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# Induction of IL-6 and inhibition of IL-8 secretion in the human airway cell line Calu-3 by urban particulate matter collected with a modified method of PM sampling

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ABSTRACT

Exposure to particulate matter (PM) induces inflammatory cytokines. In the present study, we evaluated the secretion of IL-6 and IL-8 by an airway cell line exposed to PM with a mean aerodynamic size equal to or less than 10 or 2.5 μm ($PM_{10}$ and $PM_{2.5}$, respectively) collected in Mexico City, using a modified high-volume sampling method avoiding the use of solvents or introducing membrane components into the samples. PM was collected on cellulose-nitrate (CN) membranes modified for collection on high-volume samplers. Composition of the particles was evaluated by particle-induced X-ray emission (PIXE) and scanning electron microscopy. The particles (10–160 μg/cm$^2$) were tested on Calu-3 cells. Control cultures were exposed to LPS (10 ng/mL to 100 μg/mL) or silica (10–160 μg/cm$^2$). IL-6 and IL-8 secretions were evaluated by ELISA. An average of 10 mg of PM was recovered form each cellulose-nitrate filter. No evidence of contamination from the filter was found. Cells exposed to $PM_{10}$ presented an increase in the secretion of IL-6 (up to 400%), while IL-8 decreased (from 40% to levels below the detection limit). A similar but weaker effect was observed with $PM_{2.5}$. In conclusion, our modified sampling method provides a large amount of urban PM free of membrane contamination. The urban particles induce a decrease in IL-8 secretion that contrasts with the LPS and silica effects. These results suggest that the regulation of IL-8 expression is different for urban particles (complex mixture containing combustion-related particles, soil and biologic components) than for biogenic compounds or pure mineral particles.


## 1. Introduction

It has been widely demonstrated that urban particulate matter (PM) induces the secretion of different cytokines when epithelial cells or macrophages are exposed *in vitro* (Alfaro-Moreno et al., 2007a). Some of the most frequently tested cytokines are TNF-α, IL-1β, IL-6 and IL-8 (Becker et al., 2005; Hetland et al., 2005; Veranth et al., 2006). The evaluation of pro-inflammatory effects induced by PM has been predominantly evaluated on alveolar epithelial cells and macrophages (Becker et al., 2005; Veranth et al., 2006; Ishii et al., 2005; Mazzarella et al., 2007). A large proportion of the urban PM mass is included in the coarse fraction (PM 2.5–10 μm in aerodynamic size) (de Vizcaya-Ruiz et al., 2006; Liu et al., 2003; Osornio-Vargas et al., 2003), and particles tend to preferentially deposit in airways, specifically at bifurcations (Balashazy et al., 2003). Therefore, it is necessary to evaluate the effects of PM on airway epithelial cells. So far, only a handful of articles have dealt with the effects of PM on airway epithelial cells, showing that IL-8 could present a positive correlation (Becker et al., 2005) or an inverse correlation (Veranth et al., 2006) with PM concentration.

The use of urban PM in experimentation faces several challenges, for example, how well does the collected sample represent PM suspended in the air, how efficient is PM recovered from the sampling substrate and how accurate is the dosing of PM on both *in vivo* and *in vitro* systems. Collecting samples that

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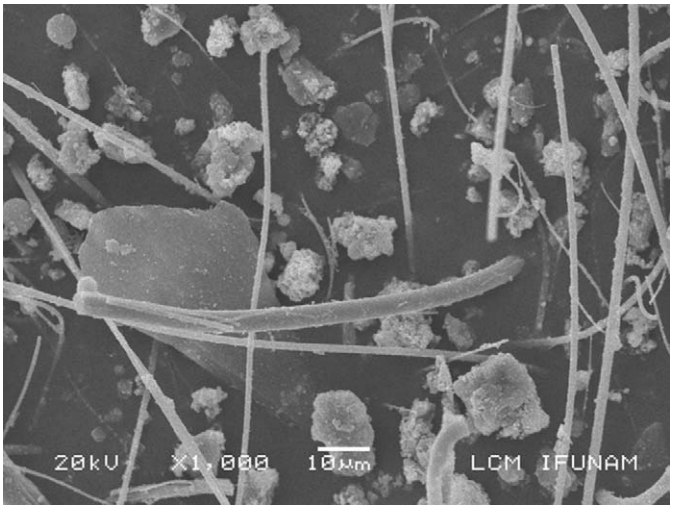


**Fig. 1.** Scanning electron microscopic image of particulate matter recovered from standard fiberglass membranes. Note the presence of long fibers from the membranes contaminating the PM.

accurately represent mass, size and composition of suspended particles remains difficult. In addition, toxicological evaluation requires the collection of sample masses in the order of hundreds of milligrams. These requirements have stimulated a series of adaptations or developments of new techniques for PM collection. Adaptations include the dislodgment of PM from sampling membranes (fiberglass, quartz, Teflon, cellulose) either by aqueous (Timblin et al., 1998) or organic solvent extraction (Hsiao et al., 2000) or by mechanical means (Alfaro-Moreno et al., 2002). New techniques also include concentrators that keep the particles in water (Kim et al., 2001), foam (Kavouras and Koutrakis, 2001) or suspended in the air (Ghio and Huang, 2004). All these methods face problems related to the efficiency of recovery and potential physicochemical modification of the PM samples. To avoid the need of using solvents it seems better to remove the particles physically from the collection substrates. However, this had the disadvantage of potentially introducing contamination from the substrate and the chance of misrepresenting the sample due to particle retention on the substrate. The introduction of membrane contaminants in the recovered sample is particularly critical when fiberglass or quartz membranes are used as these minerals have their own intrinsic toxicity (Dick et al., 2000; Li et al., 1997) (Fig. 1). Experimental evaluation of different substrates suggests that cellulose nitrate (CN) and Teflon are the best options for *in vitro* and *in vivo* studies, respectively (Dick et al., 2000). Unfortunately, these membranes are usually only available for low-volume samplers, thus making them unsuitable for collection of large samples.

The aims of the present research were as follows: (1) to develop a PM sampling method for large amounts of PM that avoids contamination of the particulate matter with sampling substrate components; (2) to evaluate the effects of urban PM on an airway cell line (Calu-3).

## 2. Material and methods

### 2.1. Air sampling

$PM_{10}$ and $PM_{2.5}$ were sampled in two regions of Mexico City: in the north, which has been related to industry (Xalostoc), and in the City Center, related to commercial activities (Merced).

High-volume $PM_{10}$ and $PM_{2.5}$ samplers (GMW Model 1200, VFC HVPM10; Sierra Andersen, Smyrna, GA, USA) were used. Samples were collected during 24 h with an airflow rate of $1.13\,m^3/min \pm 10\%$ on CN sheets ($20.3\,cm \times 25.4\,cm$) with a nominal pore size of 3 μm, cut from rolls (11302-131, Sartorius, Goettingen, Germany). Sampling was done during November–December of 2004, and January–April 2005, every Monday, Wednesday and Friday. The 24 h average concentration of $PM_{10}$ during those months was $62 \pm 19\,\mu g/m^3$ (Red Manual, SMA-GDF).

The use of CN membranes in the high-volume samplers reduces significantly the airflow rate. Thus, in order to preserve the airflow rate and particle size sampling performance, the CN membranes were perforated in a regular pattern using an industrial sewing machine (DB2-B755-3, Brother International, Mexico, DF, Mexico) making 1 mm holes (90/14 gauge needle) spaced every 3 mm. The pattern is shown in Fig. 2A, and consists of 20 intact non-perforated rectangles ($4.2 \times 5\,cm^2$) limited by a series of holes distributed in 4 rows and 6 columns. Rectangles located in columns 2 and 5, rows 2 and 3, had 65 additional perforations distributed 3 mm apart, in 5 lanes and 13 columns.

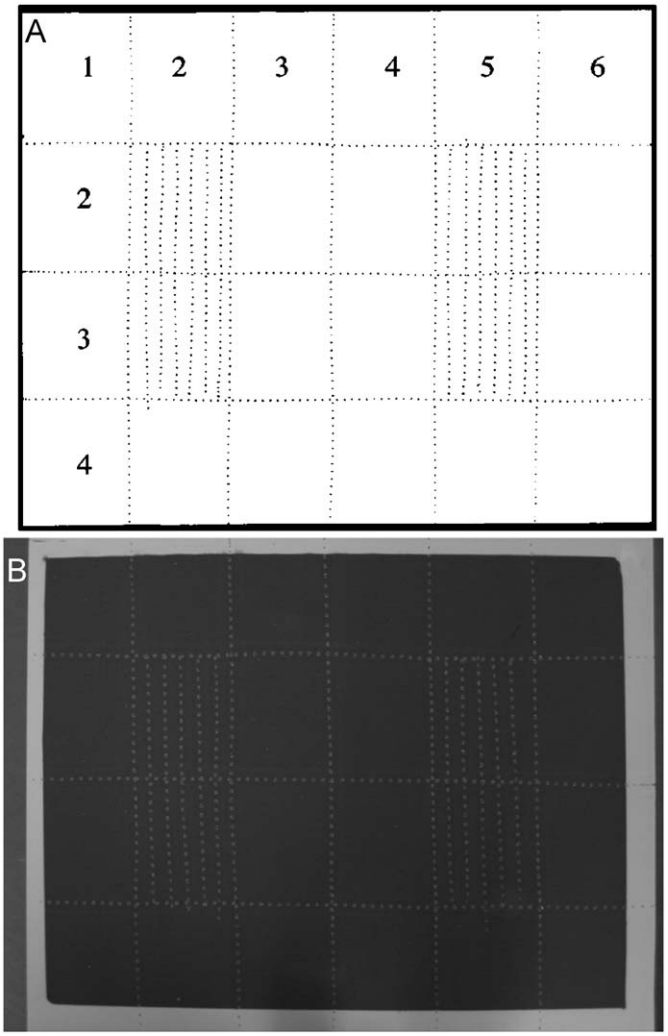


**Fig. 2.** Perforated cellulose-nitrate membranes "naked" or with $PM_{10}$: (A) perforation pattern used on a CN membrane to maintain the airflow in the high-volume sampler. (B) A membrane with a 24 h sample obtained in downtown Mexico City. Note the homogeneous distribution of $PM_{10}$ on the membrane (black) in contrast with the unexposed rim of the membrane (white).