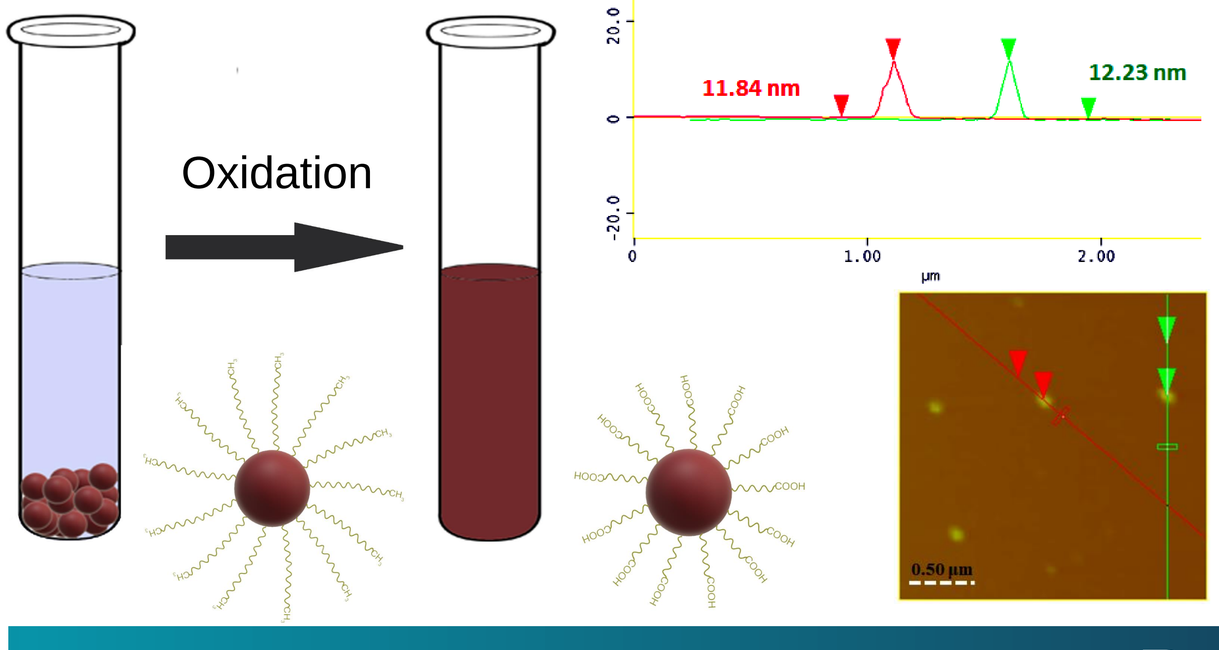
In vitro toxicity evaluation of newly synthesised iron oxide magnetic nanoparticles (IOMNPs)

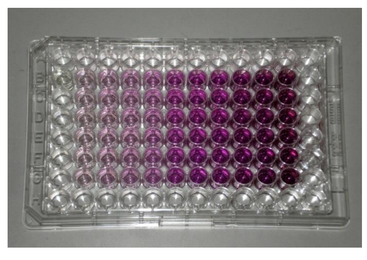
Amalia Fryda[1,2], Niki Karouta[3], Yannis V. Simos[2,*], Konstantinos Spyrou[3], Michaela Patila[1], Evangelia Dounousi[4], Dimitrios Gournis[3], Dimitrios Peschos[2], Haralambos Stamatis[1]

Introduction

Advances of nanotechnology in the past two decades have found fertile ground in the exploitation of magnetic nanoparticles for biomedical applications. Due to their physicochemical properties magnetic iron oxide nanoparticles are considered attractive candidates for cancer applications since they can be used as contrast agents in the diagnosis (magnetic resonance imaging, MRI) and treatment monitoring (magnetic hyperthermia cancer treatment), as drug or gene delivery vehicles and potentially as active medical agents. However, data regarding the *in vivo* impact of IOMNPs on tissues and cells are scarce. In the present study, the cytotoxic effects of newly synthesised IOMNPs were studied against leiomyosarcoma (LMS) cells.



Methods

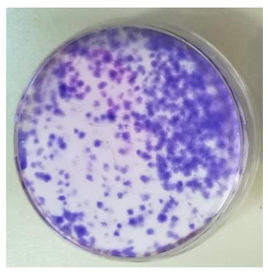


Cell viability

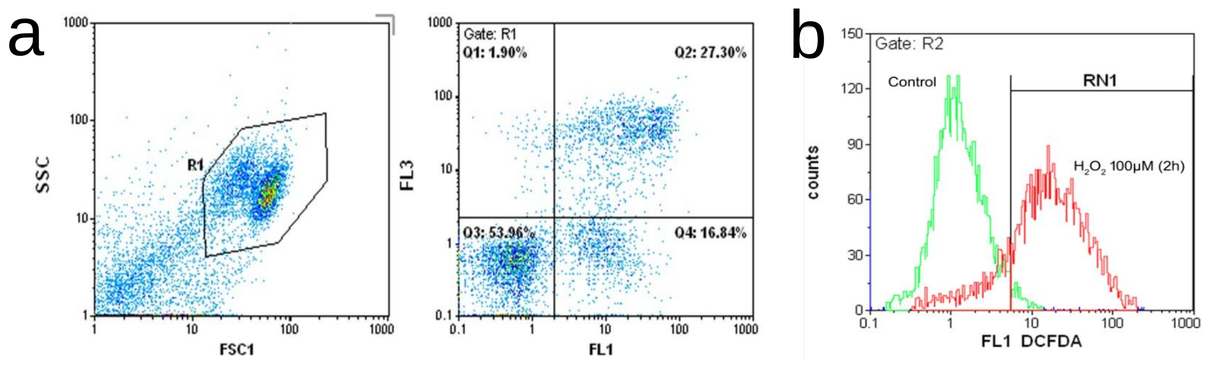
Cells were plated in 96-well plates and incubated for 24 hours before the treatment with various concentrations of the IOMNPs. Cell viability was measured by means of the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, a tetrazole). Absorbance was determined using a microplate spectrophotometer.

Colony Formation Efficiency (CFE)

The ability of single cells to grow into colonies after exposure to the IOMNPs for 24 hours was measured by clonogenic assay.

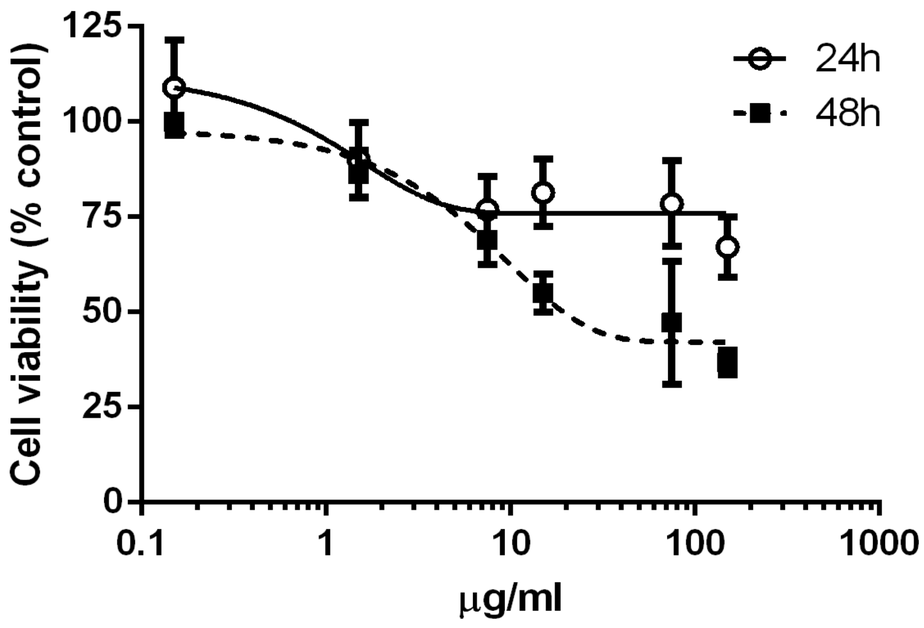


Flow cytometry

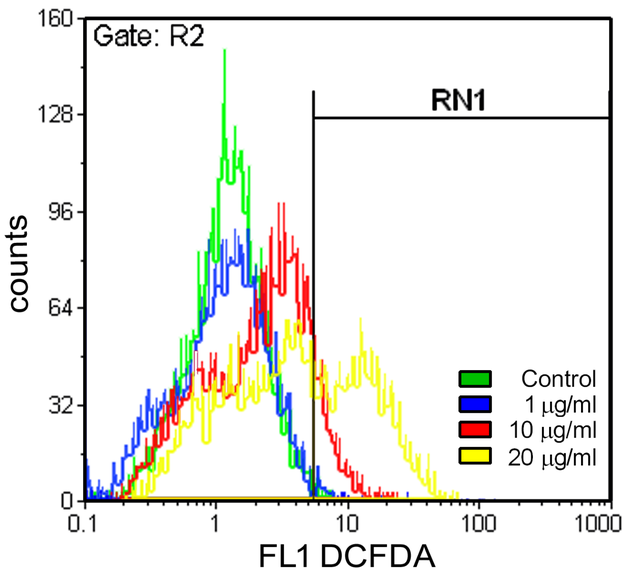


Annexin V-FITC and propidium iodide (PI) double staining was used to quantify apoptosis (a) and dichlorodihydrofluorescein diacetate (DCFH-DA) for the detection of Reactive Oxygen Species (ROS) formation (b). Percentage of apoptotic and necrotic cells was calculated overall viable cells (100 %). For both techniques the cells were analyzed on a fluorescence-activated cell sorting flow cytometer (Partec ML, Partec GmbH, Germany).

Results

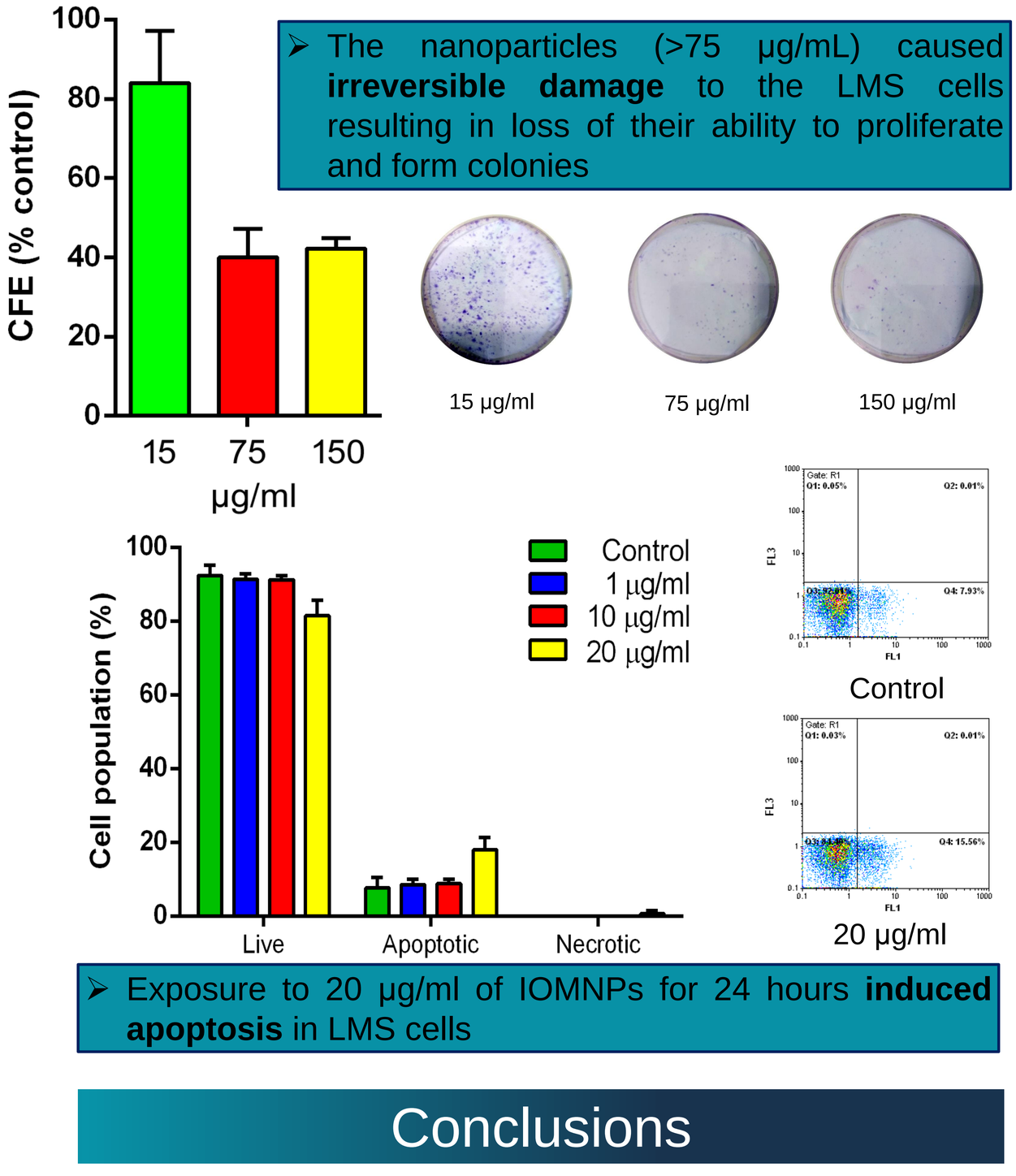


➢ IOMNPs showed a **dose- and time-dependent** cytotoxic activity.



	Mean Fluorescence Intensity
Control	72.3±4.4
1 μg/ml	72.5±7.5
10 μg/ml	96.6±19.4
20 μg/ml	100.4±11.0

➢ At low concentrations (up to 20 μg/mL) IOMNPs triggered the **formation of ROS** in cancer cells.



➢ The nanoparticles (>75 μg/mL) caused **irreversible damage** to the LMS cells resulting in loss of their ability to proliferate and form colonies

➢ Exposure to 20 μg/ml of IOMNPs for 24 hours **induced apoptosis** in LMS cells

Conclusions

Further studies are needed to **determine the cellular internalization of IOMNPs** and the **non-toxic concentrations in normal tissues** that will allow their application in treatment and diagnosis of diseases.

UNIVERSITY OF IOANNINA

[1] Laboratory of Biotechnology, Department of Biological Applications and Technologies

[2] Department of Physiology, School of Health Sciences, Faculty of Medicine

[3] Department of Materials Science and Engineering

[4] Department of Nephrology, School of Health Sciences, Faculty of Medicine