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Characterization of a multiculture in-vitro cell exposure chamber for assessing the biological impact of diesel engine exhaust

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Abstract. In order to study the various health influencing parameters related to particulate as well as to gas-phase pollutants emitted by Diesel engine exhaust, there is an urgent need for appropriate sampling devices and methods for cell exposure studies and associated biological and toxicological tests. In a previous paper [1], a specific concept for a cell culture exposure chamber was introduced to allow the uniform exposure of cell cultures to diesel aerosols. In the present work, this cell culture exposure chamber is evaluated and characterized with state-of-the-art nanoparticles measurement instrumentation to assess the local deposition of soot aggregates on the cell cultures and any losses due to particle deposition on the cell culture exposure chamber walls, and in addition an upgraded Multiculture Exposure Chamber (MEC) for in vitro continuous flow cell exposure tests is introduced with improved, compared to the previous version, features. Analysis and design of the MEC employs CFD and true to geometry representations of soot particle aggregates.

1. Introduction

Primary health concerns from airborne pollutants include lung carcinogenicity and non-malignant respiratory effects such as irritation, inflammation, and exacerbation or initiation of allergic hypersensitivity. The latter especially is an emerging area of concern [2]. As the prevalence of asthma and other allergic diseases has increased throughout the industrialized world in recent decades, air pollution, including exhaust emissions, especially in urban areas has been suggested as one possible cause. Therefore, the environmental and health related impacts of Diesel exhaust emissions continue to attract the attention of researchers, industry and legislative bodies. The Diesel engine effluent is a complex mixture of particles and gases with hundreds of chemicals, including many organics, present both in the gaseous and condensed phase. Even though advanced technology Diesel engines and emission control devices have been developed to improve their environmental performance and therefore to reduce the expected emissions, the currently prevailing approach does not guarantee that a decrease in regulated emissions will not generate compounds that might have more deleterious health.
effects, since the Diesel exhaust is experiencing a highly active catalytic environment as it passes through the different emission control devices such as Diesel Oxidation Catalysts (DOC), Diesel Particulate Filters (DPF), Selective Catalytic Reduction (SCR) systems, etc.

Specifically the particle size distribution is considered as a very pertinent factor influencing the toxic effect of exhaust emissions. The influence of particle size on the deposition rate in each region of the respiratory tract is well known. Especially particles with diameters less than 300 nm deposit with significant rates in the alveolar region. In addition, it is self-evident that the composition of particles can be also distributed with respect to size. Aerosol particle size distribution can be dramatically altered by biased sampling and/or storage/resuspension of particles. This observation justifies the strategy that toxicological assays must be carried out with “real” exhaust, including particulates sampled directly from the exhaust flow pipe of a running engine and maintained in suspension in the diluted gases, in order to minimize changes of physicochemical properties of Particulate Matter (PM) and pollutant bio-availability [3]. Measuring exposure to Diesel exhaust aerosol is challenging due to the physical characteristics and chemical complexity of particulate matter: with a mean diameter of ~100 nm, Diesel PM is composed primarily of organic elemental carbon, adsorbed and condensed hydrocarbon, sulfate and metals [4]. The ratio of organic to inorganic carbon depends upon a number of factors that include fuel, engine type, duty cycle, engine maintenance, operator habits, use of emission control devices, and lubricant oil consumption [5]. Research is highly required to assess the actual fate and bioreactivity of exhaust components with focus on particles and also on particle-associated compounds in conditions which do not alter their physicochemical properties and bio-availability.

2. Objectives
In order to study the various health influencing parameters related to particulate as well as to gas-phase pollutants created in the Diesel engine exhaust, there is an urgent need for appropriate sampling devices and methods for cell exposure studies and associated biological and toxicological tests.

In a previous paper [1], a specific concept for a cell culture exposure chamber had been introduced to allow the uniform exposure of cell cultures to diesel aerosols. In the present work, the characterization and evaluation of this cell culture exposure chamber is performed with state-of-the-art nanoparticle measurement instrumentation to assess the local deposition of soot aggregates on the cell cultures and any losses due to particle deposition on the exposure chamber walls, in order to address the establishment of the requisite cell exposure conditions and operational parameters, i.e. dilution and pretreatment of the Diesel engine exhaust. In addition, an upgraded Multiculture Exposure Chamber (MEC) for in-vitro continuous flow cell exposure tests is introduced with the following improved, compared to the previous version, features:

- An improved geometrical arrangement of exposed cell cultures to accommodate testing of up to thirty six cell cultures per MEC.
- On-line local measurement of particle number concentration (hence direct dose).

In the next sessions, a description of the methodology employed to evaluate the cell culture exposure chamber is given and the results obtained regarding the assessment of the impact of the electrostatic phenomena on the soot particles’ deposition on the walls of the exposure chamber and the overall average efficiency in capturing soot particles in the exposure chamber are presented. Finally, the analysis and design of the new generation MEC is described based on CFD and true to geometry representations of soot particle aggregates.

3. Methodology
In order to characterize and evaluate the cell culture exposure chamber, experiments with state-of-the-art nanoparticles’ measurement instrumentation, which provide the essential data to assess the local deposition of soot aggregates on the cell cultures and any losses due to particle deposition on the exposure chamber walls, are needed and therefore, the following experimental set up is considered (Figure 1).
Figure 1. Flow diagram of the experimental setup

In this experimental setup, a sample either from the Diesel engine exhaust (at a steady engine operation point: speed and torque, 2250 rpm and 101 Nm, respectively), or from the exit aerosol of the soot generator Combustion Aerosol Standard – CAST (at several operation points delivering thus aerosol streams with different average soot particle diameter at each operating point) is being diluted and thermally pretreated before it enters the exposure chamber. The dilution of the sample is considered as a crucial experimental factor and the final dilution ratio is selected in order to adequately simulate the “real” exposure of human lung cells to the environmental particulate pollution [1]. The temperature of the exposure chamber is constantly kept at 37±1°C with the aid of a specially constructed oven. Soot particles concentration and size distribution in the aerosol phase, from both Diesel engine exhaust and the CAST soot generator, are being recorded simultaneously “upstream” and “downstream” of the exposure chamber with a Scanning Mobility Particle Sizer (SMPS, TSI model 3936). The experimental conditions under which the measurements were conducted are given in Table 1.

Table 1. Experimental conditions for each case of measurement

| Experimental conditions       | CELL   | CAST   |
|------------------------------|--------|--------|
| Exhaust flowrate             | 2.2 lpm| 2.2 lpm|
| Upstream average particle concentration | $3\times10^7$#/cm$^2$ | $2\times10^5$#/cm$^2$ |
| Temperature                  | 37°C   | 38 °C  |
| Duration                     | 3 hr   | 3 hr   |

3.1. Assessment of particle losses due to particle deposition on the walls of the exposure chamber

Diesel nanoparticle formation may be attributed to nucleation, absorption or condensation mechanisms as well as to ion-induced nucleation mechanism. Therefore, charges on Diesel soot particles are expected especially if ion-induced nucleation is the dominant mechanism in the formation of nuclei mode particles during exhaust dilution. Metal additives in the fuel contribute also in the increase of the charged fraction of soot particles especially in the accumulation mode [6]. The existence of charge on soot particles may vary with the particle diameter. However, it is expected that the electric forces
would affect the deposition mechanism of soot particles in the exposure chamber. Therefore, to assess the impact of electrostatic phenomena on soot particles deposition on the walls of the cell culture exposure chamber and eventually to estimate the degree of particle losses, the whole inner surface of the chamber is covered with a grounded conductive film (aluminum foil). Figure 2 depicts the cell culture exposure chamber in a laminar flow hood that was employed for “real” in-vitro cell exposure tests in [1] is depicted, while Figure 3 illustrates the same chamber when its whole inner surface is covered with a grounded conductive aluminum foil. Under these conditions, an aerosol stream of soot particles from CAST, properly diluted and pretreated, is delivered to the exposure chamber, while soot particles concentration and size distribution are being recorded simultaneously with an SMPS "upstream" and "downstream" of the exposure chamber with:

- a non grounded inner surface
- a grounded aluminum foil covered inner surface

![Figure 2. The cell culture exposure chamber in the laminar flow hood [1]](image)

![Figure 3. Exposure chamber inner surface covered with a grounded conductive film](image)

3.2. Dose of soot particles delivered to cell cultures

In order to estimate the dose of soot particles to which cell cultures are being exposed in the normal operation of the in-vitro cell culture exposure chamber, measurements of the size specific soot particle collection efficiency of a free of cells device are performed. In this situation of exposure tests, particles from two different sources of soot (Diesel Engine exhaust and CAST) are delivered to the exposure chamber, and soot particles concentration and size distribution are being recorded simultaneously with an SMPS, as described previously.
4. Results
In Figure 4, the size specific collection efficiency in the case of the exposure chamber with grounded and not-grounded inner surface as a function of the average soot particle size $d_p$, is illustrated. The existence of electrostatic field results in the deposition of fewer soot particles in the multiculture exposure chamber, especially in the case of smaller particle diameters ($d_p < 60$ nm). The average collection efficiency of the not-grounded exposure chamber is reduced more than 26% for particles with diameters lower than 60 nm, due to the effect of electrostatic forces, in addition to diffusion mechanisms.

![Figure 4](image1.png)

**Figure 4.** Assessment of the impact of electrostatic phenomena on soot particles deposition in the multiculture exposure chamber

From the collection efficiency measurements of soot particles generated by CAST at various modes of operation (variation of the size of the soot particles delivered in the exposure chamber), it is obvious that the overall collection efficiency of the exposure chamber is approximately 76% (Figure 5). These results were being confirmed by respective measurement with a sample from the real Diesel engine exhaust (Figure 6).

![Figure 5](image2.png)

**Figure 5.** Collection efficiency of the exposure chamber with respect to soot particles generated by CAST at various modes of operation
Figure 6. Collection efficiency of the exposure chamber with respect to soot particles from Diesel engine exhaust

5. Future work
In order to optimize the operation of the cell exposure chamber, an upgraded design for multiculture exposure tests is proposed with the following improved features:
- an improved geometrical arrangement of exposed cell cultures to accommodate testing of up to thirty six cell cultures
- on-line local measurement of particle number concentration (hence direct dose estimation)
- on-line particle sampling for TEM analysis of particles depositing on the cell cultures.

CFD analysis
The performance of the proposed cell exposure chamber geometry is being studied using Computational Fluid Dynamics (CFD) and particle transport simulation within the chamber volume. The CFD analysis is developed with the aim to assess the uniformity of soot deposition among the culture medium wells, particularly the difference between upstream and downstream samples, and to study the effect of geometry modifications of the flow inlet and outlets, especially with respect to soot deposition uniformity. A number of results already obtained with this analysis indicate the potential of the updated exposure chamber configuration proposed.

The CFD analysis of the updated exposure chamber needs to consider only a sub-unit of the total geometry corresponding to two half-inserts (see Figure 7). This is possible due to symmetry of the culture wells and periodicity of the overall geometry, given a small / negligible gap between each set of (six) inserts. The CFD geometry was constructed in two versions: a baseline geometry with approx. 15 mm from inflow slit to the first culture wells and an elongated geometry with an increase of 20 mm in the distance between wells and inflow slit. The flow boundary conditions consisted of prescribed inflow velocity along the inflow slit and prescribed (atmospheric) pressure at the outlet. The inflow velocity corresponded to a flow of 0.2 lpm per culture well. Analysis of the soot particle exposure dose of each culture well was performed by using an Eulerian representation of the soot particle concentration field, i.e. the particle transport mechanisms of convection and diffusion were modeled. A boundary condition of perfect sticking, i.e. zero particle concentration, was applied on all solid and liquid surfaces within the exposure chamber. An overall grid resolution of 0.2 mm was employed throughout the model, with refinement to a 0.1 mm resolution in proximity to the culture well liquid surface. Example results for the flow and particle concentration fields can be seen in Figure 8 and Figure 9.

Despite the fine spatial resolution used for the discretisation of the flow and soot particle concentration fields, the direct calculation of deposition rate on the surfaces can be ill conditioned
because it involves the multiplication of diffusivities of the order of $10^{-9}$ kg/m-s with a concentration gradient of the order $10^5$ i.e. the very thin particle concentration boundary layer (of thickness $\approx 0.1$ mm) can only be marginally resolved in a model encompassing the exposure chamber geometry (size of order 100 mm). Therefore, soot deposition is best obtained by indirect interpretation of the concentration field and so particle concentration profiles normal to the culture medium surface are extracted at the center of each culture dish for all geometric configurations considered, with the resulting profiles shown in Figure 10. Interpretation of the concentration gradients so obtained indicates only a minor / indiscernible variation in soot deposition rate between the upstream – downstream culture wells. This result can be explained to a large extent by the small (only $\approx 5\%$) reduction in soot concentration “outside” the concentration boundary layer over the culture medium surfaces, between upstream and downstream wells, irrespective of inlet geometry. It is believed that, in future work, this difference in particle concentration can be reduced further by manipulation of the flow field through geometric structures on the exposure chamber cover, i.e. the surface facing the culture medium wells.

Figure 7. Overview of the exposure chamber geometry showing symmetric and periodic repetitions of the geometry sub-unit considered in the CFD analysis

Figure 8. Visualisation of the flow velocity field on symmetry and periodic sections through the exposure chamber (baseline) geometry. Velocity is in m/s on a logarithmic scale.
Figure 9. Visualisation of the soot concentration (normalised against inflow concentration) on longitudinal and lateral sections through the cell culture wells. The result is shown for the elongated geometry.

Figure 10. Particle concentration profiles at the center of the culture medium wells for all geometry cases and well positions considered.

6. Conclusions
A cell culture exposure chamber, designed and built in a previous work [1], was characterized and evaluated with respect to the local deposition of soot aggregates on the cell cultures and possible losses due to particle deposition on the cell culture exposure chamber walls. According to the results obtained when measuring the size specific collection efficiency of the exposure chamber with its inner surface grounded and not grounded, it was noticed that a greater selectivity in collecting smaller soot nanoparticles can be achieved, if the existence of electrostatic field in the exposure chamber is eliminated. Based on simulating exposure tests with two sources of soot particles (real Diesel engine exhaust and synthetic soot generator CAST), the average collection efficiency of the proposed
exposure chamber is estimated to be approximately 76%, independently of the specific ranges of the particle size distribution delivered to the exposure chamber.

An upgraded Multiculture Exposure Chamber (MEC) for in vitro continuous flow cell exposure tests is proposed with improved, with respect to the previous version, features. Preliminary CFD analysis of the updated/proposed exposure chamber configuration already indicates satisfactory soot deposition uniformity and the scope for further improvement by flow field manipulation.

The evaluation of the upgraded multiculture exposure chamber will address the establishment of the requisite cell exposure conditions and operational parameters, i.e. dilution and pretreatment of the diesel engine exhaust.

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