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Biocompatibility of 3D-Printed Methacrylate for Hearing 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 clear methacrylate indicated for 3DP of hearing devices was examined for biological safety using zebrafish bioassays adapted to Organization for Economic Cooperation and Development (OECD) fish embryo test. In addition, the proprietary material was characterized for composition using headspace gas chromatography–mass spectrometry (GC–MS). To initiate the biological test, newly fertilized zebrafish eggs were cultured on non-treated and ethanol-treated methacrylates in glass petri dishes with ultrapure water, incubated at 28.5 °C and assessed for developmental endpoints of toxicity at 24 h intervals until 96 h. Toxicological data indicate that non-treated methacrylate is extremely toxic in zebrafish bioassays, whereas ethanol-treated counterpart 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 devices; methacrylates; zebrafish embryo model; digital light processing

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]. 3DP comprise a host of processes and technologies that offer a diverse spectrum of capabilities for the manufacturing of end-use products and devices in different materials [2]. The digital manufacturing process simply involves feeding a virtual model (usually 'STL' file) into a designated 3D printer to build parts in successive layers until the desired 3D part is completed. Despite the potentials of 3DP offering significant benefits in terms of speed [3], independent biological evaluation of manufactured devices is highly recommended [4,5] due to the unique parameters of the manufacturing process, which can influence their physical, chemical and biological properties [6]. In this study, E-Shell 450 clear methacrylate indicated for hearing devices is examined for biological safety using zebrafish bioassays adapted to the Organization for Economic Cooperation and Development (OECD) fish embryo test [7]. The zebrafish is considered an excellent model for developmental toxicity, offering economy and ease of quantifying multiple toxicity endpoints [8], as well as fulfilling the pertinent aim to replace, reduce or refine the use of animals for the purposes of research or hazard identification [9,10]. Representative materials were built with

digital light processing (DLP) technology [11]. 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 [2]. To extrapolate toxicity effects to residual monomer and degradation products [12] that may be present in the proprietary material, it was characterized for chemical composition using headspace gas chromatography–mass spectrometry (GC–MS).

2. Materials and Methods

EnvisionTec GmbH (Brüsseler Str. 51, 45968 Gladbeck, Germany) supplied 60 × 3 mm disk-shaped samples that were built from E-Shell 450 clear resin. Postcuring (2 × 100 flashes) of samples was completed by the manufacturer in Otoflash G171 (NK-Optik GmbH, Isarstr. 2, D-82065 Baierbrunn, Germany). Table 1 shows the manufacturing parameters, hazardous composition [13] and physical properties [14] of photocured E-Shell 450 clear (Figure 1).

Table 1. Manufacturing parameters, composition and physical properties of E-Shell 450 methacrylate.

Manufacturing Parameters	Hazardous Ingredient(s) *w/w* %	Physical Properties
Photocured samples were built with Perfactory® DDP 4 M 3D printer. Manufacturing parameters are z-height: 67.98 mm, voxel: 100 µm and light power: 180 Mw/dm^2.	60–80% Proprietary methacrylate oligomers; 15–30% Proprietary methacrylate monomers; 1–2% diphenyl 2,4,6-trimethylbenzoyl.	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; Izod impact: 30 J/m; HDT: 75° at 1.82 MPa; Hardness, D Scale: 82–85; Viscosity: 320 cP at 30 °C.

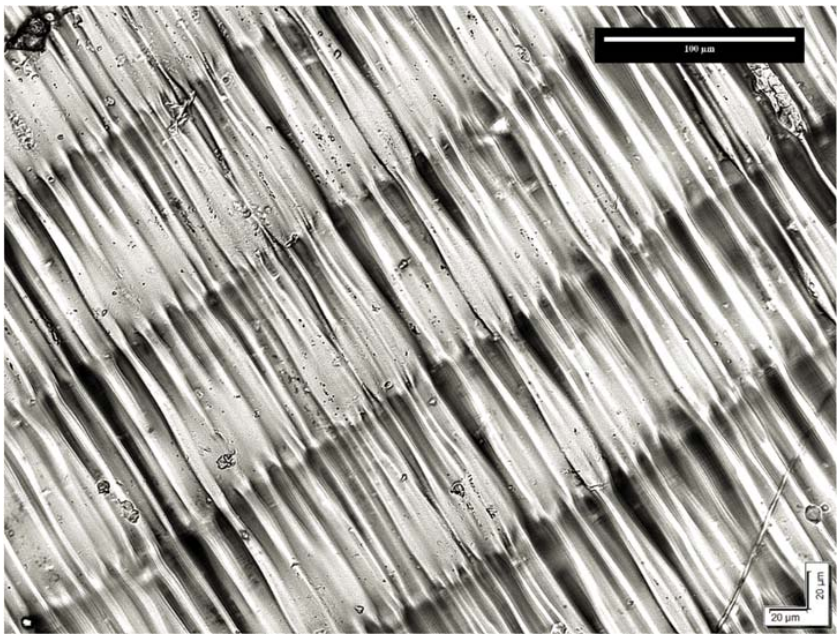

Figure 1. Surface topography of E-Shell 450 clear. Imaging was carried out with Olympus AX70 Fluorescence Microscope; Monochrome FViewII Peltier cooled digital camera (Olympus, Tokyo, Japan) and running analysis software (Soft Imaging Solutions, Münster, Germany).

To initiate the biological test, newly fertilized (1.5 h post-fertilized) zebrafish eggs (n = 20) obtained from FishCore (Australian Regenerative Medicine Institute, Monash University) were cultured on

non-treated ('as-received') and ethanol-treated [6] samples in glass petri dishes using ultrapure water (3 cm^2:1 mL ratio) [15] as test medium. The bioassays were incubated at 28.5 °C in Heracell CO_2 incubator (Thermo Fisher Scientific Inc., Waltham, MA, USA) and assessed for developmental endpoints [7,16,17] shown in Table 2 at 24 h intervals until 96 h using Olympus MVX10 Research Macro Zoom Microscope, Olympus DP 72 digital colour microscope camera and cellSens imaging software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). As per OECD test guidelines, fish is considered dead if one of the lethal endpoints is present. Ethical approval (MARP/2015/094, Approved date 8 September 2015) to use zebrafish embryos was issued by the Animal Ethics Committee in Monash University.

Table 2. Biomarkers of lethality, sublethality and teratogenicity assessed at 24 h intervals until 96 h.

	24 h	48 h	72 h	96 h
Lethal endpoints				
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				.

3. Results

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

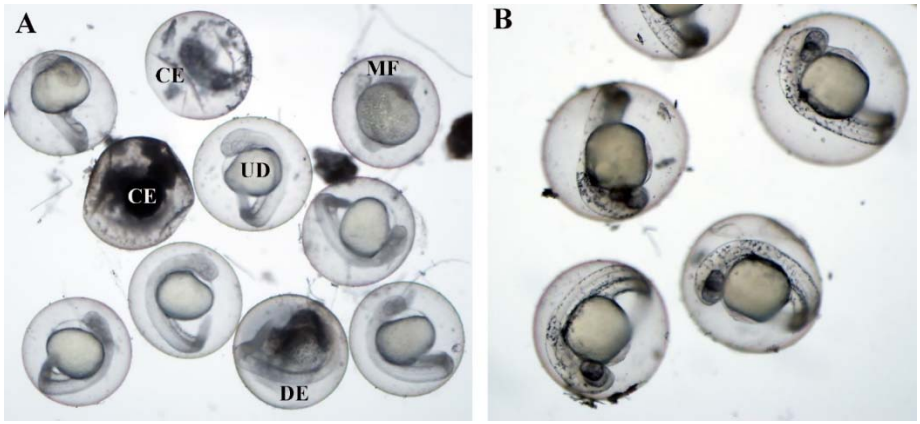

Figure 2. Toxicity effects induced by non-treated methacrylate in zebrafish embryo bioassays after 24 h (**A**): **CE** coagulated embryo, **DE** dead embryo, **MF** malformed embryo without somite and **UD** underdeveloped embryo as compared to healthy embryos in controls (**B**).

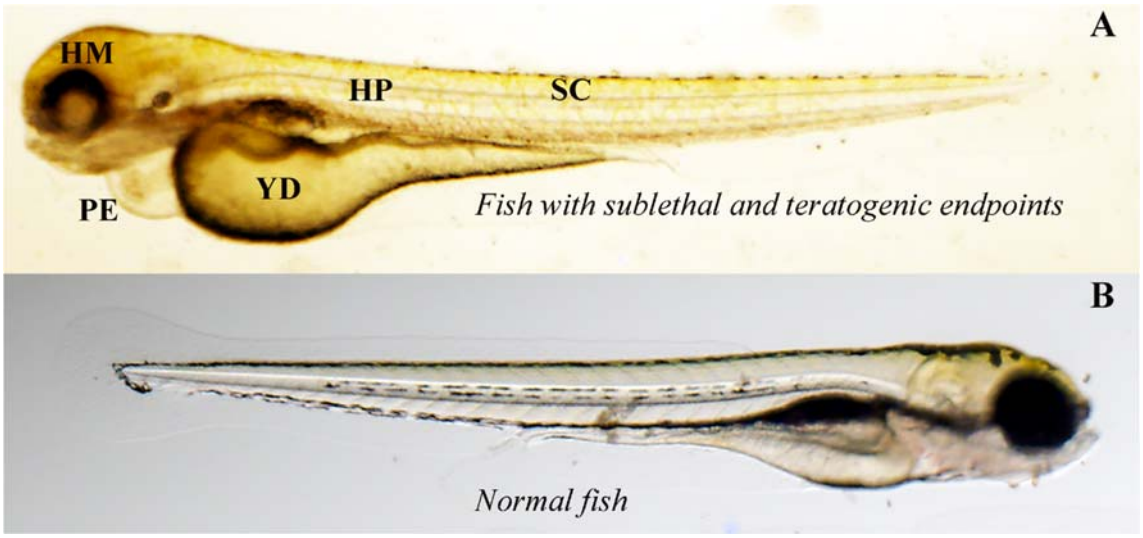

Figure 3. Fish in toxic (**A**) and control (**B**) bioassays after 96 h. Note the phenotype differences in unhealthy zebrafish (**A**): HM Head malformation, HP Hypopigmentation, SC Spinal curvature, PE Pericardial edemata, YD Yolk sac resorption delay, as compared to normal zebrafish (**B**).

Qualitative Gas Chromatography–Mass Spectrometry

Since the toxicological effects observed in bioassays are likely due to residual monomer and degradation products, the photocured materials 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 being tested in Shimadzu TQ8040 GC–MS/MS (Shimadzu Corporation, Tokyo, Japan). GC column is Agilent J&W DB5-MS 30 m 0.25 mm ID 0.25 µm 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 min. GC–MS data shown in Table 3 shows 16 chemical compounds that reduced to 5 with the applied ethanol treatment. For reliability, only chemical compounds observed in ≥75% or ≥ = 3/4 of the samples are reported.

Table 3. Chemical composition of non-treated and ethanol-treated photocured E-Shell 450 methacrylates.

Non-Treated E-Shell 450 Methacrylate	Ethanol-Treated E-Shell 450 Methacrylate
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-1,4-dihydropyrimidin-2-yl]benzamide	
Texanol	
Methyl 3-methoxy-2-methylpropanoate	
Toluene	
Undecane	

4. Discussion

Experimental results in this study indicate that non-treated methacrylate is extremely toxic in zebrafish bioassay. Ethanol-treated methacrylate, on the other hand, induced a relatively lower lethality

but surviving fish showed cumulative sublethal and teratogenic effects. The improved biological performance is likely due to induced swelling in polymeric chains, which allowed chemical compounds to diffuse in the ultrapure water [18] used to rinse the samples.

Acrylics are probably the most versatile family of monomers that can be used to prepare polymers with rigid, flexible, ionic, nonionic, hydrophobic or hydrophilic properties [19]. They are preferred in free-radical synthesis of plastics because of the high reactivity of the acrylate double bond. Under intense ultraviolet illumination, cross-linking polymerization of liquid resins proceeds extensively within a fraction of a second to generate a 3D polymer network [20]. As per standard definitions [21], E-Shell 450 is a surface device, hence it does not require stringent biological evaluation compared to methacrylates for intraoral devices. Nonetheless, uncured methacrylate monomers can be absorbed through the skin [22] and cause allergic reactions (e.g., dermatitis) [23,24]. In addition, the toxicity of methacrylate esters is theorized to involve alkylation of critical cellular nucleophiles via Michael addition [25]. 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 [22] and cardiovascular function [26,27]. The observed chemical compounds are also used in industrial applications and can be toxic to humans if present in threshold dose. For instance, cyclomethicone is used in cosmetic and personal products [28], 2-hydroxyethyl methacrylate for desensitizing teeth, benzaldehyde for pharmaceutical products and texanol as fuel additives [29]. It is worth emphasizing that inhalation toxicity [30] may result from exposure to liquid photopolymer resins, which are often characterised by unpleasant odour. For enhanced manufacturing outcomes, resins should possess high curing rate, good storage stability, low toxicity, low viscosity, and display adequate mechanical properties after photocuring [31]. Resins with relatively low viscosity are likely to produce rapid polymerisation yielding cross-linked polymers with properties suited to the demands imposed by the target application [32]. Some 3D printers have in-built heating mechanism for achieving the desired resin viscosity [33] but often not applicable to third-party materials. In general, the mechanical properties of photocured materials depend primarily on the chemical structure, functionality and concentration of the various constituents of the resin, and the degree of cure [34].

5. Conclusions

With the current influx of 3D printers and materials [35], it is imperative that the biological performance of 3D-printed medical devices is not overlooked. Users are advised to exercise caution and if necessary demand approved certification for the materials. Since 3DP is not a "one-stop" manufacturing process, operators should take cognizance of the potential toxicity of the chemicals used and implement safety measures to limit their exposure. The limitations of the study lie in the extrapolation of the toxicological data to human responses, hence quantitative analysis of the observed compounds and their throughput in zebrafish bioassays are recommended for further study.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wohlers, T.; Gornet, T. *History of Additive Manufacturing*; Wohlers Associates, INC: Fort Collins, CO, USA, 2014; p. 34.
2. 3D Printing Industry. 3D Printing Basics: The Free Beginner's Guide. Available online: http://3dprintingindustry.com/wp-content/uploads/2014/07/3D-Printing-Guide.pdf (accessed on 16 January 2016).
3. Alifui-Segbaya, F.; Williams, R.J.; George, R. Additive manufacturing: A novel method for fabricating cobalt-chromium removable partial denture frameworks. *Eur. J. Prosthodont. Restor. Dent.* **2017**, *25*, 73–78. [PubMed]
4. Stratasys. Safety Data Sheet (MED610). Available online: http://www.stratasys.com/materials/material-safety-data-sheets/polyjet/~/media/394dd5015bb6473496a19ea837881c25 (accessed on 30 November 2016).
5. Stratasys. Printing Bio-Compatible Parts on Polyjet™ 3D Printers with MED620. Available online: http://usglobalimages.stratasys.com/Main/Files/SDS/MED620_Usage_Terms_1116.pdf?v=636160085940019299 (accessed on 18 January 2016).
6. Alifui-Segbaya, F.; Varma, S.; Lieschke, G.J.; George, R. Biocompatibility of photopolymers in 3D printing. *3D Print. Addit. Manuf.* **2017**, *4*, 185–191. [CrossRef]
7. Organisation for Economic Co-Operation and Development. *Test No. 236: Fish Embryo ACUTE Toxicity (FET) Test*; OECD Publishing: Paris, France, 2013; pp. 1–22.
8. Lieschke, G.J.; Currie, P.D. Animal models of human disease: Zebrafish swim into view. *Nat. Rev. Genet.* **2007**, *8*, 353–367. [CrossRef] [PubMed]
9. International Organization for Standardization. *Biological Evaluation of Medical Devices—Part 2: Animal Welfare Requirements*; International Organization for Standardization: Geneva, Switzerland, 2006.
10. European Union. Directive 2010/63/eu of the european parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Off. J. Eur. Union* **2010**, *53*, 33–79.
11. EnvisionTec GmBH. Advanced DLP for Superior 3D Printing. Available online: https://envisiontec.com/better3dprintingtechnology/ (accessed on 5 June 2018).
12. Araújo, P.H.H.; Sayer, C.; Giudici, R.; Poço, J.G.R. Techniques for reducing residual monomer content in polymers: A review. *Polym. Eng. Sci.* **2002**, *42*, 1442–1468. [CrossRef]
13. EnvisionTec GmbH. E-shell 450: Safety Data Sheet. Available online: https://envisiontec.com/wp-content/uploads/2016/11/MK-SDS-EShell450Series-V20161114-FN-EN.pdf (accessed on 21 May 2018).
14. EnvisionTec GmbH. E-Shell® 450 Series. Available online: https://envisiontec.com/3d-printing-materials/perfactory-materials/e-shell-450-series/ (accessed on 22 July 2017).
15. International Organization for Standardization. *ISO 10993-5, Biological Evaluation of Medical Devices—Part 5: Tests for In Vitro Cytotoxicity*; International Organization for Standardization: Geneva, Switzerland, 2009.
16. 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.
17. 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? *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2009**, *149*, 196–209. [CrossRef] [PubMed]
18. 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 Res. Int.* **2013**, *2013*, 485246. [CrossRef] [PubMed]
19. Merck. Acrylic Monomers. Available online: https://www.sigmaaldrich.com/materials-science/material-science-products.html?TablePage=16397884 (accessed on 3 December 2017).
20. Decker, C. Photoinitiated crosslinking polymerisation. *Prog. Polym. Sci.* **1996**, *21*, 593–650. [CrossRef]
21. 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, Switzerland, 2009.
22. Autian, J. Structure-toxicity relationships of acrylic monomers. *Environ. Health Perspect.* **1975**, *11*, 141–152. [CrossRef] [PubMed]
23. Geurtsen, W. Polymethylmethacrylate resins. In *Biocompatibility of Dental Materials*; Springer: Berlin/Heidelberg, Germany, 2009; pp. 255–270.

24. Kanerva, L.; Estiander, T.; Jolanki, R.; Tarvainijn, K. Occupational allergic contact dermatitis caused by exposure to acrylates during work with dental prostheses. *Contact Dermat.* **1993**, *28*, 268–275. [CrossRef]
25. 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. [CrossRef]
26. Mir, G.N.; Lawrence, W.H.; Autian, J. Toxicological and pharmacological actions of methacrylate monomers i: Effects on isolated, perfused rabbit heart. *J. Pharm. Sci.* **1973**, *62*, 778–782. [CrossRef] [PubMed]
27. 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. *J. Pharm. Sci.* **1974**, *63*, 376–381. [CrossRef] [PubMed]
28. DrugBank. Cyclomethicone 5. Available online: https://www.drugbank.ca/drugs/DB11244 (accessed on 18 June 2018).
29. National Center for Biotechnology Information. Pubchem Compound Database. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 19 June 2018).
30. 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. *Environ. Sci. Technol.* **2016**, *50*, 1260–1268. [CrossRef] [PubMed]
31. 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. *Polym. Bull.* **2016**, *73*, 571–585. [CrossRef]
32. Vitale, A.; Cabral, J.T. Frontal conversion and uniformity in 3D printing by photopolymerisation. *Materials* **2016**, *9*, 760. [CrossRef] [PubMed]
33. Formlabs. *Expanding 3D Printing Technologies to High-Volume Applications and Beyond*; Mary Ann Liebert, Inc. Publishers: New Rochelle, NY, USA; pp. 1–53.
34. Decker, C. Kinetic study and new applications of UV radiation curing. *Macromol. Rapid Commun.* **2002**, *23*, 1067–1093. [CrossRef]
35. eLABORATE. *3D Printer Marketplace*; Joseph Allbeury: Cammeray, Australia, 2017; Volume 14, pp. 46–52.