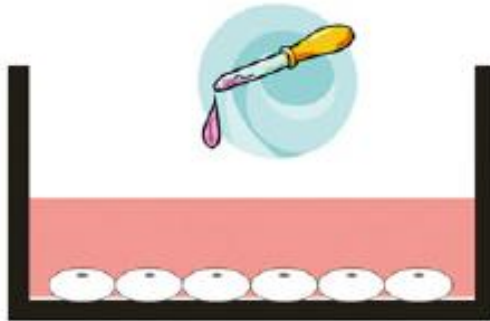


Comparing Nanomaterial Toxicity with Lung Cells Cultured under Air-Liquid Interface and Submerged Conditions

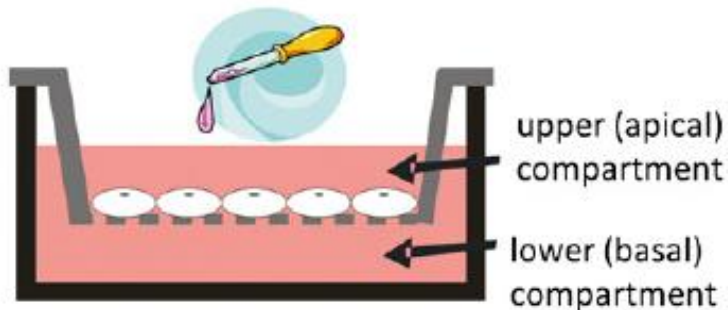
Dr. Yaobo Ding

Cell exposure at the Air-Liquid Interface

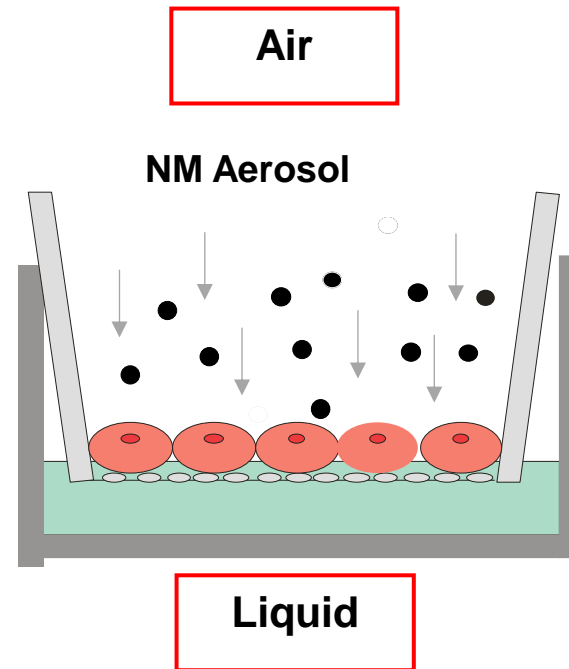
Submerged exposure



Or



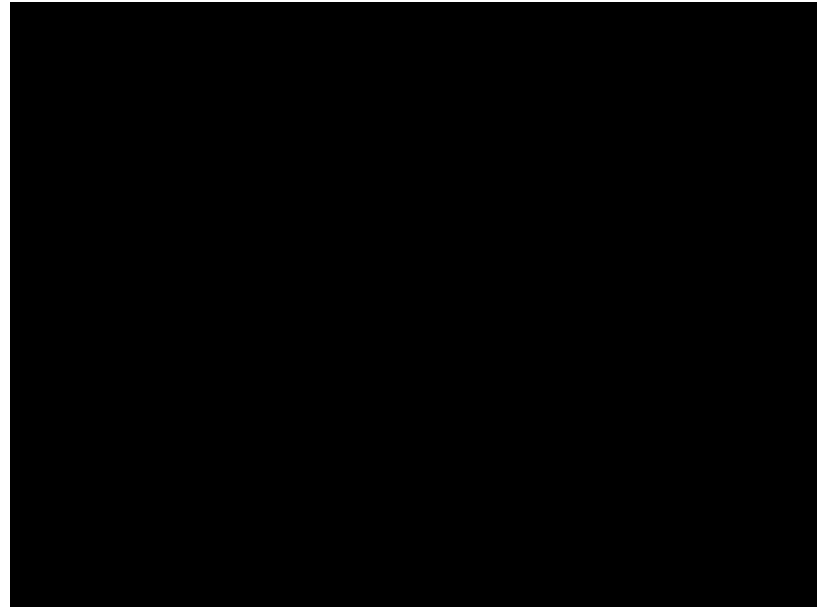
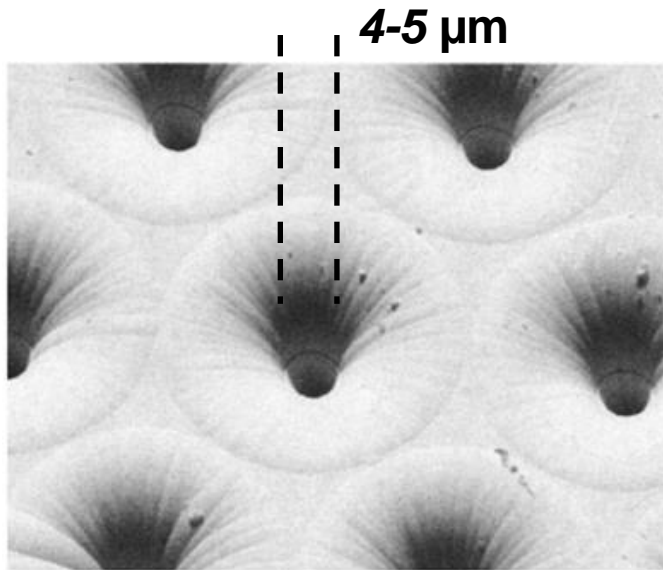
Air-liquid interface exposure



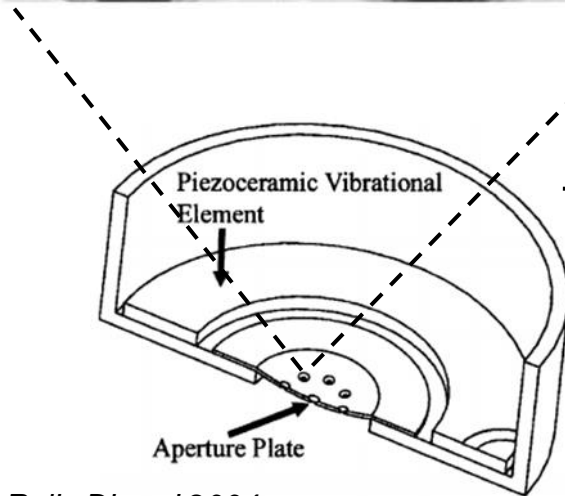
Physiological realistic testing !

Lenz et al., Am J Respir Cell Mol Biol., 2014

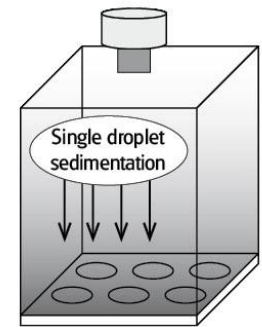
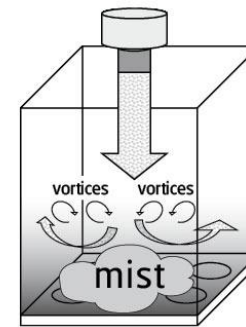
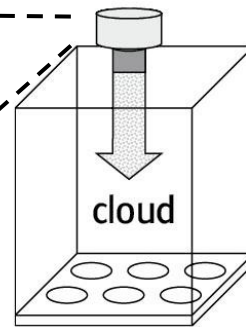
Cell exposure at the Air-Liquid Interface



<https://www.youtube.com/watch?v=1KdkmqmcxWY>
https://www.youtube.com/watch?v=1IA_SSScMeE



Lenz et al., *Am. J. Resp. Cell Mol. Biol.*, 2014

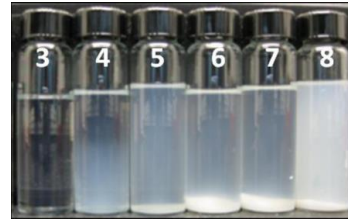
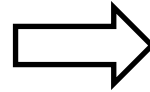


Rajiv Dhand 2004

Suspension preparation protocol

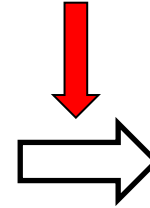


Nanopowders

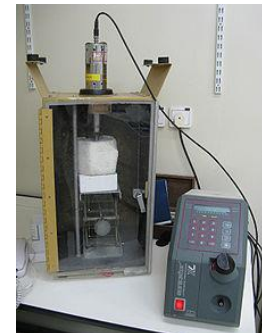
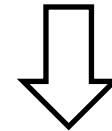


Suspended in pure water

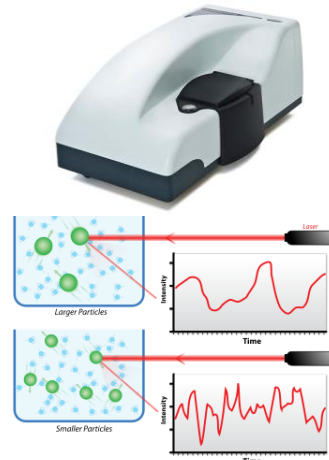
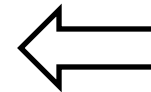
(NaCl)



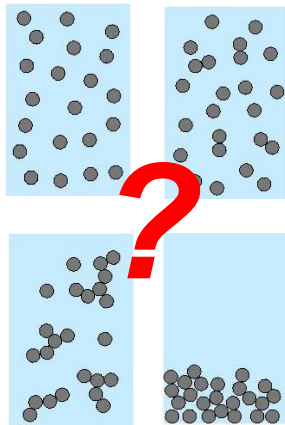
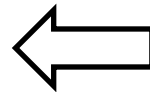
Vortex shaker



Sonication

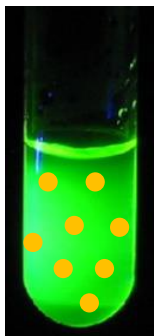


Dynamic light scattering

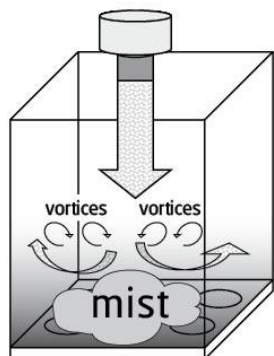


Uniform & fast delivery of NM aerosols

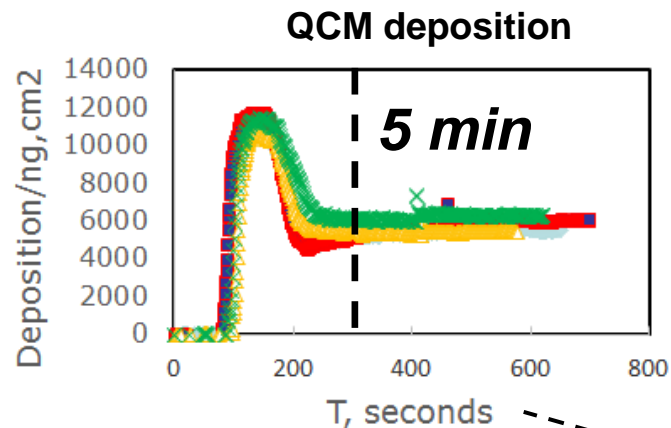
Fluorescein Concentration



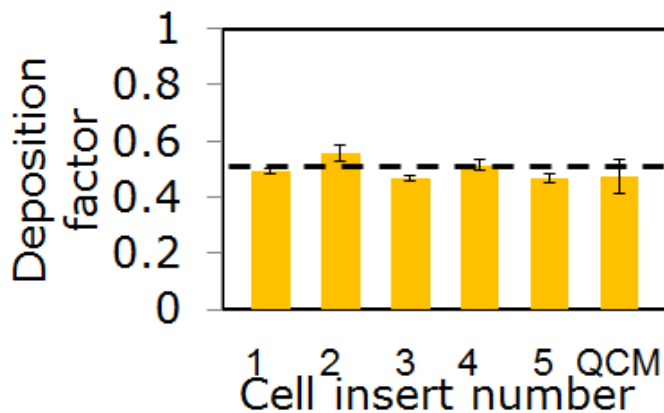
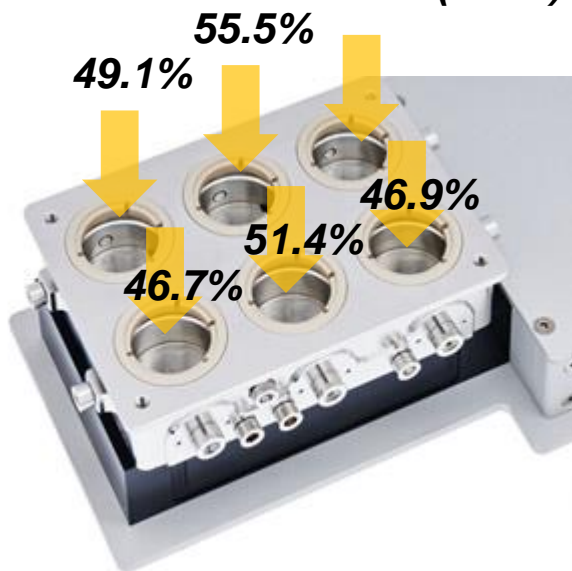
Nebulization



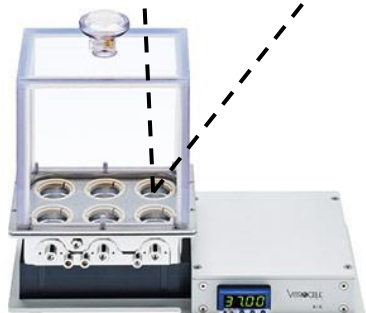
Fluorescein Concentration (plate reader)



47.5% (QCM)

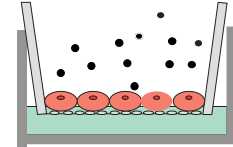
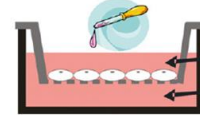
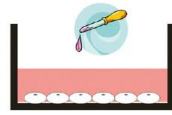


Mean deposition: 49.5%
Insert-insert variability: 3.4%



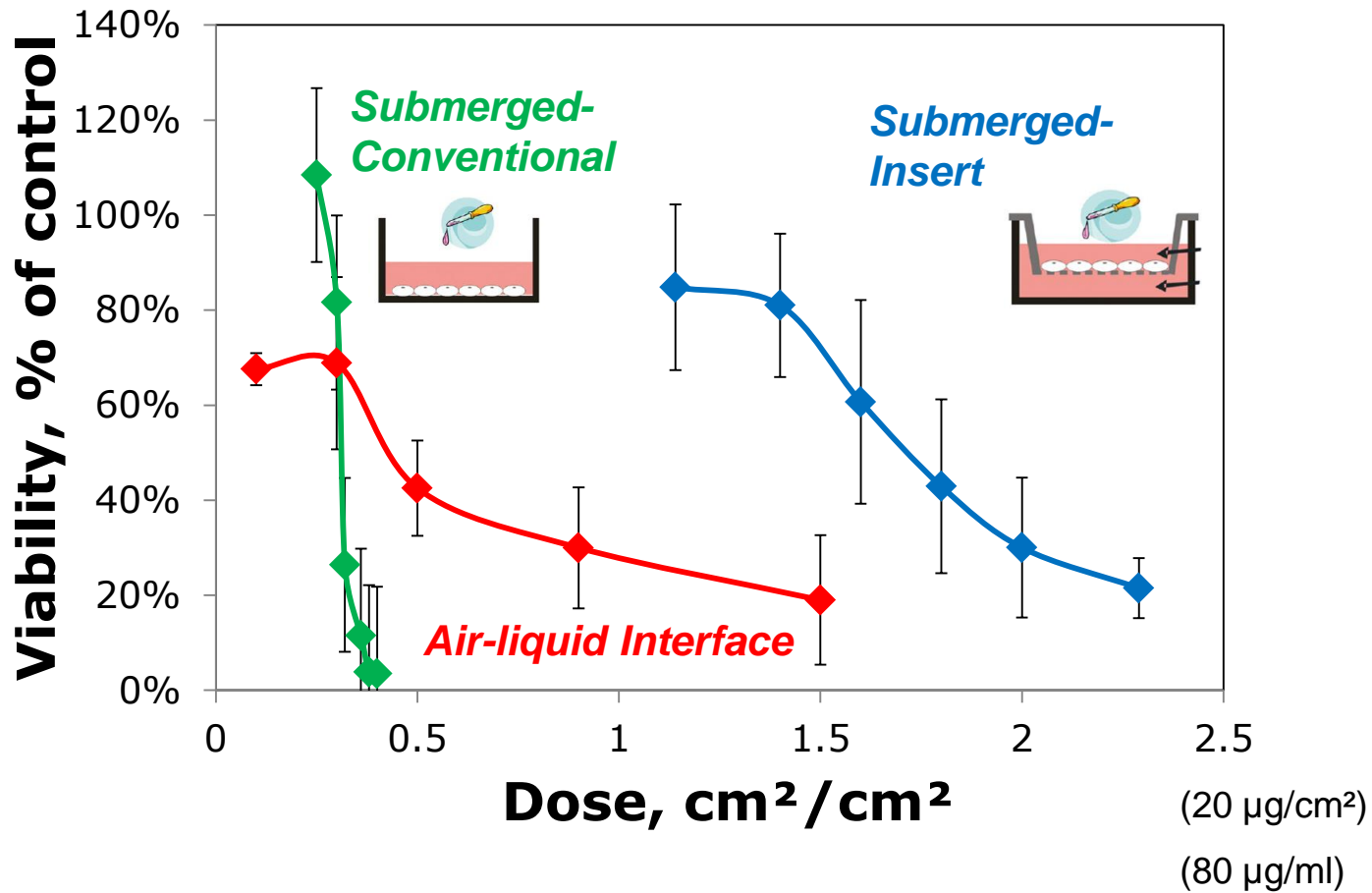
VITROCELL-CLOUD 6

Cell growth and exposure conditions

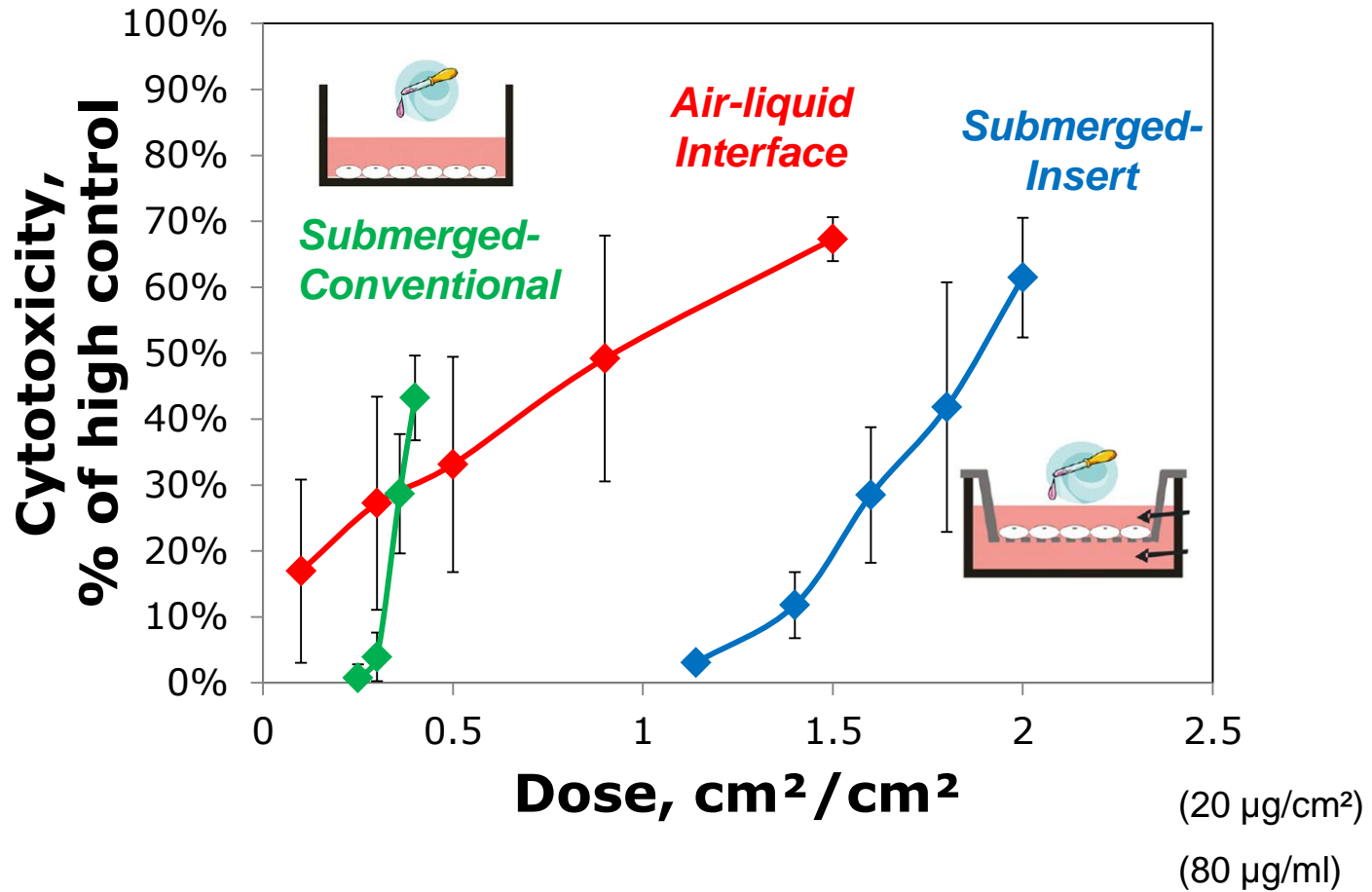


	Submerged	Sub-Insert	ALI
Plate type	6-well plate	6-well insert	6-well insert
Cell type	A549 IL-8	A549 IL-8	A549 IL-8
Seeding surface	9.6 cm ²	4.2 cm ²	4.2 cm ²
Seeding Nr.	1 mil.	1 mil.	1 mil.
Growth time	3 day	4 day	4d+1d (in air)
Apical Med.vol.exp.	1 ml	1 ml	0 ml
Med. Height	ca. 1 mm	ca. 2.4 mm	0.01 mm (ZnO suspension)
	Type	Size, TEM	BET SA
Nanomaterial	ZnO NM110	50-150 nm	12 m ² /g

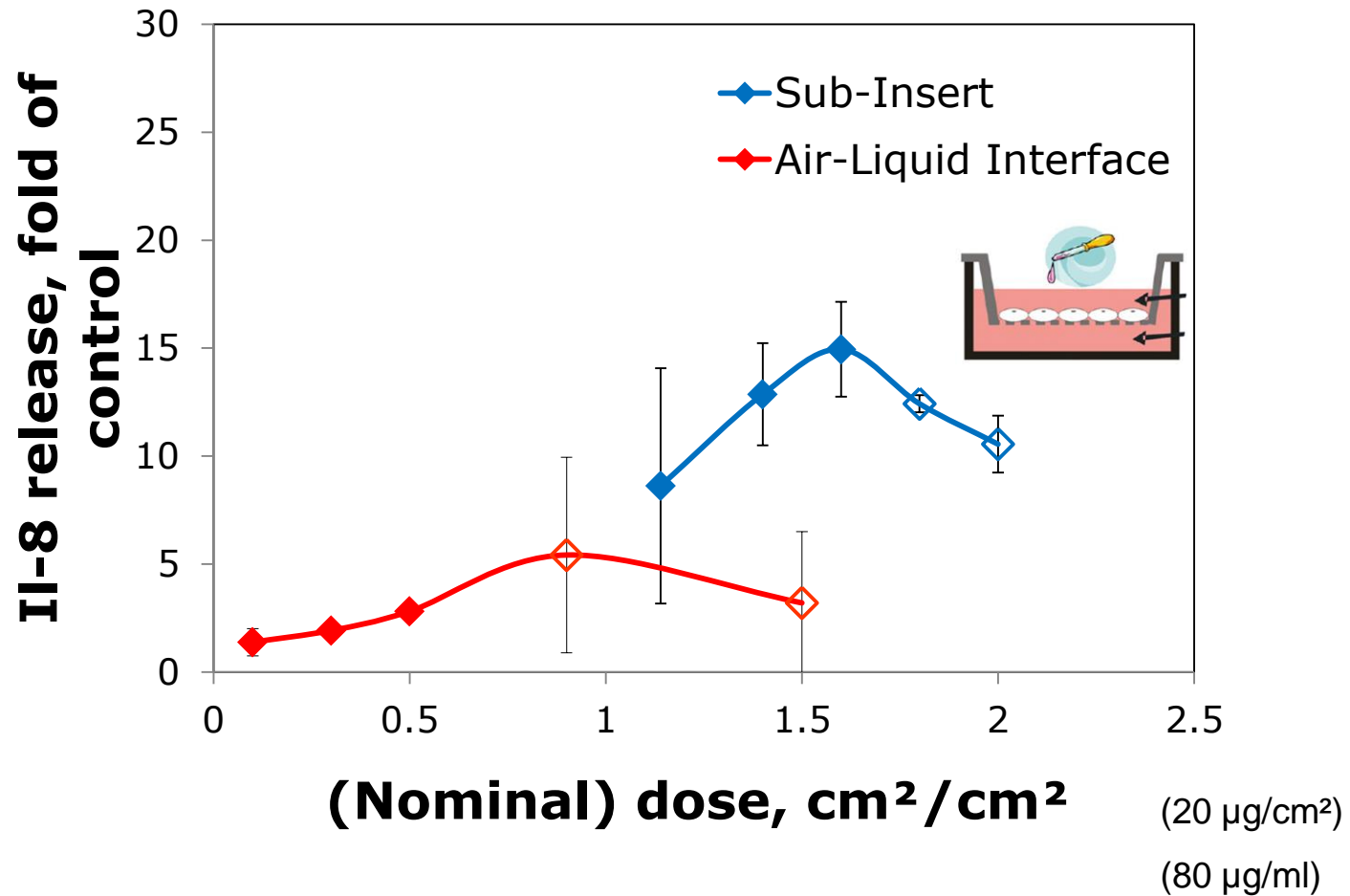
Cell viability (WST-1 assay), 24h



Cytotoxicity (Lactate Dehydrogenase release), 24h



Interleukin-8 induction, 24h

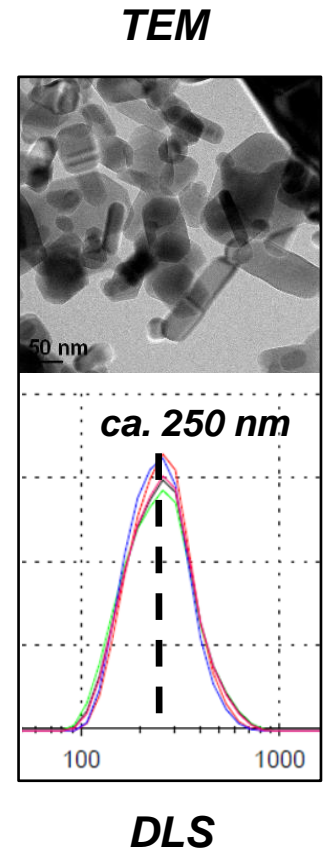
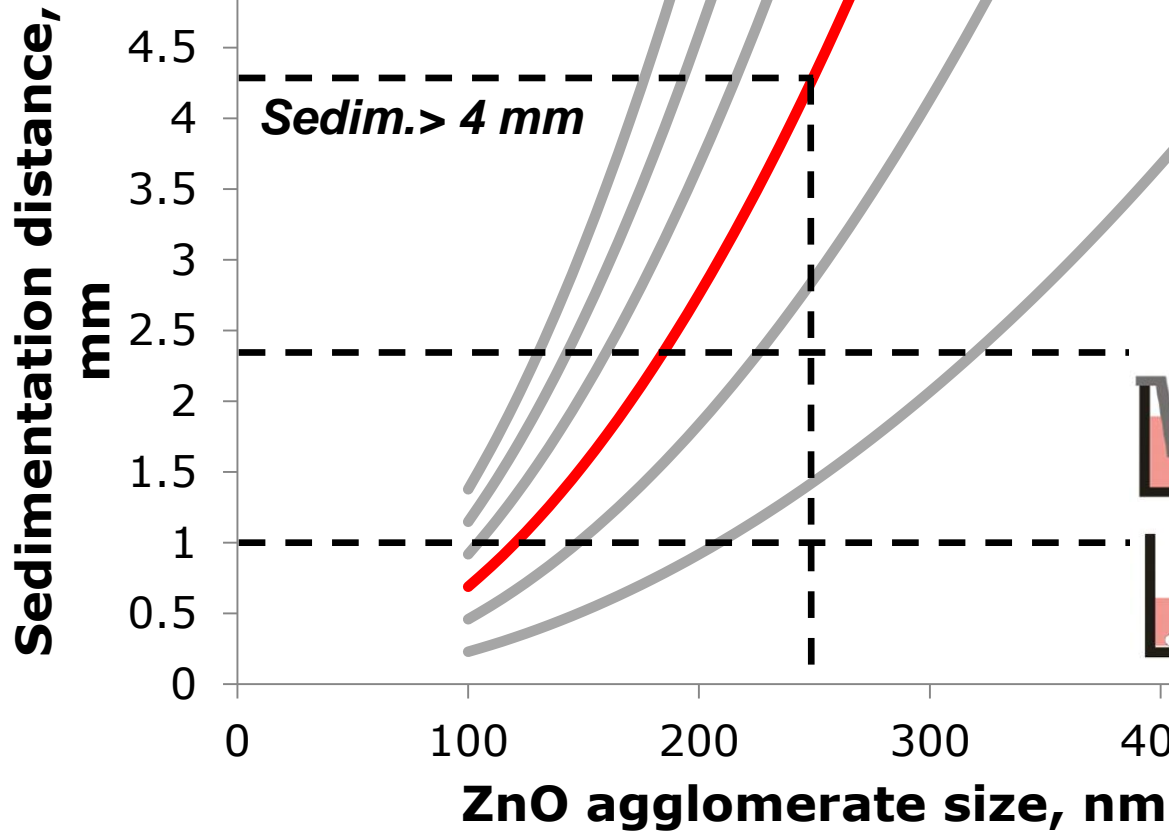


Calculation of Sedimentation

(Bulk density: 5.6 g/cm³)

Effective density 4.0 3.5 3.0 2.5 2.0

1.5 g/cm³



*Colloid and Surface Chemistry, Duncan J. Shaw, 4th edition, 1992 p.21-25

Summary

- I. Uniform and efficient aerosol delivery was achieved using VITROCELL-CLOUD (ALICE-CLOUD) with 5 min exposure time. Suspension quality is controlled for aerosolization.**
- II. Cell viability decreased and LDH/IL-8 induction increased when dose increased**
- III. ZnO nanoparticle dose-response curve of lung epithelial cells varied for submerged (sharp) and air-liquid interface (broad) exposures**
- IV. Cells grown in plastic surface (well) and at porous insert surface behaved differently:**
 - I. dose rate**
 - II. Cells are more sensitive in plastic wells**
 - III. both**

Thank you !



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German Research Center for Environmental Health

Reference

Vitro-cell ALICE video: <https://www.youtube.com/watch?v=1KdkmqmcxWY>

Vitro-cell ALICE image: <http://www.vitrocell.com/inhalation-toxicology/exposure-systems/vitrocell-cloud-system>

Aeroneb video: https://www.youtube.com/watch?v=1IA_SSScMeE