids and (b) monocytic and macrophagic THP1 cells exposed to ferrofluids.

dependent, and reversed by the use of iron chelator. Without chelator, high and low concentrations of FFDMSA showed a dramatic effect on the cell growth. The FFDX preparation (Fig. 1b) showed a limited toxicity even at high concentration, as compared to the FFDMSA. In order to distinguish between cell growth and true cytotoxicity, RAW cells were irradiated at 100 Gy, which inhibited the cell division. FFDMSA showed to be cytotoxic (Fig. 2), and iron chelation protected against cytotoxicity. The human monocytic THP1 cell line was also used to be compared to the murine cell line (Fig. 3a). In this system, FFDX showed to be slightly more toxic than FFDMSA, and the ionic ferrofluid was shown to be the most toxic of the preparations, with a dose dependency. Differentiation of monocytes to active macrophages with retinoïc acid dramatically increased the toxicity of FFDX and in a lesser extend the toxicity of FFDMSA. This effect was also dose dependent.

4. Discussion

In this work, the Trypan blue was used for estimating the viability of cells, despite some drawbacks of this method. Nonetheless, the color of ferrofluids adsorbed or internalized inside the cells impaired the use of metabolic tests based on the production of a chromogen (MTT test). Moreover, the quantification of intracellular enzymes released after cell leakage was also difficult in the presence of ferrofluids and showed not to be well suited for the monocyte/macrophage cell line. The differential toxicity observed between the RAW and THP1 cell line may be explained by differences in their cell coating (driving particles interaction with the cell surface) and their degradative pathways. This highlighted the need to use more than one cell line, in order to investigate the toxicity and inflammatory effects of ferrofluids which has been previously observed in vivo [2]. The effects of free iron are to be distinguished from the effects of non-soluble iron which is trapped in the form of iron oxide. Moreover, the intracellular events leading to the internalization of particles and their chemical modification into acidic compartment of the cell probably take part to the delayed toxicity of the ferrofluids in vivo, as activated THP1 (macrophage) are more sensitive to the toxic effects of ferrofluids. Quantification of internalized iron and Fe^{2+}/Fe^{3+} ratio may help in understanding the degradative pathways of ferrofluids, as well as concerning their long-term clearance. The THP1 model seemed to be an interesting model, as it is able to be switched between two states (pynocytosis, phagocytosis). More efforts are to be performed in order to understand the biological basis of ferrofluid's toxicity and for designing safer preparations, each of them having original properties in terms of charged surfaces, coating and chemical stability. The use of Raman spectroscopy may be of invaluable help for this purpose [4].

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