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ABSTRACT

The use of bioprinting as a powerful tool for tissue and organ fabrication has been a promising development in the field of biomedicine, offering unprecedented versatility in the fabrication of biologically and physiologically relevant constructs. Even though there are a plethora of commercial bioprinters available in the market, most of them are overly expensive. Thus, university facilities and independent research groups often find it difficult, if not impossible, to equip themselves with such machinery. In this Review, we analyze affordable alternatives to commercial bioprinters, which are presented by the Do-it-Yourself (DiY) community. First, we discuss the current state of these low-cost technologies, and the advances made to bridge the divergence between marketed bioprinters and DiY devices. Afterwards, the different bioprinting technologies that are most commonplace for these low-cost devices are examined. Additionally, an overview of the pioneering DiY bioprinters takes place, as well as the open-source software alternatives to control these bioprinters. Next, we analyze the different factors to take into consideration during the bioprinting workflow, such as bioinks, computer-aided models, and bioprinting parameters. Finally, we conclude with a brief assessment of current limitations and potential solutions, as well as future developments in the arena of bioprinting.

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I. INTRODUCTION

Tissue engineering and regenerative medicine (TERM) has rapidly become one of the most important and promising areas in biomedicine, currently pushing advancements to tackle the global need for organ transplants with the help of numerous technologies including three-dimensional (3D) bioprinting.[1] The goal of this specific approach consists of using additive manufacturing (AM)–based technology to fabricate artificial organs and tissues. This AM-enabled biotechnology utilizes biocompatible materials along with living cells, together often times termed as bioinks, to spatially construct functional structures, which sometimes can also be scaffold-free.[2,3]

On average, 20 people die every day in the United States of America (USA) because of the lack of available organs for transplant, which is even higher globally; this means that the number of donors is several times smaller than the number of patients in need of a transplant.[4] Additionally, rejection of the artificial tissue by the host often complicates the process. Therefore, the ability to reproduce healthy tissues and organs is of paramount importance in the field of bioprinting, relying on different bioprinting mechanisms and the precise deposition of bioinks typically in a layer-by-layer manner to fabricate these constructs.[5–7]

One of the most important characteristics of new trends in bioprinting includes the use of patient-specific information to generate anatomically accurate structures thanks to the ability to co-culture various cell types, paving the way for precise patterning.[8] An additional emerging application of bioprinting is *in vitro* tissue modeling, which provides an insight into cells' and tissues' behaviors as well as their responses to external perturbations, and is further aided by organ-on-a-chip systems. As succinctly described by Burdick and colleagues, as the technology advances, it will shift the paradigm on our understanding of patient-specific complexities, through the integration of genomics, bioinformatics, and big data.[9]

Computer-aided tissue engineering (CATE) allows for the design and therefore the bioprinting of well-defined tissue constructs that can be built at high resolution with personalized features. Thus, the probability of successful regeneration of tissues and organs is greatly increased.[5,6] As recently established, bioprinting further allows the intraoperative fabrication of structures, where different modeling approaches, such as image-based and organ-segmentation, are employed.[10] These techniques, in conjunction with the aid of artificial intelligence (AI) and machine-learning, will be transcendental for AM-based biotechnology.[1]

The advent of open-source, low-cost bioprinters has provided an alternative to commercial and expensive systems. This review provides a general overview of the current state of low-cost bioprinters, describing the several types of bioprinting techniques used and their advantages and disadvantages; an emphasis is made on extrusion-based, vat polymerization-based, and droplet-based. Next, an in-depth outlook and analysis of the open-source bioprinting software alternatives are explored; this includes firmware for bioprinters, slicer programs, and host software to manage the bioprinting procedures. Additionally, an overview of the steps involved in the bioprinting workflow will be discussed, which include: a brief introduction of bioinks, design considerations for CATE, and the impact of bioprinting parameters. Furthermore, the limitations the Do-it-Yourself (DiY) bioprinting community faces, as well as more technical problems, will be addressed along with possible solutions for each. Finally, we identify new and emerging advances in the bioprinting field, which will likely play a prominent role in the continued growth of this sector.

A. Current-state market of bioprinting

The advent of commercial bioprinting has rapidly become one of the most important areas of the tissue engineering field, prompting new bioprinting technologies and approaches to construct functional structures.[11] This contributes to the early adoption and democratization of bioprinting as a whole, opening exciting new opportunities for tissue reconstruction and *in vitro* drug testing studies.[2]

A market research report conducted by BCC Research claimed that the global bioprinting market is expected to reach $1.4 billion by 2024 from $306.2 million in 2019 at a compound annual growth rate (CAGR) of 35.4% for the period 2019 to 2024.[12] Bioinks, as a segment of the global bioprinting market, is prognosticated to grow from $130.6 million in 2019 to $588.4 million in 2024 at a CAGR of 35.1% for the same period.[12] The three-dimensional (3D) cell culture product market is anticipated to grow from $35.5 million in 2019 to $185.8 million in 2024 at a CAGR of 39.2% for the aforementioned period.[12] Another report projected the 3D bioprinting market to reach $1.647 billion by 2024 from $651 million in 2019, at a CAGR of 20.4% from 2019 to 2024.[13] This growth derives from the expected advancement in technology within the healthcare industry, as well as rising R&D investments.[13] Furthermore, the increase in a geriatric population base, which in turn will be susceptible to neurological and cardiac diseases, as well as cancer and muscular injuries, is a major factor contributing to the expansion of the bioprinting industry.[14]

According to another recent report conducted by Report and Data,[13] the North American 3D bioprinting market is forecasted to grow significantly from 2019 to 2027. This field is emerging as a promising technology that can revolutionize the treatment of several medical conditions as a result of the continuous innovations by current industry leaders, paving the way for better patient care, customized medical treatment, and a solution to the shortage of organ transplants eventually.[13]

The bioprinting field has gained considerable support from healthcare professionals, due to the growth of stem cell research and financial support from a considerable number of public and private organizations. In addition to this, the scientific community has accepted 3D bioprinting as an exciting new tool that offers a new method for testing drugs faster and more accurately at a lower cost, and with higher biological relevance to humans than animal testing in many cases. Moreover, the demand from academic institutes commands a major share of the market, due to its increasing adoption of the technology, resulting in a key factor driving the market demand.[15]

Extrusion-based bioprinting accounts for the majority of the market share and is expected to maintain its position over the forecast period (2024),[16] thanks to the diverse application areas and rise in the assimilation of the technique.[12,16]

Regarding the application, the market has been divided into segments such as medical, food and animal products, and consumer/personal product testing as seen in Fig. 1. The medical segment includes tissue and organ generation, prosthetics, and pills, among others, and is expected to have a market share of over 38.0% by 2026. The medical pill area is considered to be, according to the aforementioned Reports and Data document, the latest development in the market and is envisioned to be the one with the highest growth over the forecast period.[13]

Some of the leading companies in the global 3D bioprinting market include Allevi Inc. (USA; now a part of 3D Systems), CELLINK (Sweden), EnvisionTEC GmbH (Germany), and Organovo Holdings, Inc. (USA), amongst others, all of which are further examined in detail in Table I.[13,15]

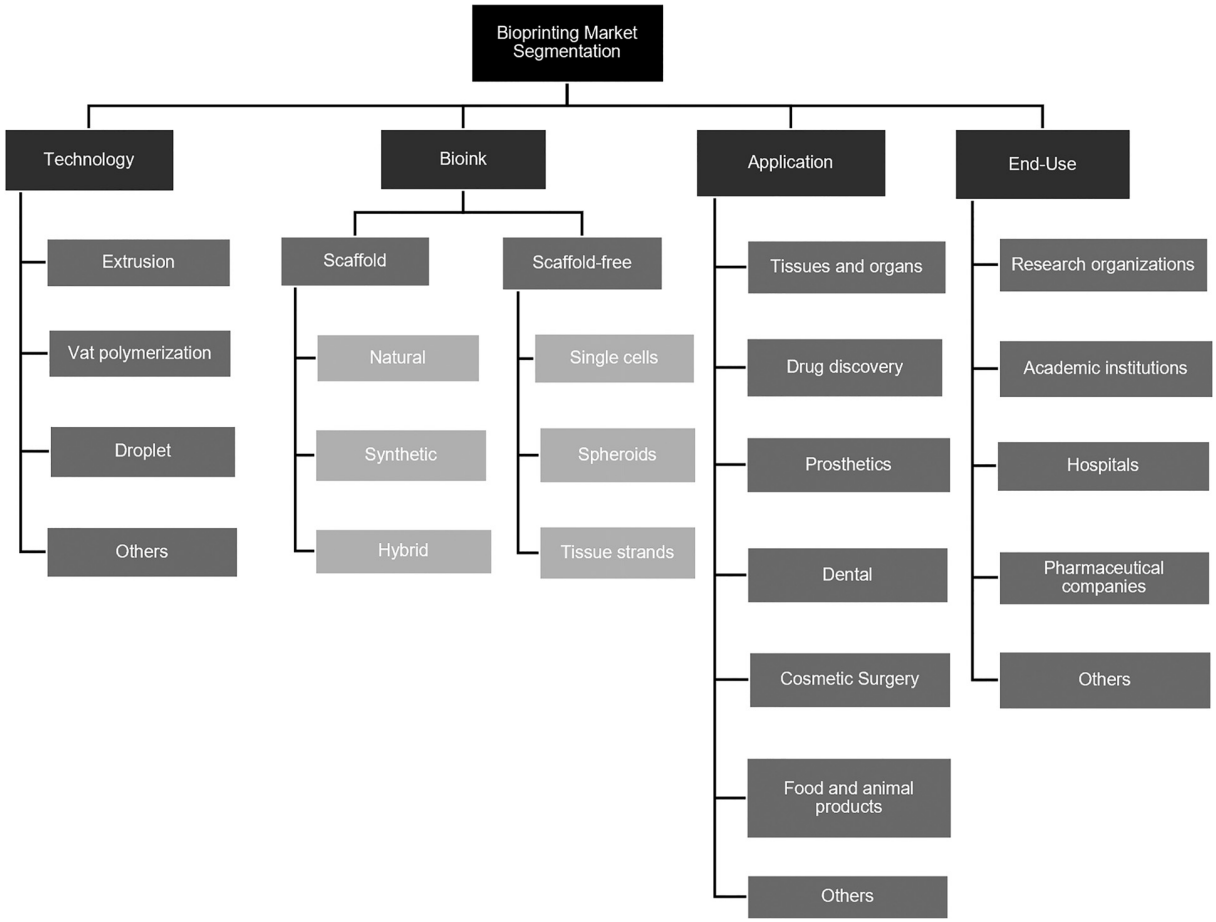

FIG. 1. Current segmentation of the bioprinting market, divided into 4 main groups: technique, bioink, application, and end-use. The subdivisions of each group are also included.

B. DiY bioprinting

In the early 2000s, the rise of rapid prototyping led to advances in many fields that include open-source microcontroller engineering as well as low-cost hardware and software.[17,18] This fact resulted in developing strategies to produce devices for self-use by the utilization of easily available products. Moreover, in recent years, a new concept has emerged based on the "values for co-creation." This concept explains that consumers are making products, goods, or services for their personal use with the help of the DiY community.[17] The maker development concept, a core value of the DiY community, makes it conceivable to engineer easy-to-use devices rapidly and effectively by using the co-creation concept.[19,20] The DiY community consists of people who are designers, builders, programmers, and users. The main objective of the community is to manufacture simple and affordable devices, with similar outputs as commercial products, while providing options for ample customization.[21]

Until around 2005, all 3D printers were made in industrial production, not affordable, and proprietary. This encouraged Adrian Bowyer, from the University of Bath in England, to develop the first low-cost DiY 3D printer named RepRap (REPlicating RAPid prototyper).[22,23] Thus, the first initiative to create affordable 3D printers was formed, ushering in a new era of open-source printers. Soon after that, a second DiY 3D printer was created from Fab@Home, at Cornell University, in 2006.[24] The launch of these two DiY 3D printers have enabled other researchers to develop more, as well as better, open-source 3D printers, and consequently, also bioprinters.[25] In 2012, Alexander B. Owczarczak *et al.* from Clemson University, developed the first DiY bioprinter, the Standard Inkjet Printer.[26] Later, other DiY bioprinters emerged such as the Biocurious Bioprinter, the high-resolution stereolithography apparatus (SLA)–based bioprinter, the Biolinker, and a DiY biofilm bioprinter, among others.[26–31] Thus, continuous work in this field has helped to further advance the technological progress, leading to more economical bioprinters ranging from $50 to $4000 (refer to Table III). After the community started to develop more economic and higher-quality bioprinters,[32–34] the utilization of terms like DiY or Do-it-With-Others, when alluding to a gathering of individuals, is now more commonplace.

The DiY community permits bioprinting designers to examine, work together, share, and convene from anywhere on the planet.[18,35,36] Specialists and researchers collaborate to develop new technologies and materials that are utilized in bioprinting for clinical applications, create new concepts, research, and fabricate bioconstructs.[37,38] This is possible due to the development of the DiY bioprinters and different bioinks, ranging from hydrogels, microcarriers, tissue strands, and cell pellets.[39–42] The advances in the DiY bioprinter community have made the printers capable of bioprinting organ-on-a-chip models such as liver-on-a-chip, vasculature-on-a-chip, heart-on-a-chip, and lung-on-a-chip, among others.[43–47] In recent days, organ-on-a-chip platforms are being increasing utilized for drug testing to combat various diseases and viral threats.[48] Fraunhofer Institute for Laser Technology used the ArtiVasc bioprinter to develop an artificial blood vessel by using two bioprinting technologies, i.e., vat polymerization-based and droplet-based in the period of 4 years from 2011 to 2015.[49] Later, some organizations, such as Wyss Institute and our own laboratory (Zhang Lab), are producing DiY bioprinters for the fabrication of organ-on-a-chip–-based or other types of tissue models.[50,51]

II. PRINTING TECHNIQUES

The importance of bioprinting resides in the fabrication of tissue-like structures that mimic the actual environment of human tissues and organs, to a good extent. For example, an organ-on-a-chip device can be used to reproduce a similar microenvironment of the target human tissue or organ outside the body, i.e., *in vitro*.[2] This becomes critical, as previously stated, in drug testing and preclinical trials, making bioprinting a key element in tissue model engineering when combined with on-chip devices. Techniques in 3D bioprinting include extrusion-based, vat polymerization–based, and droplet-based, among others.[52]

Each method has its characteristics, benefits, and drawbacks in terms of quality, printing speed, and cost, that fit different applications. For this review, the most cost-effective technologies for bioprinting, and therefore, the most common ones used in the DiY community, are analyzed: extrusion-based, vat polymerization–based, and droplet-based. A hierarchical diagram summarizing these technologies and their main subclasses is shown in Fig. 2.

As a short introduction for the reader, in an extrusion-based bioprinter, the material is dispensed through a nozzle using a pneumatic or mechanical actuator.[1] Meanwhile, a vat polymerization-based bioprinter utilizes bioinks that are light-sensitive and are cross-linked (solidified) when photoactivated.[5] On the other hand, in a droplet-based bioprinter, droplets are selectively deposited allowing high-resolution bioprinting.[8]

For a succinct summary of the characteristics of each method, Table II outlines the main features and limitations of each of the technologies. In Secs. II A–II C, these technologies will be explained in further detail.

A. Extrusion bioprinting

Extrusion-based bioprinting (EBB) is the most commonplace bioprinting technique due to its affordability, versatility, and ease of implementation.[53] Therefore, several DiY bioprinters employ this technique since it offers an economic and relatively simplistic solution for bioprinting.[53,54] As a brief overview of EBB's functionality, a pneumatic or mechanical actuator extrudes the bioink from the nozzle, as a continuous "filament" and onto a collecting bed.[53] Thus, a layer-by-layer approach is implemented, in which the path-planning of the printhead is critical.[9,53,55,56]

This technique encapsulates various advantages, one of which includes a fast deposition rate, which leads to a reduction in the bioprinting time; nevertheless, the print speed is still slow when compared to other technologies. Another benefit of EBB is the facile reproducibility of this system, a quality of particular interest to the DiY community. This allows researchers to discover a wide range of compatible bioinks with EBB-like microcarriers,[3] cell-laden hydrogels,[6,57–59] decellularized extracellular matrix (dECM) components,[60] cell aggregates,[61–63] as well as additional high-density cell constructs,[53] among others.

Nonetheless, some disadvantages are distinctive with this technique as well. Typically, the minimum achievable resolution of approximately 100 μm is comparatively lower than other technologies.[53,54] For advanced applications, this limitation would lead to an

TABLE I. Commercial bioprinters.[a]

Company	Model	Claimed resolution	Printing technique	Key features
3D Bioprinting Solutions (Russia)	Fabion 2[162]	XY: 30 μm Z: 3 μm	Extrusion-based (pneumatic)	— Triple extruders — Heating control for print bed (70 °C) — Cooling system for one of the extruders (4 °C)
3D Biotechnology Solutions (3DBS) (Brazil)	Educational Starter[163]	XY: 10 μm Z: 5 μm	Extrusion-based (mechanical)	— Heating control for print bed (60 °C) and extruder (80 °C) — Pre-curing UV system Cooling system can be installed on one of the extruders (2 °C) separately
	Genesis II[163]	XY: 10 μm Z: 5 μm	Extrusion-based (mechanical)	— Dual extruders — Heating control for print bed (60 °C) and extruders (80 °C) — Pre-curing UV system — Cooling system can be installed on one of the extruders (2 °C) separately
3DPL (Iran)	Bioprinter N1[164]	XYZ: 10 μm	Extrusion-based (pneumatic)	— Single extruder — Heating control for print bed (65 °C) and extruder (140 °C) — Chamber sterilization to reduce cell damage
	Bioprinter N2[164]	XYZ: 5 μm	Extrusion-based (pneumatic)	— Dual extruders — Heating control for print bed (65 °C) and extruders (175 °C) — Chamber sterilization to reduce cell damage — Optional cooling control for extruders (4 °C) — Photocuring capacity (using UV and blue light) while printing
	Bioprinter N2 plus[164]	XYZ: 5 μm	Extrusion-based (pneumatic)	— Dual extruder — Heating control for print bed (65 °C) and extruders (175 °C) — Chamber sterilization to reduce cell damage — Optional cooling control for extruders (4 °C) — Photocuring capacity (using UV and blue light) while printing Incorporation of live-cell imaging
Advanced Solutions (USA)	BioAssembly Bot 400[165]	XY: 20 μm	Extrusion-based (pneumatic or mechanical)	— Six-axis robotic arm — Multi-material bioprinting — Interchangeable printheads (dual extruder, UV module, and coaxial nozzle) — Integration of computer vision and AI — Temperature control for print bed (10° C to 60 °C) and extruder (−4 °C to 150 °C)
	BioAssembly Bot 200[165]		Extrusion-based (pneumatic)	— Four-axis robotic arm — Multi-material bioprinting (up to five) — Interchangeable printheads (dual extruder, UV module, coaxial nozzle) — Temperature control for print bed (10 °C to 60 °C) and printhead (−4 °C to 150 °C)
	BioBot Basic[165]	ZR (linear): 10 μm Rotary: 10 μm	Extrusion-based (pneumatic)	— Multi-material bioprinting (up to five) — Interchangeable printheads (dual extruder, UV

TABLE I. (*Continued.*)

Company	Model	Claimed resolution	Printing technique	Key features
				module, and coaxial nozzle) — Automated calibration of the system — Designed for automated direct printing in multi-well plates — Utilizes 405 nm blue light for efficient photo-polymerization; minimizes UV damage to cells — Temperature control for optimal bioinks printability
Allegro 3D (USA)	Stemaker Model D[166]	XYZ: <1 μm	Vat polymerization–-based (DLP)	
Allevi (USA)	Allevi 1[167]	XY: 7.5 μm Z: 1 μm	Extrusion-based (pneumatic)	— Adjustable photocuring mechanism — Temperature control for extruder (4 °C to 160 °C) — Automated calibration of the system
	Allevi 2[168]	XY: 5 μm Z: 1 μm	Extrusion-based (pneumatic)	— Dual extruders Adjustable photocuring mechanism — Heating control for extruders (70 °C and 160 °C)
	Allevi 3[169]	XY: 1 μm Z: 1 μm	Extrusion-based (pneumatic)	— Triple extruders — Adjustable photocuring mechanism — Temperature control for the extruders (4 °C to 160 °C) — Heating control for print bed (60 °C)
BioEdTech (Brazil)	BioEdPrinterV2[170]	XYZ: 25 μm	Extrusion-based (mechanical)	— Optional modules for temperature control of print bed and extruders — Dual extruders — Incorporated camera for analyzing prints — Photocuring capacity (using UV)
	BioEdPrinterV4[170]	XYZ: 25 μm Piezoelectric: 1 μm	Extrusion-based (mechanical) and droplet-based (drop-on-demand piezoelectric)	— Optional modules for temperature control of print bed (4 °C to 45 °C) — Optional module for multiple extruders (up to four) — Photocuring capacity (using UV) — Incorporated camera for analyzing prints
Bioprinting Solutions (Finland)	Brinter[171]	XYZ: <10 μm	Extrusion-based (pneumatic)	— Interchangeable printheads — Automated calibration of the system — Camera for online process view
Black Drop Biodrucker (Germany)	Superfill[172]	XYZ: 20 μm	Droplet-based (drop-on-demand)	— Multiple printheads (up to four) — Three-axis tabletop robotic arm — Heating control for the bioinks — Ultra-precise droplet-formation (nm size)
Cellink (USA)	Bio X[173]	XYZ: 1 μm	Extrusion-based (pneumatic) and droplet-based (electromagnetic)	— Triple extruders with interchangeable printheads — Integrated air compressor with dual filters — Adjustable photocuring mechanism — Temperature control for the printheads (4 °C to 65 °C)
	Bio X6[174]	XYZ: 1 μm	Extrusion-based (pneumatic) and droplet-based	— Sextuple extruders with interchangeable printheads — Integrated air compressor with dual filters

TABLE I. (*Continued.*)

Company	Model	Claimed resolution	Printing technique	Key features
			(electromagnetic)	— Adjustable photocuring mechanism — Temperature control for the printhead (4 °C to 65 °C)
	Inkredible[175]	XY: 10 μm Z: 2.5 nm	Extrusion-based (pneumatic)	— Dual extruders — Adjustable photocuring mechanism — Automated calibration of the system
	Lumen X+[176]	XY: 50 μm Z: 5 μm	Vat polymerization–based (DLP)	— Print with both proprietary and user-made bioinks — Utilizes a visible-light projector as the light source — Interchangeable build platforms
	Holograph X[177]	XY: 1 μm Z: 3 nm	Vat polymerization–based (holographic)	— Adjustable laser power — Fully automated system — Printing up to 250 000 voxels per second for reduced printing time (up to 90%)
Clecell (South Korea)	U-FAB Basic Desktop Bioprinter[178]	XYZ: <10 μm	Extrusion-based (pneumatic)	— Germicidal UV lamp with built-in UV LED (365 nm) — Independently controlled, liquid nebulizer for crosslinking process — Temperature control print bed (4 °C to 50 °C) — Monitoring cameras
CyFuse Biomedical K.K. (Japan)	Regenova[179] Spike[179]	Spheroid diameter: 400–600 μm XY: 10 mm Z: 8 mm Needle pitch: 400 μm	Kenzan method–based (cell mounter) Reverse Kenzan method–based (cell layering)	— Printed spheres are inserted into needlelike prongs — Places spheroids in place and then inserts a needle into the spheroids — Removes needles after fusion
Fluicell (Sweden)	Biopixlar 3D Single-cell Bioprinter[180]	XYZ: 2 μm	Extrusion-based (microfluidic)	— Ultra-high precision (up to a resolution of a single-cell) with high cell viability (>95%) — Incorporated real-time monitoring mechanism — Printing multi-cellular models (up to three different cell types using the same printhead)
FoldInk Bioprinting (Armenia)	FoldInk 3D Bioprinter[181]	XYZ: 100 μm	Extrusion-based (mechanical)	— Uses open-source software — Specially designed patented extruder (X-Trudr) and LCD screen for control
EnvisionTEC (Germany)	3D-Bioplotter Starter Series[182]	XYZ: 1 μm	Extrusion-based (pneumatic)	— Dual extruders — Heating control for extruders (30° C to 250 °C) — Option to buy different printheads with different functionalities
	3D-Bioplotter Developer Series[183]	XYZ: 1 μm	Extrusion-based (pneumatic)	— Triple extruders — Heating control for extruders (30 °C to 250 °C) — Option to buy different printheads with different functionalities — Optional temperature control for print bed