Assessing the Blockage of Toxicity of Stereolithographically 3D-Printed Parts Coated with Parylene C

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Research Article

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Assessing the Blockage of Toxicity of Stereolithographically 3D-Printed Parts Coated with Parylene C

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1. Abstract

Compared to fused deposition modeling (FDM), the stereolithography (SLA) 3D printing method can provide higher manufacturing speed and increased resolution, which is a requirement of biomedical usage. However, the additives migrating from SLA printed plastic are toxic, and the method to reduce toxicity by soaking and exposure to ultraviolet is inadequate. Moreover, current biocompatible photo-polymerizing resins are rare and expensive. Therefore, a method to negate all toxicity by coating the fabrication with parylene C is proposed. Early life zebrafish were used to assess the toxicity of the SLA printed petri dishes with and without parylene C. The result reveals that the coated petri dishes have little effect on the early life zebrafish development compared with control group, while the fertilized embryos of uncoated groups die within 24 hours. The method provides a cost-efficient and straightforward way to produce customized biocompatible fabrication, contributing to the popularization of 3D-printed products, either in daily life or in research.

2. Introduction

3D printing, or additive manufacturing, pioneered in the mid-1990s, has been widely applied in recent years [1]. Metal powder, ceram, beton, or photosensitive resin can be used as a consumable material for 3D printing. Through the patterning designed in advance on a computer, 3D printing can fabricate or accumulate consecutive layers of the consumable material and eventually form a 3D entity [2]. After decades years of development, numerous kinds of 3D printing methods have emerged, including fused deposition modeling (FDM), stereolithography (SLA), powder-liquid 3D printing (PLP), and selective laser sintering (SLS) [1-3]. The FDM method provides multiple kinds of polymers with biocompatibility [4]. However, such biocompatibility is negated when the

3D parts are with increasing complexity [5]. Particularly, SLA has its advantages of extraordinary resolution, fabrication speed, and smooth finished surface [3,6]. Therefore, SLA is more widely applied in medical science and biology in fields such as tissue engineering, organ printing, artificial bones development, and drug delivery [3]. However, for SLA, only a tiny fraction of commercially available photo-polymerizing resins have been accredited as biocompatible (i.e., according to ISO 10993-5:2009 or USP class testing). Moreover, among the imperative compounds or additives for SLA, more than 20 compounds are proved to be poisonous for creatures and be found to remain in the finished product [7]. Some of the toxic compounds are known as photo-initiators. For example, a 1-HCHPK leak from the 3D parts can induce developmental defects in zebrafish such as spine curvature, heart oedema, and an overt decrease in skin pigmentation [8]. Hence, the toxicity of SLA 3D parts was reduced by treatment with supercritical carbon dioxide [9], sonication of the material in isopropanol [10], and exposure to ultraviolet light [11]. However, the aforementioned methods are either complex, expensive, or inadequate.

To eliminate the influence of the toxicity of SLA 3D parts, the method of coating the 3D parts with parylene C to isolate the toxicity was exploited. Parylene is the generic name for the members of a xylylene family of polymers [12,13]. They are classified as thermoplastic polymers formed on substrate surfaces using the technique of vacuum deposition polymerization [12,14]. Currently, three of them are commercially valuable, i.e., parylene N and its halogenated derivatives and parylene C (p-xylylene substituted with a single chlorine molecule) and D (substituted with two chlorine molecules) [12,14]. Parylene C exhibits excellent properties for medical applications, such as low water absorption, high dielectric constant, low coefficient of

friction, and moderate elongation at break [12]. It has been successfully used as a protective coating on medical metals and metal alloys, such as gold, platinum [15-17], medical-grade stainless steel (SS 316L) [18-20], and magnesium alloys (AZ31, WE43, AZ9) [21], and ceramics such as Si_3N_4 [17] and Al_2O_3 [22]. In this study, the toxicity of the SLA printed petri dish was blocked by coating the printed parts with parylene C. The toxicity of parylene C coated petri dish was assessed compared with the uncoated groups through zebrafish acute toxicity test.

3. Materials and Methods

3.1 Producing parylene C coated STL printed petri dish.

A high-resolution stereolithography printer Lite600 (UnionTech Inc., Guangzhou, Guangdong Province, China) was used to manufacture the petri dishes. An acrylic polymer (Figure 3B) resin was used as the printing material. The photoinitiator is 2,2-bimethoxy-2-phenylacetophenone (Figure 3A). Moderate 3,4-Epoxy cyclohexylmethyl was also added to the raw material. After printing, all constructed parts were removed from the build platform, rinsed, and cleaned with ethanol and acetone. The parts were subsequently washed with water and dried with air, and the support structures were removed. Finally, all parts were cured in a UV flood chamber for a minimum of five minutes. The petri dish is coated with parylene C (Donghang Nano Technology Inc., Yingtan, Jiangxi Province, China) with a thickness of 23 μm by nano vacuum coating machine L-800 (Sante Nano Technology Inc., Dongguan, Guangdong Province, China).

3.2 Zebrafish Husbandry and Embryo Collection

Wild type AB strain zebrafish (Danio rerio) obtained from the China Zebrafish Resource Center were used in this study. The Adult zebrafish were maintained were cultured at a light/dark cycle of 14:10 with 28.5 ± 0.5 ℃ in a flow-through system (pH 7.5 ± 0.5). Males and females were

kept separately and fed twice a day with Artemia sp during light cycle. Before spawning, male and female parental zebrafish with a ratio of 1:2 (male to female) were separated in spawning boxes (Esen Corp, Beijing) overnight. The eggs were collected and incubated in egg water during the half an hour after fertilization. Only normally developing embryos were selected for the subsequent experiments.

3.3 Phenotype observation on early zebrafish development

Each treatment was exposed to 100 embryos and replicated three times, for a total of 300 embryos used to explore the effectiveness of our improved technique for 3Dprinting methods. The embryos were monitored for their survival, hatching rate, and developmental abnormalities (body length, heart edema, spinal flexure, and absence of swim bladder and heart rate) during 7 days post-fertilization by Nikon SMZ18 microscope (Nikon, Tokyo, Japan).

The numbers of dead, hatching, and developing abnormally (heart edema, absence of swim bladder) individuals were recorded daily during the tests. Embryos without heartbeat and loss of translucency under the microscope were judged to death, removed immediately from the dishes. Survival rate = survival embryos number / total embryos number during 7d exposure. The hatching success was identified as opening embryonic membrane and larva swimming up. Hatching rate = hatched embryos number/total embryos number during 7d exposure. Pericardial edema was identified as swelling due to an increased volume of fluid in the pericardium, which is a portion of the coelomic cavity separating the heart from the body wall. The rates of pericardial edema were calculated as the number of embryos with pericardial edema divided by the number of survival embryos. The HR was manually counted. Percent swim bladder was equal to the number of embryos with swim bladder divided by the total survived number of embryos.

The morphology of the larval was observed and photographed using a Nikon SMZ18 microscope (Nikon, Tokyo, Japan). The images of embryos with pericardial edema were recorded at 3 dpf, while embryos with swim bladder at 4 dpf.

3.4 Measuring the body length of the embryo

The embryos exposed to the CT group at 4 dpf were photographed by Nikon SMZ18 microscope (Nikon, Tokyo, Japan). The body length was quantified using ImageJ software, measuring the distance from the head to the tail of the embryo. About 20 embryos were detected in each group.

3.5 Statistical Analysis

Statistical analysis was performed by SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). The significant criteria were set to be $p < 0.05$, respectively. Each treatment was replicated three times. The results were reported as the average of three parallel determinations of the mixture of three replicated samples. The figures were completed by GraphPad Prism 8.0 software (GraphPad Software, San Diego, USA). The data were shown as mean ± standard error.

4. Discussion

Our 3D printing ingredient contains acrylate (monomer of photopolymer) (Figure 3B) and 2,2-bimethoxy-2-phenylacetophenone (Figure 3A) as a photo-initiator. Except for acrylate, moderate 3,4-epoxy cyclohexylmethyl was added to enhance the mechanical properties of the final product and overcome the drawbacks of a single photopolymer (e.g., low intensity and high anisotropy) [7].

The toxicity of our photopolymer and photoinitiator has been reported in numerous articles. Unpolymerized acrylate monomer is water-soluble, and therefore readily leaks into the aqueous

medium. The water solubility of an acrylate monomer may account for part of the zebrafish embryos dying by 12 hpf and all dying by 24 hpf (Figure 1A). According to recent reports, leaching of compounds to aqueous media from plastic parts is relatively high in the first 24 h [30]. C.A. Staples et al. have tested the acute and chronic aquatic toxicity for acrylic acid and it was revealed that the acute LC50 (24 h) of Rainbow trout (*Oncorhynchus mykiss*), Sheepshead minnow (*Cyprinodon variegatus*), Cladoceran (*Daphnia magna*), and Mysid shrimp (*Mysidopsis bahia*) were 45 mg/l, 236 mg/l, >110 mg/l, and 166 mg/l, respectively [31]. The investigation shows that acrylate and urethane acrylate oligomers are toxic to various species of fish, algae, and water microorganisms.

Regarding the photoinitiator, though 2,2-bimethoxy-2-phenylacetophenone is poorly soluble in water, it may damage to organs through prolonged or repeated exposure, and it is harmful to aquatic life with long-lasting effects [32]. Reactive oxygen species (ROS) are the primary metabolites of the photoinitiator; for instance, peroxides and peroxy radicals [33,34]. Otherwise, incomplete polymerization of photo-reactive resins (55-60% of polymerization under optimal conditions [35]) can cause higher levels of toxicity and leaching rate [36]. Hence, our SLA printed parts without coating parylene C are likely to have multiple toxic effects with zebrafish, for the leaching of photopolymer and photoinitiator.

However, in this study, the parylene C coated group has almost the same level compared to control group, which implies that the coating film could block the toxicity of SLA printed parts to zebrafish, despite the hatching rate of embryos exposed to the parylene C coated group being mildly lower than those of control embryos at 4 dpf and parylene C coated group exhibit a higher heart rate than that of control embryos. The differences between the two groups above

demonstrate that slight toxic compounds may exist leaching through the parylene C film, potentially caused by molecular exchange between the parylene C and the 3D-printed compounds (e.g., acrylate monomer or 2,2-bimethoxy-2-phenylacetophenone).

In the past decade, numerous studies have investigated the toxicity effects of 3D-printed parts. Methods have been exploited to modify the toxicity of 3D-printed parts, including supercritical carbon dioxide [9], sonication of the material in isopropanol [10], and exposure to ultraviolet light [11]. The recent study assessing the toxicity reduced by exposure to ultraviolet light revealed a much lower survival and hatch of embryos compared to our method of coating with parylene C [11], indicating that further strategies need to be devised to totally block or reduce the toxicity of SLA printing materials.

This study tested the toxicity of the SLA printed petri dishes with or without parylene C using early life zebrafish. The UT groups show a pronounced toxicity effect on the survival and hatch of embryos. Contrariwise, the CT group has almost the same level as the control group, implying that the coating could significantly reduce the toxicity of STL printed parts to zebrafish. However, it does not entirely negate the toxicity of these parts. Regarding its potential cardiotoxicity, different strategies for reducing the toxicity are warranted.

This technique can be applied in numerous instances. Parylene C has been proved its advantages as an inert bulk coating for metallic biomedical implants to boost their corrosion resistance [18], as it has excellent properties like low water absorption, high dielectric constant, low coefficient of friction, and moderate elongation at break [12]. Although little is known about the durability of the parylene C coated 3D-printed parts, this cheap and easy technique can be utilized to test and improve parts that need to be implanted *in vivo* as 3D printing is highly

customizable. This technique can also contribute to the popularization of SLA printed products because it can avoid toxicity from daily contact.

Using zebrafish as a model, our research provided a technique to reduce the developmental toxicity of 3D-printed parts, which currently displays an uplifting promotion to 3D printing usage. However, the whole-organism health effects of exposure to 3D-printed parts to adult zebrafish are still unknown. The potential toxicity to whole-organism in animal models and underlying mechanisms require further investigation.

References

1. Saroia, J.; Wang, Y.N.; Wei, Q.H.; Lei, M.J.; Li, X.P.; Guo, Y.; Zhang, K. A review on 3D printed matrix polymer composites: its potential and future challenges. *Int. J. Adv. Manuf. Technol.* **2020**, *106*, 1695-1721, doi:10.1007/s00170-019-04534-z.
2. Quan, H.Y.; Zhang, T.; Xu, H.; Luo, S.; Nie, J.; Zhu, X.Q. Photo-curing 3D printing technique and its challenges. *Bioactive Materials* **2020**, *5*, 110-115, doi:10.1016/j.bioactmat.2019.12.003.
3. Okolie, O.; Stachurek, I.; Kandasubramanian, B.; Njuguna, J. 3D Printing for Hip Implant Applications: A Review. *Polymers* **2020**, *12*, doi:10.3390/polym12112682.
4. Rimington, R.P.; Capel, A.J.; Christie, S.D.R.; Lewis, M.P. Biocompatible 3D printed polymers via fused deposition modelling direct C2C12 cellular phenotype in vitro. *Lab on a Chip* **2017**, *17*, 2982-2993, doi:10.1039/c7lc00577f.
5. Rimington, R.P.; Capel, A.J.; Player, D.J.; Bibb, R.J.; Christie, S.D.R.; Lewis, M.P. Feasibility and Biocompatibility of 3D-Printed Photopolymerized and Laser Sintered Polymers for Neuronal, Myogenic, and Hepatic Cell Types. *Macromol. Biosci.* **2018**, *18*, 12, doi:10.1002/mabi.201800113.
6. Okafor-Muo, O.L.; Hassanin, H.; Kayyali, R.; ElShaer, A. 3D Printing of Solid Oral Dosage Forms: Numerous Challenges With Unique Opportunities. *Journal of Pharmaceutical Sciences* **2020**, *109*, 3535-3550, doi:10.1016/j.xphs.2020.08.029.
7. Carve, M.; Wlodkowic, D. 3D-Printed Chips: Compatibility of Additive Manufacturing Photopolymeric Substrata with Biological Applications. *Micromachines* **2018**, *9*, doi:10.3390/mi9020091.
8. Walpitagama, M.; Carve, M.; Douek, A.M.; Trestrail, C.; Bai, Y.; Kaslin, J.; Wlodkowic, D. Additives migrating from 3D-printed plastic induce developmental toxicity and neuro-behavioural alterations in early life zebrafish (Danio rerio). *Aquat. Toxicol.* **2019**, *213*, 11, doi:10.1016/j.aquatox.2019.105227.
9. Popov, V.K.; Evseev, A.V.; Ivanov, A.L.; Roginski, V.V.; Volozhin, A.I.; Howdle, S.M. Laser stereolithography and supercritical fluid processing or custom-designed implant fabrication. *J. Mater. Sci.-Mater. Med.* **2004**, *15*, 123-128, doi:10.1023/B:JMSM.0000011812.08185.2a.

10. Ngan, C.G.Y.; O'Connell, C.D.; Blanchard, R.; Boyd-Moss, M.; Williams, R.J.; Bourke, J.; Quigley, A.; McKelvie, P.; Kapsa, R.M.I.; Choong, P.F.M. Optimising the biocompatibility of 3D printed photopolymer constructs in vitro and in vivo. *Biomed. Mater.* **2019**, *14*, 12, doi:10.1088/1748-605X/ab09c4.
11. Oskui, S.M.; Diamante, G.; Liao, C.; Wei, S.; Grover, W.H.J.E.s.t.l. Assessing and Reducing the Toxicity of 3D-Printed Parts. **2015**, *3*.
12. Golda-Cepa, M.; Engvall, K.; Hakkarainen, M.; Kotarba, A. Recent progress on parylene C polymer for biomedical applications: A review. *Prog. Org. Coat.* **2020**, *140*, 16, doi:10.1016/j.porgcoat.2019.105493.
13. Chang, T.Y.; Yadav, V.G.; De Leo, S.; Mohedas, A.; Rajalingam, B.; Chen, C.L.; Selvarasah, S.; Dokmeci, M.R.; Khademhosseini, A. Cell and protein compatibility of parylene-C surfaces. *Langmuir* **2007**, *23*, 11718-11725, doi:10.1021/la7017049.
14. Tan, C.P.; Craighead, H.G. Surface Engineering and Patterning Using Parylene for Biological Applications. *Materials* **2010**, *3*, 1803-1832, doi:10.3390/ma3031803.
15. Kuo, J.T.W.; Kim, B.J.; Hara, S.A.; Lee, C.D.; Gutierrez, C.A.; Hoang, T.Q.; Meng, E. Novel flexible Parylene neural probe with 3D sheath structure for enhancing tissue integration. *Lab on a Chip* **2013**, *13*, 554-561, doi:10.1039/c2lc40935f.
16. Temiz, Y.; Ferretti, A.; Leblebici, Y.; Guiducci, C. A comparative study on fabrication techniques for on-chip microelectrodes. *Lab on a Chip* **2012**, *12*, 4920-4928, doi:10.1039/c2lc40582b.
17. Hassler, C.; von Metzen, R.P.; Ruther, P.; Stieglitz, T. Characterization of parylene C as an encapsulation material for implanted neural prostheses. *Journal of biomedical materials research. Part B, Applied biomaterials* **2010**, *93*, 266-274, doi:10.1002/jbm.b.31584.
18. Cieslik, M.; Engvall, K.; Pan, J.; Kotarba, A. Silane-parylene coating for improving corrosion resistance of stainless steel 316L implant material. *Corrosion Science* **2011**, *53*, 296-301, doi:10.1016/j.corsci.2010.09.034.
19. Youssefian, S.; Rahbar, N. Nano-scale adhesion in multilayered drug eluting stents. *Journal of the Mechanical Behavior of Biomedical Materials* **2013**, *18*, 1-11, doi:10.1016/j.jmbbm.2012.11.001.
20. Bar-Cohen, Y.; Silka, M.J.; Hill, A.C.; Pruetz, J.D.; Chmait, R.H.; Zhou, L.; Rabin, S.M.; Norekyan, V.; Loeb, G.E. Minimally Invasive Implantation of a Micropacemaker Into the Pericardial Space. *Circulation-Arrhythmia and Electrophysiology* **2018**, *11*, doi:10.1161/circep.118.006307.
21. Surmeneva, M.A.; Vladescu, A.; Cotrut, C.M.; Tyurin, A.I.; Pirozhkova, T.S.; Shuvarin, I.A.; Elkin, B.; Oehr, C.; Surmenev, R.A. Effect of parylene C coating on the antibiocorrosive and mechanical properties of different magnesium alloys. *Applied Surface Science* **2018**, *427*, 617-627, doi:10.1016/j.apsusc.2017.08.066.
22. Xie, X.Z.; Rieth, L.; Williams, L.; Negi, S.; Bhandari, R.; Caldwell, R.; Sharma, R.; Tathireddy, P.; Solzbacher, F. Long-term reliability of Al2O3 and Parylene C bilayer encapsulated Utah electrode array based neural interfaces for chronic implantation. *J. Neural Eng.* **2014**, *11*, 9, doi:10.1088/1741-2560/11/2/026016.
23. Howe, K.; Clark, M.D.; Torroja, C.F.; Torrance, J.; Berthelot, C.; Muffato, M.; Collins, J.E.; Humphray, S.; McLaren, K.; Matthews, L.; et al. The zebrafish reference genome sequence and its relationship to the human genome (vol 496, pg 498, 2013). *Nature* **2014**, *505*, 248-248, doi:10.1038/nature12813.
24. McGrath, P.; Li, C.Q. Zebrafish: a predictive model for assessing drug-induced toxicity. *Drug*

Discov. Today **2008**, *13*, 394-401, doi:10.1016/j.drudis.2008.03.002.

25. Scholz, S.; Fischer, S.; Gundel, U.; Kuster, E.; Luckenbach, T.; Voelker, D. The zebrafish embryo model in environmental risk assessment - applications beyond acute toxicity testing. *Environ. Sci. Pollut. Res.* **2008**, *15*, 394-404, doi:10.1007/s11356-008-0018-z.
26. Kalueff, A.V.; Stewart, A.M.; Gerlai, R. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol. Sci.* **2014**, *35*, 63-75, doi:10.1016/j.tips.2013.12.002.
27. Zon, L.I.; Peterson, R.T. In vivo drug discovery in the zebrafish. *Nat. Rev. Drug Discov.* **2005**, *4*, 35-44, doi:10.1038/nrd1606.
28. Mu, X.Y.; Shen, G.M.; Huang, Y.; Luo, J.B.; Zhu, L.Z.; Qi, S.Z.; Li, Y.R.; Wang, C.J.; Li, X.F. The enantioselective toxicity and oxidative stress of beta-cypermethrin on zebrafish. *Environ. Pollut.* **2017**, *229*, 312-320, doi:10.1016/j.envpol.2017.05.088.
29. OECD,OECD Guidelines for the Testing of Chemicals, Fish Embryo Acute Toxicity (FET) Test OECD, Paris, France (2013)No. 236.
30. Biocompatibility of Dental Materials.
31. Staples, C.A.; Murphy, S.R.; McLaughlin, J.E.; Leung, H.W.; Cascieri, T.C.; Farr, C.H. Determination of selected fate and aquatic toxicity characteristics of acrylic acid and a series of acrylic esters. *Chemosphere* **2000**, *40*, 29-38, doi:10.1016/s0045-6535(99)00228-3.
32. https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/105868.
33. Lago, M.A.; de Quiros, A.R.B.; Sendon, R.; Bustos, J.; Nieto, M.T.; Paseiro, P. Photoinitiators: a food safety review. *Food Addit. Contam. Part A-Chem.* **2015**, *32*, 779-798, doi:10.1080/19440049.2015.1014866.
34. Sangermano, M. Advances in cationic photopolymerization. *Pure Appl. Chem.* **2012**, *84*, 2089-2101, doi:10.1351/pac-con-12-04-11.
35. Ferracane, J.L.; Condon, J.R. POSTCURE HEAT-TREATMENTS FOR COMPOSITES - PROPERTIES AND FRACTOGRAPHY. *Dent. Mater.* **1992**, *8*, 290-295, doi:10.1016/0109-5641(92)90102-i.
36. Melchels, F.P.W.; Feijen, J.; Grijpma, D.W. A review on stereolithography and its applications in biomedical engineering. *Biomaterials* **2010**, *31*, 6121-6130, doi:10.1016/j.biomaterials.2010.04.050.

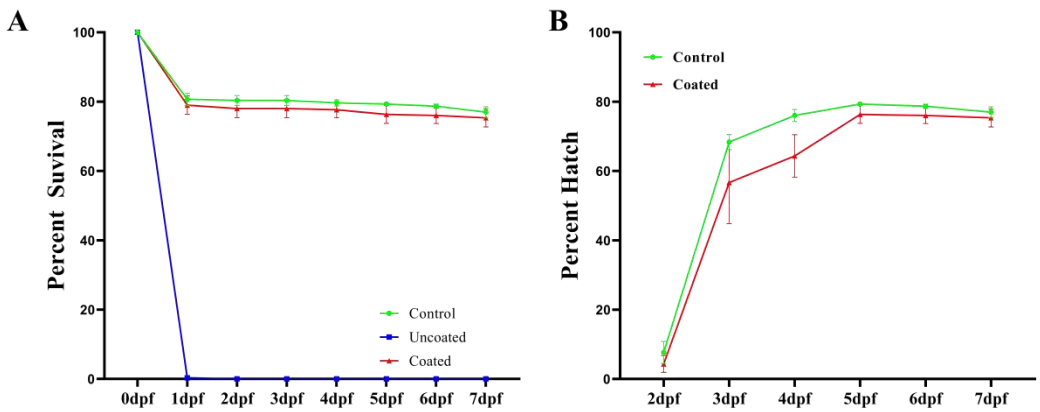

Figure 1. The survival and hatch analysis of zebrafish embryo treated after

3D-print parts.

(A) Survival rates of zebrafish embryos exposed to 3D-printed parts from CT (red green), UT (blue) and control embryos that were not exposed to printed parts (green). Each exposure represents three replicates with 100 embryos in each replicate. Embryos exposed to UT parts had significantly lower survival rates (almost 100% dead) by 12 hpf when compared to those of control embryos ($p < 0.001$), with no STL-exposed embryo surviving past day 7. Inversely, embryos exposed to parylene C coated parts did not have significantly decreased survival rates compared to those of control embryos ($p > 0.05$).

(B) Hatching rates for the CT exposed and control. We did not acquire the data of hatching rates for UT 3 D-printed parts due to its fatal effect on embryos. On the contrary, embryos exposed to CT parts did not have significantly lower hatching rates in the embryos ($p> 0.05$). These results show that coated treated STL-printed parts had almost no toxicity on the survival and hatch rate of embryos exposed to the CT parts fare almost as well as control embryos groups.

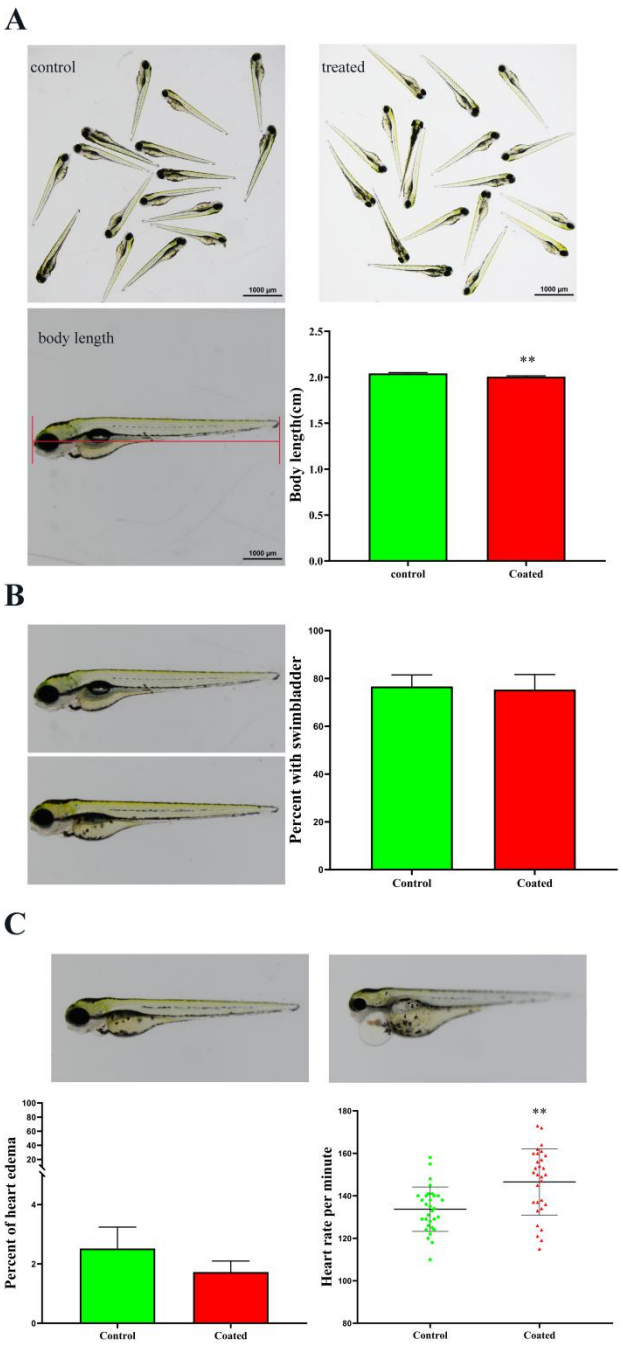

Figure 2. Effect of exposure to 3 D-printed parts printers on zebrafish malformation rate.

(A) Embryos exposed to coated 3D-printed parts printers exhibited a reduced body length compared with that of controls at 4 dpf (parylene C coated group 2.0058 ± 0.0708, control group

2.0419 ± 0.0570, p=0.0067). Body length was measured along the body axis using image J software. Statistically significant differences are indicated by asterisks ($p < 0.05$, Student's t-test).

(B) Parylene C coated parts did not have any effect on the development of swim bladders compared with the uncoated group.

(C) Pericardial edema percent in the parylene C coated group shows no difference compared with that of controls at 4 dpf. In contrast, zebrafish embryos parylene C coated parts caused an increase of the HR at 5 dpf compared to control embryos (parylene C coated group 146±16 bpm, control group 135 ± 11 bpm, p=0.0034). The scale bar represents 1000 μ m. Values are presented as mean ± S.E.

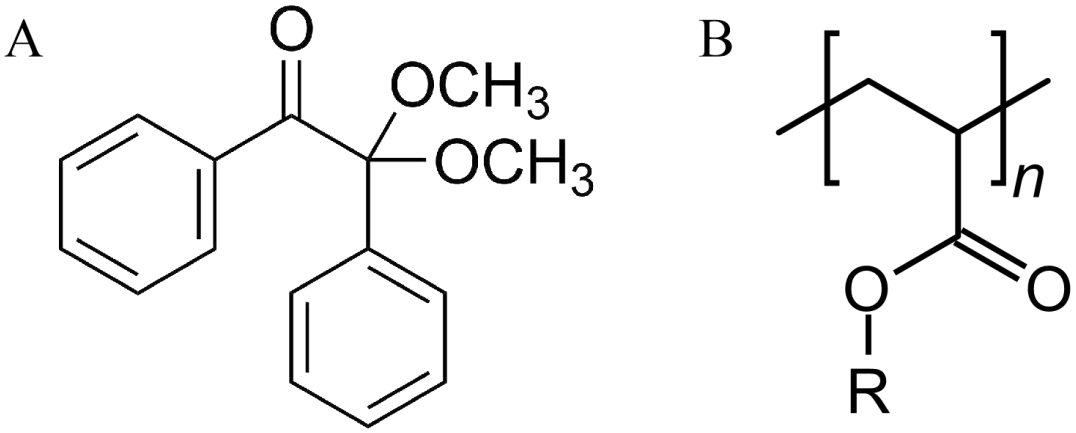

Figure 3. Structural formulas of 2,2-bimethoxy-2-phenylacetophenone and polyacrylate.

(A) Structural formulas of 2,2-bimethoxy-2-phenylacetophenone.

(B) Structural formulas of polyacrylate.

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