Cell type- and size-dependent *in vitro* toxicity of silica particles in human skin cells

Cranfield UNIVERSITY

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Presentation Overview

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Introduction and Objectives

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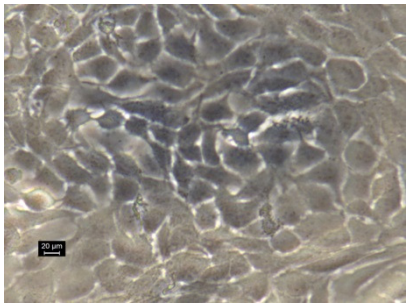
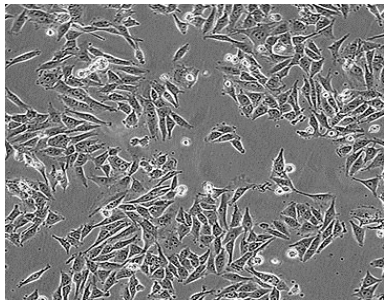
Amorphous silica particles (SiP): several applications, including biomedicine and biotechnology.

- Variable structure
- Toxicology not well studied
- Possible safety issues

- Is toxicology size-dependent for colloidal silica?
- Is toxicity cell-dependent?
- Is toxicology influenced by presence or absence of serum in culture media?



HaCaT cell line



A375 cell line

Materials and Methods

Amorphous colloidal silica particles

Particle Name	Supplier
A-SiNP 20 nm	Sigma Aldrich
A-SiNP 70 nm	Postnova Analytics
A-SMP 200 nm	Postnova Analytics
A-SMP 500 nm	Postnova Analytics

Methods:

- **Characterization:** DLS, Zeta Potential, TEM in culture medium;
- **Cell Toxicity assessment:** MTT test for mytochondrial toxicity
- **Cell death assessment**: staining of floating cells with Annexin V-FITC/Propidium Iodide and flow cytometer analysis

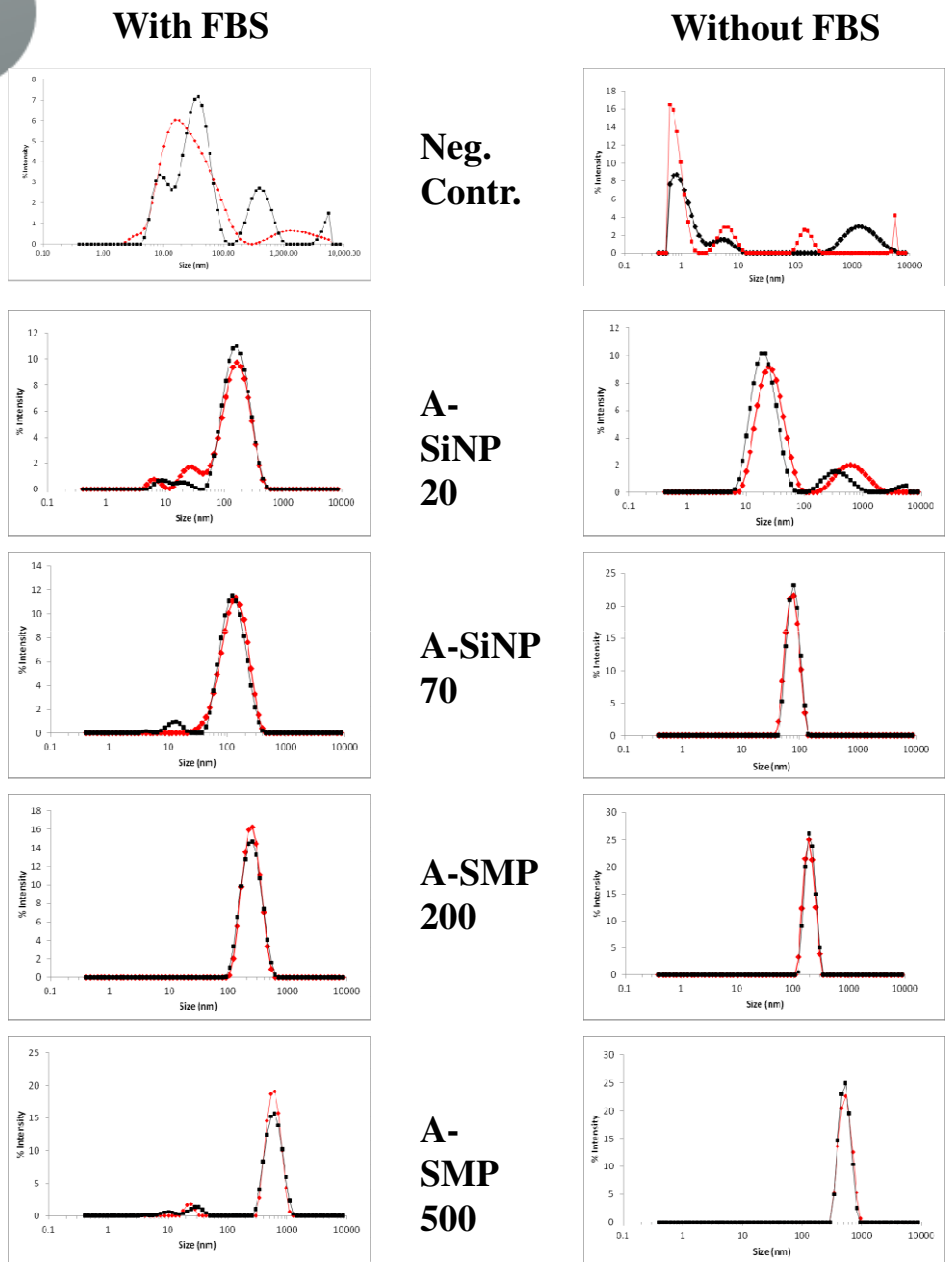
All samples were analysed in medium with and without Fetal Bovine Serum (FBS) at concentrations between 10 and 200 µg/ml.

Characterization - DLS

Particle	Size	
	W FBS	WO FBS
	0H / 24H	0H / 24H
A-SiNP 20 nm	96 / 119	29 / 22
A-SiNP 70 nm	96 / 93	71 / 72
A-SMP 200 nm	208 / 202	183 / 184
A-SMP 500 nm	426 / 216	492 / 473

- Profile didn't change overtime;
- Similar intensity at 0 and 24 hours;
- Average size was larger in the presence of FBS.

Good dispersion and serum-dependent size variation

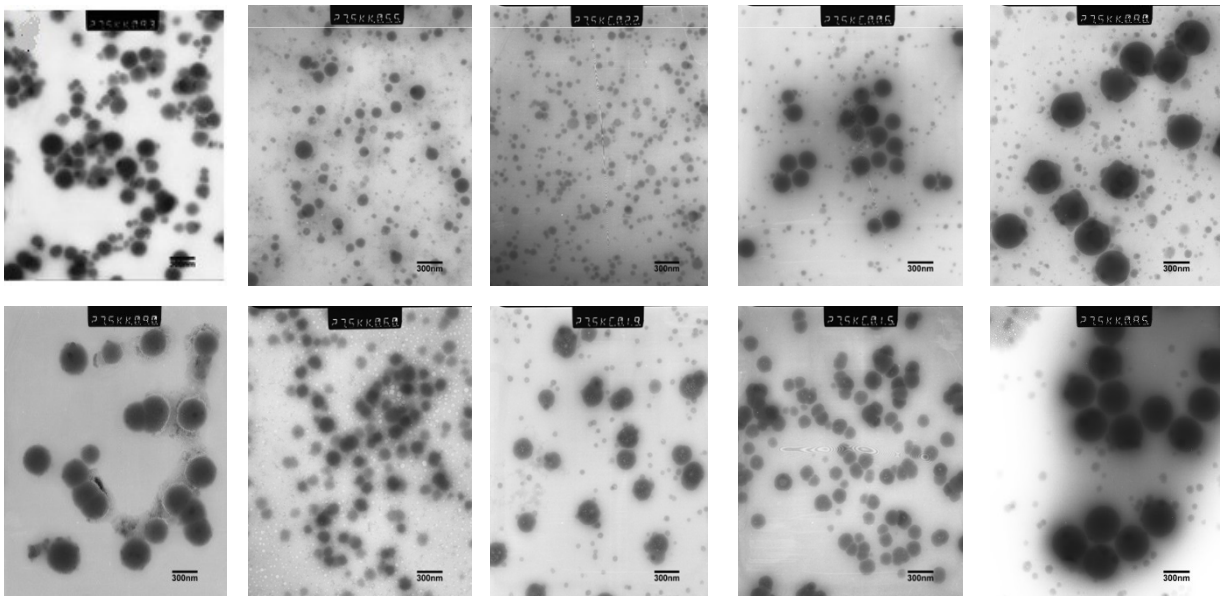


Characterization – Zeta Potential and TEM

- Negative zeta potential for all samples;
- Higher zeta potential and size-dependent change in the absence of FBS.

	With FBS	Without FBS
Neg. Contr.	-10.6	-10.4
A-SiNP 20	-12.5	-13
A-SiNP 70	-14	-17.9
A-SMP 200	-10.2	-19.2
A-SMP 500	-12.4	-13.5

Neg. Contr. **A-SiNP 20** **A-SiNP 70** **A-SMP 200** **A-SMP 500**



FBS

NO FBS

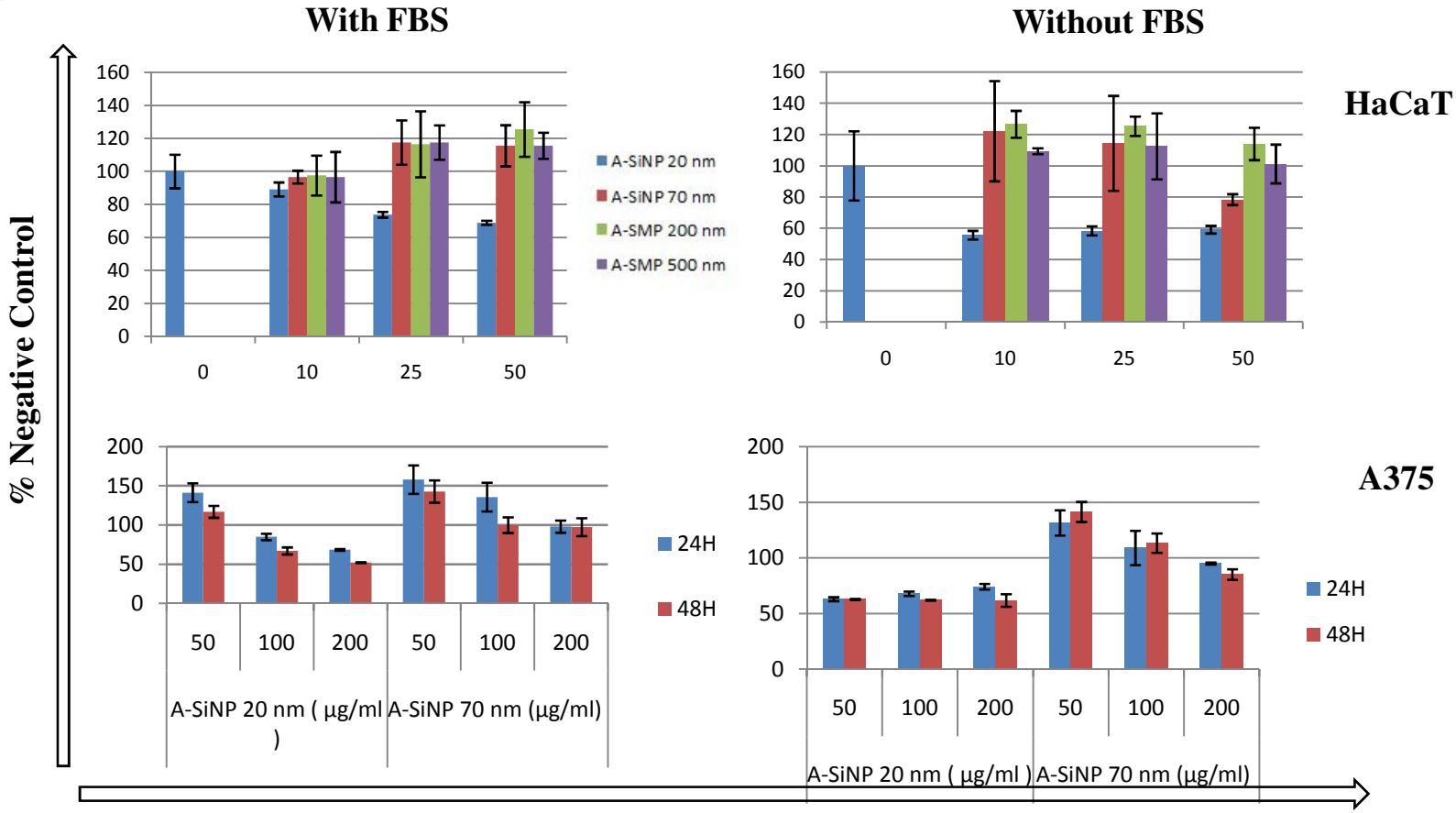
- Spherical shape
- Larger particles associated with sample preparation

Stability in medium, care should be taken when preparing samples

Cell Toxicity assessment

HaCaT cells: size- and dose-dependent effect for A-SiNP 20 nm and 70 nm both in the presence and absence of FBS.

A375 cells: size- and dose-dependent effect for A-SiNP 20 only.



Apoptosis assay on HaCaT cells

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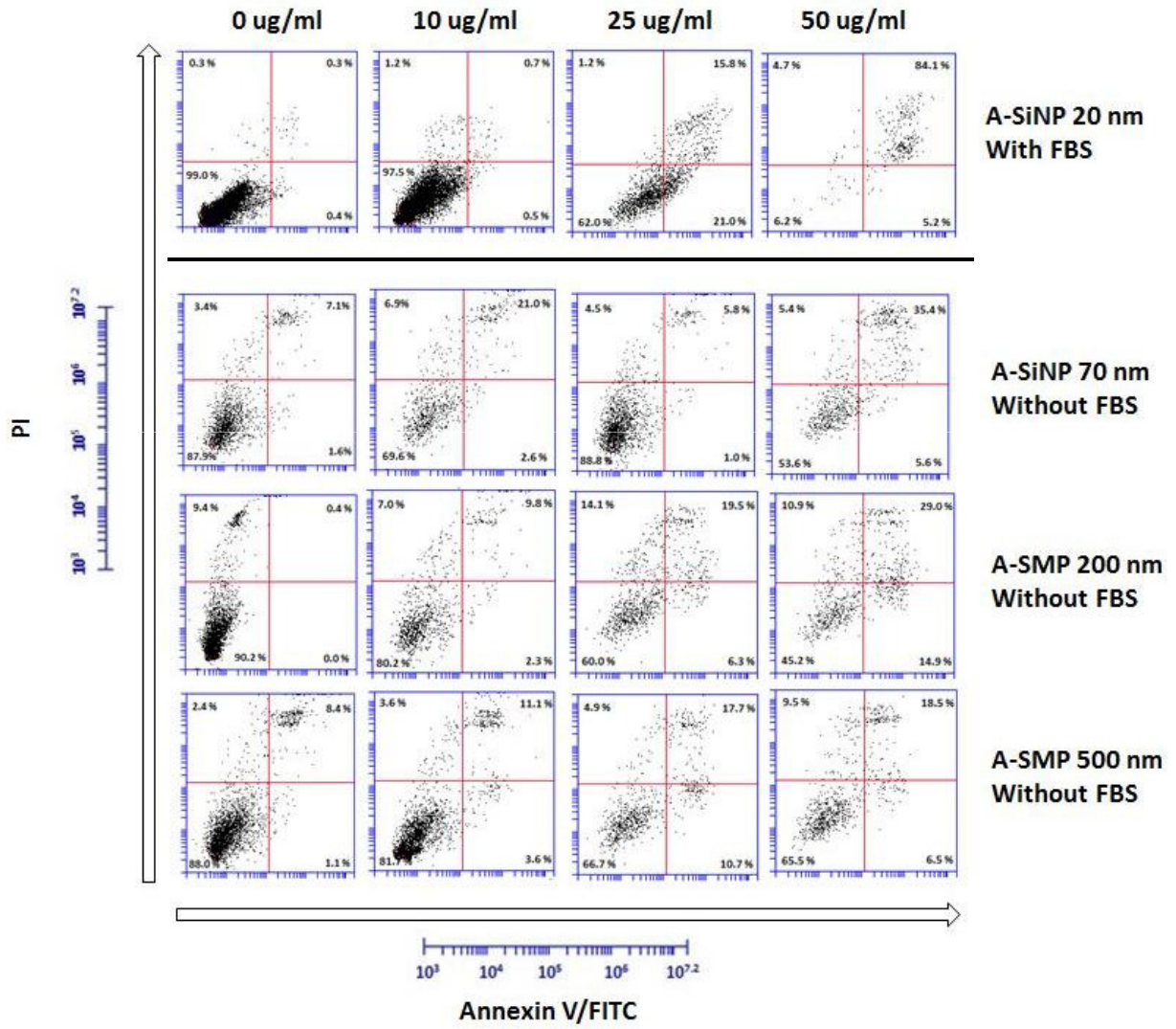
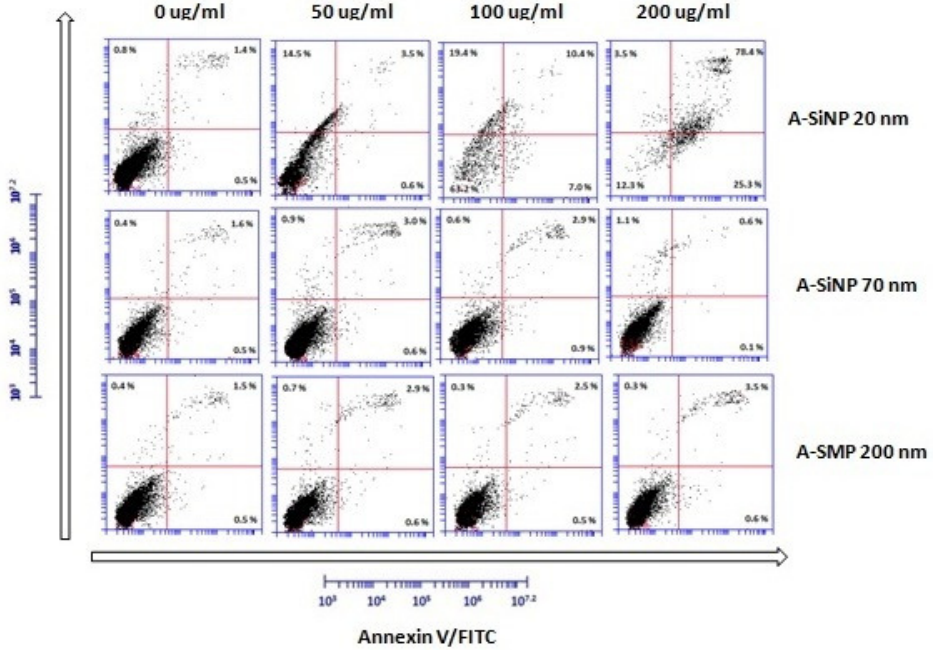
Apoptosis analysis on HaCaT cells showed a dose- and size-dependent effect, with A-SiNP 20 and A-SiNP 70 being the most toxic.



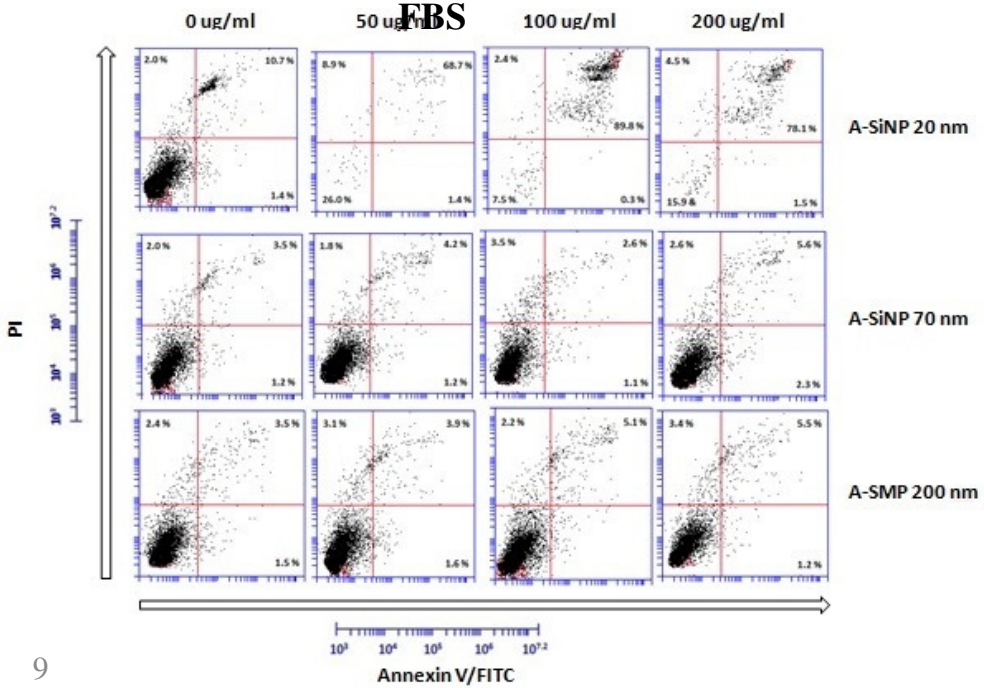
Apoptosis assay on A375 cells

Presence of FBS: dose-dependent effect for A-SiNP 20 nm; A-SiNP 70 and A-SMP 200 show little to no effect.

With FBS



Without FBS



Absence of FBS: dose-dependent effect for A-SiNP 20 nm; A-SiNP 70 and A-SMP 200 show little to no effect.

Discussion

- **Characterization:** Spherical shape, good dispersion and serum-dependent size variation.
- **Cell Viability:** A375 was sensitive only to A-SiNP 20, HaCaT to A-SiNP 20 and A-SiNP 70.
- **Apoptotic cell death:** detected for A-SiNP 20 nm both on HaCaT cells and A375 cells; other particles showed effect only on HaCaT cells in the absence of serum.

Conclusions

- Toxicity for colloidal silica is size-dependent in HaCaT cells, with smaller particles (A-SiNP 20 and 70 nm) being more toxic than its larger counterparts; on A375 cells, on the other hand, only A-SiNP 20 nm showed toxic effects;

- Toxicity is cell type dependent, as the same particles showed different behaviour on HaCaT and A375 cells. Cell death is mainly due to apoptosis;

- Nanoparticles characteristics and toxicity are influenced by serum, as particles were more toxic when it's absent from the suspension medium.

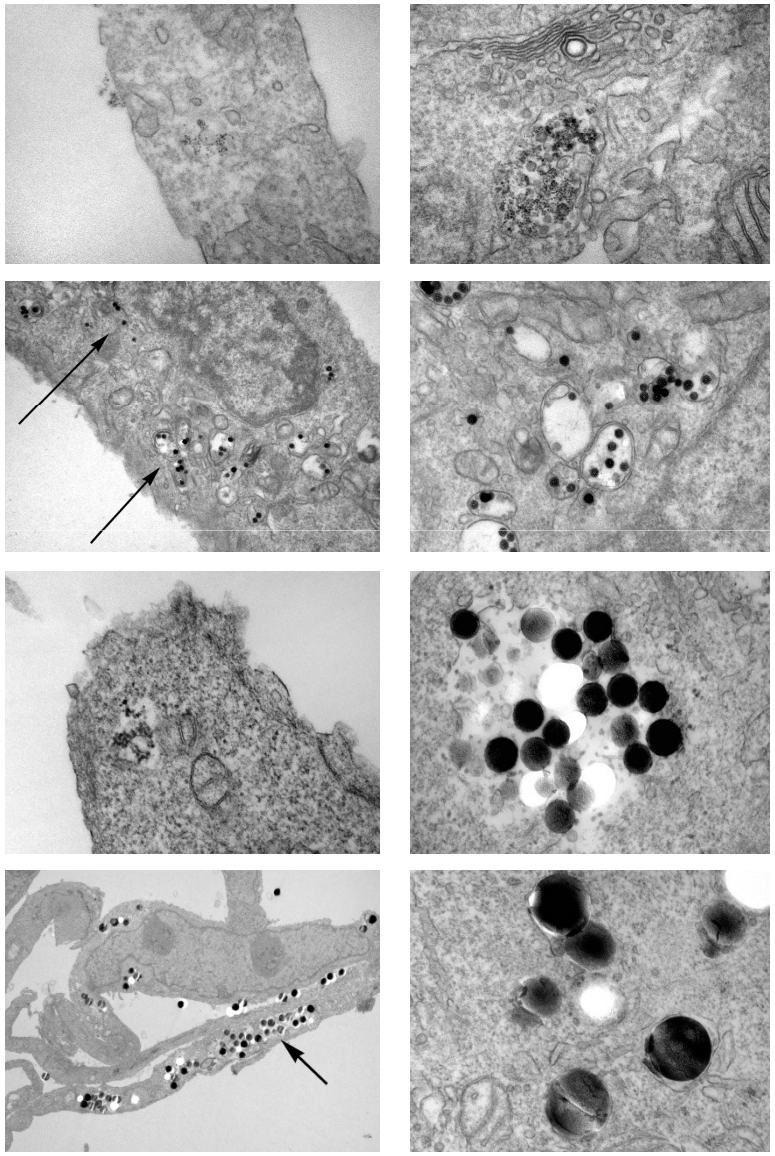
Further work

TEM Imaging on fixed HaCaT cells (in collaboration with MRC Toxicology Unit Leicester):

- Uptake at low concentration by endocytosis;
- Mostly observed in intracellular vacuoles and also free in cytoplasm;
- None of the particles was found in nucleus.

Molecular response to uptake and toxicity?

With FBS




thank you
gracias
danke
merci
obrigado
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teşekkür ederim
go raibh maith agat
mochchakkeram
sukriya
kop khun krap
terima kasih
dziękuję
bedankt
спасибо
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감사합니다
ありがとう
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