A multiculture cell exposure chamber for the assessment of airborne and engineered nanoparticles effects on health

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In order to study the various health influencing parameters related to engineered nanoparticles as well as to soot emitted by Diesel engines, there is an urgent need for appropriate sampling devices and methods for *in vitro* cell exposure studies that simulate the respiratory system and facilitate associated biological and toxicological tests.

The objective of the present work was the further advancement of a Multiculture Exposure Chamber (MEC) (initially developed in Papaioannou *et al*, see also Asimakopoulou *et al*, 2011) into a dose-controlled system for efficient delivery of nanoparticles to cells (Figure 1). It was validated with various types of nanoparticles (Diesel engine soot aggregates, SiO_2 nanoparticles) and with state-of-the-art nanoparticle measurement instrumentation to assess the local deposition of nanoparticles on the cell cultures.

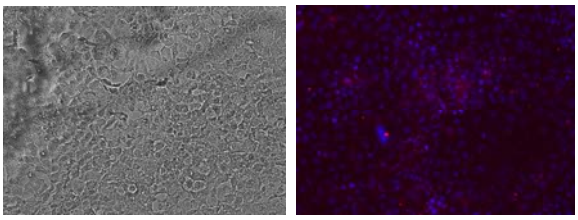
Figure 1. The upgraded Multiculture Exposure Chamber (MEC).

The dose of nanoparticles to which cell cultures are being exposed was evaluated in the normal operation of the *in vitro* cell culture exposure chamber based on measurements of the size specific nanoparticle collection efficiency of a cell-free device. The average nanoparticle deposition efficiency in the MEC is approximately 82%.

A high degree of flow homogeneity has been observed with flow visualization tests, while further design modifications in the future will provide perfect flow distribution similar to that of the Papaioannou *et al* MEC version.

The nanoparticle deposition was demonstrated by Transmission Electron Microscopy (TEM). TEM grids were placed in the 6-well plate inserts both in empty wells and in wells containing double-distilled water. The overall picture of the TEM grids used in our experiments reveal that soot particles reach the entire exposed MEC space. Further validation studies of particle transport in the MEC are performed employing Computational Fluid Dynamics (CFD).

In-use testing of the dose-controlled cell exposure system was performed by exposing A549 lung cell cultures to fluorescently labelled SiO_2 nanoparticles. Deposition of the nanoparticles on the cell cultures was demonstrated by visualization of the nanoparticle fluorescence in the cell cultures following exposure (Figure 2). The potential of the deposited nanoparticles to generate reactive oxygen species (ROS) (e.g. free radicals and peroxides) was also monitored, thus expressing the oxidative stress of the cells, which can cause extensive cellular and/or DNA damage. The efficient delivery of nanoparticles as well as the homogeneity in particle deposition among the 6-well plate inserts was demonstrated by fluorescence microscopy.



(a) (b)

Figure 2. Microscope images of A549 cells exposed to Fluoprobe532 labeled SiO_2 nanoparticles. Magnification = 200x, Blue = nuclei (DAPI), Red = nanoparticles (Fluoprobe532).

Papaioannou E., Konstandopoulos A.G., Morin J.P. and Preterre D., (2006) *SAE Tech. Paper* No. **2006-01-1075 (SP-2024)**, 389-399.

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