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Is it the time to study air pollution effects under environmental conditions? A case study to support the shift of *in vitro* toxicology from the bench to the field.

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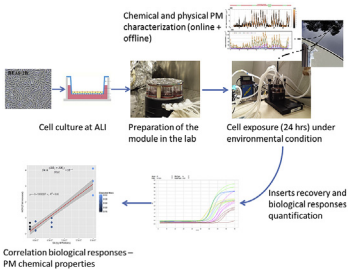
HIGHLIGHTS

- An exposure methodology for environmental exposure is presented.
- PM environmental doses of exposure cause biological responses in BEAS-2B.
- Correlation of HO1 gene with secondary aerosol is reported.
- Secondary and aged PM determine oxidative responses in cells.
- Primary PM rich in PAHs increase CYP1B1 expression.

GRAPHICAL ABSTRACT



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ABSTRACT

Air pollution and particulate matter are recognised cause of increased disease incidence in exposed population. The toxicological processes underlying air pollution associated effects have been investigated by *in vivo* and/or *in vitro* experimentation. The latter is usually performed by exposing cells cultured under submerged condition to particulate matter concentration quite far from environmental exposure expected in humans. Here we report for the first time the feasibility of a direct exposure of air liquid interface cultured cells to environmental concentration of particulate matter. Inflammatory proteins release was analysed in cell medium while differential expression of selected genes was analysed in cells. Significant association of anti-oxidant genes was observed with secondary and aged aerosol, while cytochrome activation with primary and PAHs enriched ultrafine particles. The results obtained clearly

List of abbreviation: AAE, Absorption Ångström Angstrom Exponent; BC, black carbon; Dmed, median diameter of the particle surface size distribution; CYP1B1, cytochrome 1B1 gene; IL-6, interleukin 6 protein; IL-8, interleukin 8 protein; IL-6, interleukin 6 proteingene; IL-8, interleukin 8 protein; HO1, heme oxygenase gene; NQO1, HADPH-quinone oxidoreductase gene; AhR, Aryl hydrocarbon receptor gene; PAHs, polyciclic aromatic hydrocarbons; OM, organic matter; PM, particulate matter; NRPM1, non-refractory PM1; OP, PM oxidative potential.

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show the opportunity to move from the lab bench to the field for properly understanding the toxicological effects also of ultrafine particles on selected *in vitro* models.

1. Introduction

Air pollution is among the most important environmental causes of diseases all over the world (WHO, 2014). Air pollution and in particular particulate matter (PM) exposure are classified as carcinogen for humans (IARC, 2015) and are related to several pulmonary and cardiovascular diseases beside lung cancer (Lynch et al., 2016; Dockery, 2009; Araujo et al., 2008). The relation between PM and human diseases derives mainly from epidemiological studies which allowed determining statistical association between PM concentration and increase in relative risk of developing health diseases. The mode of action underlying these correlations on the contrary has been investigated by toxicological studies both *in vitro* and *in vivo*. The latter give the researchers the opportunity to investigate the potential effects of inhaled PM on the whole organism (Davel et al., 2012; Mantecca et al., 2010) but there are limitations regarding the models normally used (i.e. rodents) which do not respond to toxicants as humans. On the other hand *in vitro* models of human cells (mainly lung epithelial cells, neuronal cells and cells of the immune system) allow the comprehension of the molecular pathways activated by airborne particles (Yan et al., 2016; Longhin et al., 2016; Dergham et al., 2015; Schwarze et al., 2013) but a significant limitation among others is related to the protocol of exposure to PM.

So far the exposure to airborne PM of *in vitro* systems has been conducted by collecting particles on supports (usually Teflon filters or metal plates), removing the particles from the support (by sonication in the case of filter or by gently brushing away the particles from plates) (Bein and Wexler, 2014, 2015), dispersing the particles in a liquid medium and treating the cells cultured in conventional submerged or air liquid interface (ALI) condition (Leclercq et al., 2017; Borgie et al., 2015; Gualtieri et al., 2011). Although this procedure is accepted by the scientific community several questions arise on the possibility that the subsequent steps, from particles collection to cells exposure, may introduce artefacts in the final PM sample. Sampling and detachment may vary the aggregation state of particles, subsequent dispersion in media may facilitate the dissolution of soluble components, the submerged condition is not representative (at least for lung epithelial cells) for real exposure conditions, while ALI exposure requires the generation of an aerosol which may be not representative of the environmental condition under which the particles are sampled. These limitations strongly reduce the impact of results obtained with *in vitro* models despite their well-recognized utility in studying PM pathways of effects and in identifying a general mode of action of PM toxicity. Finally, application of *in vitro* toxicology to airborne ultrafine particulate (UFP) matter is largely limited by the difficulty in sampling enough amount of UFP mass, in setting up a procedure of detachment that allow significant recovery of sampled mass and in avoiding physico-chemical modification of particles during the sample preparation.

During the last years, significant steps forward have been made in online procedures for PM chemical and physical properties characterization. Aerosol scientists have been developing instruments for the evaluation of particles diameter, organic and inorganic chemicals associate to solid carbonaceous particles and for the characterization of the light absorption properties of the different organic particles present in air pollution. All these instruments have provided new insight in the comprehension on the evolution of airborne particles in the atmosphere in term of mass, number and chemical properties, in the identification of the sources of pollution responsible for the worsening of air quality and in the characterization of primary and secondary particulate pollution. In parallel, *in vitro* toxicologists have taken advantage from the development of ALI exposure module that allowed the exposure of cells to controlled aerosol under culture conditions mimicking those of the alveolar epithelium. These exposure modules have been applied in controlled laboratory experiments to expose cells to cigarette smoke, nanoparticles, and organic volatile compounds of interest. However, all the applications reported refer to laboratory conditions in which the aerosols for *in vitro* model exposure are well characterized and produced *ad hoc* for the desired experiment. Few papers report the effects of PM samples re-suspended in cell medium and aerosolized on cells cultured at ALI (Roth et al., 2017; Leclercq et al., 2017).

Despite the significant improvement of knowledge and of the instruments used by aerosol scientists, the collaboration with *in vitro* toxicologists has been so far limited. As recently stated “Too little is known about how different components contribute to the overall toxicity of particulate matter. Such information could inform policy strategies to reduce deaths – for example, by rapidly setting severe limits for the most dangerous compounds.” (Lelieveld and Pöschl, 2017). The authors proposed theoretical and experimental models to cover this gap and, among others, they suggest “exposing the cell cultures to ambient air”.

Recently we have reported the preliminary results of a one month campaign held in Rome during which aerosol scientists (chemists, physicists and engineers) together with toxicologists collaborated at the evaluation of the impact of fine and ultrafine particles on human health (Costabile et al., 2017). In particular, an ALI exposure module was used to treat the bronchial epithelial cells BEAS-2B to particulate matter under environmental condition. Here we report in detail the biological results, obtained during the CARE campaign, which are, as far as we know, the first results presented on *in vitro* models exposed under environmental condition to air pollution. To support the application of the selected ALI module we compared the maximal theoretical PM deposition calculated for the module with the deposition obtained by a risk assessment developed model for lung deposition. The results clearly show the feasibility of the employed ALI module to assess the impact of air pollution under environmental conditions and without pre-sampling requirements; this approach opens the doors of a new paradigm for air pollution focused *in vitro* toxicology and will substantially improve our knowledge of the effects of airborne contaminants on lung epithelia and promote new policy strategies to prevent air pollution-associated health effects.