UiO : Universitetet i Oslo

UPO
UNIVERSITÀ DEL PIEMONTE ORIENTALE

POLITECNICO DI TORINO 1859 1906

"Jožef Stefan" Institute
Ljubljana, Slovenia

Design and biological validation of magnetic NPs conjugated with chemotherapy drugs and viral vectors for cancer therapy

Dr. Catalano Enrico, PhD

University of Oslo - UiO

AKERSHUS UNIVERSITETSSYKEHUS

Nano Innovation 2016
Rome, 20-23 September
Conference & Exhibition

NANOINNOVATION'S GOT TALENT

call for young researchers
by BRACCO FOUNDATION

fB Fondazione Bracco

Personal scientific curriculum

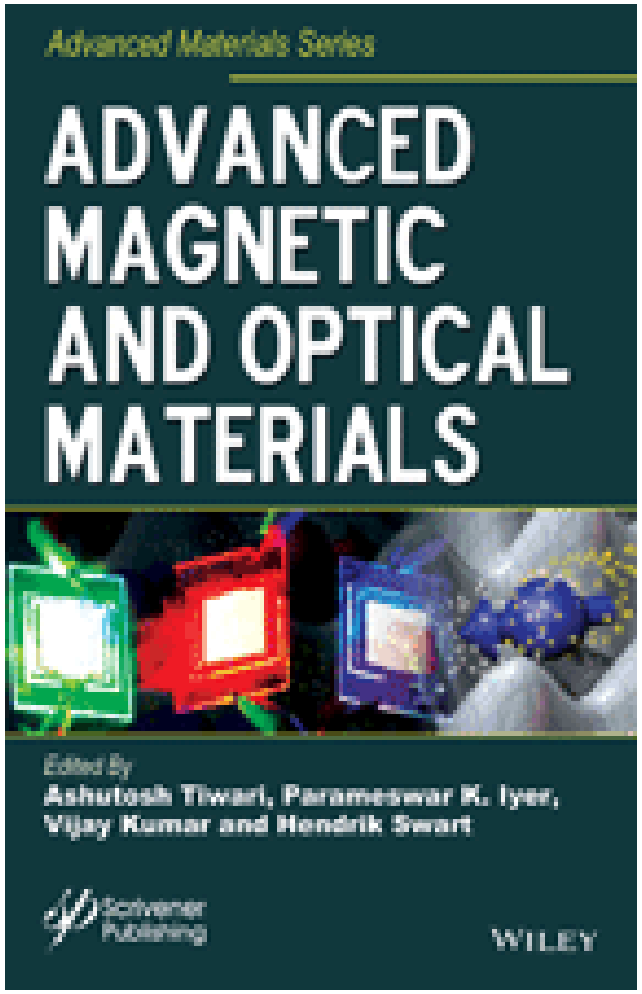
- 2009 BSc in **Pharmaceutical Biotechnology** at University of Bari
- 2011 MSc in **Medical Biotechnology and Molecular Medicine** at University of Bari
- 2015 PhD in **Biotechnologies for human health** at Univerity of Piemonte Orientale with a project about **applications of nanomedicine in biomedicine and cancer therapy**
- 2016 University of Oslo - **Marie-Curie "Scientia Fellows" postdoctoral researcher** with the project entitled: "**New therapeutic approaches for personalized breast cancer nanomedicine**"

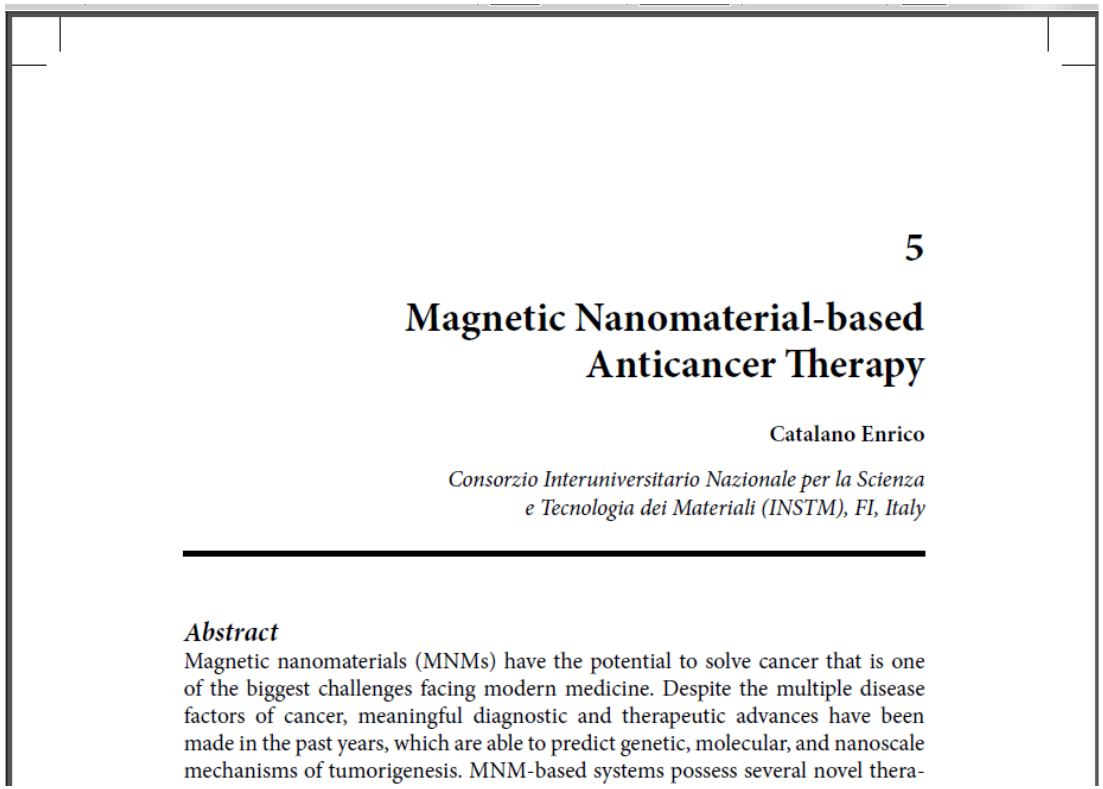
- 1 Italian patent and 1 world patent - "Compositions Comprising Rutin Useful for the Treatment of Tumors Resistant to Chemotherapy" WO/2015/036875

Scientia Fellows project –
Marie-Curie Postdoctoral programme in Health Sciences

UiO : Det medisinske fakultet

AKERSHUS UNIVERSITETSSYKEHUS





5

Magnetic Nanomaterial-based Anticancer Therapy

Catalano Enrico

Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM), FI, Italy

Abstract

Magnetic nanomaterials (MNMs) have the potential to solve cancer that is one of the biggest challenges facing modern medicine. Despite the multiple disease factors of cancer, meaningful diagnostic and therapeutic advances have been made in the past years, which are able to predict genetic, molecular, and nanoscale mechanisms of tumorigenesis. MNM-based systems possess several novel thera-

Advanced Magnetic and Optical Materials edited by Ashutosh Tiwari, Parameswar K. Iyer, Vijay Kumar and Hendrik Swart

Series: [Advanced Materials Series] **Scrivener Publishing - Wiley** Copyright: 2017

Overview

- Applications of nanomedicine
- Synthesis procedure of iron-oxide nanoparticles (MNPs)
- Physicochemical, magnetic and colloidal characterization of iron-oxide nanoparticles
- In vitro and in vivo biocompatibility evaluation of iron-oxide nanoparticles
- Nanomedicine applications on liver cancer cells
- Final results
- Nanomedicine for breast cancer treatment
- Cancer genome editing with CRISPR/Ca9 system

UNIVERSITÀ DEL PIEMONTE ORIENTALE

SUPSI
Scuola Universitaria Professionale
della Svizzera Italiana

UNIVERSITÀ DEGLI STUDI DI TORINO
ALMA UNIVERSITAS TAURINENSIS

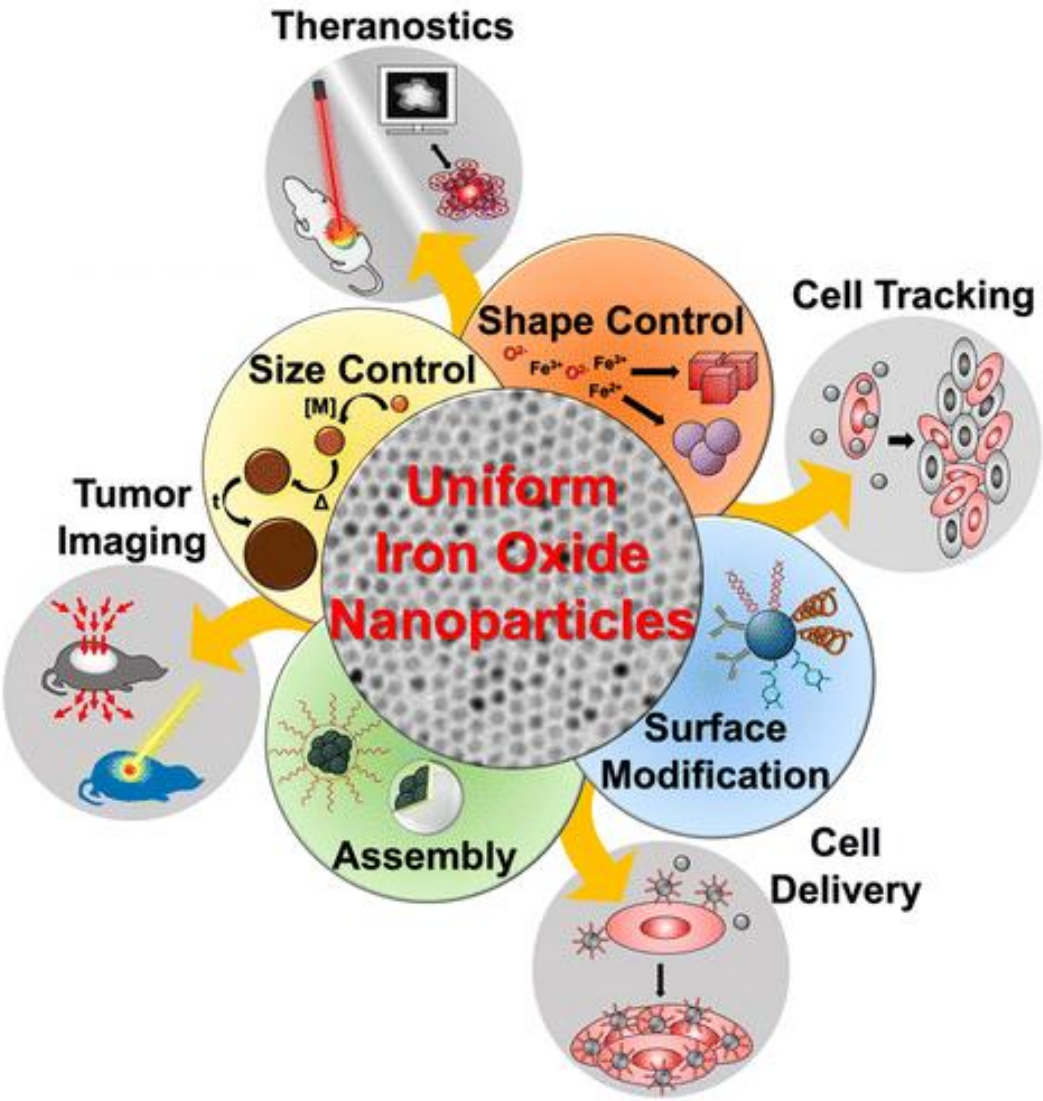
Development of engineered magnetic nanoparticles for cancer therapy

Project supported by AIRC and Compagnia San Paolo

AIRC
ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO

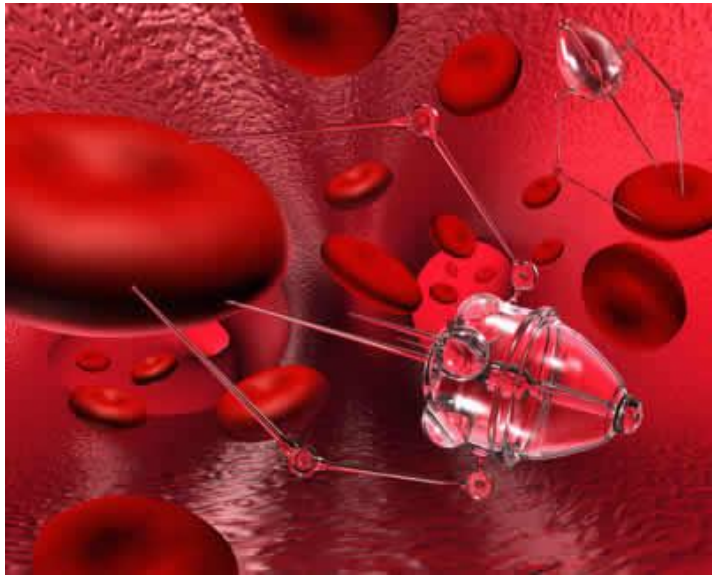
Compagnia di San Paolo

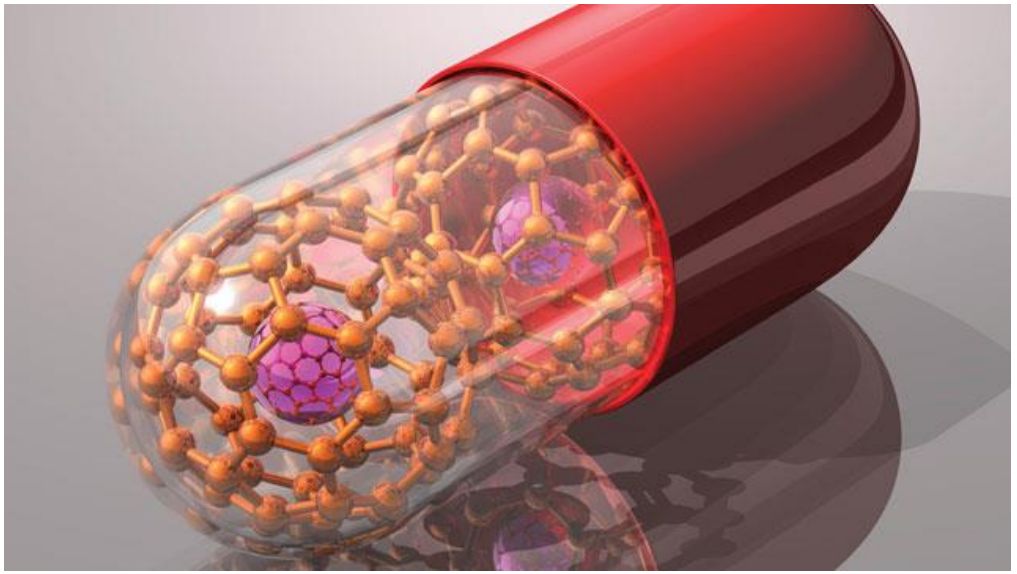
Applications of MNPs in biomedicine

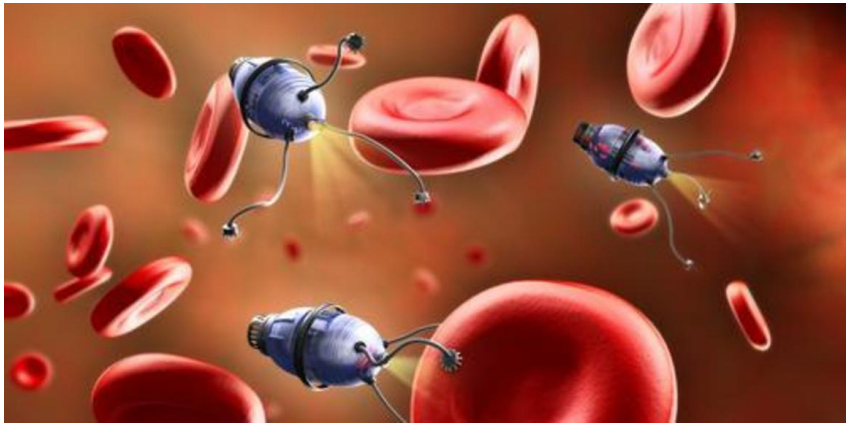


Daishun Ling et al. Acc. Chem. Res. 2015

Targeted nano-drug delivery in tumor microenvironment

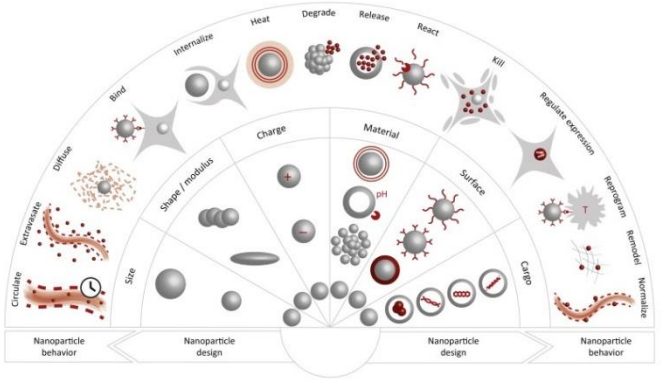


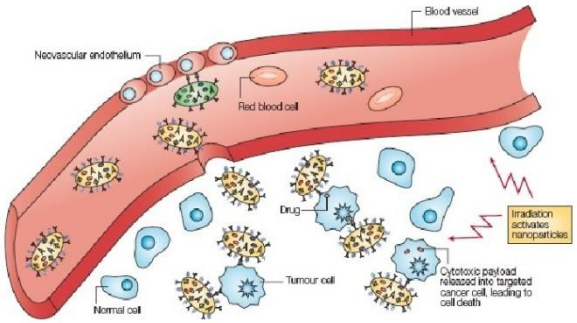


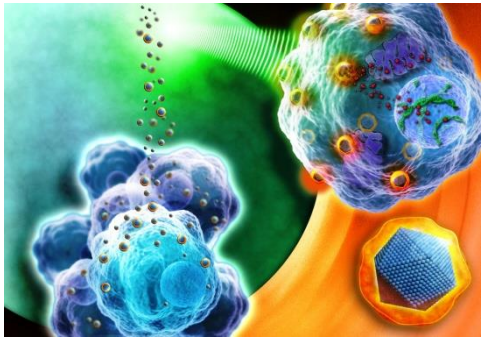


Nanomedicine and Cancer therapy

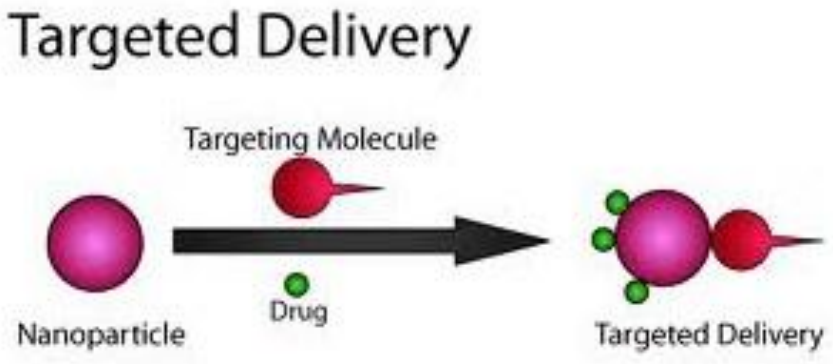
- Nanotechnology is at the forefront of both targeted drug delivery and intrinsic therapies.
- Nanoparticles can already be injected into the tumor and then be **activated to produce heat** and destroy cancer cells locally either by magnetic fields, X-Rays or light.
- The **encapsulation of existing chemotherapy drugs or genes** allows much more **localized delivery** both **reducing significantly the quantity of drugs absorbed by the patient for equal impact and the side effects on healthy tissues in the body**.

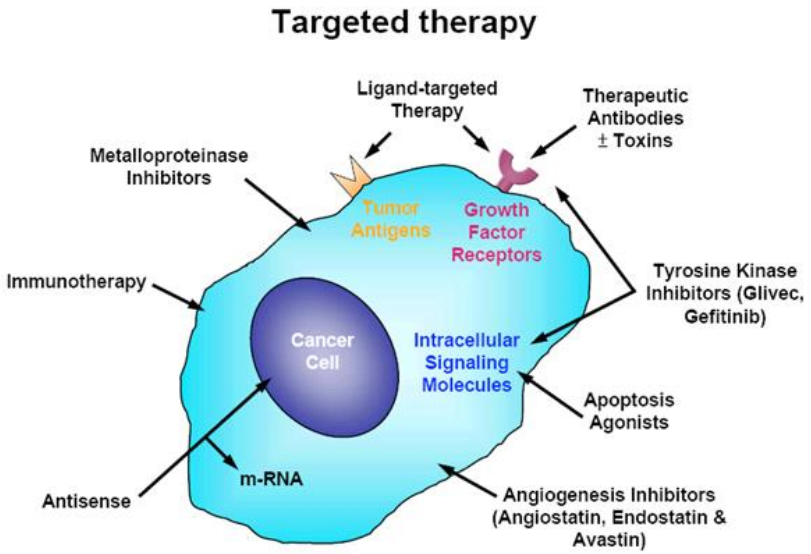


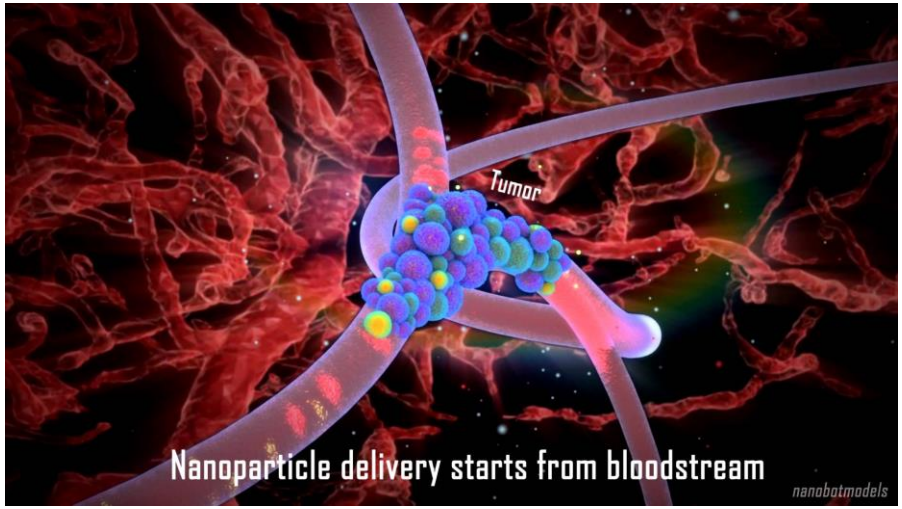




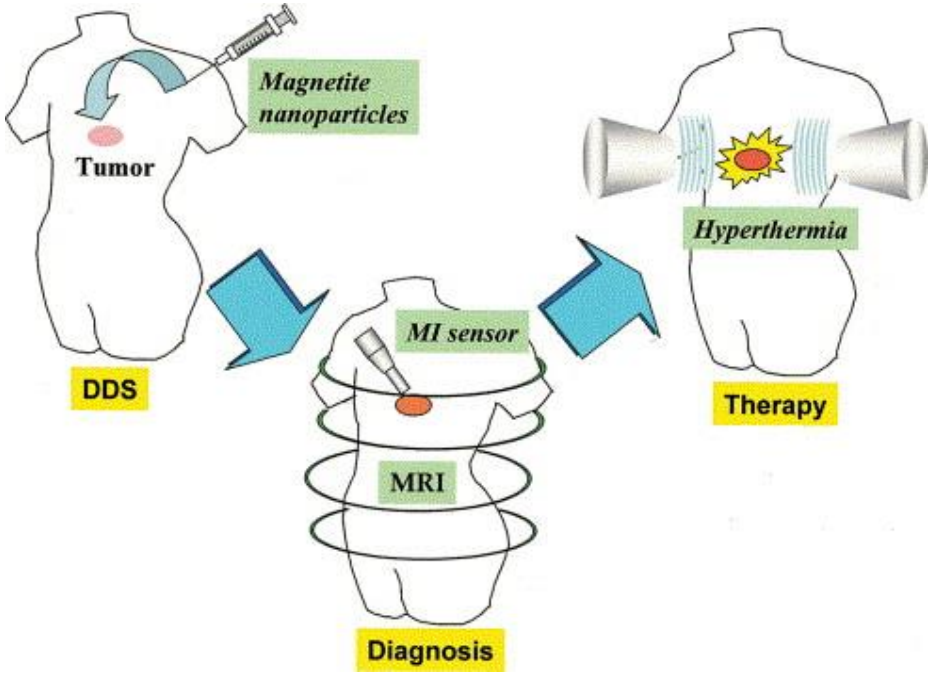
Targeted drug delivery in cancer therapy

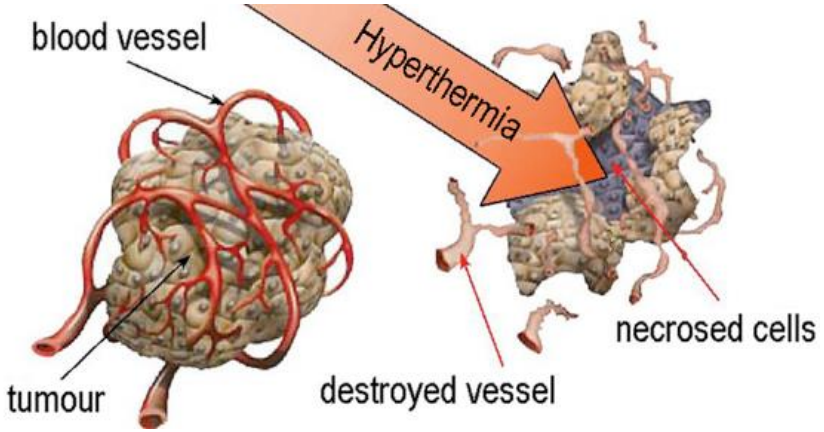






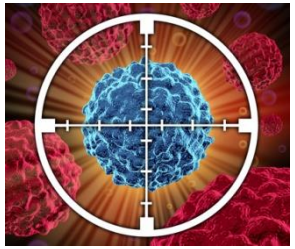
Hyperthermia to Treat Cancer by using magnetic nanoparticles



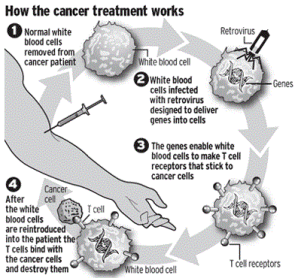


Multiple therapeutic approach of the project

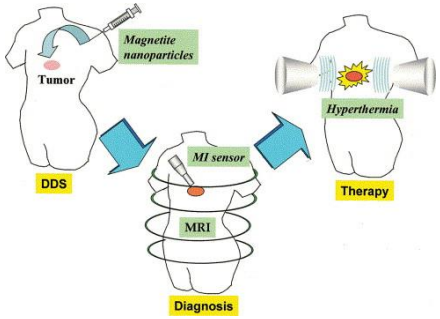
Targeted chemotherapy



Cancer gene therapy



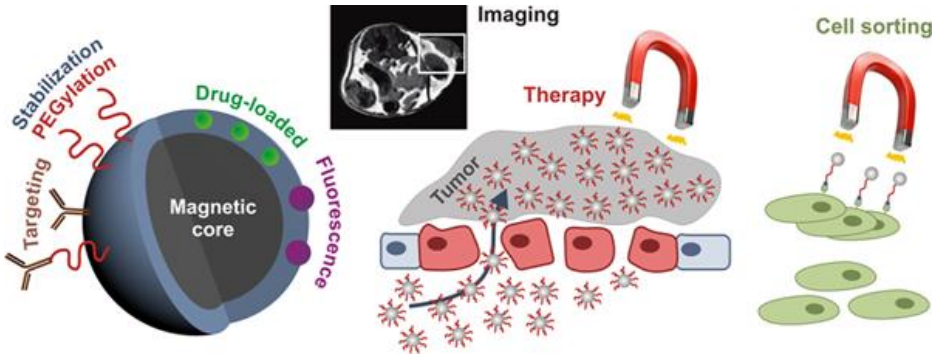
Hyperthermia in the cancer site



Objectives of my PhD work

- Biological characterization and validation of iron-oxide nanoparticles (MNPs or SPIONs) to be used in cancer therapy and biomedicine

Different applications of magnetic nanoparticles



Industrial applications

Drug delivery

Hyperthermia

Tissue engineering and repair

Magnetic resonance imaging (MRI)

Biosensing and Bioanalysis

Biochemical separations

L. Harivardhan Reddy et al., Chem. Rev. 2012

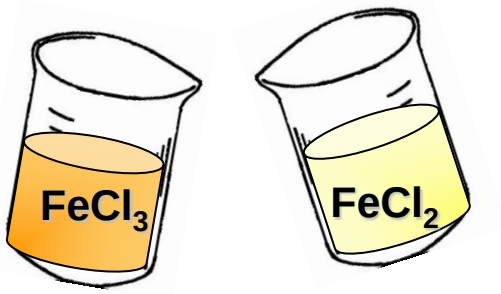
Fe_3O_4

MNPs were prepared by coprecipitation…

*37.5ml 0.1M $FeCl_2 \cdot 4H_2O$

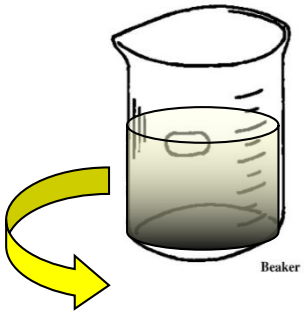
\+

50ml 0.1M $FeCl_3 \cdot 6H_2O$



pH≅10

+ NH_4OH



Mechanical stirring

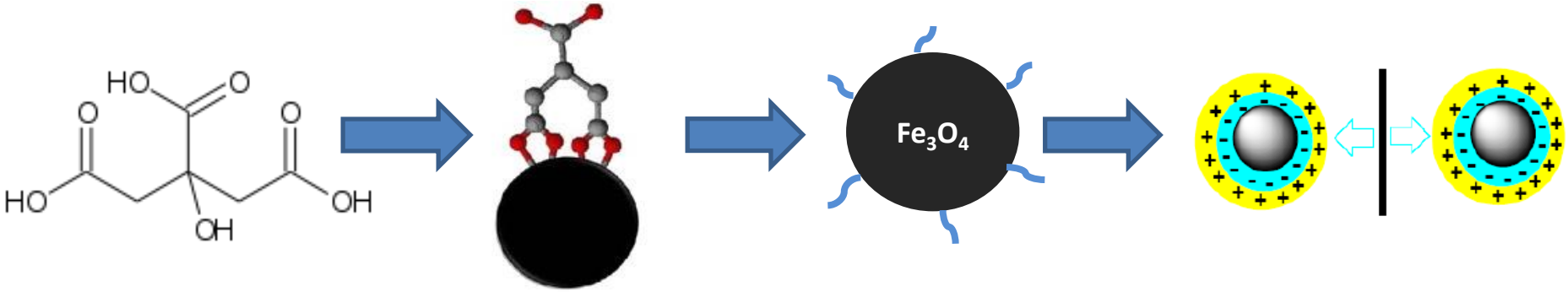
Magnetite

$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$

Maghemite

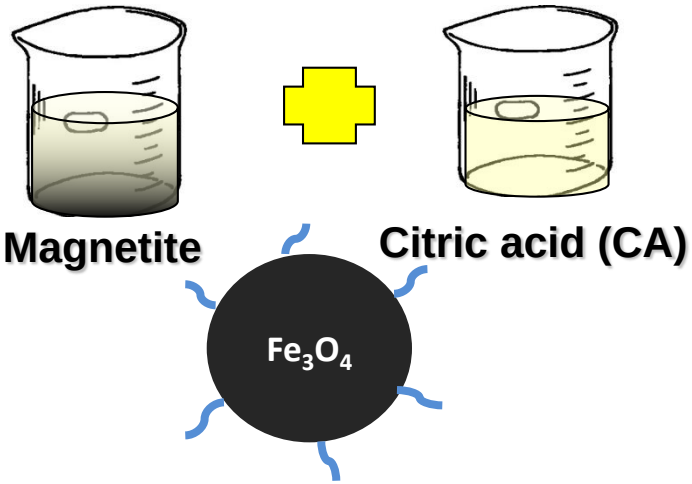
$2Fe_3O_4 + O_2 \rightarrow 3\gamma\text{-}Fe_2O_3$

…and dispersed in citric acid 0.05 M:



* Li Z., Kawashita M., Araki N., Mitsumori M., Hiraoka M., Doi M., 2010, Magnetite nanoparticles with high heating efficiencies for application in the hyperthermia of cancer. *Materials Science and Engineering C*, **30**, 990–996.

Silica shell coating (Mag-SiO_2 NPs): the silica shell was obtained by wet chemistry on the magnetic core stabilized with citric acid



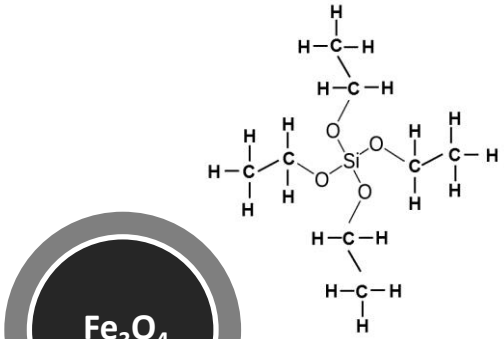
water

ethanol

TEOS

(Stöber)

NH_4OH (pH≈10)

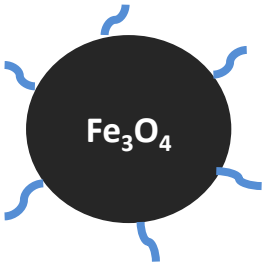


Mag-SiO_2

*[1] Singh R. K., Kim T. H., Patel K. D., 2012, *J Biomed Mater Res Part A*, published online in Wiley Online Library (wileyonlinelibrary.com)
*[2] Stöber W., Fink A., 1968, Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *Journal of colloidal and interface science*, **26**, 62-69

Silica-Calcium shell coating (Mag-SiO_2-Ca(3) NPs) was obtained using two different precursors: calcium citrate and calcium hydroxide

Magnetite



Citric Acid
0.05M

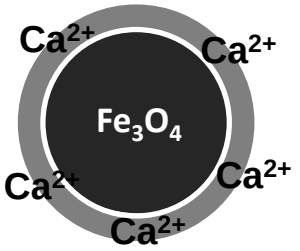
+

Ca citrate/
Ca hydroxide
water
ethanol
TEOS
(Stöber)

Si:Ca ratio 99:1

Mag-SiO_2-Ca(3)

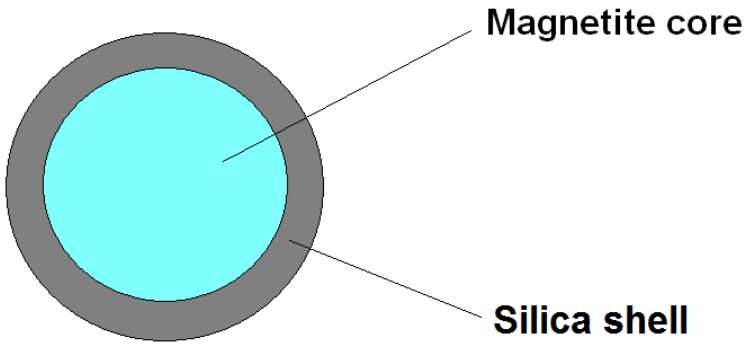
NH_4OH
(pH≈10)

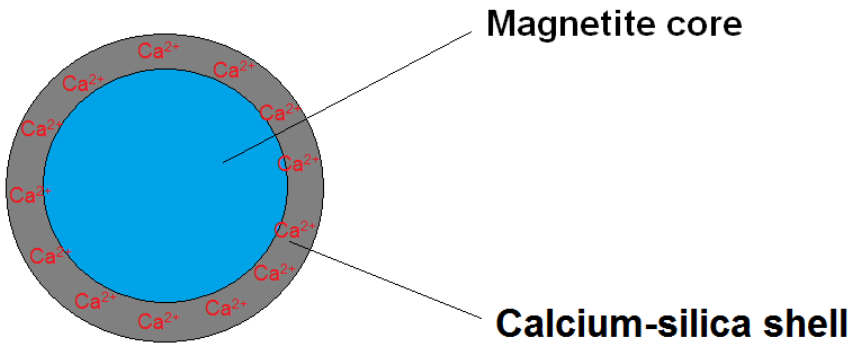


Mag-SiO_2-Ca(3)

*1 Singh R. K., Kim T. H., Patel K. D., 2012, *J Biomed Mater Res Part A*, published online in Wiley Online Library (wileyonlinelibrary.com)
*2 Stöber W., Fink A., 1968, Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *Journal of colloidal and interface science*, **26**, 62-69

General characteristics of iron-oxide nanoparticles





Type of nanoparticle	Medium	pH approx	Concentration
Magnetite (Fe_3O_4)	Water	9.6	1,8 mg/ml
Magnetite – silica (Fe_3O_4-SiO_2)	Water	7.9	3,4 mg/ml
Magnetite – silica – calcium (99:1) – Fe_3O_4-SiO_2-Ca(3) CITR	Water	9.2	4,4 mg/ml
Magnetite – silica – calcium (99:1) – Fe_3O_4-SiO_2-Ca(3) IDR	Water	8.3	4,5 mg/ml

Physicochemical characterization of MNPs

- The magnetic nanoparticles were characterized for:

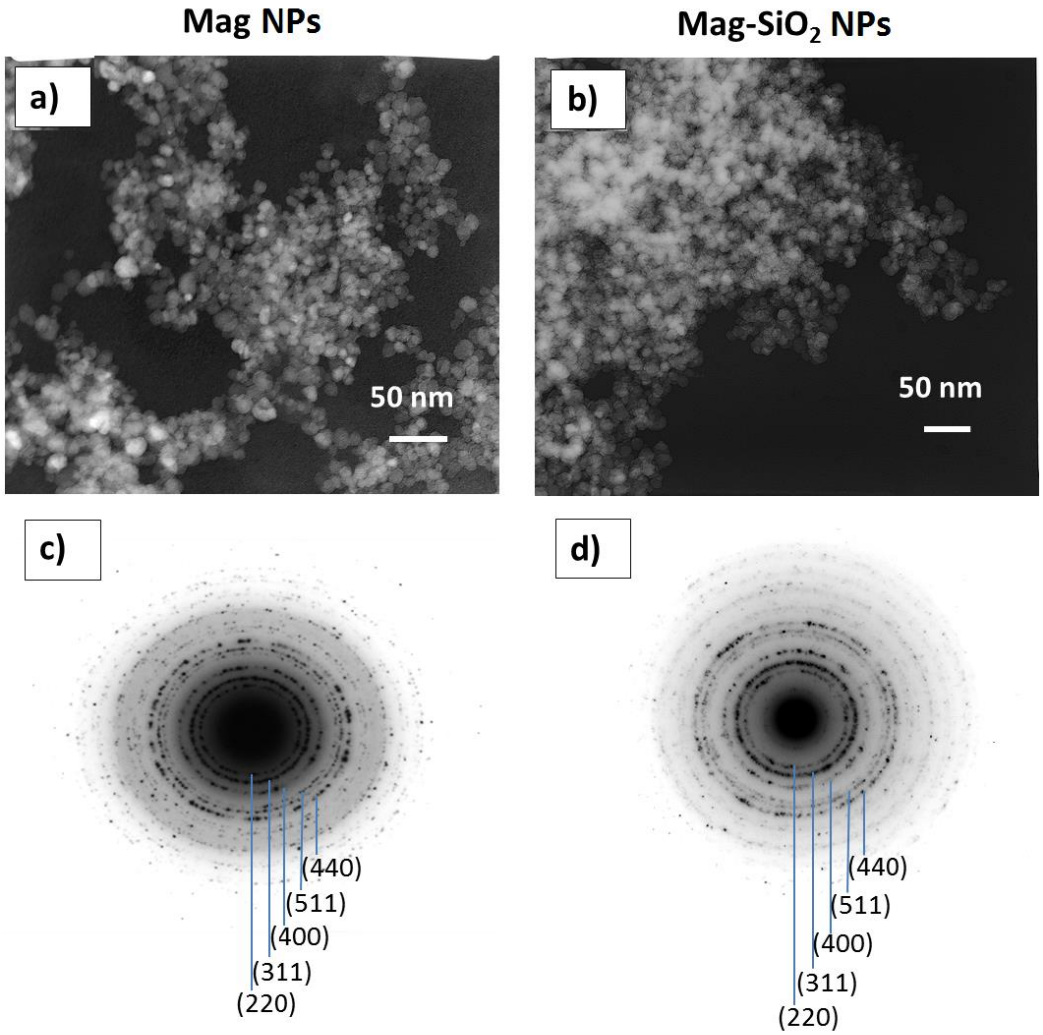
→ Morphology and size (FESEM, TEM)

→ Surface chemical properties (EDS probe)

→ Superparamagnetic behavior of MNPs (Magnetic hysteresis)

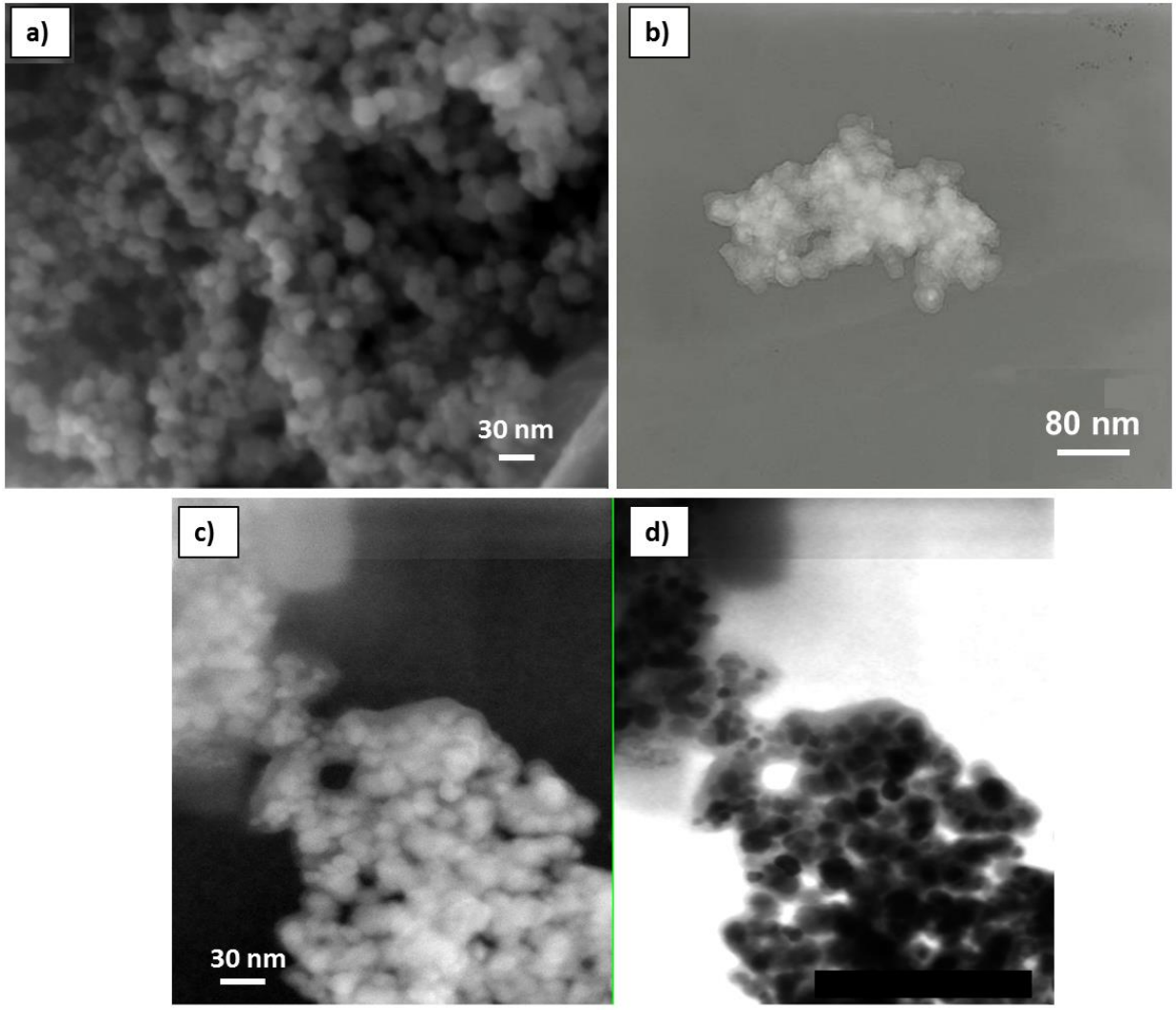
→ Colloidal stability (Zeta potential)

TEM images of MNPs



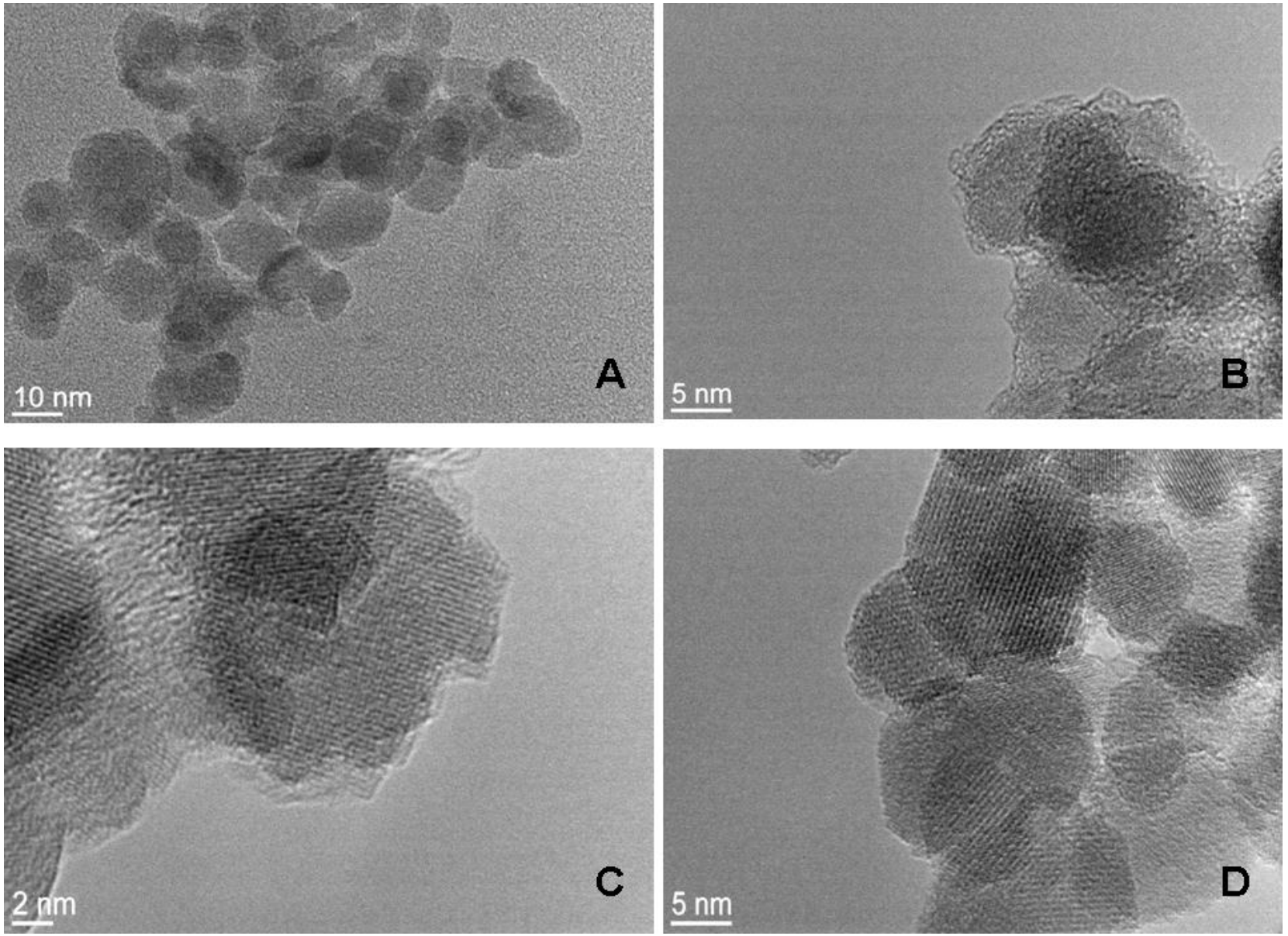
TEM Images and SAED patterns of Fe_3O_4 and Fe_3O_4-SiO_2 samples. a) TEM image of Fe_3O_4, b) SAED pattern of Fe_3O_4, c) TEM image of Fe_3O_4-SiO_2, d) SAED pattern of Fe_3O_4-SiO_2.

FESEM images of MNPs



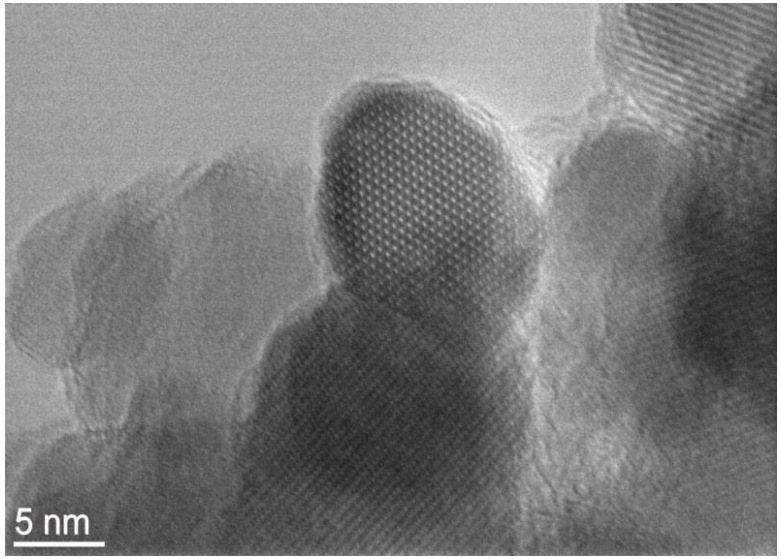
FESEM (a), **TEM** (b) and **STEM** (c - dark field mode, d - bright field mode) images of Fe_3O_4-SiO_2 NPs.

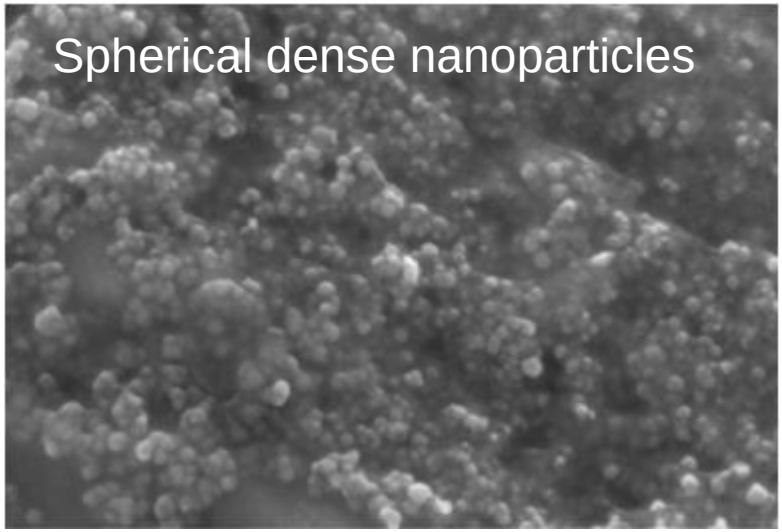
TEM images of iron-oxide nanoparticles



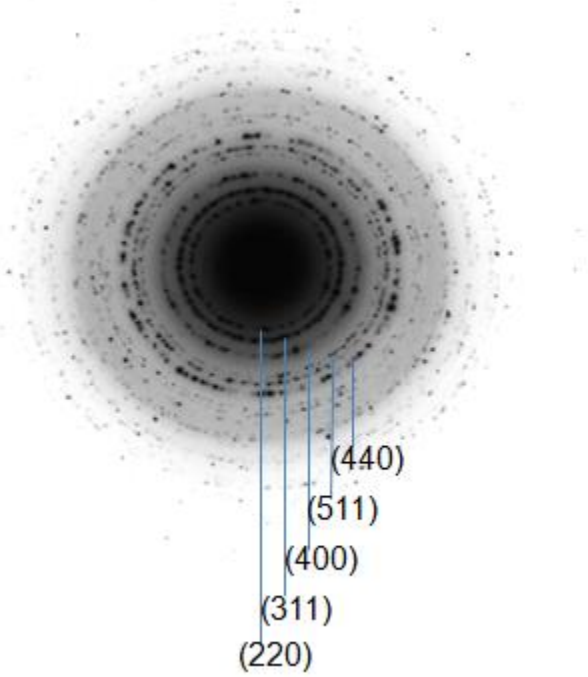
TEM images: Fe_3O_4 (A), Fe_3O_4-SiO_2 (B), Fe_3O_4-SiO_2-Ca3 (C), Fe_3O_4-SiO_2-Ca17 (D) nanoparticles.

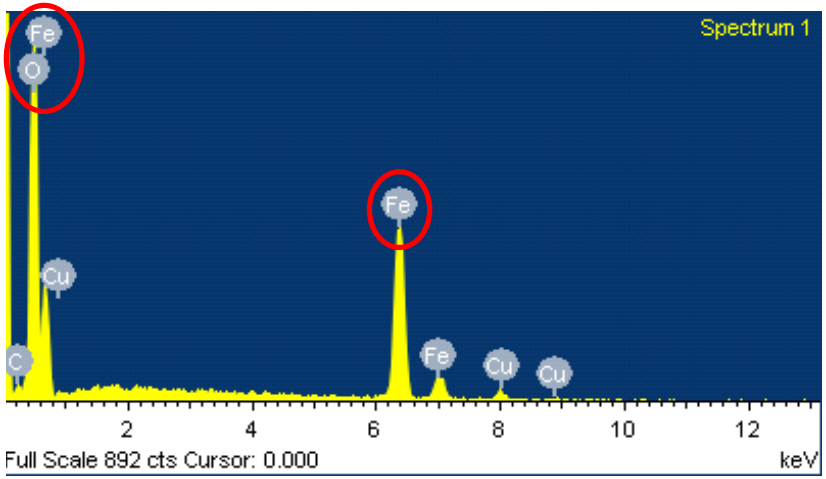
Characterization of Fe_3O_4 NPs: TEM - FESEM - EDS



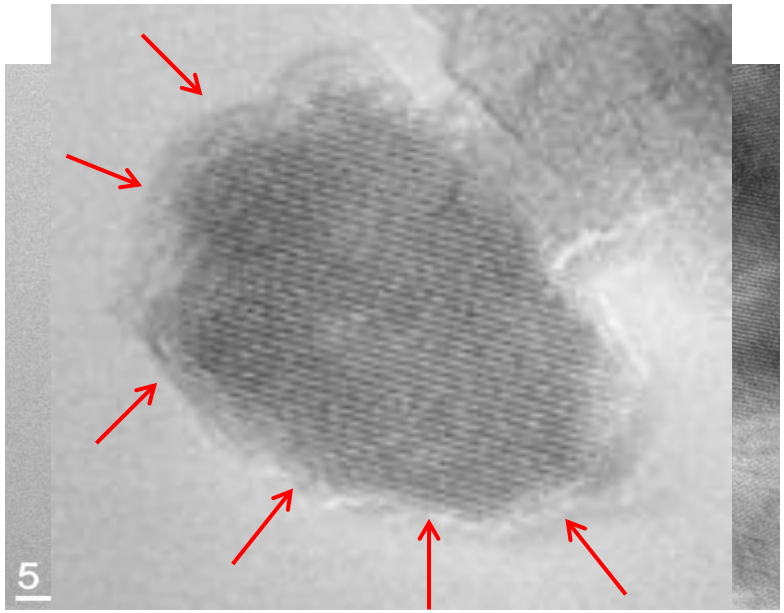


Diffraction signals of magnetite

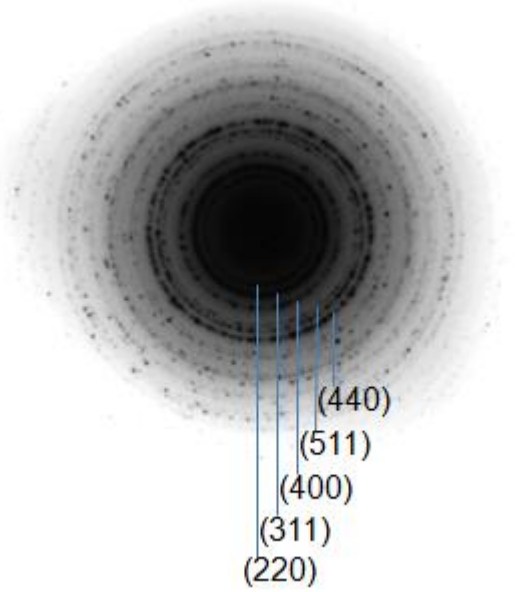


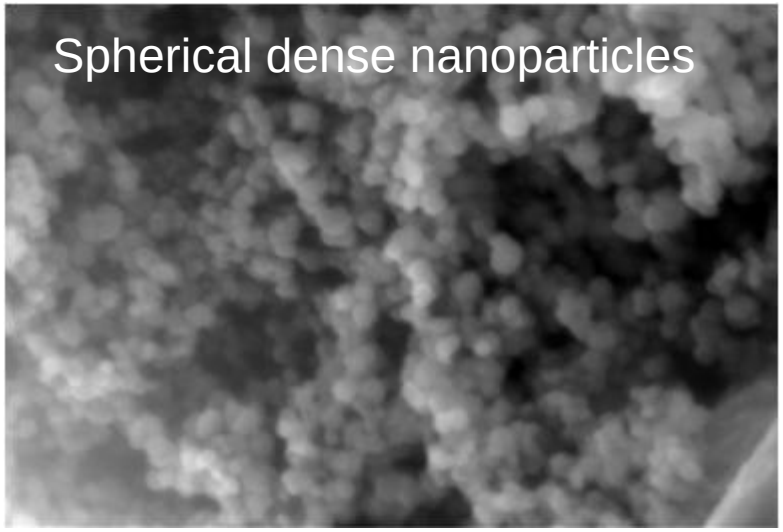


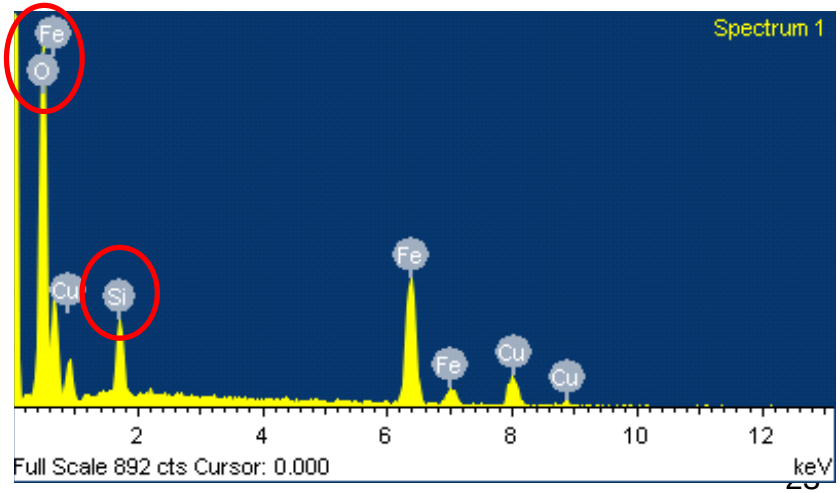
Fe_3O_4-SiO_2 NPs: TEM – FESEM - EDS



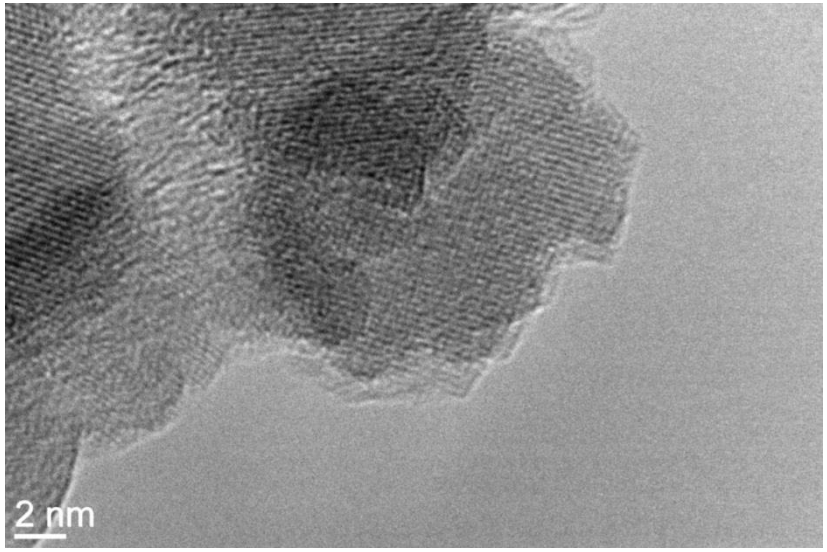
- Diffraction signals of magnetite
- Halo for the amorphous phase

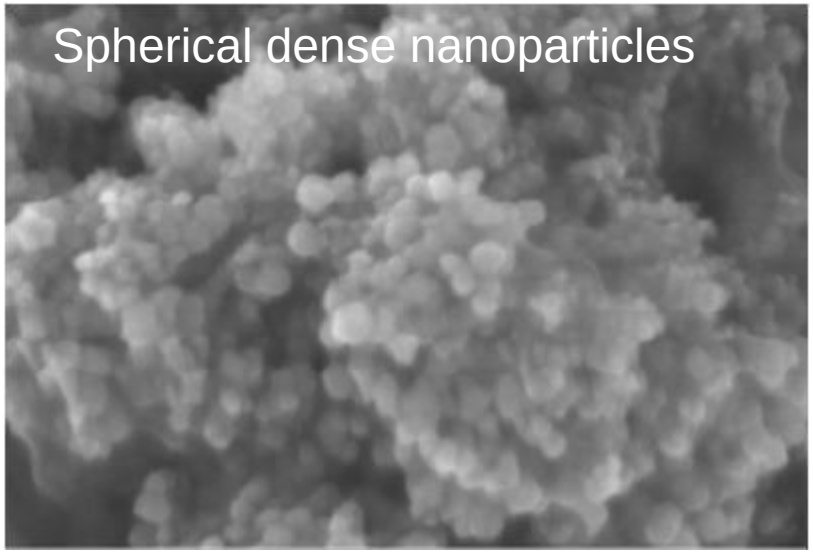




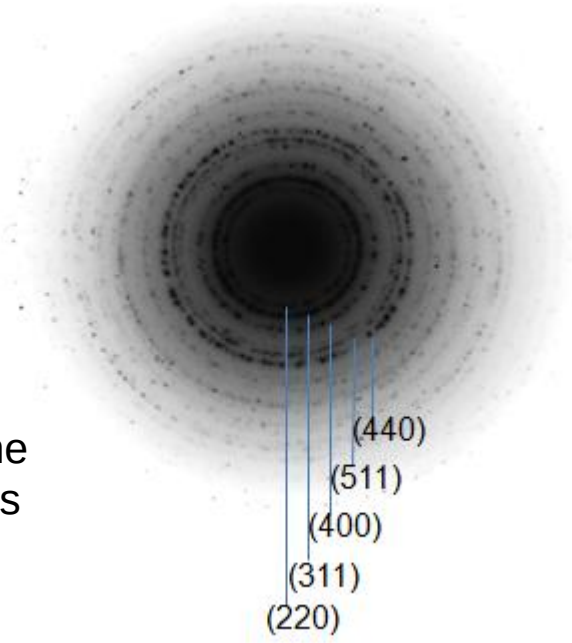


Fe_3O_4-SiO_2-Ca(3) NPs: TEM - FESEM - EDS

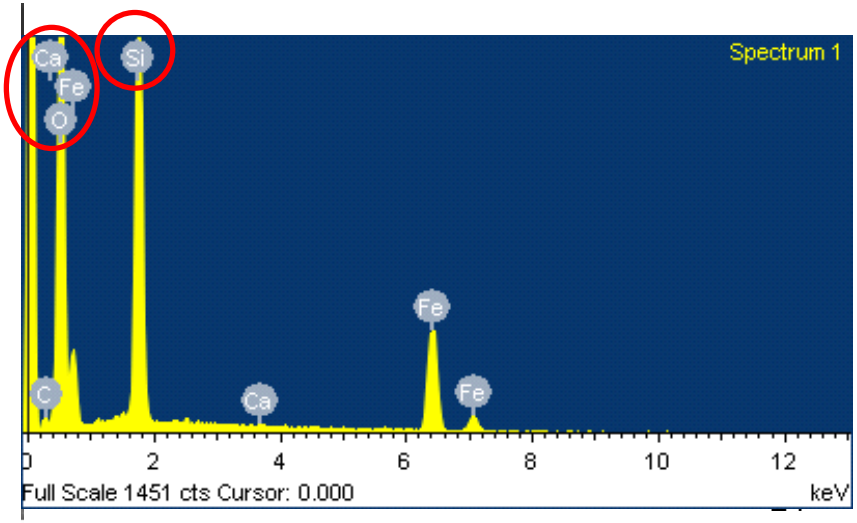




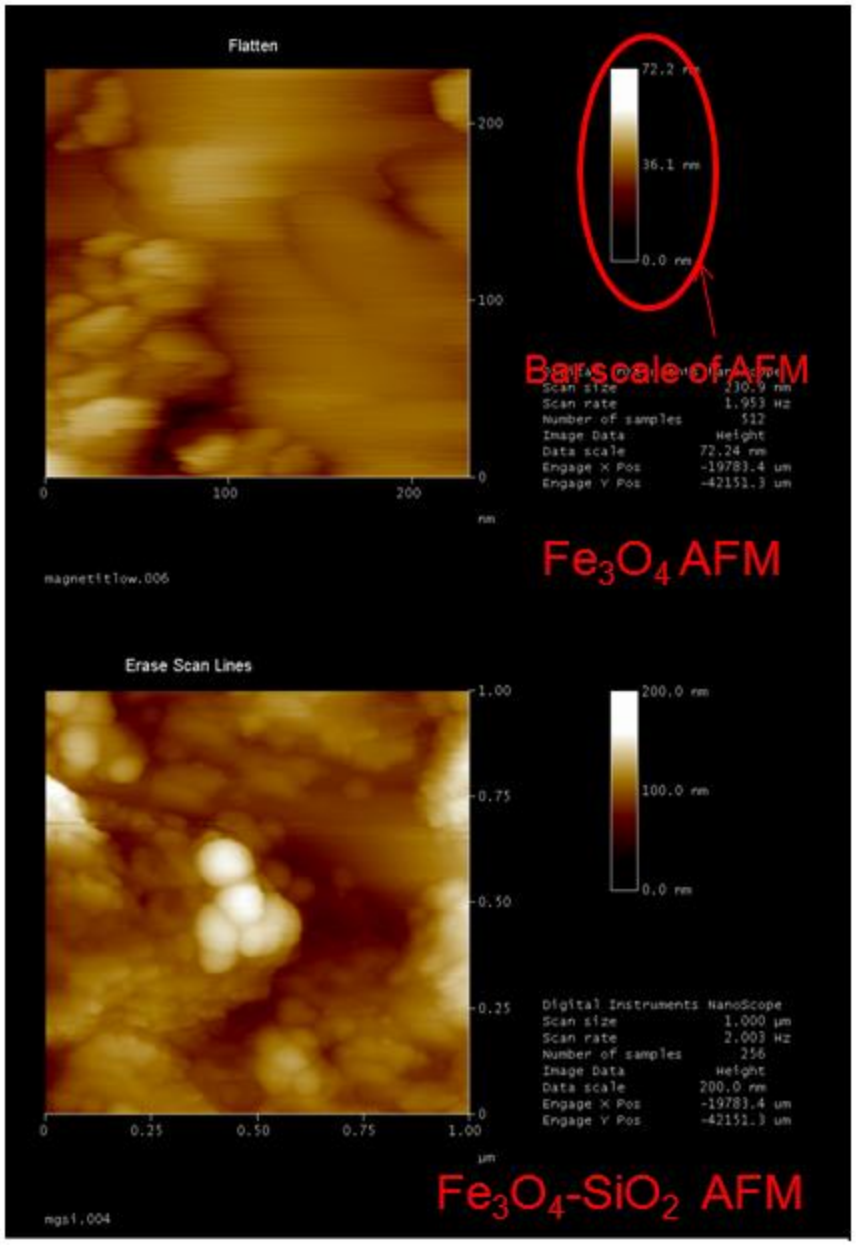
- Diffraction signals of magnetite

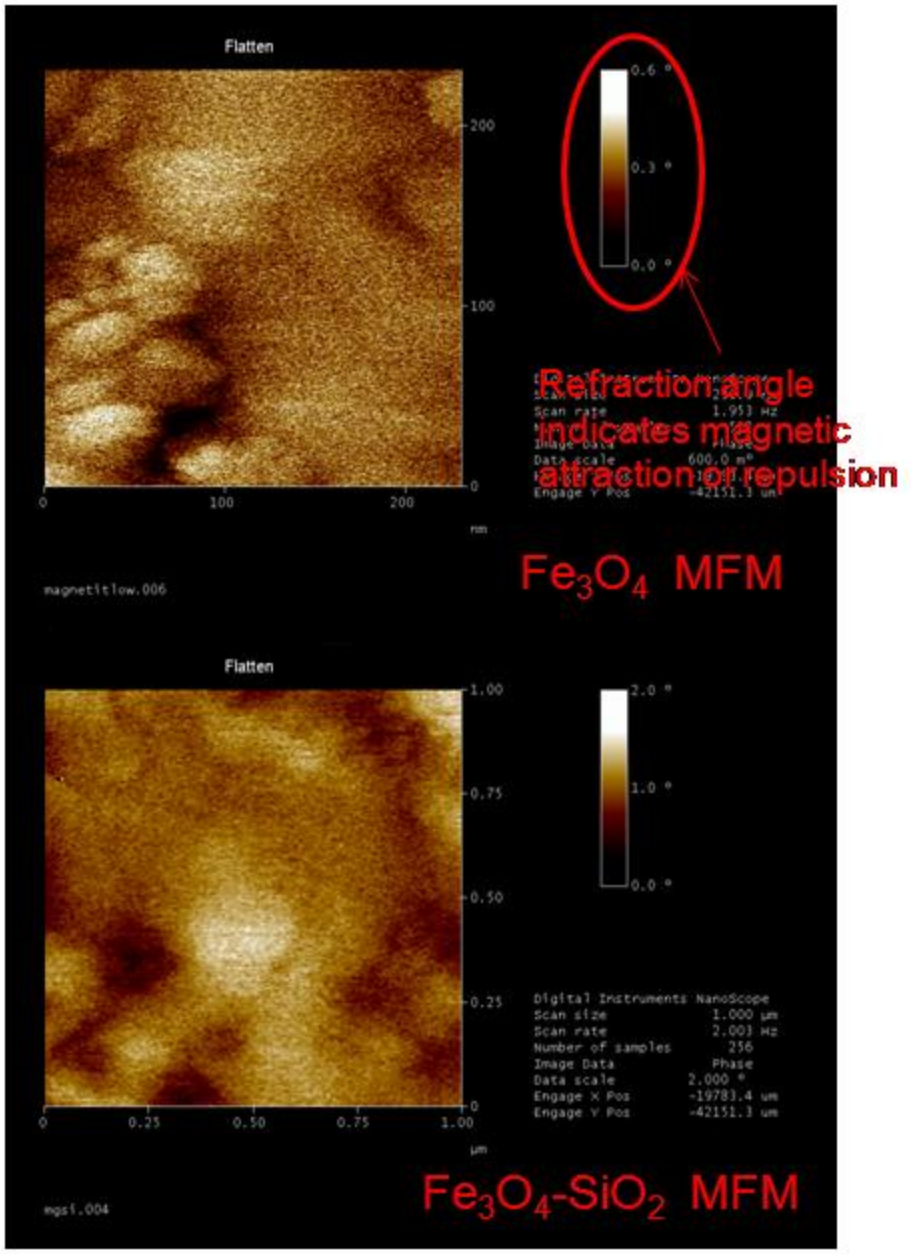


- Halo for the amorphous phase

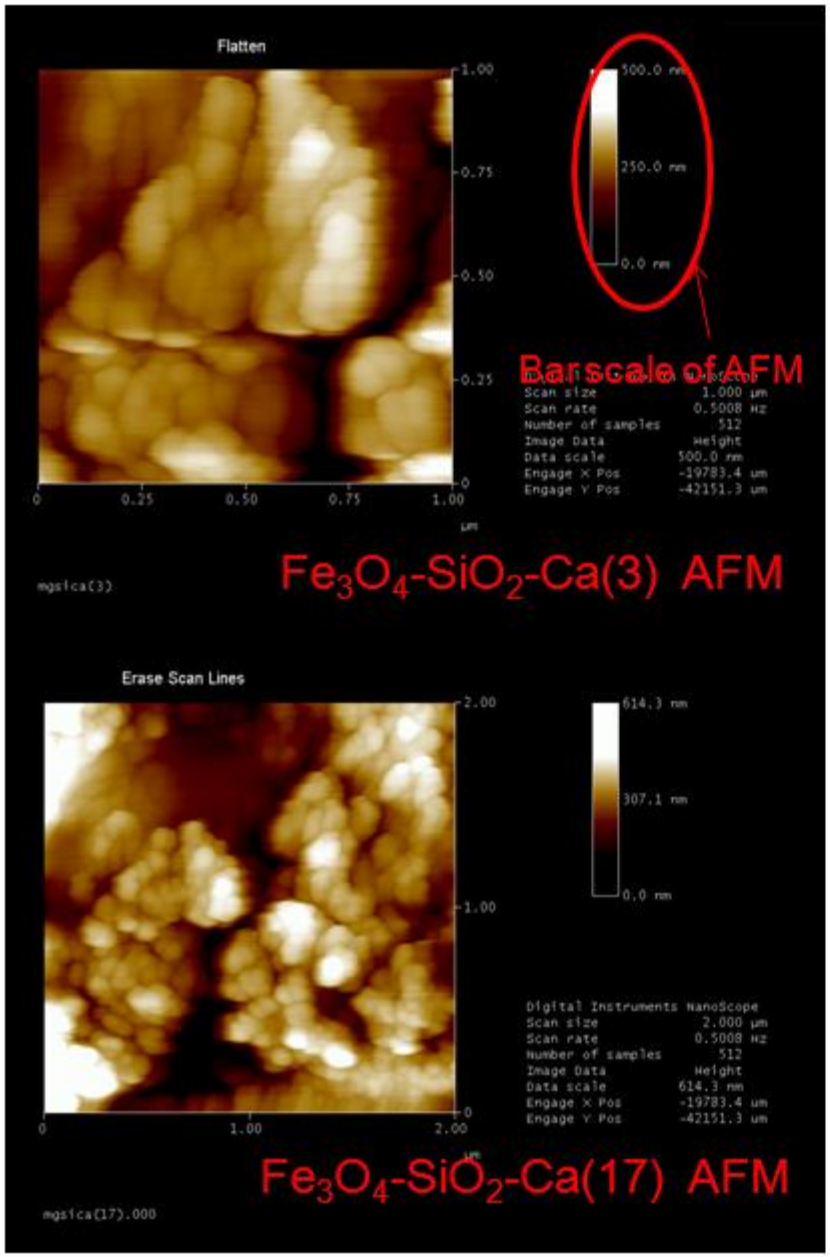


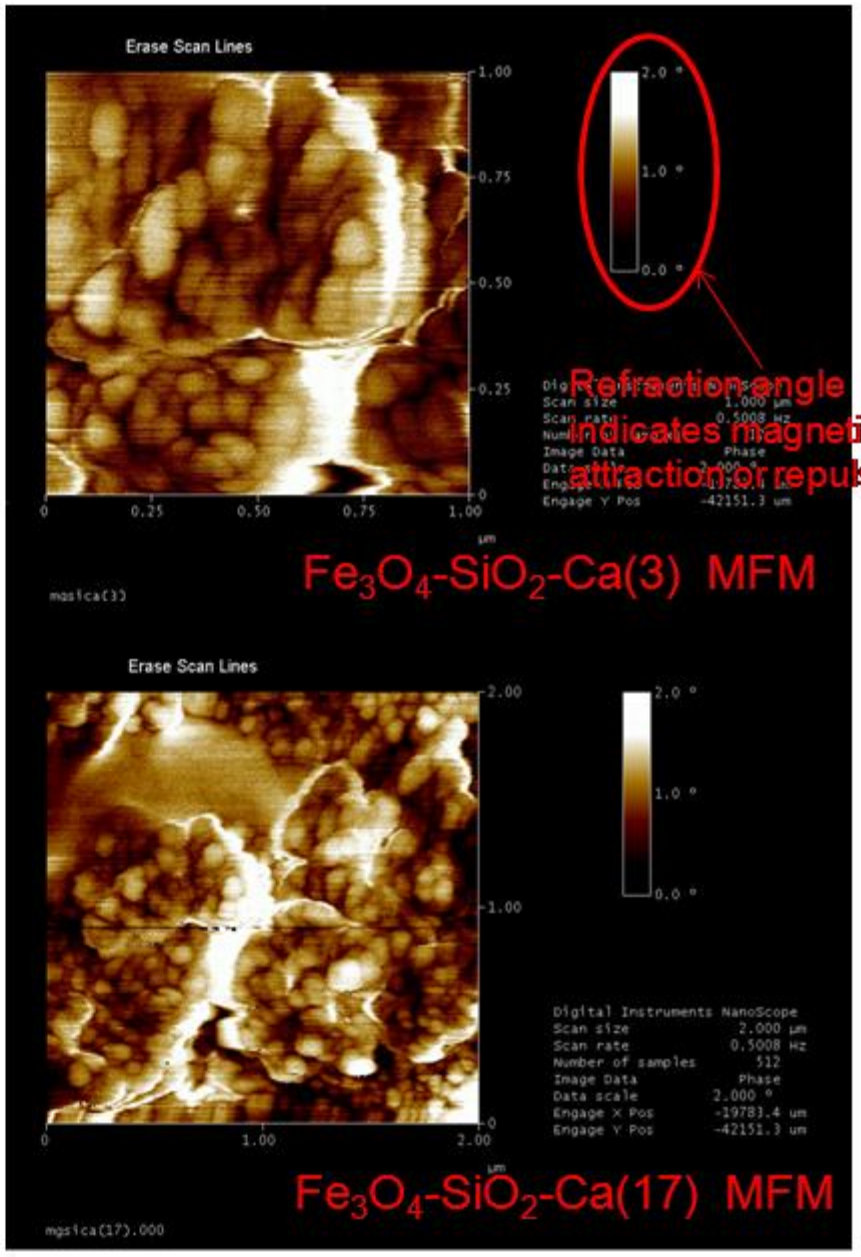
Iron oxide nanoparticles - AFM/MFM



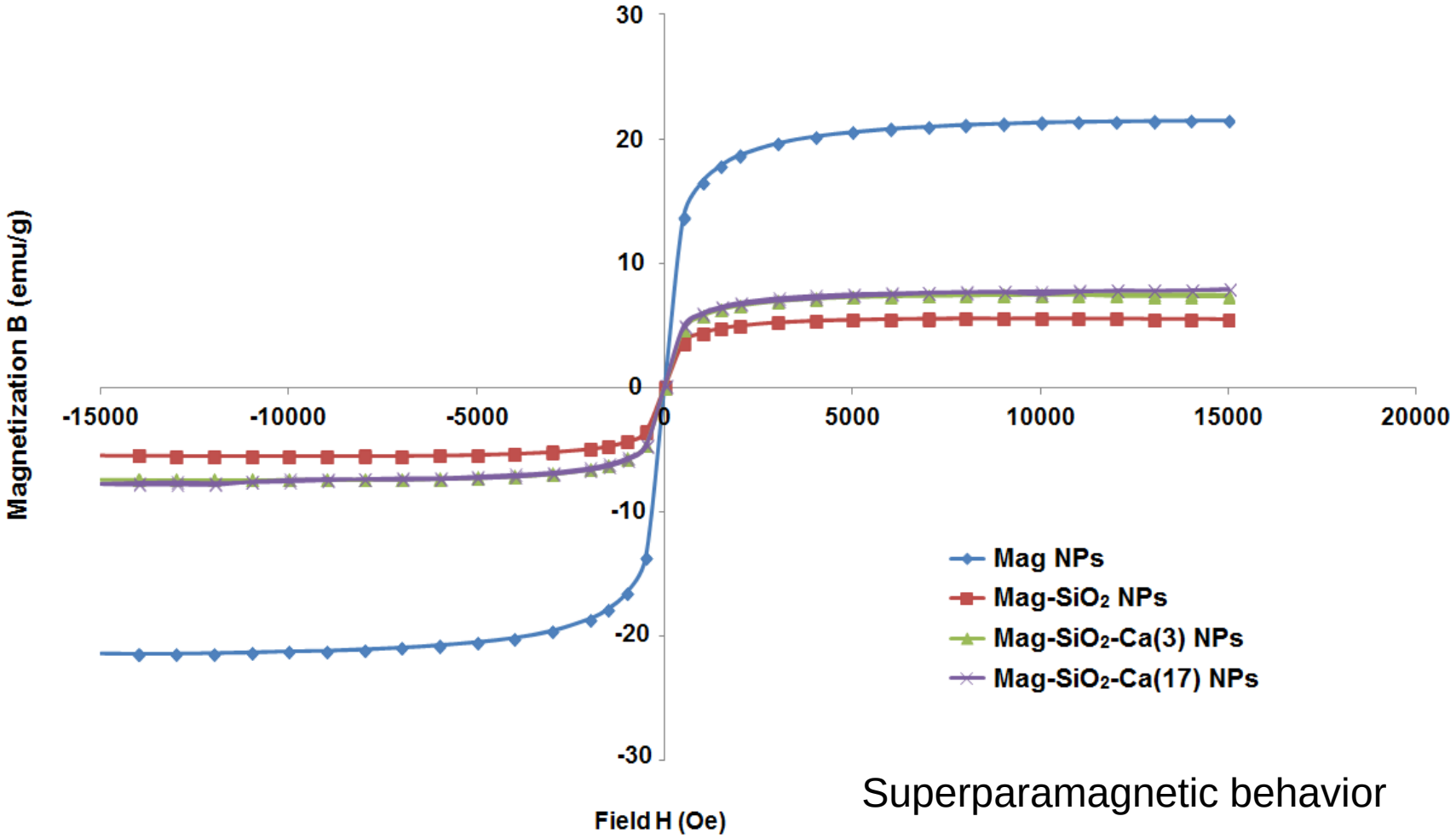


Silica-calcium core-shell iron-oxide nanoparticles - AFM/MFM





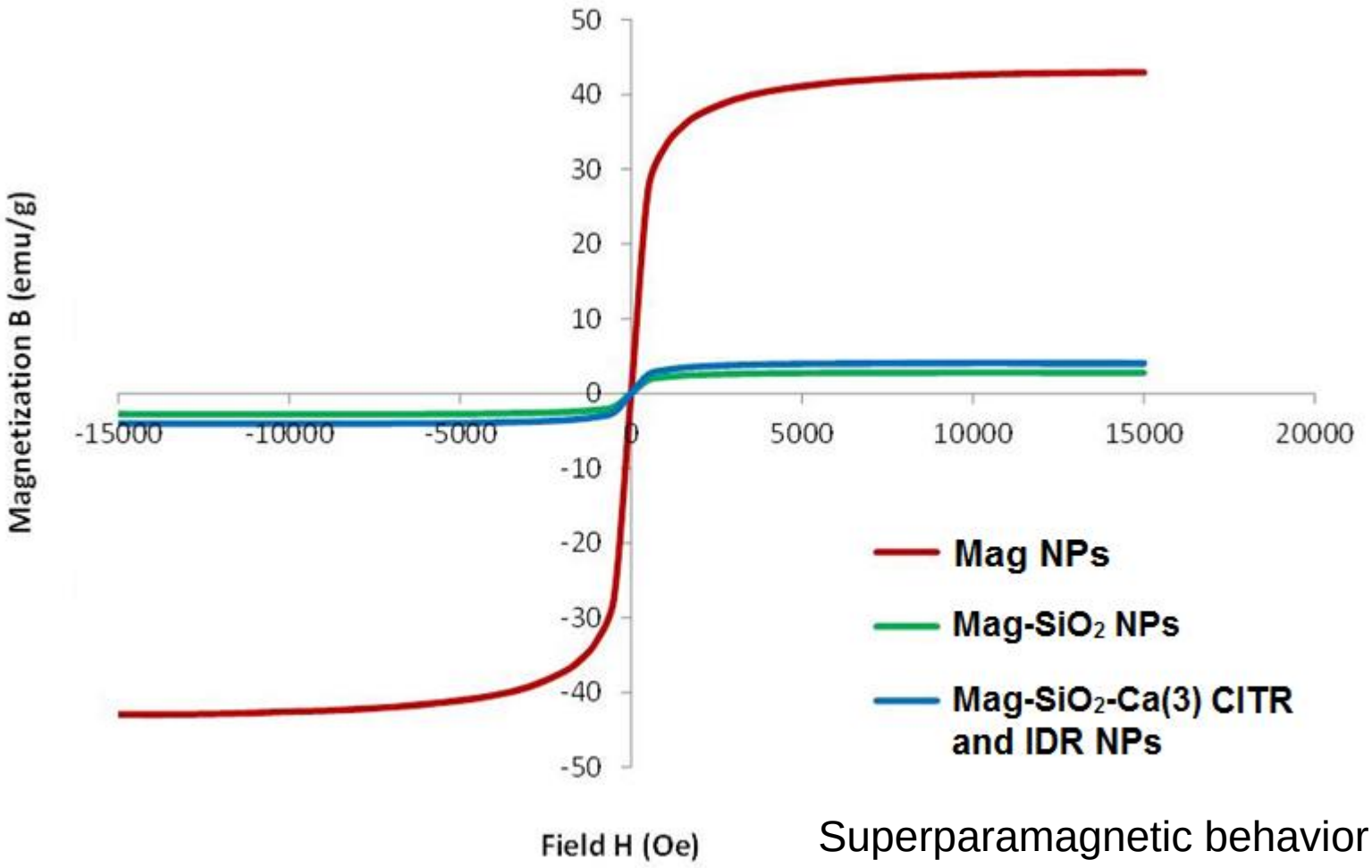
Magnetization curves of MNPs



Superparamagnetic behavior

Vibrating Sample Magnetometer (VSM-Lakeshore) was used to determine the magnetic characteristics of the samples.

Magnetization curves of MNPs



Superparamagnetic behavior

Vibrating Sample Magnetometer (VSM-Lakeshore) was used to determine the magnetic characteristics of the samples.

Definition of Zeta potential (ZP)

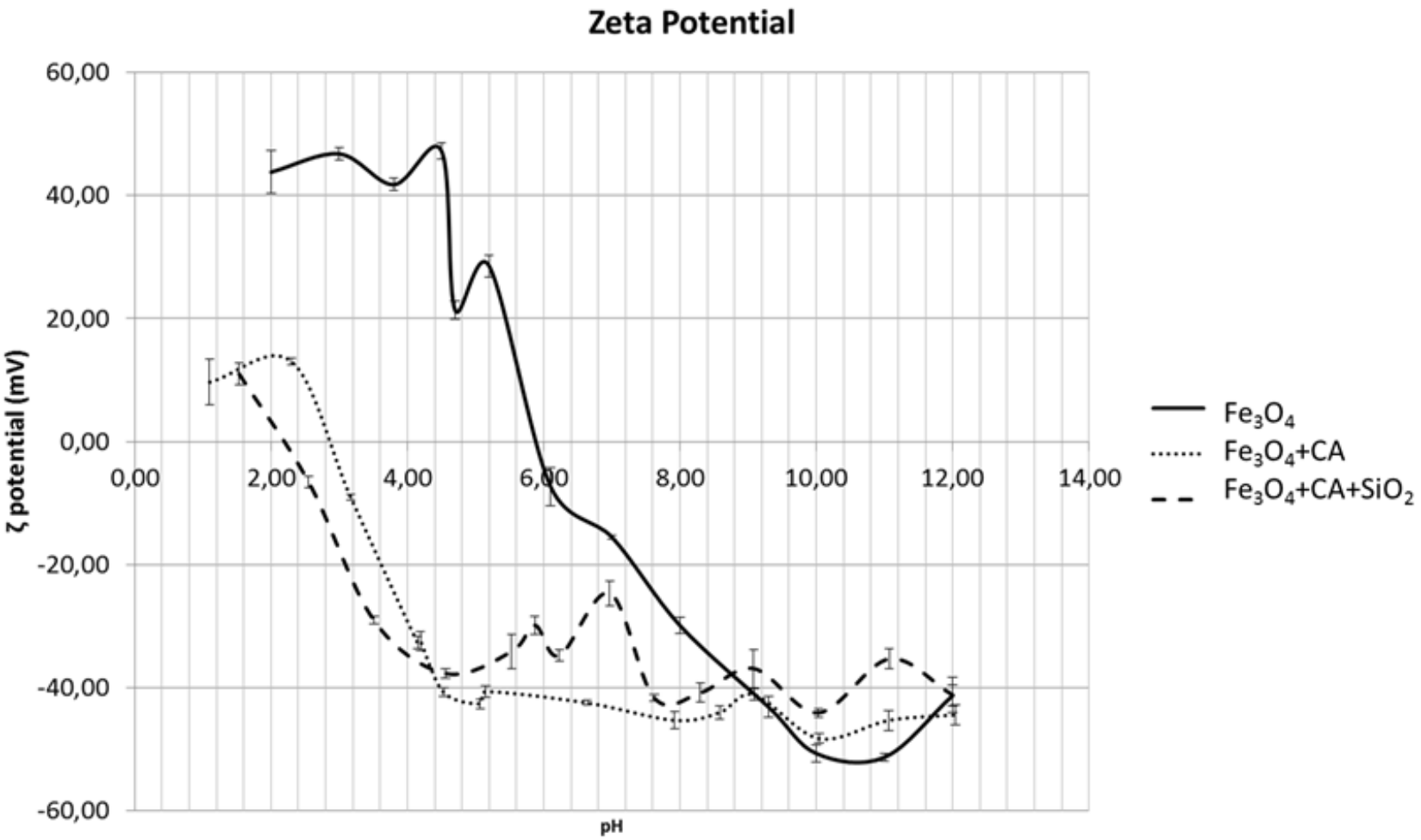
The significance of **Zeta potential** can be related to the stability of colloidal dispersions.

Relationship between the value of Zeta potential and colloidal stability

Zeta Potential [mV]	Stability behaviour of the colloid
da 0 a ±5	Rapid coagulation or flocculation
da ±10 a ±30	Incipient instability
da ±30 a ±40	Moderate stability
da ±40 a ±60	Good stability
> ±61	Excellent stability

Robert JH. Zeta Potential in Colloid Science: Principles and Applications. Colloid Sciences Series, Sydney. Academic Press 1989; 1-38.

Zeta potential of magnetic nanoparticles



Values of Zeta potential of third synthesis of magnetic nanoparticles

Type of nanoparticle	starting pH	ZP (mV)	Stability behaviour of the colloid
Fe_3O_4 NPs	4.26	- 30.23 ± 1.49 mV	Moderate stability
Fe_3O_4-SiO_2 NPs	4.70	- 30.41 ± 0.81 mV	Moderate stability
Fe_3O_4-SiO_2-Ca(3) CITR NPs	5.63	- 43.14 ± 1.95 mV	Good stability
Fe_3O_4-SiO_2-Ca(3) IDR NPs	5.54	- 42.67 ± 1.61 mV	Good stability

Cytocompatibility evaluation of iron-oxide nanoparticles

Experimental design

Sterilization of MNPs with UV for 30 minutes

Seeding of endothelial murine cells (MS1) in well plates in a defined number to obtain 90% of confluence

Indirect cytotoxicity evaluation

By soaking of MNPs in DMEM cell culture medium for 24 and 72 hours

Direct cytotoxicity evaluation

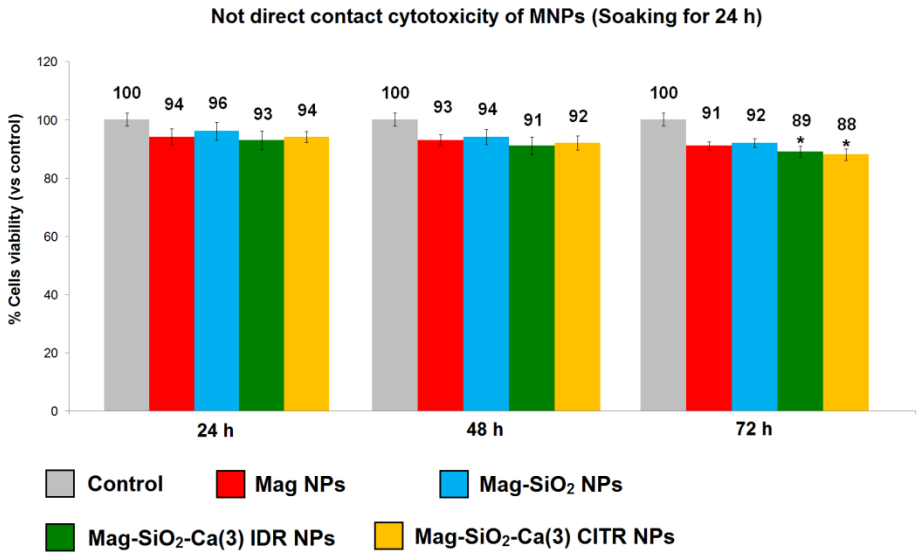
Addition of MNPs (24 hours after cell seeding) using the following concentrations: 2 and 20 µg/ml

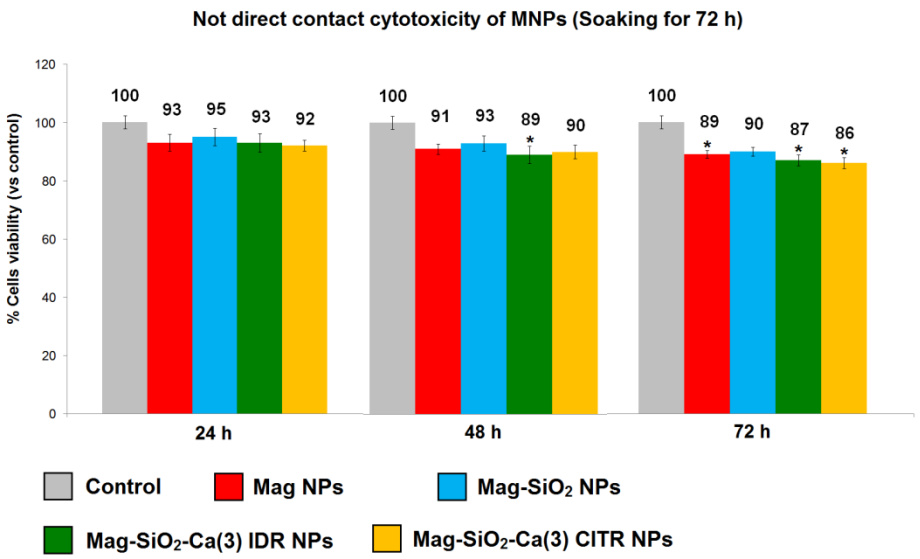
Experimental times: 24, 48 and 72 hours

Viability test: MTT assay

Brigitta Szalay et al, Journal of Applied Toxicology

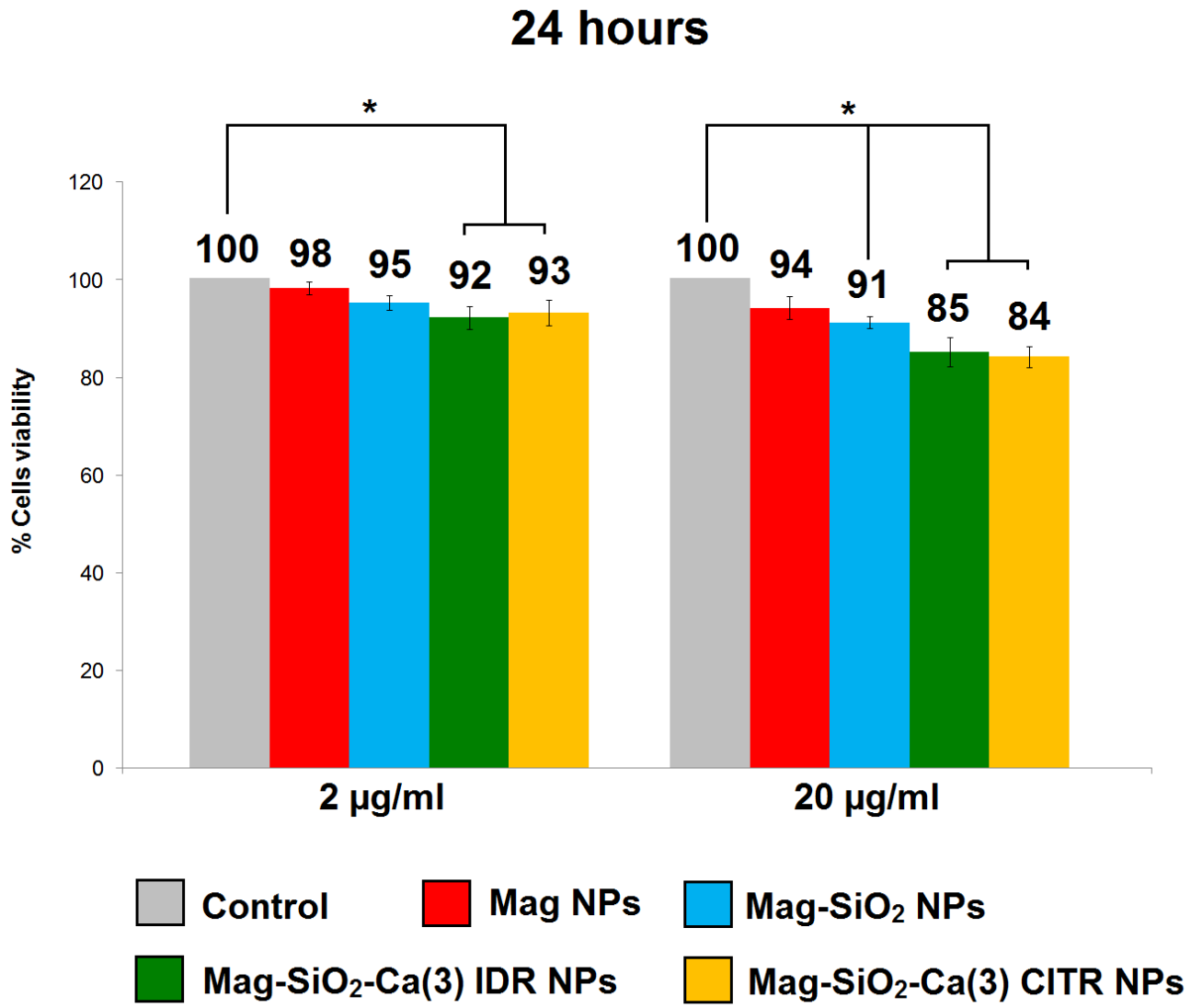
Not direct contact cytotoxicity evaluation of MNPs





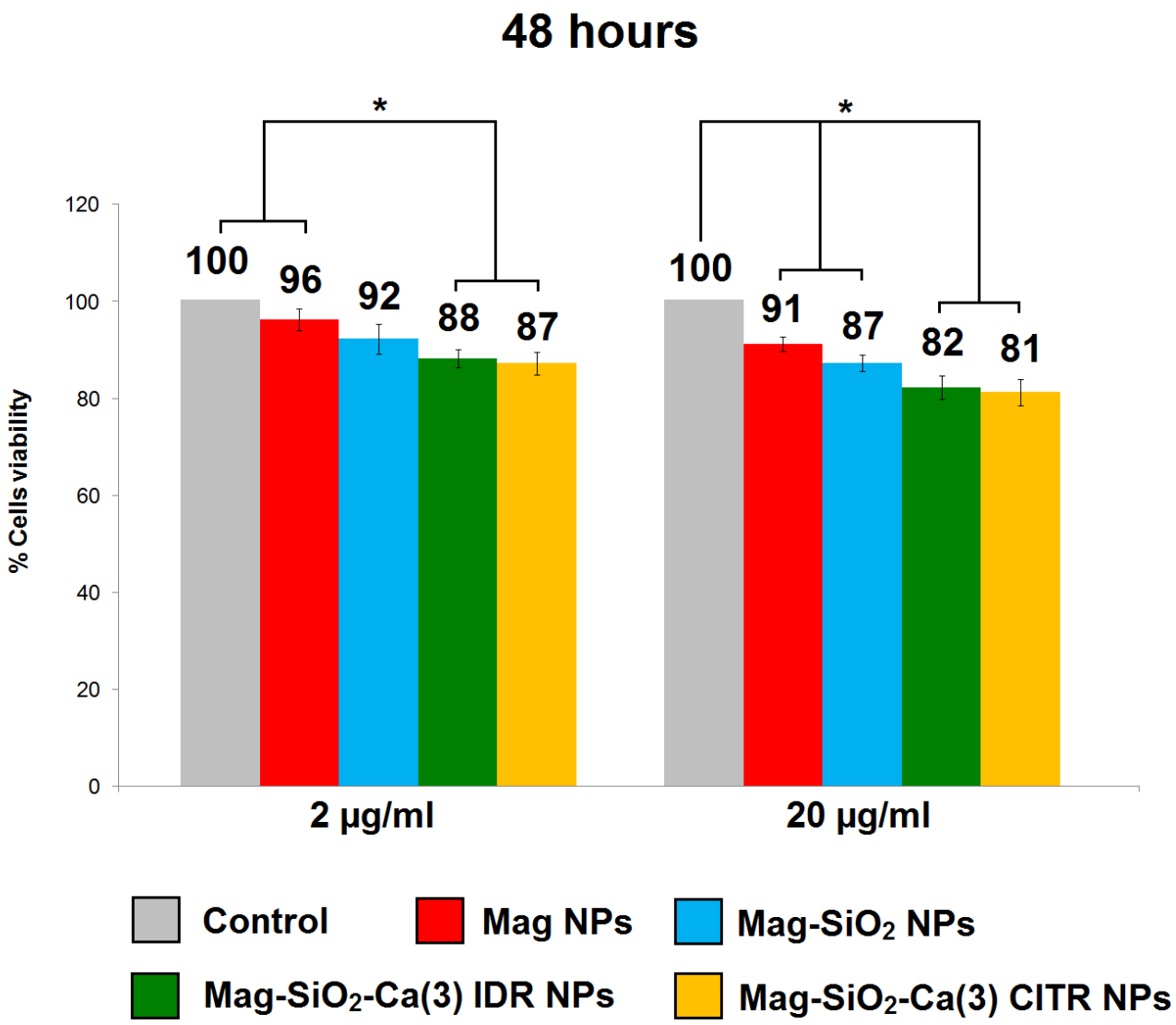
*P < 0.05 compared with control

Cytocompatibility of MNPs in static conditions (24 hours)



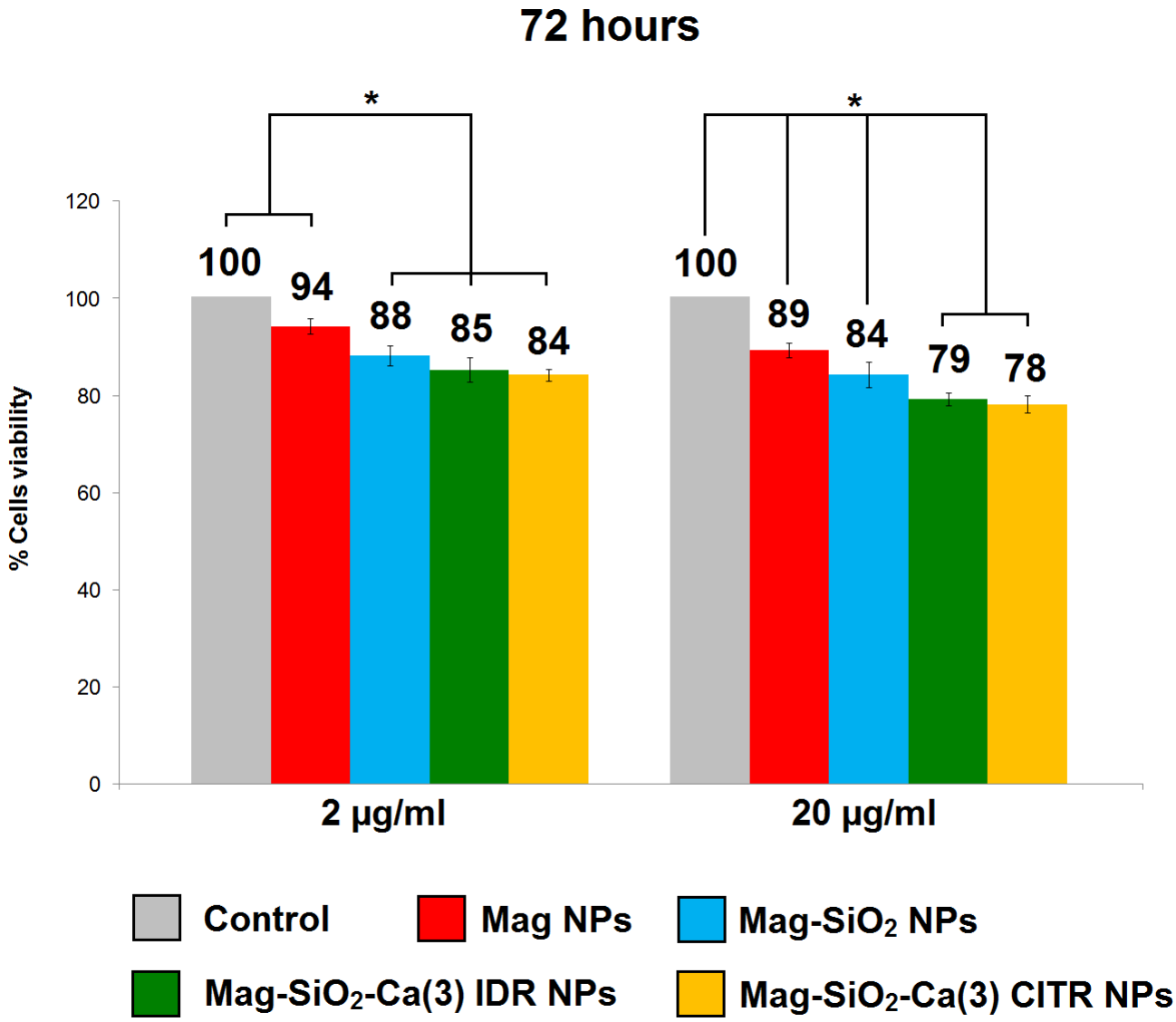
*P < 0.05 One way analysis of variance (ANOVA) followed by Scheffe's test

Cytocompatibility of MNPs in static conditions (48 hours)



*P < 0.05 One way analysis of variance (ANOVA) followed by Scheffe's test

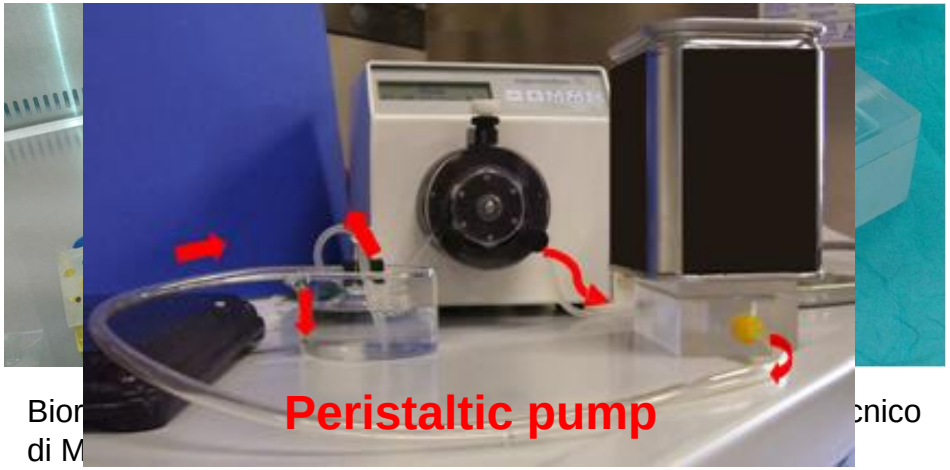
Cytocompatibility of MNPs in static conditions (72 hours)



*P < 0.05 One way analysis of variance (ANOVA) followed by Scheffe's test

Cytocompatibility of MNPs in dynamic conditions

Cytocompatibility of MNPs was investigated also in dynamic conditions



Experimental setting

- Continuous flow bioreactor with a peristaltic pump simulating cytocompatibility in dynamic conditions
- Humidified incubator at 37°C, 5% CO_2 atmosphere

MS1 cells (30.000 cells/cm^2) were seeded at confluence on a strip of electrospun polycaprolactone (PCL)

When MS1 cells were confluent → strips were inserted in the bioreactor.

MS1 cells were subjected to a continuous flow of cell culture medium (DMEM) with MNPs at the concentration of 20 µg/ml.

- **Experimental times: 2 h, 12 h and 24 h.**
- **Cell viability tests used: XTT and LDH assay.**

Ucciferri N. Nanotoxicology. 2014 Sep; 8(6):697-708.

Cytocompatibility in dynamic conditions

Cell viability was evaluated using LDH assay and XTT assay

LDH release (% of total)			
Stimulation	Fe_3O_4 NPs	Fe_3O_4-SiO_2 NPs	Control
2 h	2.77 ± 0.39%	2.59 ± 0.28%	2.24 ± 0.41%
12 h	3.19 ± 0.46%	3.04 ± 0.35%	2.48 ± 0.30%
24 h	3.56 ± 0.51%	3.98 ± 0.46%	2.74 ± 0.37%

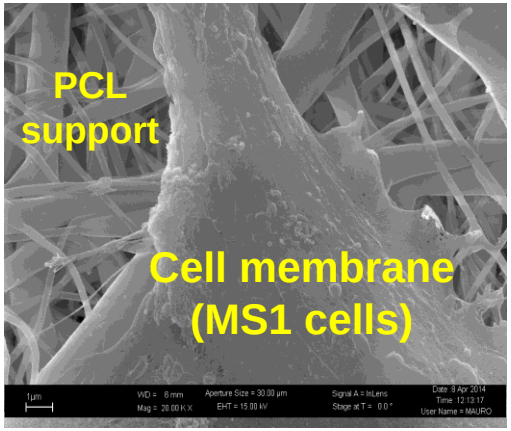
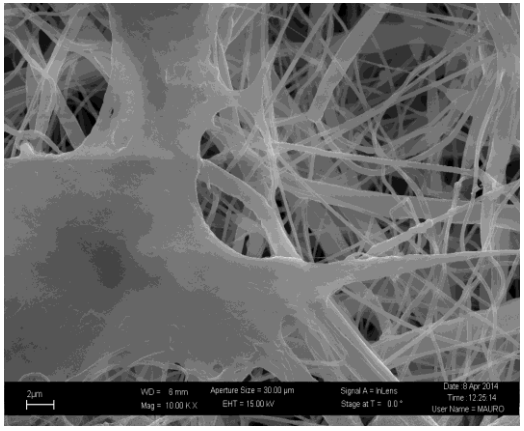
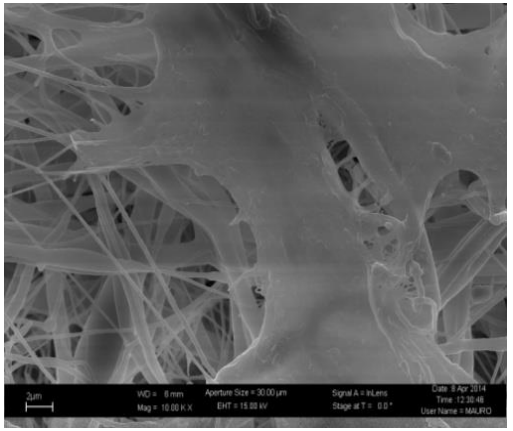
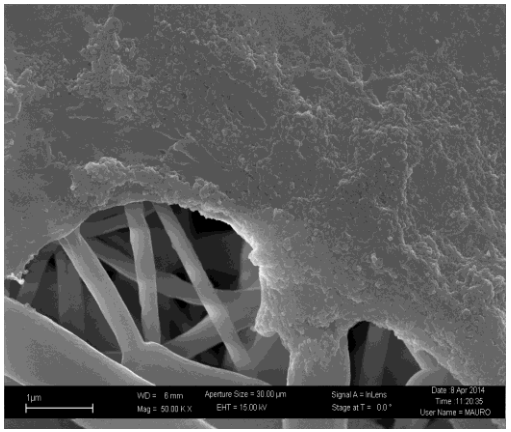
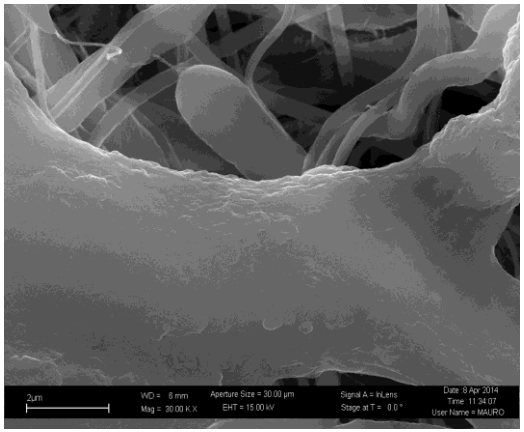
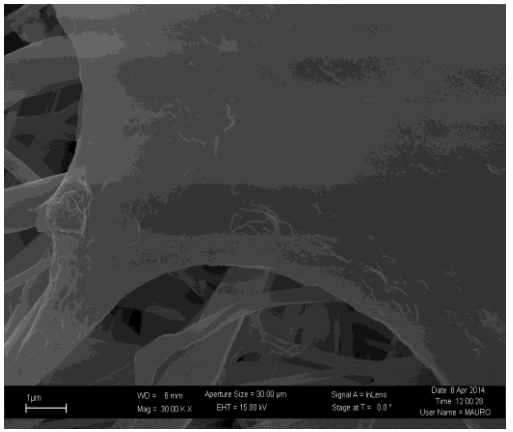
XTT assay			
Stimulation	Fe_3O_4 NPs	Fe_3O_4-SiO_2 NPs	Control
2 h	93.8 ± 4.2%	96.9 ± 3.7%	100%
12 h	89.5 ± 2.8%*	93.1 ± 3.3%	100%
24 h	86.8 ± 3.1%*	90.9 ± 2.7%	100%

The viability ranged between 86% and 97% in XTT assay and the results for both assays were comparable.

*$P < 0.05$ compared to control

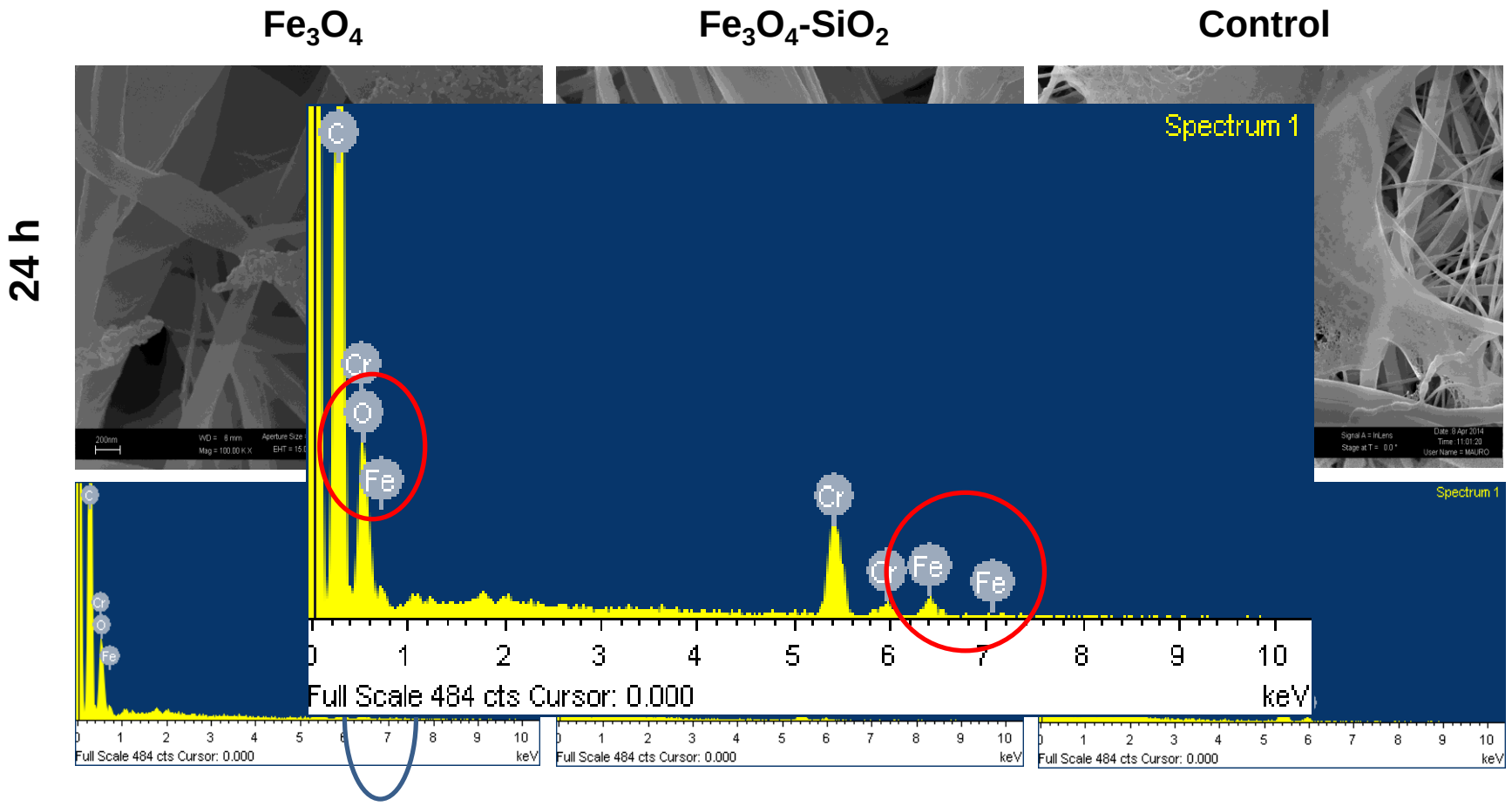
Cytocompatibility in dynamic conditions (Fe_3O_4 and Fe_3O_4 -SiO_2 NPs)

Cells were analyzed with FESEM equipped to EDS probe, in order to evaluate the presence, if any, of Fe_3O_4 and Fe_3O_4-SiO_2 nanoparticles deposits.

	Fe_3O_4	Fe_3O_4 -SiO_2	Control
2 h			
12 h			

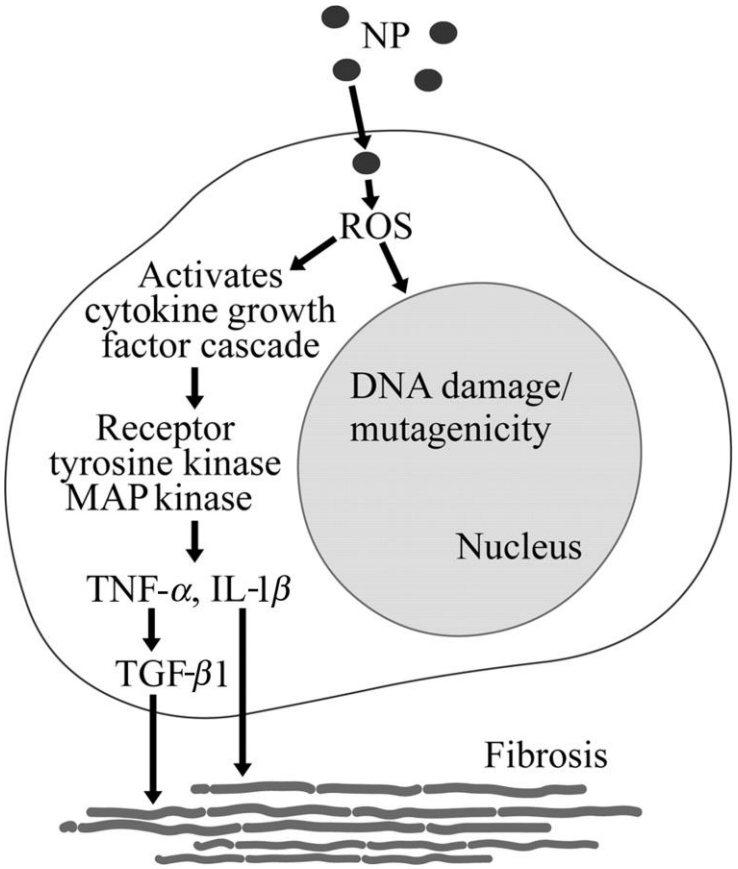
Using dynamic culture conditions, the cells morphology appeared typically elongated.

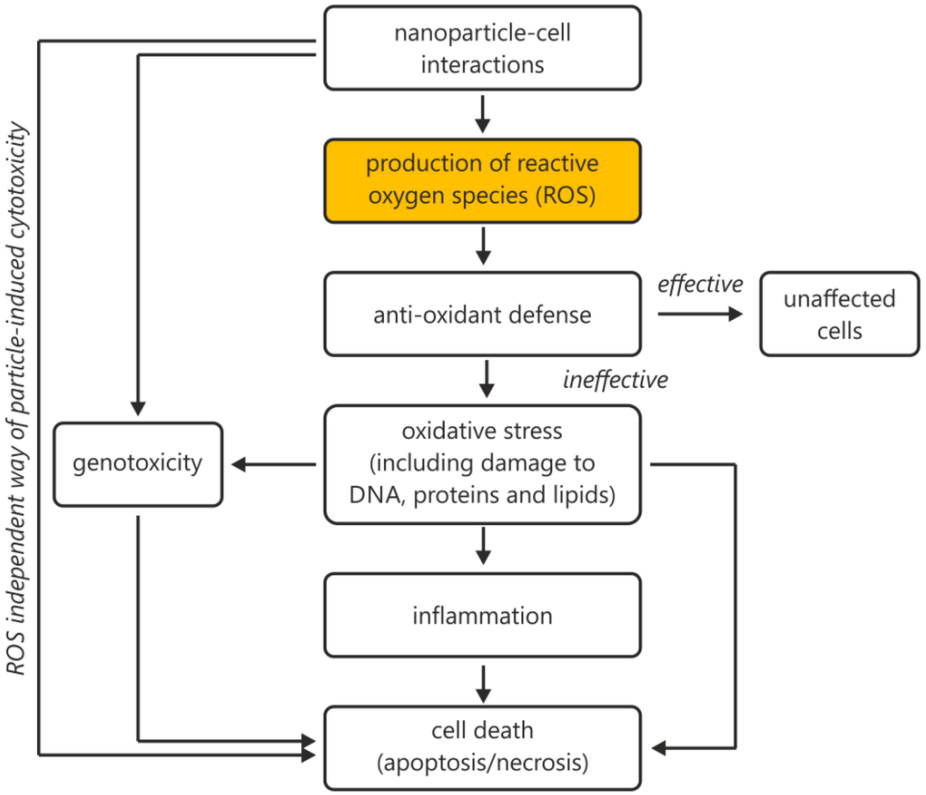
Cytocompatibility in dynamic conditions



FESEM and EDS analyses showed MNPs adsorbed onto the MS1 cell membrane. MNPs deposition was not observed when Fe_3O_4-SiO_2 nanoparticles were used

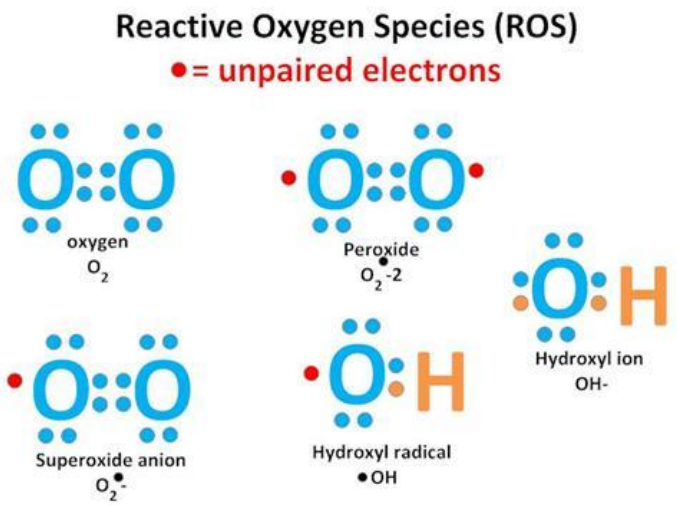
ROS generation induced by MNPs





Matthias Roesslein et al., Int. J. Mol. Sci. 2013

ROS generation induced by MNPs after 24 hours (First synthesis)

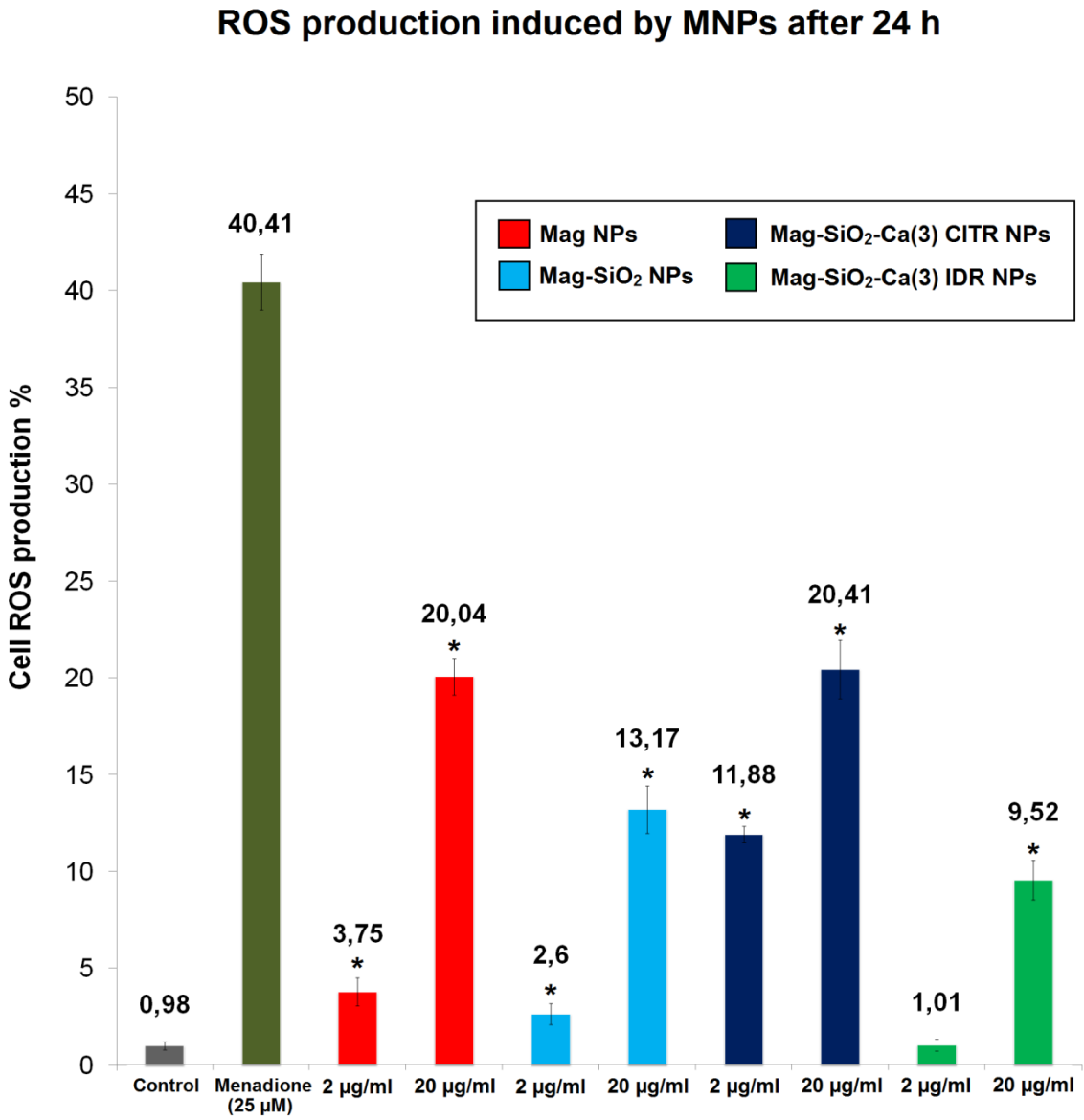


CellROX Green Reagent measures reactive oxygen species (ROS) in live cells

Protocol

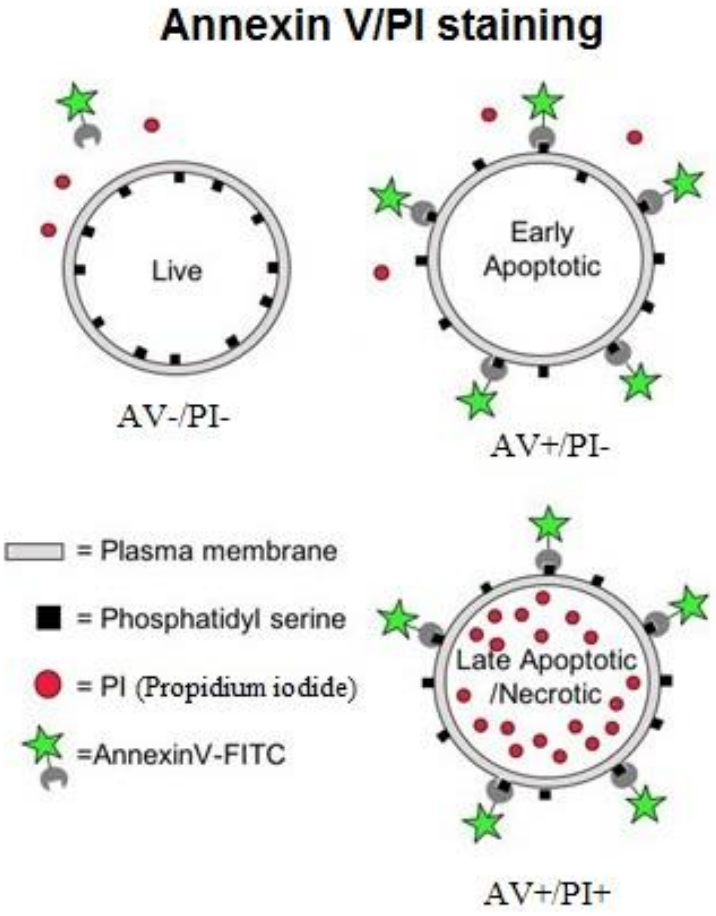
1. MS1 cells were treated for 24 h with the following concentrations of MNPs: 10, 20, 40 and 80 µg/ml.
2. Add the CellROX Green Reagent at concentration of 5 µM.
3. Incubate the cells for 30 minutes at 37°C.
4. Remove medium and wash the cells with PBS.
5. Analyze the cells to FACS.

ROS production after 24 hours induced by MNPs



*P < 0.05 compared with control untreated and positive control (Menadione 25 µM).

Apoptosis evaluation: Annexin V/PI



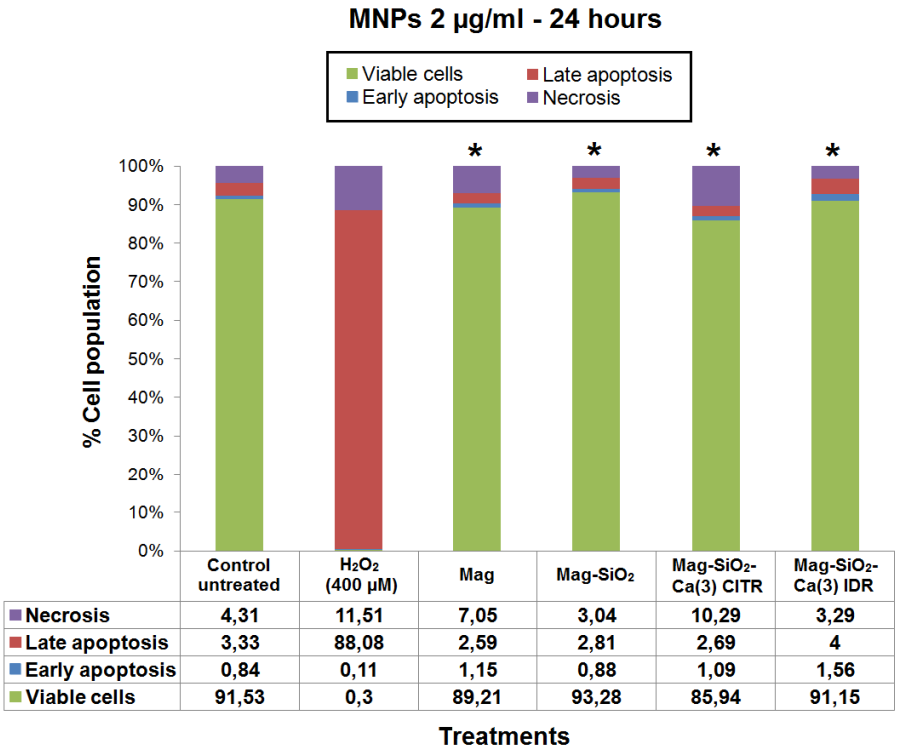
- Apoptosis evaluation of MNPs effect on MS1 cells using the following concentrations: 2 and 20 µg/ml
- Annexin V-FITC - PI (Propidium iodide) staining
- Experimental time-points: 24, 48 and 72 hours

MS1 cells were seeded

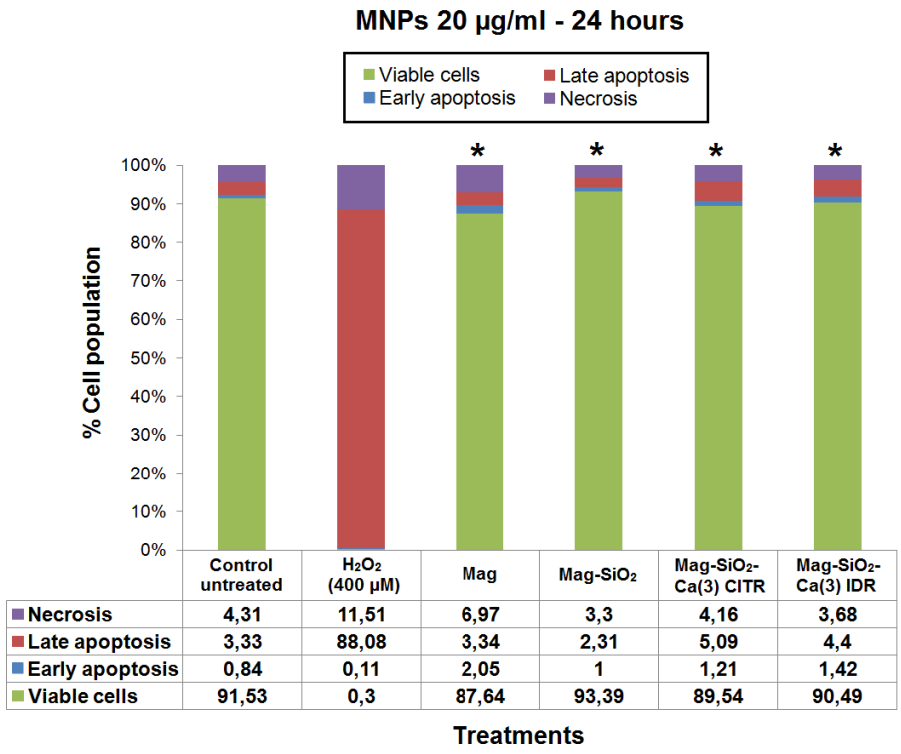
at a density of:

- $2{,}5 \times 10^5$ cells (24 hours)
- $1{,}75 \times 10^5$ cells (48 and 72 hours)
- FACS Analysis

Apoptosis evaluation after 24 hours



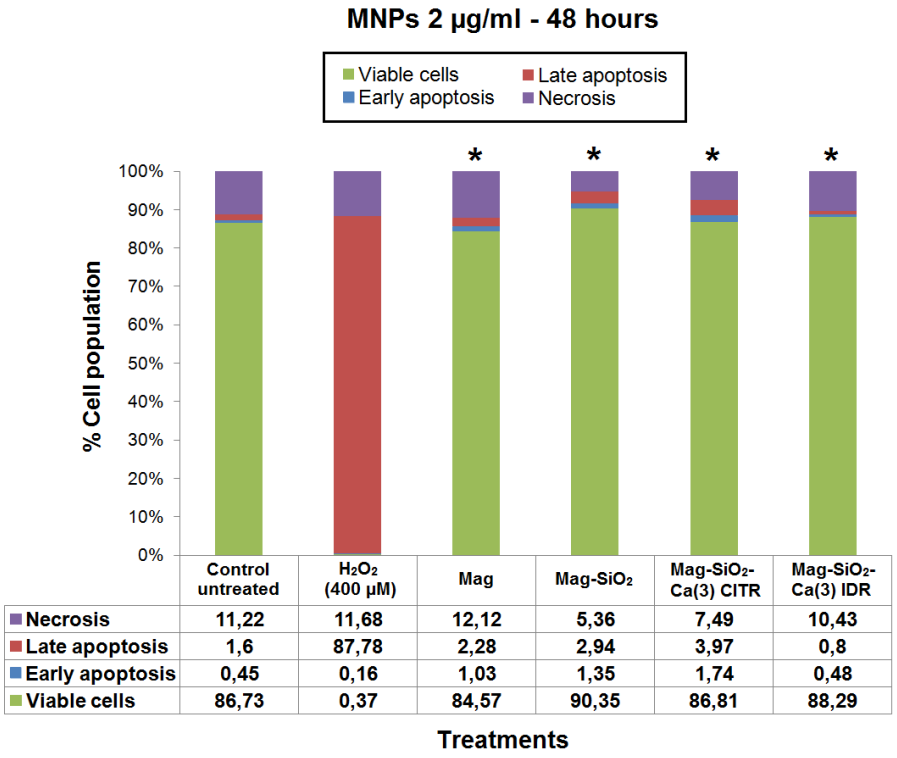
	Control untreated	H_2O_2 (400 µM)	Mag	Mag-SiO_2	Mag-SiO_2-Ca(3) CITR	Mag-SiO_2-Ca(3) IDR
Necrosis	4,31	11,51	7,05	3,04	10,29	3,29
Late apoptosis	3,33	88,08	2,59	2,81	2,69	4
Early apoptosis	0,84	0,11	1,15	0,88	1,09	1,56
Viable cells	91,53	0,3	89,21	93,28	85,94	91,15



	Control untreated	H_2O_2 (400 µM)	Mag	Mag-SiO_2	Mag-SiO_2-Ca(3) CITR	Mag-SiO_2-Ca(3) IDR
Necrosis	4,31	11,51	6,97	3,3	4,16	3,68
Late apoptosis	3,33	88,08	3,34	2,31	5,09	4,4
Early apoptosis	0,84	0,11	2,05	1	1,21	1,42
Viable cells	91,53	0,3	87,64	93,39	89,54	90,49

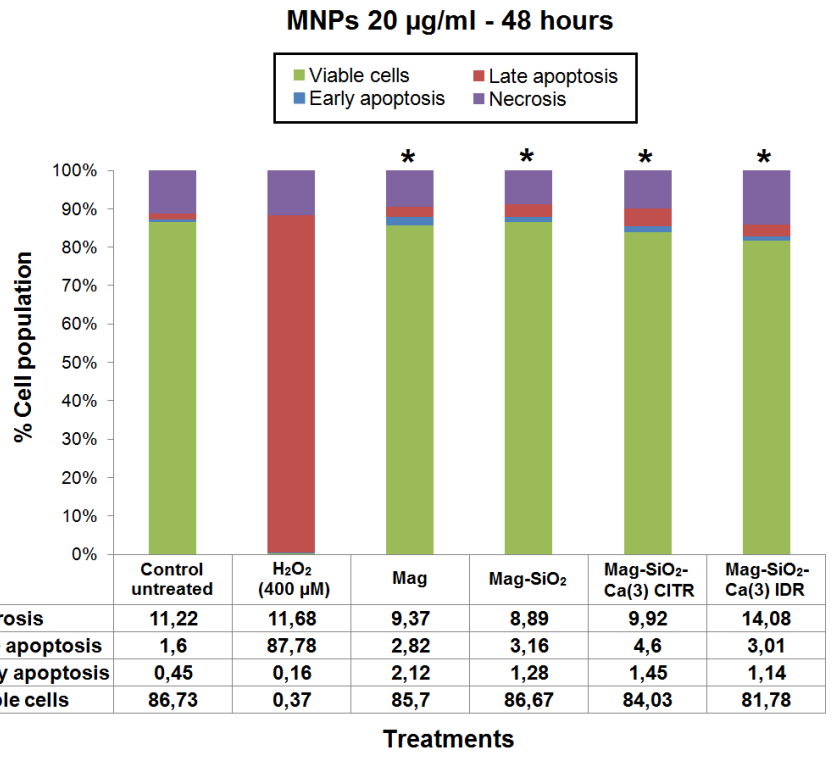
*P < 0.05 compared to control (Chi-square test)

Apoptosis evaluation after 48 hours



	Control untreated	H_2O_2 (400 µM)	Mag	Mag-SiO_2	Mag-SiO_2-Ca(3) CITR	Mag-SiO_2-Ca(3) IDR
Necrosis	11,22	11,68	12,12	5,36	7,49	10,43
Late apoptosis	1,6	87,78	2,28	2,94	3,97	0,8
Early apoptosis	0,45	0,16	1,03	1,35	1,74	0,48
Viable cells	86,73	0,37	84,57	90,35	86,81	88,29

Treatments

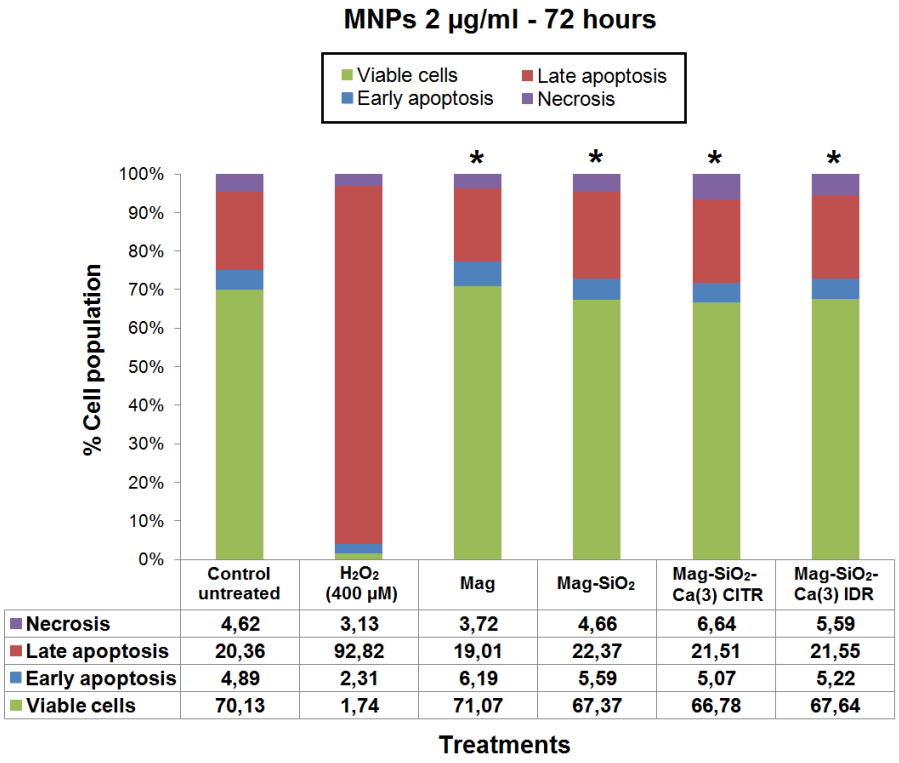


	Control untreated	H_2O_2 (400 µM)	Mag	Mag-SiO_2	Mag-SiO_2-Ca(3) CITR	Mag-SiO_2-Ca(3) IDR
Necrosis	11,22	11,68	9,37	8,89	9,92	14,08
Late apoptosis	1,6	87,78	2,82	3,16	4,6	3,01
Early apoptosis	0,45	0,16	2,12	1,28	1,45	1,14
Viable cells	86,73	0,37	85,7	86,67	84,03	81,78

Treatments

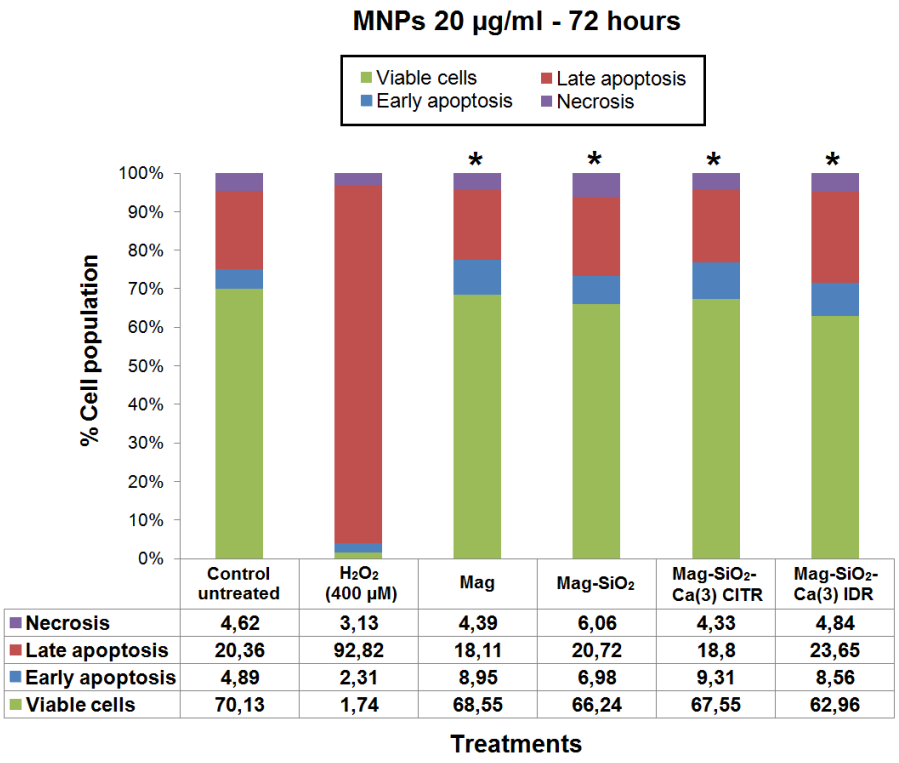
*P < 0.05 compared to control (Chi-square test)

Apoptosis evaluation after 72 hours



	Control untreated	H_2O_2 (400 µM)	Mag	Mag-SiO_2	Mag-SiO_2-Ca(3) CITR	Mag-SiO_2-Ca(3) IDR
Necrosis	4,62	3,13	3,72	4,66	6,64	5,59
Late apoptosis	20,36	92,82	19,01	22,37	21,51	21,55
Early apoptosis	4,89	2,31	6,19	5,59	5,07	5,22
Viable cells	70,13	1,74	71,07	67,37	66,78	67,64

Treatments



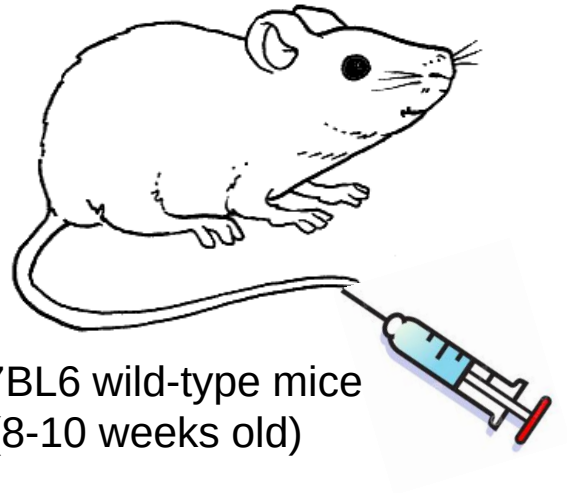
	Control untreated	H_2O_2 (400 µM)	Mag	Mag-SiO_2	Mag-SiO_2-Ca(3) CITR	Mag-SiO_2-Ca(3) IDR
Necrosis	4,62	3,13	4,39	6,06	4,33	4,84
Late apoptosis	20,36	92,82	18,11	20,72	18,8	23,65
Early apoptosis	4,89	2,31	8,95	6,98	9,31	8,56
Viable cells	70,13	1,74	68,55	66,24	67,55	62,96

Treatments

*P < 0.05 compared to control (Chi-square test)

In vivo evaluation of iron-oxide nanoparticles

Biodistribution of MNPs - 7 days (Short term evaluation)



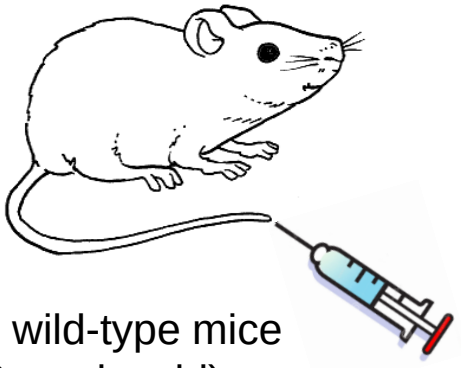
C57BL6 wild-type mice
(8-10 weeks old)

- **Fe_3O_4 and Fe_3O_4-SiO_2 nanoparticles were tested using the following concentration: 2 mg Fe/kg body weight**
- **Evaluation of hematological parameters after 3 and 7 days (May-Grunwald-Giemsa staining for counting of blood cells)**
- **Evaluation of biochemical parameters of renal and hepatic functionality after 7 days: ALT, AST, CRE, LDH**

Aspartate transaminase (AST), alanine transaminase (ALT), creatinine (CRE), lactic acid dehydrogenase (LDH)

In vivo evaluation of MNPs

Biodistribution of MNPs - 7 days



C57BL6 wild-type mice
(8-10 weeks old)

Explant of organs after 7 days

Macroscopic evaluation (organ weights)

Histological evaluation (Perls' Prussian blue staining)

Compositional evaluation for iron accumulation (Inductively coupled plasma-atomic emission spectrometry (ICP-AES))

Serum biomarkers analysis (2 mg Fe/kg - 7 days)

	Control	Fe_3O_4 NPs	Fe_3O_4-SiO_2 NPs
ALT (U/l)	39.42 ± 2.69	43.15 ± 2.72	44.26 ± 2.06*
AST (U/l)	127.35 ± 12.07	129.34 ± 7.12	134.81 ± 6.83*
CRE (µmol/l)	19.21 ± 1.25	21.91 ± 1.03	23.48 ± 1.47*
LDH (U/l)	833.75 ± 53.28	841.67 ± 57.91	843.46 ± 46.35

ALT alanine transaminase, AST aspartate transaminase, CRE creatinine, LDH lactic acid dehydrogenase

Data are presented as mean ± SD (n = 4).

*P < 0.05 compared with control

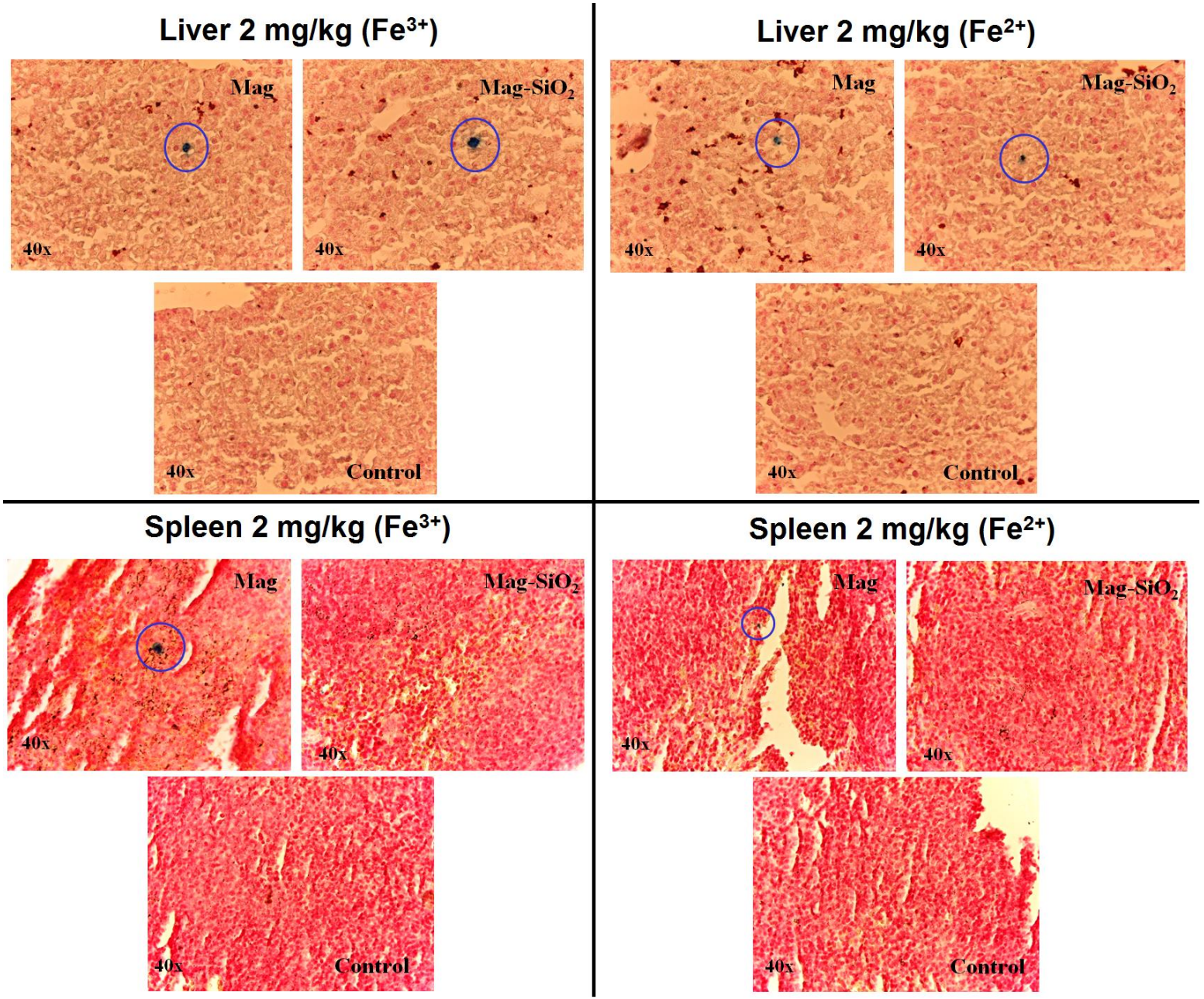
Hematological parameters (2 mg Fe/kg)

	Control	Fe_3O_4 NPs	Fe_3O_4-SiO_2 NPs
Red blood cell (10^6/µl) after 3 days	**9.83 ± 0.52**	**9.76 ± 0.93**	**9.71 ± 1.19**
Red blood cell (10^6/µl) after 7 days	**9.75 ± 0.83**	**9.69 ± 1.07**	**9.64 ± 0.71**
White blood cell (10^3/µl) after 3 days	**6.51 ± 0.73**	**6.63 ± 1.18**	**6.72 ± 1.26**
White blood cell (10^3/µl) after 7 days	**6.57 ± 1.14**	**6.73 ± 1.22**	**6.88 ± 1.61**

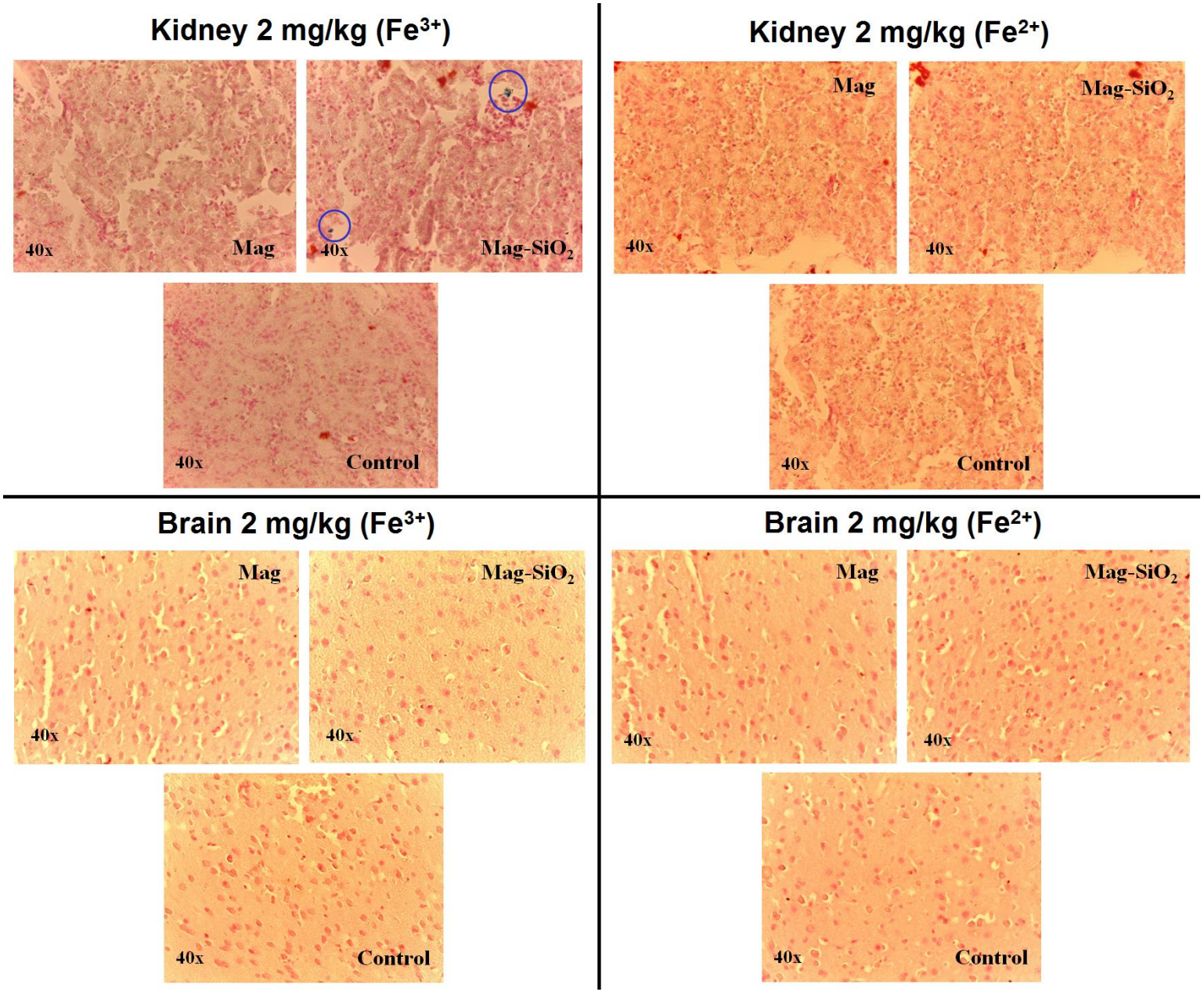
Organ weights (2 mg Fe/kg)

Organ	**Fe_3O_4 NPs**	**Fe_3O_4-SiO_2 NPs**	**Control**
Liver	1,37 ± 0,18 g	1,23 ± 0,22 g	1,46 ± 0,07 g
Spleen	94 ± 12,9 mg	102 ± 6,4 mg	100,3 ± 15,5 mg
Left Kidney	151,5 ± 20,8 mg	144,5 ± 22,2 mg	176,8 ± 8,3 mg
Right Kidney	165,2 ± 27,5 mg	164,5 ± 36,8 mg	177,5 ± 4,2 mg
Brain	421,5 ± 41,3 mg	415 ± 52,5 mg	441,5 ± 18,3 mg
Lung	157,5 ± 11,1 mg	166,8 ± 6,5 mg	170 ± 7,4 mg
Heart	167 ± 29,7 mg	157,8 ± 37,8 mg	173,8 ± 8,2 mg

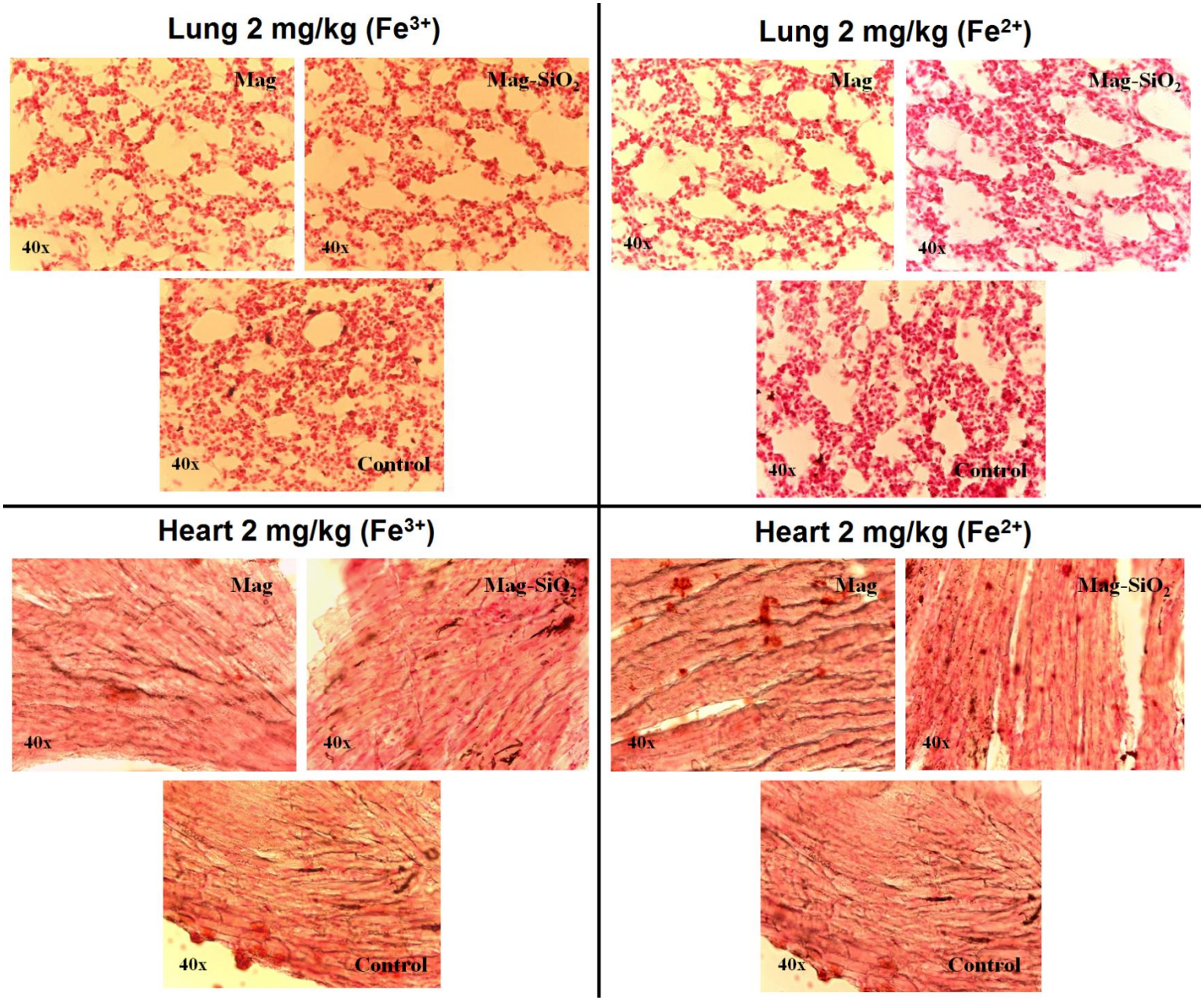
Prussian Blue staining (2 mg Fe/kg - Liver, Spleen)



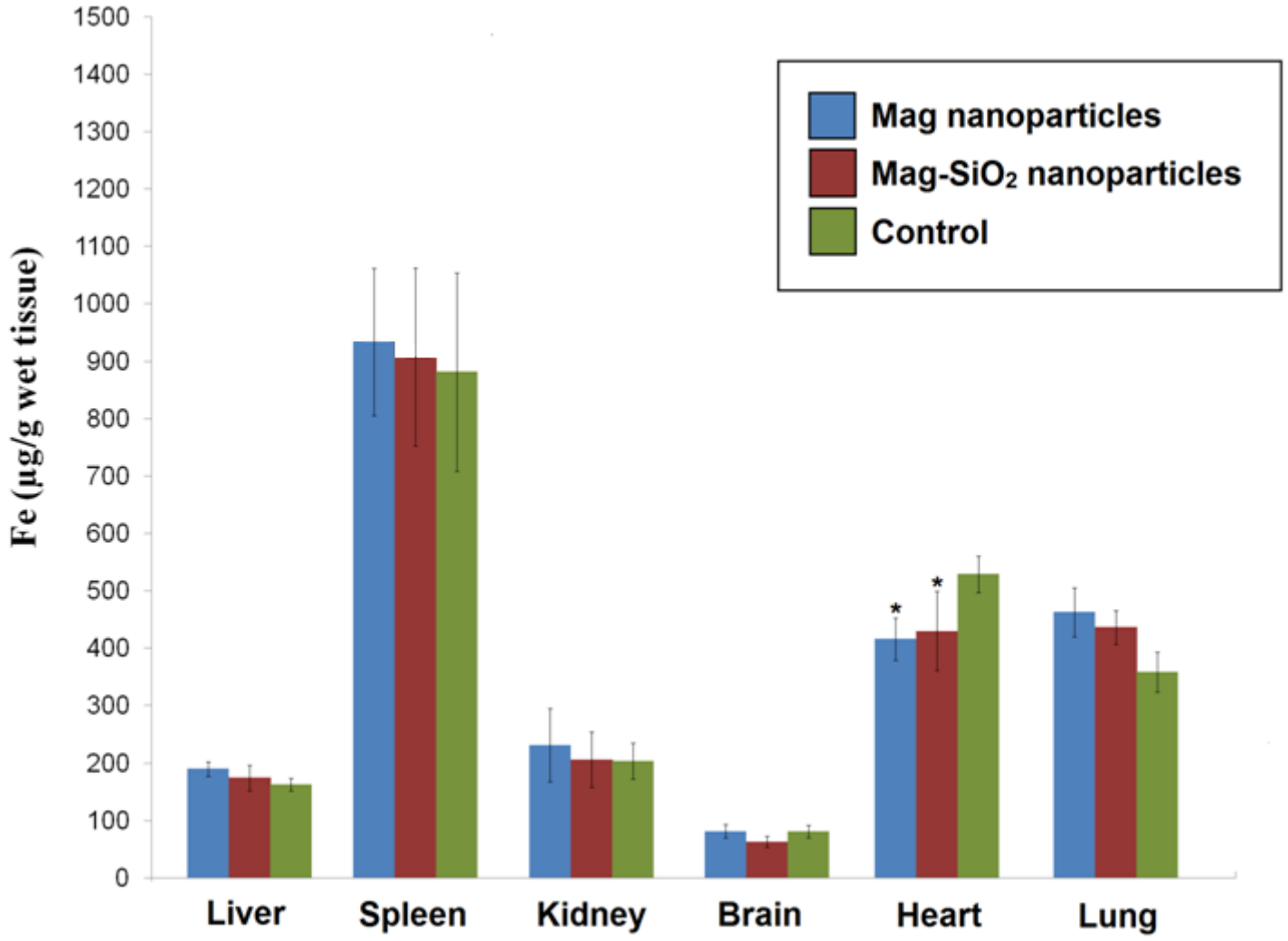
Prussian Blue staining (2 mg Fe/kg - Kidney, Brain)



Prussian Blue staining (2 mg Fe/kg - Lung, Heart)



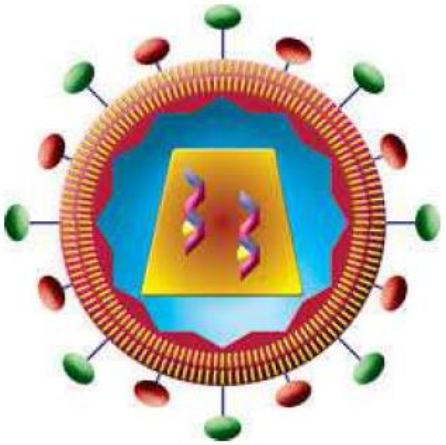
ICP-AES analysis results (2 mg Fe/kg)

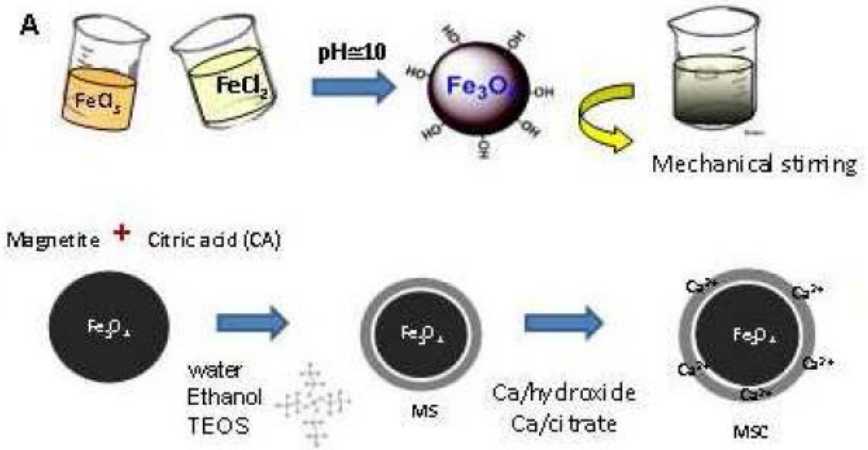


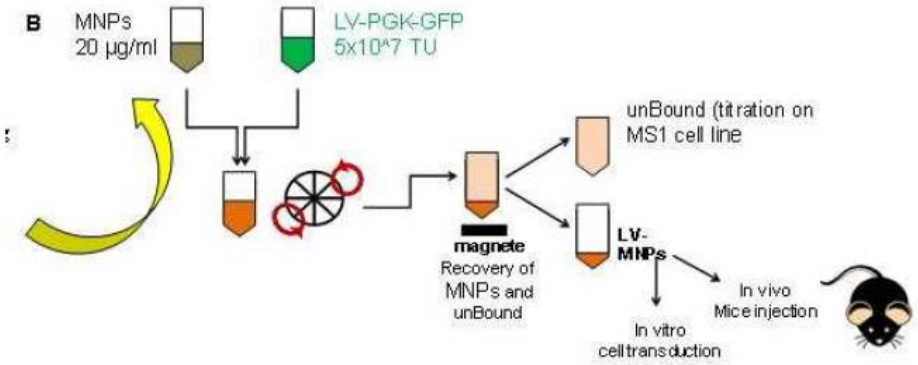
*P < 0.05 compared to control - One way analysis of variance (ANOVA) followed by Scheffe's test

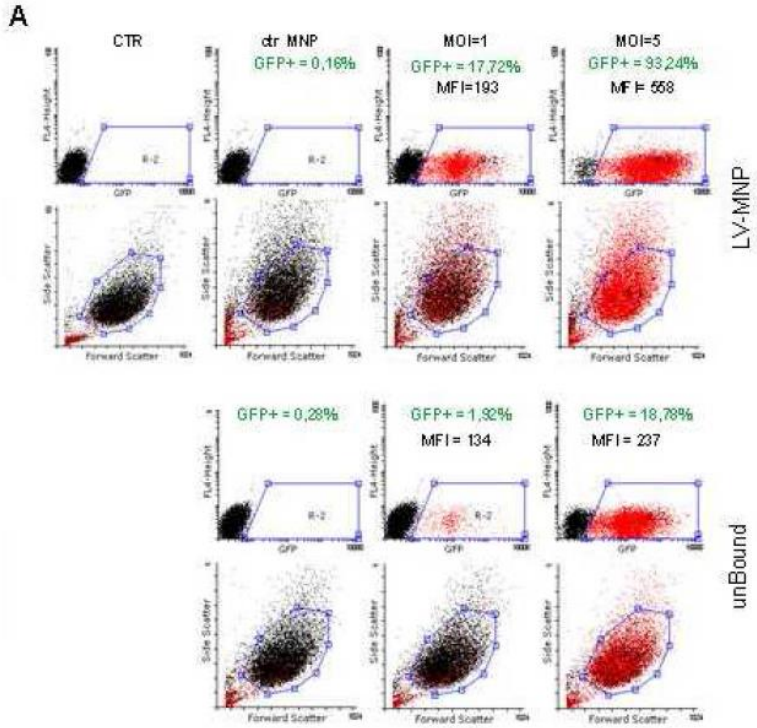
Cell/MNPs interaction via Lentivirus coupling

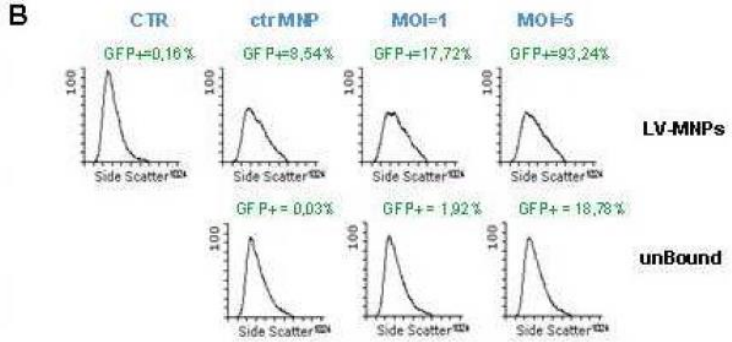
- Retrovirus (RNA)
- Peri-capside
- Used in cell trasduction as a vector
- Different types of MNPs were assembled with lentiviral vectors with different MOI (multiplicity of infection) ratios

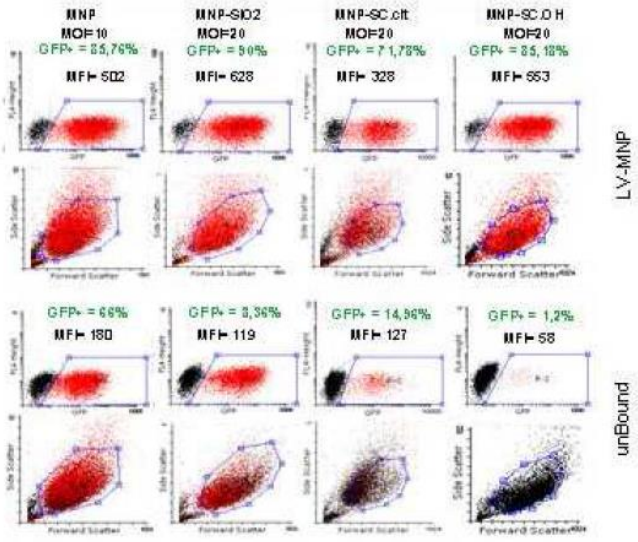







A
CTR
ctr MNP
GFP+ = 0,16%
MOI=1
GFP+ = 17,72%
MFI=193
MOI=5
GFP+ = 93,24%
MFI = 558
LV-MNP
GFP+ = 0,28%
GFP+ = 1,92%
MFI = 134
GFP+ = 18,78%
MFI = 237
unBound
Forward Scatter
Side Scatter


B
CTR
ctrMNP
MOI=1
MOI=5
GFP+=0,16%
GFP+=8,54%
GFP+=17,72%
GFP+=93,24%
LV-MNPs
GFP+ = 0,03%
GFP+ = 1,92%
GFP+ = 18,78%
unBound
Side Scatter


C
MNP
MOI=10
GFP+ = 85,76%
MFI= 502
MNP-SiO2
MOI=20
GFP+ = 90%
MFI= 628
MNP-SC.cit
MOI=20
GFP+ = 71,78%
MFI= 328
MNP-SC.OH
MOI=20
GFP+ = 85,12%
MFI= 553
LV-MNP
GFP+ = 66%
MFI= 180
GFP+ = 8,36%
MFI= 119
GFP+ = 14,96%
MFI= 127
GFP+ = 1,2%
MFI= 58
unBound
Forward Scatter
Side Scatter

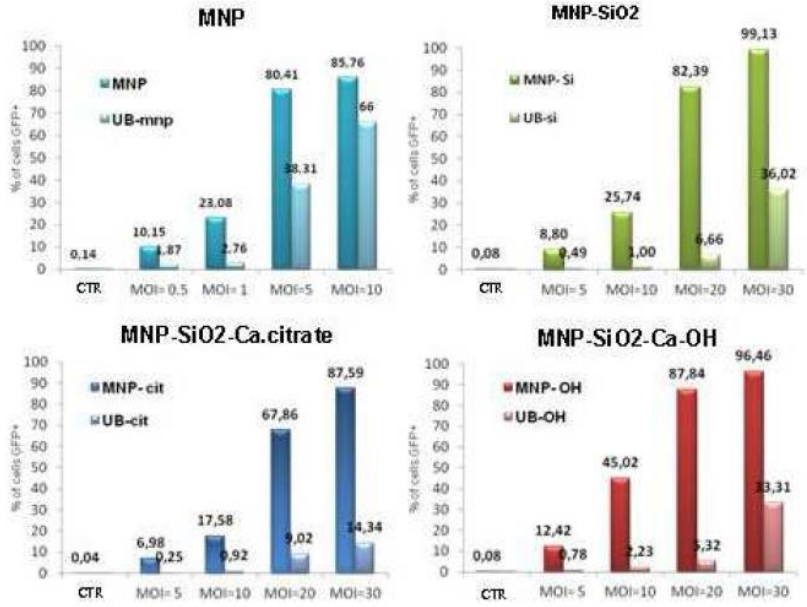
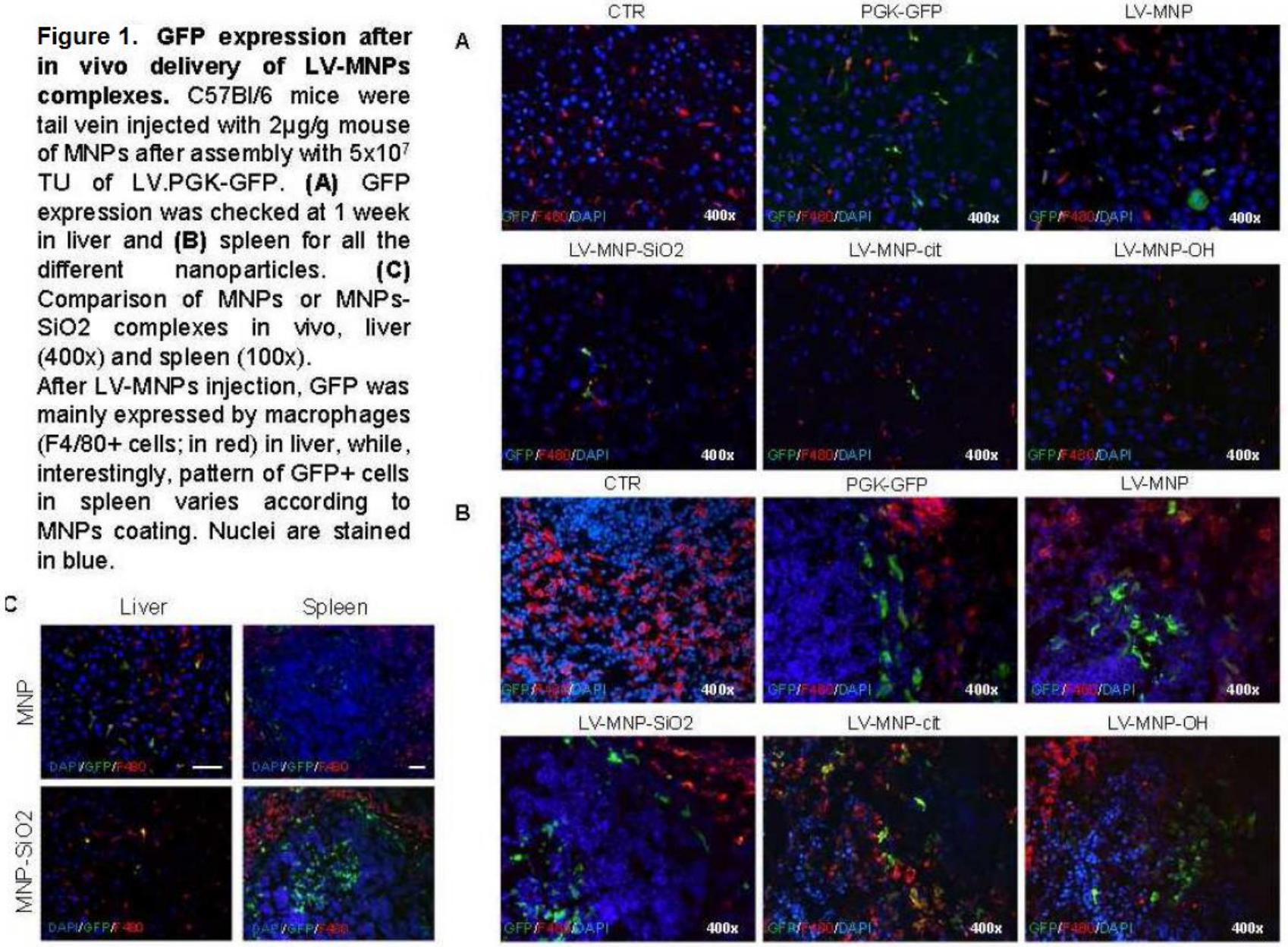

D
MNP
MNP
UB-mnp
% of cells GFP+
0,14
10,15
1,87
23,08
2,76
80,41
38,31
85,76
66
CTR
MOI= 0.5
MOI= 1
MOI=5
MOI=10
MNP-SiO2
MNP- Si
UB-si
0,08
8,80
0,49
25,74
1,00
82,39
6,66
99,13
36,02
CTR
MOI=5
MOI=10
MOI=20
MOI=30
MNP-SiO2-Ca.citrate
MNP- cit
UB-cit
0,04
6,98
0,25
17,58
0,92
67,86
9,02
87,59
14,34
CTR
MOI= 5
MOI=10
MOI=20
MOI=30
MNP-SiO2-Ca-OH
MNP- OH
UB-OH
0,08
12,42
0,78
45,02
2,23
87,84
5,32
96,46
33,31
CTR
MOI=5
MOI=10
MOI=20
MOI=30

Figure 1. GFP expression after in vivo delivery of LV-MNPs complexes. C57Bl/6 mice were tail vein injected with 2µg/g mouse of MNPs after assembly with $5x10^7$ TU of LV.PGK-GFP. **(A)** GFP expression was checked at 1 week in liver and **(B)** spleen for all the different nanoparticles. **(C)** Comparison of MNPs or MNPs-SiO2 complexes in vivo, liver (400x) and spleen (100x).
After LV-MNPs injection, GFP was mainly expressed by macrophages (F4/80+ cells; in red) in liver, while, interestingly, pattern of GFP+ cells in spleen varies according to MNPs coating. Nuclei are stained in blue.



Magnetic hyperthermia induced by iron-oxide nanoparticles on liver cancer cell line (Hep G2)

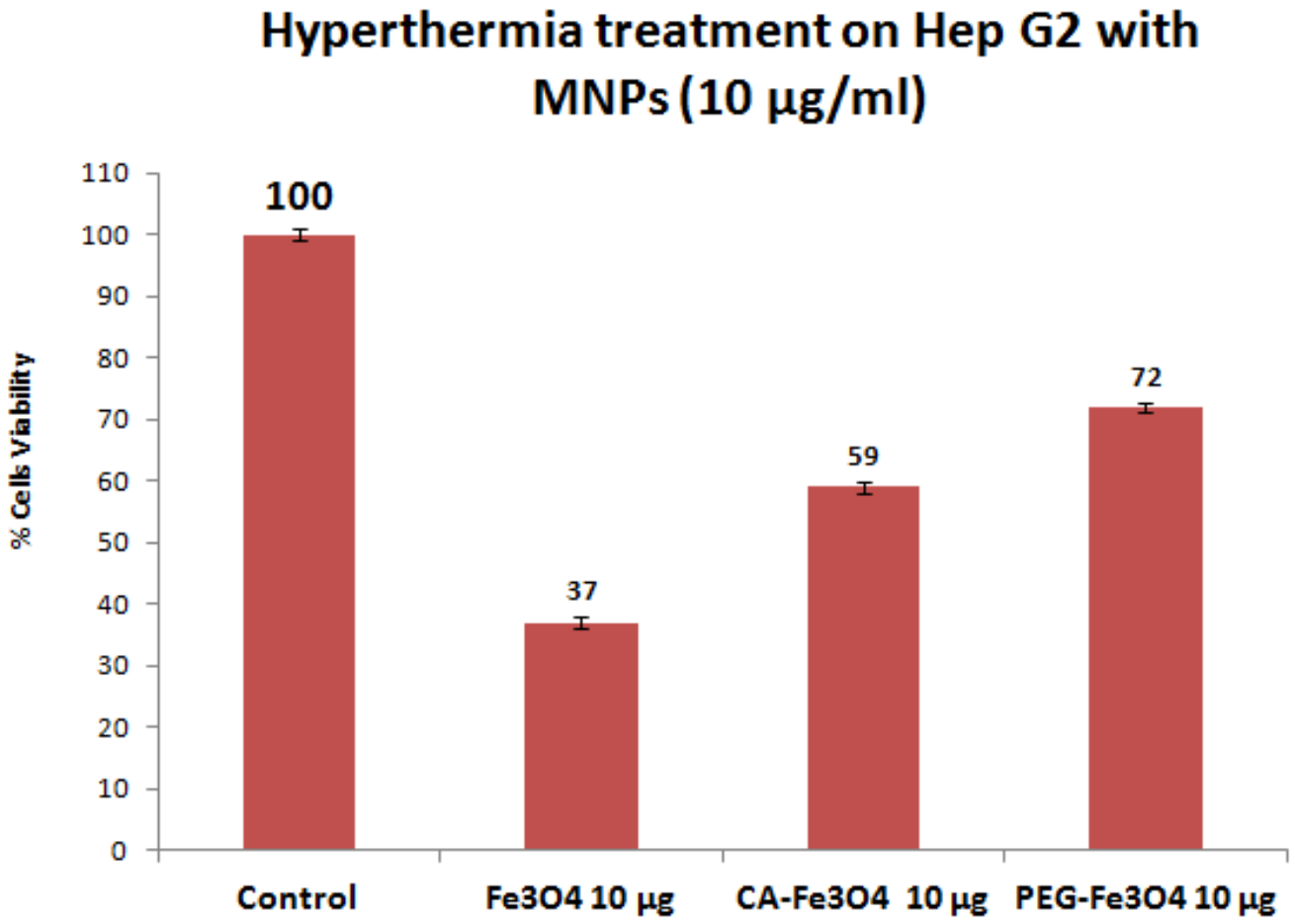
- Three types of iron-oxide nanoparticles: pure Fe_3O_4 NPs, PEG functionalized Fe_3O_4 NPs, carboxylic acid (CA) functionalized Fe_3O_4 NPs
- For hyperthermia treatment, cells will be put in the incubator at 46°C for 30 minutes, corresponding to a temperature dosage of 90 cumulative equivalent minutes at 43°C

- **Hyperthermia treatment on the human liver cancer cell line (Hep G2)**
- **THLE-3 cells were used as cell model of normal liver cells**
- Two concentrations of nanoparticles used: 10 µg/ml, 20 µg/ml

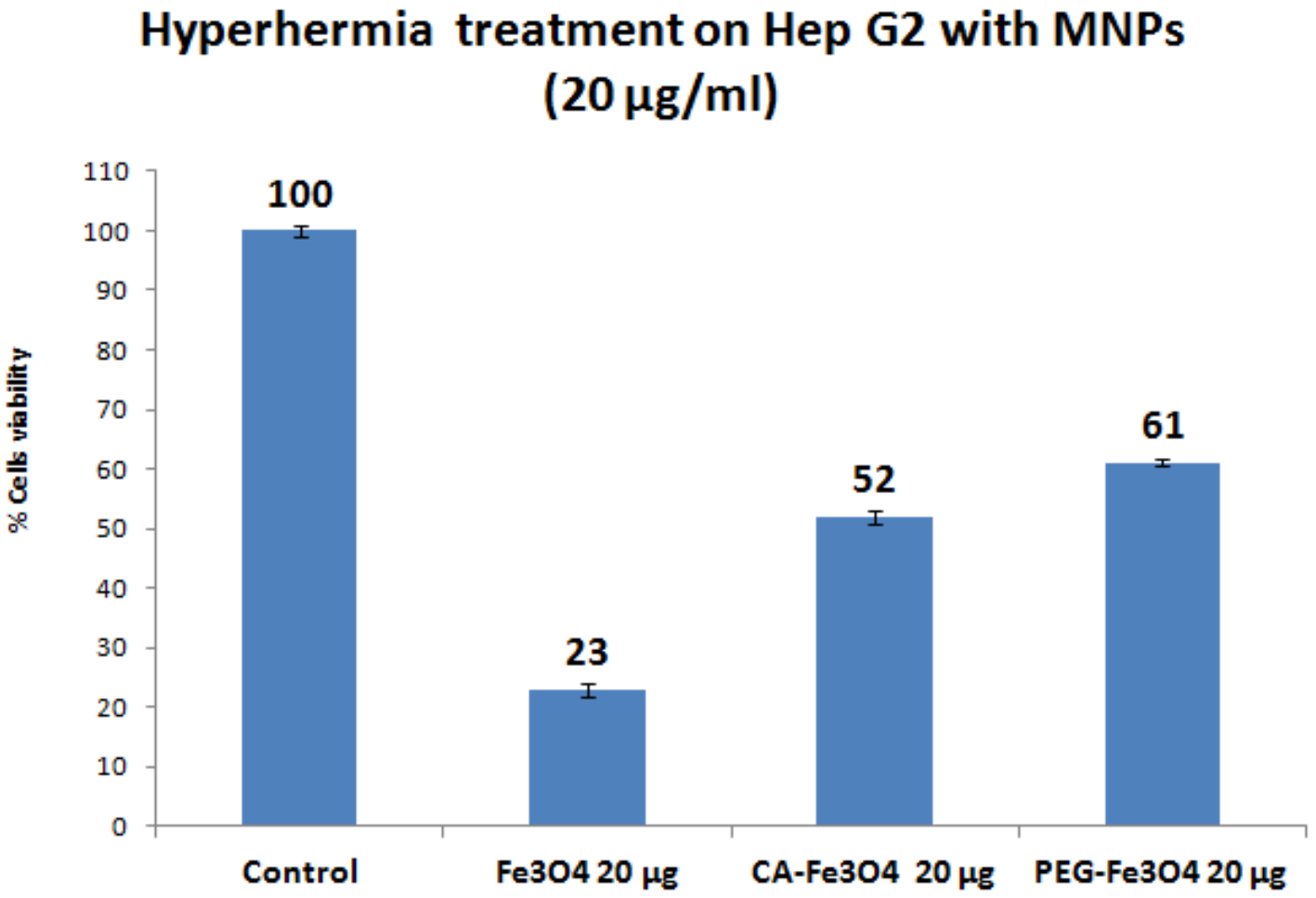
- MTT assay

Brigitta Szalay et al, Journal of Applied Toxicology

Hyperthermia treatment induced by MNPs (10 µg/ml) on liver cancer cell line (Hep G2)



Hyperthermia treatment induced by MNPs (20 µg/ml) on liver cancer cell line (Hep G2)



Final results

- The MNPs used in this study demonstrated to be cytocompatible in both static and dynamic conditions of cell viability.

- ROS generation induced by MNPs was in a concentration-dependent manner comparable to controls.

- MS1 cells in contact with MNPs showed a small percentage of apoptotic and necrotic cells comparable to untreated control.

Final results-2

- The in vivo data confirmed a good performance of the nanoparticles using a concentration of 2 mg Fe/kg body weight for biomedical applications.

- Lentiviral vectors coupled with SPIONs increased gene expression in liver and spleeen. Thus it can be used for applications of cancer gene therapy.

- Magnetic hyperthermia induced by iron-oxide nanoparticles was efficient on killing liver cancer cell cells in *in vitro* conditions in a target way

Take home massage

- The final set-up of magnetic nanoparticles was optimized to be used like a carrier for drug targeting/gene therapy or for cancer therapy and thermoablative therapies.

- The tools developed in this work spanned a range of physical-chemical, biological and magnetic aspects and incorporate innovations on a nanometric range of scales.

- The magnetic nanoparticles could be in future one of the field with higher perspectives of development for different scientific applications, especially such as a smart targeted drug delivery platform for in vivo disease therapies.

UiO : University of Oslo

New therapeutic approaches for personalized breast cancer nanomedicine

Catalano Enrico, PhD and Prof. Vessela N. Kristensen

Scientia Fellows project –
Postdoctoral fellowship programme in Health Sciences

breast cancer campaign
Research that saves lives

MARIE CURIE ACTIONS

Breast cancer

- **The breast is made up of glands called lobules that can make milk and thin tubes called ducts that carry the milk from the lobules to the nipple. Breast tissue also contains fat and connective tissue, lymph nodes, and blood vessels.**

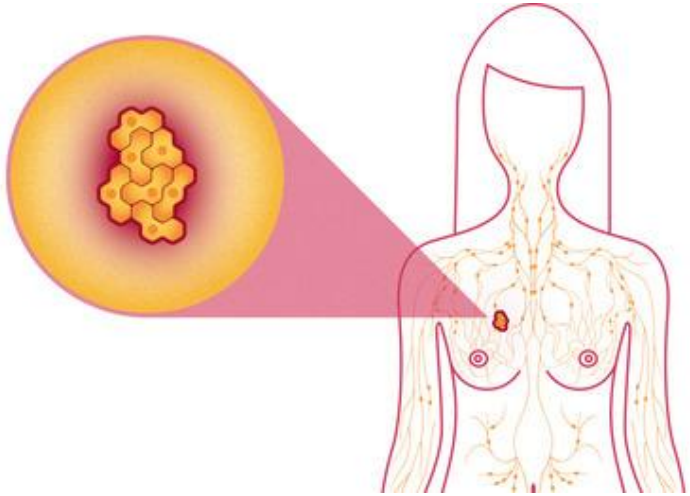
- **The most common type of breast cancer is ductal carcinoma, which begins in the cells of the ducts. Breast cancer can also begin in the cells of the lobules and in other tissues in the breast. Invasive breast cancer is breast cancer that has spread from where it began in the ducts or lobules to surrounding tissue.**

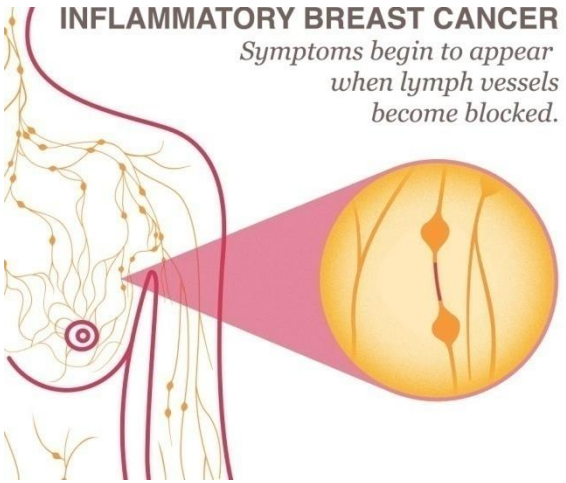
- **In the U.S., breast cancer is the second most common cancer in women after skin cancer. It can occur in both men and women, but it is very rare in men. Each year there are about 2,300 new cases of breast cancer in men and about 230,000 new cases in women.**

Key statistics about breast cancer from the SEER Cancer Statistics Review, 1975-2010

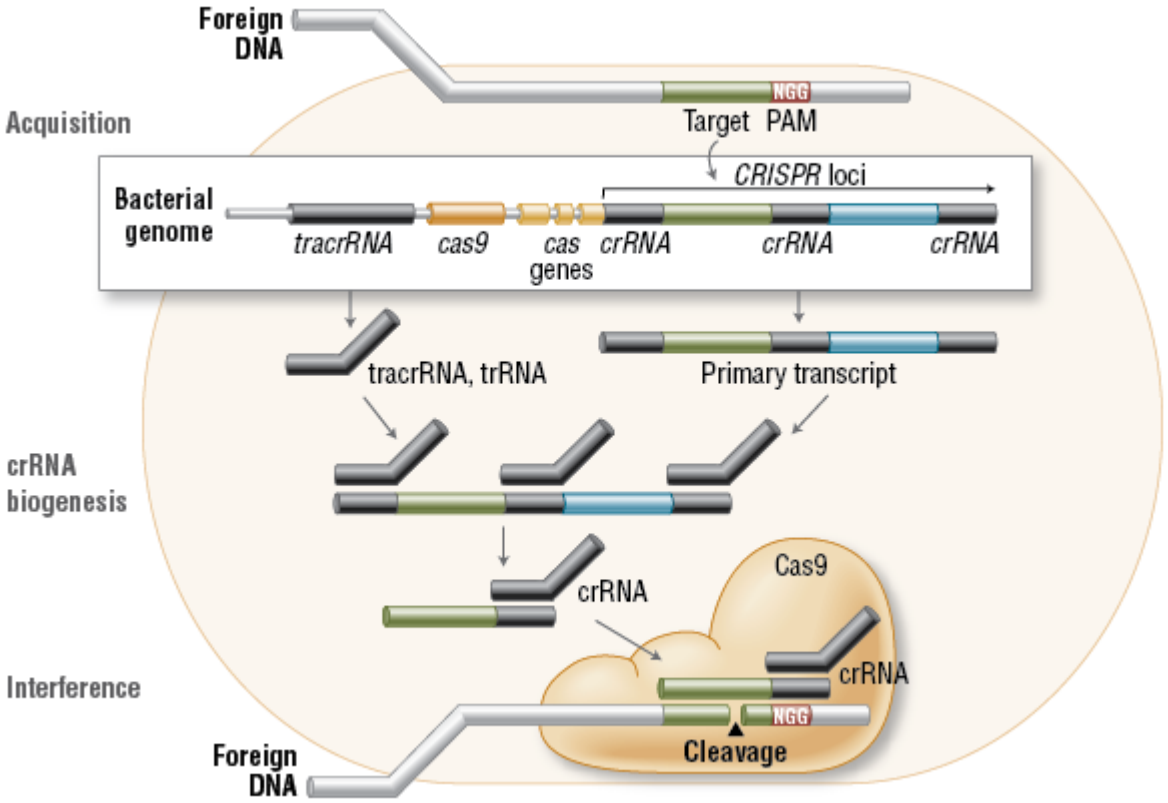
Breast cancer

- Breast cancer is the most common invasive cancer in women worldwide. It affects about 12% of women worldwide. Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancers. In 2012, it comprised 25.2% of cancers diagnosed in women, making it the most common female cancer.

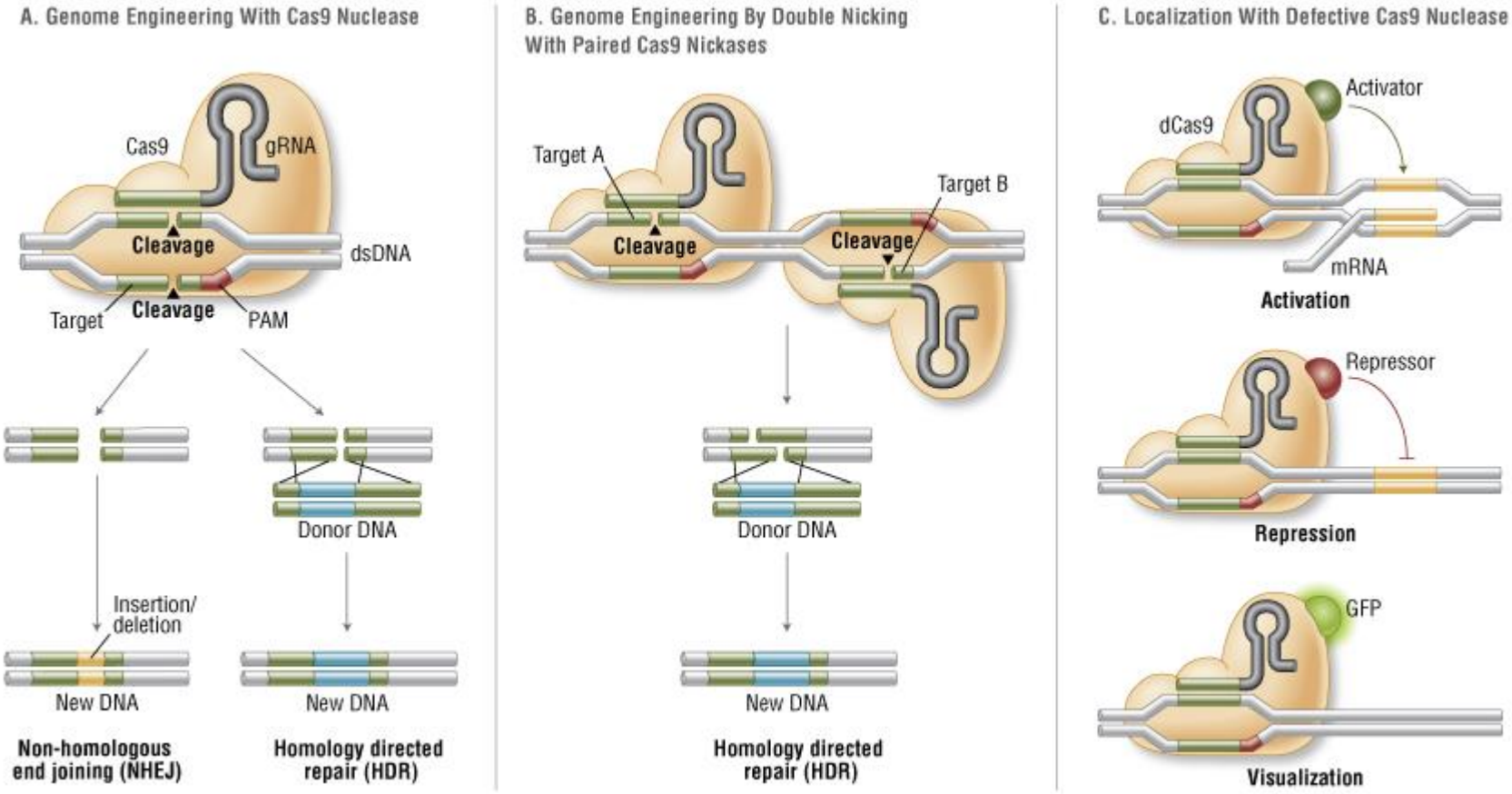




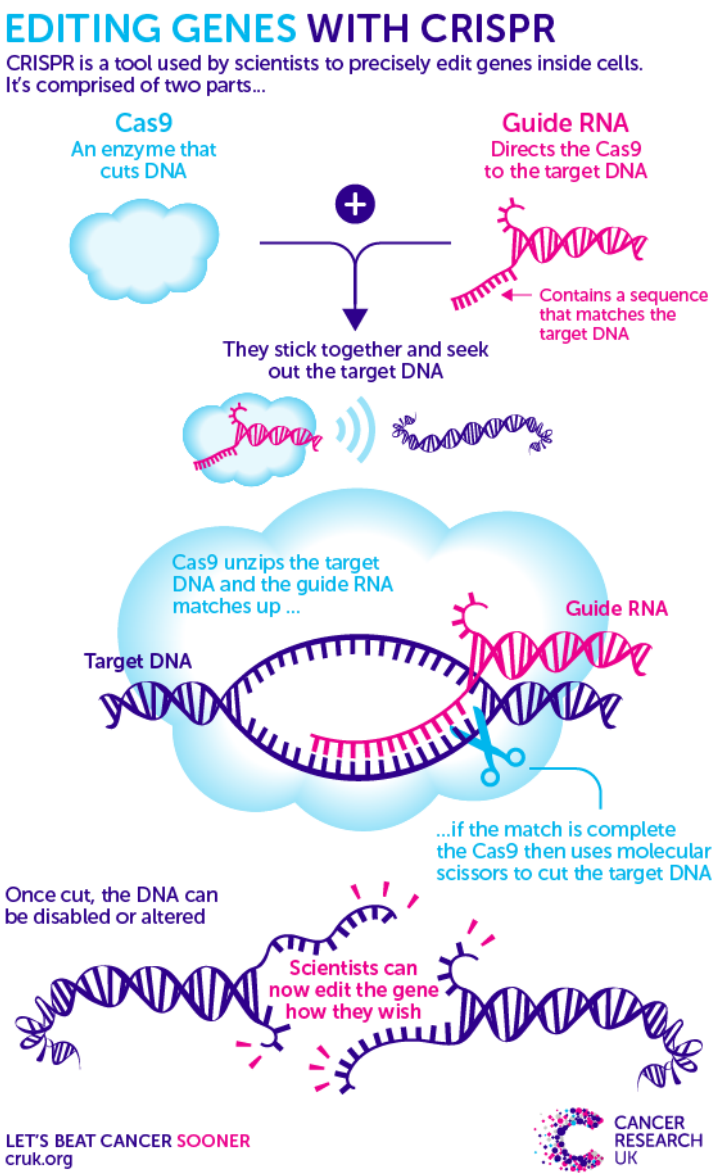
CRISPR/Cas9 and Targeted Genome Editing



CRISPR/Cas9 and Targeted Genome Editing



Editing genes with CRISPR

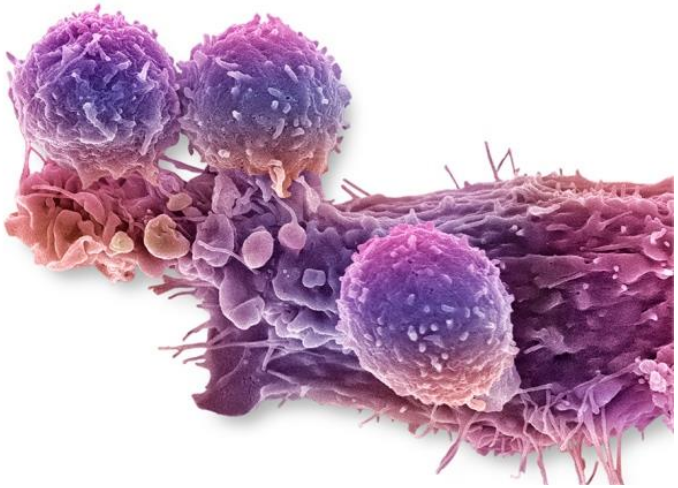


Editing cancer-associated genes with CRISPR-Cas9

- CRISPR-Cas9 genome editing tool is capable of inducing loss of function (LOF) mutations, gain of function (GOF) mutations and chromosomal rearrangements in vitro and in vivo.

- It is simple-to-design, easy-to-use and multiplexing nature streamlines the generation of animal and cellular cancer models, enabling rapid functional interrogation of cancer-associated genes.

First trial of CRISPR/Cas9 sytem in people suffering from lung cancer



BIOMEDICINE

First trial of CRISPR in people

Chinese team approved to test gene-edited cells in people with lung cancer.

BY DAVID CYRANOSKI

Chinese scientists are on the verge of being first in the world to inject people with cells modified using the CRISPR–Cas9 gene-editing technique.

A team led by Lu You, an oncologist at Sichuan University's West China Hospital in Chengdu, received ethical approval to test the cells in people with lung cancer on 6 July, and plans to start the trial next month.

That timeline puts the proposal ahead of a planned US trial to test CRISPR–Cas9-modified cells, also for the treatment of cancer.

"It's an exciting step forward," says Carl June, a clinical researcher in immunotherapy at the University of Pennsylvania in Philadelphia.

Last month, the US trial was approved by an advisory panel of the US National Institutes of Health (NIH) but had yet to receive a green light from the US Food and Drug Administration (FDA) and a university review board. There have also been a number of human clinical trials using an alternative gene-editing technique, including one led by June, that have helped patients to combat HIV — but none so far has used CRISPR.

The Chinese trial will enrol patients who have metastatic non-small cell lung cancer and for whom chemotherapy, radiation therapy and other treatments have failed. "This technique is of great promise in bringing benefits to patients," says Lu.

CHROMOSOME SNIP

Lu's team will extract immune cells called T cells from the participants' blood, and use CRISPR–Cas9 technology — which pairs a molecular guide able to identify specific genetic sequences on a chromosome with an enzyme that can snip the chromosome at that spot — to knock out a specific gene in the

CRISPR/Cas 9 genome editing for breast cancer

- CRISPR/Cas 9 genome editing could be associated with Next-Generation DNA Sequencing (NGS) to control the outcome of DNA editing of targeted genes.
- In this perspective DNA sequencing will allow to control the success of insertion or deletion of target genome sequences of interest in genes related to breast cancer.
- This strategy can be implemented to modify in a target way oncogenes, tumor suppressor genes and genes involved in breast cancer development.

CRISPR/Cas9 genome editing for breast cancer

- CRISPR/Cas9 genome editing will be used to act on genes involved in cancer development process and drug resistance and on high-penetrance genes of breast cancer susceptibility of (BRCA1, BRCA2, p53, PTEN, STK11, CDH1) that increase breast cancer risk more than four-times.

- (Epi)genetic modifications of cancer microenvironment with CRISPR-Cas9 systems for therapeutic purposes could represent a promising area in cancer research.

Multiple therapeutic approach for breast cancer

This innovative therapeutic nanomedicine solution for breast cancer treatment will be based on

- **Hyperthermia effect**
- **Efficient localized and targeted drug delivery of MNPs assembled with anticancer drugs**
- **CRISPR/Cas9 cancer genome editing**

Goal of the project

- This project aims to implement an extremely **innovative multi-therapeutic strategy that combines targeted drug delivery of chemotherapeutics, the use of MNPs properties of hyperthermia to target *in vitro* breast cancer drug-resistant and not drug-resistant cell lines and cancer genome editing by CRISPR/Cas9 system.**

Main objectives of the project

The project will be structured into the following tasks:

1. **In vitro efficacy of MNPs conjugated to chemotherapy drugs without or under hyperthermic conditions on breast cancer cell lines.**

2. **Preliminary approach of cancer genome editing of genes involved in breast cancer development process and drug resistance.**

Impact of the project

- **The application of magnetic nanoparticles are widely expected to change the landscape of breast cancer therapy for foreseeable future.**

- **This project will be focused on application of multifunctional therapeutic treatments for targeted drug delivery, cancer nanotheranostics, genome editing of high-penetrance genes involved in breast cancer susceptibility and combination therapy towards breast cancer.**

- **This approach of nanomedicine in cancer treatment could be also applied to modulate transcription factors and epigenetics of breast cancer cells.**

Thanks To:

COST Committee for the economical support, AIRC project, Compagnia San Paolo project.

Prof. Enrica Vernè, Dott.ssa Sara Ferraris, Dott.ssa Marta Miola.

Prof. ssa Lia Rimondini, Prof.ssa Antonia Follenzi, Dott.ssa Barbara Azzimonti, Prof.ssa Maria Prat, Dott.ssa Francesca Oltolina and all the people of the Biomedical materials Lab (Dott. Andrea Cochis, Dott.ssa Elena Varoni).

and all of You for the attention!!