

## Assessing and Reducing the Toxicity of 3D-Printed Parts

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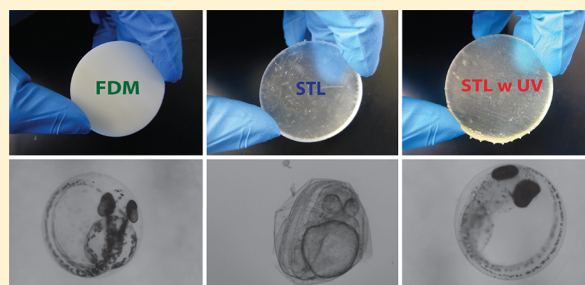
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### S Supporting Information

**ABSTRACT:** 3D printing is gaining popularity by providing a tool for fast, cost-effective, and highly customizable fabrication. However, little is known about the toxicity of 3D-printed objects. In this work, we assess the toxicity of printed parts from two main classes of commercial 3D printers, fused deposition modeling and stereolithography. We assessed the toxicity of these 3D-printed parts using zebrafish (*Danio rerio*), a widely used model organism in aquatic toxicology. Zebrafish embryos were exposed to 3D-printed parts and monitored for rates of survival, hatching, and developmental abnormalities. We found that parts from both types of printers were measurably toxic to zebrafish embryos, with STL-printed parts significantly more toxic than FDM-printed parts. We also developed a simple post-printing treatment (exposure to ultraviolet light) that largely mitigates the toxicity of the STL-printed parts. Our results call attention to the need for strategies for the safe disposal of 3D-printed parts and printer waste materials.



## INTRODUCTION

Even though additive manufacturing or “3D printing” was first introduced in 1983,<sup>1</sup> the technology has become widespread only in the past few years. The value of the 3D printing market grew from \$288 million in 2012 to \$2.5 billion in 2013 and is projected to grow to \$16.2 billion by 2018.<sup>2</sup> Much of this growth has occurred in the life sciences, where 3D printing has found applications in dentistry,<sup>3,4</sup> prosthetics and implantable devices,<sup>5,6</sup> surgical instruments,<sup>7</sup> and even tissue and organ replacement.<sup>8</sup> By providing businesses, researchers, physicians, and hobbyists with custom objects and tools quickly and inexpensively, 3D printers are revolutionizing manufacturing, accelerating research, and changing how medicine is practiced.

In spite of the growing popularity of 3D printers, relatively little is known about the toxicity of 3D-printed parts. Previous work has found that 3D-printed parts can be toxic to cancer cells<sup>9</sup> and may cause allergic or inflammatory responses<sup>5,10</sup> and infections<sup>11</sup> in patients. Additionally, some 3D printers release potentially hazardous particles into the air during operation.<sup>12</sup> However, the whole-organism health effects of exposure to 3D-printed parts remain largely unexplored. As 3D-printed parts find increasing use in the medical and life science fields, the effects of exposure to these parts need to be understood. Additionally, as consumer-grade 3D printers become more widespread, the amount of 3D-printed parts and printer waste being released into the environment will also grow, and the toxicity of these materials in the environment remains largely unexplored.

With little known about the toxicity of 3D-printed parts, there are consequently few techniques for reducing the toxicity of these parts. Researchers have found that heating a 3D-printed part can reduce its toxicity to cancer cells, but heating also adversely affects the appearance of the part.<sup>9</sup> Treating 3D-printed parts with supercritical carbon dioxide can reduce the inflammation caused when the parts are implanted in the body,<sup>5</sup> but this technique requires a specialized instrument that is more expensive than many 3D printers. There is an unmet need for simple and accessible techniques for reducing the toxicity of 3D-printed parts in research, healthcare, and commercial applications.

In this work, we assessed the effects of 3D-printed parts on an organism’s health and developed a simple technique for reducing the toxicity of these printed parts. We chose zebrafish (*Danio rerio*) as the model organism for this study. Zebrafish are widely used vertebrate model organisms that, because of their ability to reproduce quickly and in large numbers, make high-throughput screening of potential toxicants feasible and affordable.<sup>13</sup> There are many genetic similarities between humans and zebrafish, and the relatively fast development of sophisticated cardiovascular, nervous, and endocrine systems in these animals makes them a very popular developmental

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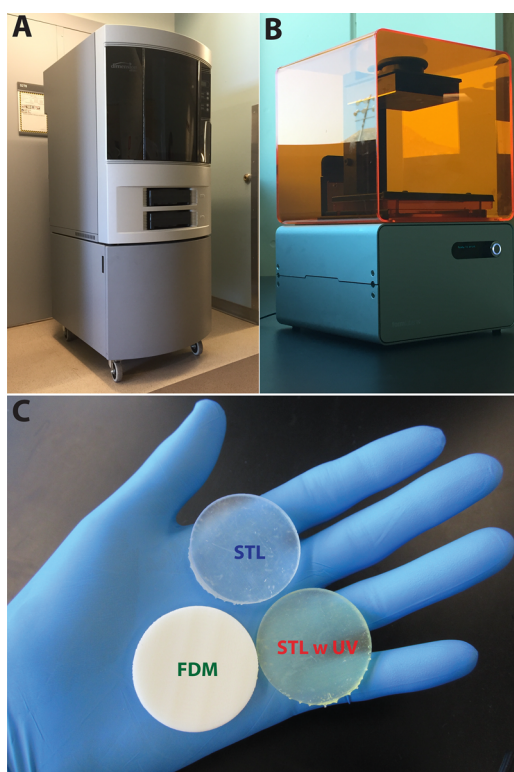
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model.<sup>14</sup> As aquatic organisms, zebrafish are also a relevant model for understanding the bioavailability and bioaccumulation of chemical and biological toxicants<sup>15</sup> and overall environmental toxicity. Finally, zebrafish are optically transparent throughout their development (embryonic and adult stage) and can be analyzed using imaging techniques to identify developing pathologies and phenotypic changes in real time.

## METHODS

**3D Printers.** We studied the toxicity of printed parts from the two main commercially available types of 3D printers, fused deposition modeling (FDM) and stereolithography (STL) printers. FDM printers feed a polymer filament into a heated nozzle that melts the polymer and deposits it layer by layer onto the growing part.<sup>16</sup> In this study, we used the Dimension Elite printer (Stratasys, Eden Prairie, MN) (Figure 1A), which prints parts out of acrylonitrile butadiene styrene (ABS).



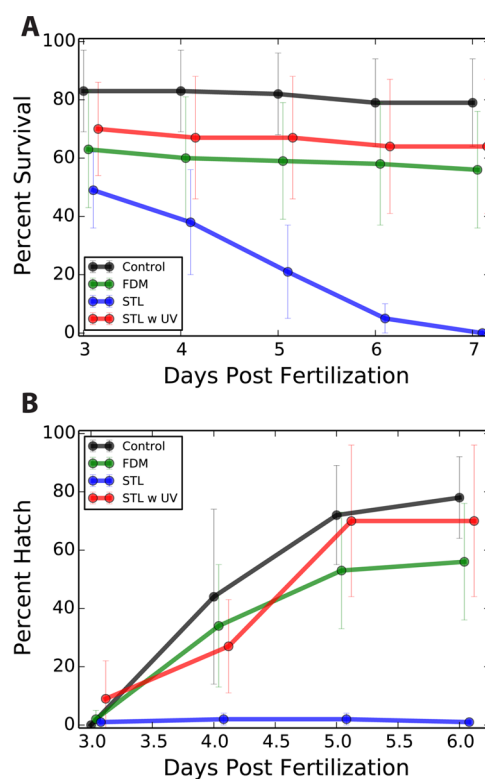
**Figure 1.** Commercial 3D printers and test pieces. (A) A commercial fused deposition modeling (FDM) printer (Dimension Elite printer from Stratasys), which deposits melted acrylonitrile butadiene styrene (ABS) layer by layer onto a stage to build a 3D-printed part. (B) A commercial stereolithography (STL) printer (Form 1+ printer from Formlabs, Cambridge, MA), which uses a light source to polymerize a liquid resin to form a printed part. (C) Examples of the FDM- and STL-printed test parts used in this study (40 mm diameter and 4 mm height). Also shown is an STL-printed part that was treated with ultraviolet light (STL w UV) to reduce its toxicity. The UV treatment has little effect on the appearance of the printed part.

In contrast, STL printers use a light source to polymerize a bath of photocurable liquid resin layer by layer to form a finished part.<sup>1</sup> Because the chemical compositions of the photocurable resins are typically not provided by printer manufacturers, little is known about the chemical and biological compatibility of STL-printed parts. In this study, we used the Form 1+ printer (Figure 1B); this printer uses a 405 nm Class 1

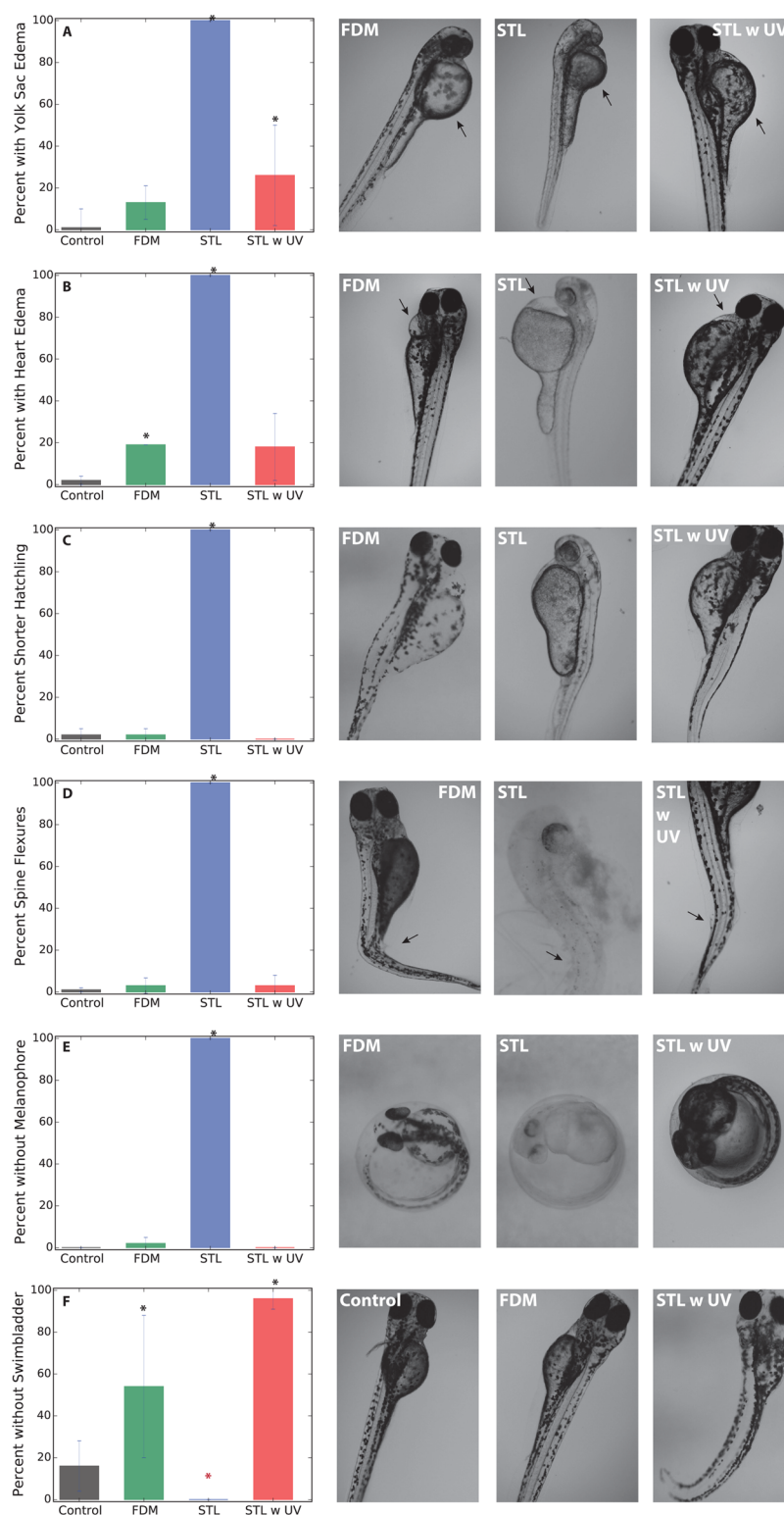
laser to cure a resin that is a combination of methacrylated oligomers and monomers and photoinitiators.<sup>17</sup>

## 3D-Printed Test Parts and Cleaning Procedures.

Cylindrical test parts (40 mm diameter and 4 mm thick, shown in Figure 1C) were designed using SolidWorks (Dassault Systèmes, Vélizy-Villacoublay, France), exported as an .STL file, and printed using the FDM and STL printers. The 3D-printed parts used in toxicity tests in Figures 2 and 3 were cleaned according to the printer manufacturers' specifications. FDM-printed parts were submerged in a 2% (w/v) sodium hydroxide solution for 4 h to dissolve the temporary polylactic acid supports, then rinsed with ultrapure water, and air-dried. STL-printed parts were washed in two consecutive baths of isopropyl alcohol for 5 min each and then air-dried.



**Figure 2.** Survival and hatching rates of exposed zebrafish embryos compared to control unexposed embryos. (A) Survival rates of zebrafish embryos exposed to 3D-printed parts from a FDM printer (green), embryos exposed to parts from a STL printer (blue), embryos exposed to STL-printed parts that were treated with ultraviolet light (red), and control embryos that were not exposed to printed parts (black). Each exposure represents three replicates with 30 embryos in each replicate. Embryos exposed to STL-printed parts had significantly lower survival rates by day 3 post-fertilization when compared to those of control embryos ( $p \leq 0.05$ ), with no STL-exposed embryo surviving past day 7. However, embryos exposed to FDM- and UV-treated STL-printed parts did not have significantly decreased survival rates compared to those of control embryos ( $p \geq 0.05$ ). (B) Hatching rates for the same four exposure types as in panel A. Embryos exposed to STL-printed parts had significantly lower hatching rates by day 4 post-fertilization compared to those of control embryos ( $p = 0$ ); virtually none of the STL-exposed embryos hatched. However, embryos exposed to FDM- and UV-treated STL-printed parts did not have significantly lower hatching rates in the embryos ( $p \geq 0.05$ ). These results show that after STL-printed parts had been treated with UV light, embryos exposed to the treated parts fare almost as well as control embryos that were not exposed to printed parts.



**Figure 3.** Malformation rates in exposed zebrafish embryos compared to those of unexposed control embryos. Rates of six types of malformations in zebrafish embryos exposed to FDM-printed parts (green), embryos exposed to STL-printed parts (blue), embryos exposed to STL-printed parts that received UV treatment (red), and control embryos that were not exposed to printed parts (black). The observed malformations were (A) yolk sac edema, (B) heart edema, (C) embryo length deformation, (D) spine flexures, (E) a lack of melanophore development, and (F) a lack of swim bladders. Malformations A–E were monitored at day 4 and day 7 post-fertilization, and malformation F was monitored at day 7. Unexposed control embryos (black) had low levels of malformation in all six categories, and embryos exposed to FDM-printed parts (green) had elevated rates of malformations in three of the six categories (A, B, and F). However, 100% of embryos exposed to STL-printed parts had a significantly higher rate of malformations in all six categories (blue). Because the embryos that were exposed to STL-printed parts did not survive past 7 days, these embryos were not checked for the development of swim bladders (red asterisk in panel F). Embryos exposed to STL-printed parts that received UV treatment (red) had rates of malformations that were comparable to those of embryos exposed to FDM-printed parts for malformations A–E, though they did have significantly slower swim bladder development (F).



To determine the effects of different part cleaning techniques on the toxicity of the printed parts, additional 3D-printed parts were cleaned using alternative cleaning procedures with little or no effect on the toxicity results of the printed parts (Supporting Information).

**UV Light Exposure of STL-Printed Parts.** Exposure to ultraviolet light was used to detoxify some STL-printed parts in this study. An Intelli-Ray 400 UV light source (Uvitron International, Inc., West Springfield, MA) with a peak irradiance of 100–120 mW/cm<sup>2</sup> was used. Each STL-printed part was exposed to UV light at 50% lamp power for an exposure time on each side of 30 min, for a total of 1 h of exposure time per part.

**Animal Husbandry and Exposure to 3D-Printed Parts.** We assessed the toxicity of 3D-printed parts using zebrafish (*D. rerio*) following a specific protocol approved by the University of California, Riverside's Animal Care and Use Committee (approval number 20130005). The zebrafish were wild-type AB strain and approximately 16 months old at the time of spawning. The fish cultures were kept in aerated aged tap water (dechlorinated) at 27 °C with a 14 h/10 h light/dark cycle. Males and females were kept separately and fed twice a day on *Artemia* sp. until the night before spawning, when they were transferred to breeding aquaria. Eggs were collected the next morning, examined, and separated on the basis of the stage of development. All embryos were directly exposed to their respective 3D-printed parts at 2 h postfertilization. Each printed part was placed in a large sterile Petri dish (90 mm in diameter and 15 mm in height) and surrounded with approximately 45 mL of ultrapure water (resistivity of 18.2 MΩ cm at 25 °C).

Each printed part was exposed to 30 embryos and replicated three times, for a total of 90 embryos used to study the effectiveness of each cleaning technique for both printing methods. The embryos were monitored for their survival, hatching rate, and developmental abnormalities (reduced length, yolk sac edema, heart edema, spinal flexure, absence of swim bladder, and discoloration) at days 4 and 7 postfertilization by visual inspection. Dead embryos were identified by the loss of translucency and removed from the dish before further inspection of the health of the remaining embryos.

**Statistical Analysis and Data Visualization.** The significance of the results was tested using the Wilcoxon Rank Sum nonparametric test with appropriate assumptions on R programming language. The *p* values were set to 0.05 to test for the significance of treatments. The results were visualized using the Matplotlib package in the Python programming language.

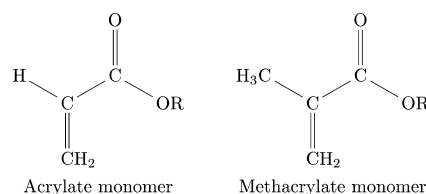
## RESULTS AND DISCUSSION

**Assessing the Toxicity of 3D-Printed Parts.** Figure 2A shows the percent survival of embryos exposed to 3D-printed parts from FDM (green) and STL (blue) printers compared to that of unexposed control embryos (black) through 7 days postfertilization. While the embryos exposed to FDM-printed parts had slightly decreased average survival rates compared to those of control embryos, the embryos exposed to STL-printed parts had significantly decreased survival rates ( $p \leq 0.05$ ), with more than half of the embryos dead by day 3 and all dead by day 7. The percent of exposed embryos that hatched followed a similar trend (Figure 2B): embryos exposed to FDM-printed parts had hatching rates slightly lower than those of unexposed embryos, but embryos exposed to STL-printed parts had

significantly decreased ( $p \leq 0.05$ ) hatching rates (essentially zero hatching).

We also used six deformities as markers to assess the health of embryos after they hatched. We monitored hatchlings for yolk sac edema (Figure 3A), heart edema (Figure 3B), reduced hatchling length (Figure 3C), the presence of spine flexures (Figure 3D), and a lack of melanophores (Figure 3E) throughout the monitoring period of 7 days, and the lack of a swim bladder (Figure 3F) at day 7 postfertilization. The zebrafish micrographs in Figure 3 show the most severe cases of deformity in each category, for embryos exposed to parts from each of the 3D printer types. Of the few zebrafish embryos that hatched after exposure to STL-printed parts, 100% of the hatchlings had all six malformations (blue in Figure 3). In contrast, zebrafish embryos exposed to FDM-printed parts had significantly lower rates of malformations, although FDM-exposed embryos still exhibited deformities at a rate higher than that of unexposed control embryos (especially for yolk sac edema) and a statistically significant increase ( $p \leq 0.05$ ) in heart edema (green in Figure 3). Embryos exposed to FDM-printed parts also exhibited significantly delayed swim bladder development ( $p \leq 0.05$ ) compared to that of the control embryos.

**Reducing the Toxicity of STL-Printed Parts.** While the exact chemical compositions of the resins used in STL printers are usually trade secrets, the resins' Material Safety Data Sheets indicate that they usually contain acrylate and/or methacrylate monomers:



Specific members of these classes of compounds are already known to be toxic in some situations. For example, acrylate monomers can be acutely toxic if they are inhaled, are swallowed, or come into contact with skin.<sup>18</sup> If the R group is a hydrogen, the resulting compounds (acrylic acid and methacrylic acid) have been shown to have toxic effects on embryonic and fetal development in rat fetuses.<sup>19</sup> If the R group in the methacrylate monomer is a methyl group, the resulting compound (methyl methacrylate) and its polymerized form [poly(methyl methacrylate) or PMMA] have been associated with irreversible cardiovascular failure when they are used as scaffolds.<sup>19</sup> Finally, exposure to methacrylate monomers with a variety of other R groups (ethyl, *n*-butyl, isobutyl, and isodecyl) has been observed to cause cytotoxicity, cardiovascular failure, gastrointestinal problems, respiration issues, and developmental malformations.<sup>19</sup> In summary, while we do not know the exact composition of STL printer resins, ample evidence of the toxicity of the monomers in these resins exists.

On the basis of the known toxicity of acrylate and methacrylate monomers, we hypothesized that monomers or short-chain polymers may be leaching out of the STL-printed parts and contributing to the extreme toxicity of those parts. To test this hypothesis, we performed gas chromatography–mass spectrometry (GC–MS) analysis of water samples left in contact with STL-printed parts. The results suggest that at least three different chemical species are present in the leachate; these species have different retention times in GC but very

similar fragments in MS (Supporting Information). This supports our hypothesis that monomers or short-chain polymers are present in the leachate from STL-printed parts, although additional analysis is necessary for a definitive identification.

If monomers or short-chain polymers are indeed leaching out of STL-printed parts, additional photoinduced polymerization of the 3D-printed part might reduce the amount of these species leaching out of the printed part and thus reduce the toxicity of the part. To test this hypothesis, we exposed STL-printed parts to ultraviolet light (wavelength of 350–400 nm, peak irradiance of 100–120 mW/cm<sup>2</sup>) for 30 min on each side of the printed part. As shown in Figure 1C, this UV exposure treatment has a minimal effect on the appearance of the 3D-printed part. Embryos exposed to STL-printed parts that were UV-treated fared much better than embryos exposed to untreated parts. As shown in panels A and B of Figure 2 (red), the survival and hatching rates of embryos exposed to treated parts recovered to almost control levels. Embryos exposed to UV-treated STL-printed parts also showed a significantly lower incidence of spine flexures (Figure 3D, red). All hatchlings exposed to UV-treated parts were normal in length (Figure 3C, red) and developed normal levels of melanophores (Figure 3E, red). However, embryos exposed to UV-treated parts still had significantly elevated rates of yolk sac edema ( $p \leq 0.05$ ) and heart edema compared to those of control embryos (Figure 3A,B, red) and most of the embryos exposed to UV-treated parts had not developed swim bladders by the end of day 7 (Figure 3F, red). Therefore, while our UV treatment appears to significantly reduce the toxicity of STL-printed parts to zebrafish, it does not completely eliminate the toxicity of these parts, and additional research into detoxification strategies is merited.

Our findings have important consequences in several different communities. Physicians and nurses using 3D-printed parts in clinical applications need to consider the consequences of patient exposure to these parts; researchers using 3D-printed parts in life science experiments should be on the lookout for artifacts caused by exposures of organisms to these objects, and waste collection agencies should develop strategies for the safe collection and disposal of parts and waste materials generated by 3D printers. The cost of 3D printers has dropped dramatically—FDM printers are currently available for as little as \$200, and the STL printer used in this study can be bought for \$3299—and this trend is expected to continue in the coming years. Consequently, 3D printers are spreading beyond industry and research laboratories and into homes and small businesses. The individuals using these printers may not have the training necessary to use these printers safely and dispose of their wastes responsibly, and municipal waste disposal agencies may not have resources for collecting and treating 3D printer waste. This situation is particularly worrisome for STL printers, which can generate liters of solvent waste contaminated with resin monomers during post-printing part cleanup. The potential for 3D printer toxic waste to enter waterways is alarming and deserves additional study.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.5b00249.

Results from testing the toxicity of 3D-printed parts subjected to alternative postprint cleaning techniques and GC–MS analysis of leachates from STL-printed parts (PDF)

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### Notes

The authors declare no competing financial interest.

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