Biomedical photopolymers in 3D printing

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Abstract

Purpose

3D printing of acrylic-based medical devices is projected to grow exponentially despite the limitations of vat photopolymerization and the lack of information on the clinical performance of the materials. The purpose of this article is to address an issue of critical importance in the translation of the 3D printed structures to the clinic, which is assessing the toxicity of the polymers and their precursors.

Design

This review highlights the different manufacturing processes, challenges, and novel experimental work including the zebrafish embryo model which offers a potential method for toxicity profiling of biomedical photopolymers and their precursors due to its high genetic similarity to humans.

Findings

Materials information and experimental data available so far suggest that there is a need for regular and rigorous evaluation of new materials to establish their safety and protect users engaging in biomedically-related 3D printing activities.

Originality

The review identifies stringent, contemporary, and cost-effective analytical methods for assessing the safety of biomedical photopolymers and their precursors.

Keywords

Photopolymer; Photopolymerization; 3D Printing; Zebrafish embryo model; Medical devices; Biomaterials.

1. Introduction

Although natural polymers such as proteins, cellulose, silk and natural rubber have existed since the dawn of time, it was the revolutionary discovery of synthetic polymers in the nineteenth century that led to plastics which have become an essential element of modern life (Fried, 2014). The word "polymer" is derived from Greek: "polys" and "meros" meaning "many" and "part", respectively (Jensen, 2008). Polymers are identified by the structural and repeating units in their chains. A structural unit in a polymer chain represents a residue from a monomer used in the synthesis of the polymer, whereas a repeating unit refers to a structural unit or a covalently bonded combination of two complementary structural units, which is repeated many times to make the whole chain (Mandal, 2014). In general, polymers are molecules composed of repeating units connected by covalent bonds in a variety of ways (Ravve, 2012). Photopolymers, on the other hand, are polymeric materials that change their structural and chemical properties when exposed to light, usually within the ultraviolet (100-400 nm) and visible light (400-740 nm) region of the electromagnetic spectrum (Chua *et al.*, 2017) by a process known as photopolymerization or photoinitiated polymerization.

2. Photopolymerization in 3D Printing

Photopolymerization is considered to be the most effective way to transform solvent-free resins into solid polymers, at ambient temperature (Decker, 1996). Compared to thermally-activated polymerization, it is more economical and offers a myriad of practicalities in imaging, microelectronics, graphic arts, printing plates, photoresists, laser direct imaging, computer-to-plate technology, holographic optical elements and dentistry, to name a few (Tehfe *et al.*, 2013). In 3D printing (3DP), the capacity to use photopolymerization to initiate speedy polymerization of solvent-free liquid resins accounts for its utility in the manufacturing of medical devices. 3DP simply involves importing a virtual model (usually "STL" file) into a designated 3D printer

to build parts in successive (two-dimensional) layers until the desired 3D part is completed (Alifui-Segbaya *et al.*, 2017b). 3DP also comprises a host of processes and technologies that offer a diverse spectrum of capabilities for the manufacturing of parts, end-use products and medical devices using different materials (3d Printing Industry, 2015). For this review, stereolithography, digital light processing and material jetting being photopolymerization processes currently used for acrylic-based medical devices are discussed. The complete manufacturing process is discussed in detail elsewhere in literature (Stansbury and Idacavage, 2016, Lee *et al.*, 2017, Ligon *et al.*, 2017).

2.1 Stereolithography

Stereolithography (SL) is considered the pioneer of 3DP processes (Hull, 1986, 3d Systems, 2017a). In a traditional SL system shown in **Figure 1A** (3d Printing and Additive Manufacturing, 2017), layers of liquid photopolymer resin from a vat are selectively cured or solidified with ultraviolet (UV) laser beam to form physical parts. For each printing step, the laser scans across the surface of the resin and solidifies it in predetermined x-y directions, leaving the surrounding area in a liquid form. The build platform in the vat then drops fractionally (z-direction), submerging the solidified layer into the resin to be recoated and irradiated (3d Systems, 2015). In an inverted SL system **(Figure 1B)**, the incident light is emitted from the bottom of the vat to solidify the liquid resin. The penetration depth i.e., photosensitive energy that goes into the resin is dependent on the type of photoinitiator, concentration of photoinitiator, and wavelength of the laser. The cure depth defines the thickness of each solidified layer and is influenced by the irradiation dose, critical energy and concentration of the resin (Tran and Wen, 2014). In general, the functionality of parts manufactured by SL is controlled by parameters that include physicochemical properties of the resins, speed and resolution of the optical scanning systems, the power, wavelength and types

of laser used, the spot size of the laser, the recoating system and the postprocessing steps (Chua and Leong, 2015).

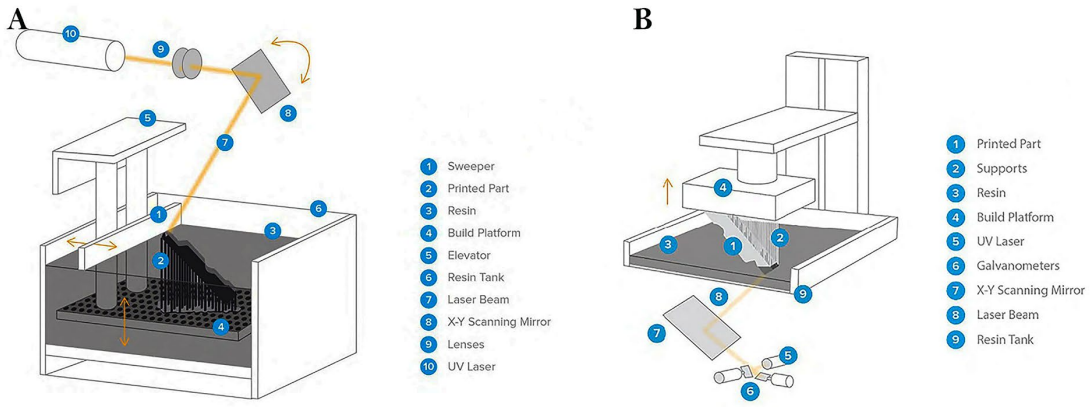

Figure 1 Schematic of traditional (A) and inverted (B) stereolithography 3D printers. Republished with permission of Mary Ann Liebert, Inc., New Rochelle, NY, from Expanding 3D printing technologies to high-volume applications and beyond, 3D Printing and Additive Manufacturing, eBook, 2017.

2.2 Digital light processing

Digital light processing (DLP) is similar to SL in that both are vat photopolymerization processes that require washing built parts in organic solvents to remove wet resin remnants, followed by postcuring in a UV oven to harden them. DLP, however, 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 (3d Printing Industry, 2015). Perfactory DLP®, for instance, uses high-definition projectors that deliver light in pixels, creating volumetric pixels or voxels in the resin. The projector emit light in multiple intensities along a spectrum that goes from white to grey and black, thus enabling parts to be built in varying depths at fixed x-y dimensions (Envisiontec Gmbh, 2016). The newly introduced continuous liquid interface production (CLIP), combines

DLP technology with oxygen permeable optics and programmable resins to build 3D parts: incident light is projected through oxygen-permeable window ("dead zone") to selectively cure layers of resin flowing continuously above it (Carbon Inc.).

2.3 Material Jetting

In material jetting (MJ), liquid photopolymer resin is selectively squirted through multiple jet heads, and then cured with a passing of UV light as each layer is deposited. The jet heads are fixed (in x-y directions) whereas the build tray is lowered (z-direction) for resin to be added and solidified. MJ also allows for simultaneous deposition of materials with multi-coloured attributes and physical properties. Depending on the technology, support structures can be melted away in a heated oven before cleaning (3d Systems, 2017b) or cautiously removed with a water jet or by hand (Stratasys, 2015). Unlike SL and DLP, no post-curing is required for MJ parts.

3. Basic chemistry of phopolymerization

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 (Merck, 2017). They are preferred in free-radical polymerization (FRP) because of the high reactivity of the acrylate double bond. Under intense illumination, crosslinking polymerization of resins proceeds extensively within a fraction of a second to generate a three-dimensional polymer network. The speedy polymerization of the resins occurs by virtue of aromatic ketone photoinitiators undergoing fast homolytic cleavage upon irradiation (Decker, 1996, Decker, 2002). Liquid photopolymer resins for 3DP of medical devices (e.g., **Figure 2** (Alifui-Segbaya, 2018)) are largely proprietary but are usually composed of photoinitiators, mono-or-multifunctional monomers and functionalized oligomers. The photoinitiators absorb the incident light and generate reactive radicals or ions; mono-or-multifunctional monomers act as

reactive diluents to adjust viscosity; and functionalized oligomers constitute the backbone of the polymer after polymerization (Decker, 2002). Monofunctional monomers in the resins, can either act as a crosslinker or reactive diluent to adjust formulation viscosity while multifunctional monomers act concurrently as a crosslinker and diluent (Pandey, 2014).

Polymerization reaction consists of three dinstinct stages: initiation, propagation and termination. Initiation involves the formation of radicals that react with vinyl monomers. Propagation describes the rapid and progressive addition of monomers to growing polymer chain without changes to active centers. Termination occurs when free-radical chain reactions end either by combination (e.g., styrene) or disproportionation (e.g., methyl methacrylate). By combination, two growing polymer chains react with each other to form a single nonreactive polymer whereas by disproportionation, a hydrogen atom is transferred from one radical to another to form polymers with saturated and unsaturated ends. Additionally, a transfer of a growth active site from active chain to a previously inactive one might occur (Polymer Properties Database, 2018). To illustrate, the photoinitiator (PI) generates free radicals (PI $\rightarrow$ PI*($h\nu$) $\rightarrow$ 2R$\bullet$), which are then transferred to active groups on the monomer (M$\bullet$) chains (R$\bullet$ + M $\rightarrow$ RM$\bullet$) to form a crosslinked phase in repeated addition $(RM\bullet)_n \rightarrow$ polymer) (Fouassier *et al.*, 2010). For enhanced clinical outcomes, resins should possess a high curing rate, good storage stability, low viscosity, low toxicity, and display adequate mechanical properties after polymerization (Li *et al.*, 2016). Resins with relatively low viscosity are likely to produce rapid polymerisation yielding crosslinked polymers with properties suited to the demands imposed by the target application (Vitale and Cabral, 2016). The mechanical properties of photocured materials thus depend primarily on the chemical structure, functionality and concentration of the various constituents of the resin, and the degree of cure (Decker, 2002). Despite the high reactivity of acrylate double bond, oxygen inhibition due to excited triplet state quenching by

O_2 and scavenging of initiating R•, as well as propagating RM_n• radicals by O_2 (Tehfe *et al.*, 2013) occur as drawbacks of the FRP process. Likewise, the relatively low thermal resistance, glass transition temperature and physical properties of the resultant polymers (Crivello and Reichmanis, 2014).

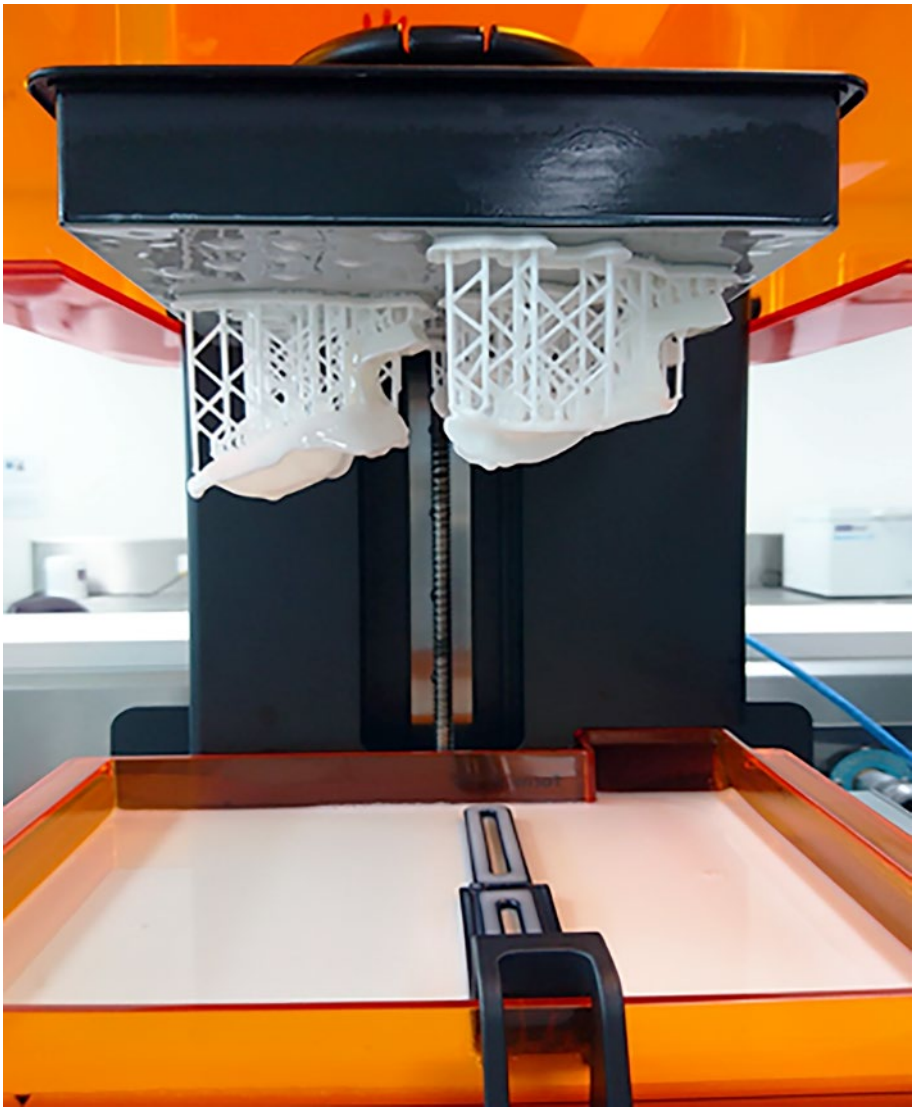

Figure 2 Custom impression trays built from a liquid photopolymer resin in "Form 2" inverted SL printer. Republished from Toxicological assessment of photopolymers in additive manufacturing using the innovative zebrafish embryo model, Alifui-Segbaya, F. PhD Thesis, Griffith University, 2018.

4. Biocompatibility of acrylic-based medical devices in 3D printing

In FRP, polymerization of carbon-carbon double bond is critical since the carbonyl group is not prone to polymerization by radical initiators due to its polarized nature (Odian, 2004). Free radicals attack on the 'unsaturated' vinyl double bond (π-bond) forms a single bond (σ-bond) to another carbon and an unpaired electron (Kim and Watts, 2008). As polymerization progresses, the amount of aliphatic double bonds decreases but in practice, the process does not guarantee a 100% monomer conversion rate (Duray *et al.*, 1997). 3DP being an iterative process could also result in undesired material chemistries. The quality of additively manufactured parts may vary significantly regardless of whether they were manufactured using different 3D printers or with the same 3D printer and a standardised workflow of parameters, process steps, and materials (Food and Drug Administration, 2016). It is important to note that the dimensional accuracy of the parts is also of paramount importance, for instance, medical or anatomical models for diagnosis, surgical planning, and the reconstruction of posttraumatic defects, tumoral resections, and other complex craniofacial defects (Mazzoli *et al.*, 2007).

Apart from the medical applications of acrylic materials, their widespread use also raises questions pertinent to their toxicological properties, and their short-and long-term health effects on persons exposed to them (Autian, 1975). For medical devices, it is recommended to use approved materials, apposite manufacturing parameters and postprocessing techniques to minimise the potential effects to residual monomer and degradation products (Alifui-Segbaya *et al.*, 2018). Interestingly, disclaimers for some photopolymers state that, "it is the responsibility of the customer, its respective customers and end-users to determine the biocompatibility of all of the components, printed parts, and all other materials used in the finished product for their respective purposes, including prolonged skin contact (of more than 30 days) and short-term mucosal-membrane contact (of up to 24 h)" (Stratasys, 2016a,

Stratasys, 2016b). **Table 1** shows compositional differences between liquid and photocured resins; data for the latter were obtained in-house using gas chromatography - mass spectrometry (GC-MS) protocols (Alifui-Segbaya and George, 2018). Also, the gradations of toxicity (**Figure 3)** elicited by the photocured materials in zebrafish bioassays (Alifui-Segbaya *et al.*, 2017a) were possibly influenced by their physicochemical characteristics, before and after immersion in ethanol (Alifui-Segbaya *et al.*, 2018).

Table 1 Composition of liquid and photocured[1] biomedical photopolymers

MED610	**Liquid resin (w/w %):** 20-30% Exo-1,7,7-trimethylbicyclo [2.2.1] hept-2-yl acrylate; 5-10% Tricyclodecane dimethanol diacrylate and ≈ 60% is proprietary. **Photocured resin [2]:** 2-Propanol, 1-methoxy-; Cyclohexanone; Benzaldehyde; 2-Propyl-1-pentanol and 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl ester, exo- **Photocured resin with ethanol treatment:** 2-Propanol, 1-methoxy-; Cyclohexanone; Benzaldehyde; Cyclohexene, 1-methyl-4-(1-methylethenyl)-; Linalool and 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl ester, exo-
MED620	**Liquid resin (w/w %):** <30% Acrylic monomer; <25% Exo-1,7,7 Trimethylbicyclo [2.2.1] hept 2-yl acrylate ; <15% Acrylic oligomer; <3% Photoinitiator; <0.8% Titanium dioxide; <0.3% Acrylic acid ester; the rest is proprietary. **Photocured resin [3]**: 2-Propanol, 1-methoxy-**;** Acetic acid, butyl ester**;** Ethylbenzene**;** Benzene, 1,3-dimethyl-; Cyclohexanone; Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-methylene-; Benzaldehyde; 2-Vinylfuran; Benzene, 1-ethyl-2-methyl-; Pentanedioic acid, dimethyl ester; 2-Oxepanone; Isoborneol; N-Acryloylmorpholine; 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl ester, exo- and Furan, 2-butyltetrahydro- **Photocured resin with ethanol treatment:** 2-Propanol, 1-methoxy-; Ethylbenzene; p-Xylene; Cyclohexanone; Benzaldehyde and 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl ester, exo-
Visijet SL Clear	**Liquid resin (w/w %):** 60-75% 4,4' Isopropylidenedicyclohexanol oligomeric reaction products with 1-chloro-2,3 epoxy propane; 15-25% 3-ethyl-3-hydroxymethyl-oxetane and Mixture containing triarylsulfonium salt: 50% propylene carbonate and 50% mixed triarylsulfonium salts (1-5%). **Photocured resin [4]:** Methyl Isobutyl Ketone; Cyclohexanone**;** Benzaldehyde**;** Propylene Carbonate**;** 1-Hexanol, 2-ethyl-**;** 1,4-Dioxaspiro (4.5) decane-2-one**;** 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl ester, exo- and Methanone, (1-hydroxycyclohexyl) phenyl- **Photocured resin with ethanol treatment:** 2-Propanol, 1-methoxy-; Methyl Isobutyl Ketone; Cyclohexanone; Ethanol, 2-butoxy-; Benzaldehyde; Propylene Carbonate**;** 1,4-Dioxaspiro (4.5) decane-2-one; 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1]hept-2-yl ester, exo- and Methanone, (1-hydroxycyclohexyl)phenyl-
Visijet M3 Crystal	**Liquid resin (w/w %):** 15-25% Ethoxylated bisphenol A diacrylate; 14-24% Urethane acrylate oligomers and 5-11% Tripropyleneglycol diacrylate. **Photocured resin [5]**: 2-Propanol, 1-methoxy-; Toluene; Cyclopentanol, 2-methyl; Cyclohexanone; Ethanol, 2-butoxy-; Benzaldehyde; 2-Propyl-1-pentanol; 2-Oxepanone; 2-Propenoic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl ester, exo-; Tetrahydro [2,2'] bifuranyl-5-one and Methanone, (1-hydroxycyclohexyl) phenyl- **Photocured resin with ethanol treatment:** 2-Propanol, 1-methoxy-; Toluene; Cyclopentanol, 2-methyl-, cis; Cyclohexanone; Ethanol, 2-butoxy-; Benzaldehyde; 1-Hexanol, 2-ethyl-; 2-Oxepanone; 2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo; Furan, 2-butyltetrahydro- and Methanone, (1-hydroxycyclohexyl)phenyl-

[1] Analysed by headspace GC-MS: Samples were frozen in liquid nitrogen at -196 °C, ground into powder and tested in GC-Shimadzu TQ8040 GC-MS/MS (Shimadzu Corporation, Tokyo, Japan). The GC column used was Agilent J&W DB5-MS 30m 0.25mm ID 0.25um film thickness. Test parameters are, 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.

[2] Photocured MED610 was built by Stratasys (C-BONS International Center 108, Hong Kong, China) using Objet Eden260VS 3D printer.

[3] Photocured MED620 was built by Stratasys (C-BONS International Center 108, Hong Kong, China) using Objet Eden260VS 3D printer.

[4] Photocured Visijet SL Clear was built by 3D Systems (3D Systems, Rock Hill, SC) using ProJet 7000 HD printer parameters.

[5] Photocured Visijet M3 Crystal was built by (3D Systems, Rock Hill, SC) using MJP 3600 3D printer.

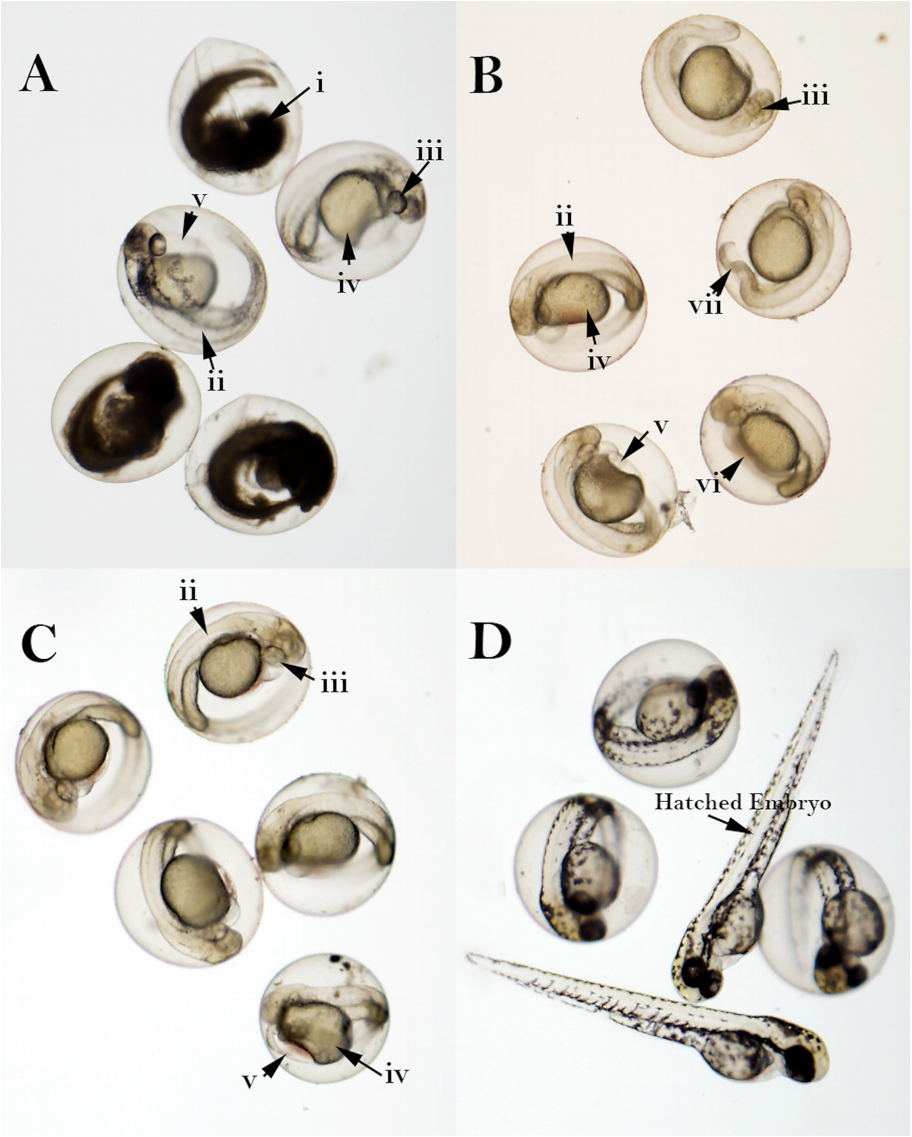

Figure 3 Zebrafish (AB/Tü) bioassays containing a: photocured and ethanol-treated visijet clear b: photocured visijet crystal c: photocured and ethanol-treated visijet crystal and d: control. The toxicity endpoints in "a", "b" and "c" are i mortality ii hypopigmented body iii hypopigmented eye iv deformed yolk v pericardial edema vi blood pooling vii. malformed tail. Republished with permission of Mary Ann Liebert, Inc., New Rochelle, NY, from Biocompatibility of Photopolymers in 3D Printing, Alifui-Segbaya, F., et al., Volume 4, Number 4, 2017; permission conveyed through Copyright Clearance Center, Inc.

5. Zebrafish as an alternative animal model for evaluating biocompatibility

Since it is generally accepted that no biomaterial is 100% safe (Wataha, 2012), toxicological tests are designed in order to help the identification of toxicity mechanisms of chemical compounds, damage that may ensue in their exposure, and the necessary preventive measure to ameliorate their adverse effects (United States National Library of Medicine, 2004). Several tests are recommended by the International Organization for Standardization (ISO) based on intended use of materials or devices (Iso 7405, 2008). To determine acute toxicity, *in vitro* cytotoxicity assays rely on immortalized cell lines that have little metabolic capability and often lack information on organ-specific or cell-type specific physiology (Committee on Predictive Toxicology Approaches for Military *et al.*, 2015). Animal tests, on the other hand, are expensive, complex, and present laborious administrative challenges (Wataha, 2012). In this regard, zebrafish (non-mammalian *in vivo*) bioassay offers economy and ease of quantifying multiple toxicity endpoints, dose-response relationship, and toxicodynamics of chemicals or leachable substances (Hill *et al.*, 2005, Strähle *et al.*, 2012).

The use of zebrafish (*Danio rerio*) as a laboratory animal was pioneered by George Streisinger and others (Streisinger *et al.*, 1981). Zebrafish are small benthopelagic cyprinid fish that originates from the Ganges River system, Burma, the Malakka peninsula and Sumatra. It has an average adult length ranging between 3 and 5cm, and an approximate generation time of 3 to 4 months at 26 °C in both soft and hard waters. As an aquatic vertebrate species, it is easily obtainable and spawned, and under appropriate conditions will provide many non-adherent and transparent eggs (Braunbeck and Lammer, 2006, Belanger *et al.*, 2010). Despite the physiological differences between fish and humans, 70% of protein-coding human genes are related to genes found in zebrafish. Likewise, 84% of genes known to be associated with human disease have a zebrafish counterpart (Howe *et al.*, 2013). These similarities make zebrafish

appropriate for research in cancer aetiology (Pei and Strauss, 2013), developmental biology, embryogenesis, pharmaceutical drug discovery (Detrich *et al.*, 1999, Delvecchio *et al.*, 2011, Kari *et al.*, 2007, Gustafson *et al.*, 2012) and assessing teratogenic effects (Sipes *et al.*, 2011, Selderslaghs *et al.*, 2012), to name but a few.

The Organization for Economic Cooperation and Development (OECD) fish embryo toxicity (FET) test designed to determine acute toxicity of chemicals on embryonic stages of zebrafish (Oecd Test Guideline 236, 2013) has been successfully applied to toxicity profiling of photopolymers in 3DP (Oskui *et al.*, 2016, Macdonald *et al.*, 2016, Alifui-Segbaya *et al.*, 2017a, Alifui-Segbaya and George, 2018, Alifui-Segbaya *et al.*, 2018). The wild-type strain recommended for chemical screening is also popular for research related to sequencing, genetic screening, gene expression and transgenesis (Haper and Lawrence, 2011). It is important to understand that, the FET test is not considered an animal experiment since fish in their early stage are "not sufficiently aware" that they will suffer when a procedure is carried out on them"(Braunbeck *et al.*, 2015). Nonetheless, some developmental endpoints induced by additively manufactured methacrylates in fish bioassays are comparable to those reported in animal studies that linked methacrylic esters to embryonic fatal toxicity, teratogenicity, and cardiovascular function (Alifui-Segbaya *et al.*, 2018). In general, the zebrafish is an excellent model for developmental toxicity and offers advantages such as external fertilization, high fecundity, transparent embryos**,** that permit 'whole organism' assessment through direct observation of internal organs and ease of phenotype assessment over other vertebrates or rodents models, in which aspects of organogenesis and disease pathology cannot be examined without interventions such as surgery or post-mortem examination (Lieschke and Currie, 2007).

6. Conclusions

Currently, very little data is published on systematic studies of the biocompatibility of 3D printable polymers and their precursors despite the increasing popularity of 3DP and contemporaneous influx of new materials. Thus, there is a need for methods to stringently evaluate these materials to establish their safety and protect users engaging in biomedically-related 3DP activities. In the long term, standards for preclinical evaluation could be revised to reflect the current trends in biomaterials, manufacturing techniques, and biological evaluation of medical devices and precursors they are generated from.

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