Nanotoxicologie et cytométrie en flux

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Château de Bouthéon
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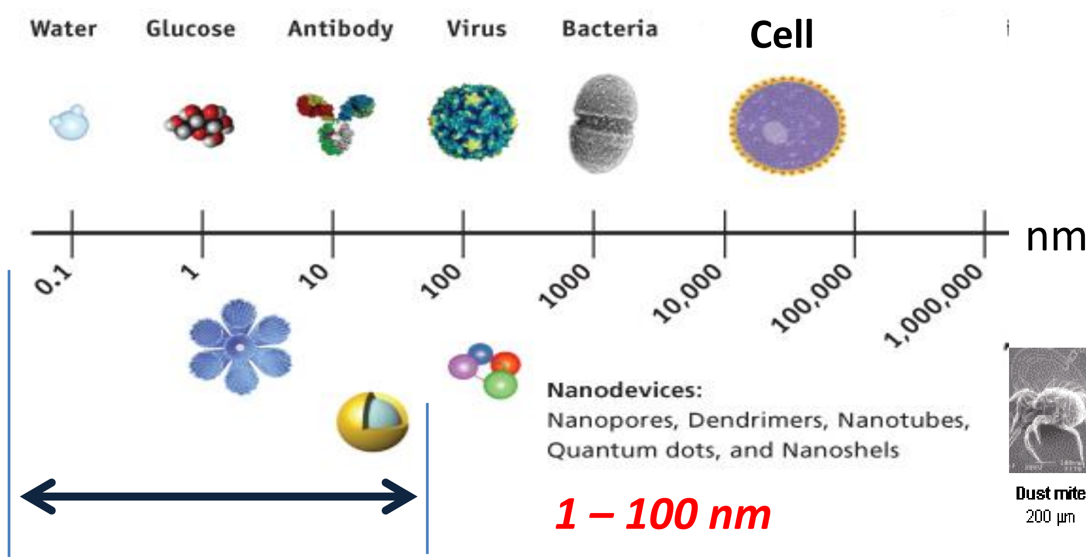
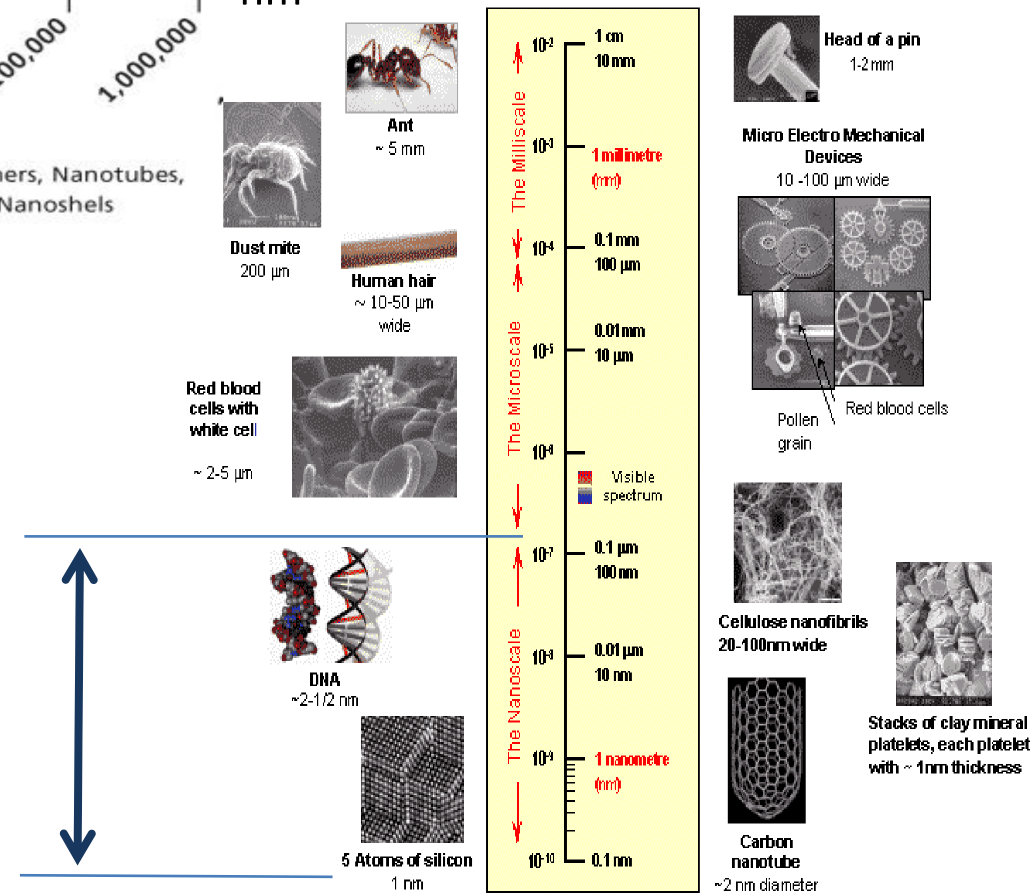
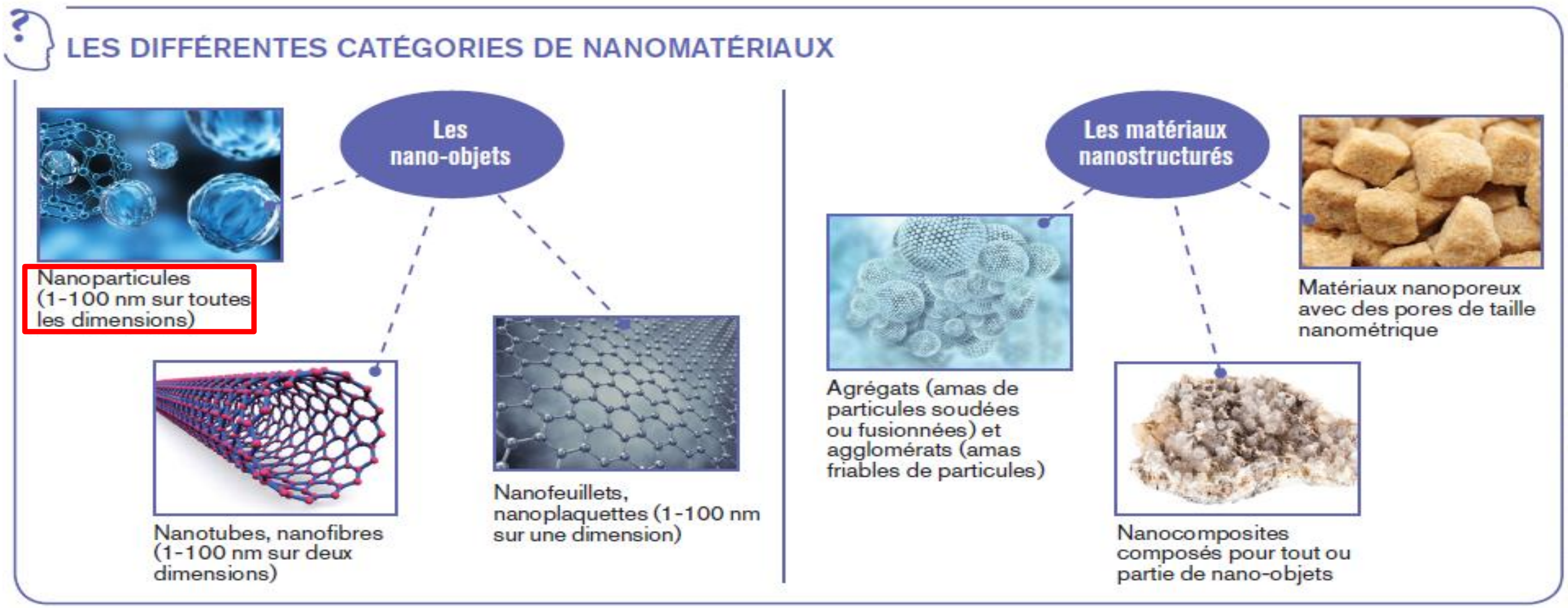
Living scale



Nanometric scale
0.1 - 100 nm



Different categories of nanomaterials



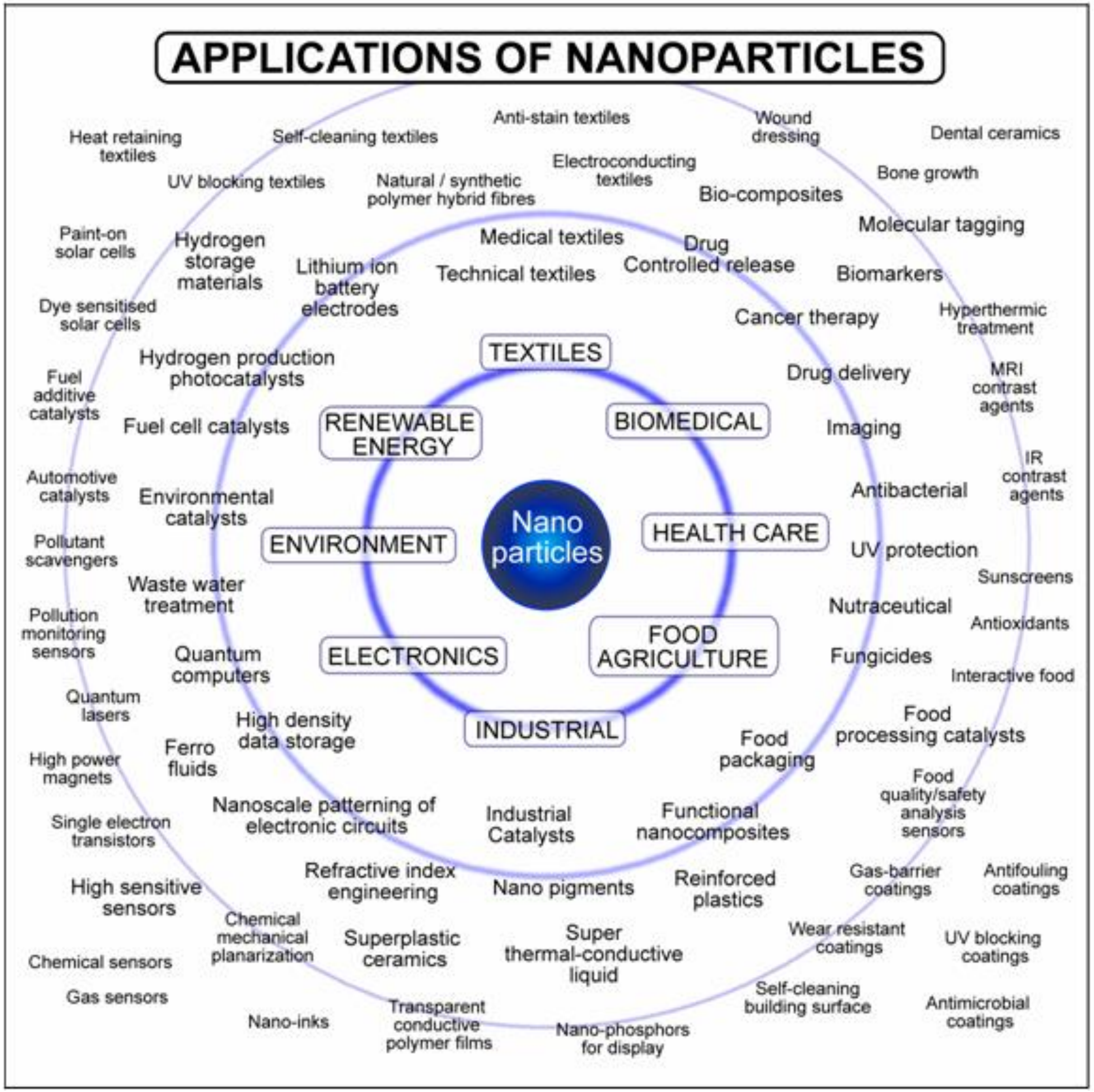
Quels sont les nanomatériaux :

Selon la CEE, il s'agit ' d'un matériaux soit naturel, soit formé accidentellement, soit manufacturé et constitué de particules libres ou sous forme d'agrégat ou d'agglomérat, dont au moins 50% des particules présentent une ou plusieurs dimensions externes se situant entre 1 nm et 100 nm.'

http://extranet.inserm.fr/objectif-sante-securite; 2015; Les nanomatériaux à l'Inserm

❑Nanoparticles/Nanotubes

- **Natural**
- **Synthetic**
- **Nanoparticles interactions**



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Nanoparticles characteristics

❑ General features

- Nanoparticles

❖ "Nanosized particles" (NSP)

❖ "Engineered nanoparticles" (NP)

❖ "**Ultrafine particles**" (UFP)

- **Size**: at least one dimension between **1-100 nm**

- **High surface reactivity**

- Exposure: accidental or intentional

-**Routes of exposure**: inhalation, ingestion, skin uptake, and injection

The toxicity concern arises due to the fact that the particle are of size that of biomolecules and can interact with them in unanticipated ways

Nanoparticles characterization

- Transmission Electron Microscopy (TEM)
- Scanning electron microscopy (SEM)
- X Ray Diffraction (XRD)
- X ray photoelectron spectroscopy (XPS)
- Dynamic Light Scattering (DLS)
- Zeta potential
- Thermogravimetric analysis (TGA)
- Raman spectroscopy
- FTIR (Fourier transform infrared spectroscopy)

Nanotoxicology

❑ Nanotoxicology definition

Nanotoxicology is the branch of science that deals with the study and application of toxicity of nanomaterials

❑ Ratio Benefit / Risk of nanomaterials : unknown

Due to the wide range of application of nanoparticles, to their presence in our environment, and of their different accumulation processes, there is a need to develop appropriated methodologies allowing to evaluate their toxicity

Among these methodologies, **flow cytometry** constitutes a powerfull method

Which toxic effects?

- ❑ Inflammation
- ❑ Oxidative stress
- ❑ Genotoxicity
- ❑ Cell death

✓ The toxicity can depend on: chemical composition, size, size distribution, shape, agglomeration, surface properties, solubility, crystallinity of nanoparticle, and of nanoparticles interactions

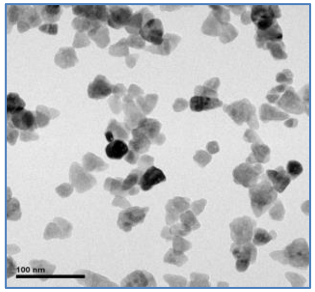
✓ Thus, **each nanoparticle constitutes a particular case**

✓ Consequently, **there is a real need to develop reliable, efficient, and rapid methods allowing to evaluate the toxicity of nanoparticles**

Flow cytometry offers these possibilities

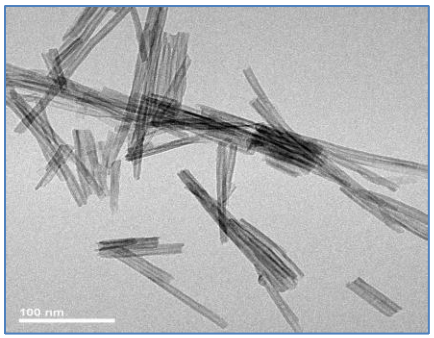
Nanoparticles studied

- **TEM (ZnO NPs)**



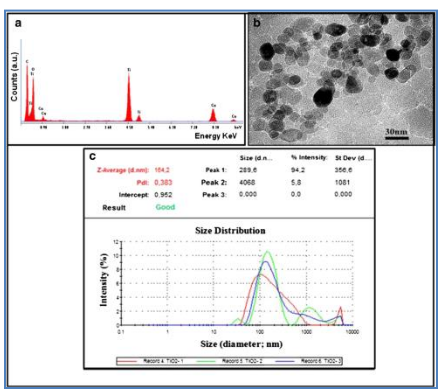
ZnO NPs
Precursors: $ZnSO_4$ and NaOH
Conditions: 90°C, atmospheric pressure
Size:
TEM 30-40nm
DLS (111.0±0.7) nm (H_2O)
(633±6) nm (medium)

- **TEM (TiONts-APTES)**

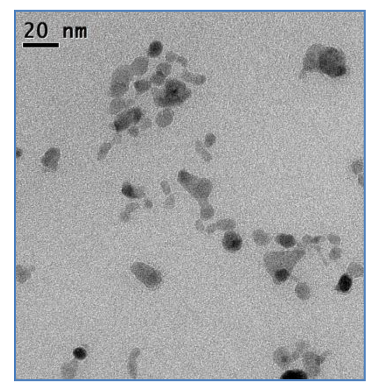


TiONts
Precursors: TiO_2 and NaOH 10M
Conditions: 150°C, hydrothermal reactor, 36h
Functionalization: APTES
Size:
TEM 10 nm (diameter)
about 150 nm in length

- **TEM (TiO2-NPs)**



- **TEM : Ultrasmall Super Paramagnetic Iron Oxide (USPIO)**



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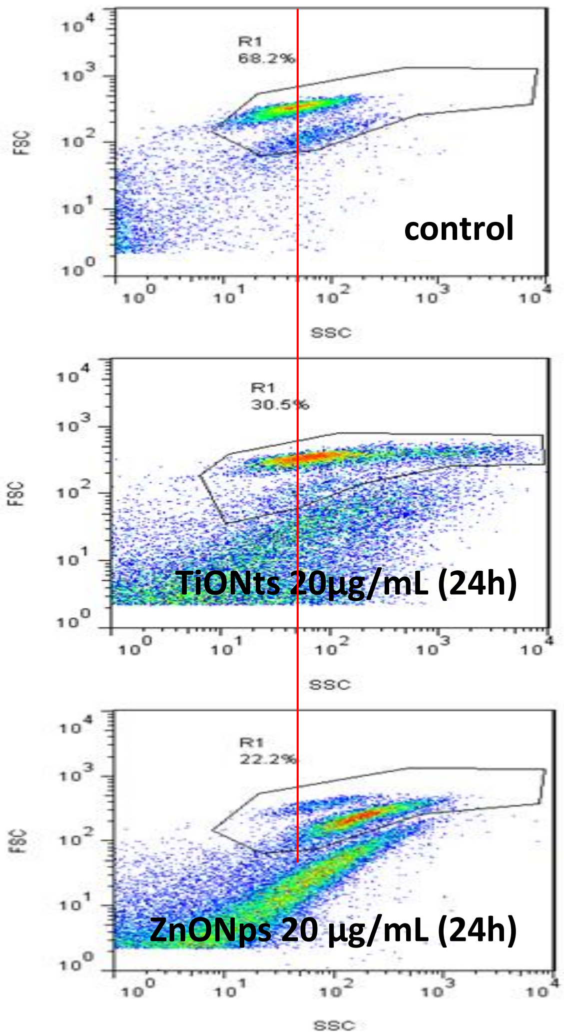
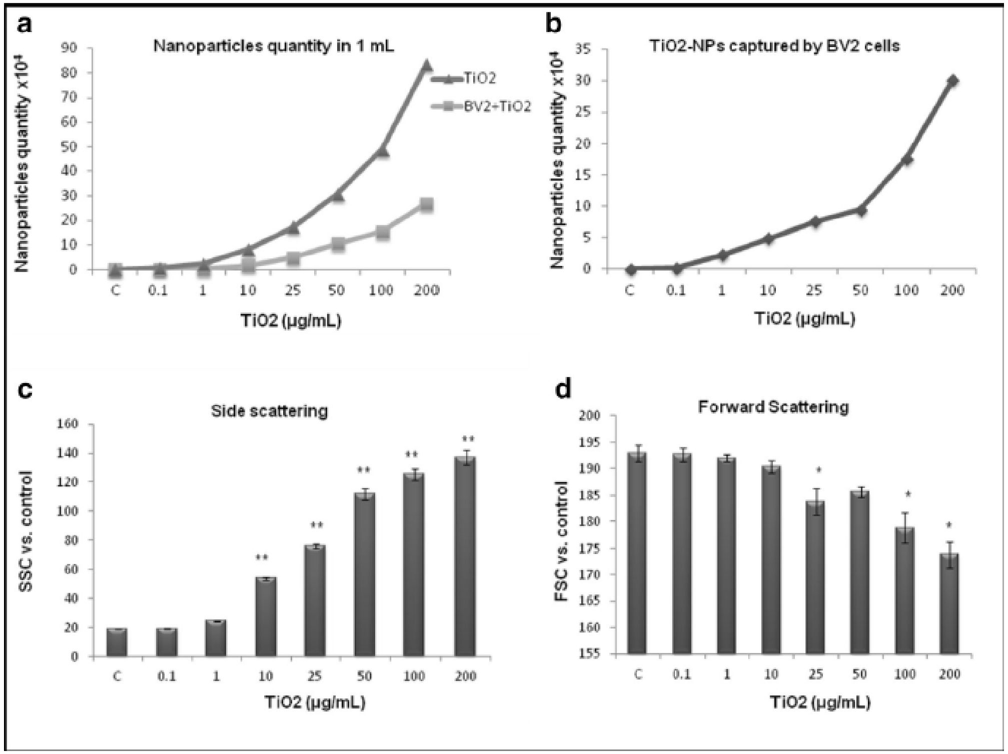
Absorption and / or internalisation of nanoparticles

Murine microglial BV-2 cells



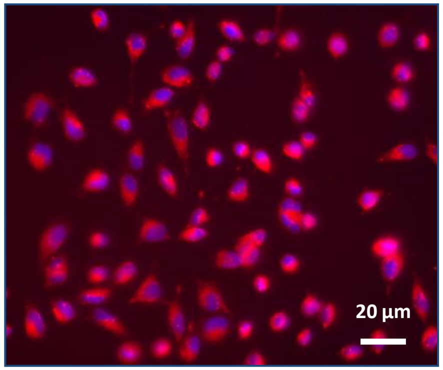


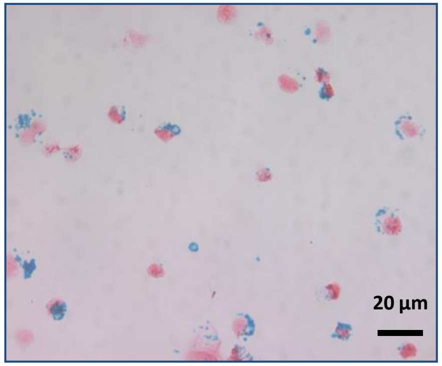
Rihane N, Nury T, M'rad I, El Mir L, Sakly M, Amara S, Lizard G. Microglial cells (BV-2) internalize titanium dioxide (TiO2) nanoparticles: toxicity and cellular responses. Environ Sci Pollut Res Int. 2016; 23(10): 9690-9.

Evidence of nanoparticles internalization

❑ **Fluorescence microscopy : conventional and confocal microscopy**

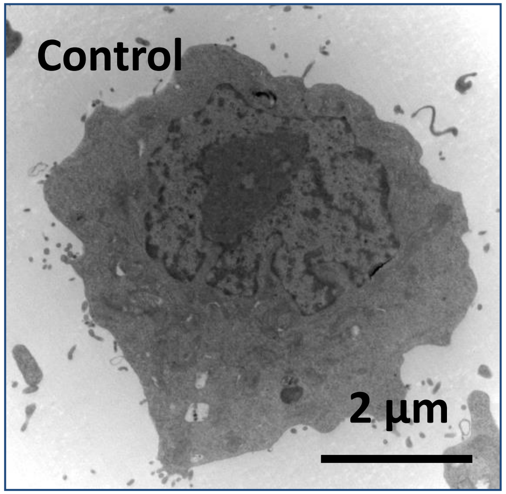
USPIO (HB95)
with Rhodamine B
Oligodendrocytes
(158N cells)

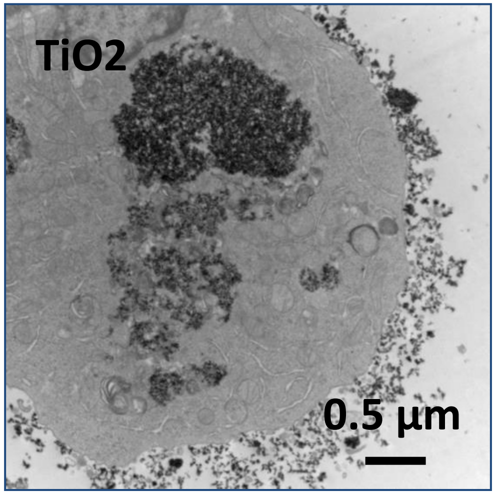


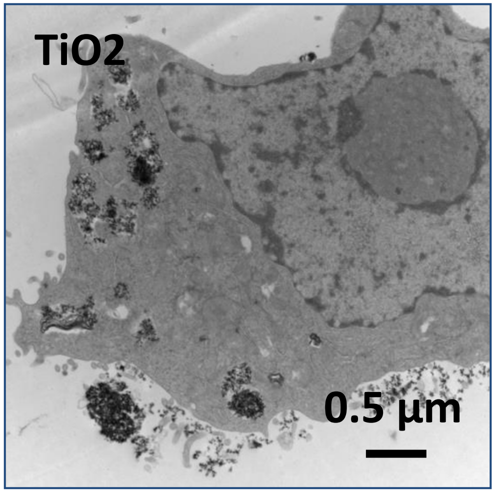


USPIO (HB95)
Perls staining
(158N cells)

❑ **Transmission electron microscopy (cellular localization, interaction and impact on organelles, nucleus, …)**







TiO2, BV-2 cells

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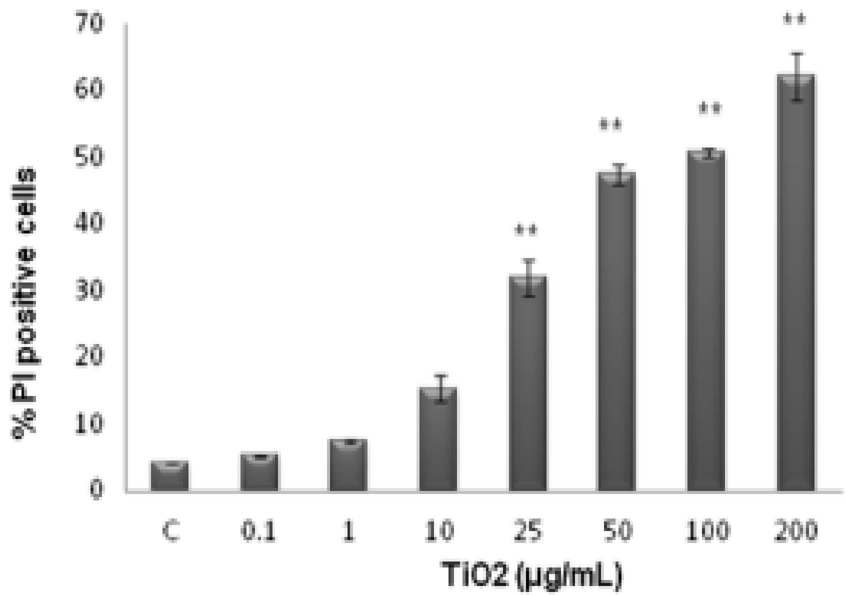
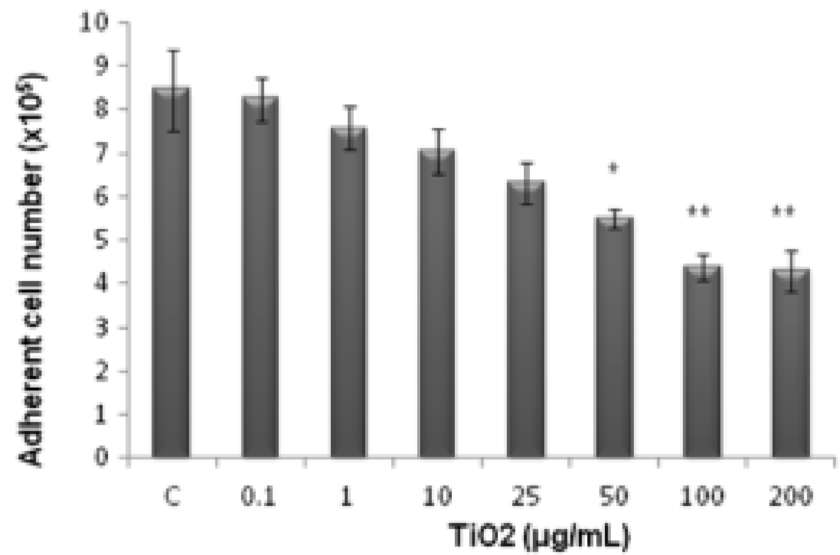
Impact on cell viablity and / or cytoplasmic membrane damage

Staining with propidium iodide





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Impact on organelles: mitochondria, lysosomes, peroxisomes

❑ **Mitochondria**

✓ Transmembrane mitochondrial potential (staining with DiOC6(3))
✓ Mitochondrial mass (nonyl acridine orange and mitotracker)
✓ Apo2.7 (mitochondrial membrane protein; apoptotic mitochondria?)

❑ **Lysosome**

✓ Lysosomal integrity (acridine orange)
✓ Lysosomal mass (lysotracker)
✓ Lysosomal pH (LysoSensor Yellow/Blue DND-160)
✓ Antibodies anti-lysosome (LAMP)

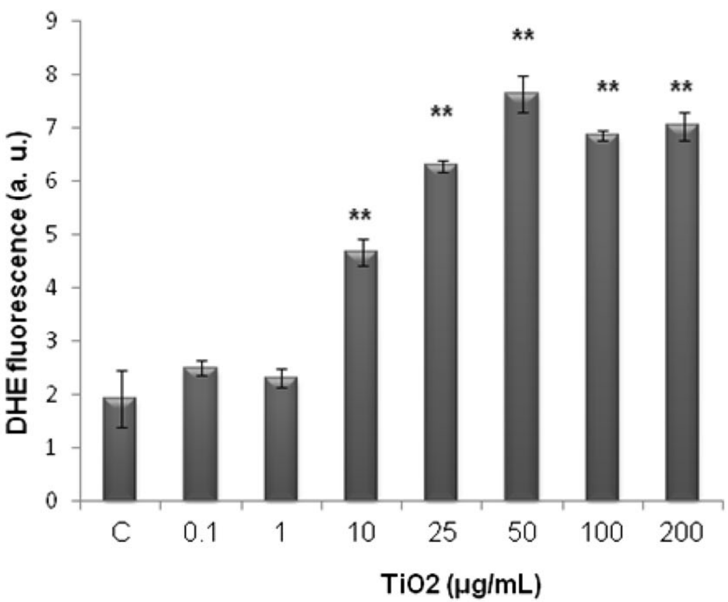
❑ **Peroxisome**

✓ Peroxisomal mass (ABCD3, Pex14)

Impact on oxidative stress and inflammation

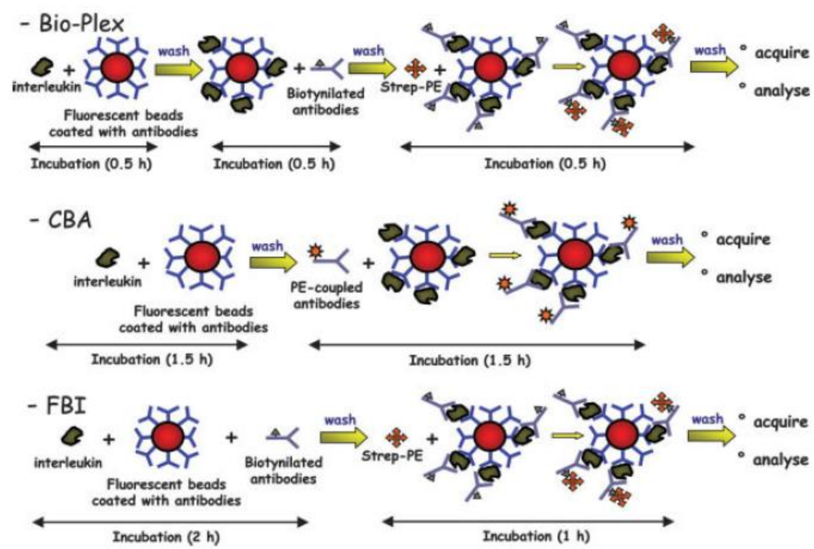
❑ ***Oxidative stress***

- **ROS:** (DHE, H_2DCFDA)
- **RNS:** (DAF2)
- **lipid peroxidation**: cis-parinaric acid; antibodies (4-HNE)



❑ ***Inflammation***

Multiplexed flow cytometric analyses



Prunet C *et al*. Cytometry A. 2006 May;69(5):359-73.

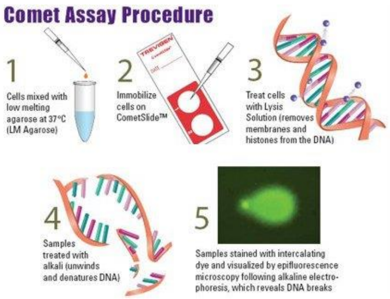
Nanoparticles and genotoxicity

❑ **DNA damage**

✓ SubG1 peak (apoptotic cells; internucleosomal DNA degradation) staining with propidium iodide

✓ TUNEL method (apoptotic cells, internucleosomal DNA degradation)

✓ COMET test



✓ Oxidative DNA damage (8-oxo-guanine)

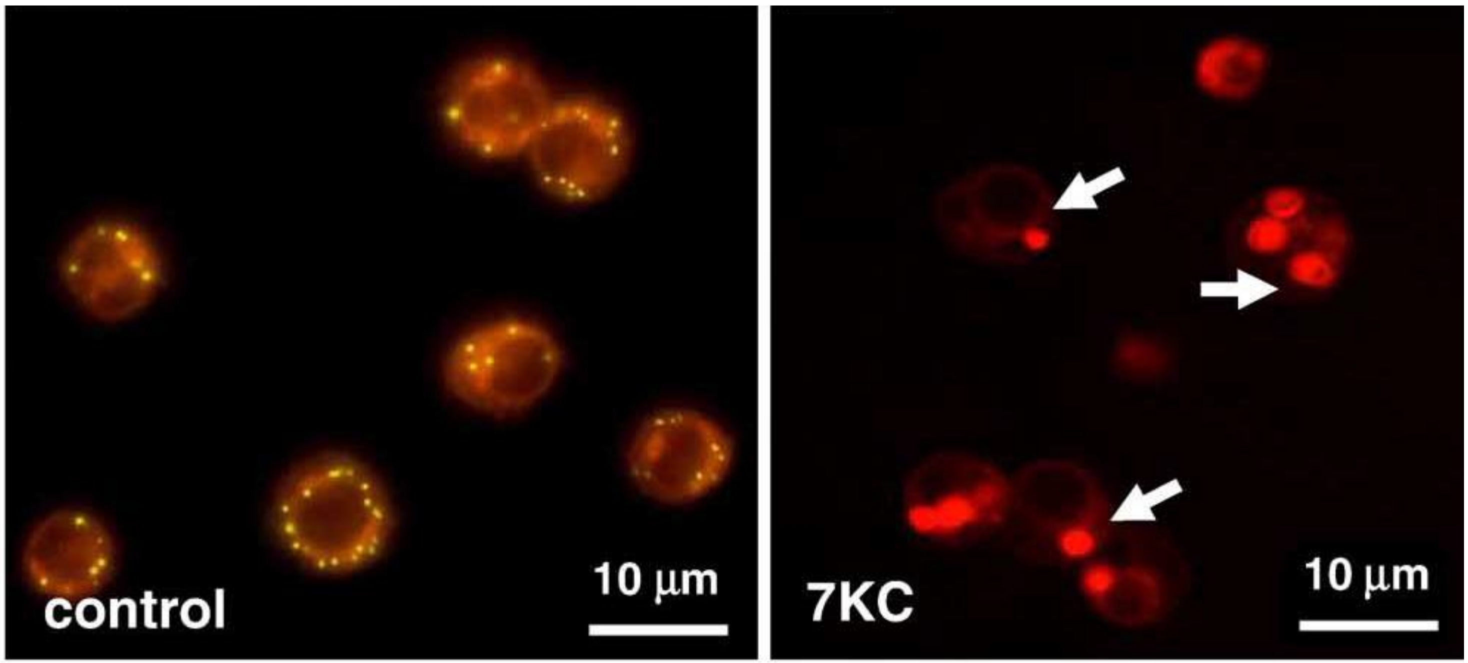
❑ ***Impact on cell proliferation***

✓ Cell cycle analysis (propidium iodide; BUdR)

✓ Antibodies Ki67, PCNA.

Impact on lipid metabolism

To evaluate the impact of Nts and Nps on lipid profil (lipid metabolism), staining with Nile Red could be used



Excitation 488 nm : neutral lipids (yellow); polar lipids (orange / red)

Potentialisation of cytotoxic effects in aged related diseases

International Journal of Nanomedicine

Dovepress
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Open Access Full Text Article

ORIGINAL RESEARCH

Iron nanoparticles increase 7-ketocholesterol-induced cell death, inflammation, and oxidation on murine cardiac HL1-NB cells

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Sophie Pelloux[3]
Jean-Marc Riedinger[4]
Frédérique Frouin[1]
Yves Tourneur[3]
Gérard Lizard[2]

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
12 March 2010

Kahn E *et al.* Int J Nanomedicine. 2010; 5: 185-95.

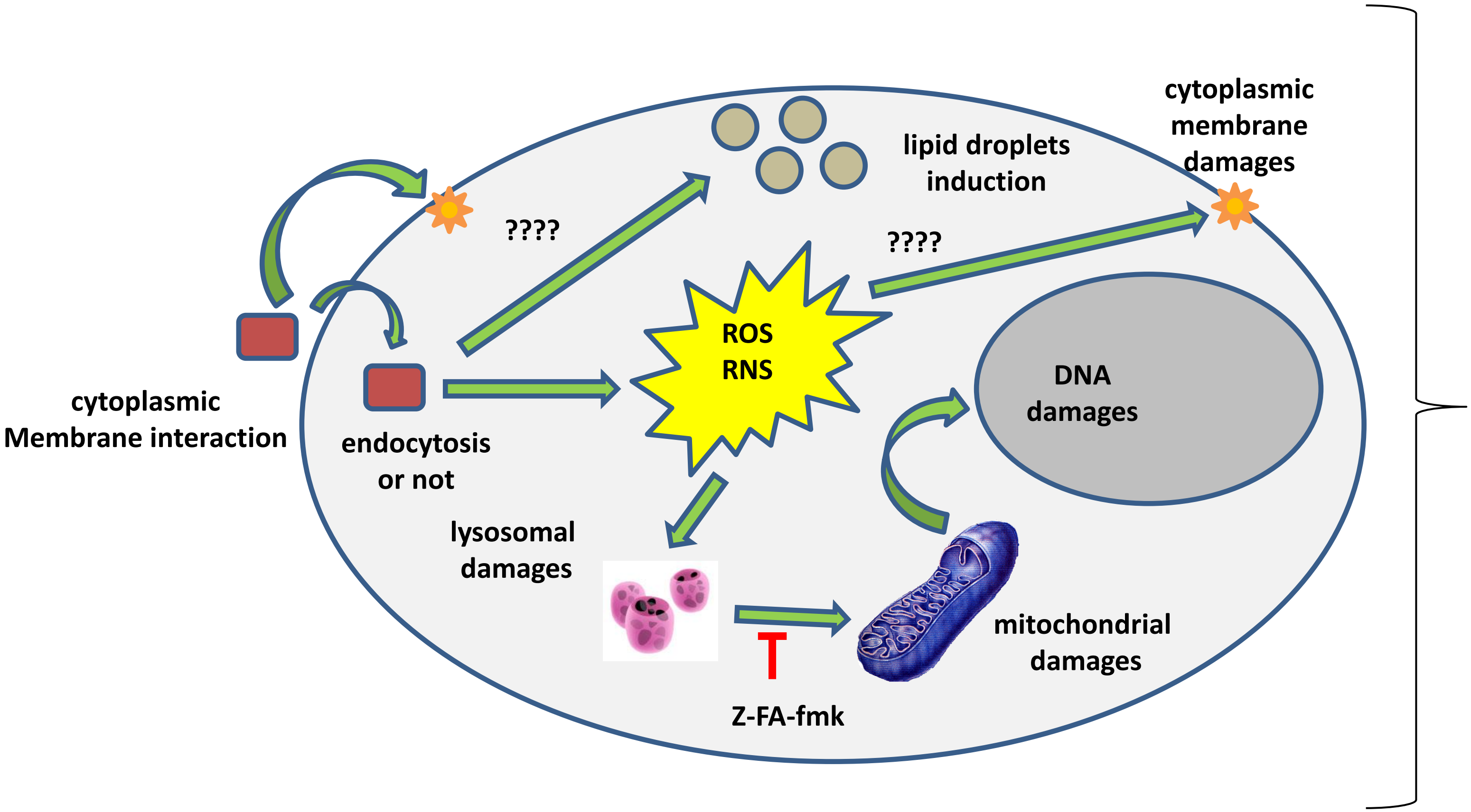
- OBJECTIVE

To evaluate the cytotoxicity of iron nanoparticles on cardiac cells and to determine whether they can modulate the biological activity of 7-ketocholesterol (7KC) involved in the development of cardiovascular diseases. Nanoparticles of iron labeled with Texas Red are introduced in cultures of nonbeating mouse cardiac cells (HL1-NB) with or without 7-ketocholesterol 7KC, and their ability to induce cell death, pro-inflammatory and oxidative effects are analyzed simultaneously.

- CONCLUSION

The designed protocol makes it possible to show how Iron Texas Red nanoparticles are captured by cardiomyocytes. Interestingly, whereas these fluorescent iron nanoparticles have no cytotoxic, pro-inflammatory or oxidative activities, **they enhance the side effects of 7KC.**

Flow cytometry permits the characterization of NPs or NTs – induced cytotoxicity



* Apoptosis
* Necrosis
* Autophagy
...

NPs and NTs – induced cytotoxicity:
Precised mecanisms can be deduced from flow cytometric analyses

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