NanoImpact 1 (2016) 1–8

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.journals.elsevier.com/nanoimpact

Full Length Paper

Effects of intratracheally instilled laser printer-emitted engineered nanoparticles in a mouse model: A case study of toxicological implications from nanomaterials released during consumer use

Sandra V. Pirela [a], Xiaoyan Lu [a], Isabelle Miousse [b], Jennifer D. Sisler [c], Yong Qian [c], Nancy Guo [d], Igor Koturbash [b], Vincent Castranova [d], Treye Thomas [e], John Godleski [a], Philip Demokritou [a,*]

[a] Department of Environmental Health, Center for Nanotechnology and Nanotoxicology, T. H. Chan School of Public Health, Harvard University, Boston, MA, United States
[b] Department of Environmental and Occupational Health, College of Public Health, University of Arkansas for Medical Sciences, Little Rock, AR, United States
[c] Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, United States
[d] Department of Pharmaceutical Sciences/Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV, United States
[e] U.S. Consumer Product Safety Commission, Office of Hazard Identification and Reduction, Rockville, MD, United States

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 4 December 2015
Accepted 18 December 2015
Available online 31 December 2015

Keywords:
Nanoparticles
Laser printers
Inflammation
Epigenetics

ABSTRACT

Incorporation of engineered nanomaterials (ENMs) into toners used in laser printers has led to countless quality and performance improvements. However, the release of ENMs during printing (consumer use) has raised concerns about their potential adverse health effects. The aim of this study was to use "real world" printer-emitted particles (PEPs), rather than raw toner powder, and assess the pulmonary responses following exposure by intratracheal instillation. Nine-week old male *Balb/c* mice were exposed to various doses of PEPs (0.5, 2.5 and 5 mg/kg body weight) by intratracheal instillation. These exposure doses are comparable to real world human inhalation exposures ranging from 13.7 to 141.9 h of printing. Toxicological parameters reflecting distinct mechanisms of action were evaluated, including lung membrane integrity, inflammation and regulation of DNA methylation patterns.
Results from this in vivo toxicological analysis showed that while intratracheal instillation of PEPs caused no changes in the lung membrane integrity, there was a pulmonary immune response, indicated by an elevation in neutrophil and macrophage percentage over the vehicle control and low dose PEPs groups. Additionally, exposure to PEPs upregulated expression of the *Ccl5* (*Rantes*), *Nos1* and *Ucp2* genes in the murine lung tissue and modified components of the DNA methylation machinery (*Dnmt3a*) and expression of transposable element (TE) LINE-1 compared to the control group. These genes are involved in both the repair process from oxidative damage and the initiation of immune responses to foreign pathogens. The results are in agreement with findings from previous in vitro cellular studies and suggest that PEPs may cause immune responses in addition to modifications in gene expression in the murine lung at doses that can be comparable to real world exposure scenarios, thereby raising concerns of deleterious health effects.

1. Introduction

The use of laser printers leads to exposure to various pollutants, including ozone, volatile organic compounds and particulate matter (PM), among other pollutants (He et al., 2007; Morawska et al., 2009; Wang et al., 2012). In particular, the release of a significant number of particles, the majority of which are nanoparticles, during the use of this growing technology has become a reason for concern. More recently, in order to assess the complex chemistry of printer emitted particles (PEPs) and their potential health hazards, a Printer Exposure Generation System (PEGS) was recently developed to generate and sample airborne PEPs for subsequent physicochemical, morphological and toxicological analyses (Pirela et al., 2014a). The PM emission profiles from commonly used printers were evaluated and further characterization was performed on both raw toner powder and PEPs. The detailed analysis showed that laser printers emit up to 1.3 million particles/cm^3 with modal diameters of <200 nm (Pirela et al., 2014a). More importantly, Pirela et al. (2014b) found nanoscale materials used in the toner formulation that become airborne during the use of a printer, thus, classifying toners as nano-enabled products (NEPs). Additionally, the authors found that toner powders and PEPs share a complex chemistry and contain elemental and organic carbon, as well as inorganic compounds such as nanoscale metals and metal oxides.

* Corresponding author at: Department of Environmental Health, Center for Nanotechnology and Nanotoxicology, T. H. Chan School of Public Health, Harvard University, 665 Huntington Avenue, Room 1310, Boston, MA 02115, United States. Tel.: +1 917 432 3481.
E-mail address: pdemokri@hsph.harvard.edu (P. Demokritou).

http://dx.doi.org/10.1016/j.impact.2015.12.001
2452-0748/

While the physicochemical and morphological properties of PEPs have been studied in detail, their toxicological profiles remain largely unknown. In a series of recently published papers, several physiologically relevant cell lines (i.e., human small airway epithelial cells, microvascular endothelial cells, macrophages and lymphoblasts) were treated with various doses of PEPs using both mono- and co-culture exposure systems (Sisler et al., 2014; Pirela et al., 2015). In both studies, it was shown that PEPs triggered an unfavorable series of biological responses in macrophages, small airway epithelial cells and microvascular endothelial cells at doses comparable to approximately 8 h or more of consumer inhalation of PEPs. Specifically, cell treatment with PEPs led to significant changes in cell viability, hereditary genetic material changes, reactive oxygen species (ROS) generation and release of inflammatory mediators, among other adverse effects. Moreover, recent findings suggest that PEPs can also influence the cellular epigenome. Particularly, a 24-hour exposure to PEPs caused altered expression of DNA methylation machinery in small airway epithelial cells, in turn leading to changes in global DNA methylation and reactivation of transposable element (TE) LINE-1 and *Alu* (Pirela et al., 2015; Lu et al., 2015a).

Notably, the toxicity of PEPs remains poorly characterized in vivo with only a few published studies. Major discrepancy on those in vivo studies is the use of toner powders rather than the PM and gaseous pollutants emitted from laser printers. For example, Bai, Zhang (Bai et al., 2010) reported that mice exposed to printer toner particles showed significant pulmonary inflammation, damage to the epithelial-capillary barrier and enhanced cell permeability. Comparable inflammatory and fibrotic responses were also observed in rats exposed to toner powders (Morimoto et al., 2013). A historic rodent chronic inhalation exposure concluded that toner led to a substantial increase in lung weight, a chronic inflammatory response, pulmonary fibrosis and increased incidence of primary lung tumors in exposed rats (Muhle et al., 1991). However, as extensive as these studies were in identifying the biological response in the rodent lung following exposure to toner, they are limited by addressing only the toxicity of toner powder, which may be relevant to occupational settings and workers directly handling toner powders but is not applicable to consumers using laser printers.

In this study, we sought to further expand on the latest cellular toxicology studies performed by our group on PEPs (Sisler et al., 2014; Pirela et al., 2015; Lu et al., 2015b). Particularly, we present findings on the murine responses to intratracheal instillation exposures to various doses of PEPs. The endpoints evaluated included bronchoalveolar lavage (BAL) levels of lactate dehydrogenase, myeloperoxidase, cytokines and white blood cell differentials, as well as lung tissue expression of a number of genes involved in immune responses, cell survival and signaling, among other important biological processes.

2. Material and methods

2.1. Experimental design

Fig. 1 shows the experimental setup of the previously developed Printer Exposure Generation System (PEGS, (Pirela et al., 2014a)) used in this study. It consists of: a) a glovebox type environmental chamber to house the printer used in this study (Printer B1 in our previous publications: (Pirela et al., 2014a; Pirela et al., 2014b)) for uninterrupted operation; b) real time and time-integrated PM sampling and monitoring instrumentation to quantify particle size distribution and collect size-fractionated PEPs for analysis; and c) an animal inhalation exposure system for toxicological evaluation.

Groups of mice were exposed to various exposure doses of the smallest size fraction of PEPs (particles with an aerodynamic diameter smaller than 0.1 μm, $PM_{0.1}$) by intratracheal instillation. Following the exposure, animals were sacrificed and BAL was performed. The BAL fluid (BALF), blood and lung tissue were subsequently used to measure biochemical markers of inflammation, albumin and hemoglobin levels, white blood cell differentials and expression of a number of genes in addition to epigenetic analyses. In more detail:

2.2. Exposure characterization, sample preparation of size-fractionated airborne PM for intratracheal instillation exposures

2.2.1. Real time instrumentation for PM

A water-based condensation particle counter (WCPC Model 3785, TSI Inc., Shoreview, MN) was used to monitor the number concentration of particles sized from 5 to 1000 nm. A scanning mobility particle sizer (SMPS Model 3080, TSI Inc., Shoreview, MN) was also used in order to measure the particle size distribution (ranging from 2.5 to 210 nm) in the chamber. All the instruments were calibrated and background tests were performed at the beginning of each sampling experiment.

2.2.2. Size-selective integrated PM sampling and colloidal suspension preparation

The Harvard compact cascade impactor CCI, (Demokritou et al., 2004) was used to size fractionate and collect PM samples. The CCI operates with four stages and allows for collection of moderately large amounts of particles (mg level) for the following size fractions: $PM_{2.5–10}$, $PM_{0.1–2.5}$ and $PM_{0.1}$. The main advantage of CCI is the fact that size-fractionated PM is collected on pre-cleaned adhesive-free polyurethane foam (PUF) impaction substrates and Teflon filters from which the particles can be efficiently extracted using a water-based protocol. In summary, particles in the sampling substrates are extracted in deionized water

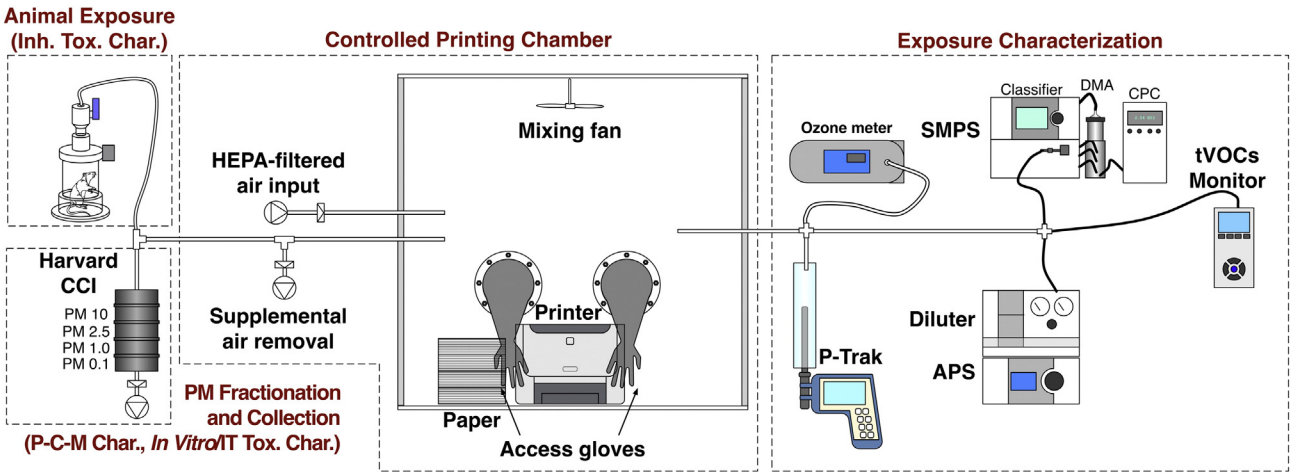

Fig. 1. Printer Exposure Generation System used to collect freshly generated PEPs for subsequent intratracheal instillations.