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Short Communication

# Inhalation exposure to three-dimensional printer emissions stimulates acute hypertension and microvascular dysfunction

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ABSTRACT

Fused deposition modeling (FDM™), or three-dimensional (3D) printing has become routine in industrial, occupational and domestic environments. We have recently reported that 3D printing emissions (3DPE) are complex mixtures, with a large ultrafine particulate matter component. Additionally, we and others have reported that inhalation of xenobiotic particles in this size range is associated with an array of cardiovascular dysfunctions. Sprague-Dawley rats were exposed to 3DPE aerosols via nose-only exposure for ~3 h. Twenty-four hours later, intravital microscopy was performed to assess microvascular function in the spinotrapezius muscle. Endothelium-dependent and -independent arteriolar dilation were stimulated by local microiontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). At the time of experiments, animals exposed to 3DPE inhalation presented with a mean arterial pressure of 125 ± 4 mm Hg, and this was significantly higher than that for the sham-control group (94 ± 3 mm Hg). Consistent with this pressor response in the 3DPE group, was an elevation of ~12% in resting arteriolar tone. Endothelium-dependent arteriolar dilation was significantly impaired after 3DPE inhalation across all iontophoretic ejection currents (0–27 ± 15%, compared to sham-control: 15–120 ± 21%). Endothelium-independent dilation was not affected by 3DPE inhalation. These alterations in peripheral microvascular resistance and reactivity are consistent with elevations in arterial pressure that follow 3DPE inhalation. Future studies must identify the specific toxicants generated by FDM™ that drive this acute pressor response.

## 1. Introduction

Fused deposition modeling (FDM™) is a type of material extrusion three-dimensional (3D) printing technology in which a polymer filament is heated and extruded through a nozzle to create an object. We have previously characterized the aerosols generated and emitted during FDM™ 3D printing (Yi et al., 2016; Stefaniak et al., 2017). These studies have demonstrated that thermal decomposition of polymer filaments during 3D printer operation releases billions of ultrafine particles ($PM_{0.1}$, particles with at least one dimension < 100 nm) per minute, with emission rates being higher for acrylonitrile butadiene styrene (ABS) polymer compared to poly lactic acid (PLA) polymer. During thermal decomposition, numerous volatile organic chemicals (VOCs) are released into air, including aldehydes, ketones, alcohols, and aromatics (benzene, toluene, ethylbenzene, xylenes, and styrene). In addition to these primary emissions, some secondary reaction products were formed by the interaction of ozone with certain VOCs to produce carbonyl compounds.

$PM_{0.1}$ is generated largely from combustion-related processes (Kuwayama et al., 2013), and inhalation is the primary exposure route (Oberdorster et al., 1995). $PM_{0.1}$ inhalation has been positively associated with cardiovascular morbidity (Liu et al., 2013) and mortality (Su et al., 2015). Experimentally, these outcomes have been characterized as ischemia/reperfusion injury, conduction abnormalities, vascular dysfunction and hemostasis (Cascio et al., 2007; Courtois et al., 2008; Samet et al., 2009). We have documented that FDM™ 3D printing emissions (3DPE) are complex particulate aerosols with a significant fraction of particles in the ultrafine range (Yi et al., 2016). Therefore, it is reasonable to anticipate that alterations in cardiovascular function may follow 3DPE inhalation exposures.

Over the past decade, we have reported robust systemic microvascular dysfunction after inhalation of fine particulate matter

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(Nurkiewicz et al., 2004; Nurkiewicz et al., 2006) and nanoparticles (Nurkiewicz et al., 2008; Nurkiewicz et al., 2009). While all nanoparticles are not ultrafine PM, the two particles do share physical commonality in their defined sub 100 nm size. Cardiovascular function has not been assessed after 3DPE inhalation exposure. Therefore, we hypothesized that a similar biological response should follow 3DPE inhalation. However, these effects may not be implicitly similar as 3DPE are a more dynamic mixture of solids and gases, undergoing phase changes. Therefore, the purpose of this study was to initiate assessments of the acute cardiovascular and systemic microvascular consequences of 3DPE inhalation.

## 2. Methods

### 2.1. Animal model

All procedures and experiments in this study conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th Edition) and were approved by the West Virginia University Animal Care and Use Committee. Male, Sprague-Dawley rats (7–8 weeks of age) were purchased from Hilltop Laboratories (Scottdale, PA), and housed in an AAALAC approved animal facility at the West Virginia University Health Sciences Center. All animals were maintained on a 12-hour light/dark cycle, provided food and water ad libitum, and acclimated for at least 72 h prior to training and/or use. Animals were then randomly assigned to either the Sham-Control (filtered air) or 3DPE inhalation exposure groups.

### 2.2. 3DPE generation and aerosol inhalation exposure

All rats were progressively trained daily for ~2 weeks prior to nose-only exposures. During these sessions, rats were placed in restraining tubes (Allay®, DSI, St. Paul, MN) initially for ~5 min, and progressively increased in 15–20 minute intervals for up to 4 h. Tubes were wrapped in red polyurethane sheets to reduce visual stress. At any point during training or exposure, if rats displayed distress, they were immediately removed. On experimental days, rats were placed in a nose-only exposure system (Inhalation Tower, DSI, St. Paul, MN) for 3–4 h. The stainless steel, nose-only exposure device is designed to accommodate up to 14 rodents. The tower has two levels of seven exposure ports, radially positioned around the aerosol delivery components of the system.

A desktop 3D printer (Replicator® 2×, MakerBot Industries, Brooklyn, NY) was placed in a 500 L stainless steel chamber. Black ABS is among the more common filaments used in 3D printing, and was used for all rat inhalation exposures herein. The printer was operated continuously during rat exposures. Emissions from the chamber were pumped into the nose-only exposure tower. Real-time and time-integrated aerosol sampling and monitoring instrumentation analyzed the emissions in the generation chamber and nose-only exposure tower as previously described (Yi et al., 2013). Briefly, 3DPE particle size distribution and mass concentration were measured continuously with a scanning mobility particle sizer (SMPS, TSI Inc., Shoreview, MN), and an electric low-pressure impactor (ELPI, Dekati, Ltd., Kangasala, Finland). The aerosol mass concentration was verified gravimetrically.

### 2.3. Intravital microscopy

At 24 h. post-exposure, rats were anesthetized with thiobutabarbital sodium (Inactin, 100 mg/kg, i.p.), placed on a heating plate, connected to a thermocouple probe to maintain a 37 °C rectal temperature with an Animal Temperature Controller (World Precision Instruments, Sarasota, Florida). The trachea was intubated to ensure a patent airway, and the right carotid artery was cannulated to measure arterial pressure. The right spinotrapezius muscle was then exteriorized for microscopic observation, leaving its innervation and all feed vessels intact. After exteriorization, the muscle was gently secured over an optical pedestal at its in situ length. The muscle was next enclosed in a tissue bath for transillumination and observation. Throughout the surgery and all experimental periods, the muscle was continuously superfused with an electrolyte solution (119 mM NaCl, 25 mM $NaHCO_3$, 6 mM KCl and 3.6 mM $CaCl_2$), warmed to 35 °C, and equilibrated with 95% $N_2$ – 5% $CO_2$ (pH = 7.35–7.40). Superfusate flow rate was maintained at 4–6 mL/min to minimize equilibration with atmospheric oxygen (Boegehold and Bohlen, 1988).

The animal preparation was then transferred to the stage of an intravital microscope, coupled to a CCD color video camera (BX51WI and DP71, respectively, Olympus, Tokyo, Japan). Observations were made with a 20× water immersion objective (final video image magnification = 1460×). One to three arterioles were studied per rat. Real-time images were displayed on a high-definition computer monitor and digitally captured for off-line analysis (Nurkiewicz et al., 2008, 2009). Arteriolar inner diameters were measured with Image-J software (National Institutes of Health, Bethesda, MD) calibrated with a stage micrometer.

### 2.4. Microiontophoresis

Micropipettes were custom fabricated with a Flaming/Brown Micropipette Puller (P-97, Sutter Instruments, Novato, CA). Aluminosilicate glass capillary tubes (A100-64-10, Sutter Instruments) were pulled to an inner diameter of 2–4 μm and subsequently double-beveled (BV-10, Sutter Instruments), as previously described (Nurkiewicz and Boegehold, 2004). Micropipettes were then backfilled with the muscarinic agonist acetylcholine (0.025 M; ACh), or the nitric oxide donor sodium nitroprusside (0.05 M; SNP). Backloaded pipettes were attached to a microelectrode holder with an indwelling Ag/AgCl wire that was connected to a Dual Microiontophoresis Current Programmer (SYS-260; World Precision Instruments, Sarasota, FL). The microelectrode holder was attached to a three-axis hydraulic micromanipulator, combined with a one-axis hydraulic micromanipulator (MMO-203, and MMO-220A respectively, Narishige, Tokyo, Japan) to enable four-dimensional controlled movements. The entire apparatus was mounted on a motorized stage platform (GMHB-BX, Gibraltar Industries, Buffalo, NY), adjacent to the animal preparation. Finally, an additional Ag wire was submerged in the intravital tissue bath to complete the electrical circuit. The micropipette was positioned with the tip within the arteriolar adventitial layer, slightly superior to the vessel wall to prevent accidental puncture associated with tissue movement and dilation. Holding currents of 200–500 nA were used to contain agonists in the micropipette during all control and recovery periods. Microiontophoretic ejection currents were randomly generated to evaluate endothelium-dependent dilation (ACh; 20, 40, and 100 nA), and endothelium-independent dilation (SNP; 5, 10 and 20 nA). At the end of all experiments, passive maximum arteriolar diameter was established by superfusing the tissue with $10^{-4}$ M adenosine (ADO).

### 2.5. Formulas, data and statistical analysis

Mean arterial pressure (MAP) was calculated as: MAP = diastolic pressure + (systolic pressure − diastolic pressure) / 3. Arteriolar diameter (D, μm) was sampled at 10-second intervals during all control and ejection periods. Resting vascular tone was calculated for each vessel as follows: Tone = $[(D_{pass} - D_c) / D_{pass}] \times 100$, where $D_{pass}$ is passive diameter under ADO and $D_c$ is the diameter measured during the control period (resting diameter). A tone of 100% represents complete vessel closure, and 0% represents the passive state. To evaluate arteriolar responsiveness between individual groups with subtle differences in resting diameter, arteriolar diameter was normalized. In this case, arteriolar diameter was expressed as a percent change from control and was calculated for each vessel as follows: Diameter (% change from control) = $[(D_{SS} - D_c) - 1 \times 100]$, where $D_{SS}$ is the steady state