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BIOCOMPATIBILITY OF 3D PRINTED METHACRYLATE FOR HEARING AIDS AND INNER EAR DEVICES

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ABSTRACT

The capacity of 3D printing (3DP) technologies to initiate speedy polymerization of solvent free resins accounts for their utility in the manufacturing of medical devices. Nonetheless, independent biological evaluation of 3D printed materials is recommended due to the unique parameters of the manufacturing process, which can influence their physical, chemical, and biological properties. In this study, E-Shell 450 material indicated for 3DP of hearing aid shells and inner ear devices was examined for biological safety using zebrafish bioassays adapted to OECD fish embryo test. In addition, the proprietary material was characterized for composition using headspace gas-chromatography mass spectrometry (GC-MS). To initiate test, newly fertilized zebrafish eggs were cultured on non-treated and ethanol-treated materials in glass petri dishes with ultrapure water, incubated at 28.5°C and assessed for developmental endpoints of toxicity at 24h interval until 96h. Data confirmed non-treated material was extremely toxic in bioassays within 24h whereas ethanol-treated material showed a relative lower toxicity possibly due to ethanoic-aqueous interactions as observed by GC-MS. With the current influx of 3D printing materials, users are urged to exercise caution. Operators must also take cognizance of the potential toxicity of the chemicals used in 3DP and implement safety measures to limit their exposure.

Keywords: 3D printing; biocompatibility; hearing aids; methacrylates; zebrafish embryo model.

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1 INTRODUCTION

The recent hype surrounding 3D printing (3DP) attests to its growing popularity in almost every manufacturing sector including medicine, architecture, sports, aerospace and automotive engineering and contemporary arts [1]. The different technologies in 3DP offer a spectrum of capabilities for the manufacturing of polymeric medical devices such as hearing aid shells and inner ear devices. The digital manufacturing process simply involves feeding a virtual model (usually 'STL' file) into a designated 3D printer to build parts in successive layers and the desired 3D part is completed. The capacity of the technologies to initiate speedy polymerization of solvent free resins primarily accounts for their utility in 3DP. Nonetheless, independent biological evaluation of the devices is highly recommended [2,3] due to the unique parameters of the manufacturing process, which can influence their physical, chemical, and biological properties [4]. In this study, E-Shell 450 material [5] indicated for hearing aid shells and inner ear devices is examined for biological safety using zebrafish bioassays adapted to the Organization for Economic Cooperation and Development (OECD) fish embryo test [6]. Representative materials were built with Digital Light Processing (DLP) technology. DLP is similar to stereolithography (SL) in that both are vat photopolymerization processes that require washing built parts in organic solvents to remove any wet resin remnants, followed by postcuring to harden them. However, DLP uses a more conventional light source such as an arc lamp, with a liquid crystal display panel or a deformable mirror device, which is applied to the entire surface of the vat of resin in a single pass, relatively making it faster than SL [7]. To extrapolate toxicity effects to residual monomer and degradation products that may be present in the proprietary material, it was characterized for composition using headspace gas-chromatography mass spectrometry (GC-MS).

2 MATERIALS AND METHODS

EnvisionTec GmbH (Brüsseler Str. 51, 45968 Gladbeck, Germany) supplied 60x3 mm disk-shaped samples built from E-Shell 450 Clear resin. Samples were built from using Perfactory® DDP 4M 3D printer (Z-height: 67.98mm; Voxel: 100µm; Light power: 180 Mw/dm^2). Postcuring (2 x 100 flashes) was completed by the manufacturer in Otoflash G171 (NK-Optik GmbH, Isarstr. 2, D-82065 Baierbrunn, Germany). E-Shell 450 Clear is composed of 60-80% proprietary methacrylate oligomers; 15-30% proprietary methacrylate monomers and 1-2% diphenyl (2,4,6 trimethyl benzoyl) phosphine oxide. Physical properties of the material in photocured sate are, Flexural Strength: 60-80 MPa; Flexural Modulus: 1200-1500 MPa; Elongation at Break: 2-4%; Tensile Strength: 40-48 MPa; Tensile Modulus: 2150-3250 MPa; Impact: 30 J/m; HDT: 75° at 1.82 MPa; Hardness, D Scale: 82-85; Viscosity: 320 cP at 30°C. **Figure 1** shows the surface topography of photocured E-Shell 450 Clear material. Imaging was carried out with Olympus AX70 Fluorescence Microscope, Monochrome FViewII Peltier cooled digital camera (Olympus. Tokyo, Japan) and running Analysis Software (Soft Imaging Solutions, Münster, Germany). One batch was ethanol-treated as described in Alifui-Segbaya et al. [4] while the other batch was tested 'as-received'. To initiate the test, newly fertilized (1.5-hour postfertilized) zebrafish eggs (n=20) obtained from FishCore (Australian Regenerative Medicine Institute, Monash University) Australia were cultured on samples in glass petri dishes using ultrapure water as test medium. The bioassays were incubated at 28.5 °C in Heracell CO_2 incubator (Thermo Fisher Scientific Inc.) and assessed for developmental endpoints [6,8,9] at 24h interval until 96h (**Table 1)** using Olympus MVX10 Research Macro Zoom Microscope, Olympus DP 72 digital colour microscope camera and cellSens imaging software (Olympus Soft Imaging Solutions GmbH). Fish is considered dead if one of the lethal endpoints is present between 24h and 96h. Ethical approval (MARP/2015/094) to use embryos was issued by Animal Ethics Committee in Monash University.

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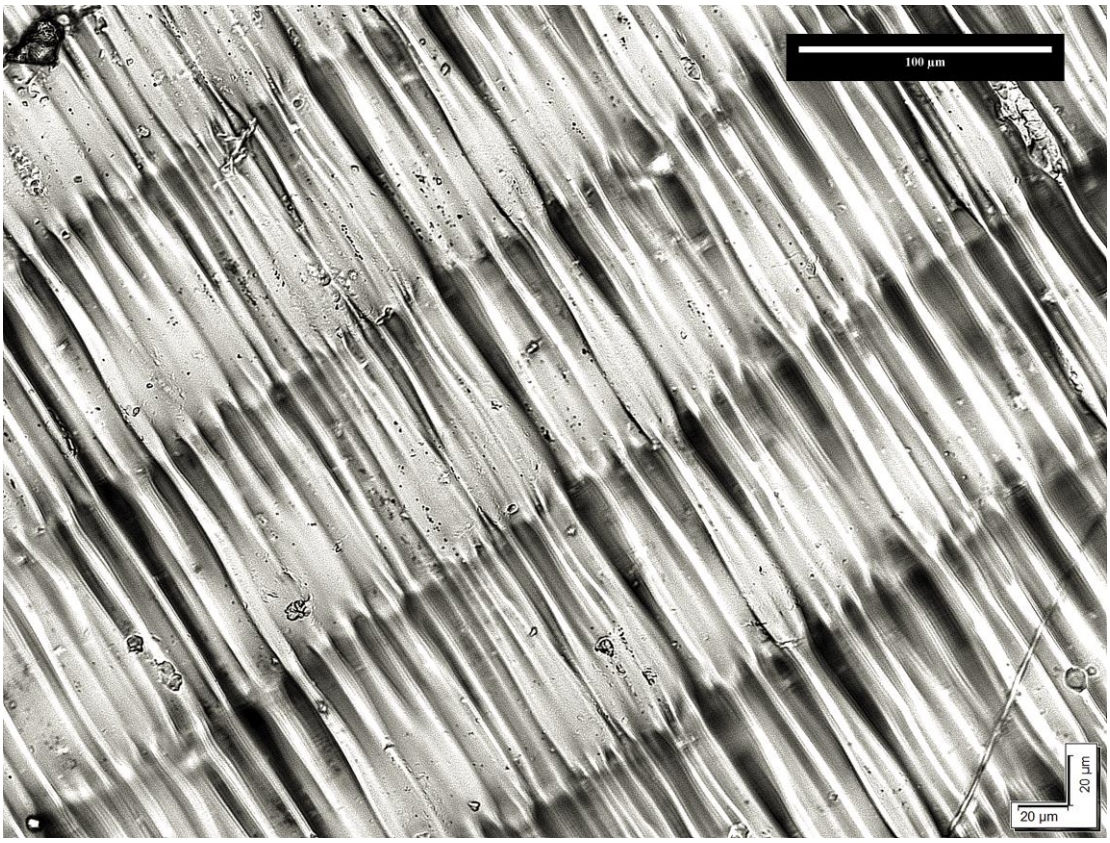

Figure 1. Surface topography of E-Shell 450 Clear

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Table 1. Biomarkers of lethality, sublethality and teratogenicity

	DURATION OF EXPOSURE			
LETHAL ENDPOINTS	24H	48H	72H	96H
Coagulation	•	•	•	•
Lack of somite formation	•	•	•	•
Non-detachment of tail-bud	•	•	•	•
Lack of heart-beat	•	•	•	•
SUBLETHAL DEVELOPMENTAL ENDPOINTS				
Development of eyes	•	•	•	•
Spontaneous movement	•	•	•	•
Hypopigmentation		•	•	•
Formation of edemata		•	•	•
ENDPOINTS OF TERATOGENICITY				
Spinal curvature and malformation of tail	•	•	•	•
Yolk deformation	•	•	•	•
Growth retardation				•

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3 RESULTS

After 24h, non-treated materials induced ≈70% embryo death or lethality while surviving embryos **(Fig. 2)** were largely unhealthy, hence test was discontinued. Although ethanol-treated materials recorded only 5% mortality after 24h, additional 50% with increased hypopigmentation, pericardial edema, yolk sac resorption delay and hypoactive behavior were observed in surviving fish by 96h (**Fig. 3).** Average growth length in surviving fish after 96h was 3241.30µm compared to 3590.33 µm in controls. At the end of the test, fish were euthanized in 0.4 % anaesthetic tricaine mesylate solution.

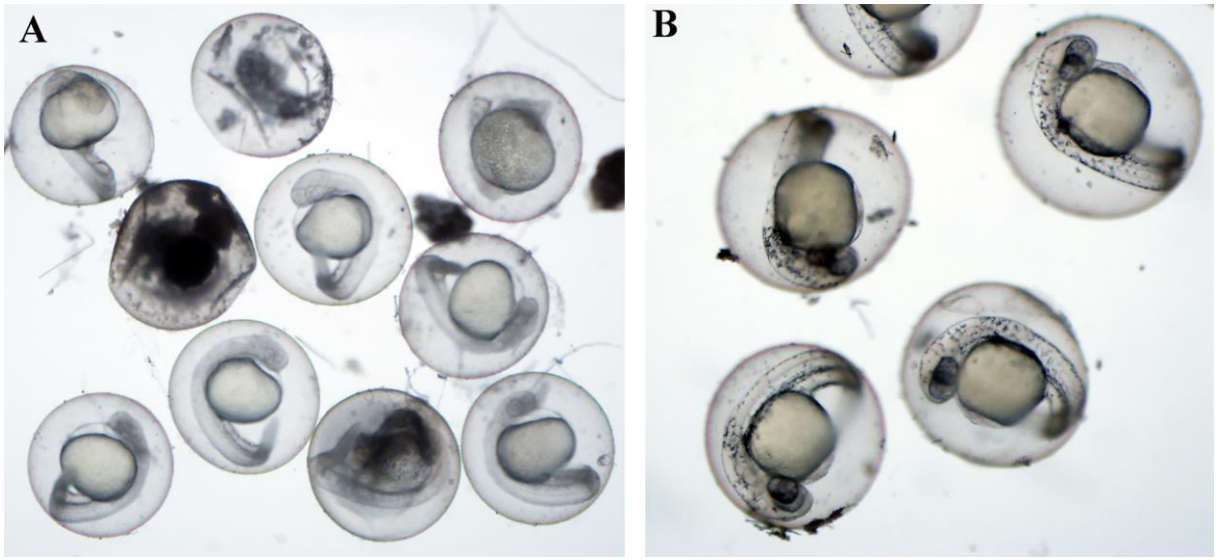

Figure 2. Toxicity effects induced by non-treated E-Shell 450 in zebrafish bioassay (**A**) compared to healthy embryos in control (**B)**

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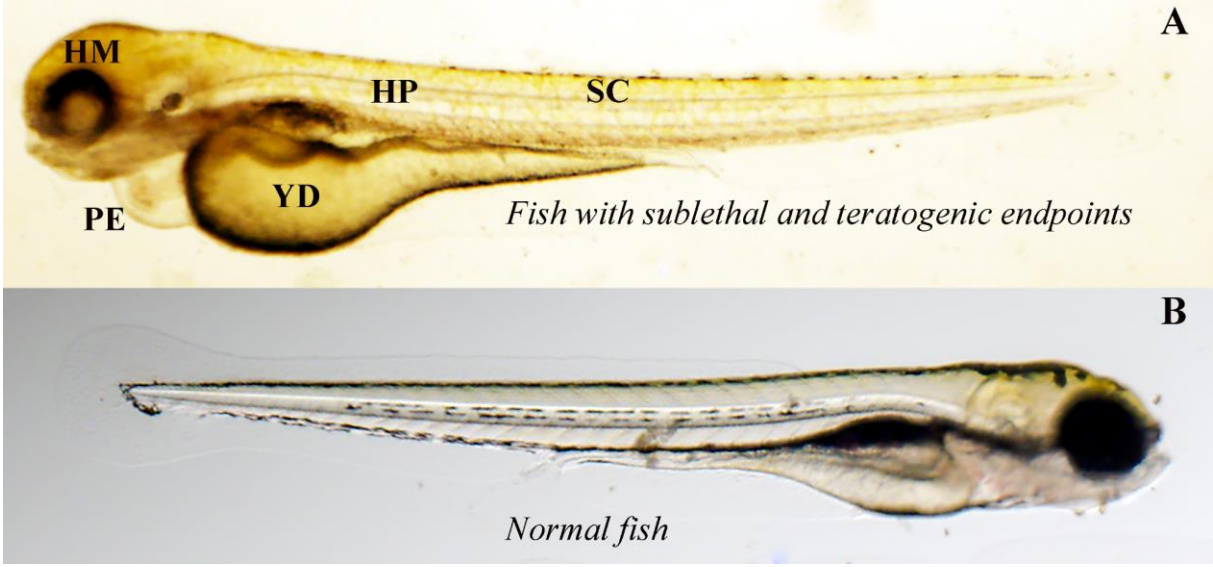

Figure 3. Fish in toxic (**A**) and control (**B**) bioassays. Note the phenotype differences in A: **HM** Head malformation **HP** Hypopigmentation **SC** Spinal curvature **PE** Pericardial edemata **YD** Yolk sac resorption delay.

3.1 Qualitative gas chromatography-mass spectrometry

Since the toxicity effects observed in the materials are likely due to residual monomer, samples were examined for chemical composition using headspace GC-MS. Prior to analysis, they were frozen in liquid nitrogen at -196 °C and ground into powder before placed in Shimadzu TQ8040 GC-MS/MS (Shimadzu Corporation, Japan). GC column is an Agilent J&W DB5-MS 30m 0.25mm ID 0.25um film thickness. Test parameters were, column oven temperature at 40.0 °C, injection temperature at 250 °C, column flow rate at 1.16 mL/min**,** split ratio of 5.0 and a total run time of 15 minutes. **Table 2** shows 16 chemical compounds that reduced to 5 with the applied ethanol treatment. For reliability, only chemical compounds observed in ≥ 75% or n=3/4 of the photocured samples are reported.

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Table 2. Chemical composition of non-treated and ethanol-treated photocured materials

NON-TREATED E-SHELL 450	ETHANOL-TREATED E-SHELL 450
2-Hydroxyethyl methacrylate	Propylene glycol methyl ether
Propylene glycol methyl ether	m-Xylene
Octyl Acrylate	Methyl 3-methoxy-2-methylpropanoate
2-Propyl-1-pentanol	Toluene
Benzaldehyde	Undecane
Tetrahydrofufuryl Butyrate	
Cyclohexanone	
Cyclomethicone 5	
Cyclomethicone 6	
2,6,11-Trimethyldodecane	
Ethylbenzene	
N-[1-(4-Hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-4-oxo-	
Texanol	
Methyl 3-methoxy-2-methylpropanoate	
Toluene	
Undecane	

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4 DISCUSSION

Experimental results in this study relate specifically to the photopolymer examined based on composition, manufacturing parameters, postprocessing and the test protocols used. Toxicological data indicate non-treated E-Shell 450 was extremely toxic in zebrafish bioassay whereas ethanol-treated E-Shell 450 showed a relative lower toxicity but also, severe cumulative sublethal and teratogenic effects in surviving fish. The improved biological performance in treated materials is likely due to induced swelling in polymeric chains, which allowed insoluble substances, in this case, chemical compounds to diffuse in the water used to rinsed them [10]. As per standards definitions [11], E-Shell 450 is a surface device hence does not require stringent biological evaluation compared to methacrylates for intraoral devices . Nonetheless, it is worth emphasising that uncured methacrylate monomers can be absorbed through the skin [12] and cause allergic reactions (e.g. dermatitis) [13,14]. In addition, the toxicity of methacrylate esters is theorized to involve alkylation of critical cellular nucleophiles via Michael addition [15]. Some of the developmental endpoints observed in toxic bioassays are comparable to those reported in animal studies that linked methacrylic esters to embryonic fetal toxicity, teratogenicity [12] and cardiovascular function [16,17]. Furthermore, some of chemical compounds observed are used in industrial applications and can be toxic if present in threshold dose. For instance, cyclomethicone is used in cosmetic and personal products [18], 2-hydroxyethyl methacrylate for desensitizing teeth, benzaldehyde for pharmaceutical products and texanol as fuel additives [19]. Similarly, inhalation toxicity [20] may result from liquid photopolymers, which are often characterised by unpleasant odour. For enhanced manufacturing outcomes, liquid photopolymers should possess high curing rate, good storage stability, low viscosity, low toxicity, and display adequate mechanical properties after photocuring [21,22]. Interestingly, heating functions in some ‘closed’ 3DP systems do not work with third-party resins [23], hence desired functional properties may not be guaranteed. With the current influx of 3D printers and materials, it is

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imperative that the biological performance of 3D printed materials is not overlooked. Users are advised to exercise caution and if necessary demand approved certification for these materials. Since 3DP is not a “one-stop”, manufacturing process operators must therefore take cognizance of the potential toxicity of the chemicals used and implement safety measures to limit their exposure.

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6 Conflict of interest statement

No competing financial interests exist.

7 REFERENCES

1. Wohlers, T.; Gornet, T. History of additive manufacturing. 2014; p 34.
2. Stratasys. Safety data sheet (med610). http://www.stratasys.com/materials/material-safety-data-sheets/polyjet/~/media/394dd5015bb6473496a19ea837881c25 (accessed on 30 November 2016),
3. Stratasys. Printing bio-compatible parts on polyjet™ 3d printers with med620. http://usglobalimages.stratasys.com/Main/Files/SDS/MED620_Usage_Terms_1116.pdf?v=636160085940019299 (accessed on 18 January 2016),
4. Alifui-Segbaya, F.; Varma, S.; Lieschke, G.J.; George, R. Biocompatibility of photopolymers in 3d printing. *3d Print Addit Manuf* **2017**, *4*, 185-191.
5. EnvisionTec GmbH. E-shell® 450 series. https://envisiontec.com/3d-printing-materials/perfactory-materials/e-shell-450-series/ (accessed on 22 July 2017),
6. Organisation for Economic Co-operation and Development. Test no. 236: Fish embryo acute toxicity (fet) test. OECD Publishing: Paris, 2013; pp 1-22.
7. 3D Printing Industry. 3d printing basics: The free beginner's guide. http://3dprintingindustry.com/wp-content/uploads/2014/07/3D-Printing-Guide.pdf (accessed on 16 January 2017),
8. 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.
9. Lammer, E.; Carr, G.J.; Wendler, K.; Rawlings, J.M.; Belanger, S.E.; Braunbeck, T. Is the fish embryo toxicity test (fet) with the zebrafish (danio rerio) a potential alternative for the fish acute toxicity test? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **2009**, *149*, 196-209.
10. Neves, C.B.; Lopes, L.P.; Ferrao, H.F.; Miranda, J.P.; Castro, M.F.; Bettencourt, A.F. Ethanol postpolymerization treatment for improving the biocompatibility of acrylic reline resins. *BioMed research international* **2013**, *2013*, 485246.
11. International Organization for Standardization. Iso 10993-1: Biological evaluation of medical devices -part 1: Evaluation and testing within a risk management process. International Organization for Standardization: Geneva, 2009.
12. Autian, J. Structure-toxicity relationships of acrylic monomers. *Environmental Health Perspectives* **1975**, *11*, 141-152.
13. Geurtsen, W. Polymethylmethacrylate resins. In *Biocompatibility of dental materials*, Springer Berlin Heidelberg: Berlin, Heidelberg, 2009; pp 255-270.

14. Kanerva, L.; Estiander, T.; Jolanki, R.; Tarvainijn, K. Occupational allergic contact dermatitis caused by exposure to acrylates during work with dental prostheses. *Contact Dermatitis* **1993**, *28*, 268-275.
15. McCarthy, T.J.; Witz, G. Structure-activity relationships in the hydrolysis of acrylate and methacrylate esters by carboxylesterase in vitro. *Toxicology* **1997**, *116*, 153-158.
16. Mir, G.N.; Lawrence, W.H.; Autian, J. Toxicological and pharmacological actions of methacrylate monomers i: Effects on isolated, perfused rabbit heart. *Journal of Pharmaceutical Sciences* **1973**, *62*, 778-782.
17. Mir, G.N.; Lawrence, W.H.; Autian, J. Toxicological and pharmacological actions of methacrylate monomers iii: Effects on respiratory and cardiovascular functions of anesthetized dogs. *Journal of Pharmaceutical Sciences* **1974**, *63*, 376-381.
18. DrugBank. Cyclomethicone 5. https://www.drugbank.ca/drugs/DB11244 (accessed on 18 June 2018),
19. National Center for Biotechnology Information. Pubchem compound database. https://pubchem.ncbi.nlm.nih.gov/ (accessed on 19 June 2018),
20. Azimi, P.; Zhao, D.; Pouzet, C.; Crain, N.E.; Stephens, B. Emissions of ultrafine particles and volatile organic compounds from commercially available desktop three-dimensional printers with multiple filaments. *Environmental Science & Technology* **2016**, *50*, 1260-1268.
21. Li, J.; Cui, Y.; Qin, K.; Yu, J.; Guo, C.; Yang, J.; Zhang, C.; Jiang, D.; Wang, X. Synthesis and properties of a low-viscosity uv-curable oligomer for three-dimensional printing. *Polymer Bulletin* **2016**, *73*, 571-585.
22. Vitale, A.; Cabral, J.T. Frontal conversion and uniformity in 3d printing by photopolymerisation. *Materials* **2016**, *9*, 760.
23. eLABORATE. 3d printer marketplace. 2017, pp 46-52.