UNIVERSITÀ DEL PIEMONTE ORIENTALE

Super-paramagnetic iron oxide nanoparticles (SPIONs) as functionalizing platforms for delivering and tissue regeneration

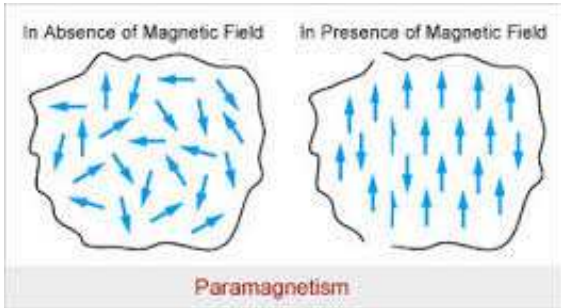
Lia Rimondini

Magnetic properties of the materials

ferrimagnetic

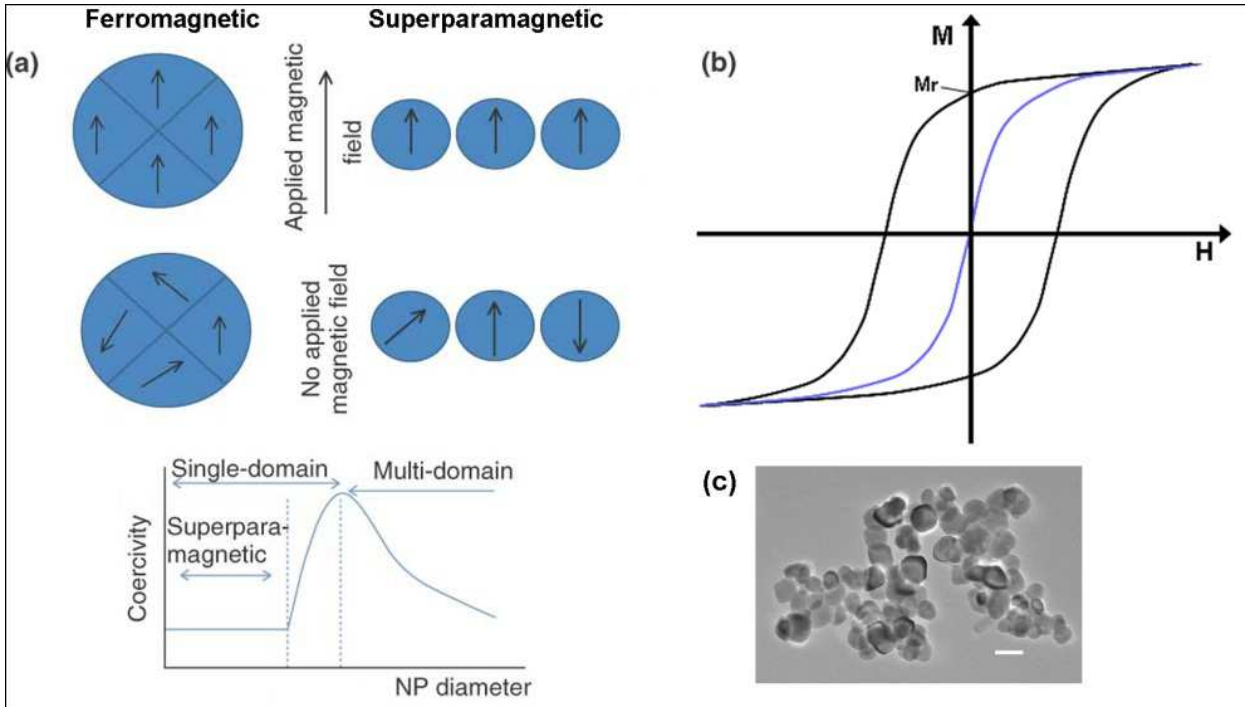
paramagnetic

diamagnetic



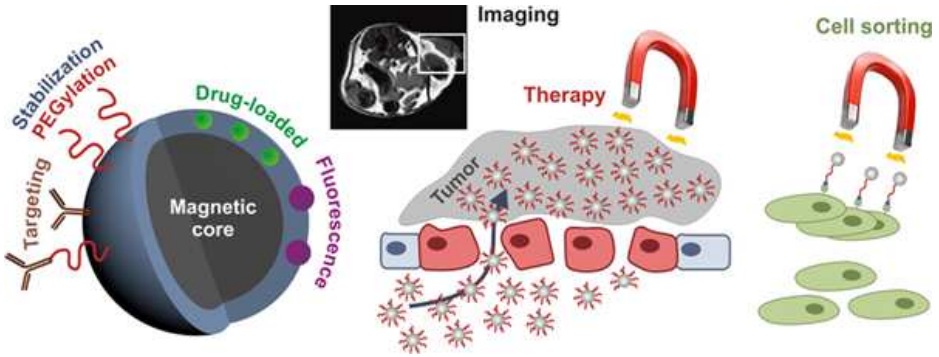
Residual magnetic moment remains at zero field at room T

Magnetic properties of the materials at the nanosize



- No residual magnetiation at field zero
- Single magnetic domain
- High magnetic susceptibility
- Low energy required for spin inversion
- T generation

Different applications of magnetic nanoparticles



Industrial applications

Drug delivery

Hyperthermia

Tissue engineering and repair

Magnetic resonance imaging (MRI)

Biosensing and Bioanalysis

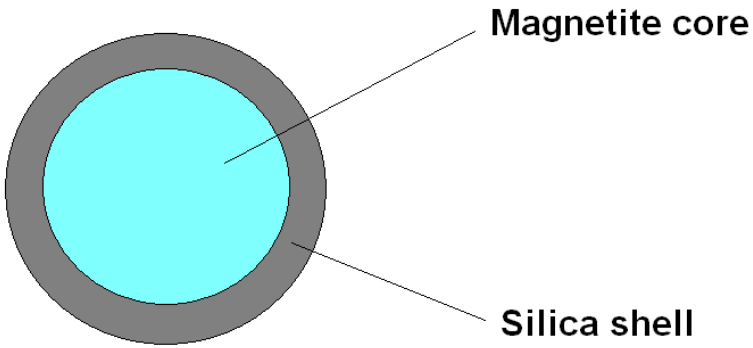
Biochemical separations

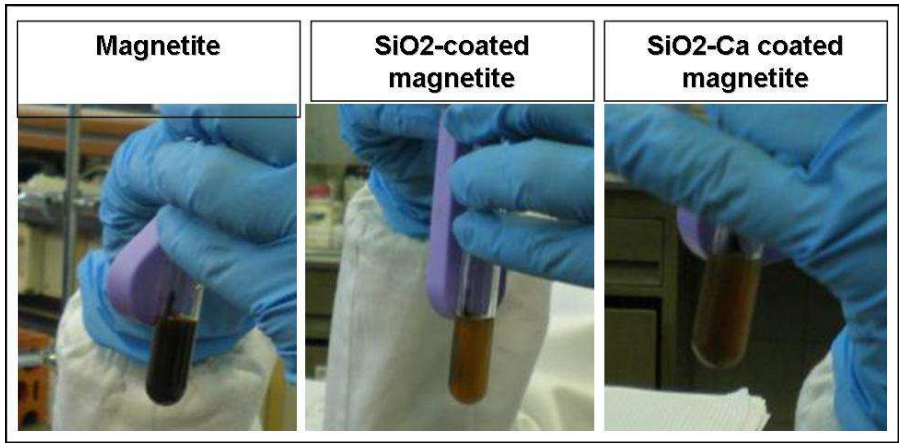
L. Harivardhan Reddy et al., Chem. Rev. 2012

Synthesis of MNPs via wet-chemistry

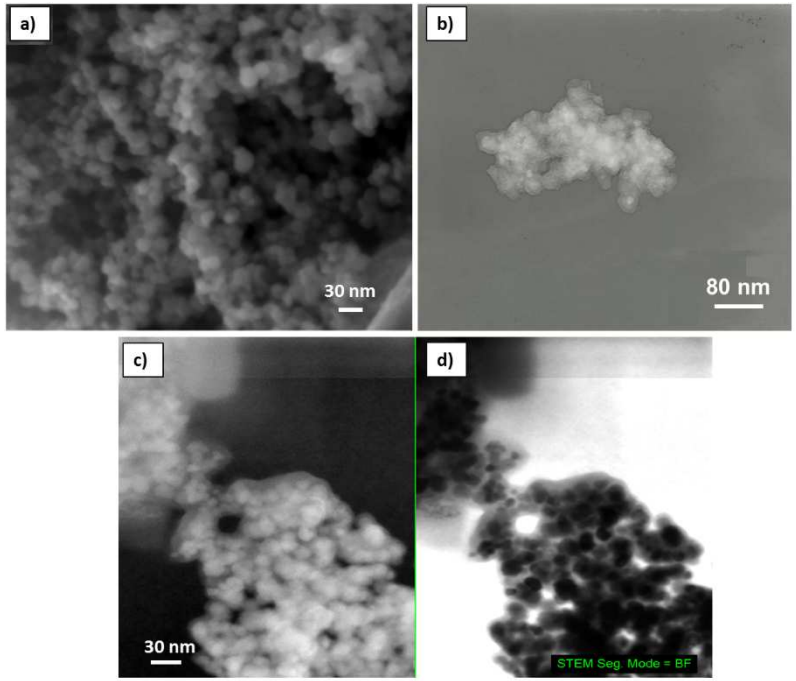
Mag NPs: Magnetite nanoparticles

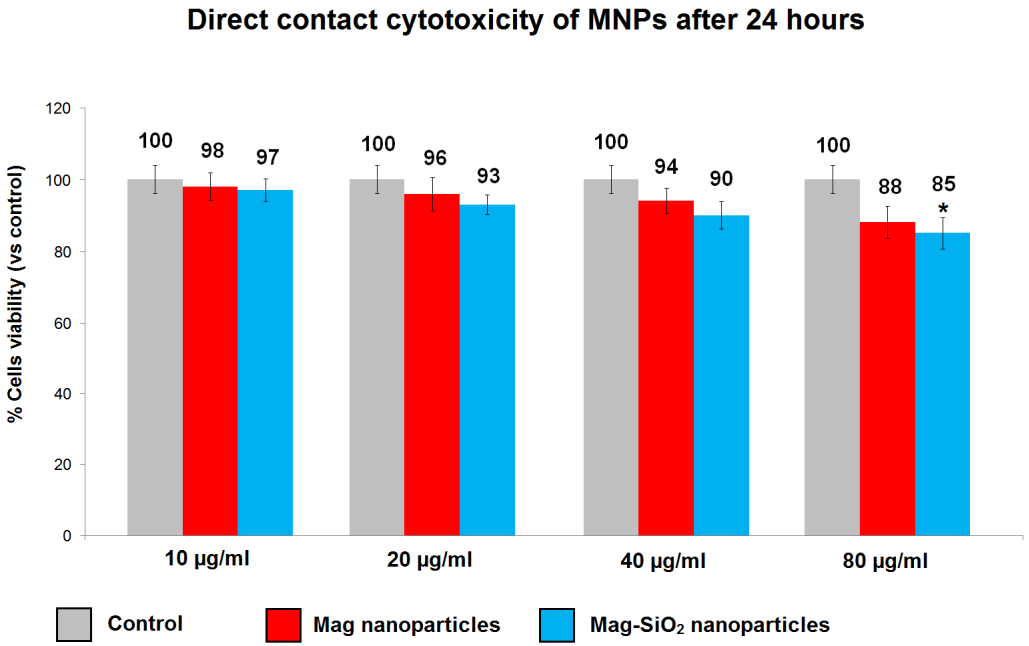
Mag-SiO_2 NPs: SiO_2 coated magnetite nanoparticles



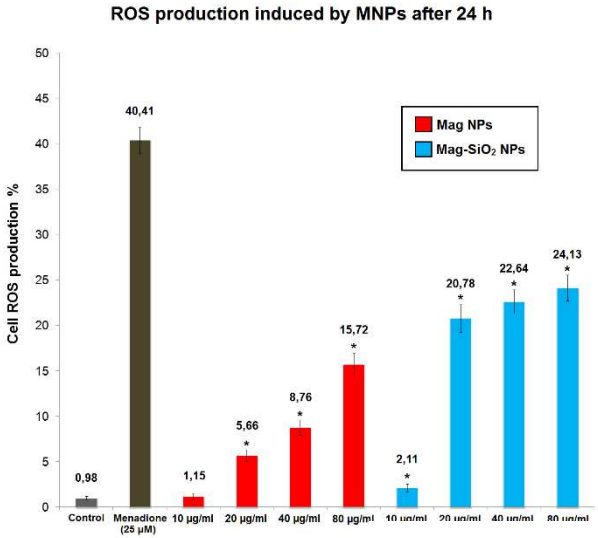


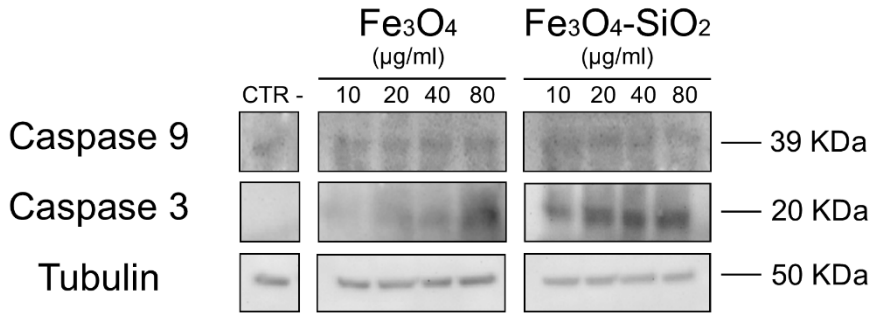
Characterizations / Chemical-physical
Biological: endothelial cells (MS1)

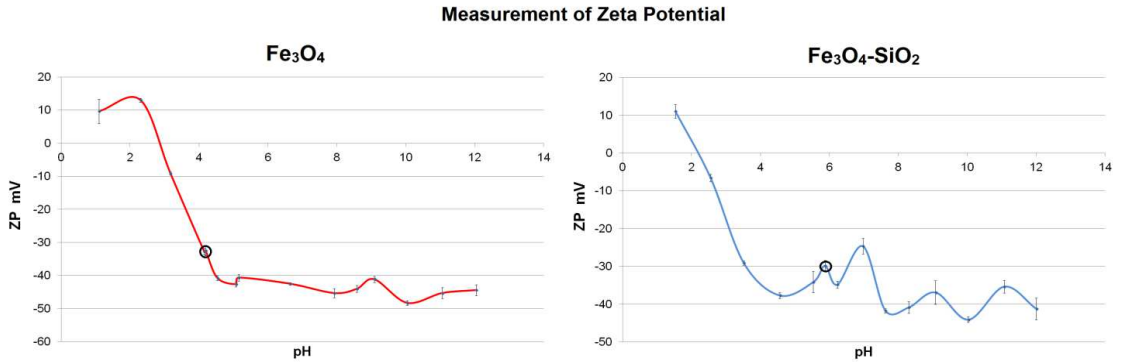




Characterizations / Chemical-physical
Biological: endothelial cells (MS1)



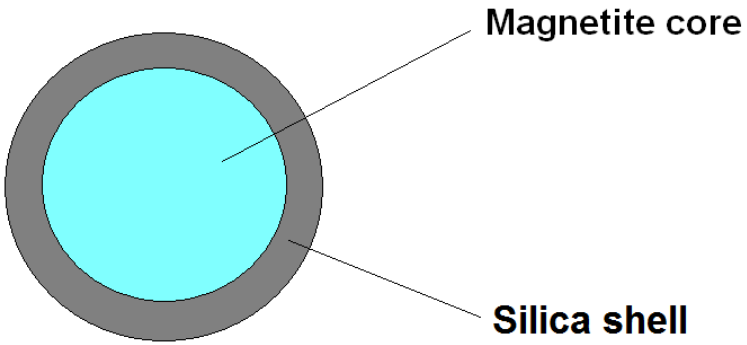


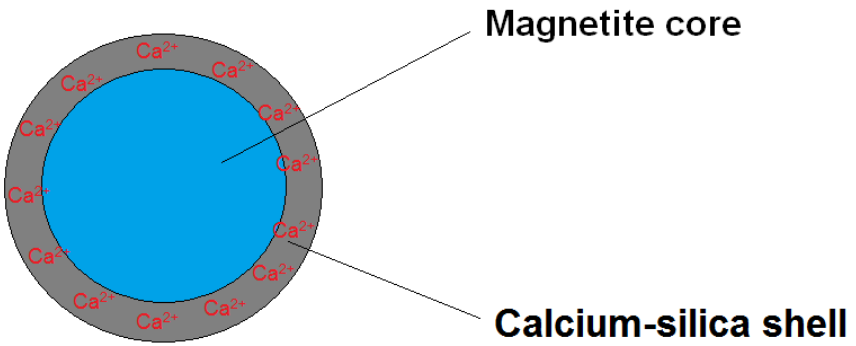


Sample	Starting pH	ZP (mV)	Stability behaviour of the colloid
Fe_3O_4 NPs	4.19	- 32.46 ± 1.61 mV	Moderate stability
Fe_3O_4-SiO_2 NPs	5.87	- 29.88 ± 1.42 mV	Incipient instability

Verné E, et al. Composite bone cements loaded with a bioactive and ferrimagnetic glass-ceramic: Leaching, bioactivity and cytocompatibility. Mater Sci Eng C Mater Biol Appl. 2015 ;53:95-103.

General characteristics





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

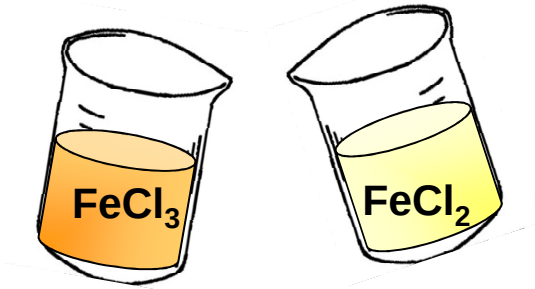
Fe_3O_4

MNPs were prepared by coprecipitation…

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

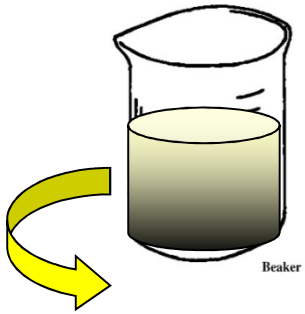
+

50ml 0.1M $FeCl_3 \cdot 6H_2O$



pH≅10

+ NH_4OH



Mechanical stirring

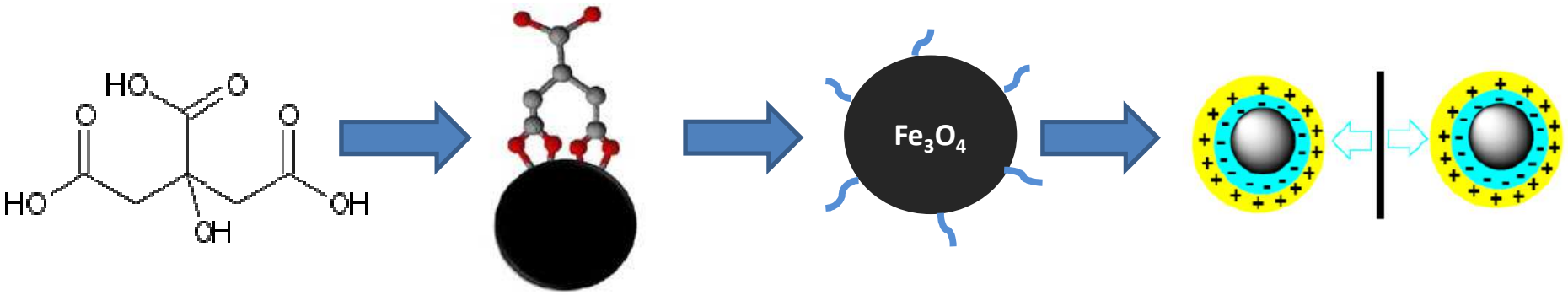
Magnetite

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

Maghemite

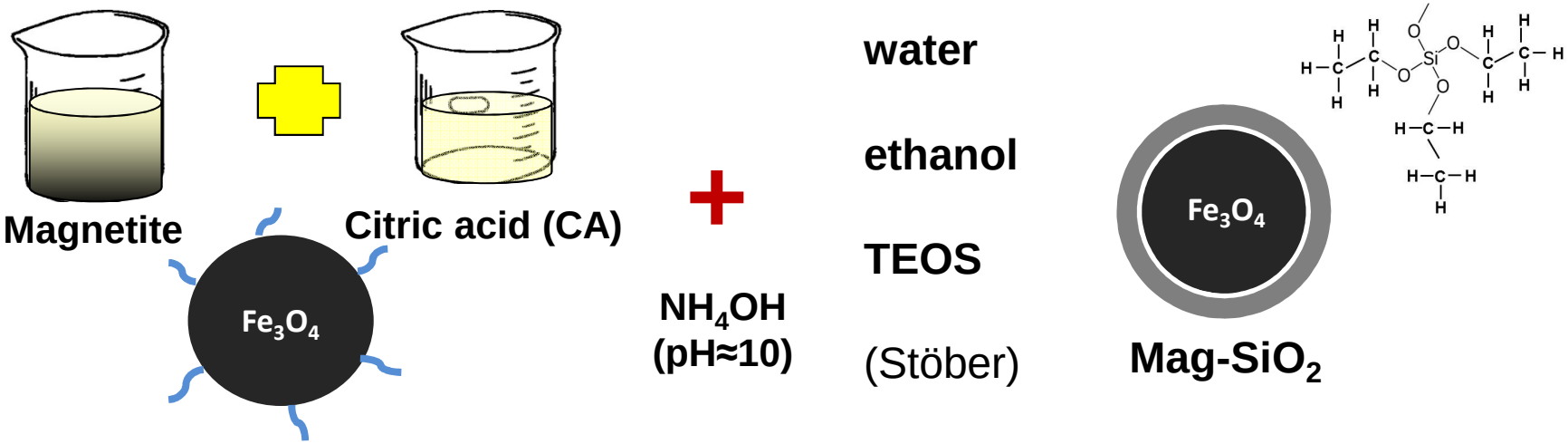
$2Fe_3O_4 + O_2 \rightarrow 3\gamma\text{-}Fe_2O_3$

…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.

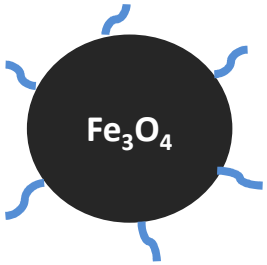
Silica shell coating (Mag-SiO_2 NPs): the silica shell was obtained by wet chemistry on the magnetic core stabilized with citric acid



*[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

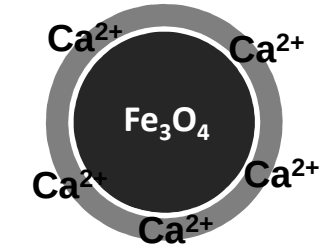
+

Ca citrate/
Ca hydroxide
water
ethanol
TEOS
(Stöber)

Si:Ca ratio 99:1

Mag-SiO_2-Ca(3)

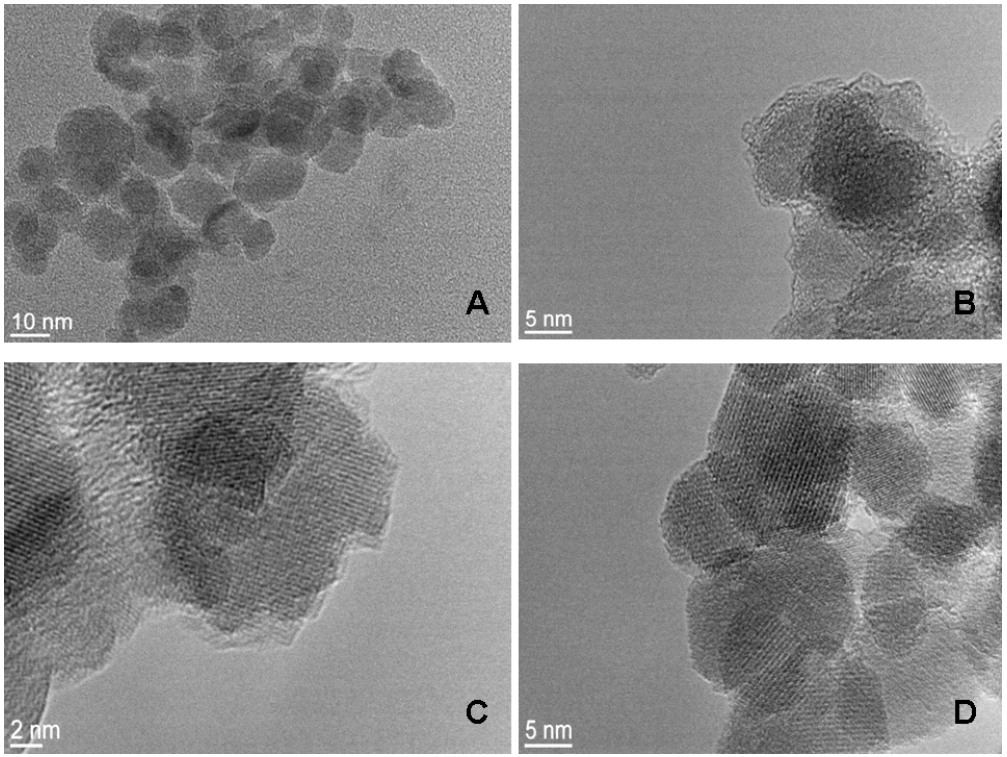
NH_4OH
(pH≈10)

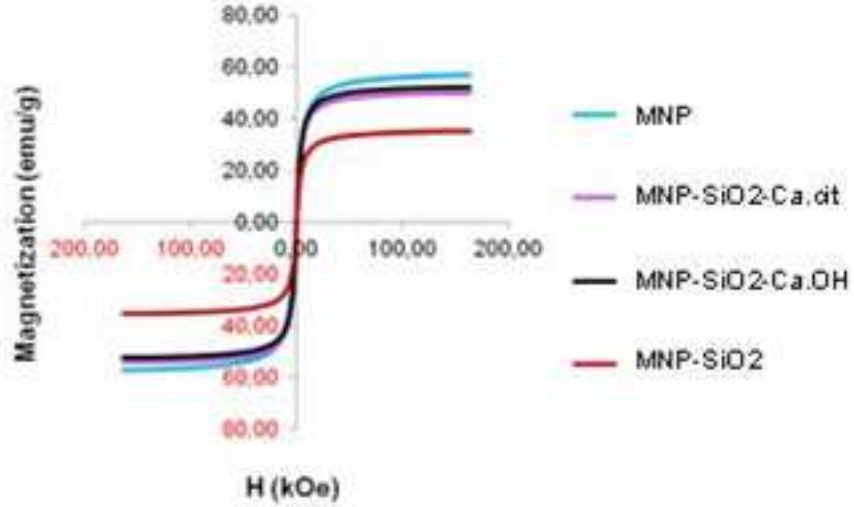


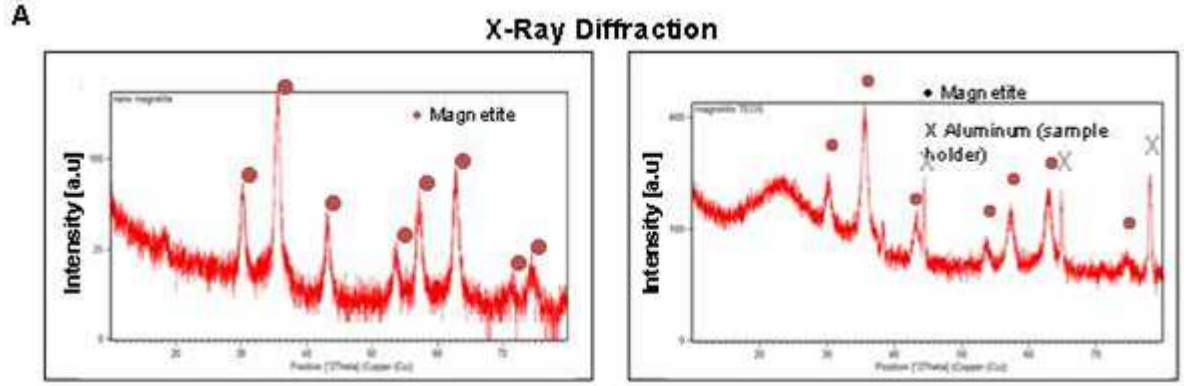
Mag-SiO_2-Ca(3)

*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

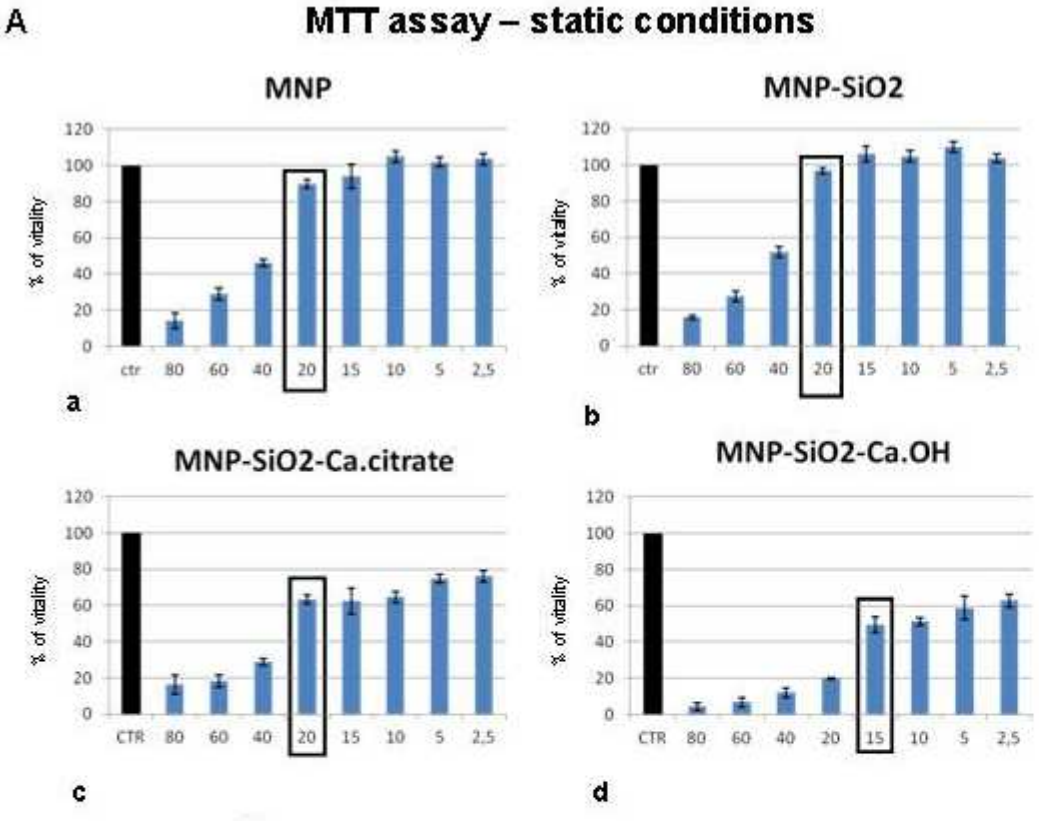
Characterizations / Chemical-physical
Biological: endothelial cells (MS1)



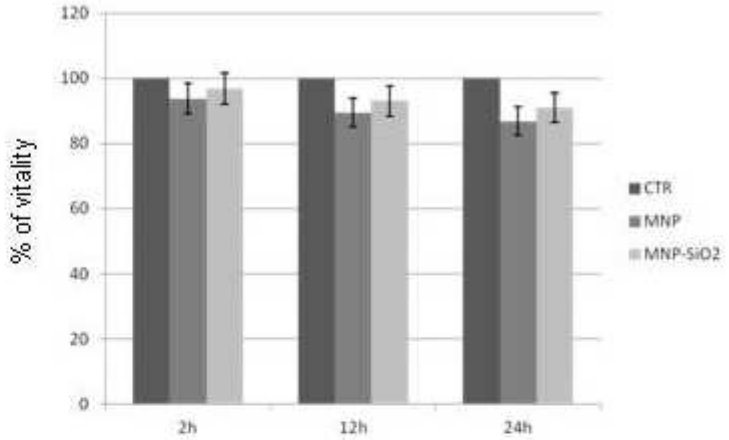




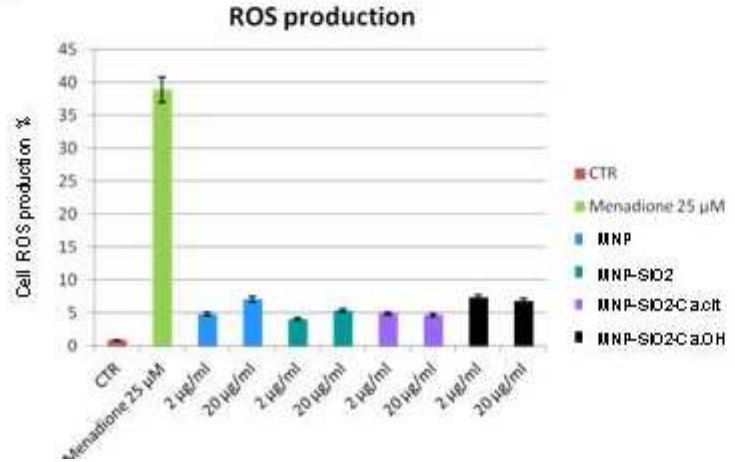
A


MTT assay – static conditions
MNP
MNP-SiO2
MNP-SiO2-Ca.citrate
MNP-SiO2-Ca.OH
% of vitality
ctr
CTR
80
60
40
20
15
10
5
2,5
a
b
c
d

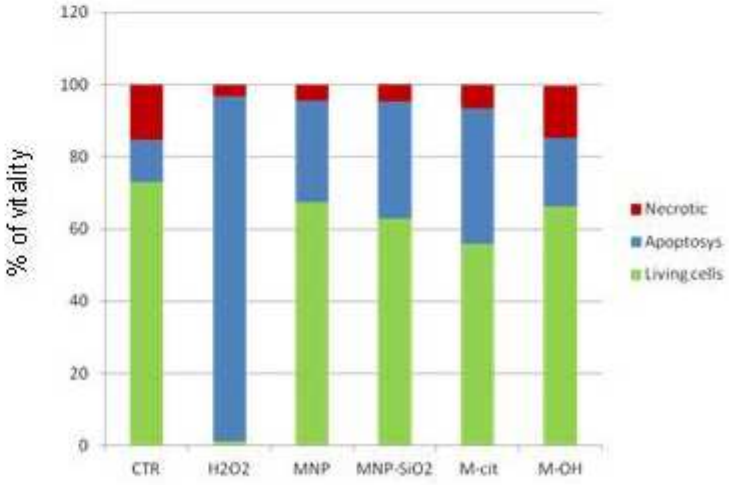
B


XTT assay – dynamic conditions
% of vitality
2h
12h
24h
CTR
MNP
MNP-SiO2

C

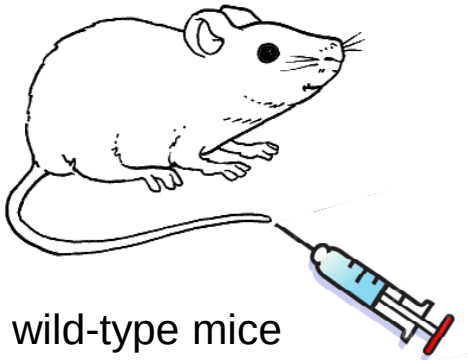

ROS production
Cell ROS production %
CTR
Menadione 25 µM
2 µg/ml
20 µg/ml
MNP
MNP-SiO2
MNP-SiO2Ca.cit
MNP-SiO2Ca.OH

D


Apoptosys assay
% of vitality
CTR
H2O2
MNP
MNP-SiO2
M-cit
M-OH
Necrotic
Apoptosys
Living cells

In vivo evaluation of MNPs

Biodistribution of MNPs - 7 days



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

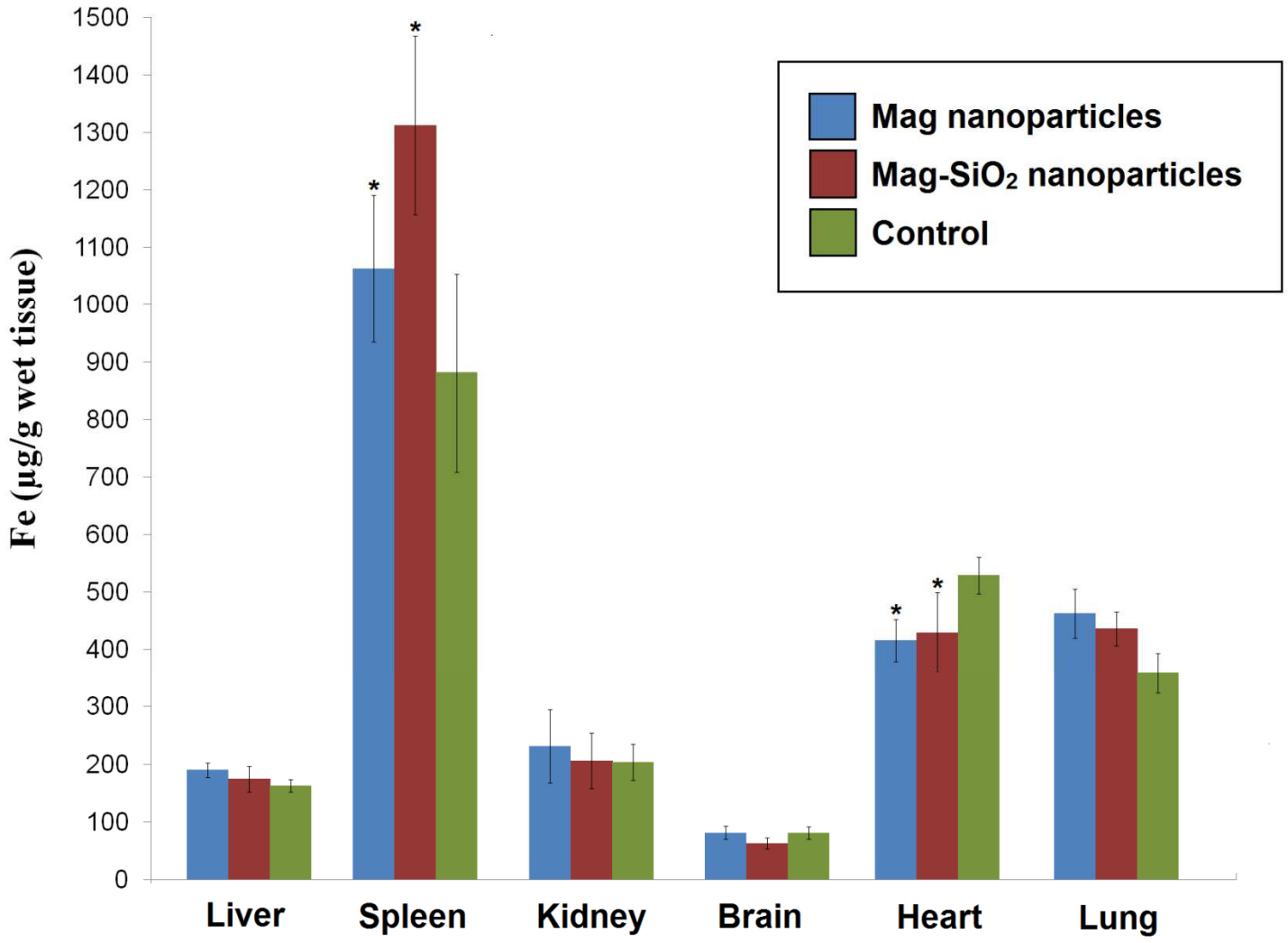
Explant of organs after 7 days

Macroscopic evaluation (organ weights)

Histological evaluation (Perls' Prussian blue staining)

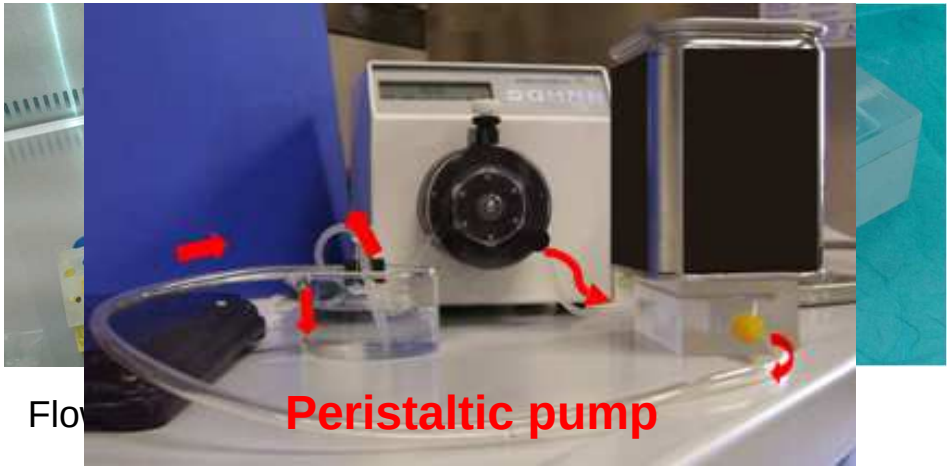
Compositional evaluation for iron accumulation (Inductively coupled plasma-atomic emission spectrometry (ICP-AES))

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

Cytocompatibility of MNPs in dynamic conditions



Experimental setting

- Continuous flow bioreactor with a peristaltic pump simulating the blood stream in a capillary
- 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)

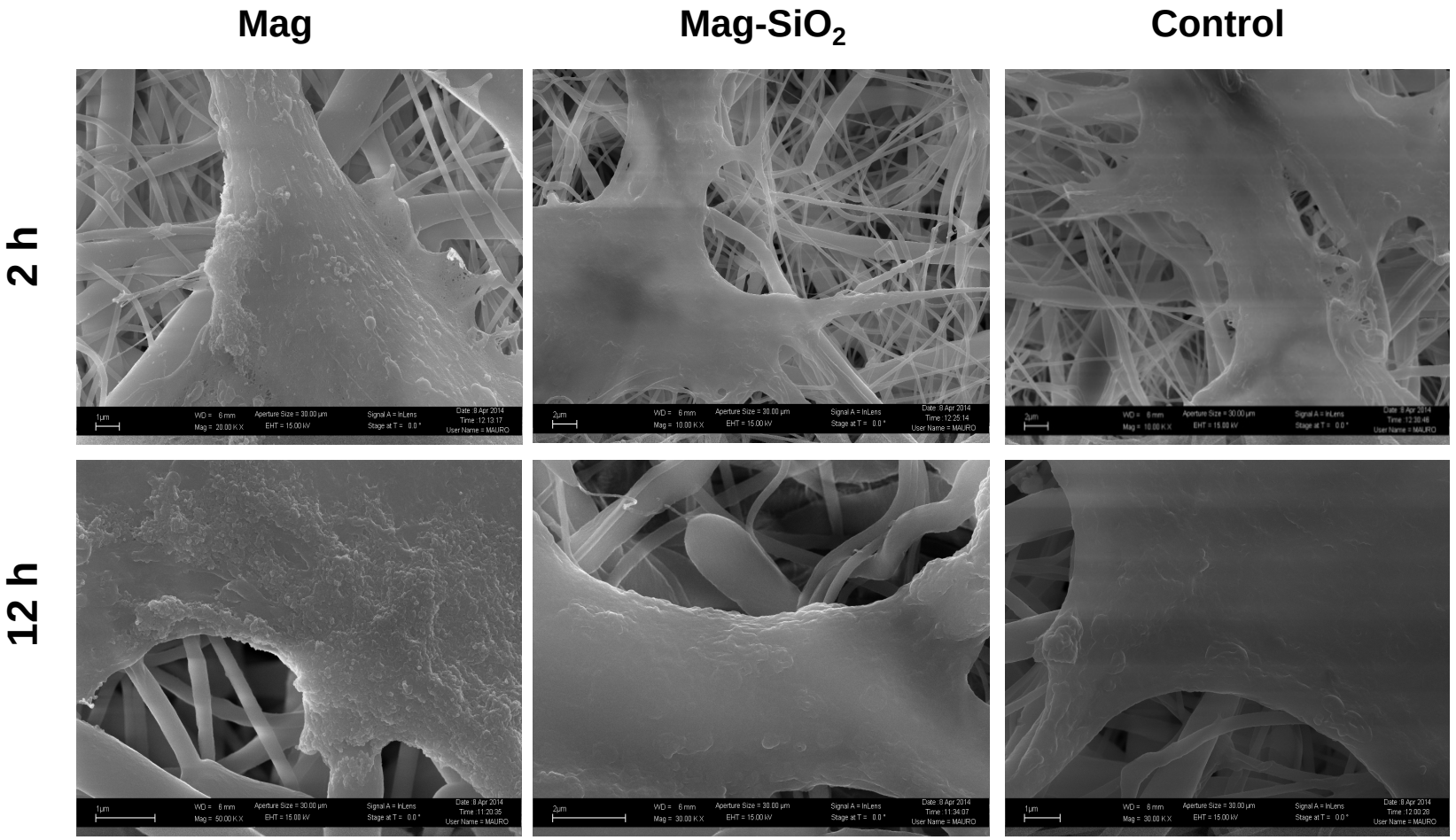
When MS1 cells were confluent → strips were inserted in the bioreactor.

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.**

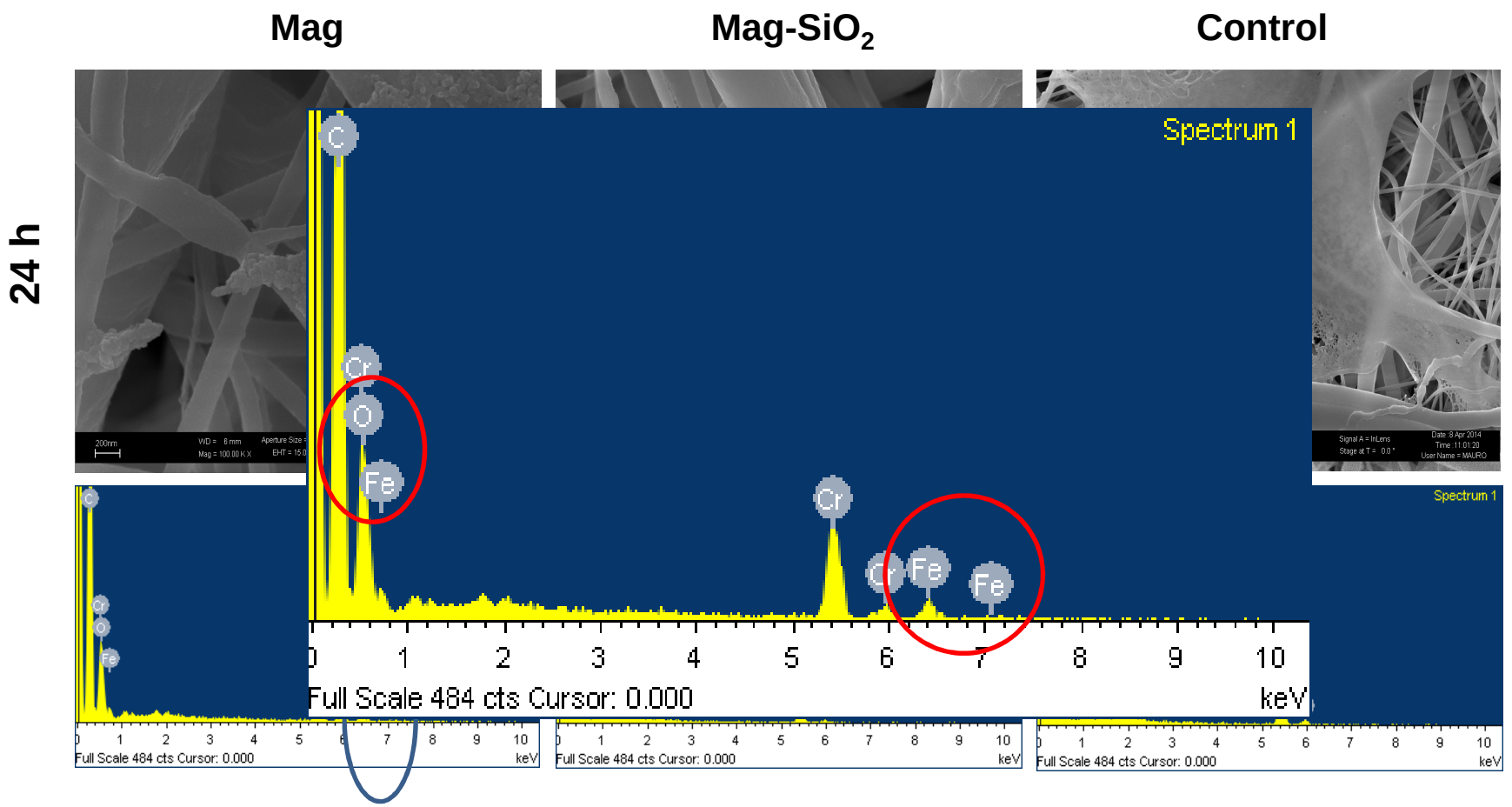
Ucciferri N. Nanotoxicology. 2014 Sep; 8(6):697-708.

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



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

Cytocompatibility in dynamic conditions



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

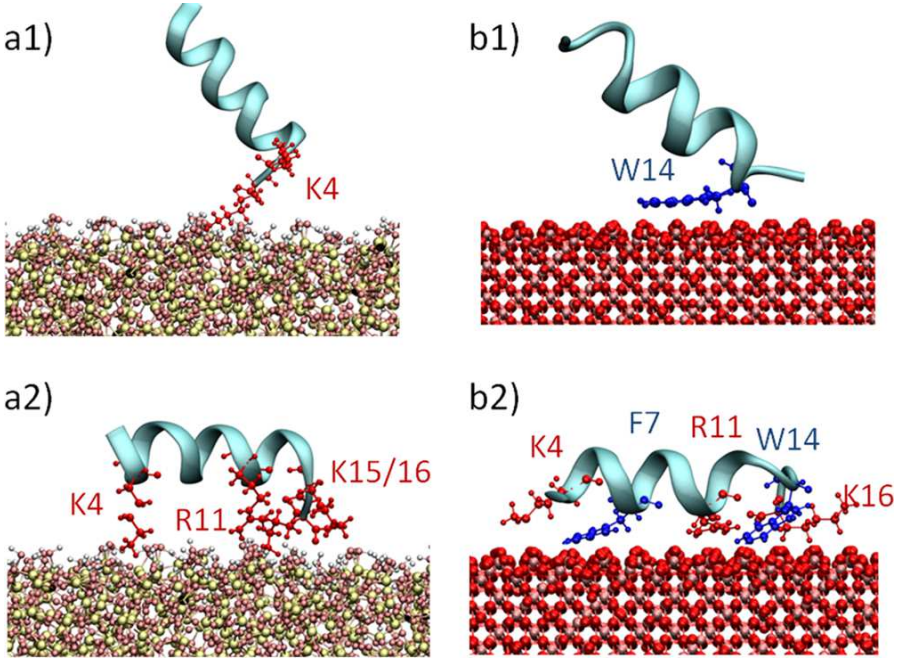
Conclusion I

- Size and shape control
- Colloidal stability and dispersibility in solution
- Biocompatibility

SPIONs / Cell membrane interaction

Cell/SPIONs interaction via Cell penetrating peptide absorption

- Cell penetrating peptides
- TAT peptide
- Drosophila Antennapedia homodomain –derived penetrating peptide (p Antp)
- 16 AA residues
- Positively charged at neutral pH
- No interaction with negatively charged molecules

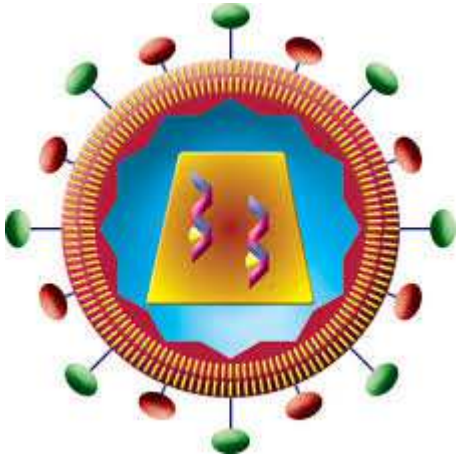


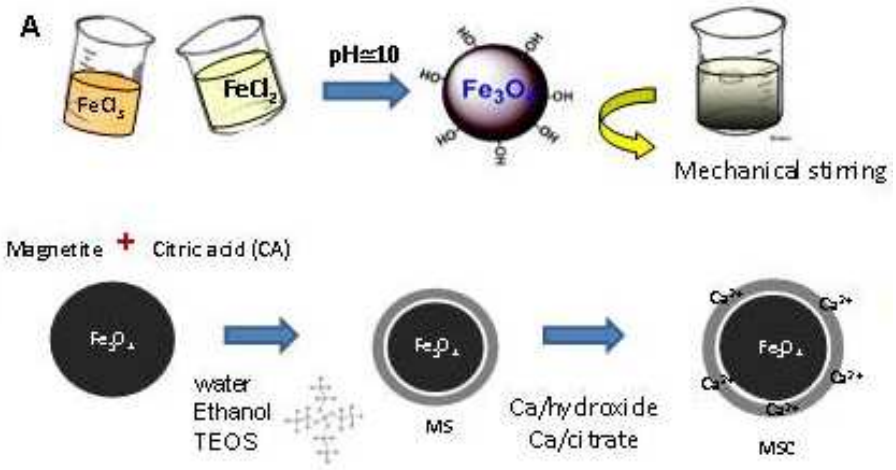
- Electrostatic interations
- Alanine and lysine competitive subtraction

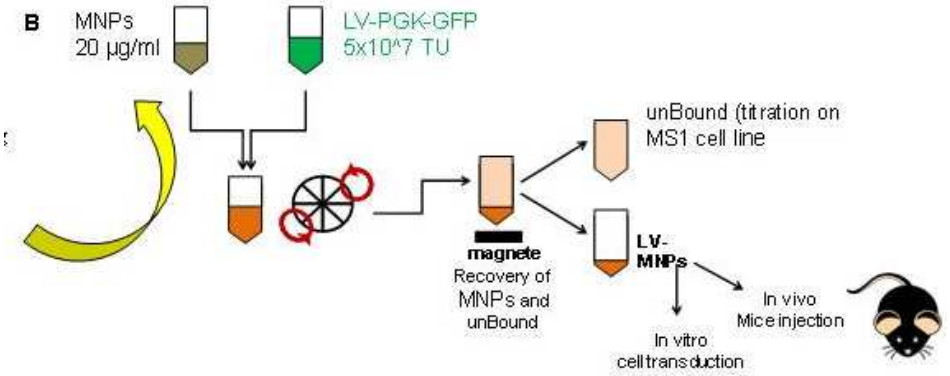
Grasso G, Deriu M, Prat M, Rimondini L, Vernè E, Follenzi a, Danani A. Cell penetrating peptide adsorption on magnetite and silica surfaces: a computaional investigation. J Phys Chem B 2015, 119, 8239-8246

Cell/SPIONs interaction via Lentivirus coupling

- Retrovirus (RNA)
- Peri-capside
- Used in cell trasduction as a vector







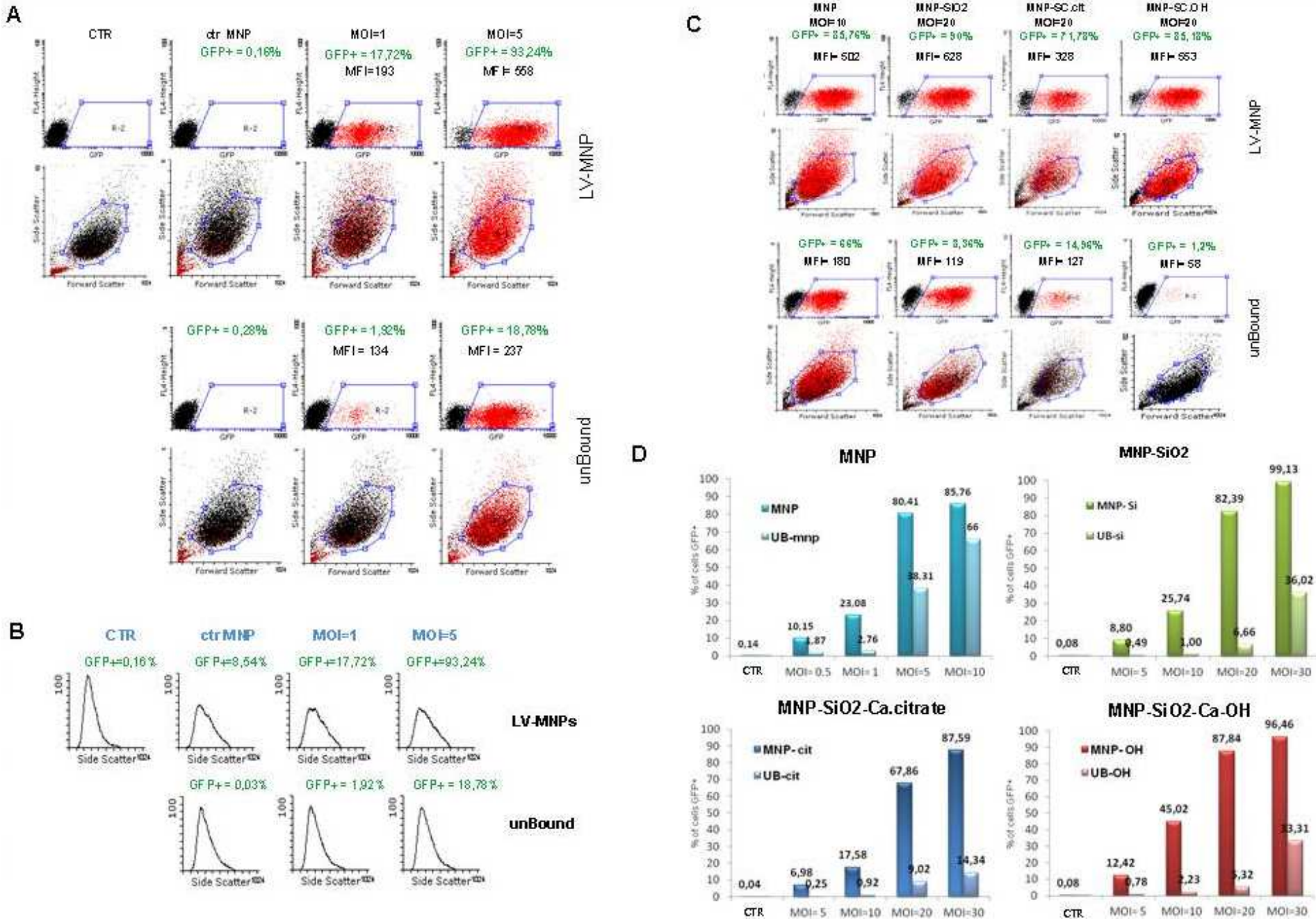
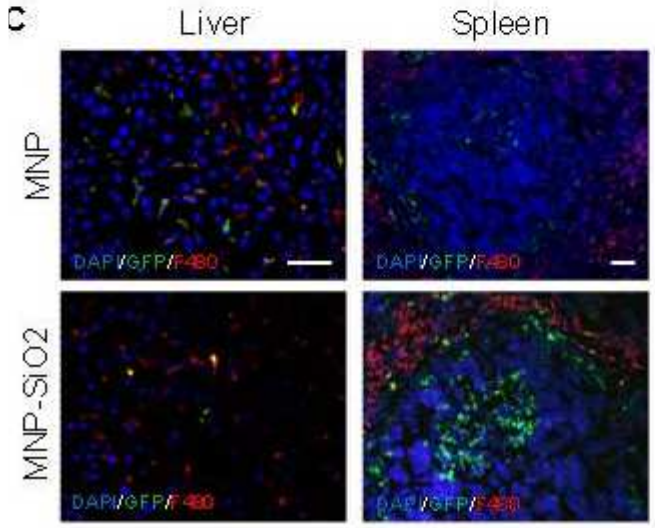
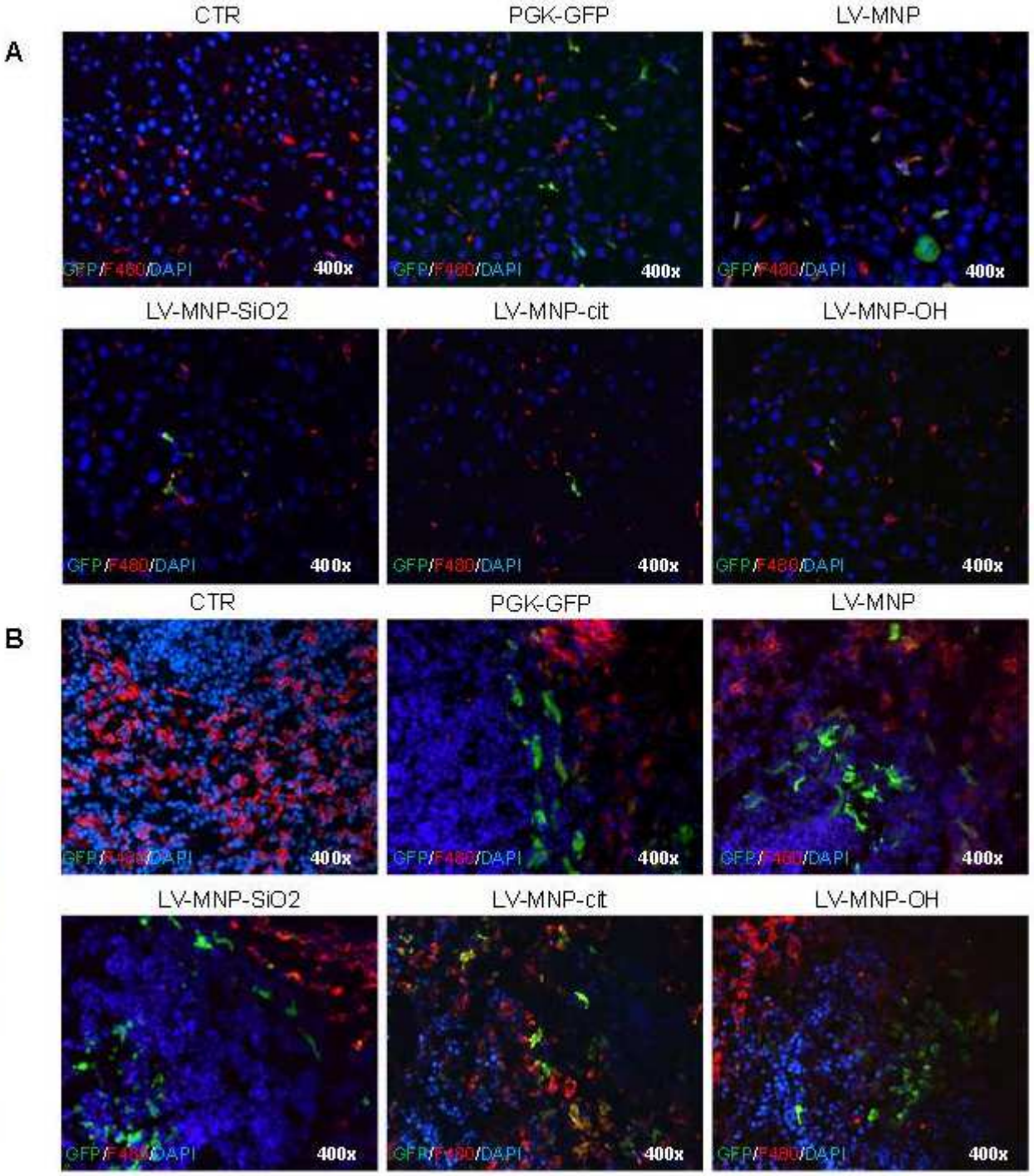

A
CTR
ctr MNP
GFP+ = 0,16%
MOI=1
GFP+ = 17,72%
MFI=193
MOI=5
GFP+ = 93,24%
MFI = 558
LV-MNP
GFP+ = 0,28%
GFP+ = 1,92%
MFI = 134
GFP+ = 18,78%
MFI = 237
unBound
B
CTR
ctrMNP
MOI=1
MOI=5
GFP+=0,16%
GFP+=8,54%
GFP+=17,72%
GFP+=93,24%
LV-MNPs
GFP+=0,03%
GFP+=1,92%
GFP+=18,78%
unBound
Side Scatter
C
MNP
MOI=10
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+ = 14,94%
MFI= 127
GFP+ = 1,2%
MFI= 58
unBound
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 6. 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.





Conclusions

- The MNPs used in this study demonstrated to be cytocompatible in both static and dynamic conditions.
- SiO_2 and Ca ions increase colloidal stability of SPIONs but reduce interaction with membranes
- pAntp is adsorbed onto SPIONs but probably it is unable to improve cell interactions when SiO_2 shelled
- Lentivirus vector coupled with SPIONs increases gene expression in liver and spleeen

UNIVERSITÀ DEL PIEMONTE ORIENTALE

Proff. Antonia Follenzi and Maria Prat
Drs. Ester Borroni, Andrea Cochis, Francesca Oltolina

Prof. Enrica Vernè
Drs. Sara Ferraris and Marta Miola

"Jožef Stefan" Institute
Ljubljana, Slovenia

Dr. Sasa Novak

SUPSI
University of Applied Sciences
of Southern Switzerland

Prof. Andrea Danani
Drs. Gianvito Grasso and Marco Deriu

Thanks To:

AIRC

Compagnia di San Paolo

cost

"Jožef Stefan" Institute
Ljubljana, Slovenia

SUPSI
University of Applied Sciences
of Southern Switzerland

and all of You for the attention!!