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Toxicity testing of combustion aerosols at the air–liquid interface with a self-contained and easy-to-use exposure system

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ABSTRACT

In vitro toxicity testing of airborne particles usually takes place in multi-well plates, where the cells are exposed to a suspension of particles in cell culture medium. Due to the artefacts caused by particle collection and preparation of suspensions, the air–liquid interface (ALI) exposure is challenging this conventional exposure technique to become the method of choice. The ALI technique allows for direct sampling of an aerosol and exposure of cell cultures to airborne particles. At the same time, it reflects the physiological conditions in the lung to a greater extent. So far, the available ALI systems have mostly been laboratory set-ups of the single components. Here, we present a mobile and complete system providing all process technology required for cell exposure experiments at dynamic aerosol sources. The system is controlled by a human machine interface (HMI) with standard routines for experiments and internal testing to assure reproducibility. It also provides documentation of the exposure experiment regarding process parameters and measured doses. The performance of this system is evaluated using fluorescein-sodium dosimetry, which is also used to determine the factor of dose enhancement by optional electrostatic deposition. The application of the system is shown for two different technical aerosol sources: wood smoke particles emitted by a household log wood stove

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and emissions from a ship diesel engine. After exposure of lung cells, cytotoxicity and gene regulation on a genome-wide scale were analysed.

Nomenclature

A	surface area of cell culture [cm²]
$c_{m,SMPS}$	aerosol mass concentration calculated from SMPS measurement [mg/cm³]
N_i	number concentration in channel i of the SMPS measurement [1/cm³]
d_i	particle diameter in channel i of the SMPS measurement [nm]
ρ_P	particle density [g/cm³]
f	deposition efficiency, deposited particle fraction [%]
RID	relevant in vitro dose (Cohen, Teeguarden & Demokritou, 2014)
$RID_{m,FSD}$	deposited particle mass measured by fluorescence spectroscopy [μg/cm²]
$RID_{SMPS,diff}$	diffusional deposited dose calculated from SMPS data [μg/cm²]
$RID_{TEM,diff}$	diffusional deposited dose calculated from TEM data [μg/cm²]
$RID_{SMPS,HV}$	electrostatic deposited dose calculated from SMPS data [μg/cm²]
$RID_{TEM,HV}$	electrostatic deposited dose calculated from TEM data [μg/cm²]
$t_{exposure}$	duration of exposure [h]
$V_{exposure}$	aerosol flow rate [l/h]

1. Introduction

1.1. Toxicity testing of submicron particles

During the first half of the 20th century, several episodes of extreme air pollution in European and US cities demonstrated that airborne particulate matter adversely affects human health (Dockery & Pope, 1994). Since then, many epidemiological studies have consistently linked air pollution to higher morbidity and mortality (Anderson, Thundiyil & Stolbach, 2012; Dockery, 2009). *In vivo* and *in vitro* data available on the toxicity of aerosols from specific sources generally support the epidemiological findings and give important insights into molecular mechanisms and the effects of specific physical and chemical properties of aerosol components, as was summarised by recent reviews (Kelly & Fussell, 2012; Nemmar, Holme, Rosas, Schwarze & Alfaro-Moreno, 2013; Schwarze et al., 2006).

Toxicity of airborne particles following inhalation can be studied either by *in-vitro* or by *in-vivo* experiments. The advantages and limitations of both test methods have been discussed in detail elsewhere (Maier et al., 2008; Sayes, Reed & Warheit, 2007). *In-vitro tests* are conducted with organ-specific, often human, test cells. The deposition of originally airborne particles onto test cells is carried out either from the liquid phase (submerged exposure) or from the gas phase at the air–liquid interface (ALI). Classical submerged testing of particles allows for straightforward analyses of a large number of different particles, concentrations, and time points within a short period in particular when high-throughput methods are applied (Nel et al., 2013). However, this test method has several limitations with respect to particles and cells:

(1) It is not representative of the conditions in the lung, because the cells are covered by a few millimetres of culture medium. This changes the oxygen partial pressure in comparison to the lung surface, where the layer of lung-lining fluid covering the cells is extremely thin (Blank, Rothen-Rutishauser, Schurch & Gehr, 2006).
(2) For submerged exposure of particles, which are components of complex aerosols, the particles must be separated from the gas phase by filtration. Collection of the solid particles, however, may change their agglomeration state and their chemical composition. Semi-volatile compounds in the filtered gas may adsorb to the deposited particles or be removed partly (Subramanian, Khlystov, Cabada & Robinson, 2004).
(3) The particle properties will be changed by dispersion in cell culture medium, which contains a large number of biomolecules, including serum proteins. Proteins are known to adsorb to the particles, form a corona, and may prevent adverse effects to the cells (Monopoli, Wan, Bombelli, Mahon & Dawson, 2013; Panas et al., 2013).
(4) In submerged exposure the dose cannot be determined correctly because of several reasons: as the agglomeration state is unknown, settling velocity is not defined; particles may also dissolve partially in the culture medium (Teeguarden, Hinderliter, Orr, Thrall & Pounds, 2007). For submerged exposure the particle dose is often delivered as a bolus. During inhalation of aerosols, by contrast, the particles are deposited linearly over a defined period. This may have an effect on the quality and intensity of the biological effects.