

Biocompatibility of Photopolymers in 3D Printing

Frank Alifui-Segbaya ^{1, *} Sony Varma ² Graham J. Lieschke ^{3¶} Roy George ^{4¶}

DOI: <https://doi.org/10.1089/3dp.2017.0064>

¹ School of Dentistry and Oral Health, Griffith University, Australia.

Email: f.alifui-segbaya@griffith.edu.au

² Australian Regenerative Medicine Institute, Monash University, Australia.

Email: sony.varma@monash.edu

³ Australian Regenerative Medicine Institute, Monash University, Australia.

Email: graham.lieschke@monash.edu

⁴ School of Dentistry and Oral Health, Griffith University, Australia.

Email: r.george@griffith.edu.au

*Corresponding Author

Frank Alifui-Segbaya

School of Dentistry and Oral Health,

Gold Coast campus, Griffith University, Australia.

Email: f.alifui-segbaya@griffith.edu.au

¶These authors contributed equally to this work.

Abstract

The biocompatibility of photopolymers in additive manufacturing (AM) often referred to as 3D printing (3DP) is an issue of concern due to, among other things, the unique parameters of the manufacturing process, which can influence the physical, chemical, and biological properties of AM-produced devices. The quality of AM-produced devices may consequently vary when identical parts are built using different 3D printers or even when the same 3D printer, parameters, process steps, and materials are used. In this novel study, representative materials built with stereolithography and material jetting processes were subjected to biological evaluation using the Organization for Economic Cooperation and Development (OECD) fish embryo test designed to determine acute toxicity of chemicals on embryonic stages of fish. The study demonstrates that the AM materials are toxic in zebrafish assays; however, the adverse effects of toxicity in some materials were reduced significantly after treatment with ethanol. Within the limitations of the study, it is evident that material composition and cleaning method are significant parameters by which the biological risks of photopolymers in 3DP can be assessed. Furthermore, the zebrafish biocompatibility assay is a reliable assessment tool for quantifying the toxicity of leachates in AM materials.

Keywords

Biocompatibility; Toxicity; Photopolymer; 3D Printing; Additive manufacturing

1 Introduction

In photopolymerization-based additive manufacturing (AM) or 3D printing (3DP), layers of liquid photopolymer resin undergo a chemical reaction upon irradiation, usually in the ultraviolet (UV) range to become solid ¹⁾. Despite the advantages the manufacturing process offers in the fabrication of bespoke medical devices, it also presents several unique parameters ²⁾ that can influence the physical ^{3, 4)}, chemical ³⁾ and biological properties ⁵⁻⁷⁾ of AM-produced devices. The quality of the AM-produced devices may consequently vary when identical parts are built using different 3D printers or even when the same 3D printer, parameters, process steps, and materials are used ²⁾. An issue of concern is recent studies highlighting their potential toxicity ⁸⁻¹¹⁾. By being toxic, there is a relative ability of the materials to cause injury to biological tissues, ranging from improper biochemical function, organ damage, and cell destruction, to death ¹²⁾. Interestingly, a disclaimer for medical photopolymers state that, ‘it is the responsibility of the customer, its respective customers and end-users to determine the biocompatibility of the printed parts product for their respective purposes’ ^{13, 14)}. In this study, representative materials built with stereolithography (SLA) and material jetting (MJ) processes are subjected to biological evaluation using the OECD fish embryo test designed to determine acute toxicity of chemicals on embryonic stages of fish ¹⁵⁾. In a typical SLA system, layers of liquid photopolymer resin from a vat are selectively cured layer-by-layer with a UV laser beam to form a solid polymer. In MJ, the liquid photopolymer resin is selectively squirted through multiple jet heads, and then cured with a passing of UV light as each layer is deposited. Unlike SLA, post-curing is not required in MJ ¹⁶⁾. In designing this study, the hypothesis that material composition and/or post-processing methods will influence toxicity was proposed.

2 Materials and Methods

2.1 Specimen preparation

Disk-shaped 15 x 3 mm samples were built from CAD models sent to Stratasys (C-BONS International Center 108, Hong Kong, China) and 3D Systems (3D Systems, Rock Hill, SC, USA) in materials listed in TABLE 1. Based on the composition of the materials^{13, 14, 17, 18}), a cross-linked polymethylmethacrylate (PMMA) material produced by injection moulding was included in the test for comparison purpose. The PMMA material was tested in ‘as-built’ (ASB) form, whereas the AM materials were tested in both “ASB” and treated “Rx” forms. “ASB” specimens were rinsed with ultrapure water (complying with grade 1 of ISO 3696) and air-dried for 30 minutes. ‘Rx’ specimens were soaked in ethanol absolute for analysis (purity $\geq 99.9\%$, Merck KGaA, Darmstadt, Germany) for 3 min, rinsed five times with ultrapure water, and air-dried for 30 min.

TABLE 1 Photopolymer, Application and Composition

Photopolymer	Application	Composition
MED materials (Stratasys, Airport Boulevard B 120, 77836 Rheinmünster, Germany) for Stratasys' MJ system. MED samples were built in Objet Eden260VS 3D printer.	MED610 is ISO 10993 and USP Class VI approved transparent acrylic for orthodontic appliances, implant surgical guides, partial denture try-ins and, delivery and positioning trays for temporary placement in the mouth for up to 24h.	Exo-1,7,7-trimethylbicyclo [2.2.1] hept- 2-yl acrylate: 20-30%; Tricyclodecane Dimethanol Diacrylate: 5-10%; \approx 60% is proprietary.
	MED620 is ISO 10993 and USP Class VI approved opaque acrylic for veneer try-ins and diagnostic wax-ups for temporary placement in the mouth for up to 24h.	Acrylic monomer (<30%); Exo-1,7,7 Trimethylbicyclo [2.2.1] hept 2-yl acrylate (<25%); Acrylic Oligomer (<15%); Photo Initiator (<3%); Titanium dioxide (<0.8%); Acrylic acid ester <0.3%. The rest is proprietary.
Visijet materials (3D Systems, Rock Hill, SC, USA) for 3D Systems' SLA and MJ systems.	Visijet SL Clear is USP Class VI capable material. It is transparent and polycarbonate-like material for medical models. Samples were built by SLA in and ProJet 7000 HD printer.	Dangerous components are listed as: 4,4'. Isopropylidenedicyclohexanol; oligomeric reaction products with 1-chloro-2,3-epoxypropane (60-75%); 3-ethyl-3-hydroxymethyl-oxetane (15-25%); Mixture containing triarylsulfonium salt: 50% propylene carbonate and 50% mixed triarylsulfonium salts (1-5%).
	Visijet M3 Crystal is USP Class VI capable material. It is a translucent material for medical applications. Samples were built by Material Jetting in ProJet MJP 3600 printer.	Dangerous components are listed as: Ethoxylated bisphenol A diacrylate (15-25%); Urethane acrylate oligomers (14-24%); Tripropyleneglycol diacrylate (5-11%).
Telio CAD polymethylmethacrylate, PMMA (Wieland Dental + Technik GmbH & Co. KG, Germany) block for subtractive manufacturing.	Opaque material for long-term provisional crowns and bridges. Samples were milled in Zenotec mini 4-axis geometry machine.	Contains >98% cross-linked PMMA ¹⁹⁾ . Ref. 663618 Lot. TP1118

2.2 Test procedures

Test procedures and toxicological analyses in this study were informed by stringent OECD test guidelines ¹⁵⁾ that require newly fertilised zebrafish eggs exposed to test chemicals for a maximum period of 96 h. Ethical approval (MARF/2015/094) for the study was issued by the Animal Ethics Committee in Monash University, Australia.

Embryos for the experiment were obtained by pair-wise mating and natural spawning from 5-7 months old wild-type (AB/Tü strain) zebrafish maintained at 28 °C, pH 7.2 and 14h light/10h dark photoperiod (FishCore, Australian Regenerative Medicine Institute, Monash University). In the preliminary test (Test 1) test, five randomly selected 1.5-hour post-fertilized (hpf) embryos were placed in test chambers containing specimens and 1.2 ml of transparent E3 medium and incubated at 28.5 °C in Heracell CO₂ incubator (Thermo Fisher Scientific, Inc.). Controls assays comprised only embryos in E3 medium. Each test was performed in triplicates and repeated for reliability. Phenotype assessment was carried out at 24h intervals using Olympus MVX10 Research Macro Zoom Microscope and cellSens imaging software (Olympus Soft Imaging Solutions GmbH) to identify lethal endpoints: coagulation of the embryo, non-detachment of the tail-bud, lack of somite formation and lack of heartbeat. These apical endpoints indicate acute toxicity and, consequently, death of the embryos ¹⁵⁾. Selected sublethal developmental endpoints (development of eyes, spontaneous movement, heartbeat/blood circulation, pigmentation, formation of edema) and teratogenic effects (malformation of the head, malformation of tail, yolk deformation, general growth retardation) in Nagel ²⁰⁾ were also assessed and recorded. At the end of the test, larvae were euthanized in 0.4 % anaesthetic tricaine mesylate solution. In this study, "embryo" denotes 24 - 72 hpf fish whereas "larva" denotes 96 hpf fish ²¹⁾.

3 Results

3.1 Toxicity data for preliminary test

In addition to embryo deaths recorded in some Visijet assays on day 1, surviving embryos in “ASB” Visijet Clear (60%), “Rx” Visijet Clear (13%), “ASB” Visijet Crystal (67%) and “Rx” Visijet Crystal (100%) recorded severe sublethal and teratogenic effects (FIGURE 1) on day 2 and test was discontinued. Representative larvae in Visijet assays are shown in Supplementary Figure S1. All embryos in “ASB” MED assays coagulated on Day 1, in contrast to normal embryonic developments in “Rx” MED assays. However, sublethal and teratogenic effects were observed in these assays from day 2. On day 3, a 100% hatching rate in all assays was recorded, in addition to minor behaviour perturbations, yolk sac edema, pericardial edema, and tail malformations. These endpoints were seemingly pronounced by day 4, preceding larvae mortality in MED610 (\approx 15%) and MED620 (\approx 30%) assays. Figure 2 compares 96h representative larvae in MED620 and control assays.

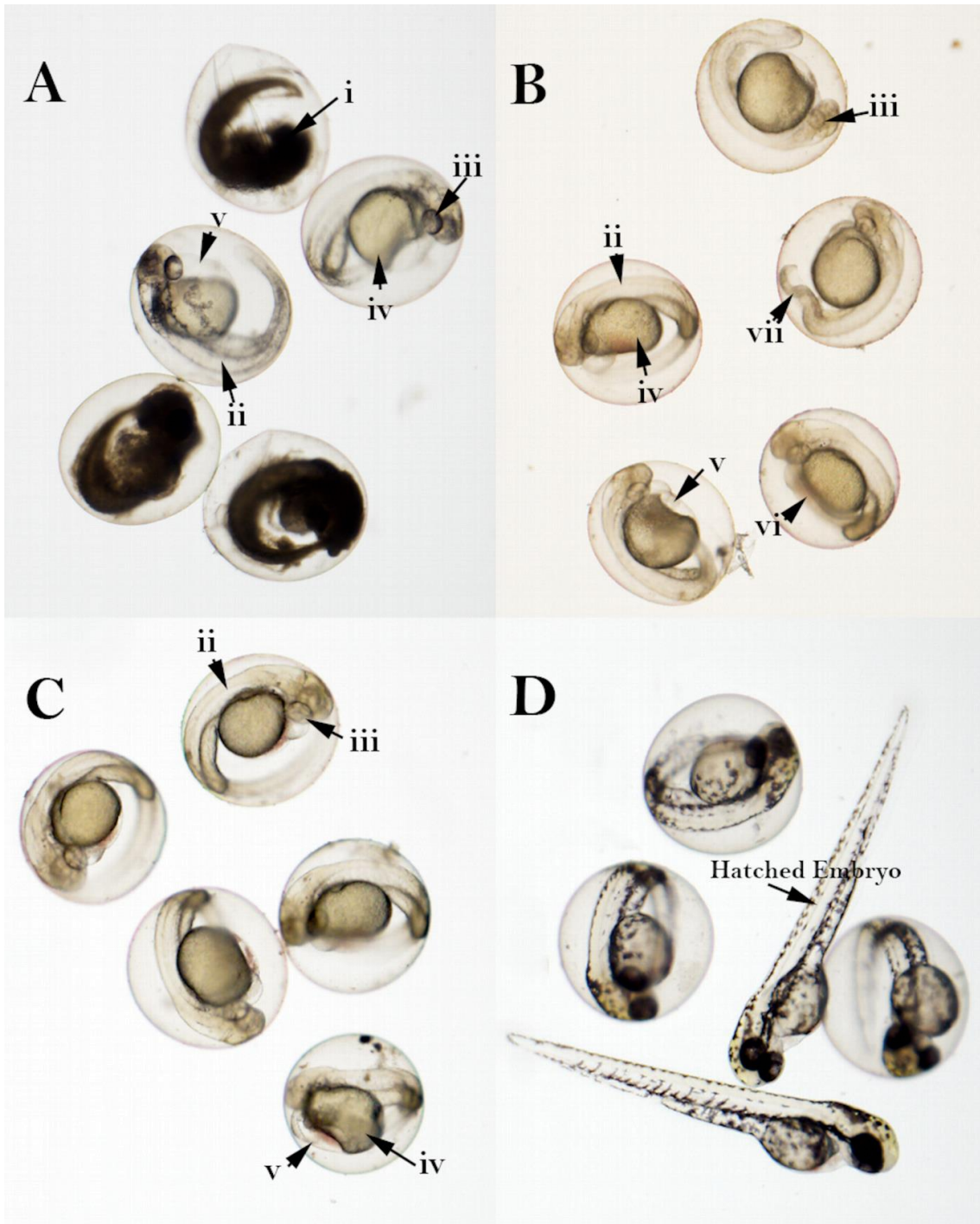


FIGURE 1: Day 2 embryos in Rx Visijet Clear (A), ASB Visijet Crystal (B), Rx Visijet Crystal (D) showing developmental endpoints: i. mortality ii. hypopigmented body iii. hypopigmented eye iv. deformed yolk v. pericardial edema vi. blood pooling vii. malformed tail in comparison to embryos in Control (E) without developmental endpoints.

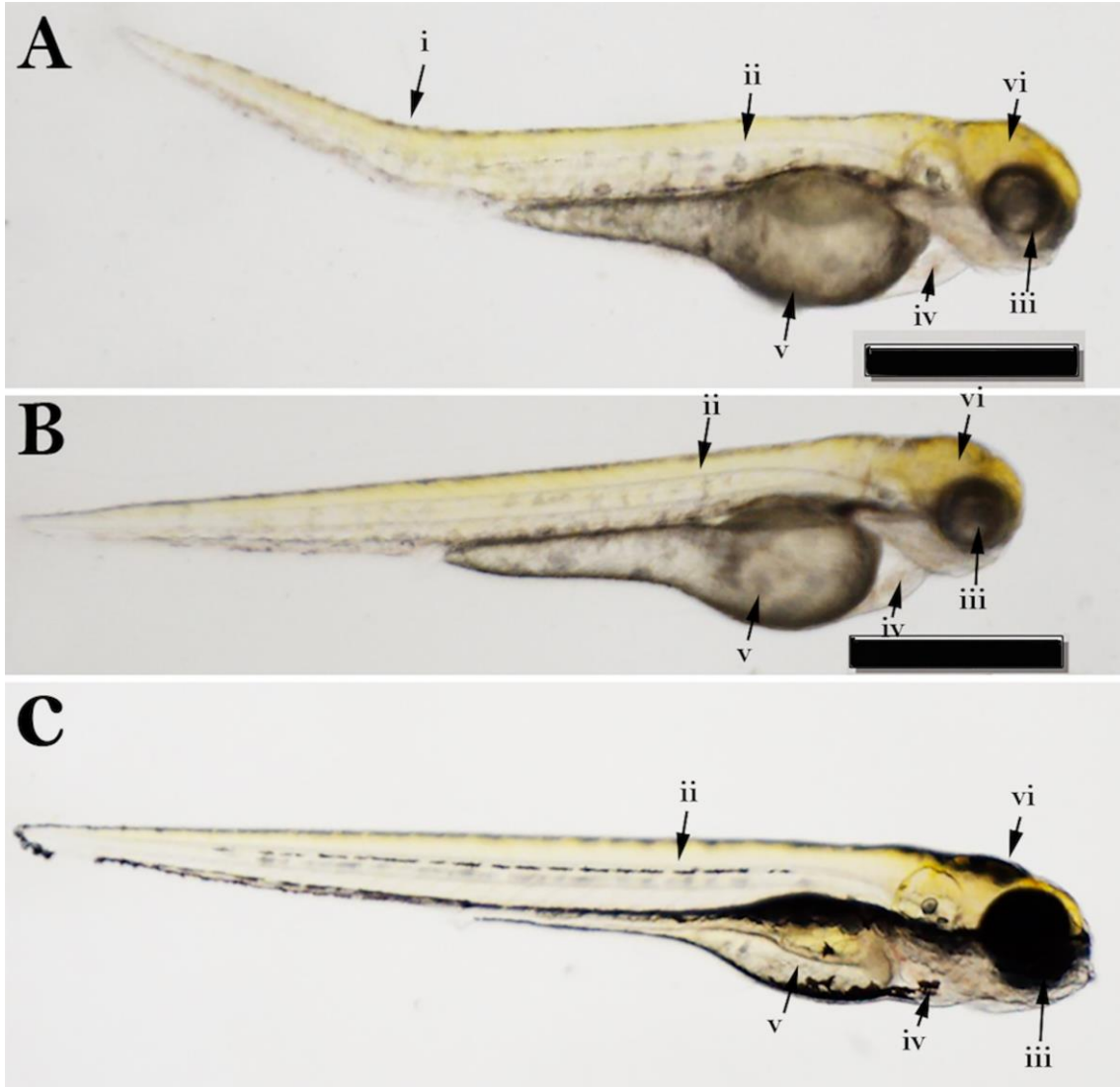


FIGURE 2: Day 4 representative larvae in MED620 assay (A and B) showing i. malformed tail ii. hypopigmented body iii. malformed head iv. pericardial edema v. yolk sac resorption delay vi. malformed head in comparison with control (C).

3.2 Additional test

Since toxicity is dose dependent, additional test (Test 2) was performed to measure the effects of leachate concentration on the fertilized embryos. Assays in this test batch comprised 10 embryos, 2 AM-produced specimens and 10ml E3 medium in soda lime silica petri dish. Tests were repeated for reliability. No lethal endpoint was observed in Visijet Clear assays; however, 96h larvae in “ASB” Visijet Clear developed pericardial edema a day; this occurred a day after 72h embryos in “Rx” Visijet Clear developed severe pericardial edema. In Visijet Crystal assays, $\approx 70\%$ lethality was recorded in “ASB” Visijet Crystal on day 1 and test was discontinued. Although only 5% lethality was observed in “Rx” Visijet Crystal, the embryos were lethargic with pericardial edema, yolk deformations, and severe hypopigmentation on day 2 and test was concluded. More than 50% lethality was observed in ‘ASB’ MED assays on Day 1. No lethal endpoint was recorded in ‘Rx’ MED; however, behaviour perturbations observed from day 2 to day 3 and the darkening of yolk sacs (SUPPLEMENTARY FIGURES S2, S3) in 96h larvae indicated some degree of toxicity ⁸⁾ in the assays. FIGURE 3 shows the survival rate of embryos and larvae exposed to AM materials within the 96h period in Test 1 and Test 2. The survival rates are however, not indicative of the overall biological performance of the materials.

No developmental endpoint was observed in Telio CAD (SUPPLEMENTARY FIGURE S4) and control assays. As per OECD guidelines, the results are valid as the overall survival rates in the control groups were $\geq 90\%$. Larvae length (FIGURE 4) was measured at the end of the test on day 4 in “Rx” MED, Telio CAD and Control assays in Test 1. There was a significant difference ($p \leq 0.05$, unpaired, two-tailed student’s t-test) between ‘Rx’ MED materials vs. control, and between “Rx” MED materials vs. Telio CAD. In Test 2, a significant difference ($p \leq 0.05$) was found between “Rx” MED610 vs. control and between “ASB” Visijet Clear vs. control.

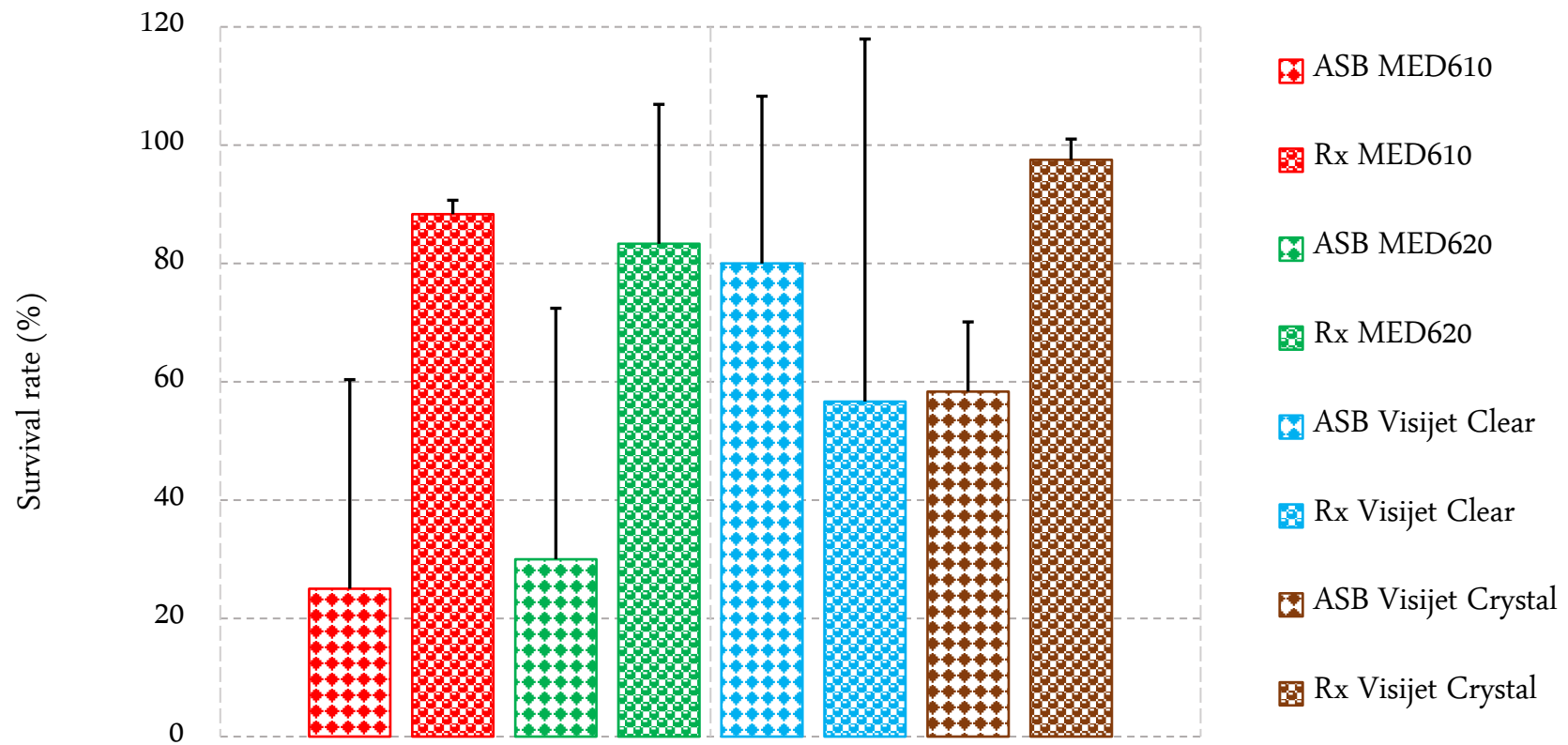


FIGURE 3: Survival rate of embryos and larvae exposed to AM materials within the 96h period in Test 1 and Test 2. Error bars show standard deviation from the mean.

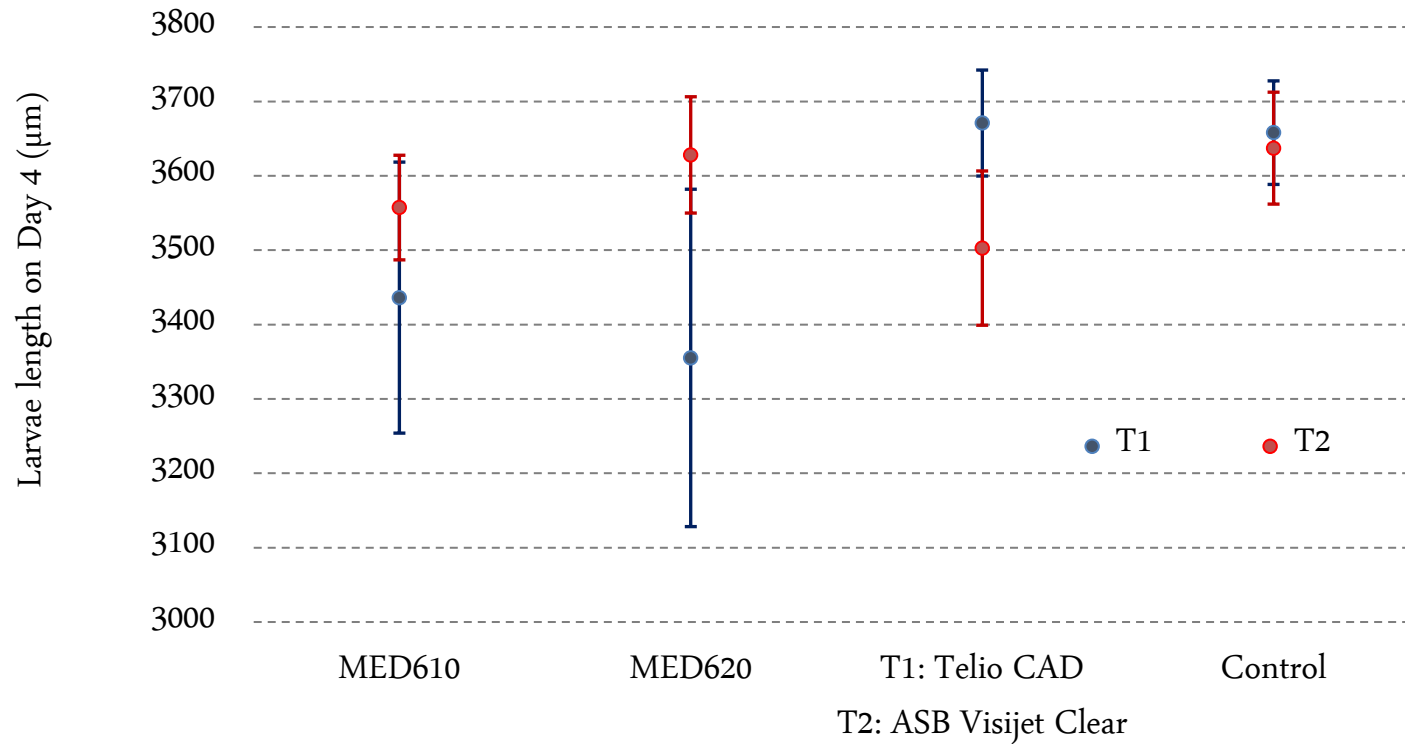


FIGURE 4: Growth retardation measured on day 4 in Test 1 (T1): MED610 (A), MED620 (B), TelioCAD (C) and control, (D) and Test 2 (T2): MED610 (A), MED620 (B) and ASB Visijet Clear, (C) and control (D). Error bars show standard deviation from the mean.

4 Discussion

The safety data sheets for MED ^{13, 14)} and Visijet Crystal materials ¹⁸⁾ indicate they are acrylic formulations whereas Visijet Clear is a polycarbonate-like polymer ¹⁷⁾. The United States Pharmacopeia (USP) protocols are used to classify plastics in Classes I - VI, based on end use, type and time of exposure of human tissue to plastics, of which Class VI requires the most stringent testing of all the six classes ²²⁾. As per this classification, Visijet photopolymers are USP Class VI-capable materials which according to the manufacturer, are ideal for dental and orthopedic surgical guides, one-day crown preparation guides and parts in other medical applications ²³⁾. MED photopolymers, in contrast, are USP Class VI (and ISO 10993) certified materials with similar applications to Visijet polymers. Likewise, they are only approved for up to 24 h in the mouth.

Toxicological data show that 'as-built' AM materials are generally unsafe in zebrafish assays; comparatively, Visijet materials performed better than MED materials. Ethanol treatment; however, proved effective in enhancing the biocompatibility of the MED materials but produced contrasting results in Visijet materials. The improved tolerance from ethanol treatment corroborates similar findings by Macdonald et al. ⁸⁾ The biological performance of Visijet Clear improved in Test 2 and, surprisingly, was safer in 'ASB' form than in 'Rx' form. Although 'Rx' Visijet Crystal recorded almost 100% survival rates in Test 1 and Test 2, tests were concluded on day 2 due to the severity of sublethal and teratogenic effects observed in embryos. Previous studies ^{8, 10)} have also reported Visijet materials to be unsafe in zebrafish assays. Although it is not unusual for zebrafish under 120 hpf to spend most of their time lying inactively on the bottom of the tanks until the inflation of their swim bladder ²⁴⁾, those exposed to AM materials showed increased lethargy or behavioural perturbations, which often precede mortality. This sublethal endpoint according to Zhu et al. ¹⁰⁾ indicates that photopolymer leachates directly affect the central nervous system or muscle contraction in zebrafish larvae.

Hypopigmentation in terms melanophore development and retinal pigment epithelium was a common sublethal effect observed in assays exposed to AM materials, with the most severe occurring in 48h Visijet Crystal embryos. Chemicals such as anilines, phenols and, p-tert-

butylphenol have also been linked to hypopigmentation in zebrafish embryos ²⁰⁾. The photopolymer leachates also induced various degrees of teratogenic effects in the assays within the duration of test. The striking phenotypic similarities observed in Telio CAD and control assays confirm the absence of toxic residual monomer in the former ²⁵⁾. Residual monomer refers to substances such as monomers, additives and reaction products that are not firmly incorporated in the polymer network and may therefore leach and cause local and/or systemic side effects ⁷⁾. The acute systemic toxicity test is designed accordingly to determine the toxic potential or the irritant effects of toxic leachables that may be present in extracts of these biomaterials over a relatively short time ²⁶⁾. In general, the zebrafish excels as a model system for developmental toxicity and offers advantages such as external fertilization, high fecundity, and ease of phenotype assessment over other vertebrates including the mouse, in which aspects of organogenesis and disease pathology cannot be examined without interventions such as surgery or post-mortem examination ²⁷⁾.

Researchers have attempted to improve the biocompatibility of photopolymers in 3DP. Inoue and Ikuta ²⁸⁾ were able to detoxify SLA parts with high temperature heat process in nitrogen atmosphere but the procedure also impaired the transparency of the materials. Leonhardt et al ²⁹⁾ suggested extraction of residual monomers with supercritical CO₂ to improve biocompatibility. Oskui et al. ⁹⁾ and Leonhardt et al. ²⁹⁾ reported improved biocompatibility outcomes for materials cleaned with isopropanol and post-cured. Schmelzer et al. ¹¹⁾, in contrast, reported in their in vitro study that ethanol treatment was ineffective in improving the biocompatibility of MED610. It is worth emphasizing that although in vitro tests are faster, less expensive, more reproducible, and more scalable than other types of tests, they may suffer from a lack of relevance to the clinical use of materials, and this weakness is not trivial ³⁰⁾. Nevertheless, the conflicting results, further demonstrate the need for continual biological assessments of photopolymers especially those for clinical applications as often, the medical profession is overly complacent in its acceptance of new materials without demanding proof of their safety and efficacy ¹²⁾.

5 Conclusion

Within the limitations of the study, it is evident that the zebrafish biocompatibility assay is a reliable assessment tool for quantifying the toxicity of leachates in AM materials. Although ethanol proved an effective detoxicant for MED materials, conclusive evidence cannot be drawn regarding its use in a broader context due to the unique parameters (physical and chemical structure of the materials, printing process and post-processing methods) of the manufacturing process that eventually influence the clinical performance of the end-use devices. As a result, extreme caution must be exercised with the increased use of AM materials particularly with 3D printers that may lack apposite settings to ensure the integrity of built parts. Furthermore, as the 3DP industry continues to grow exponentially with contemporaneous influx of new materials, it is imperative for practitioners and patients to understand both the advantages and limitations of the new technologies and materials.

Acknowledgements

The authors would like to thank Objective 3D (Australia) for kindly supplying the MED materials and 3D Systems (Australia) for supplying the Visijet materials for this study. G.J.L was supported by the NHMRC (1044754, 1069284). The Australian Regenerative Medicine Institute is supported by funds from the State Government of Victoria and the Australian Federal Government.

Author Disclosure Statement

No competing financial interests exist.

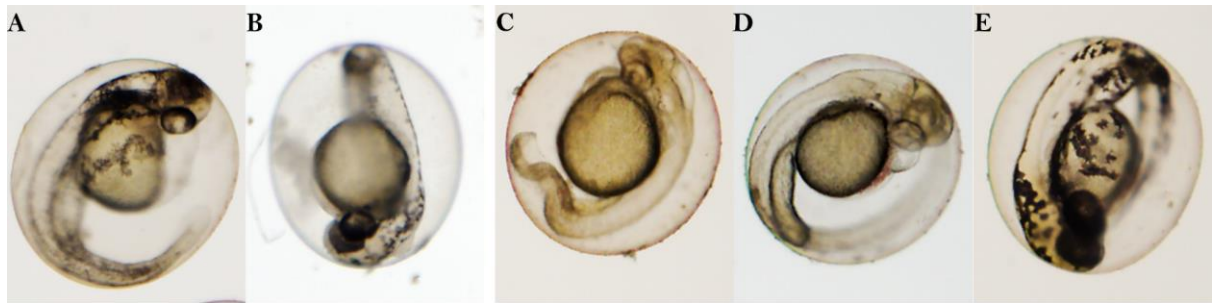
6 References

- 1) Chua CK, Leong FK. 3D Printing and Additive Manufacturing : Principles and Applications. 4 ed. Singapore: World Scientific Publishing Co. Pte. Ltd.; 2015. p. 31-229.
- 2) Food and Drug Administration. Technical Considerations for Additive Manufactured Devices:Draft Guidance for Industry and Food and Drug Administration Staff. Rockville, MD:: Food and Drug Administration; 2016. p. 1-28.
- 3) Decker C. Kinetic Study and New Applications of UV Radiation Curing. *Macromolecular Rapid Communications* 2002; 23: 1067-93.
- 4) Crivello JV, Reichmanis E. Photopolymer Materials and Processes for Advanced Technologies. *Chem Mater* 2014; 26: 533-48.
- 5) Powers JM, Wataha JC. *Dental Materials: Foundations and Applications. Properties and Manipulation.* 11 ed. Philadelphia, PA: Elsevier Health Sciences; 2017.
- 6) Autian J. Structure-toxicity relationships of acrylic monomers. *Environmental Health Perspectives* 1975; 11: 141-52.
- 7) Geurtsen W. *Polymethylmethacrylate Resins. Biocompatibility of Dental Materials.* Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. p. 255-70.
- 8) Macdonald NP, Zhu F, Hall CJ, Reboud J, Crosier PS, Patton EE, et al. Assessment of biocompatibility of 3D printed photopolymers using zebrafish embryo toxicity assays. *Lab on a Chip* 2016; 16: 291-7.
- 9) Oskui SM, Diamante G, Liao C, Shi W, Gan J, Schlenk D, et al. Assessing and Reducing the Toxicity of 3D-Printed Parts. *Environmental Science & Technology Letters* 2016; 3: 1-6.
- 10) Zhu F, Friedrich T, Nugegoda D, Kaslin J, Wlodkowic D. Assessment of the biocompatibility of three-dimensional-printed polymers using multispecies toxicity tests. *Biomicrofluidics* 2015; 9.
- 11) Schmelzer E, Over P, Gridelli B, Gerlach JC. Response of Primary Human Bone Marrow Mesenchymal Stromal Cells and Dermal Keratinocytes to Thermal Printer Materials In Vitro. *Journal of Medical and Biological Engineering* 2016; 36: 153-67.
- 12) Anusavice KJ, Shen FC, Rawls FHR. *Phillips' Science of Dental Materials.* 12 ed. St. Louis: Elsevier Saunders; 2013. 592 p.

- 13) Stratasys. Safety Data Sheet (MED610): Stratasys; 2016 [cited 2016 30 November]. Available from: <http://www.stratasys.com/materials/material-safety-data-sheets/polyjet/~media/394dd5015bb6473496a19ea837881c25>.
- 14) Stratasys. Printing Bio-compatible Parts on PolyJet™ 3D Printers with MED620: Stratasys; 2016 [cited 2018 18 January]. Available from: http://usglobalimages.stratasys.com/Main/Files/SDS/MED620_Usage_Terms_1116.pdf?v=636160085940019299.
- 15) Organisation for Economic Co-operation and Development. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. Paris: OECD Publishing; 2013. p. 1-22.
- 16) Alifui-Segbaya F, Williams RJ, George R. Additive Manufacturing: A Novel Method for Fabricating Cobalt-Chromium Removable Partial Denture Frameworks. *European Journal of Prosthodontics and Restorative Dentistry* 2017; 25: 73-8.
- 17) 3D Systems. VisiJet SL Clear (SLA) 2017 [cited 2017 18 May]. Available from: <https://au.3dsystems.com/materials/visijet-sl-clear/safety-data-sheets>.
- 18) 3D Systems. VisiJet® EX 200, VisiJet Crystal 2017 [cited 2017 18 May]. Available from: http://infocenter.3dsystems.com/materials/sites/default/files/sds-files/professional/24184-s12-02-asds_ghsenglishvisijet_ex_200_and_crystal.pdf.
- 19) Inc.. IV. Telio CAD 2016 [cited 2016 29 August]. Available from: http://www.ivoclarvivadent.us/en-us/products/chairside-cad_cam-blocks/telio-cad.
- 20) Nagel R. DarT: The embryo test with the zebrafish *Danio rerio* - a general model in ecotoxicology and toxicology. *Altex-Altern Tierexp* 2002; 19: 38-48.
- 21) Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Developmental Dynamics* 1995; 203: 253-310.
- 22) Niazi SK. Disposable Bioprocessing Systems: CRC Press Taylor and Francis Group; 2012. Available from: <http://go.galegroup.com.libraryproxy.griffith.edu.au/ps/i.do?p=EAIM&sw=w&u=griffithh&v=2.1&it=r&id=GALE%7CA277538041&sid=summon&asid=d72a4ab852aa7a77afd6b66713fcef3e>.
- 23) 3D Systems. Class VI Certification for VisiJet Materials 2017 [cited 2017 28 September]. Available from: <http://infocenter.3dsystems.com/materials/classvi>.
- 24) Braunbeck T, Kais B, Lammer E, Otte J, Schneider K, Stengel D, et al. The fish embryo test (FET): origin, applications, and future. *Environmental Science and Pollution Research* 2015; 22: 16247-61.
- 25) Williams DF. On the mechanisms of biocompatibility. *Biomaterials* 2008; 29: 2941-53.

- 26) Rauckman EJ, Soiefer A. Acute Toxicology. Toxicological Testing Handbook: CRC Press; 2006. p. 135-47.
- 27) Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet 2007; 8: 353-67.
- 28) Inoue Y, Ikuta K. Detoxification of the Photocurable Polymer by Heat Treatment for Microstereolithography. Procedia CIRP 2013; 5: 115-8.
- 29) Leonhardt S, Klare M, Scheer M, Fischer T, Cordes B, Eblenkamp M. Biocompatibility of photopolymers for additive manufacturing. Current Directions in Biomedical Engineering 2016. p. 113-6.
- 30) Wataha JC. Predicting clinical biological responses to dental materials. Dental Materials 2012; 28: 23-40.

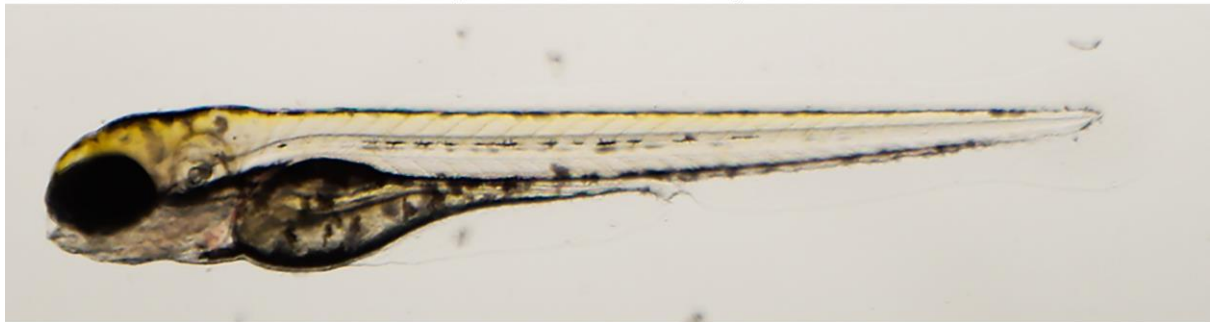
7 Supplementary Figures



S 1. Day 2 embryos in Visijet assays: ASB Clear (A), Rx Clear (B) ASB Crystal (C) Rx Crystal (D) and Control (E) in Test 1



S 2. Day 4 larva in Rx MED610 assay in Test 2



S 3. Day 4 larva in Rx MED620 assay in Test 2



S 4. Day 4 larva in Telio CAD assay in Test 1

