Contribution ID: **33** Type: **Poster**

Aerosol Sampling and Cytotoxicity Assessment on a Single Filter: „Cells on Particles“ Platform

Introduction/Objective:
Air pollution significantly harms human health, contributing to respiratory and cardiovascular diseases as well as overall declines in well-being. There is a growing need for more effective and comprehensive methods to assess the toxicity of air pollutants. Typically, the evaluation of particle toxicity has relied on in vitro methods, using cell cultures exposed to particles (such as air-liquid interface) or their extracts (submerged exposure). However, these approaches result in trade-offs in terms of particle transformations during sampling/delivery to cells. To address this gap, new methods for capturing particles and assessing their direct impact on living cells may be suggested. This study aimed to test and validate a novel “Cells-on-Particles” integrated aerosol sampling and in vitro cytotoxicity testing platform under both indoor and outdoor conditions.
Methods:
The “Cells-on-Particles” integrated platform serves the dual purpose of particulate sampling and cytotoxicity testing. The key element of the platform is a nanofibrous layer, consisting of fibres smaller than 1 μm in diameter. This creates a fibre network sufficiently dense to capture fine particles with high efficiency. Simultaneously, the nanofibrous matrix provides a cell-friendly surface for attachment while preventing cell penetration into deeper layers. Selecting the optimal dose of pollutants for the cytotoxicity study is paramount to achieving a meaningful cell response. In the case of the “Cells-on-Particles” platform, the dose is adjusted by controlling the amount of aerosol particles deposited on the platform, as cells are placed directly onto the platform without further extraction or dilution.
This platform was tested under two types of aerosols –exhaled tobacco smoke and urban outdoor aerosol. The platform was inserted as a 37 mm sampling filter aerosol to Personal Environment Monitors (SKC Inc, USA) with a cut-off size of 2.5 um. The generation and sampling of exhaled tobacco aerosol were performed in a small-scale chamber. A human subject used either a conventional cigarette (CC) or a heated tobacco product (HTP) and exhaled the aerosol through an inlet into the chamber. For outdoor conditions, fine particulate matter (PM2.5) samples were during winter period near a averagely loaded urban street.
The human bronchial epithelial cell line (BEAS-2B) was used for cytotoxicity assessments. Fibrous substrates exposed to aerosols were cut into 6.4 mm diameter discs. These discs were placed at the bottom of 96-well plates, with the particle-coated surface facing upward. BEAS-2B cells were seeded directly onto the fibrous matrix discs at a density of 6,000 cells per well in 100 μl of culture medium. Cell viability was assessed using the RealTime-Glo™ MT Cell Viability Assay. The cytotoxicity of the particles was evaluated by measuring the release of lactate dehydrogenase using the LDH-Glo™ Cytotoxicity Assay.
Results:
Exhaled HTP aerosol particles at doses of 49.2 and 73.5 μg/cm² did not reduce cell viability. After 48 hours of exposure, viability remained higher compared to untreated (control) BEAS-2B cells but slightly decreased compared to 24-hour exposure. Exhaled aerosol particles from CC and HTP had significant but opposing effects on cell viability-complete loss of cell viability with CC versus stimulation of proliferation and metabolic activity with HTP. The results showed that for HTP aerosols, particle surface densities in the range of 50-70 μg/cm² appeared suitable to obtain biological indicator values. For CC, the dose had to be adjusted to less than 30 μg/cm² to avoid complete cell death.
In the case of outdoor aerosol, the viability of BEAS-2B cells exposed to varying concentrations of PM2.5 demonstrates a clear dose-dependent cytotoxic effect. After exposure, cell viability decreased progressively

with increased concentrations of PM2.5, with a decline observed even at 11 μg/cm² and a near complete loss of viability at 33 μg/cm². Concentrations of 93 μg/cm² and higher result in complete cell death, highlighting the severe impact of PM2.5 on cell health.
Conclusions:
The "Cells-on-Particles" platform enables the direct exposure of cells to particles, eliminating the need for extraction or resuspension processes. This approach not only provides a more rapid and sensitive toxicity screening but also allows for a more representative comparisons across various particle types and exposure conditions, including complex environmental aerosols.

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