Cytotoxicity of metal oxide nanoparticles to the lung and immune cells *in vitro*: a contribution to safer nanoantibacterials

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Outline

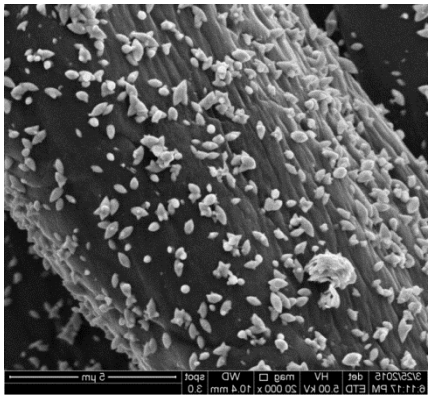
- Nano-antibacterials
- Sonochemically synthesized CuO, ZnO NPs
- Cytotoxicity of sonochemically synthesized NPs to the lung and immune cells
- Conclusions

Nano-antibacterials

CuO
ZnO
TiO_2
Ag

Textile industry

Antibacterial textiles



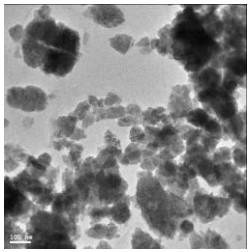
Mode of action:

- Release of ions → ionic stress
- Production of ROS → oxidative stress

Novel nano-antibacterials

Target organims (e.g., bacteria, yeast)

Toxic by design



Safe by desing

Non-target organisms (e.g., human cells)

Ionic stress ↑
Size
Coating
Charge

Oxidative stress↑
Size
Defects in the crystalline structure

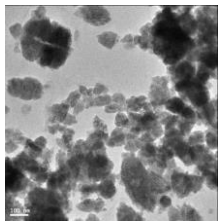
- Defects sites in the NPs crystal lattice are responsible for the formation of ROS (Malka et al., 2013).
- It was found that the crystallites which have more defects and less organized structure are more toxic to bacteria (Perelshtein et al., 2014).

Sonochemical method for the NPs synthesis

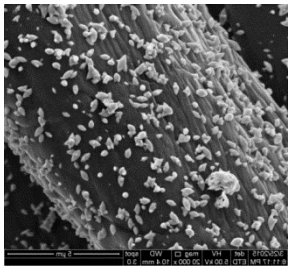
Zn/Cu atcetate

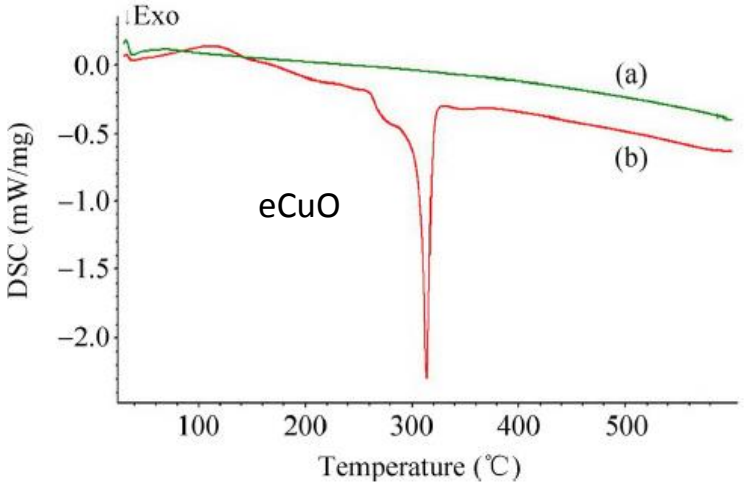
Water | Ethanol/water

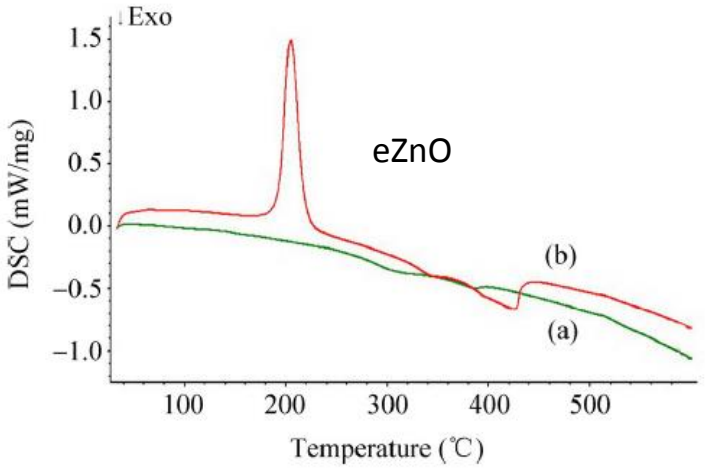
Ultrasounds



Water-based
ZnO and CuO
Ethanol-based
ZnO andCuO and
Zn-doped CuO
($Cu_{0.88}Zn_{0.12}O$)







DCS of NPs (Perelsthein et al. 2014)

Research group of Prof. A. Gedanken (Bar-Ilan University, Isreal)

Antibacterial properties

Escherichia coli
Pseudomonas aeruginosa
Staphylococcus aureus

Regular
Multi-drug resistant bacteria

10 minutes in 0.85% NaCl

ZnO and CuO NPs → 1-2 log reduction
Zn-doped CuO NPs → 5-6 log reduction

⇒ It was found that the crystallites which have more defects and less organized structure are more toxic.

⇒ Since modulation of the nanoparticles defects influenced their toxicity, the possibility of engineering **safer** nano-antibacterials is indicated.

Malka et al., 2013; Perelsthein et al., 2014

Aim of the study

Was to evaluate the **cytotoxic** and **pro-inflammatory effect** of the sonochemically synthesized CuO, ZnO and Zn-CuO NPs to the human lung and immune cells.,

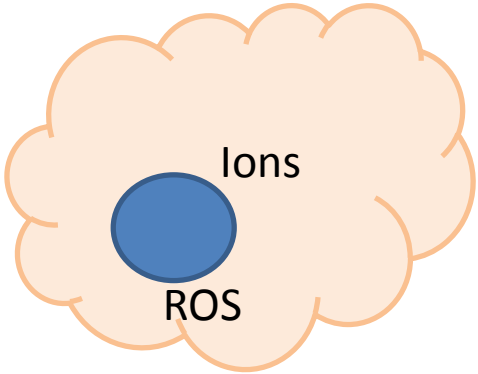
Toxicity of NPs

Bacteria

Ions

ROS

Mammalian cells



Ions

ROS

Project: Do new generations of nano-antibacterials overcome the epithelial barriers posing human health at risk? A predictive nanotoxicology study (OVERNanoTOX), 2014-2016, Dr. P. Mantecca

Antibacterial NPs synthesis and coating of the textiles
Bar-Ilan University, Israel

In vitro nanotoxicology

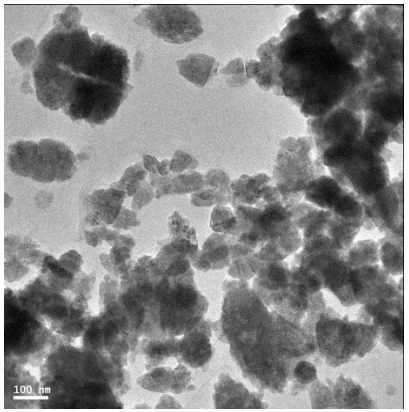
Estimation of human exposure
Abrasion tets
EMPA, ETH, Zùrich

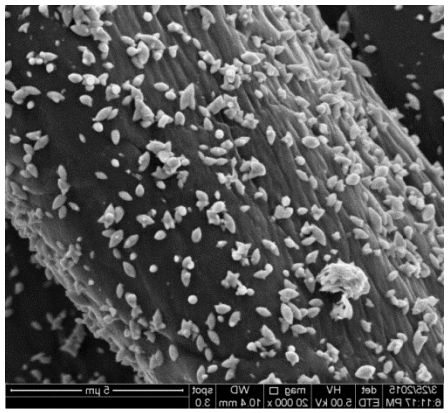
Ingestion toxicty:
Intestine epithelial barrier
Milano University

Inhalation toxicity:
Lung and immune cells
Milano-Bicocca University

Safety of the sonochemically synthesized NPs to human cells *in vitro*

Safety/risk = toxicity and exposure

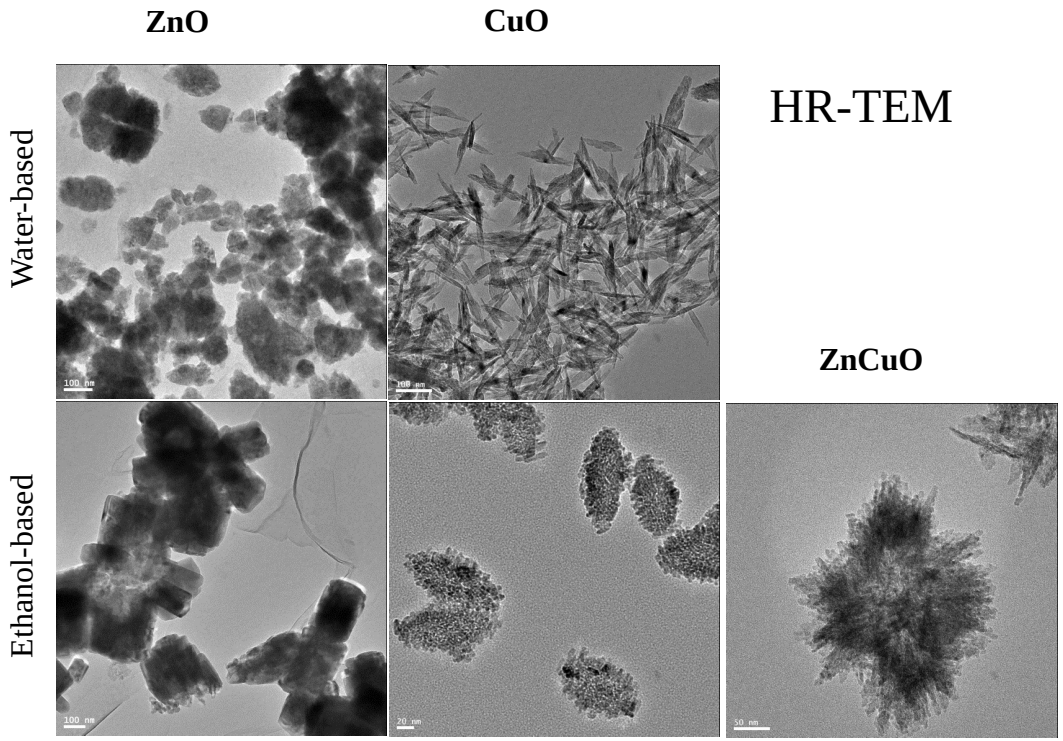




Adverse cellular effect?
Concentration?

Release of the particles?
Concentration?

Characterization of the particles

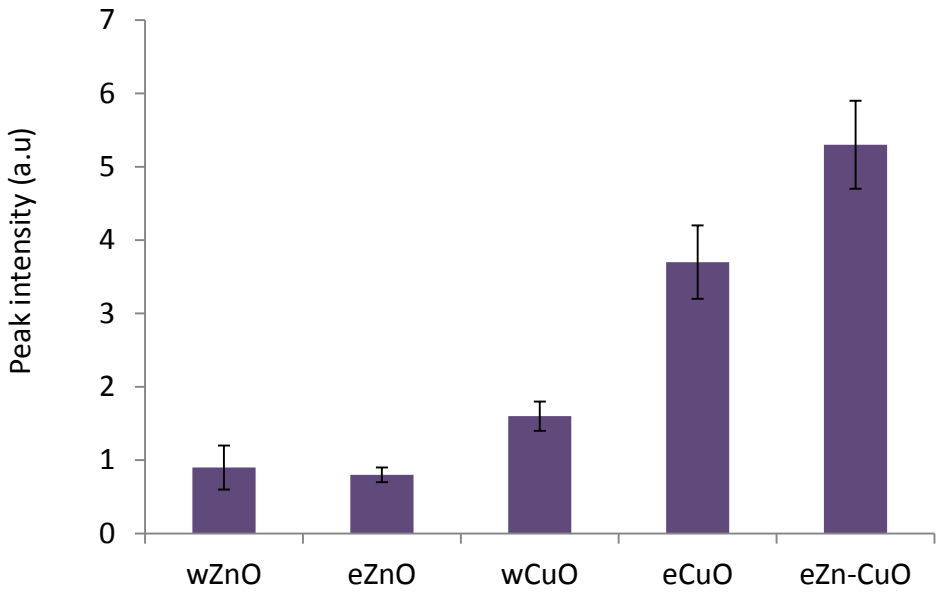


NPs	Diameter, nm
wZnO	70
wCuO	25
eZnO	160
eCuO	50 (5)
eZnCuO	30

➢The type of the solvent during the hydrolysis of metal acetates has an impact on the shape and the size of the sonochemically synthesized NPs

ROS production in cell-free system

Radical detection by the electron spin resonance (ESR) study



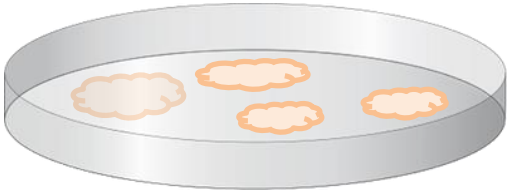
- Main detected radical was hydroxyl radical (·OH).
- Correlation between the ROS production and antibacterial activity.

Inhalation toxicity *in vitro*: lung and immune cell lines

A549 - human adenocarcinomic **alveolar basal epithelial** cells

THP-1 - human acute **monocytic** leukemia cell line (monocytes)

A549

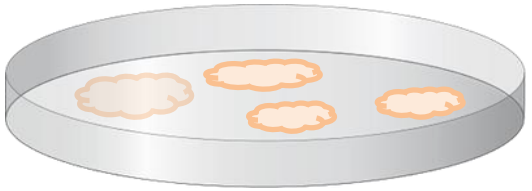


Adherent cell line

THP-1

Monocytes - suspension cells

Differentiataion by 200 nM PMA for 72 h



Macrophages - adherent cells

PMA -phorbol 12-myristate 13-acetat

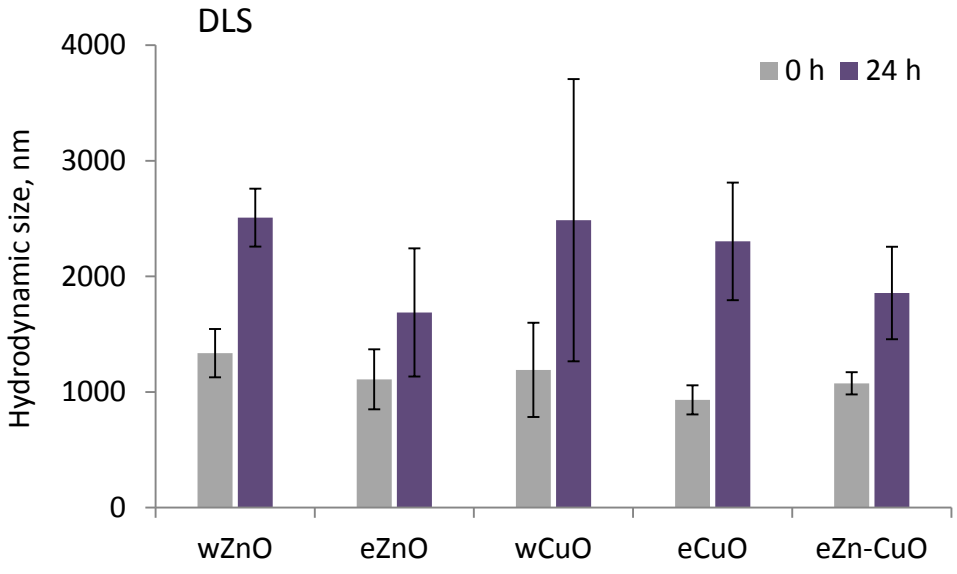
Toxicity assessment

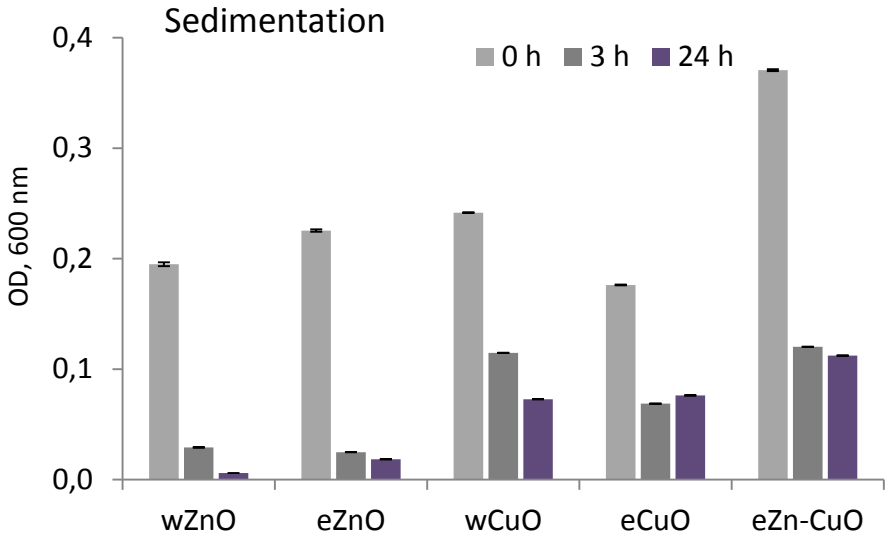
- On 6-multiwell plates (growth area).
- Cell culture medium (OptiMEM, Invitrogen) without serum at 37°C, 5%CO_2
- Studied NPs concnetration ranged from 0.1 to 100 mg/L
- Exposure time 3 h and 24 h

NPS Effects endpoints:

- Viability, MTT-viability assay
- Pro-inflammatory effect, release of the interleucin IL-8
- Oxidative stress (NAC experiment)

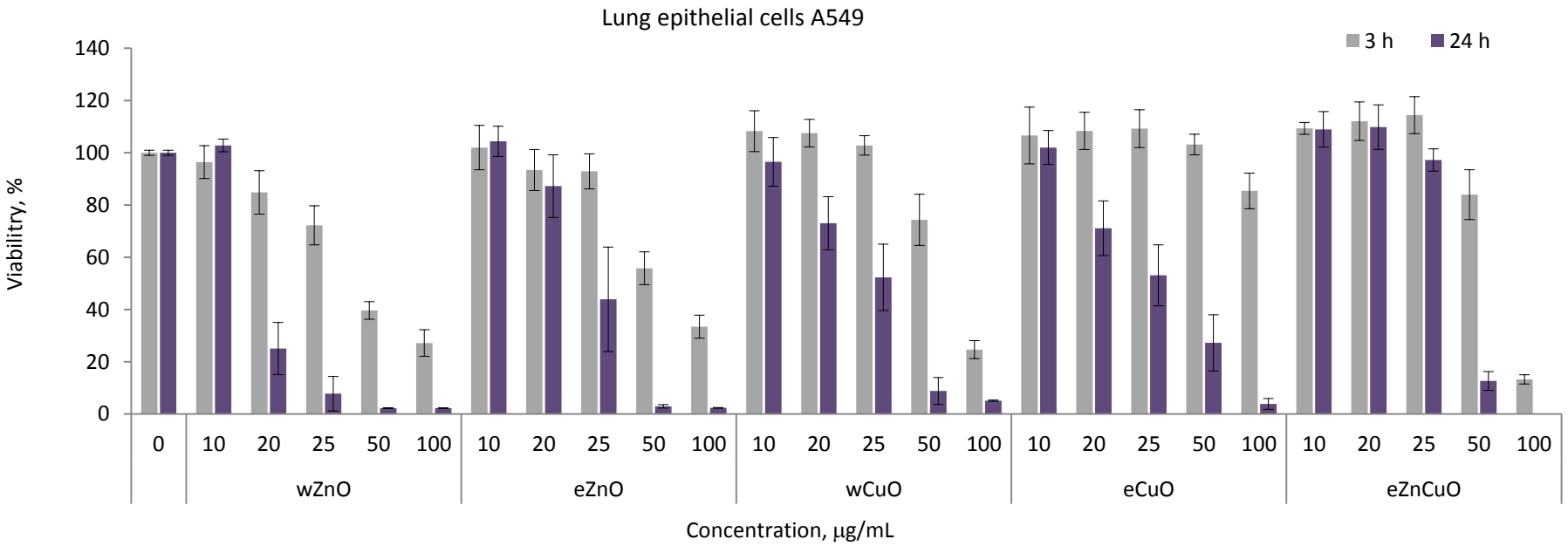
NPs suspension characterization in culture medium





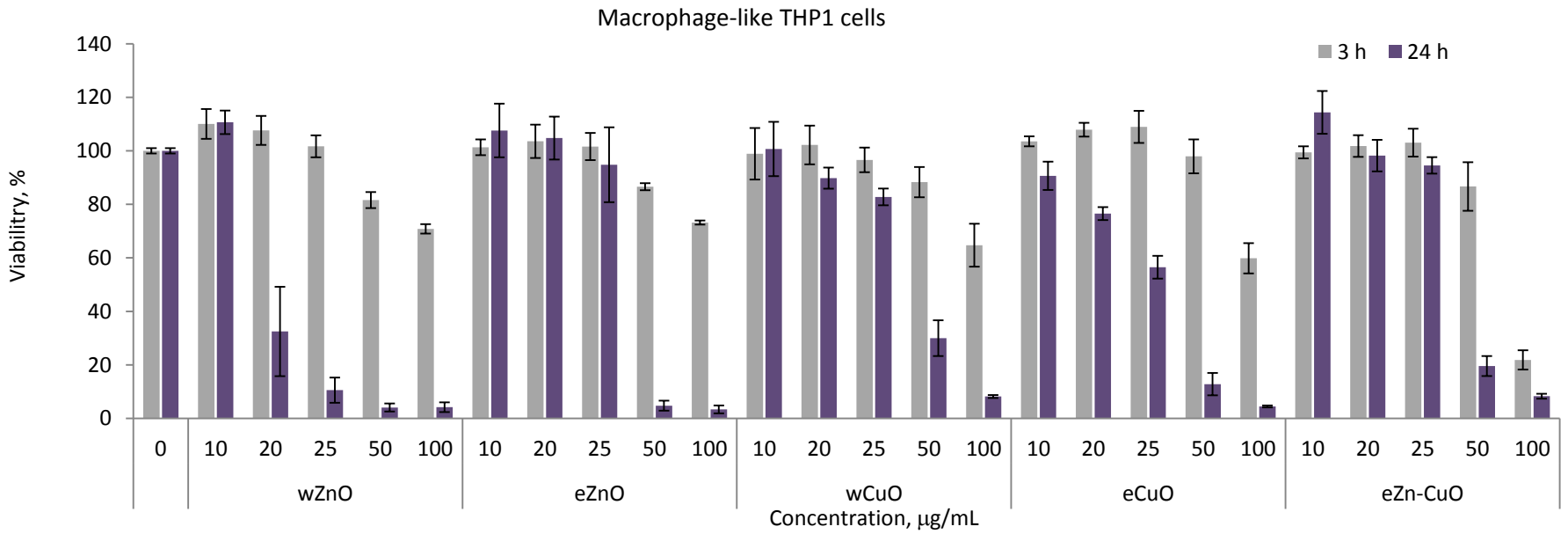
In the cell culture medium NPs were agglomerated and tended to settle

Cell viability of lung epithelial cells

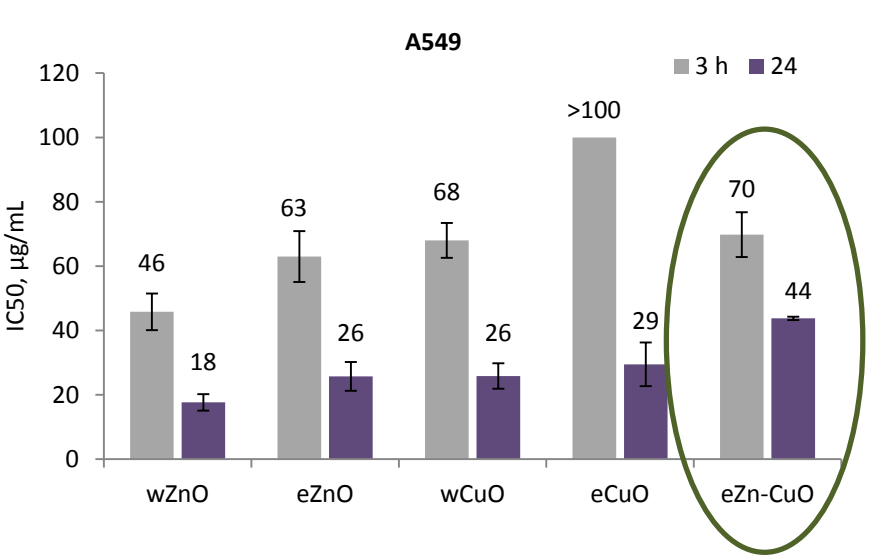


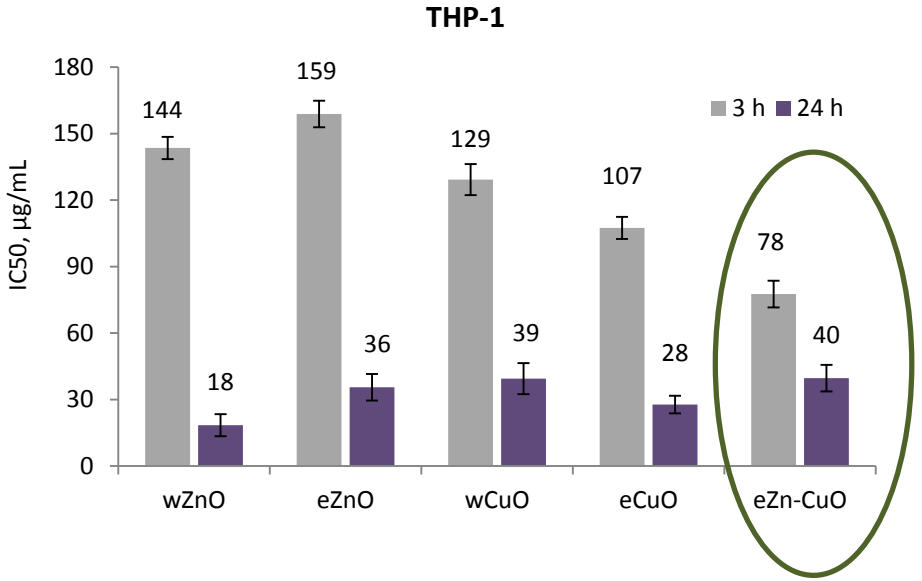
- After 3 h exposure ZnO NPs had more pronounced effect on the cell viability
- After 24 h exposure all the NPs had comparable effect

Cell viability of macrophage-like THP-1 cells



IC_{50} values (µg/mL)

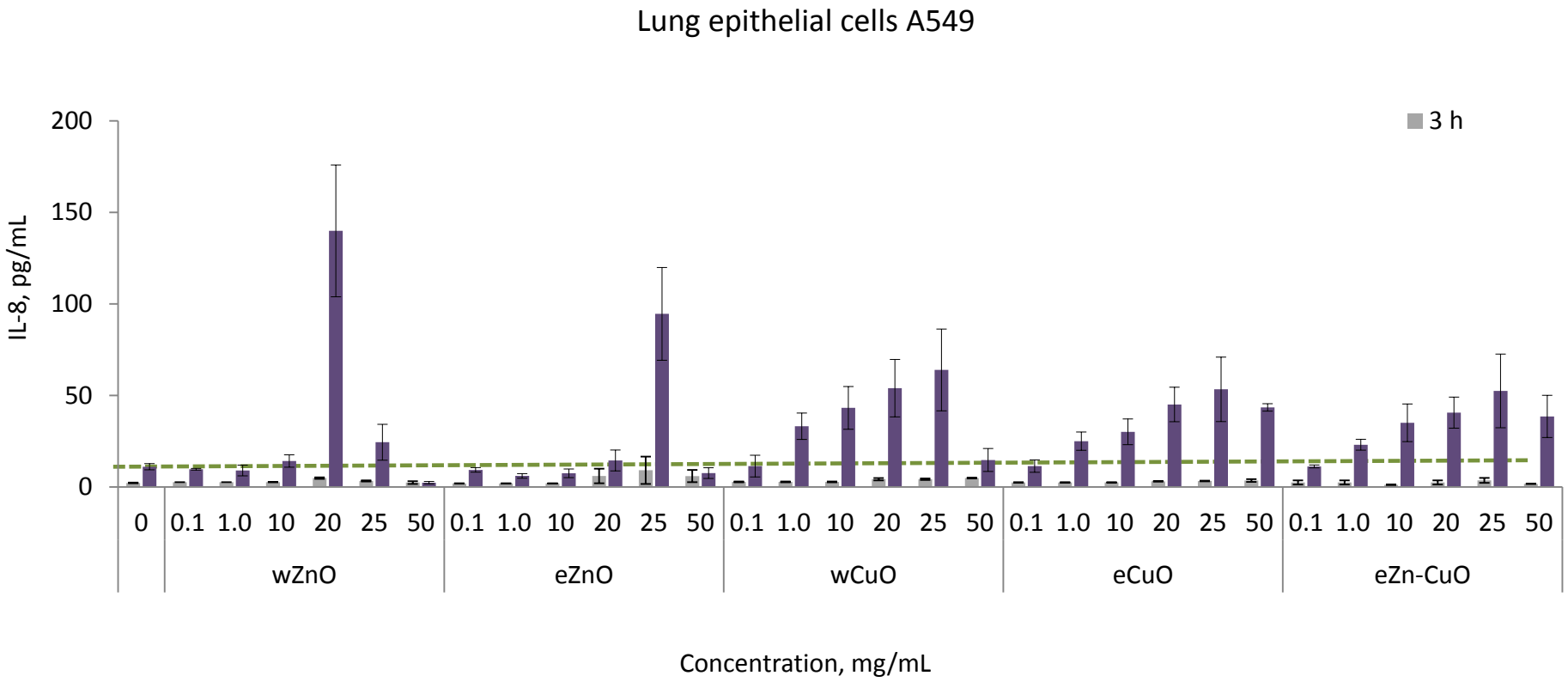




No clear difference in the cell response can be associated to NPs shape, size and ROS production

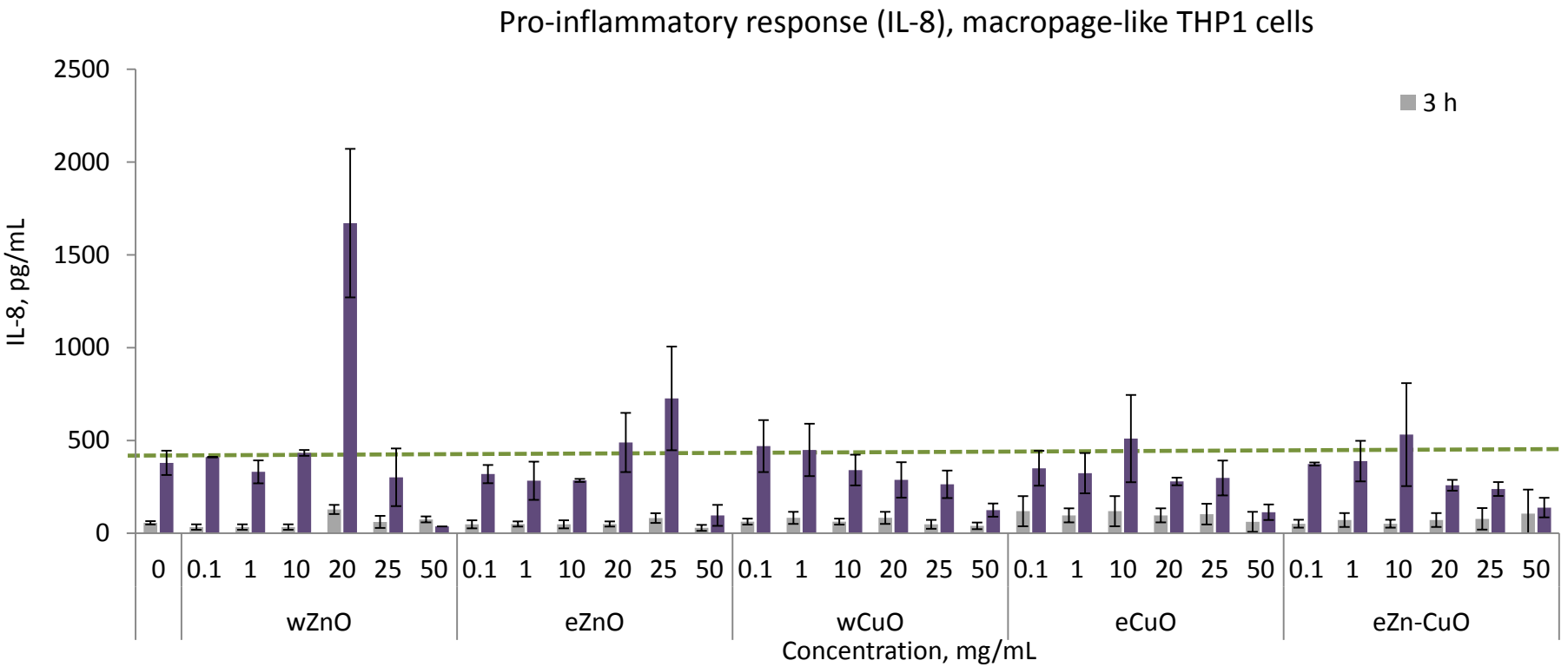
IC_{50} – half-effective concentration

Pro-inflammatory effect (IL-8)



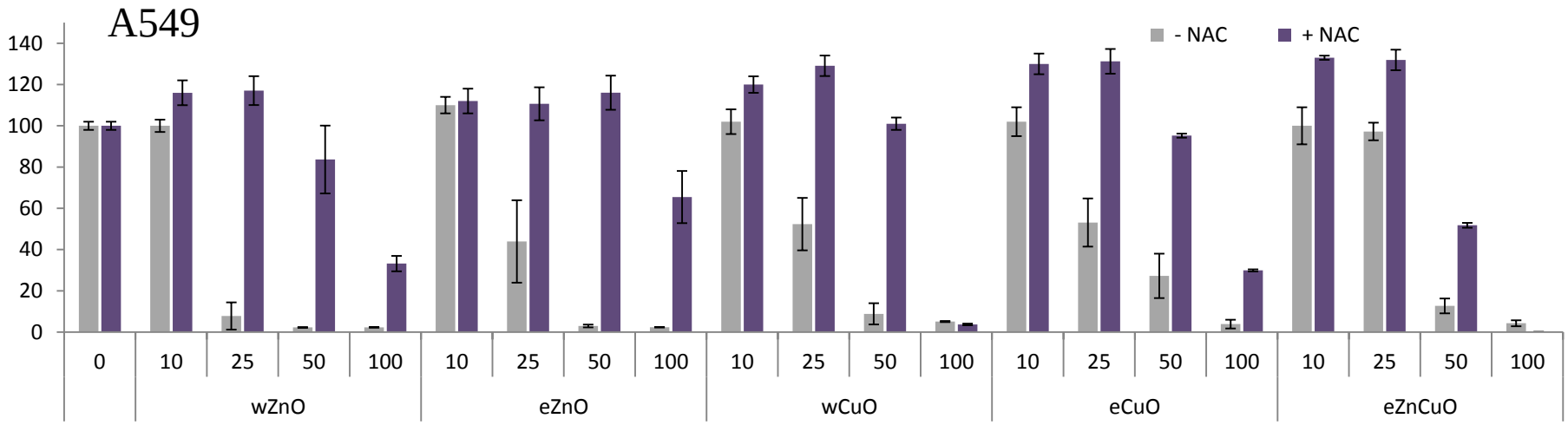
- Differently from the ZnO NPs, CuO NPs (wCuO) induced IL-8 release in the lung epithelial cells already at sub-toxic concentrations

Pro-inflammatory effect (IL-8)

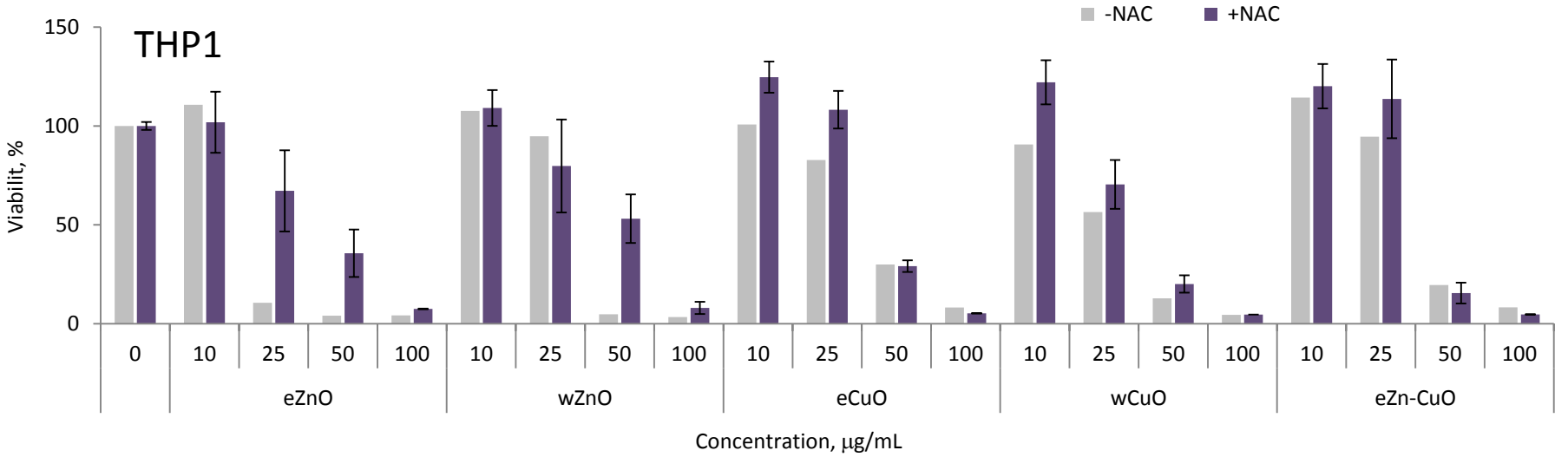


- In the immune cells only nZnO (wZnO) induced pro-inflammatory response (but only at higher toxic concentration).

Oxidative potential to lung and immune cells



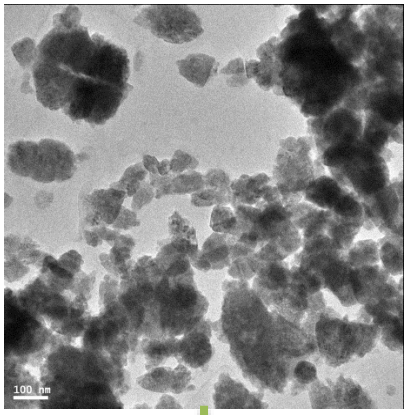
THP-1, without and with NAC

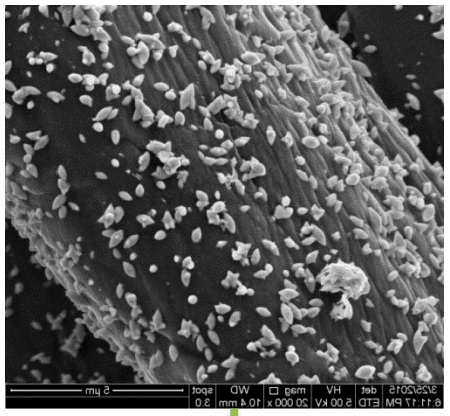


NAC - N-acetyl-L-cysteine (ROS scavenger)

Safety of the sonochemically synthesized NPs to human cells *in vitro*

Safety/risk = toxicity and **exposure**

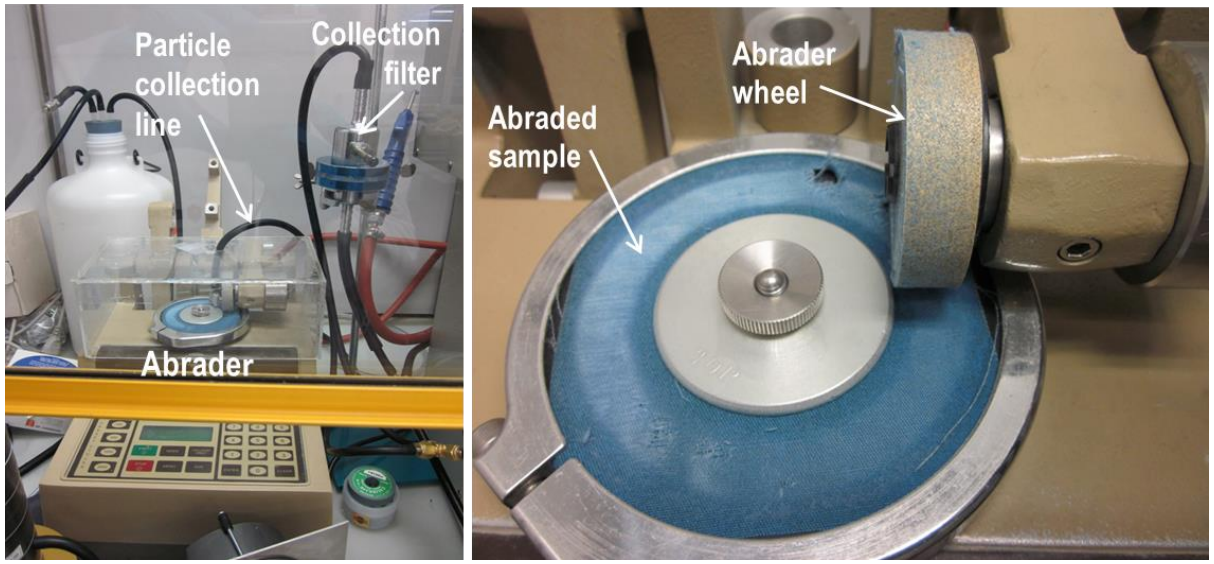




Toxic
Pro-inflammatory effects
Oxidative stress

Release of the particles?
Concentration?

Airborne NPs monitoring: abrasion test



Cotton and cotton/polyester NPs coated textiles

- The coated samples with NPs in water showed higher particle release than NPs in ethanol
- Ethanol based NPs size was 100-200 nm and for water-based NPs 1-3 µm
- Released particles concentration was low, in the range of hundreds of particles per cm^3 or less

Conclusions

1. Sonochemically synthesized NPs were toxic to the lung and immune cells after 3 h exposure only at the high concentrations (50-100 mg/L)
2. After 24 h exposure the IC_{50} values for the studied NPs were 20-40 mg/L
3. No clear differences in the cell response can be associated to the size and shape and ROS production of the NPs
4. Even the highly antimicrobial eZnCuO had similar toxicity to lung and immune cells
5. NPs had different pro-inflammatory effects on the lung and immune cells
6. Very little amount of particles were released from the coated fabrics in abrasion test.
7. At the low concentrations (up to 10 mg/L) the NPs were not toxic to the lung and immune cells.
8. According to the pro-inflammatory response profile the CuO NPs may be consider less safe tan ZnO NPs for antibacterial application

Acknowledgments

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