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Bio-kinetics of single ultrafine particles and agglomerates at the lung barrier

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Motivation

Air pollution is a predominant and recurring concern in our modern society associated to lung and cardiovascular diseases.[1,2] Increased concern has been expressed regarding the adverse health effects elicited by exposure to ultrafine particles (UFP) fraction (<100 nm) of the ambient particulate showing specific toxicological effect.[3,4] In order to better understand the risk associated to UFP inhalation, a clear understanding on their bio-kinetics at the air-blood barrier must be gained. To date, however, the correlation of primary and secondary particle size, i.e. single particles vs. agglomerates, and their cellular uptake and / or translocation across the air-blood tissue barriers are not yet understood.

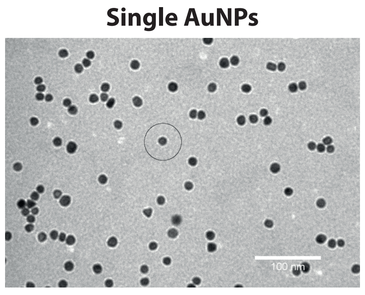
GOAL: STUDY BIO-KINETICS OF NANOPARTICLES AT THE HUMAN LUNG TISSUE IN VITRO BARRIER AND COMPARE SINGLE TO AGGLOMERATE PARTICLES

taken from: http://www.theguardian.com/uk/2013/jan/27/diesel-engine-fumes-worse-petrol

Method

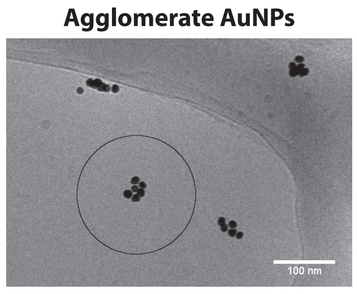
Particles model: Engineered gold nanoparticles[5] (AuNPs)

Single AuNPs

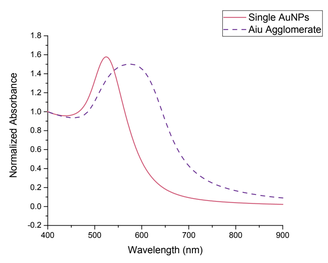


Single AuNPs TEM image, solid circle represents the hydrodynamic diameter (56 nm) measured by DLS.

Agglomerate AuNPs



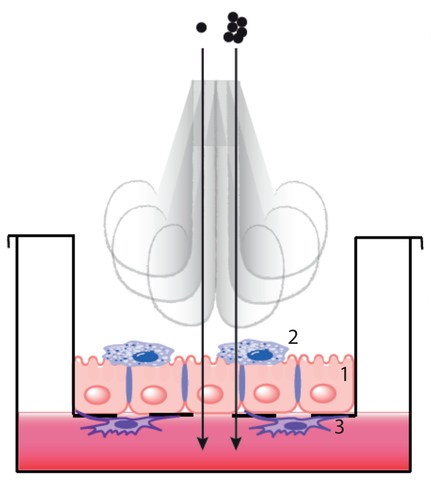
AuNPs Agglomerates cryoTEM image, solid circle represents hydrodynamc diameter (192 nm) measured by DLS.



Extinction spectra of the single AuNPs and the AuNPs agglomerates. The spectra were normalized based on their absorbance at 400 nm.

In vitro approach to simulate realistic exposure environment

The aim is to analyse the deposition, internalisation and translocation of **nebulized nanoparticles** (single and agglomerates AuNPs) in the human lung by adopting a sophisticated *in vitro* approach that realistically mimics the inhalation of UFP.



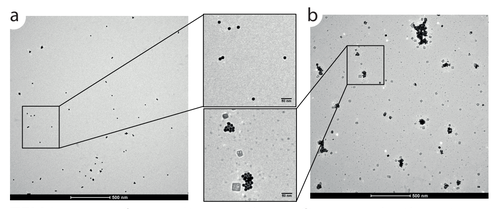
Single and agglomerate AuNPs were deposited using an air-liquid interface exposure system enabling a dose-controlled deposition.[6]

Advanced 3D lung model composed of human lung alveolar cells (A549) (1), macrophages (2) and dendritic cells (3) (human monocytes derived) which was cultured at the air-liquid interface.[7]

Results

Deposition

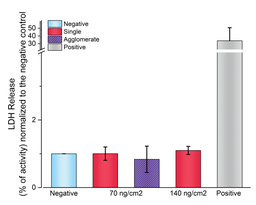
The nebulized particles were homogenously deposited at a dose of 70 and 140 ng/cm^2 as measured by ICP-OES.*



TEM images of deposited particles after nebulization at a dose of 70 ng/cm^2 of (**a**) single nanoparticles and (**b**) agglomerates

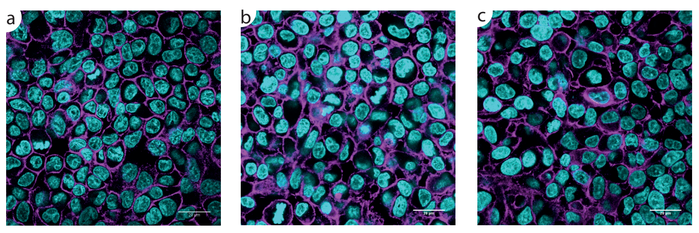
Biological Impact

Cell viability



LDH assay 24 h after exposure, data expressed as mean: n = 5 for single AuNPs at 70 and 140 ng/cm^2; n = 2 for AuNP agglomerates at 70 ng/cm^2; Positive control 0.1% Triton X-100, n=7

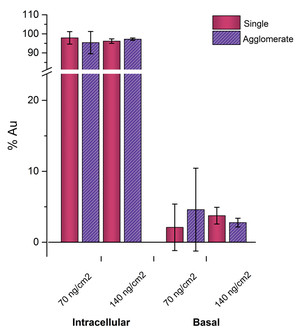
Cell morphology



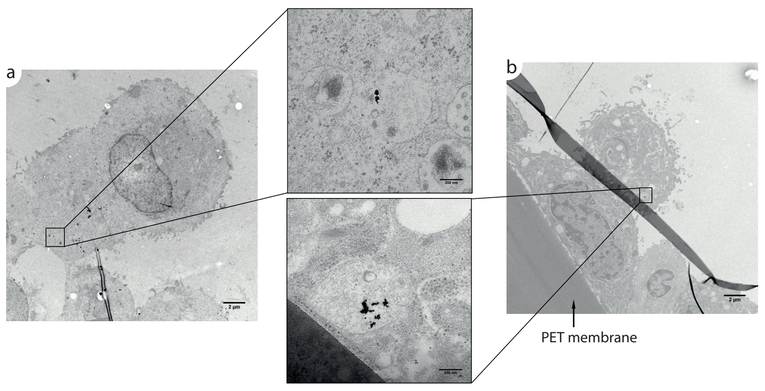
Laser Scanning Microscopy (LSM) of triple cell co-culture 24 h after exposure to **a**) NaCl-water solution, **b**) single AuNPs, 70 ng/cm^2 and **c**) AuNP agglomerates, 70 ng/cm^2. Immunofluorescence labelling of F-actin (magenta) and nuclei (cyan).

Bio-ditribution

Translocation of Au



Data obtained by ICP-OES measurement 24 h after exposure. Data expressed as mean: n = 2, except for single AuNPs at 70 ng/cm^2 n=6.



TEM images of the triple cell co-culture 24 h after exposure to 140 ng/cm^2 of (**a**) single and (**b**) agglomerate particles. In (**a**) and (**b**) the particles were found in the cytoplasm within a vesicle. In (**b**), on the bottom left can be seen the PET membrane on which the triple cell co-culture were grown.

* The dose 140 ng/cm^2 represents the mean of single particles at 130 ng/cm^2 and agglomerates at 150 ng/cm^2.

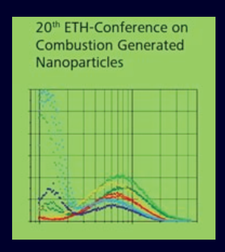
Conclusion

No apparent cytotoxicity, cell layer damage or pro-inflammation was observed after exposure to single nanoparticles or agglomerates at a concentration of 70 and 140 ng/cm^2. The biological kinetics revealed that the majority of the nanoparticles, singles or agglomerates, were taken up by cells and could be found in macrophages, epithelial and dendritic cells. Only a minor fraction, i.e. less than 3-5 %, was found in the basolateral side for both particles types, which is also corresponding to the translocation rate found *in vivo* for single gold nanoparticles.[8]

A longer exposure time to assess the nanoparticles fate, and exposure to bigger agglomerates should be assessed to see how agglomeration of singles particles can influence the cell up-take and the translocation of nanoparticles across the air-blood tissue barrier. It will broaden our general knowledge on nanoparticle-cell interactions and help to further understand the biological impact of agglomeration of UFPs in comparison to single particles after deposition on the lung cell surface.

References

(1) C. A. Pope, D. W. Dockery and J. Schwartz, Inhal. Toxicol., 1995, 7, 1–18.
(2) K. Donaldson, L. Tran, L. A. Jimenez, R. Duffin, D. E. Newby, N. Mills, W. MacNee and V. Stone, Part. Fibre Toxicol., 2005, 2, 10.
(3) A. Peters, H. E. Wichmann, T. Tuch, J. Heinrich and J. Heyder, Am. J. Respir. Crit. Care Med., 1997, 155, 1376–1383.
(4) H. Schulz, V. Harder, A. Ibald-Mulli, A. Khandoga, W. Koenig, F. Krombach, R. Radykewicz, A. Stampfl, B. Thorand and D. A. Peters, J. Aerosol Med., 2005, 18, 1–22.
(5) V. Hirsch, C. Kinnear, L. Rodriguez-Lorenzo, C. A. Monnier, B. Rothen-Rutishauser, S. Balog and A. Petri-Fink, Nanoscale, 2014, 6, 7325–31.
(6) A. G. Lenz, E. Karg, B. Lentner, V. Dittrich, C. Brandenberger, B. Rothen-Rutishauser, H. Schulz, G. A. Ferron and O. Schmid, Part. Fibre Toxicol., 2009, 6, 32.
(7) B. M. Rothen-Rutishauser, S. G. Kiama and P. Gehr, Am. J. Respir. Cell Mol. Biol., 2005, 32, 281–289.
(8) W. G. Kreyling, S. Hirn, W. Möller, C. Schleh, A. Wenk, G. Celik, J. Lipka, M. Schäffler, N. Haberl, B. D. Johnston, R. Sperling, G. Schmid, U. Simon, W. J. Parak and M. Semmler-Behnke, ACS Nano, 2014, 8, 222–33.



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