REVIEW ARTICLE

3D-printed Bioreactors for *In Vitro* Modeling and Analysis

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Abstract: In recent years, three-dimensional (3D) printing has markedly enhanced the functionality of bioreactors by offering the capability of manufacturing intricate architectures, which changes the way of conducting *in vitro* biomodeling and bioanalysis. As 3D-printing technologies become increasingly mature, the architecture of 3D-printed bioreactors can be tailored to specific applications using different printing approaches to create an optimal environment for bioreactions. Multiple functional components have been combined into a single bioreactor fabricated by 3D-printing, and this fully functional integrated bioreactor outperforms traditional methods. Notably, several 3D-printed bioreactors systems have demonstrated improved performance in tissue engineering and drug screening due to their 3D cell culture microenvironment with precise spatial control and biological compatibility. Moreover, many microbial bioreactors have also been proposed to address the problems concerning pathogen detection, biofouling, and diagnosis of infectious diseases. This review offers a reasonably comprehensive review of 3D-printed bioreactors for *in vitro* biological applications. We compare the functions of bioreactors fabricated by various 3D-printing modalities and highlight the benefit of 3D-printed bioreactors compared to traditional methods.

Keywords: Cell culture, Bacteria, Three-dimensional-printed chip, Three-dimensional-printed devices, Three-dimensional-printed bioreactors

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Received: March 12, 2020; ***Accepted*:** June 03, 2020; ***Published Online:*** August 18, 2020

Citation: Priyadarshini BM, Dikshit V, Zhang Y, 2020, Three-dimensional-printed Bioreactors for *In Vitro* Modeling and Analysis, *Int J Bioprint*, 6(4): 267. DOI: org/10.18063/ijb.v6i4.267.

1 Introduction

1.1 Bioreactor

Bioreactors are essential tools that not only guide and support the development of *in vitro* live tissues but also act as culture vessels to study the biological response of the tissues to physiologically relevant conditions[1]. In the context of this review, bioreactors refer to devices for cellular and biochemical assays. The design and configuration of a bioreactor should complement the requirements of biological systems. For example, bioreactors for the study of vascularization and cardiac regeneration are coupled with the pulsatile flow to augment cell differentiation and maturation[2]. Similarly, bioreactors for lung tissue models are often linked to airflow setup to imitate native lung functions[3]. In addition, various operational parameters related to the flexibility, design, and other characteristics of bioreactors greatly influence the biological performance of bioreactors[4]. In the past few years, modeling and applications of bioreactors have evolved in various fields of research. Due to their enormous versatility, bioreactors have been employed in many industries, including biological,

biomedical, pharmaceutical, food, wastewater treatment, chemical, and fermentation[5]. This review focuses on biological applications in detail.

1.2 Three-dimensional (3D)-printed bioreactor

Conventional bioreactors grant operators the convenience of controlling the environment and experimental manipulation of two-dimensional tissue models[6]. However, their incompatibility with *in vivo* systems and their inability to reflect true cell traits and tissue morphology has necessitated 3D systems which exhibit better spatial distribution and structurally complex tissue architecture. Nevertheless, it is challenging to produce 3D bioreactors with complex geometry using conventional manufacturing methods[7].

Additive manufacturing (AM), also known as 3D-printing technology, has shown enormous potential in the fabrication of complex, low-cost, and custom-designed structures constructed by depositing a layer on top of earlier printed layers[5]. Over the past three decades, several 3D-printing strategies have been established with a focus on the fabrication of bioreactors of various shapes and sizes[8,9]. Through 3D-printing, specialized bioreactors can be engineered with high performance in terms of experimental throughput, liquid controllability, and stability[10]. 3D-printing not only grants freedom to optimize new bioreactor designs but also enhances cellular functionality and suitability of bioreactor for specific applications such as *in vitro* culturing and testing[11].

In view of this article, any 3D-printed culture apparatus, including chip, culture chamber, or filters that directly contact the cells, are considered as 3D-printed bioreactors. Moreover, various customized components and accessories of bioreactors such as culture tube holders, test parts, chamber inserts, and sensors fabricated with various 3D-printing modalities have been discussed. Several bioreactor models were designed to encourage the flow of culture medium for even distribution of nutrients throughout the culture vessel. The fluid flow in bioreactors could be manipulated at the micro-level by coupling bioreactors with microfluidic networks. The compartmentalized microfluidic devices with interconnected microchannels created cellular environments confined in a culture vessel that directed fluid flow through the cell culture[12,13]. In addition, these devices were shown to emulate physiological relevance by creating *in vitro* microenvironments on the same scale of cells. However, devices with challenging functionalities and dimensional specifications, such as channel height and aspect ratio, are difficult to achieve by conventional microfluidic techniques. Recent advancements have led to the development of 3D-microfluidics with intricate detailing, greater accuracy, and better resolution[14] using 3D-printing techniques.

1.3 Methods for fabricating 3D-printed bioreactors

Features of 3D-printed devices rely primarily on the chosen printing method. Some applications only 3D-printed the substrate in cell culture for *in vitro* analysis, whereas other applications embedded living cells into biocompatible printable materials (bio-inks)[15]. In this review, we primarily focus on the 3D-printed bioreactors for *in vitro* studies, not including the direct printing of cells. Various 3D-printing methods have been used to fabricate 3D structures and devices based on various printing techniques including selective laser melting (SLM), direct metal laser sintering (DMLS), fused deposition modeling (FDM), fused filament fabrication (FFF), inkjet, PolyJet, material jetting, stereolithography (SLA), digital light processing (DLP), micro-SLA (μSLA), and multiphoton lithography, each with their own advantages and disadvantages[16]. These 3D-printing processes are also used to fabricate bioreactors. However, none of these 3D-printing processes are ideal due to their specific limitations such as biocompatibility issues, difficulty in removing support materials, low printing resolution, poor dimensional accuracy, and rough surface texture[17-19]. Considerations for the choice of 3D-printing methods are shown

in **Tables 1 and 2**. 3D-printing process can be selected based on the manufacturing capability of the printer and the type of material used by printer[20,21]. However, 3D-printing process can be also selected according to the given main design requirements of bioreactor, including functionality and visual appearances[9,18].

Different laser sintering approaches such as SLM and DMLS are highly reproducible AM techniques used to fabricate porous and 3D

Table 1. 3D-printed bioreactors used in mammalian cell culture applications for assessing of cell viability, cell encapsulation, cell/tissue models, cell imaging, cell therapy, and organ-on-chip applications.

Printing technique	Printer model	Possible reason for choice of printer	Material	3D construct developed	Application	Cells used	Ref.
Cell viability							
SLA	ILIOS 3D-printer	High precision, transparency	PEG-DA-250 resin	Transparent disks	Bioreactor for studying resin compatibility on cells	Chinese hamster ovary cells (CHO-K1), Primary hippocampal neurons	[18]
SLA	Form 1+3D-printer	High accuracy, high resolution	Photocurable liquid resin	Cylindrical test parts	Bioreactor accessory for studying resin compatibility on cells	Zebrafish	[17]
FDM	Dimension Elite 3D-printer	Affordable, easily available	ABS				
Material jetting	Objet350 Connex 3D-printer	Dimensional accuracy	Objet Vero Clear	Microfluidic chip	Bioreactor for resin compatibility on cells	Bovine pulmonary artery endothelial cells	[31]
Cell encapsulation							
SLA	Commercial 3D-printer (Proto Labs)	High accuracy	3D Systems Accura® 60	Pump-free perfusion cell culture device	Bioreactor for cell encapsulation	Oral squamous cell carcinoma tumor, Liver cells	[32]
Material jetting	Objet260 Connex3 3D-printer	Dimensional accuracy	VeroClear-RGD810				
Cell/tissue models							
SLA	Commercial 3D-printer (EnvisionTEC)	Structural robustness	Eshell 300	Chamber and insert	Bioreactor accessory for tissue interactions	Human bone marrow stem cell	[33]
Testing of therapeutics							
Material jetting	Objet Connex 350 3D-printer	Droplet precision	Objet Vero White Plus	Device	Bioreactor for cell toxicity+drug transport	Endothelial cells	[34]
Material jetting	Objet Connex 350 3D-printer	Geometrical precision	Objet VeroClear	Microfluidic chip	Bioreactor for drug metabolism	Red blood cells	[13]
Material extrusion	MakerBot Replicator 2X 3D-printer	Good mechanical properties	ABS	Cartridges	Accessory item for cell toxicity	Human embryonic kidney cells	[35]

(Contd...)

Table 1. *(Continued)*.

Printing technique	Printer model	Possible reason for choice of printer	Material	3D construct developed	Application	Cells used	Ref.
Organ-on-a-chip							
Material jetting	Objet30 Pro 3D-printer	Rigidity, transparency	Objet VeroClear	Modular chamber	Bioreactor for blood-brain-barrier environment	Endothelial cells, rat primary astrocytes	[36]
SLA	Cellbricks 3D-bioprinter	High resolution	Gelatin and polyethylene glycol	Liver lobule	Bioreactor for characterization of liver organoid under static conditions	Human hepatoma cell line, human stellate cells	[37]
SLA	Perfactory 3 Mini-Multi Lens 3D-printer	High resolution	PIC100 resin	3D vessel	Bioreactor that mimics healthy and stenotic blood vessels	Human umbilical vein endothelial cells	[38]
Material extrusion	MakerBot Replicator 2 3D-printer	Affordable, geometrical precision	Polylactic acid	Input/output multiplexer	Bioreactor for endocrine tissue function	Endocrine cells	[39]
Material extrusion	ROSTOCK MAX V2 Desktop 3D-printer	Affordable, geometrical precision	Polymer	Molds	Bioreactor for bone metastasis	MC3T3-E1 cells	[40]
Material extrusion	Printrbot Simple Metal 3D-printer	Affordable, geometrical precision	Thermoplastic	Conformal device	Bioreactor for whole organ biomarker profiling	Microfluidic devices that interface with surface of whole organs	[41]
Material extrusion	Custom microextrusion-based 3D-printer	Micro-extrusion	Silicone, sodium polyacrylate hydrogel				
Cell observation							
DLP	Micro Plus Hi-Re 3D-printer	High resolution	HTM140	Microscopy chamber	Bioreactor accessory for multidimensional imaging	Human cell lines infected by membrane-GFP lentivirus, nuclear-tdTomato	[42]
DLP	EnvisionTEC Perfactory 3D-printer	High resolution	Eshell® 300	Fluidic culture chamber	Bioreactor for cell imaging	hMSCs	[43]
SLA	3D Systems Viper SLA system	Affordable, high accuracy, high resolution	WaterShed XC 11122 resin	Cell perfusion system (valves and pumps)	Bioreactor for cellular calcium imaging	CHO-K1 cells	[44]
SLA	PicoPlus 27 3D-printer	High accuracy, high resolution	Polypropylene/ acrylnitril-butadien-styrol	Semiconductor-based biosensors	Bioreactor for cell growth and metabolism imaging, resin compatibility on cells	CHO-K1 cells	[45]

3D: Three-dimensional, FDM: Fused deposition modeling, SLA: Stereolithography, DLP: Digital light processing, ABS: Acrylonitrile butadiene styrene

uniform metal structures with distinct cavities, and precisely control geometric parameters down to several hundred microns using laser power as low as 90 W[22,23]. Extrusion printing and its common variants such as FDM and FFF extrude molten polymer in a layer-by-layer manner to construct 3D objects[17,24]. This 3D-printing technique is cost-effective and could be easily adopted as a viable manufacturing option to create 3D constructs with high resolution, structural integrity, and transparency. Jetting-based methods, including inkjet, PolyJet, and material jetting deposit fluidic materials in a controlled fashion through a nozzle onto a 3D platform and are used to create highly complex constructs[25]. These direct cell printing techniques will not be discussed in this review. Another widely used 3D-printed method is the vat photopolymerization, including SLA and DLP, which prints by curing photosensitive resins with ultraviolet light[17,26]. SLA uses a laser beam that scans line-by-line to cure the photosensitive resin, whereas DLP uses a digital light projector to cure each layer of photoreactive resin in one go. Compared to DLP, SLA-based printers offer a higher spatial resolution, resulting in structures with dimensions <10 μm. μSLA-based systems that utilize two-photon optics further improve the resolution to submicrons[17]. The resulting ultrafine features may influence the mechanistic properties of cells in tissues. Nevertheless, resins used for SLA printers often contain methacrylate and/or acrylate monomers that have a reputation to be cytotoxic[17].

2 3D-printed bioreactor for biological applications

3D-printing is a rapidly evolving technology that provides an opportunity to fabricate complex 3D structures for biological applications[5,27]. It is an important tool for translational research that focuses on the *in vitro* biology and disease models in bioreactors. The increasing accessibility to 3D-printing has spurred substantial efforts toward many creative developments of 3D-printed bioreactors for the cultivation of mammalian as well as microbial cells. Various bioreactors have been fabricated with 3D-printing to study the response of these cells to the smallest details of their local environments such as substrate geometric arrangement, chemistry, and mechanics[28,29].

Much of our understanding of fundamental cellular mechanisms is garnered from the aberrant interactions of cells on 2D substrates. As we move toward more-compliant microenvironment, it is vital to demystify exactly what factors are operative in 3D systems rather than simply considering a dimensionality factor at play[30]. The increased capabilities of 3D-printers have resulted in well-architecture constructs with fine features and application-specific geometries. The key challenge here lies in achieving the geometry that provides the correct degree of biomimicry, mechanical and chemical cues needed for sufficient cell-cell signaling, cell development, and gene expression. Indeed, surface parameters such as porosity, roughness, and curvature are tunable according to experimental needs, and their effect on the collective cell behavior including adhesion, growth, alignment, proliferation, and differentiation has been demonstrated as well. Ideally, the role of 3D-printing is to provide cells a suitable environment supporting their transition into functional tissue *in vitro*. With 3D-printing, we are able to fabricate bioreactors of different sizes and shapes and introduce cells into the bioreactors post-printing for *in vitro* testing. Overall, this article aims to cover 3D-printed bioreactors for the *in vitro* study of both mammalian and bacterial cell culture.

2.1 3D-printed bioreactor for *mammalian cell culture*

3D-printed bioreactors used in mammalian cell culture applications for assessment of cell viability, cell encapsulation, cell/tissue models, cell imaging, testing of therapeutics, and organ-on-chip applications are discussed below and summarized in **Table 1**.

2.1.1 Cell viability in 3D-printed bioreactors

Bioreactors are an indispensable tool for maintaining cellular microenvironment to promote cell viability, growth, and proliferation.

The biocompatibility of cells with the materials used for 3D-printing also affects cell viability and survivability. Biocompatibility could be achieved with post-printing modification and has already been reviewed earlier[46]. The compatibility of zebrafish larvae on parts 3D-printed by FDM (using acrylonitrile butadiene styrene, [ABS]) and SLA (using photocurable liquid resin) (**Figure 1A**) indicated that materials used for FDM were less toxic compared to SLA evidenced by significantly lower rates of malformations. Following the UV treatment of SLA parts, the toxicity was significantly reduced but not completely eliminated[17]. In contrast, a concurrent study indicated the potential of transparent PEG-DA-250 resin disks (printed by SLA) for supporting the long-term culture of

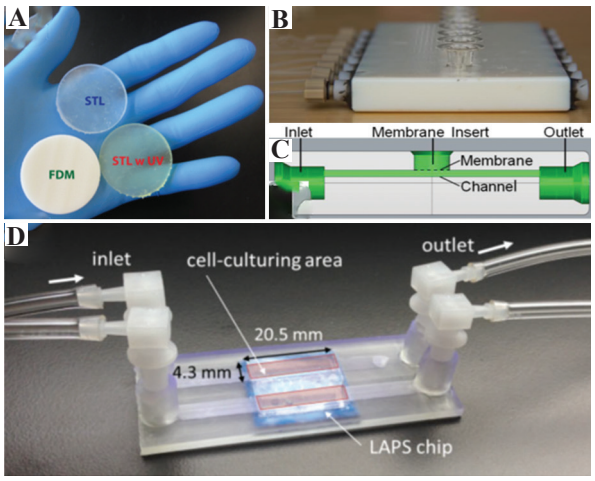

Figure 1. (A) Resin disks three-dimensional (3D)-printed by fused deposition modeling, stereolithography (SLA), and SLA w UV used for testing resin toxicity on zebrafish (40 mm diameter and 4 mm height)[17]. (B) 3D-printed device design showing adapters for syringe-based pumps, channels, membrane insertion port, and outlets. (C) The side view schematic of the 3D-printed device to understand the channel and fluid to flow under the membrane. The membrane is manually inserted into the port on top of the device. Finally, there is an outlet to allow fluid to leave the device[34]. (D) Potentiometric sensor-based biosensor chip showing inlet, outlet, and sensing area (20.5 mm × 4.3 mm) with attached microfluidic channels[45].

adherent CHO-K1 cells and primary hippocampal neurons[18]. Elsewhere, bovine endothelial cells were immobilized on a 3D transparent microfluidic chip made from photocurable resins by material jetting. Owing to unknown resin properties, the internal channels of the chip were coated with polydimethylsiloxane (PDMS) and polystyrene, respectively. Cell adherence and survival were favorable to PDMS, in comparison to polystyrene-coated, polished, and untreated samples[31].

2.1.2 3D-printed bioreactor for cell encapsulation

A pump-free perfusion device was fabricated by SLA (3D Systems Accura 60) and material jetting (VeroClear-RGD810) for immobilizing multicellular spheroids and maintaining their viability. Even though SLA resulted in cell-immobilizing microstructures with smoother surfaces, good spheroid functionality, and prolonged viability compared to PolyJet printing, the inferior optical properties restricted sample visualization by microscopy[32]. Despite a conducive capsule housing for cell culture, it remains a challenging task to entrap certain cell models with biocompatible substrates and mandates optical transparency of capsules due to their suitability for cell imaging.

2.1.3 3D-printed bioreactor for cell/tissue models

In addition to providing a complex yet controlled ambient for cell viability and cell encapsulation through spatial and temporal control of cell growth, the increasing versatility of 3D-printing also enables the development of tissue culture constructs that mimic specific biological functions and capture cell-tissue interactions inside the culture system. For example, the pathogenesis associated with a tissue can be studied. A 3D-printed multichambered bioreactor fabricated with non-cytotoxic Eshell 300 resin using SLA was fitted into a microfluidic base, creating tissue-specific environments for the study of interactions between chondral and osseous tissues during osteochondral differentiation[33]. This system

provided opportunities to investigate the tissue physiology and the role of each tissue in the pathogenesis of osteoarthritis.

2.1.4 3D-printed bioreactor for testing of therapeutics

3D-printed bioreactors are also useful in the clinical translation and commercialization of standardized cell-based products for cell-based therapies and drug-testing. A reusable material jetted (Objet Connex 350) fluidic device incorporated a porous polycarbonate membrane not only enabled molecular transport and drug migration through the membrane (**Figure 1B and C**) but also indicated drug susceptibility of mammalian cells[34]. Moreover, collecting analytes while simultaneously measuring the release stimulus was also possible with this 3D-printed bioreactor[13]. Electrodes and other additional functionalities such as membrane inserts and fluidic interconnects were integrated to ensure signal detection and flow control. A compact ready-to-use material extruded (MakerBot Replicator 2X) cartridge containing assay reagents was integrated with genetically engineered sentinel cells and interfaced with a custom-developed smartphone Tox-App for rapid quantification of cellular toxicity[35].

2.1.5 3D-printed bioreactor for organ-on-chip applications

An organ-on-a-chip device fabricated by 3D-printing aims to assemble organ models in 3D specific architecture on a microfluidic chip. By virtue of precise geometrical features attained by 3D-printing coupled with controlled flow dynamics and imaging compatibility of microfluidics, a continuous perfusion model had been developed to imitate the blood-brain barrier environment[36]. This setup consisted of a porous membrane that allowed coculture of different cell types across the membrane and a 3D-printed cell insert module that accommodated cell monolayers which formed a fully functional closed-loop perfusion model. This 3D-printed bioreactor was able to overcome the limitations faced by static culture models and demonstrated the synergy between microfluidics and 3D-printing[47]. Similarly, a 3D bone-on-a-chip device used coculture strategies to study disease mechanisms of the metastasis of breast cancer cells to bone marrow[40]. In the study, transparent PDMS chambers for cell growth casted from a 3D-printed mold (Rostock MAX V2 Desktop printer) were separated from the media reservoir by a membrane. 3D-printing of this geometrical design enabled frequent monitoring of interactions between cancer cells and the bone matrix *in vitro* and eliminated the need to take bone metastasis samples from patients.

Another study demonstrated the use of a perfusion-type liver organoid model using a sinusoidal liver lobule on a chip 3D-printed by SLA (Cellbricks bioprinter) with polyethylene glycol and gelatin containing bio-inks[37]. Cells cultured within the liver organoid model revealed high-yield protein expression compared to monolayer cultures. This *in vitro* model in 3D-printed bioreactor ensured hepatocyte functionality and could be modified to accommodate nutritional supply for larger tissue models to explore the mechanistic properties. The organ-on-a-chip systems could also be personalized by integrating additional systems to emulate the complexities of an organ. To design a 3D arterial thrombosis model, anatomical models were obtained from imaging scans and converted into a printable 3D model. The molds for chips with miniaturized healthy and stenotic vasculatures were then developed using a Perfactory 3 SLA 3D-printer with PIC100 resin. The vascular structures incorporated on-chip successfully mimicked vessel environments, showing human blood flow at physiologically relevant conditions and with artificially induced thrombosis[38]. Another system non-invasively interfaced a 3D-printed microfluidic device with a porcine kidney model to isolate and profile biomarkers from whole organs in real-time. From the cortex of the kidney, relevant metabolic and pathophysiological biomarkers were transported to the microfluidic device by virtue of the fluid flow in the microchannel. Hence, the 3D organ-on-a-chip could perhaps overcome the drawbacks of whole organ structures[41]. For a complex organ model, a multi-channel perfusion-type chamber was developed to assess endocrine secretions, due to their multiple inlet and outlet needs[39]. The

device warranted precise control of nutrient inputs, hormone outputs, and permitted observation by fluorescence imaging.

2.1.6 3D-printed bioreactor to facilitate cell observation

The visualization of real-time cellular response to a 3D culture environment through imaging facilitates the monitoring of specific cellular processes. Another research group proposed a multidimensional observation chamber (the UniveSlide) with an SLA 3D-printed frame for medium/high throughput long-term imaging in controlled culture environments, which was also compatible with different microscopy techniques[42]. Moreover, this all-in-one device may be suitable for automatized multi-position imaging of thick samples. The use of agarose gel with imprinted microwells as a base support frame was a convenient addition for trapping cells and subsequent 3D viewing. A 3D-printed fluidic culture chamber was used to dynamically culture hMSCs, study the mechanical behavior of the cells in a controlled microenvironment, and visualize cells within 3D-printed constructs without sectioning using imaging techniques such as confocal or fluorescence laminar optical tomography[43]. Bioreactor accessories such as 3D-printed valves and pumps used for cell culture were also fabricated with SLA (3D Systems Viper system) using WaterShed XC 11122 resin. This study demonstrated controlled adenosine triphosphate (ATP) stimulation of live cells in an incubation chamber for observation of Ca2+ response[44]. Recently, a semiconductor-based biosensor chip was fabricated using Asiga Pico Plus 27 by DLP (**Figure 1D**) to facilitate the observation of cell metabolism on the microfluidics-based light-addressable potentiometric sensor chip[45].

2.2 3D-printed bioreactor for microbial cell culture applications

In the recent past, several studies have attempted to unravel the gaps of our understanding of bacteria survival mechanisms in complex microenvironments. AM offers an opportunity to reproduce the geometry of actual environments. The focal point of this section revolves around the use of 3D-printed bioreactors for various microbial applications such as long-term microbial culture, pathogen detection, pathogen phenotypic study, and antibacterial assays, which are summarized in **Table 2**.

2.2.1 3D-printed bioreactor for long-term microbial culture

Tracking the bacterial cell growth for a prolonged period provides crucial information on cell survival and proliferation conditions in addition to their nutrition and energetic physiology[64]. A number of bioreactors were built by 3D-printing to assist in monitoring the growth of bacteria in liquid cultures. A customized FDM-printed culture tube holder (**Figure 2A**) was interfaced with a mini-spectrophotometer connected to a light source through optical fibers to monitor bacteria growth in liquid culture through turbidimetric measurement[20]. Elsewhere, 3D-printed

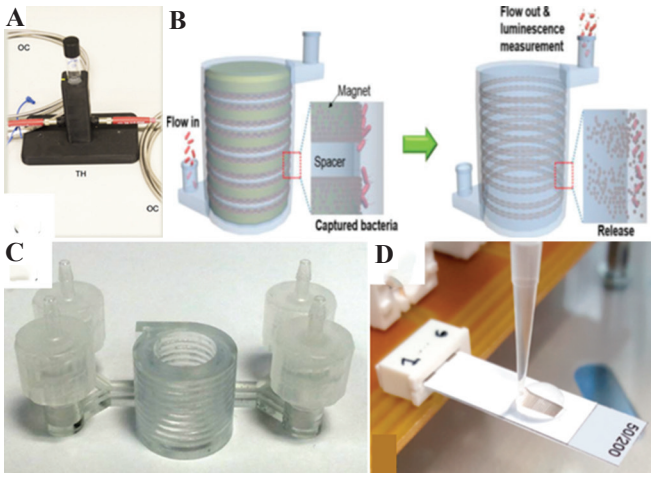

Figure 2. (A) Three-dimensional (3D)-printed culture tube holder for monitoring the bacterial growth of liquid microbial cultures (OC: Optical cable; TH: Tube holder)[49]. (B) 3D-printed magnet-spacer assembly showing bacterial separation by 3D immunomagnetic flow assay[58]. (C) 3D-printed vertically designed cylindrical chamber was developed for bioluminescent bacterial detection[7]. (D) Inkjet-printed interdigitated electrode sensor for phage detection[26].

Table 2. 3D-printed devices for various microbial applications such as long-term microbial culture, pathogen detection, pathogen phenotype study, antibacterial assays, and 3D-printing of bacteria and biofilms.

Printing technique	Printer model	Possible reason for choice of printer	Material	3D construct developed	Application	Bacteria used	Ref.
Long-term microbial culture							
FDM	Fortus 250 mc 3D-printer	Good mechanical properties	ABS plus	Culture tube holder	Accessory item for bacterial growth monitoring	*E. coli, S. aureus*	[20]
SLA	Form 2 3D-printer	High resolution, high accuracy	STL 3D-printed resins	Culture millifluidic disks	Bioreactor for biotoxicity of 3D-printing resin on bacteria	*P. putida*	[48]
SLA	Form 1+ 3D-printer	Affordable, high resolution, high accuracy	Formlabs: ClearV2 and flexible, Shapeways: Elastoplastic, extreme detail, frosted acrylic, white strong, and flexible	Culture tubes	Accessory item for microbial liquid culture	*E. coli*	[49]
Material jetting	Connex350 3D-printer	High precision	Stratasys: MED610, VeroClear, TangoBlack, Tango Plus				
Pathogen detection							
Material extrusion	Profi3Dmaker 3D-printer	Affordable, optimal printing quality	ABS	Chip	Bioreactor for colorimetric detection	*S. aureus, E. coli, S. typhimurium, L. rhamnosus, Methicillin-resistant S. aureus*	[50]
Material extrusion	Easy 3D Maker 3D-printer	Affordable, geometrical precision	Polylactide	Bead-based microfluidic chip	Bioreactor for electrochemical detection	Influenza virus	[51]
Material extrusion	UPBOX+ 3D-printer	Affordable, optimum resolution	ABS	Smartphone platform	Accessory item for detection of viable algae in fresh and marine water	*C. reinhardtii, M. aeruginosa, Amphiprora sp., and C. closterium*	[52]
Material jetting	3D Systems ProJet 3000UHD 3D-printer	Ultra-high resolution	VisiJet EX200 polymer	Finger actuated microfluidic device	Bioreactor for colorimetric detection	*E. coli*	[53]

(Contd....)

Table 2. *(Continued).*

Printing technique	Printer model	Possible reason for choice of printer	Material	3D construct developed	Application	Bacteria used	Ref.
Material jetting	Stratasys Objet24 3D-printer	High precision	Vero White Plus	Microfluidic chip	Bioreactor for colorimetric detection	*E coli*	[54]
Material jetting	Objet30 Pro 3D-printer	Accuracy, versatility	Vero™ Black material	Micro-millifluidic bioreactor	Bioreactor for microalgal culture	*D. tertiolecta*	[55]
SLA	3D Systems Viper SLA system	High resolution, high accuracy	DSM Somos WaterShed XC 11122	Cylindrical microchannel device	Bioreactor for detection by ATP bioluminescence assay	*Salmonella*	[56]
SLA	3D Systems Viper SLA system	High resolution, high accuracy	DSM Somos WaterShed XC 11122 resin	Helical microchannel device	Bioreactor for detection and separation by ATP bioluminescence assay	*E. coli*	[7]
DLP	Carima DP 110 3D-printer	Dimensional precision, good reproducibility	Acrylic resin	Trapezoidal-shaped concentration chamber	Bioreactor for detection by ATP luminescence assay	*E. coli*	[57]
DLP	Carima IM-96 3D-printer	Dimensional precision	Photocurable resin	Millifluidic device	Bioreactor for detection in blood by PCR and qPCR	*E. coli* and *S. aureus*	[8]
Ink jetting	Fuji Dimatix printer	Controllability, efficiency	Polyethylene terephthalate	Microfluidic device	Bioreactor for electrochemical detection	*Salmonella*	[58]
Ink jetting	Epson ET-2550 EcoTank® printer	Low-cost	Conductive inks based on silver nanoparticles and transparent and flexible polyethylene terephthalate sheets	Interdigitated electrode sensor	Accessory item for electrochemical detection of bacteriophages contamination	*L. lactis* subsp. *Lactis* and its phage	[25]
FDM	Prusa MovtecH model open-source 3D-printer	Rapid fabrication	ABS	T-junction device	Bioreactor for bacterial detection	*E. coli*	[59]
Pathogen phenotype analysis							
Material jetting	ProJet™ MJP 2500 Plus 3D-printer	High resolution	VisiJet M2 RCL, 3D Systems	Microfluidic chip	Bioreactor for live/dead bacterial cell differentiation with propidium monoazide pretreatment	*E. coli*	[60]
SLA	Form 2 3D-printer	Affordable, high resolution	Resin	Incubation/diffusion chamber	Bioreactor for pathogen phenotype analysis in soil matrices	*B. cereus*, *E. coli*	[61]
Wastewater treatment							
SLA	028 J Plus 3D-printer	High resolution	Urethane-acrylate based resin with 20% silica-alumina powder	Magnetic cylindrical (planar and spiral) microrobots	Bioreactor for antibacterial activity, biocompatibility	*S. aureus*	[26]

(Contd....)

Table 2. *(Continued).*

Printing technique	Printer model	Possible reason for choice of printer	Material	3D construct developed	Application	Bacteria used	Ref.
SLA	Form 1 3D-printer	Affordable, high resolution, high accuracy	Formlabs Clear FLGPCL02	40-mL anaerobic digester	Bioreactor for wastewater treatment	Microbial inoculum	[62]
SLS	FARSOON 251 3D-printer	Good mechanical properties	Nylon FS3200PA	Bio-carrier sphere	Biofilm reactor for wastewater treatment	Biofilm	[21]
Material jetting	Objet30 3D-printer	High resolution	Acrylate-based monomer resin	Gyroid media carrier	Biofilm reactor for wastewater treatment	Biofilm of nitrifying bacteria	[63]

3D: Three-dimensional, FDM: Fused deposition modeling, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *S. typhimurium*: *Salmonella typhimurium*, *P. putida*: *Pseudomonas putida*, *L. rhamnosus*: *Lactobacillus rhamnosus*, Methicillin-resistant *S. aureus*: Methicillin-resistant *Staphylococcus aureus*, *C. reinhardtii*: *Chlamydomonas reinhardtii*, *M. aeruginosa*: *Microcystis aeruginosa*, *D. tertiolecta*: *Dunaliella tertiolecta*, *C. closterium*: *Cylindrotheca closterium*, *L. lactis*: *Lactococcus lactis*, *B. cereus*: *Bacillus cereus*, ABS: Acrylonitrile butadiene styrene

bioreactors built by SLA and material jetting were used for long-term culture that mimicked the shape and dimensions of a standard commercial polystyrene tube[49]. Ten different 3D-printable resin materials were tested, of which only MED610 (ISO-certified biocompatible), VeroClear, and Frosted Acrylic exhibited no significant bacteria growth inhibition. Other materials were unsuitable because of rapid media evaporation (elastoplastic), sticky residue formation (Extreme Detail), deposition of particles inside the bioreactor after storage (White Strong and Flexible), physical instability (TangoBlack), and drastically reduced growth rates of bacteria cells (ClearV2, Flexible and Tango Plus). In a later study, an SLA-printed bioreactor in the form of a disk (Formlabs) was tested for biotoxicity effects of resins on bacteria and suggested a dose-response relationship to resin[48].

2.2.2 3D-printed bioreactor for pathogen detection

Undesired pathogen contamination in water, food, and blood poses a great public health threat. Rapid detection and separation of bacterial pathogens are therefore necessary in the field of food industry, clinical diagnostics, and environment quality control to ensure safety[65]. To monitor and quantify the presence of microbes, the design and fabrication of new 3D-printed diagnostic devices have been the focus of these areas. Considering the importance of an appropriate pathogen detection system, several studies had combined the 3D-printed bioreactors with detection methods such as calorimetry, bioluminescence, polymerase chain reaction (PCR), electrochemical, and contactless conductivity for bacteria sensing. Colorimetric detection is a rapid, easy-to-operate technique capable of simple visual detection. A material extrusion-based 3D-printed chip using ABS was made from Profi3Dmaker for bacteria culture, DNA isolation, and colorimetric detection of mecA genes, specific to the presence of methicillin-resistant *Staphylococcus aureus*. The entire chip was placed in a thermostatic box for maintaining a homogenous magnetic field and facilitating non-crosslinking aggregation of nanoparticle

probes with bacterial DNA for *in vitro* diagnostic applications[50]. Another study used a manually actuated miniature 3D-printed device fabricated using VisiJet EX200 polymer by material jetting for rapid on-site multiplexed bacterial detection using calorimetric measurement[53]. The finger-actuated pumping membrane seated on the pumping chamber was connected to individual enrichment/detection chambers through serpentine channels for bacteria detection in drinking water. Upon depressing and releasing the membrane, a vacuum pressure filled in each chamber and sucked in the sample. The lowest detection limit of $1e^6$ colony forming units (CFU)/mL was observed in approximately 6 hours. Furthermore, these pathogen detection devices were also connected to accessories for colorimetric readout, which would improve the limit of detection[54].

Some groups have resorted to combined ATP bioluminescence and magnetic particle-based immunomagnetic separation for bacteria sensing. This is a more rapid and efficient approach for increasing the sensitivity and specificity of pathogen identification. A 3D-printed bioreactor with cylindrical hollow microchannel and high-capacity efficient magnetic O-shaped separator (HEMOS) was designed for Salmonella detection in large-volume samples (**Figure 2B**). The magnet-spacer feature in the central area of HEMOS maximized the magnetic field, thereby allowing ultra-rapid capture of 10 CFU/mL of nanocluster-immobilized bacteria within 3 min[56]. Similarly, a 3D-printed bioreactor with helical chambers (**Figure 2C**) was developed for bioluminescent *Escherichia coli (E. coli)* detection in milk[7]. The device enabled sheath inlet flow for improved size-dependent separation of bacteria-nanocluster complexes in the helical microchannel. A number of studies employed 3D-printed millifluidic platforms to process samples larger than 1 mL. At sub-millimeter scale, recyclable, 3D-printed trapezoidal preconcentration chamber built by DLP (acrylic resin) was used to isolate *E. coli* in blood samples[57]. Another 3D-printed millifluidic device preconcentrated bacterial DNA by sequential isolation using magnetic silica beads was also developed for improved pathogen detection in blood. This method extracted bacterial DNA in 10 mL of buffer and 10% blood within 30 min and detected as low as 1 CFU bacterial using either PCR or quantitative PCR[8].

Electrochemical detection has also been accepted as a powerful tool for bacterial and viral detection in 3D-printed biomarkers by identifying disease-related biomarkers and environmental hazards. A pump-free bioreactor used for electrochemical detection of Salmonella consisted of two flexible polyethylene terephthalate layers with sintered inkjet-printed electrodes directly bonded to the channel-containing layer, forming a sealed microfluidic device[58]. This high throughput device accommodated immunomagnetic bacterial separation. Similarly, a material-extruded bead-based microfluidic chip with a three-electrode setup was used for the detection of influenza hemagglutinin[51]. Elsewhere, a prototype system with real-time impedance measurements was used to detect phage infection of cultured *Lactococcus lactis*[25]. The two standard microbiological testing methods used for comparison were based on plaque assay and turbidity measurements. Only the inkjet-based biosensor system showed a greater sensitivity to phage infection with a response within the first 3 h of phage inoculation. Another study described a T-junction microfluidic device with integrated sensing electrodes developed by FDM (using ABS) for label-free counting of *E. coli* cells incorporated in spherical oil droplets. Cells were counted using a single-step contactless conductivity system and quantified by plate counting method. This approach offered noticeable advantages as a single-step method with minimal incubation time before detection[59]. Studies have also explored the use of 3D-printed bioreactors for the culture of microbes other than bacteria, such as algae. A material jetted milli-microfluidic device (Vero™ Black material) with growth chambers, microchannels, and semi-integrated optical detection system was used for algal culture[55]. Even though the growth was unsuccessful due to poor microalgal retention resulting from photopolymer incompatibility with cells, other metrics observed during the culture offered a mechanical perspective that indicated the 3D-printed architecture posed promising advantages in comparison to other

complex microfabrication processes. Another group developed a 3D-printed smartphone platform integrated with an optoelectrowetting-operated microfluidic device for on-site detection of viable algae cells[52]. The collected data were wirelessly transmitted to a central host for real-time monitoring of water quality with reduced analysis time. Given its sensitivity, this chip allowed sample preparation methods such as droplet immobilization and mixing, target cell counting, and fluorescent detection.

2.2.3 3D-printed bioreactor for pathogen phenotypic analysis

Profiling pathogen phenotypes is important in decoding the virulence and interaction of pathogen with its surroundings. A propidium monoazide (PMA) pretreatment was carried out in a 3D-printed bioreactor to efficiently discriminate live waterborne bacterial pathogens in natural pond water samples[60]. The material jetted bioreactor was designed with an inlet, splitter, and mixers for proper sample-PMA mixing followed by incubation in serpentine channels containing herringbone structures for alternating dark and light incubation. The results obtained from this 3D-printed bioreactor suggested the need for species-specific optimization of pretreatment performance. Elsewhere, an SLA-printed incubation/diffusion chamber was designed for culturing bacteria from soil samples to study their interaction dynamics. The chamber facilitated diffusion of soil components with target cells and also allowed single-cell and ensemble bacterial phenotypic analyses[61].

2.2.4 3D-printed bioreactor for wastewater treatment

Several 3D-printed bioreactors have demonstrated great potential in water treatment applications that were difficult to be achieved by conventional wastewater treatment systems. Cylindrical microrobots printed by SLA conveyed excellent water purification capability and great biocompatibility with mammalian cells[26]. Other intricate 3D-printed bioreactor designs, including fullerene-shaped bio-carriers[21] and gyroid-shaped carrier[63], have been shown to stimulate microbial assemblages for improved organic matter removal and better performance of biofilm reactors. Other studies employed SLA-printed miniature anaerobic digester reactors as a process screening tool for sustainable treatment of wastewater and biowaste[62].

3 Conclusions and future directions

In recent years, significant advances have been made in 3D-printed bioreactor technologies. Bioreactors have been tailored to easy online monitoring and automated bioprocesses, thereby closing the gap between conventional bioreactors and their miniature 3D-printed counterparts. However, in addition to their basic functions, other design aspects, such as flexible operation and process optimization, should be taken into account, especially for devices used to study complex physiological phenomena. It is noteworthy to mention that there has been limited clinical translation of 3D-printed bioreactors. This could be attributed to the lack of optimized protocols that are fine-tuned to respective 3D-printing methods and materials. The reproducibility of certain 3D-printing processes is suboptimal.

At present, 3D-printing research for *in vitro* biological applications focuses mostly on relatively simple systems that only incorporate a limited number of cells and cell types. Future studies should aim to attend to relatively complex tissues and organs. Moreover, several concerns such as 3D-printing compatible design, removal of support structures, the choice of appropriate cell lines, better cocultivation concepts, establishment of optimal conditions, and protocol standardization remain to be resolved and should be the focus of future research. With advances in various aspects of 3D-printing, one would be able to design and manufacture customized bioreactors with tailored functionalities using 3D-printing in laboratory settings, which would significantly drive future biomedical research by offering on-demand *in vitro* testing.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

The authors are thankful for the support by HP-NTU Digital Manufacturing Corporate Lab, Nanyang Technological University, Singapore. The content is solely the responsibility of the authors. This research was conducted in collaboration with HP Inc. and supported/partially supported by the Singapore Government through the Industry Alignment Fund-Industry Collaboration Projects Grant.

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