RESEARCH ARTICLE

Bioprinting of Multimaterials with Computer-aided Design/Computer-aided Manufacturing

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Abstract: Multimaterials deposition, a distinct advantage in bioprinting, overcomes material's limitation in hydrogel-based bioprinting. Multimaterials are deposited in a build/support configuration to improve the structural integrity of three-dimensional bioprinted construct. A combination of rapid cross-linking hydrogel has been chosen for the build/support setup. The bioprinted construct was further chemically cross-linked to ensure a stable construct after print. This paper also proposes a file segmentation and preparation technique to be used in bioprinting for printing freeform structures.

Keywords: Three-dimensional bioprinting, Bioprinting, Hydrogel, Three-dimensional printing, Rapid prototyping, Additive manufacturing, Computer-aided design, Support structure generation

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Received: August 11, 2019; ***Accepted:*** January 03, 2020; ***Published Online:*** January 22, 2020

Citation: Lee JM, Sing SL, Yeong WY, 2020, Bioprinting of multimaterials with computer-aided design/computer-aided manufacturing. *Int J Bioprint*, 6(1):245. DOI: 10.18063/ijb.v6i1.245.

1 Introduction

Bioprinting can be considered as a derivative technique that has evolved from three-dimensional (3D) printing, also known as additive manufacturing[1-3]. Through computer assistive technology, different bioprinting techniques can be integrated together for multimaterial printing. Material jetting and material extrusion are two technologies that have been commonly used in bioprinting due to the ease of setup. Moreover, these processes are relatively biocompatible as opposed to other additive manufacturing technologies. In material jetting bioprinting, biomaterials, cells, and growth factors are dispensed as droplets[4-7]. Comparatively, the material extrusion setup uses a combination of an automated robotic system for controlling platform movement and a dispensing system for deposition of cell-hydrogel constructs in forms of either strands or droplets[8-17]. In addition, valves can be placed at the nozzle to create droplets by regulating the flow of the hydrogel within the syringe[18].

2 Limitations of bioprinting in forming structural stable construct

Hydrogels[19], commonly used as vehicles for cell delivery in bioprinting, are high water content polymers with hydrophilic polymer chains that can be cross-linked to form 3D matrices[20]. Naturally derived hydrogels, such as collagen, gelatin, hyaluronic acid, chitosan, alginate, and cellulose, are used for tissue engineering and in bioprinting[21-23]. However, the naturally derived polymers have certain drawbacks including relatively weak mechanical property, fast degradation, and sometimes may cause allergic reactions[21,23]. Materials that are used for bioprinting are usually limited by their

viscosity and gelling speed, hence reducing the process window to bioprint freeform biological structures[24]. Different bioprinting strategies have been used to overcome such material-based limitation for bioprinting freeform constructs[25]. One such strategy is through the use of support materials that help assist in forming structural integrity for the build materials.

2.1 Support structure generation in additive manufacturing

The use of build/support configuration is prevalent in 3D printing. 3D printing technologies, such as inkjet printing[26-28], and material extrusion such as fused deposition modeling make use of multimaterials to differentiate the part from the support materials[29,30]. The software involved has the capability to generate the support structures needed for the part and to assign a secondary material to the supports. Using materials of different properties, the supports can be removed preferentially during post-processing[31].

In general, the purpose for support structure in additive manufacturing is to provide structural integrity where regions of object display overhanging or floating features. Support structure generation in additive manufacturing can be distinguished according to the density difference between build and support parts. For instance, support structure generation for metal powder bed fusion and stereolithography (STL) is designed as struts[32]. Comparatively, support material can

Table 1. Comparison of the current technology for multimaterial deposition and support structure generation across additive manufacturing technologies.

Material	Form	AM technologies		Multimaterial deposition	Support structure generation	Function of support structure
Metal	Powder	Powder bed fusion[40-43]	Selective laser melting	×	Struts	- Support floating and overhanging objects - Melt pool heat dissipation - Prevent thermal warping prevention
			Electron-beam melting	×		
		Directed energy deposition[44]	LENS	✓	NA	
Polymer	Powder	Powder bed fusion[45-47]	Selective laser sintering	×	NA	
	Powder	Binder jetting[45-47]	Indirect inkjet printing (Binder 3DP)	✓	NA	
	Liquid, photopolymer	Material jetting[45,47]	Polyjet/inkjet printing	✓	Partially or fully encapsulate build part	- Support floating and overhanging objects
	Solid, thermoplastic	Material extrusion[45,47]	Filament deposition modeling	✓	Lattice scaffolding structures	- Support weight imbalance
	Liquid, hydrogel		Bioprinting	✓	Partially or fully encapsulate build part	- Support floating and overhanging objects - Improve print fidelity
	Liquid, photopolymer	Vat polymerization[45,47]	Stereolithography	✓	Struts	- Support floating and overhanging objects

LENS: Laser engineered net shaping

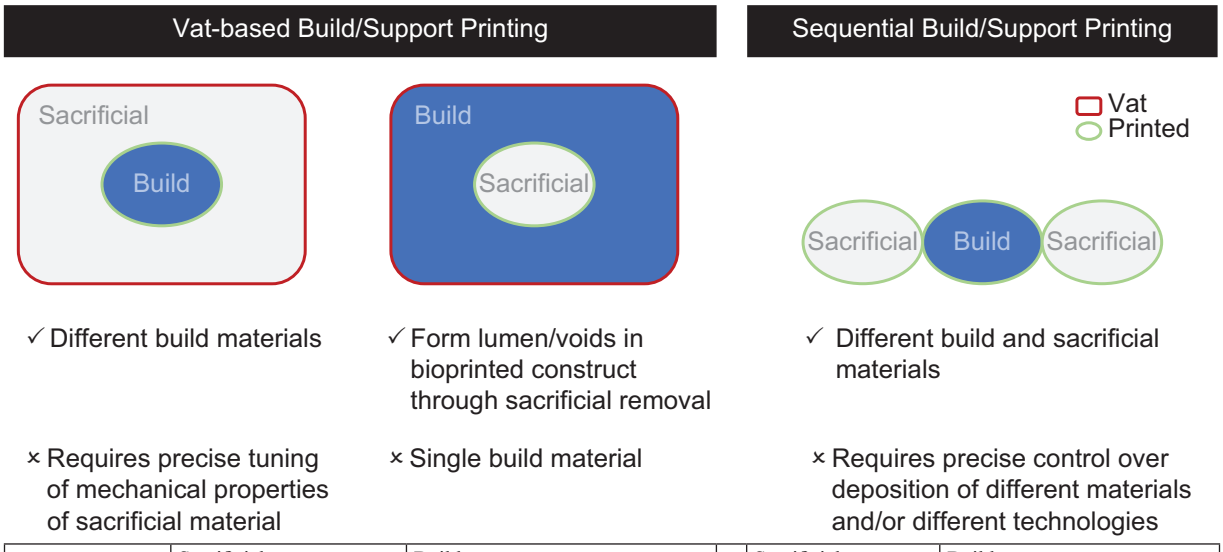
be generated to partially or fully encapsulate the 3D-printed part commonly used in material jetting and material extrusion techniques[33,34]. Support structures are also generated in response to include technology-specific consideration. For instance, the support structure in selective laser melting[35-38] or electron-beam melting[39] functions is strategically placed to improve heat dissipation and prevent print jobs from thermal warping[32].

The prevalence of multimaterial deposition and build/support printing configuration has been demonstrated across additive manufacturing technologies (**Table 1**). With an increase interest in multimaterial bioprinting for use in build-support configuration, it is imperative to develop a systematic framework for file processing method in multimaterial bioprinting. Considerations in segmenting computer-aided design (CAD) files for bioprinting are also discussed in the following section.

2.2 Use of build/support strategies in bioprinting

Bioprinting allows the deposition of heterogeneous materials and cells in a controlled manner to form an engineered construct that recaptures the complexities of native tissues[25,48,49]. The capability of depositing multimaterial in bioprinting is in line with build/support strategies commonly found in 3D printing. Increasing research interest lies in bioprinting cell-hydrogel materials using a build/support configuration[4,50-56]. In build/support configuration, support materials are used to provide mechanical strength to hold the structure giving the overall form for the engineered construct. In many cases, these materials are eventually removed from the printed constructed (i.e., sacrificial). On the other hand, cells and/or hydrogels (build materials) provide the functional components in the bioprinted construct.

Such printing strategy can be either vat-based or sequential printing of build/support materials (**Figure 1**). In such build/support configuration, the



	Sacrificial	Build		Sacrificial	Build
Natural	- Alginate[4] - Collagen[4,47] - Gelatin[47,55,56]	- Gelatin and derivatives[46] - Collagen[56] - Alginate[47,56] - Fibrinogen[47] - HA[47,56] - Spheroids[55] - Dextran and derivatives[57]		- HA and derivatives[44]	- HA and derivatives[44,52,54] - Gelatin and derivatives[44,52,54] - Fibrinogen[54] - Alginate[51] - Atelocollagen[52] - dECM[58]
Synthetic	- Pluronic F127[46] - PEO and derivatives[57]			- Pluronic F127[54] - PCL[46,51,52,54,58] - PLGA[52]	

Figure 1. Schematic illustration on the different configurations in build/support printing.

support material can be a temporary scaffold[57,58] that is either manually remove or dissolved away (i.e., sacrificial); while support material such as poly(epsilon-caprolactone) and poly(lactic-co-glycolic acid) thermoplastic provides mechanical integrity and eventually degrades through biological process[9,15,59-62]. Pluronic F127 and gelatin are commonly used in vat-based build/support printing such that bioink containing cells are extruded and embedded within the vat of support material[55].

Optimizing build orientation and topology of support structure aims at reducing support material usage and total build time. Algorithms have been developed to design tool path for strategically positioning build parts, minimizing support material wastage, and decreasing both build and post-processing time[34,66]. In this article, a novel file processing method is introduced. In brief, CAD files used for bioprinting are segmented into different sections. Build or support material printing reaches a certain z-layer before the printhead is changed for the alternating materials.

3 Methodology

3.1 File preparation

A series of CAD segmentation method is used in the novel approach in preparing CAD files for bioprinting. This method has several advantages such as (i) improve structural stability of bioprinted construct, (ii) deposit multimaterial, (iii) optimize overall printing time, and (iv) overcome machine limitations. Overview of the novel approach as compared to conventional file segmentation approach is illustrated in **Figure 2**.

To assess the functionality of the novel file segmentation approach, two models are demonstrated (i) freestanding coil (15 mm) and (ii) left ventricle wall. Regenhu bioprinter with multi-printhead channels is used for the printing.

3.2 Synthesis of gelatin methacrylate

All materials are purchased from Sigma-Aldrich unless otherwise stated. Gelatin methacrylate (GelMA) was synthesized as previously described with slight modification[67]. About 10% w/v Gelatin Type A (porcine skin) was dissolved in 1× phosphate-buffered saline (PBS) (Vivantis) stirred at 600 rpm keeping temperature at 60°C for 1 h. Methacrylate anhydride was added at 1.4% v/v dropwise into the solution and the reaction is continued for 2 h at 50°C. The reaction is quenched by adding pre-warmed 1× PBS at 40°C. The mixture was transferred into dialysis tubing (MWCO: 12400) for dialysis in distilled water for 4 days at 40°C. Finally, the solution was lyophilized for 7 days to obtain pure GelMA and was stored at −20°C until further use.

3.3 Preparation of bioink (build and support material)

The build material contains 5% w/v Gelatin Type A, 5% w/v GelMA, and 2% w/v sodium alginate which were dissolved in 1× PBS. Photoinitiator containing 10% w/v 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Sigma-Aldrich) was dissolved in ethanol and added to the mixture of build material at 0.02% v/v. The support material consists of Pluronic F-127 and 1 M calcium chloride dissolved in 10 mM HEPES buffer.

4 Results and discussion

Hydrogels that rapidly cross-link are chosen as the complimentary pairs in a build/support configuration[25]. As such, build material comprising gelatin methacrylate and sodium alginate is coupled with support material comprising of Pluronic F127/calcium chloride. Primary cross-linking occurs when alginate is in contact with $CaCl_2$ from the support material. Pluronic F127 comprises a hydrophobic core conjugated with hydrophilic segments at the two ends[68]. However, Pluronic F127 has weak mechanical properties specifically with rapid dissolution when in contact with aqueous media or biological fluid. Hence, it is essential to ensure that support material is structurally stable before secondary polymerization of GelMA without being dissolved. A layer of alginate hydrogel is formed between the build/support interface that acts as a barrier to slow the

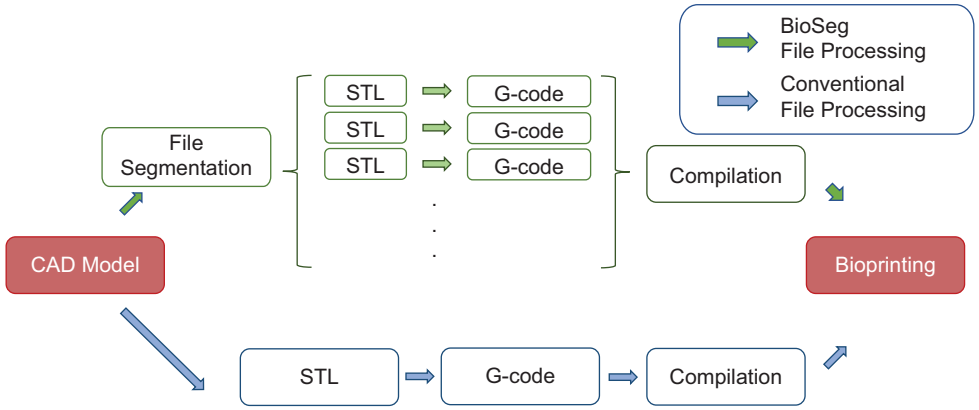


Figure 2. Process flow for file preparation for bioprinting, comparing between the two different file processing methods.

rate of dissolution, providing sufficient time before secondary cross-linking through ultraviolet (UV) cross-linking of GelMA.

4.1 Freestanding coil

The coil is designed with both materials interlacing each other, as shown in **Figure 3A**. The segments in red and gray regions correspond to the build and support material, respectively.

As the coil has materials overlapping each other at multipoints, the CAD model was sliced into different segments to be fabricated, **Figure 3B**. The prepared CAD file is then fabricated using both the conventional and new proposed file preparation methods. The fabricated coils are shown in **Figure 4**.

Comparing the two file processing methods, each file preparation method has its respective consideration factors, as shown in **Table 2.** As conventional file processing approach deposits different materials according to each z-layer, the printing process is significantly longer as compared to the printing files that have been segmented using BioSeg file processing. The BioSeg files minimize time required to interchange between different printheads. Such printing approach first completes the print of a single material until critical parameters such as material instability and height limitation of printhead are reached before switching to the alternative material. One distinct advantage of processing CAD models for bioprinting is to improve on the design freedom of printed construct. For instance, it has been found

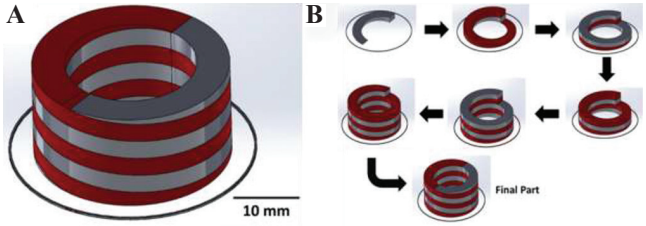


Figure 3. Freestanding coil computer-aided design (CAD) model (A) multimaterial (Red: Build material and gray: Support material) CAD model, (B) slicing of CAD files into segments.

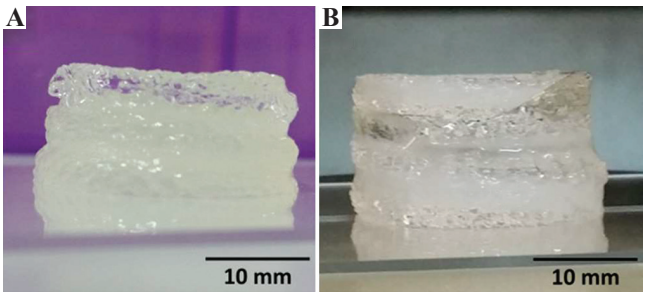


Figure 4. Coil fabricated using (A) conventional file processing, (B) BioSeg file processing.

that intrinsic staircase features are observed in filament deposition modeling[45]. These features arise from (i) the basic structure of a filament and (ii) processing of 3D CAD files using STL format. The use of STL format for 3D printing requires the deposition of material in a planar form, layer by layer. Using BioSeg method of printing, materials can be divided into smaller fragments and will not be restricted by the layer-by-layer printing of STL format.

Table 2. Consideration factors and comparison between the different file processing methods.

Intentional spacing	BioSeg file processing	Conventional file processing
Consideration factors	Bioprinter's compatibility issues Axis of movement Height of platform Distance between printed model and print head Material's compatibility issues Curing mechanism of build material Stability of material prior to curing Interaction between different materials	Material's compatibility issues Curing mechanism of build material Stability of material prior to curing Interaction between different materials
Advantages	Shorten printing duration Greater design of freedom in depositing build material	Straightforward process

4.2 Left ventricle

A model of the heart's left ventricle is fabricated to show the capability of proposed new method for realistic bioprinting applications. Based on the file segmentation criteria, the current model does not require fragmenting the CAD model into smaller part files. The 3D CAD images of the structure fabricated, together with the generated support structure, are shown in **Figure 5**.

The printed construct was cured under exposure of UV lamp before support material removal by washing with cold water. The curvature of the left ventricle printed was present even without the support structure, as shown in **Figure 5C**.

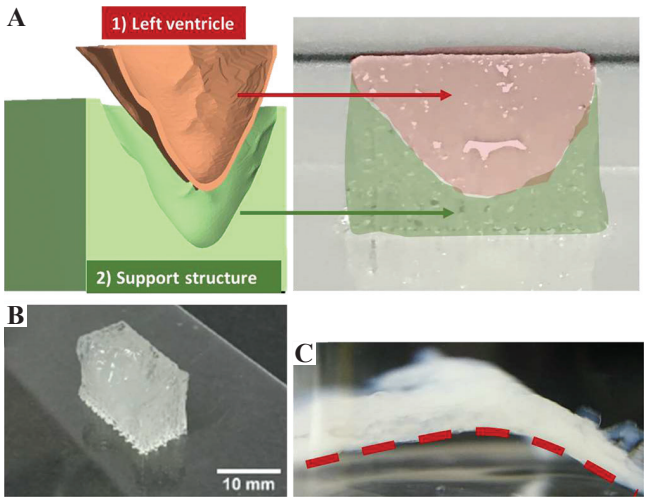


Figure 5. Three-dimensional image of the left ventricle (A) computer-aided design model with build (red) and support (green) materials corresponding with the respective red and green area of printed construct, (B) side profile of printed construct, (C) curvature of printed left ventricle preserved after support material removal (top view, curvature mapped with red dotted line).

5 Conclusion

Multimaterial deposition is of increasing interest in bioprinting for improving print fidelity in 3D bioprinting. Specifically, the use of build and support materials has been demonstrated in several researches to build engineered tissue constructs with structural stability. The study on support material generation and print path optimization is of great relevance for bioprinting as demonstrated across other additive manufacturing technologies.

Material selection is imperative in a build/support printing setup for building biological constructs with shape fidelity. Build materials have been chosen to facilitate cross-linking at different degrees. The first degree of cross-linking uses hydrogel with rapid gelation mechanism (sodium alginate and calcium chloride) to provide partial mechanical stability before fully cross-linking the printed construct through the formation of chemical bonds between polymer chains (gelatin methacrylate).

In this paper, we have also demonstrated a proof of concept to highlight the novelty in file segmentation for multimaterial deposition in bioprinting. This method can be used to fully utilize the tool changing capabilities of bioprinter to print multimaterials at a reduced print time. Considerations in terms of machine and materials

compatibility are needed when adopting such file segmentation method across different bioprinter setup. Fragmenting CAD models and printing 3D bioprinted models into small section invoke novel bioprinting approaches for structures that are more organic and closer to nature. Future studies may include cells in the build material printing to study cell responses with the improved BioSeg file processing method.

Acknowledgment

This research is supported by the National Research Foundation, Prime Minister's Office, Singapore under its Medium-Sized Centre funding scheme and by NTU startup grant.

Conflicts of interest

There are no conflicts of interest to declare.

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