# Effects of brake wear nanoparticles on respiratory cells

Chloé PUISNEY, Evdokia OIKONOMOU, Sophie NOWAK, Alexandre CHEVILLOT, Jean-François BERRET and Armelle BAEZA-SQUIBAN

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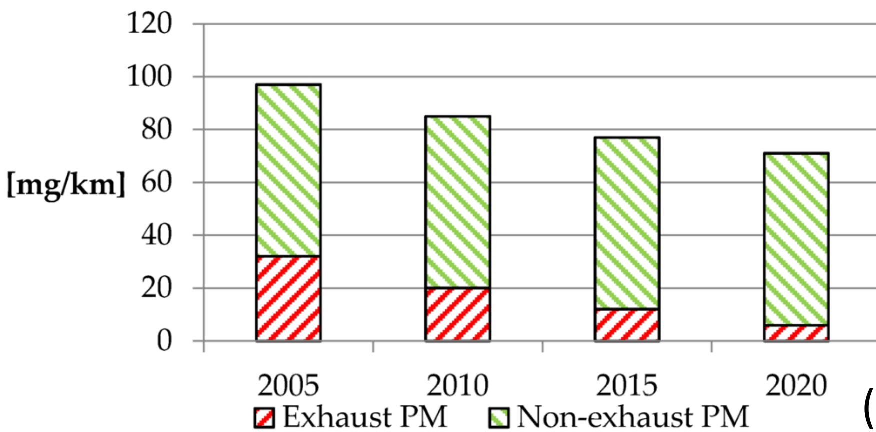
# General considerations (1)

Exhaust

Non-exhaust

Average urban PM10 emissions by passenger cars




(Hooftman *et al*, Energies, 2016)

- Brake wear contribution (Grigoratos *et al*, Environmental Science Pollution Research, 2015) :
  - up to 55 % by mass to total non-exhaust traffic-related $PM_{10}$
  - up to 21 % by mass to total traffic-related $PM_{10}$ emissions

# General considerations (2)

## What is already known about brake wear particles ?

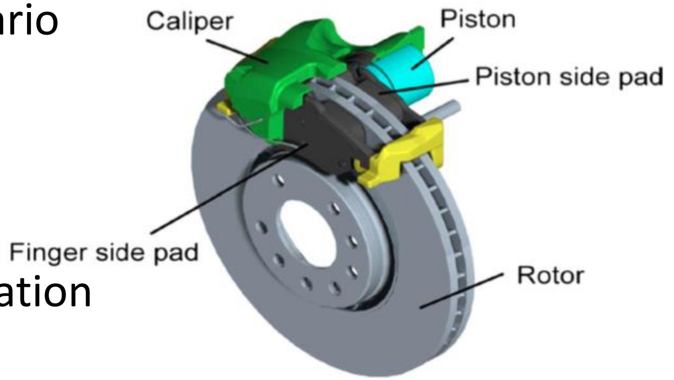
- Braking scenario associated with temperature ↑ + friction strength
  - →Surface chemistry alteration
  - →Material degradation
    - →Favorable to particles generation

- Wide range of size : from few nanometers to several micrometers

(Thorpe and Harrisson, Science of Total Environment, 2008)

  → Direct link between particles size and braking scenario

- Due to brake linings composition :
  - →Known as non-exhaust linked pollution sources
  - →Important source of environmental metal contamination




(Wahlström *et al*, 2009)

# Experimental strategy

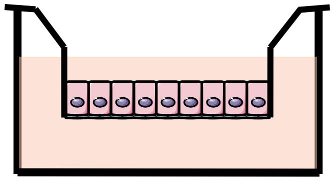
Testing center

Brake drum

Brake pad

Physico-chemical characterization

Toxicological study



# Physicochemical characterization (1)

X-ray Fluorescence

| Chemical element | Composition (%) |
|---|---|
| Mg | 1,6 |
| Al | 11,0 |
| Si | 13,1 |
| S | 6,9 |
| K | 2,1 |
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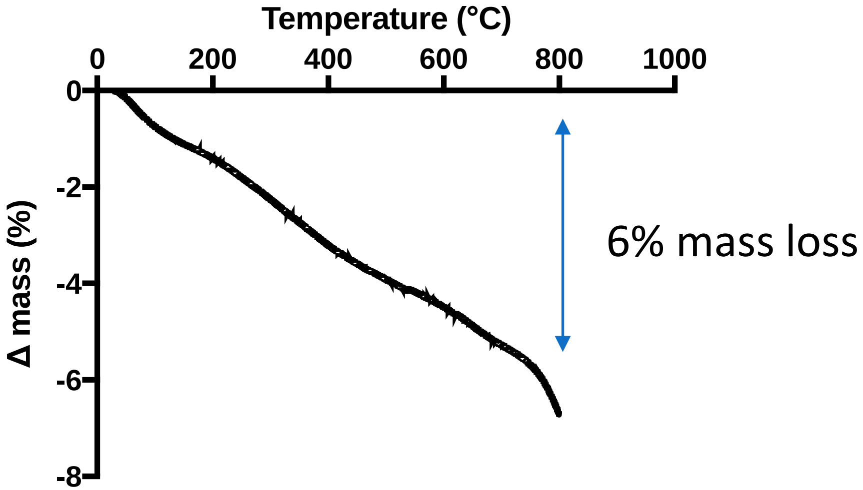
Thermogravimetric analysis




Organic : 6%
Inorganic : 94%

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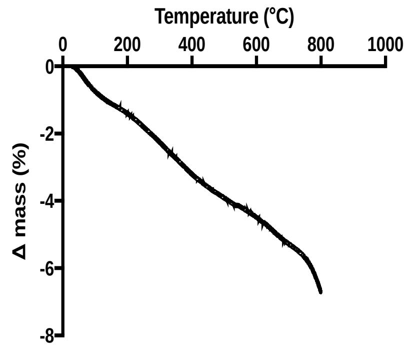


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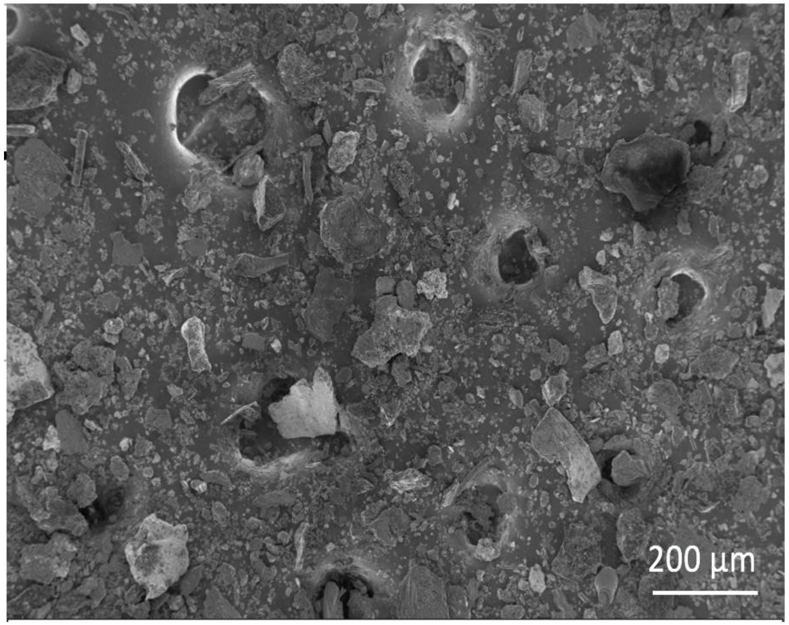
Scanning Electron Microscopy



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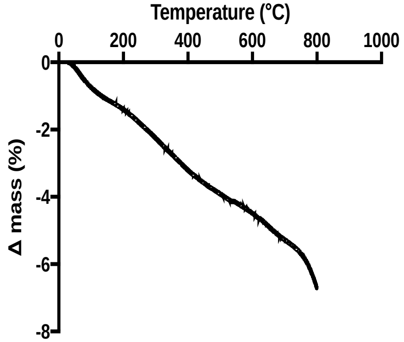


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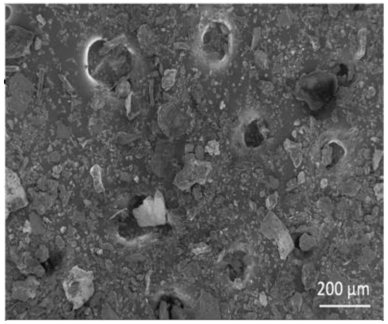


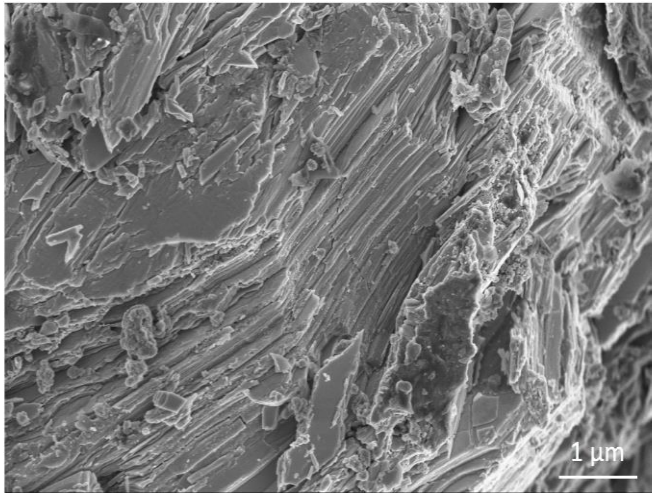

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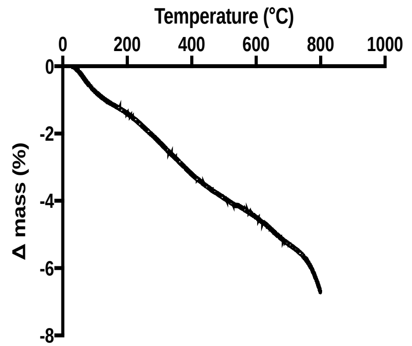


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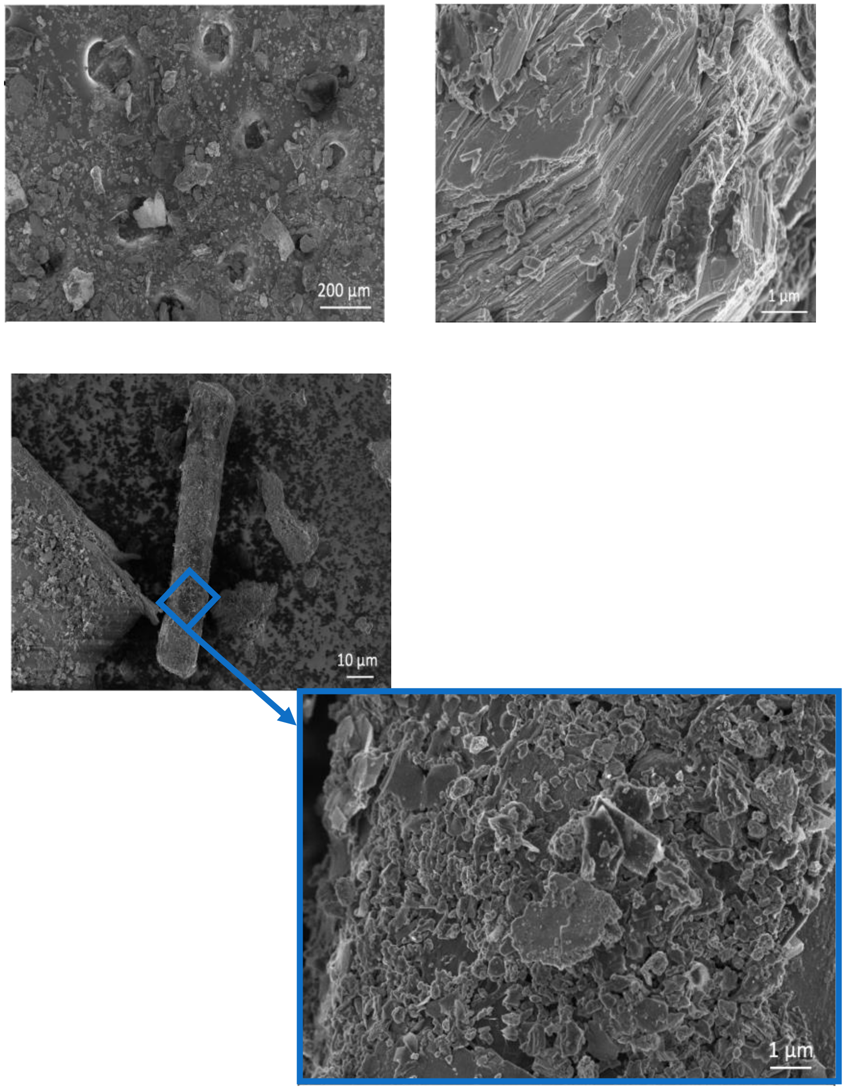

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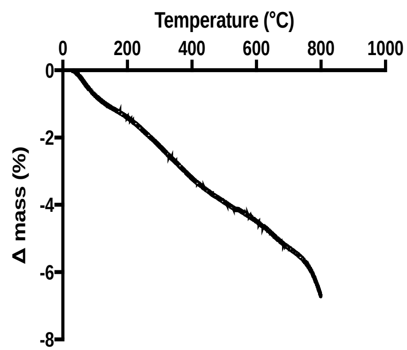


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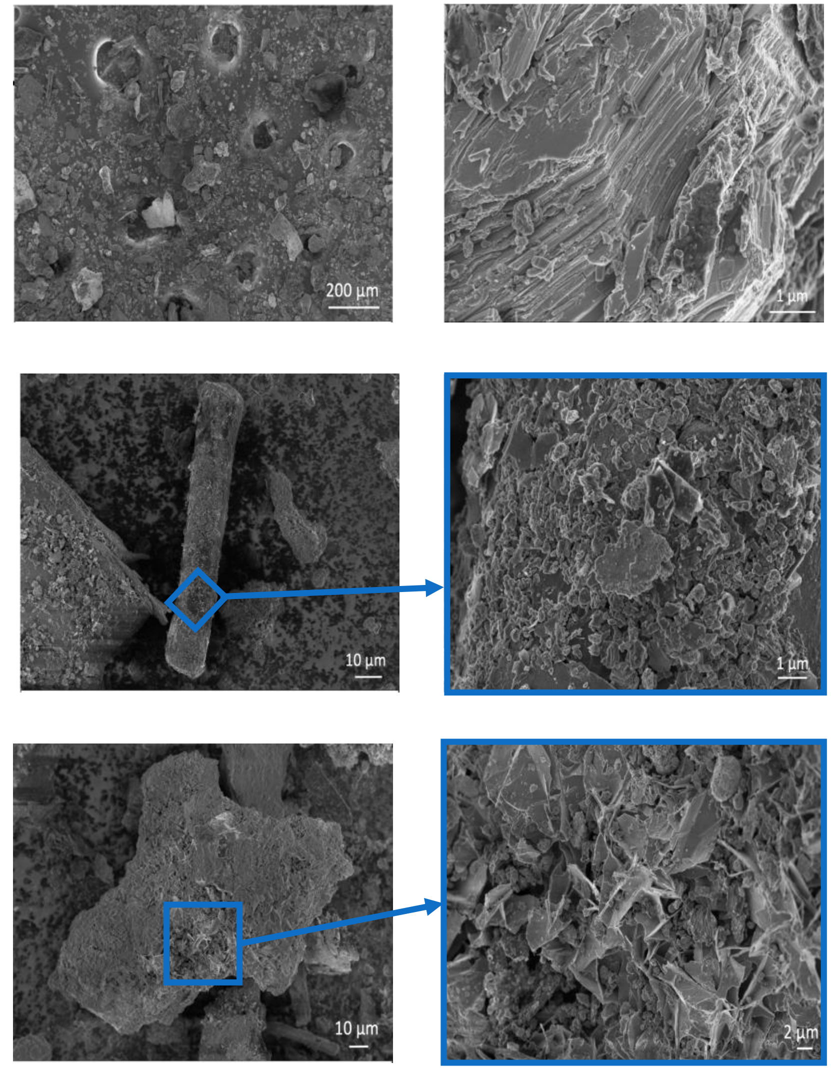
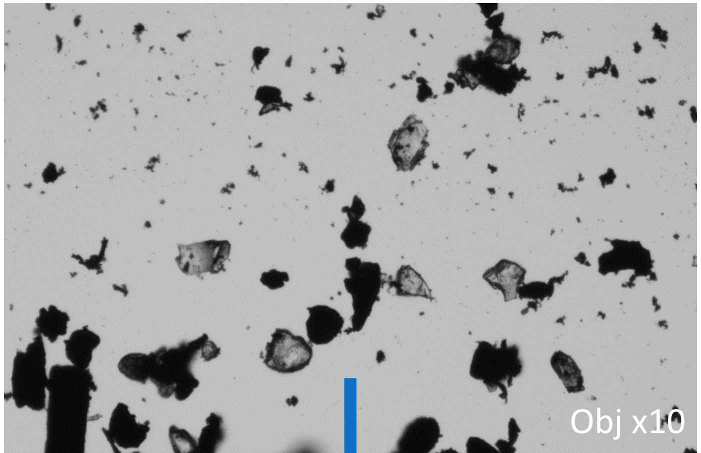
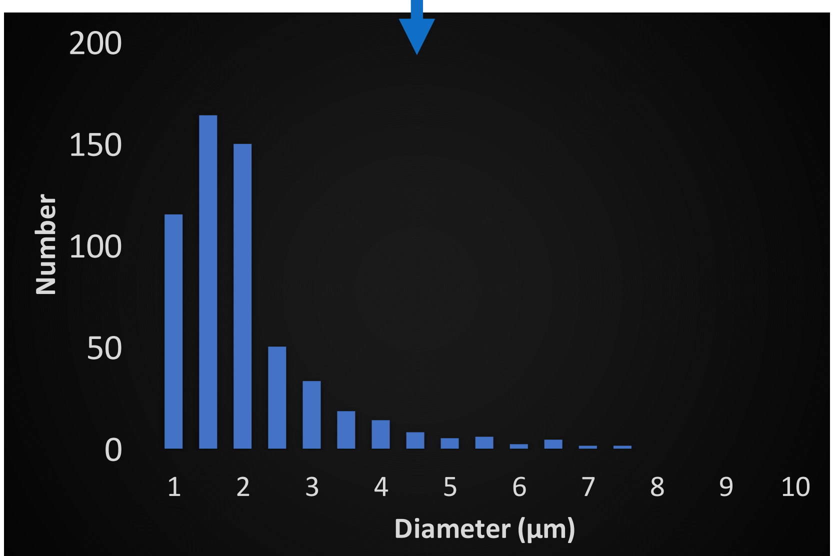


# Physicochemical characterization (2)

**Optical microscopy**

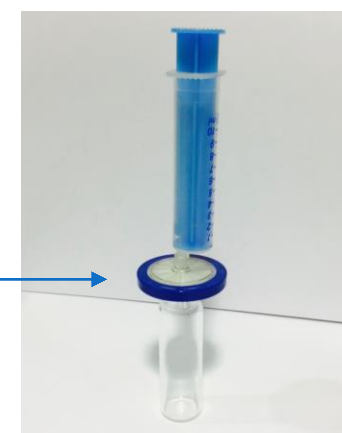



0 → 10 min

Sonication
+
Filtration process

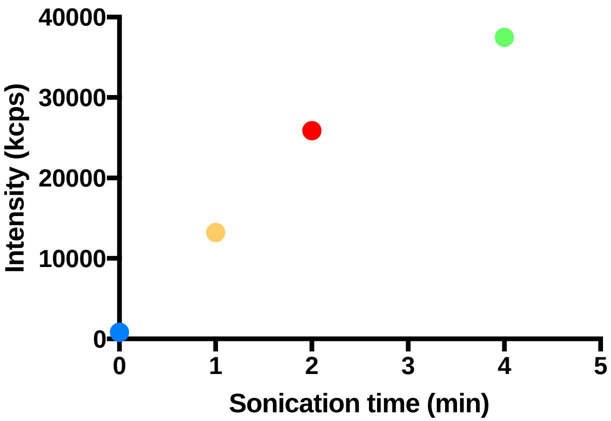
0,45 µm filter

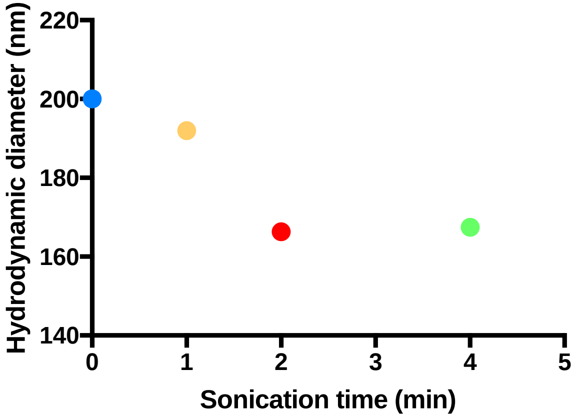


# Physicochemical characterization (3)

## Characterization of brake wear powder suspensions

| | Sonication time (min) | Unifltered | Filtered |
|---|---|---|---|
| Z-average (d.nm) | 0 | 12000 | 200 |
| | 1 | 1600 | 192 |
| | 2 | 1200 | 166 |
| | 4 | 621 | 167 |
| Intensity (kcps) | 0 | $3,4 \times 10^5$ | $8,1 \times 10^2$ |
| | 1 | $6,7 \times 10^5$ | $1,3 \times 10^5$ |
| | 2 | $5,2 \times 10^5$ | $2,5 \times 10^5$ |
| | 4 | $1,8 \times 10^6$ | $3,7 \times 10^5$ |




**Sonication + filtration process :**
**Size decrease**
**Intensity increase**

- ✓ Selection of nanometer size fraction
- ✓ Stability study

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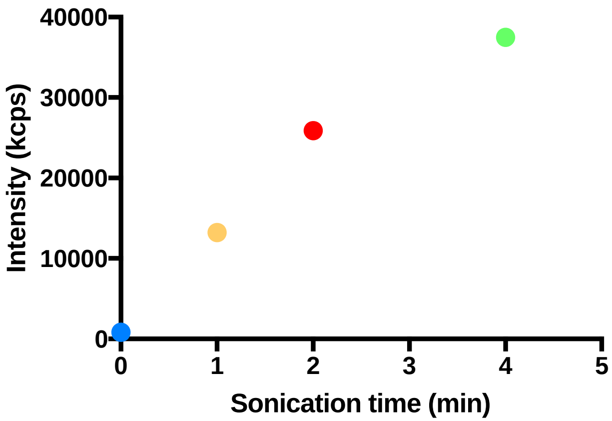


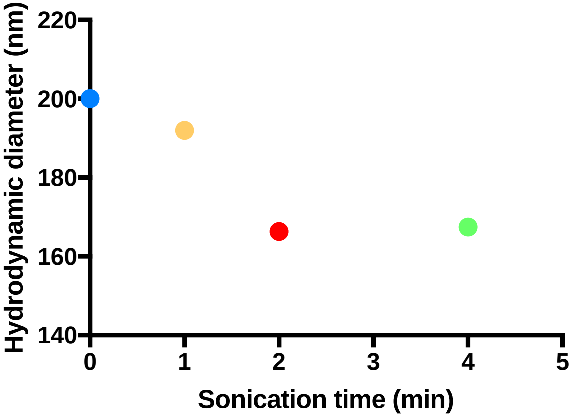


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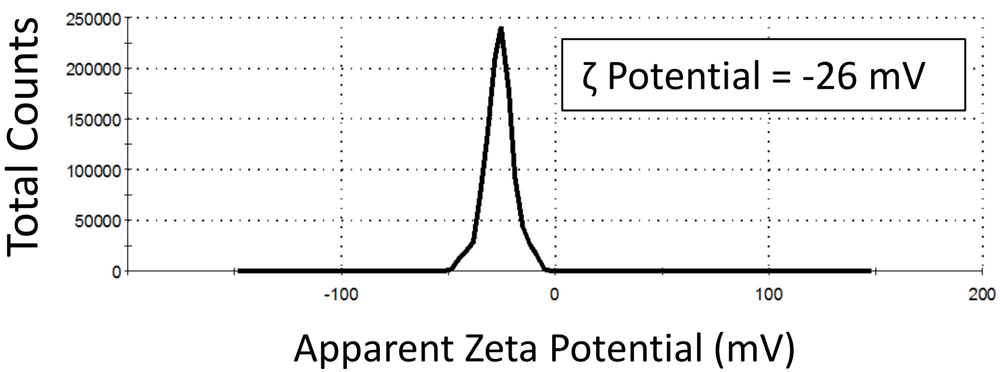


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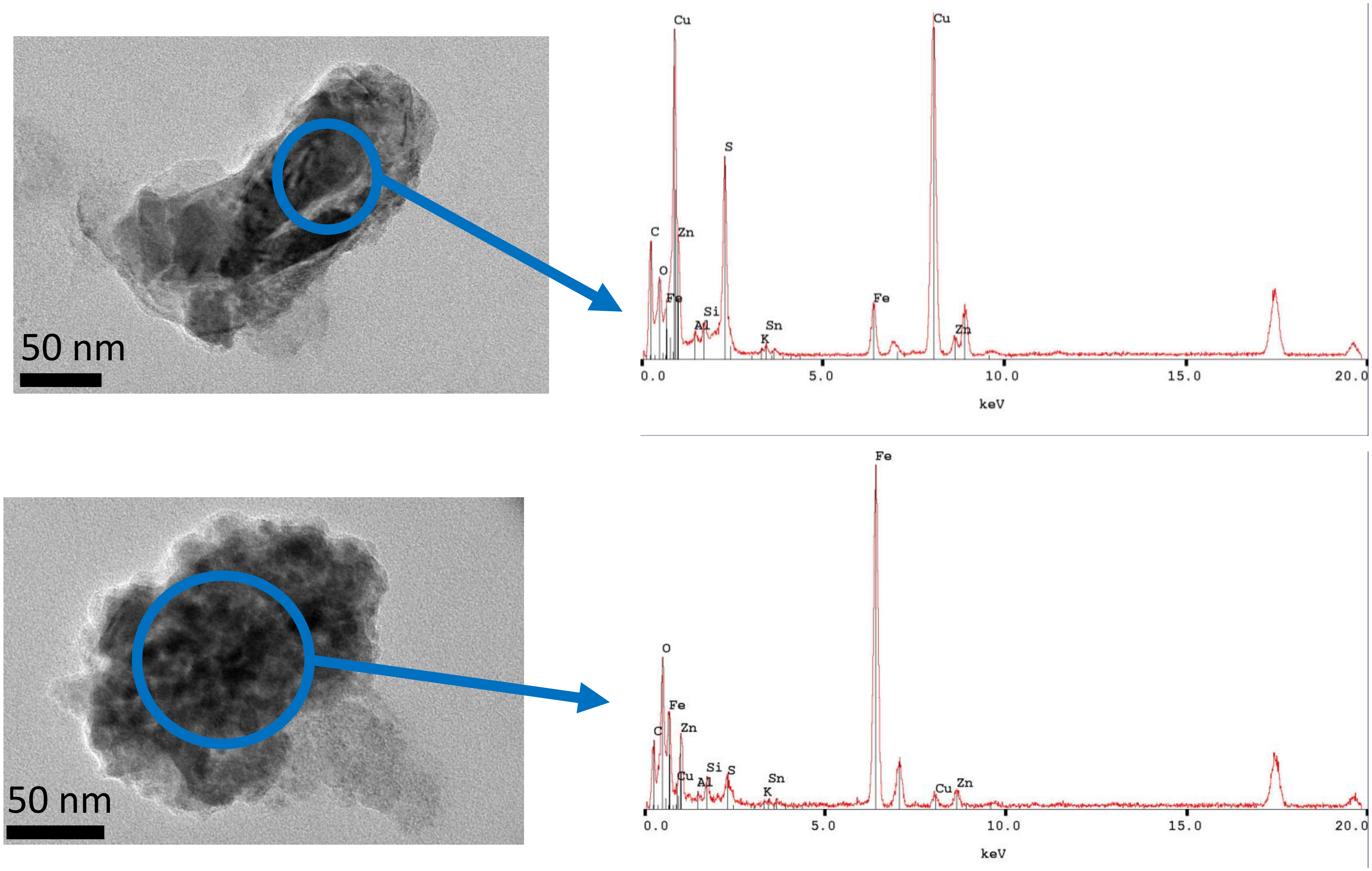
# Physicochemical characterization (4)

Transmission electron microscopy coupled with energy dispersive X-ray spectroscopy of filtered suspensions

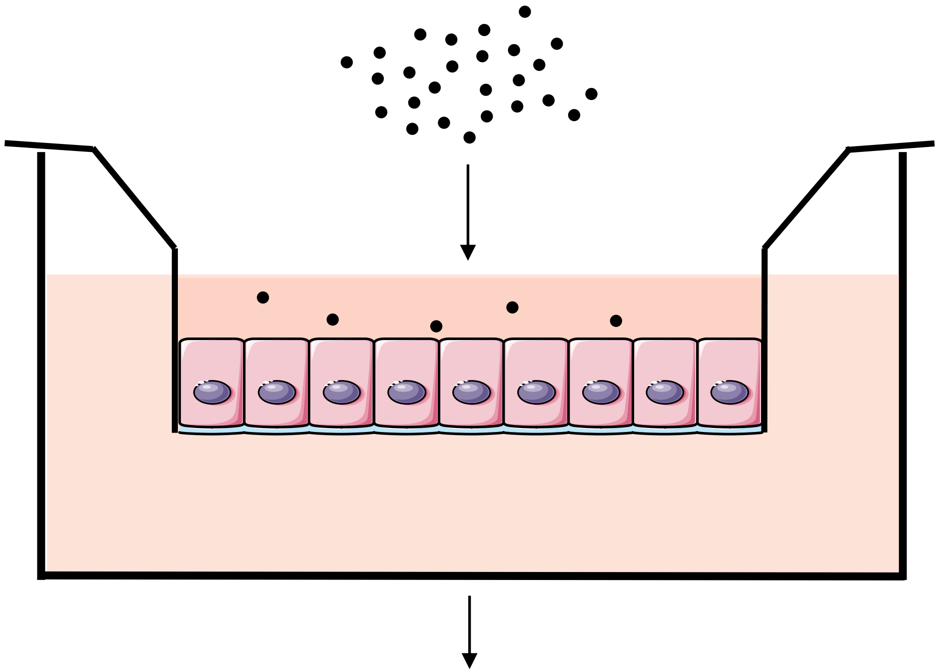


# Toxicological study (1)

24h exposure to :

Brake wear powder suspensions

Benchmark = $Fe_2O_3$ nanoparticles (50 nm)



*Calu-3 cells*
*Human bronchial epithelial cells*
*TW : Polarized differentiated epithelium*

Epithelial barrier impact ?

→ **Barrier Integrity study** : Lucifer Yellow permeability assay, Transepithelial electric resistance measurement (TEER)

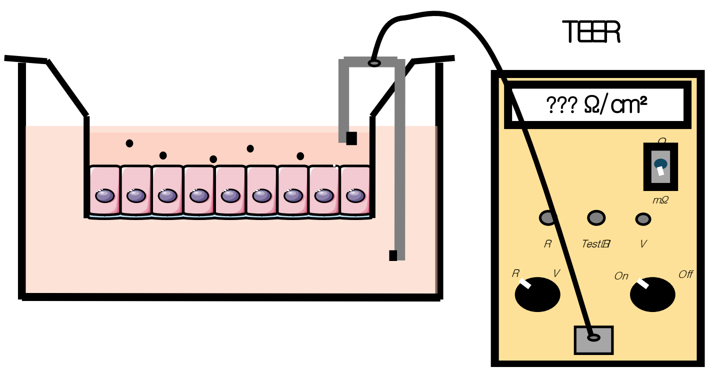
→ **Cellular morphology** : Immunostaining (Actin, ZO-1, MUC5AC)

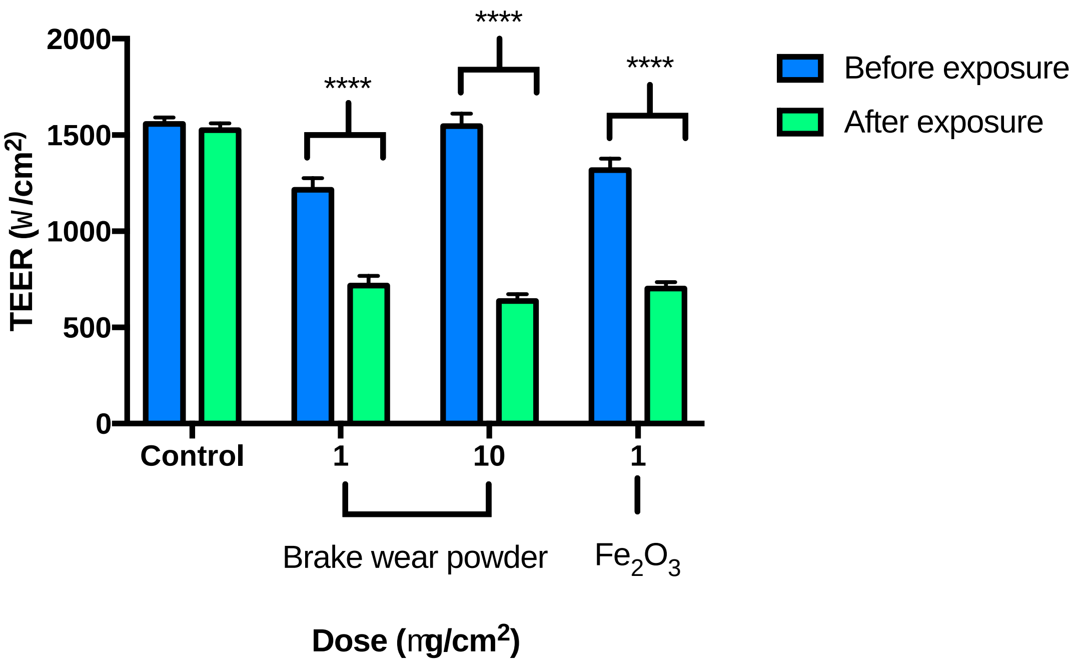
→ **Proinflammatory response** : ELISA (IL-6, IL-8, TNF)

# Toxicological study (2)

Epithelial barrier integrity assessment following 24h exposure to particles

TEER measurement



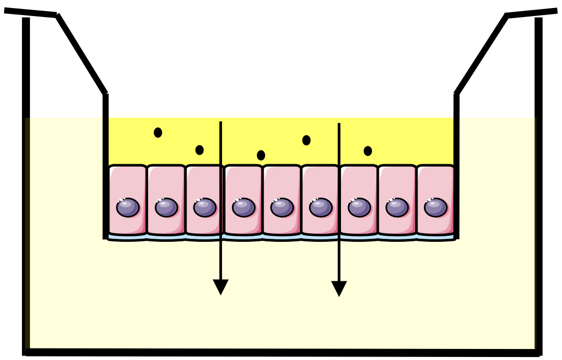



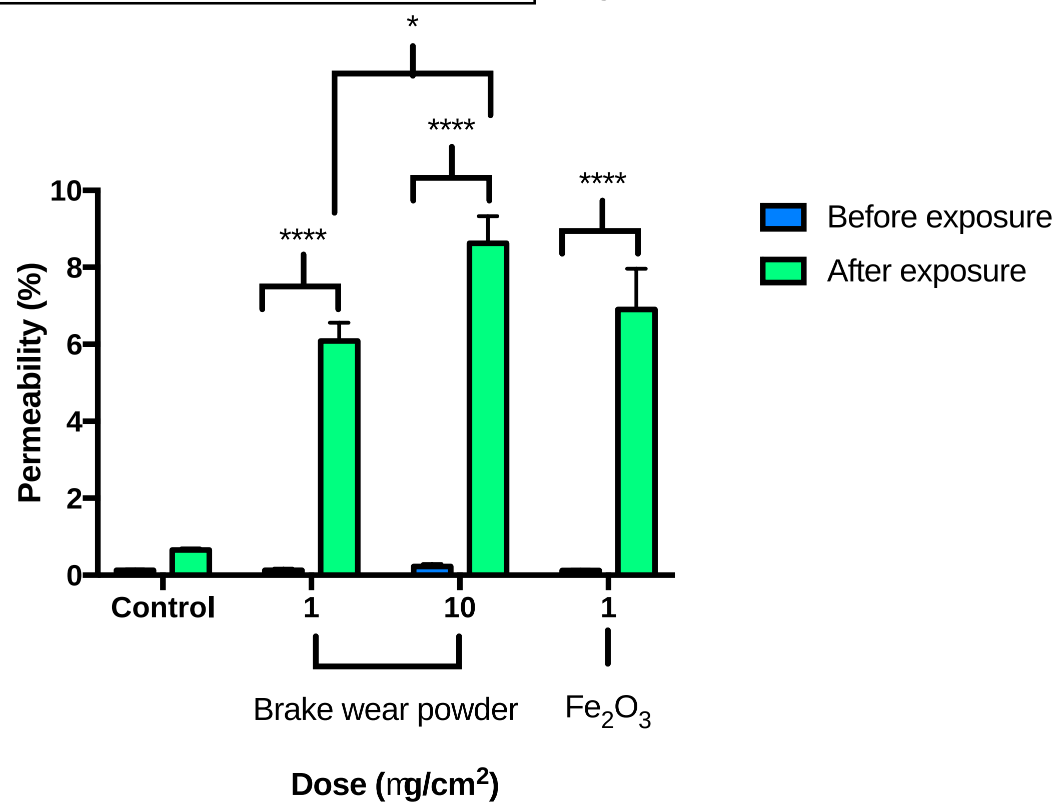

Statistical analysis : ANOVA – Dunnett's post test, compared to vehicle, P<0,05 : *, P < 0,01 : **, P<0,001 : ***, P<0,0001 : ****

# Toxicological study (2)

Epithelial barrier integrity assessment following 24h exposure to particles

Lucifer Yellow permeability assay



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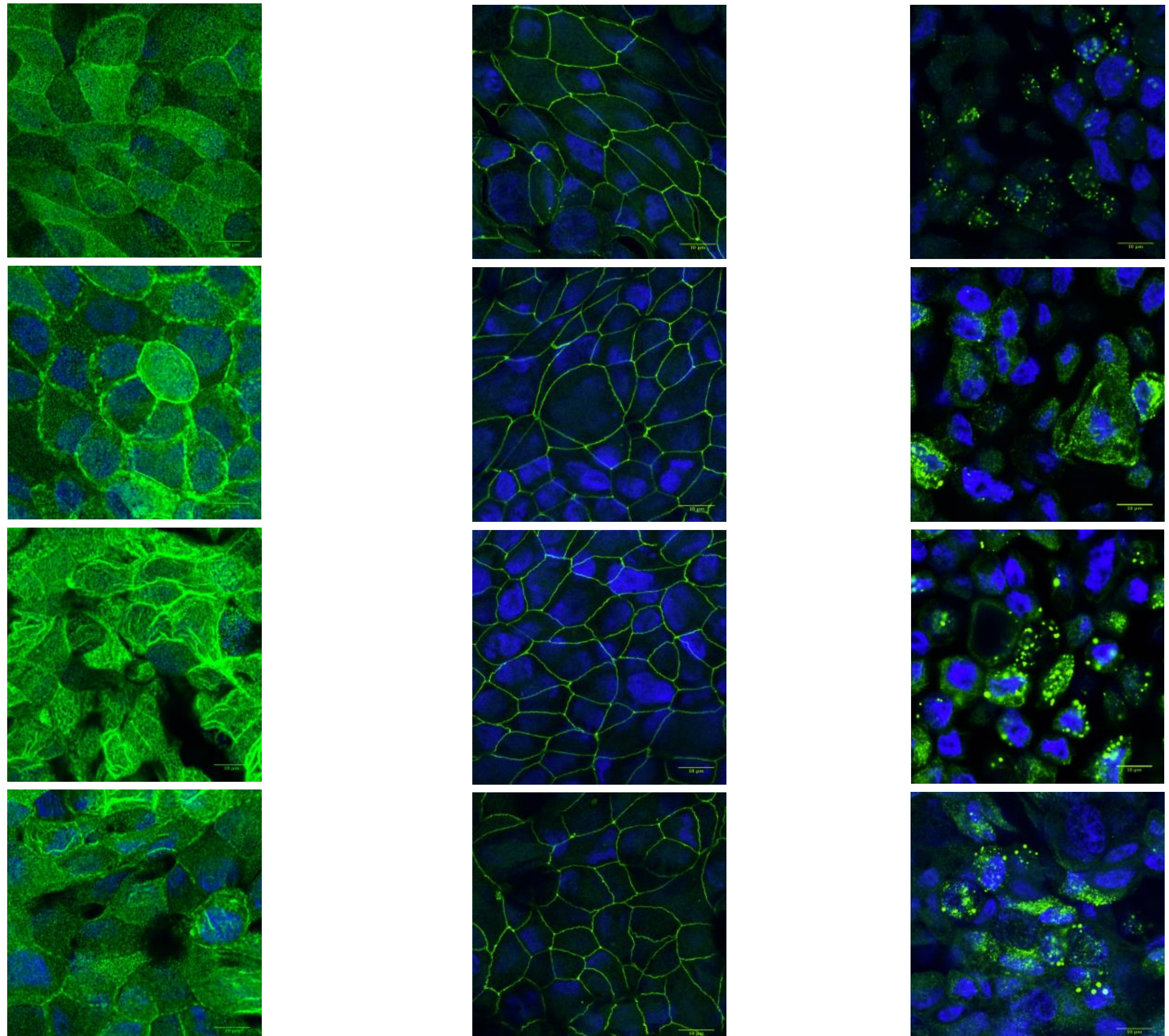
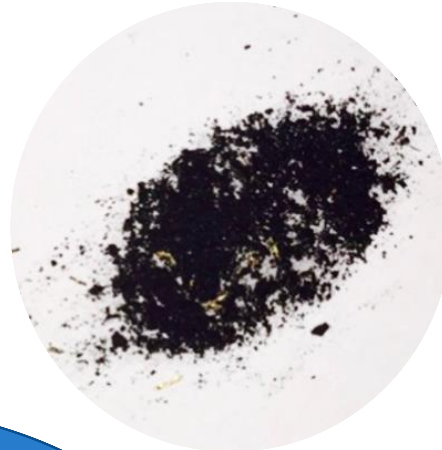
# Toxicological study (3)



# Conclusions



## Physico-chemical characterization

→ Complex mixture
→ Major components : Fe, Cu
→ Micro- and Nano-sized fractions
→ Different amorphous particles

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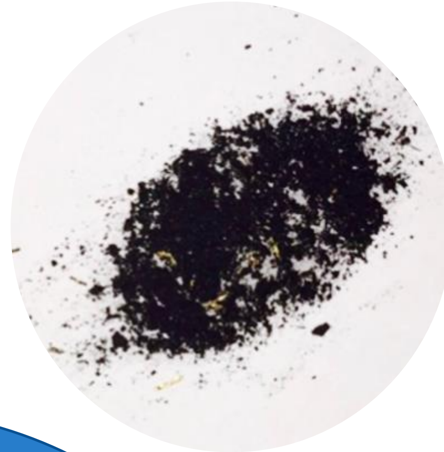

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## Toxicological study

→ Permeability ↑ = Barrier functions ↘
= tight junctions injury
→ No pro-inflammatory response (data not shown)
→ Mucus production ↑
→ Response similar as benchmark particles

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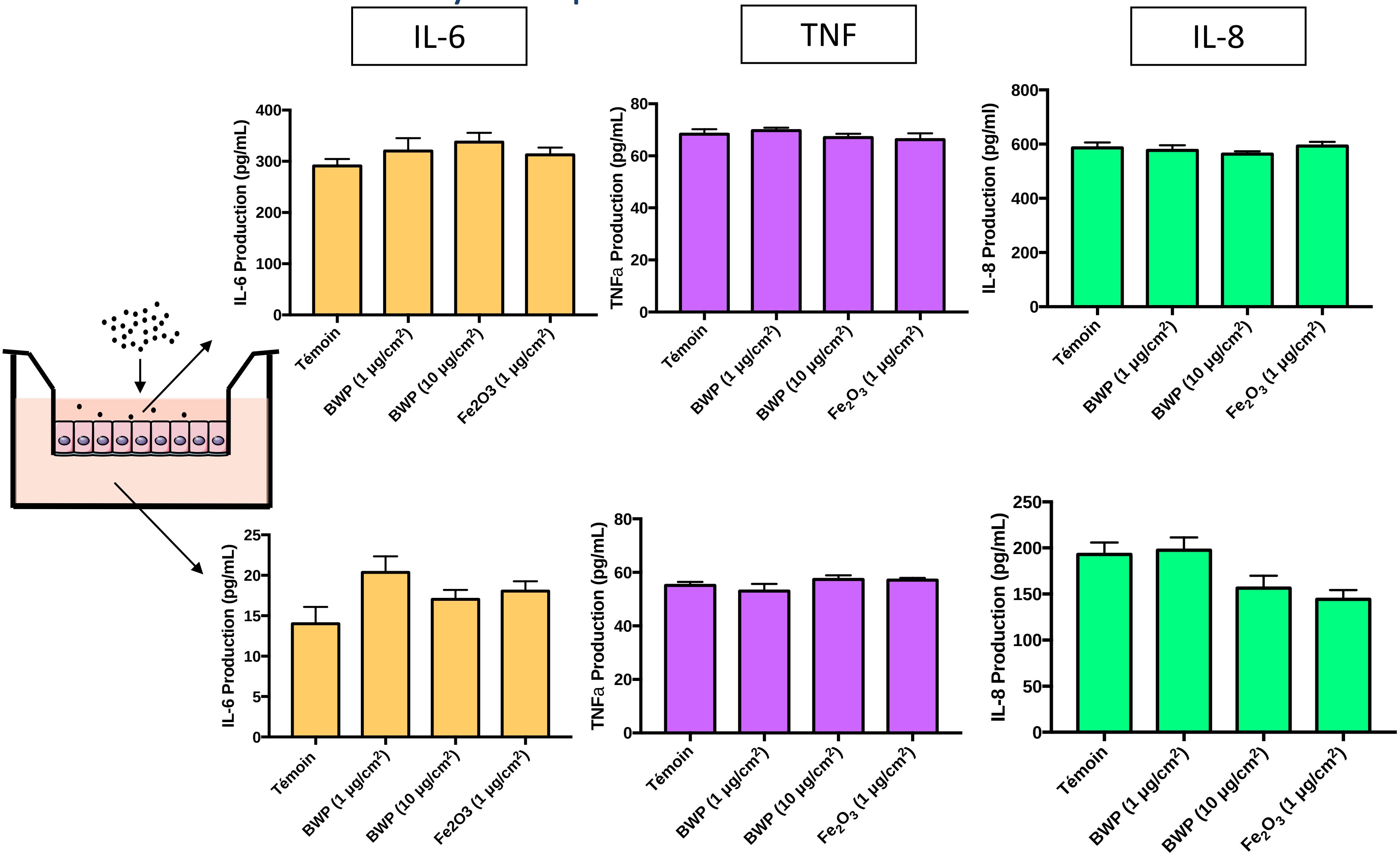
Sophie NOWAK

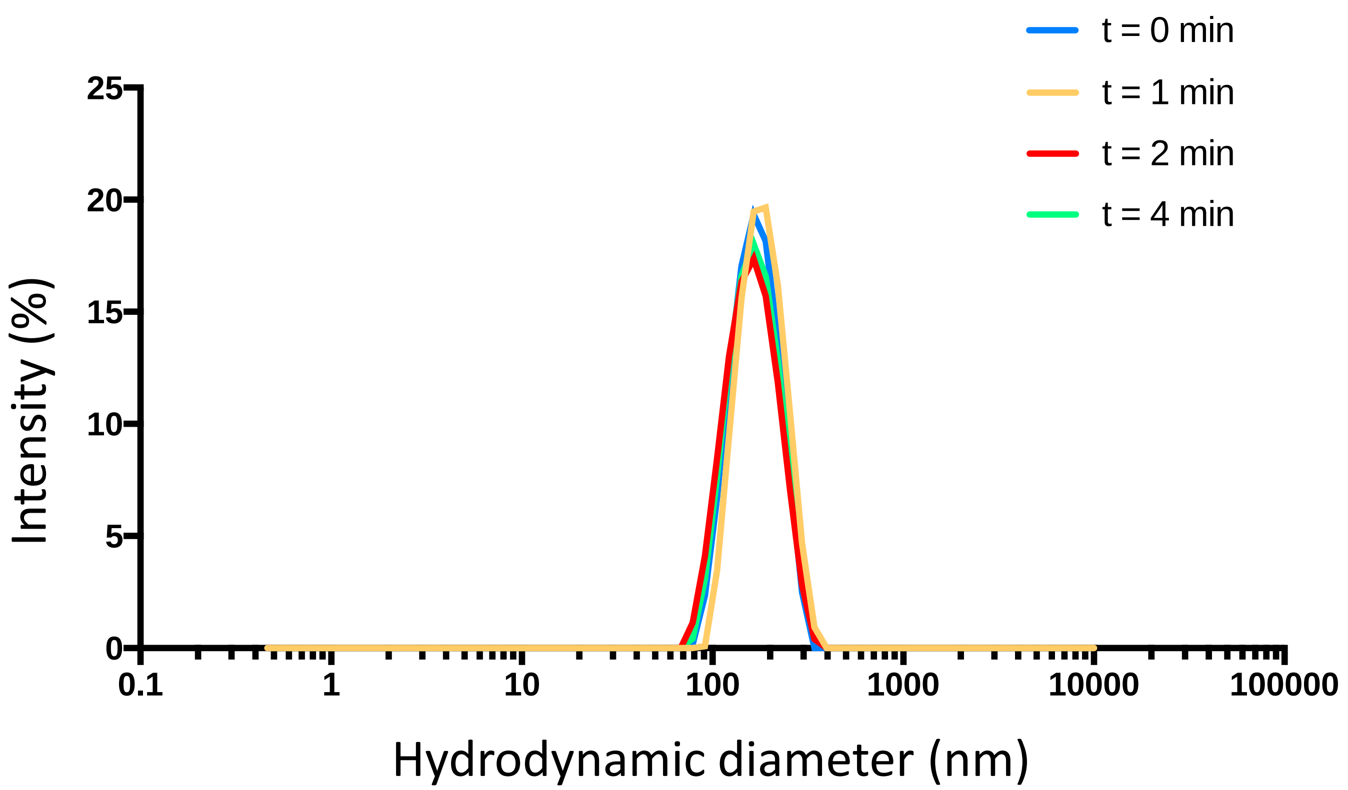
**Centre d'essais de Juvincourt**
Daniel GOMES
Frédéric RICHARD

**Services de microscopie électronique**
Sandra Casale (UPMC – TEM-EDX)
Ludovic MOUTON (UPD - SEM)

# Pro-inflammatory response



# Size distribution following sonication and filtration process



# Stability study