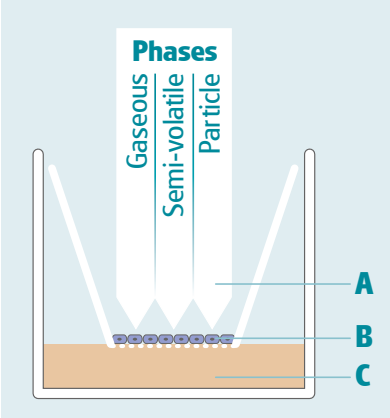
Novel TEM Grid holders for dose determination of airborne particles during *in vitro* Exposure at the air / liquid interface

Background

The assessment of toxicological effects of airborne particles to the human organism is of major importance in disease research. The presence of nano and ultrafine particles can be found both in indoor and outdoor atmospheres which include workplaces and living areas.



Picture 1: Air / Liquid Cultivation and Exposure in Exposure Module

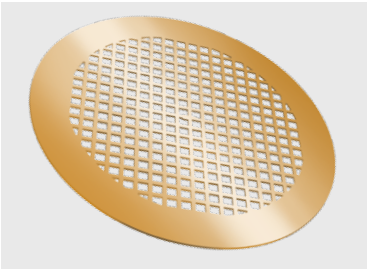
- **A** Direct and controlled exposure of test atmosphere to cells
- **B** Cells on membrane
- **C** Medium below cells

Over the last years, the degree of contribution to diseases of such particles is analyzed ever more frequent by the use of *in vitro* methods. The required equipment for these studies consisting of aerosol generation and exposure systems are nowadays developed more and more to proven levels. Here the exposure of lung cells to particles at the air/liquid interface is the prime choice over submerse exposure conditions as interaction of particles with cell culture media is avoided. So the air/liquid interface is a physiologic relevant exposure technique (picture 1).

Furthermore, dose determination under submerse conditions, thus which amount of the substance has reached the cells, is extremely difficult.

For over 10 years, VITROCELL® air/liquid interface Exposure Modules can be equipped with VITROCELL® Crystal Quartz Microbalances to assess the deposited mass in ng/cm² in real time.

The subsequent required information is related to size, shape and distribution of



Picture 2
TEM grid sample carrier

the particles at the level of the cell culture insert. For this analysis Transmission Electron Microscopy (TEM) is a proven method. Here a beam of electrons is transmitted through a specimen to form an image. The resolution is significantly higher than of usual light microscopes.

For this purpose, particles are captured on TEM grid sample carriers (picture 2) and then transferred to the TEM microscope. Due to the small size of the grids, placement in cell culture inserts is extremely difficult and it is almost impossible to position them always at the same location.

Solution

For application inside the Vitrocell® exposure modules, a new insert with integrated TEM grid holders was developed together with the Karlsruhe Institute of Technology (KIT).

The TEM grid holders can be equipped with standard sample carriers to analyse number, morphology and distribution of deposited particles. The grids are located on the same level as the cells during a regular exposure. Furthermore, the grids can be fixed on different radii on the insert surface to evaluate spatial distribution. The fixation also prevents the grid from any motion on the insert during the experiment. The VITROCELL® TEM Grid Holders are commercially available for the 6- and 12-well sizes and fit into the VITROCELL® 6 and 12 module series.

12- and 6-well sized holder equipped with TEM-Grids

VITROCELL® Systems GmbH
Waldkirch / Germany
Advanced in vitro exposure systems.

Components with highest precision

1

2

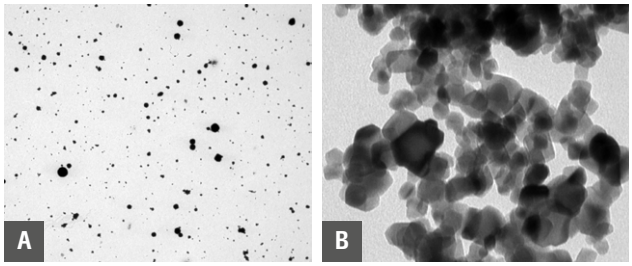
3

1. Holder base plate (left) with counterplate (right) in mounting position
2. Holder base plate with inserted grids / with counterplate and TEM grids in receiving position
3. Complete TEM Grid Holder

Results

Picture 3 shows images of particles deposited on sample grids inside the VITROCELL® Exposure Modules. While image 3A shows an overview of the deposition pattern, picture 3B gives detailed information regarding size, shape and agglomeration state of the particles of the investigated aerosol.

A recent publication in Nature Scientific Reports (Mülhopt, S. et al. 2020) demonstrated usability as well as reliability of the novel TEM Grid holder system.



Picture 3: Distribution of TiO_2 Particles in a 800-fold (A) and in a 125 000-fold (B) magnification (images courtesy of KIT)

References

Nature Scientific Reports, volume 10, Article number: 8401 (2020)
Published: 21 May 2020, Open Access: **"A novel TEM grid sampler for airborne particles to measure the cell culture surface dose"**

Sonja Mülhopt, Christoph Schlager, Markus Berger, Sivakumar Murugadoss, Peter H. Hoet, Tobias Krebs, Hanns-Rudolf Paur & Dieter Stapf

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