EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Nanobiosciences

ESRF Beamtime: MD 543

EXPERIMENTAL REPORT

Summary:
The main objective of the study was to investigate the possibility of producing a controlled drug release in neuron cells by using nanoparticles coated with an acid-sensitive polymer (PVP) and a drug and/or an imaging molecule for MRI investigations: Gd-DTPA.
Results indicate that nanoparticles (NPs) are confined in the perinuclear region of the cells for all concentration tested.

Introduction

One of the main limitations in treating solid tumors with conventional chemotherapeutics is the difficulty in really accessing cancer cells, due particularly to defective vasculature in tumors and a sort of barriers that reduce a sufficient drug delivery. In addition, cancer drug resistance can develop rapidly for some aggressive tumors. A big help in treating cancer diseases could come from synergic cytotoxic activities and from attempts of imaging guided therapy. This is a recent concept in the expectations of nanomedicine, referred as *theranostic* approach.[1] Amongst the different nanoparticles (NPs), magnetic and core-shell magnetic nanoparticles with different composition aimed to kill cancer cells by magnetically induced hypertermia have been developed. It is relevant that, magnetic nanoparticles could be suitable for treating brain tumors, because they can cross blood brain barrier.[2] Moreover, multifunctional magnetic nanoparticles for drug and gene delivery and for *in vivo* applications in cancer diagnostic and therapy have also been investigated.[35] We previously successfully applied XRF to reveal the presence of Gd containing drugs in different cell and tissue models for other purposes (and MD375, MD462 reports). Moreover, we have also used both ID21 at ESRF and TwinMic at ELETTRA to investigate the uptake and distribution of magnetic nanoparticles in Balb 3T3 mouse fibroblasts.[4]

Experimental approach

Different types of magnetic nanoparticles have been used in this study. In particular, FeSi(Gd) NPs (15-80nm in diameter) have been synthesized at JRC. These nanoparticles consist of a Fe core (about 5-7 nm) surrounded by a silica other shell of ~20nm in diameter where the Gd-DTPA (Diethylenetriaminepentaacetic acid gadolinium(III) dihydrogen salt hydrate) compound has been inserted.
Two different cell lines have been investigated: U87 human glioblastoma astrocytoma cell line and Balb 3T3 (mouse, fibroblasts) cells used as positive control. The cells were exposed to nanoparticles for 24 h at different concentrations (between 2 and 22 μg/mL). Other magnetic nanoparticles were also used for comparison: Fe_2O_3 (maghemite) NPs of about 30 nm in diameter[5] and cobalt ferrite ($CoFe_2O_3$) of about 35nm (Colorobbia, Italy).

Results

TEM picture of U87 glioblastoma astrocytoma cell exposed to SiFe(Gd) NPs are reported in figure whilst XRF maps of Ca, P, Fe, and Gd are reported in figure 2.
As can be seen, TEM results indicate that the nanoparticles are able to enter different cell compartment (vesicles and endosomes) but not the nucleus. This is also confirmed by the XRF data (Figure 2). The Fe and Gd maps show the confinement of the NPs in the perinuclear region of the cell, whilst the Ca and P signal are uniformly distributed in the cell indicating a cell healthy status. Moreover, the colocalization of Fe and Gd indicate that most of the Gd-DTPA is not released by the nanoparticles, probably because the entrapment into the silica outer shell is too strong and does not allow the release of Gd. On the other hand the nanoparticles seem well tolerated by the cells. In fact, the shape and morphology of cells after exposure to the FeSi(Gd) NPs are every similar to that of the control (Figure 2) and the Ca, S and P (not shown) are

uniformly distributed in the cell. XRF maps of U87 cells exposed to pure Gd-DTPA at the same concentrations and exposure times show a low but uniform Gd signal in the entire cell, with no accumulation of Gd in specific cell compartments. No major differences have been observed when increasing the NPs concentration 2μg/mL. However, in this case the Gd signal is very low and almost indistinguishable from the background.

A similar behaviour has been observed for the other nanoparticles tested. However, in the case of the $CoFe_2O_4$ the presence of Fe and Co in the nucleus of the cell has been detected by XRF spectroscopy. Moreover at high concentrations (>115μg/mL) an excess of Co in the nuclear compartment together with a colocalization of Fe and Ca have been observed.[4] This indicate that the $CoFe_2O_4$ are much less stable than the FeSi(Gd) nanoparticles when in contact with biological environment.

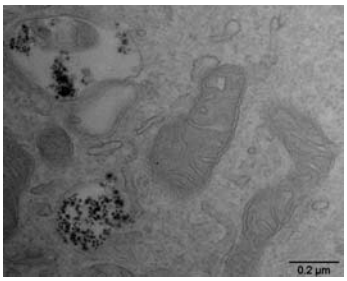
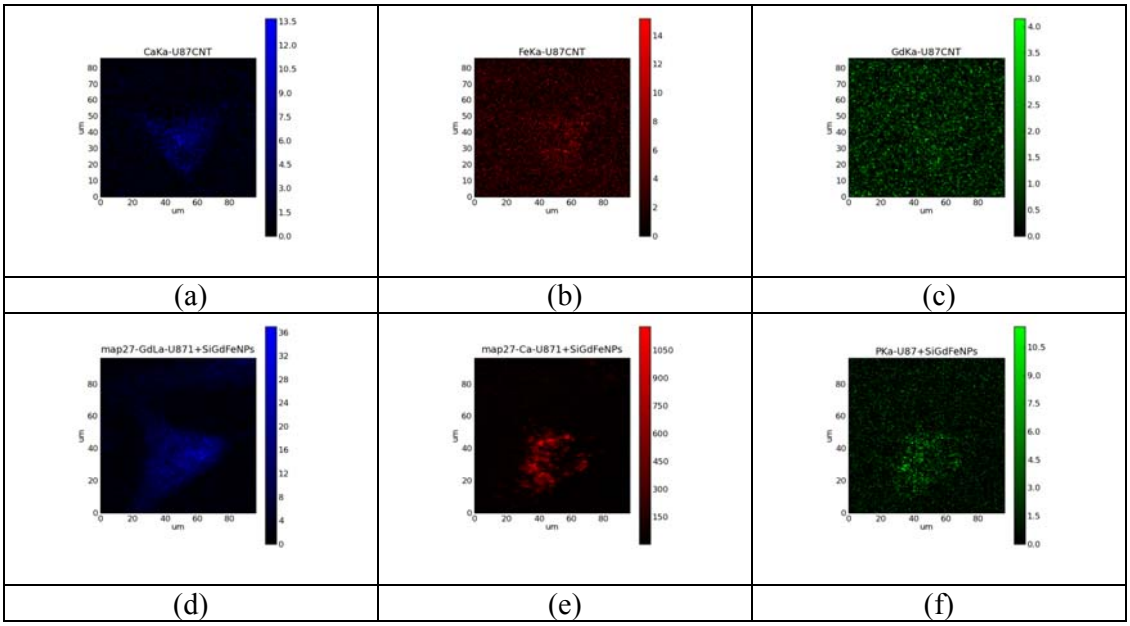


Figure 1: TEM picture of a U87 cell exposed to FESi(Gd) nanoparticles (22μg/mL for 24h)



(a) (b) (c)

(d) (e) (f)

Figure 2: SR-XRF maps of Ca (a, d), Fe (b, e) and Gd (c, f) of U87 glioblastoma cells: control (a, b, c) and exposed to FeSi(Gd) NPs (22μg/mL for 24 h).

References

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