Summary Report

Chemical Composition and Toxicity of Particles Emitted from a Consumer-Level 3D Printer Using Various Materials

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SUMMARY REPORT

Chemical Composition and Toxicity of Particles Emitted from a Consumer-level 3D Printer using Various Materials

Qian Zhang

School of Civil and Environmental Engineering

Dr. Rodney J. Weber

School of Earth and Atmospheric Sciences

Georgia Institute of Technology

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INTRODUCTION

Fused deposition modeling (FDM) 3D printing heats a thermoplastic material and deposits it by layers to build an object. Concerns of potential exposure to 3D printer emissions have been raised since consumer level 3D printers are widely used in small-scale indoor environments, especially when vulnerable populations like children are involved. Among a wide range of materials, acrylonitrile butadiene styrene (ABS) and polylactic acid (PLA) are commonly used. The average particle emission rates during 3D printing were comparable or lower than those of laser printers, and most of the emitted particles were ultrafine particles (UFPs, less than 0.1 μm in size).[1] However, details about particle chemical composition and potential toxicity are lacked.

UFPs are potentially hazardous because they can deposit in the respiratory tract, enter the blood stream, translocate to remote organs, and damage mitochondria, due to their specific properties.[2] A well-established mechanism associated with particle adverse biological effects, is the generation of reactive oxygen species (ROS), the excess of which causes cellular damage and induces oxidative stress.[3,4] Previous studies that exposed various cell types to nanoparticles (NPs) or ambient particulate matter (PM) reported corresponding formation of ROS and oxidative stress, which can trigger redox-sensitive pathways that lead to biological responses, such as inflammation,[4] cell death,[5] and diseases.[3,6] In this study, we assessed particle toxicity via multiple analyses based on ROS mechanism.

METHODS

The 3D printer was operated in a 1 m^3 well-mixed stainless-steel emission test chamber according to standards.[1] Particle number concentrations in the chamber were monitored with online particle measurement instrumentation including a scanning mobility particle sizer (SMPS, TSI Inc.) and an optical particle counter (OPC, TSI Inc.); particle mass concentrations were calculated assuming particles were spheres of unit density. Aerosol Chemical Speciation Monitor (ACSM, Aerodyne Research, Inc.) was used to analyze submicron particle chemical composition during printing. Pyrolysis gas chromatography mass spectrometry (GC-MS, Agilent Technologies) was performed on both the filament and the emitted particles collected on a quartz filter. Particles for offline toxicity analysis were collected on filters during printing.

Three filament materials were tested; a high particle number emitting ABS filament (High ABS), an ABS filament that emitted particles of more typical ABS filaments (Regular ABS), and a PLA filament (PLA) and a nylon filament (Nylon).

Three *in vitro* cellular assays were performed to assess cytotoxicity of 3D printer emitted particles, which included cell viability, assessed by WST-1 assay,[7] cell death mechanism, assessed by flow cytometry, and intracellular ROS generation, assessed by DCF assay.[8] All assays were applied to both rat alveolar macrophages (NR8383) and human tumorigenic lung epithelial cells (A549). Exposure time was 24 h, except for intracellular ROS assay which was 6 h. *In vivo* exposure was done on mice using an intra-tracheal model.[6] After exposure of 24 h, cells in the mice broncho alveolar lavage fluid (BALF) were studied by multispectral imaging flow cytometry. The Animal Care and Use Committee of the Weizmann Institute of Science approved the experiments. Biological analyses were done using particle suspensions (i.e., filter extracted in deionized water).

Oxidative potential (OP), an integrative measure of a particle's ability to induce oxidative stress, of 3D printer emitted particles was assessed using the dithiothreitol (DTT) cell-free assay. Both water-soluble and total DTT assays were applied;[9,10] the difference between the two assays was whether the sample filter with water insoluble particles were involved in the reaction with DTT.

RESULTS and DISCUSSION

Particle chemical composition

The ACSM results showed the particles emitted from Regular ABS and PLA filaments were largely organic in composition. The mass spectra of Regular ABS emitted particles were different from those of the raw filament material monomers (Figure 1A), while the mass spectra of PLA emitted particles were mostly similar to those of the PLA monomers (Figure 1B). Pyrolysis GC-MS results also showed the spectra of High ABS emitted particles were substantially different from those of High ABS raw filament, consistently indicating that the ABS particles are not formed directly from the bulk ABS material, but potentially from some additives.[11] Particle chemistry analyses imply that the toxicity of particles emitted from 3D printing could vary widely amongst filaments, and may not be directly related to the toxicity of the bulk filament materials.

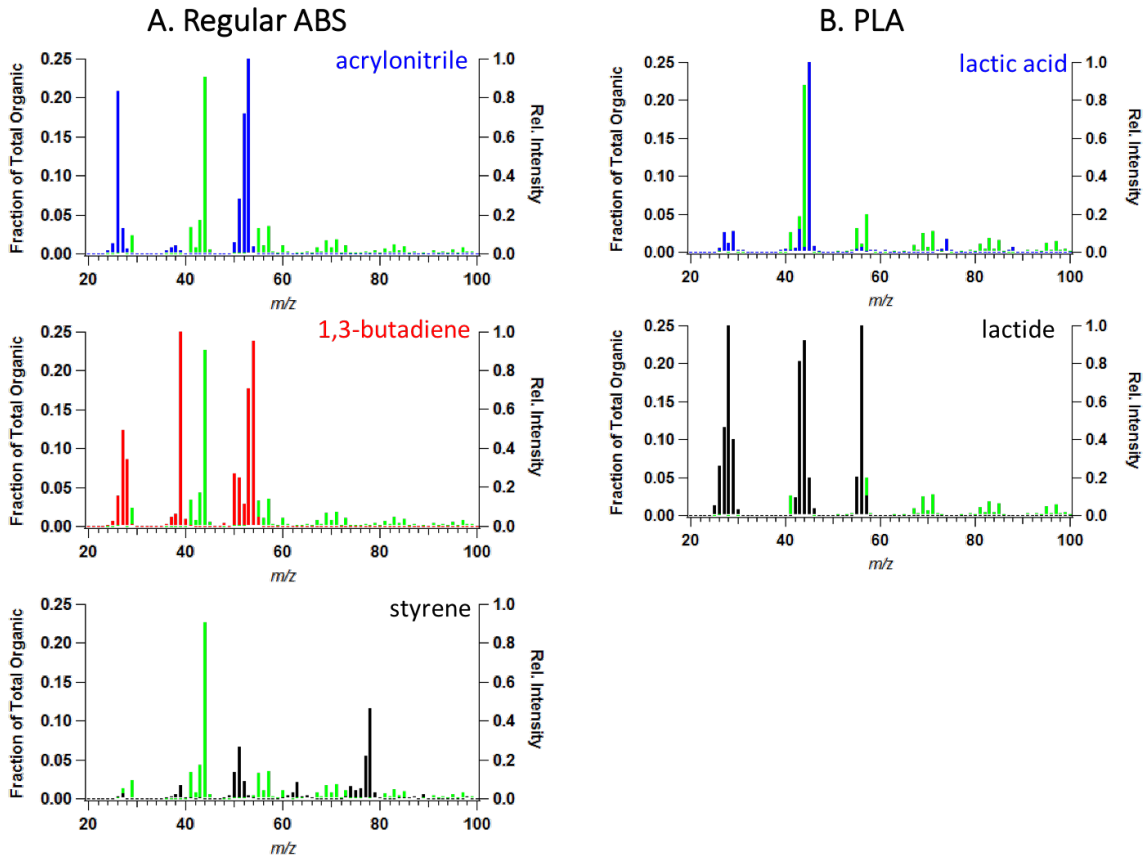

Figure 1. Particle mass spectra measured by ACSM (shown in green) of particles emitted from Regular ABS (A) and PLA (B). Reference spectra of monomers corresponding to the raw filament materials for ABS (acrylonitrile, blue; 1,3-butadiene, red; styrene, black) and PLA (lactic acid, blue; lactide, black) are included for comparison.[12]

Particle cytotoxicity

WST-1 assay results showed all three types of particles induced statistically significant decreases in cell survival rates for A549 and NR8383 cells compared to the blanks at the indicated concentrations, while no significant differences were found between cell lines or among different particle types (Figure 2A). Total cell death increased significantly after 24 h of exposure to all three types of particles compared to the blanks for NR8383, but not for A549 cells (Figure 2B). In addition, necrosis was involved in cell death, which is found to be associated with exposure to metal NP and smoke.[5,13] High ABS and PLA emitted particles increased intracellular ROS generation by 13% – 24% compared to the blanks for both cell types with no statistical differences between the two cell lines (Figure 2C). These observations were in agreement with studies showing that PM or NP can increase ROS and oxidative stress, and thereby may contribute to the adverse health effects.[2–4]

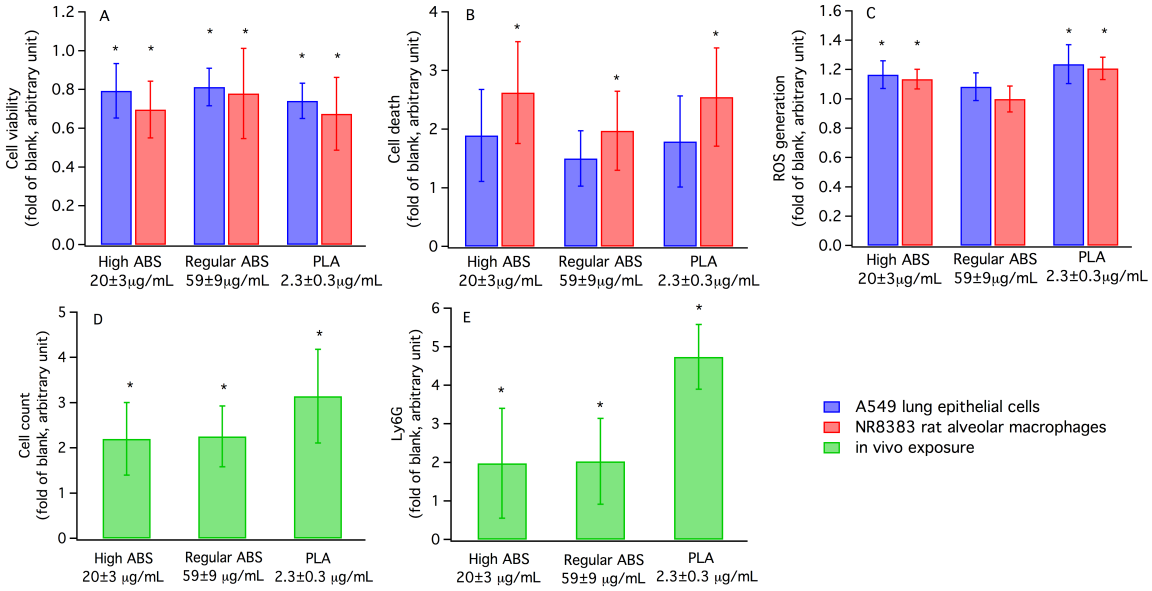

Figure 2. Biological toxicity responses for *in vitro* (A. cell viability, B. cell death, C. intracellular ROS generation) and *in vivo* (D. cell count, E. neutrophils) analyses. Data expressed as fold change compared to blank filter extracts (blank). For the *in vitro* assays, error bar represents standard deviation, and for the *in vivo* standard error of the mean. Asterisks indicate significantly ($p < 0.05$) different from the blanks. Note that the estimated doses (shown in the plots) were different for the three different particle types, e.g., the dose for PLA-generated particles is much lower.

Inflammatory responses

For all the 3D printer emitted particles tested, a single dose increased the total cell numbers significantly in the BALF of mice after 24 h (Figure 2D), which is associated with defense against intrusion of particles. In addition, all tested particles induced recruitment of neutrophils compared to the blank (Figure 2E), which is an important feature of lung injury. The PLA-emitted particles produced the strongest inflammatory response, followed by Regular ABS and High ABS. This inflammatory response in mice lungs shows the potential adverse health effects of 3D printer emitted particles, consistent with other studies that showed asthma development,[14] or increased rates of respiratory symptoms[15] for human exposed to 3D printer emissions.

Oxidative potential

The cell-free DTT assay showed that the water-soluble OP^{DTT}_{m} was below limit of detection (LOD), while the total particle OP^{DTT}_{m} was above LOD for all the particles tested (Figure 3). The OP^{DTT}_{m} of 3D printer emitted particles were compared with the OP^{DTT}_{m} of aerosols from various sources, as well as ambient PM in different locations. Only PLA emitted

particles had OP^{DTT}_m as high as combustion emitted aerosols, while other tested materials were generally lower than ambient PM (Figure 3). In average, OP^{DTT}_m for nylon and ABS emitted particles were 4 to 30 factors lower than that for ambient $PM_{2.5}$ (PM less than 2.5 µm in size) in Atlanta, where large population epidemiological studies have suggested links between DTT assay measured OP and adverse cardiorespiratory effects,[16] while that for PLA emitted particles was about a factor of 3 higher (Figure 3).

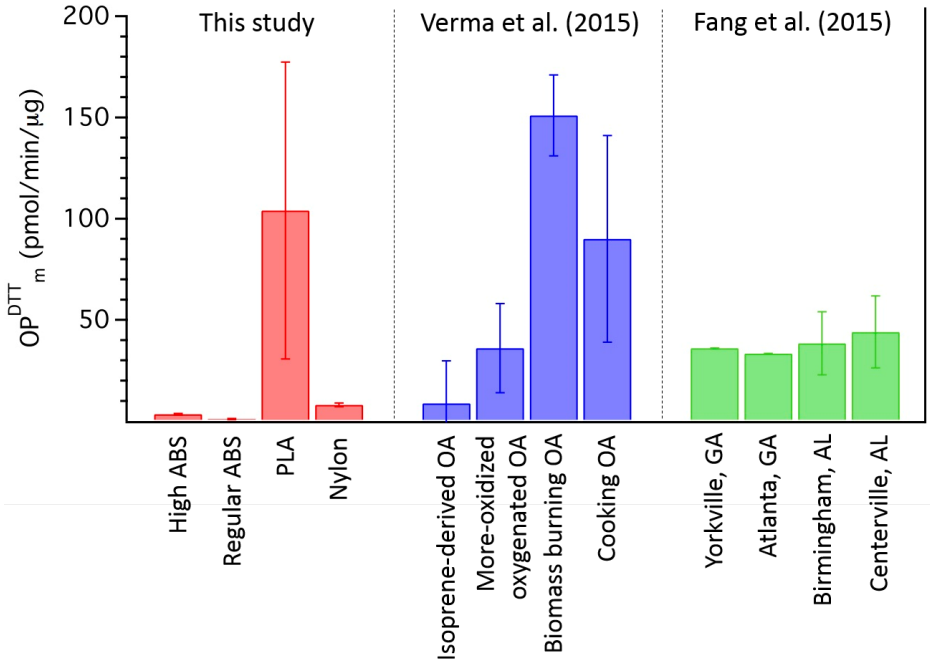

Figure 3. OP^{DTT}_m measured in this study (High ABS, Regular ABS, PLA, Nylon), compared to previous ambient studies on specific sources of organic aerosol (OA),[17] and $PM_{2.5}$ at various locations.[9] Each error bar is the standard deviation of data in each group.

Although OP^{DTT}_m provides some indication of the aerosol toxicity, potential adverse health effects depend on actual exposures, which are associated with particle emissions during printing. To consider exposures, measures of toxicity (e.g., OP^{DTT}_m) can be multiplied with particle emissions from the corresponding filaments (e.g., mass yield, which is the mass of emitted particles per mass of object printed).[1] The product is the assay response per mass of the object printed (i.e., $OP^{DTT}_m \times$ [mass yield] = OP^{DTT}_{om}). By this analysis, ABS filaments are potentially greater health concerns since their emissions are orders of magnitude higher than PLA, resulting in OP^{DTT}_{om} of ABS factors of 5 – 10 higher than that of PLA (Figure 4).

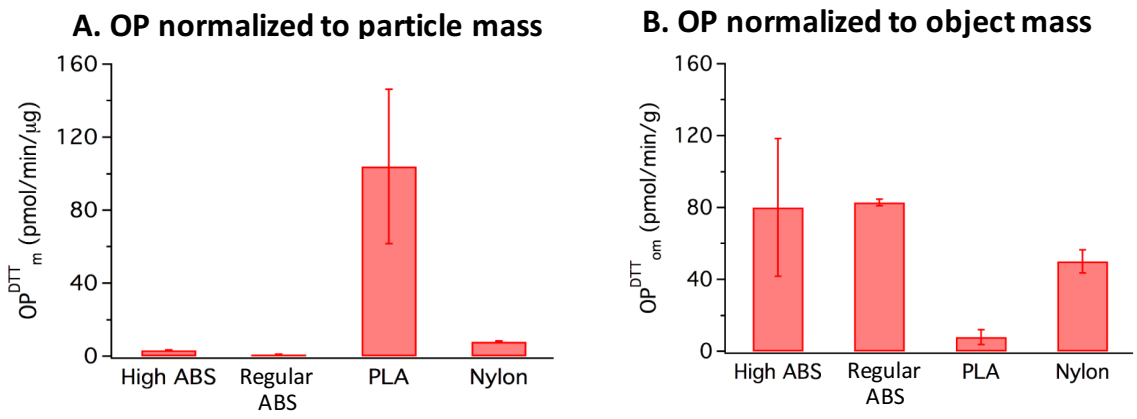

Figure 4. Total DTT assay results presented as OP normalized to particle mass (A) or OP normalized to print object mass (B). The difference between the two is whether particle emission levels were considered.

CONCLUSIONS

The chemical composition of 3D printer emitted particles may be similar to their raw filament material (e.g., PLA) or different (e.g., ABS), indicating particle formation may be associated with the bulk filament material or minor additives. Particles emitted from 3D printers have the potential to produce adverse health impacts that depend on filament materials used. A consistency of various biological responses showed that PLA-emitted particles induced similar levels of responses as ABS-emitted particles, but at much lower doses, indicating that PLA-emitted particles are potentially more toxic on a particle mass basis. However, overall exposure toxicity also depends on emission levels, resulting in ABS filaments of more concerns due to their much higher emission levels.

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2211 Newmarket Parkway, Suite 106
Marietta, Georgia 30067
E: UL.ChemicalSafety@ul.com
W: www.ulchemicalsafety.org