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A Review of the Current State of Bioprinting Technology

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ABSTRACT

The field of bioprinting has witnessed remarkable advancements in recent years, revolutionizing the fabrication of biological tissues and organs for research, pharmaceutical testing, and potential clinical applications. This progress is attributed to the development of diverse bioprinting technologies, ranging from low-cost models utilizing microextrusion-based technology to high-end systems employing droplet-based and laser-assisted techniques. These technologies offer varying levels of precision, build volume, and material compatibility, catering to a wide spectrum of research needs. Notably, microfluidic-based bioprinting has emerged as a transformative approach, enabling fast, continuous switching and mixing of materials, achieving nearly single-cell printing resolution. Moreover, support-free multiaxial printing and high-resolution printing using focused light have shown promise in enhancing geometric complexity and cell viability. The integration of modular print heads and the potential for in situ bioprinting are poised to further expand the capabilities of bioprinting technologies. Despite these advancements, current bioprinting systems exhibit certain limitations, including constraints in motion axes, printing volume, and material compatibility. Addressing these challenges will be crucial in realizing the full potential of bioprinting for tissue engineering and regenerative medicine applications. This review provides valuable insights into the diverse range of bioprinting methods, systems and their collective potential to advance tissue engineering and regenerative medicine.

Keywords: 3D Bioprinter • Bioprinting Methods • Bioprinter Modules • Bioink

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INTRODUCTION

Bioprinting is a groundbreaking fusion of technology, biology, and medicine. This method crafts biological tissues with the precision of an artist creating a masterpiece [1]. Central to bioprinting is specialized equipment enabling controlled material deposition, especially bioinks [2]. These bioinks, composed of cells, growth factors, and biomaterials, form living structures mirroring natural tissues [3]. Unlike traditional methods, which use plastic or metal, bioprinting harnesses the regenerative potential of biological components, holding great promise for regenerative medicine and personalized body part fabrication [4]. It also plays a vital role in understanding disease progression, providing controlled lab environments for research on disease mechanisms, drug testing, and personalized medicine approaches [5]. Bioprinting is a paradigm shift in medicine and tissue engineering, offering innovative solutions for tissue repair and replacement. Its transformative potential is evident in the seamless integration of cutting-edge technology and intricate biology, promising a future of precision-manufactured, functional, living tissues [6]. A key challenge involves creating bioinks that support living cells, ensuring both structural integrity and post-fabrication cell viability and growth [7]. Materials like hydrogels, collagen, and alginate are explored for

their unique properties and applications [4]. Precise control of factors like temperature, pH, and oxygen levels is vital to safeguard cell health during printing [8]. Differentiation of printed cells into desired tissue types without aberrant growth is crucial [1]. Establishing functional vascular networks within printed tissues is another pivotal aspect, essential for tissue survival and function [9]. Researchers are pushing bioprinting boundaries to create intricate organs like the heart, liver, and lungs, addressing challenges like diverse cell integration and functional vasculature systems [3].

REVIEW OF LITERATURE

History of Bioprinting

Bioprinting, a fusion of 3D printing and biology, began in the late 20th century [10]. A milestone came in the early 2000s when Dr. Thomas Boland and the team at Clemson University pioneered the first bioprinting technology, incorporating cells into hydrogels [11]. Initially, it focused on rudimentary structures like cell layers and small tissues [12]. In the late 2000s and early 2010s, there was a surge in research activity, exploring various printing methods for biomaterials like hydrogels, ceramics, and metals [6]. Precision and resolution improved, allowing printing of intricate structures with 3D imaging and CAD software, like blood vessels and heart tissues [3]. The evolution continued, expanding to different cell types, particularly stem cells, crucial for generating functional tissues and organs [1]. New bioink classes, mainly hydrogels and alginates, enhanced versatility [7]. Extrusion-based bioprinting techniques further refined precision and complexity [4]. Beyond its origins, bioprinting found applications in pharmaceuticals and cosmetics, contributing to drug discovery, development, and customized skincare [13]. Recently, there's been a surge in using bioprinting to create functional tissues and organs, addressing the organ shortage crisis [3]. Integration of machine learning and AI is being explored to enhance efficiency and precision [14,15]. Bioprinting, at the intersection of biology and engineering, has the potential to revolutionize various industries and healthcare by fabricating personalized, functional replacement tissues and organs, marking a remarkable journey from inception to continuous evolution.

Current Bioprinting Methods

Inkjet bioprinting

Inkjet bioprinting is an innovative method that has emerged as a cornerstone in the field of tissue engineering and regenerative medicine [16]. This technique leverages the principles of traditional inkjet printing, allowing for the precise deposition of bioinks containing living cells and biomaterials in a layer-by-layer fashion, ultimately enabling the creation of complex 3D tissue structures [17-18]. There are several modalities employed in inkjet bioprinting. The thermal method relies on localized heating to generate pressure pulses, forcing droplets out of the nozzle. Despite brief exposure to high temperatures (ranging from 200 to 300 °C), studies have shown minimal impact on biological molecules or cell viability [19-20]. On the other hand, piezoelectric-based printers utilize a piezoelectric crystal to induce rapid shape changes, generating an acoustic wave that breaks the liquid into droplets. While capable of precise droplet formation, concerns regarding potential cellular damage arise due to the employed high frequencies [21-22]. One of the strengths of inkjet bioprinting lies in its ability to precisely control droplet size and density [23-24]. Electronic adjustments can vary the drop size from minuscule picoliters to larger volumes, allowing for the creation of intricate concentration gradients of cells, materials, or growth factors within the 3D structure. This technology can yield high-resolution structures, typically ranging from 20 to 100 micrometers, instrumental in fabricating complex tissues with fine details [25]. Inkjet bioprinting has found significant application in functional skin and cartilage regeneration. By depositing primary cells and/or stem cells with precise density and subsequently crosslinking the cell-containing material, inkjet bioprinting enables the rapid production of viable and functional tissue constructs. This approach holds great promise for treating skin defects and joint injuries [26-27]. Moreover, inkjet bioprinting is revolutionizing drug testing and disease modeling. By creating biomimetic tissue models with patient-specific cells, researchers can study drug responses and disease progression in a controlled, physiologically relevant environment. This advancement has the potential to significantly reduce the time and cost associated with drug development [28-29]. Another transformative aspect of inkjet bioprinting lies in the ability to create patient-specific implants and prosthetics. By utilizing patient-derived cells and bioinks, it is possible to fabricate implants tailored to individual anatomies. This holds particular relevance in areas like orthopedics and craniofacial surgery [30-31].

Microextrusion bioprinting

Microextrusion bioprinting encompasses various sub-methods, including piston-driven, pneumatic, and screw-driven systems, all contributing to the fabrication

of intricate biomaterial structures [32]. This technique involves the controlled dispensing of biomaterials through nozzles or needles, widely 210 μm and 400 μm, connected to cartridges filled with specialized ink [33]. The adaptability of microextrusion bioprinting allows for the integration of multiple cartridges in a single printer, enabling the creation of heterogeneous tissue constructs. Prior to commencing the bioprinting process, it is imperative to ascertain crucial parameters such as printing speed, dispensing pressure, and movement distance. These determinants are significantly influenced by the specific properties of both the selected cell line and bioink. Printability, a vital aspect, is evaluated based on the ease with which the bioink can be printed with good resolution, while still maintaining its structural integrity post-printing. This assessment encompasses considerations of shape fidelity, resolution, biocompatibility, and cell supportive capabilities [34-36]. Despite notable progress, achieving optimal printability and cell function in bioinks continues to be an active area of research. Balancing bioink viscosity for cell support and printability is of paramount importance [37]. In some cases, this may entail subjecting cells to a certain degree of stress during printing or accepting a compromise in printability. Researchers have explored various strategies, including adjustments to printing parameters and alterations in printing techniques such as FRESH bioprinting, to enhance the printability of bioinks with optimal cell support [38]. Both Newtonian and non-Newtonian bioinks have been employed, with meticulous optimization of their printable viscosity. For shear-thinning bioinks, viscosity decreases with increasing strain rate, ultimately enhancing cell protection and resolution [39]. The achievable resolution typically spans from 5 μm to millimeters in width, while cell viability falls within the range of approximately 40–80% [40-41]. Previous observations indicated that microextrusion was associated with lower cell survival compared to inkjet and laser-based bioprinting (40–86%) due to the extrusion pressure and shear stress [42]. However, studies have reported improved cell viability with pressure extrusion printing, reaching as high as 97% on day 7, 64.4% with a piezoelectric inkjet on day 21, and 98% with a thermal inkjet on day 21 post-printing [43-45].

Laser assisted bioprinting

Laser-Assisted Bioprinting (LAB) leverages Laser-Induced Forward Transfer (LIFT) technology, originally designed for precise metal patterning in semiconductor manufacturing [46]. This advanced process involves three core components: a pulsed laser source, a ribbon, and a receiving substrate [47]. By utilizing nanosecond lasers at specific wavelengths, such as 193 nm, 248 nm, or 1064 nm, LAB achieves controlled energy deposition, typically ranging from 1-20 μJ per pulse [48]. The ribbon, a multi-layered structure with transparent glass, a laser-absorbing metal layer (commonly gold or titanium), and a suspended bioink layer comprising cells, hydrogels, and bioactive factors, plays a pivotal role. When the laser impinges on the ribbon, the metal layer vaporizes, leading to the creation of a high-pressure bubble, ejecting bioink droplets onto the receiving substrate [49]. This process ensures a remarkable resolution, varying from picometer to micrometer size, influenced by factors like bioink layer thickness, viscosity, surface tension, substrate wettability, laser parameters, and air gap [50]. LAB stands out for its ability to print high-density bioinks (up to ~108 cells mL-1) with resolution ranging from 10-100 μm, achieving this without imposing mechanical stress on cells [51]. It excels in printing individual cells or cell aggregates per droplet with exceptional accuracy and cell viability. In the realm of tissue engineering, LAB's precision and resolution hold significant promise. It facilitates the accurate reproduction of internal tissue structures, cellular orientation, and arrangement in various tissues and organs. For instance, in bone engineering, LAB significantly improves vascularization and tissue integration in 3D bone-engineered constructs [52]. Mesenchymal stromal cells were in situ printed within a collagen and nHA matrix, leading to substantial bone formation [53]. In skin tissue engineering, LAB enables the construction of tissue-engineered skin, closely mimicking native skin composition and spatial arrangement. This approach shows promise in grafting and epidermis-like tissue formation [54]. Similarly, LAB aids in fabricating cornea-mimicking structures, offering potential solutions for corneal diseases in cornea regeneration. The 3D printed scaffolds exhibit promising integration with host tissue [55]. On the other hand, in adipose tissue engineering, LAB allows for the printing of human adipose-derived stem cells (hASCs) in a 3D grid pattern, demonstrating proliferation and differentiation without compromising viability while it demonstrates the printing of multiple cell types in a 3D array, fostering vascular-like structure formation in vascular engineering [56-57]. However, despite its potential, LAB does face challenges, including cost and limitations in printing mechanisms. Ongoing research aims to overcome these hurdles and further integrate LAB with other 3D bioprinting techniques for enhanced tissue constructs [58]. Additionally, LAB offers a unique capability to create artificial cell niches, crucial for cancer and drug research. The fabricated constructs closely mimic the structural and functional orientation of native tissues, providing a

powerful tool for in vitro drug screening and toxicological testing [59].

Stereolithography bioprinting

Stereolithography (SLA) bioprinting has emerged as a highly promising technique for fabricating scaffolds, offering versatility for both cell-free and cell-laden forms [60-61]. This method leverages digital micromirror arrays to precisely modulate light intensity, allowing for the layer-by-layer polymerization of light-sensitive polymer materials [62]. It presents substantial advantages over other bioprinting methodologies. One of the pivotal strengths of SLA lies in its capacity to sequentially print light-sensitive hydrogels, irrespective of their complexity or size. This results in consistent printing times for each layer, with the total duration primarily hinging on the structure's thickness. Studies have noted that the printing time with stereolithography averages around 30 minutes [63]. This efficiency can significantly expedite the overall printing process, rendering it an enticing choice for bioprinting applications. Furthermore, SLA distinguishes itself as a nozzle-free printing technique, leading to high cell viability, often exceeding 90%, and an impressive resolution down to 10 μm [64-65]. This heightened cell viability is a crucial factor in bioprinting, ensuring that the printed cells remain viable and functional. When comparing various bioprinting systems, a succinct assessment highlights stereolithography as an exceptionally competitive technique, characterized by a significant combination of high resolution, speed, and cell viability [66]. However, it is crucial to recognize specific limitations linked to current SLA bioprinting methods. For instance, some implementations incorporate UV light sources for polymerization, which have been reported to potentially harm cells [67]. Consequently, researchers have sought alternative strategies to mitigate these concerns. One such approach involves the utilization of an eosin Y based photoinitiator, specifically engineered for crosslinking hydrogels under green light (around 514 nm) [68]. Eosin Y has been recognized for its reduced toxicity compared to other photoinitiators like Irgacure 2959, rendering it an excellent choice for bioprinting systems [69]. This technique enables visible light stereolithography-based bioprinting, substantially minimizing potential risks associated with UV light exposure.

Mask projection stereolithography bioprinting

Mask-Image Projection Stereolithography (MPSL) is a cutting-edge 3D printing technique that employs Liquid Ultraviolet (UV) curable photo-polymer and a UV laser to construct solid objects through the layer-by-layer polymerization of thin liquid layers [70]. This method stands out for its ability to create intricate shapes with internal structures, alongside easy removal of unpolymerized resin and an impressive feature resolution of approximately 1 μm [71]. Manufacturing with MPSL necessitates a photo-crosslinkable site within the polymeric (or monomeric) material. This involves integrating an inert core with photo-crosslinkable moieties like acrylates or epoxies into the polymeric design [72]. Recent applications of MPSL have ventured into the fabrication of complex scaffolds designed for tissue and cell growth [73-74]. However, it's important to note that the biological focus of these applications has primarily limited the field to aliphatic polymers and oligomers, characterized by relatively lower thermal decomposition temperatures (Td) below 400 ℃ and glass transition temperatures (Tg) typically under 100 ℃ [75]. The availability of high-Td, 3D printable polymers remain limited, with a few exceptions like cyanate ester resins [76]. Furthermore, the range of engineering polymers suitable for MPSL-based 3D printing is primarily confined to thermosets due to inherent molecular design constraints. The development of new functional polymeric materials is crucial for unlocking the full potential of 3D printing with MPSL. In addition to MPSL, other techniques within stereolithography like Laser Direct Writing (LDW) or beam scanning rely on a laser to solidify liquid-based resins within a bio-ink reservoir, with resolution being exposure-dependent [77]. However, it's worth noting that earlier printed layers can be repeatedly exposed to the laser in LDW, potentially leading to uneven mechanical strength of the final structure [78]. In contrast, MPSL utilizes a digital-light procession technique, allowing for the simultaneous solidification of an entire patterned layer [79]. This approach is notably faster compared to the laser beam technique. On the other hand, Continuous Liquid Interface Production (CLIP) technology has significantly increased the fabrication speed of MPSL by continuously building the layers of a 3D part above a "dead zone" formed by oxygen inhibition of photopolymerization [80]. While this has drastically enhanced the speed of MPSL, it is important to note that CLIP may have limitations when it comes to fabricating larger-sized parts, as the heat generated during the polymerization process may not dissipate adequately in time [81-82].

Digital light processing bioprinting

Digital Light Processing (DLP) bioprinting is an innovative additive manufacturing technique that employs digitalized light, including Ultraviolet (UV), blue, Near-Infrared (NIR), or other visible light, to induce in situ

photopolymerization. This process facilitates the conversion of liquid polymer materials into solid 3D structures with exceptional resolution and intricate architectures [83-84]. The DLP printer, driven by a Digital Micromirror Device (DMD) projector, plays a pivotal role in this process. It enables the rapid solidification of an entire layer of monomer at a time, marking a significant advancement over extrusion-based bioprinting methods [85]. This attribute grants DLP technology a substantial advantage in achieving complex structures. DLP bioprinting has been successfully employed in fabricating a diverse range of tissues, including heart, blood vessels, bone, cartilage, liver, lung, eye, neuronal tissue, and pancreatic tissue [86-88]. This showcases the broad applicability of DLP technology in various biomedical contexts. One of the key advantages of DLP bioprinting lies in its ability to customize the stiffness of printed scaffolds. By adjusting exposure time and light intensity, it becomes possible to encapsulate different cell types within the scaffold matrix [84,89]. This dynamic capability further enhances the utility of DLP bioprinting in tissue engineering applications. Moreover, DLP bioprinting has enabled the creation of multi-material components within 3D tissue constructs. This is made possible through novel Extracellular Matrix (ECM) improvements, expanding the repertoire of achievable tissue models [90-91]. In practice, DLP bioprinters are characterized by their user-friendliness, cost-effectiveness, and high accuracy/efficiency. They have found wide-ranging applications in drug screening, disease modeling, and tissue regeneration. Notably, they have been instrumental in designing liver-inspired 3D tissue models for efficient toxin trapping [83]. Additionally, hydrogel-based microfluidic chips have been employed to simulate fluid-solid interactions for drug delivery studies [92-93]. Furthermore, DLP bioprinting has enabled the fabrication of heart valve hydrogels with complex structures, showcasing high fatigue resistance [94]. These achievements underscore the transformative potential of DLP bioprinting in various biomedical domains. To achieve successful DLP-based bioprinting, meticulous consideration must be given to the selection of suitable photopolymers, photoinitiators, and photoabsorbers [95]. Various photopolymerizable polymer systems, including PEGDA, PEGMA, GelMA, and others, have been utilized, each possessing distinct mechanical and biocompatible properties [96]. This underscores the pivotal role of material selection in optimizing DLP bioprinting processes.

Kenzan bioprinting

The Kenzan bioprinting method, conceived by Koich Nakayama, stands as a remarkable leap forward in scaffold-free 3D bioprinting [97]. This technique hinges on the intrinsic tendency of cells to self-aggregate, forming high-density cellular structures by positioning cell spheroids on a fine needle array [98]. The methodological principle is rooted in the fusion of cell spheroids, facilitated by the temporary support of stainless-steel microneedles known as “kenzans” [99]. These kenzans, organized in patterns of either 9x9 or 26x26, play a crucial role as a scaffold during the fusion process [100]. The Regenova bioprinter, designed by Cyfuse Biomedical K.K., is purpose-built for Kenzan bioprinting, employing a sophisticated system featuring a camera-based machine vision system, a plate handling platform, a disposal chamber, and a container holding the Kenzan needle array submerged in PBS [101]. During the bioprinting procedure, the Regenova system employs the vision system to identify and inspect a spheroid. This selected spheroid is then retrieved from the culture plate using suction from the nozzle and precisely positioned onto a needle on the Kenzan array, adhering to a preprogrammed 3D pattern. This precision placement allows for the fusion of adjacent spheroids, culminating in the creation of a tissue construct. This method enables the fabrication of high-density cellular structures with exceptional precision [102-103]. It’s worth noting that the efficiency and resolution of Kenzan bioprinting are influenced by factors such as the size and distribution of spheroids, as well as their compaction. While the technique is potent, it’s important to acknowledge its associated challenges. It can be relatively high-cost and time-consuming, especially for larger tissue constructs [104]. Moreover, careful consideration must be given to the post-bioprinting and implantation workflow to ensure the integrity and functionality of the printed tissue constructs [105]. On the other hand, Kenzan method has found diverse applications, ranging from the fabrication of nerve conduits, cardiac patches, and bone constructs, to the reconstruction of tissues like liver, tendon/ligament, and bladder tissues [106-108]. Researchers have even pushed the boundaries of the method by using it for the creation of trachea-like tubes, offering promising prospects for the restoration of lost epithelium and capillaries due to surgical resection [109].

Acoustic bioprinting

Acoustic bioprinting, also referred to as Acoustic Droplet Ejection (ADE), stands out as a highly promising platform for droplet generation in biological applications. This method harnesses ultrasonic waves focused at the interface of fluid and air, resulting in radiation pressure that expels droplets from the surface. The size of the ejected droplet is inversely proportional to the frequency of the transducer, ranging

from 300 µm with 5 MHz waves to 5 µm with 300 MHz waves [111]. Unlike conventional inkjet printers, which rely on physical nozzles, ADE controls droplet characteristics entirely through sound waves, offering unprecedented advantages in handling biological samples. It mitigates issues such as clogging, sample contamination, and damage to cells or biomarker structures due to shear forces [112]. This nozzleless technology has been pivotal in achieving high-throughput droplet generation, processing fluids at impressive rates of up to 25000 droplets/s, or roughly 50 nL/s per ejector head. Notably, Micro-electromechanical System (MEMS) based arrays with 1024 ejector heads have demonstrated the potential to process over 180 mL of fluid in under an hour, a significant improvement over existing microfluidic cell separation method [113]. Similarly, ADE's reliance on acoustic waves, enabling them to propagate through a matched coupling media with minimal loss of acoustic energy, while ensuring no direct contact between the sample and the transducer. This effectively eliminates the risk of cross-sample contamination and maintains sterility [114]. Conversely, traditional nozzle-based printing methods encounter limitations related to print resolution, particularly due to nozzle size, which leads to frequent clogging, especially when processing cell-laden bioinks. Moreover, as the nozzle diameter decreases, the shear stress on cells escalates, potentially resulting in irreversible damage and cell death [115].

Magnetic bioprinting

Magnetic bioprinting, a cutting-edge technique in tissue engineering, leverages the power of magnetic forces to manipulate and assemble cells into desired configurations. This method relies on two primary strategies. The first strategy involves the incubation of cells with nanoparticles, specifically utilizing Fe_3O_4 magnetic fields to induce gel formation through electrostatic interactions [116]. This process initiates the binding of cells to a nanoparticle assembly known as a Nanoshuttle [116]. This assembly comprises magnetic iron oxide nanoparticles that render the cells magnetic. Subsequently, the magnetized cells are cultured in an incubator, a crucial step in cell cultivation [117]. During this phase, the cells self-organize, forming 3D structures at the air-liquid interface. This dynamic allows for the rapid creation of dense cultures capable of synthesizing extracellular matrix [118-119]. The second strategy is characterized by the combination of label-free cells with a paramagnetic buffer in the presence of an external magnetic field [120-121]. This interaction prompts cell movement towards regions of lower magnetic field strength, enabling precise control over the shape of 3D cell assemblies. The spatial arrangement of these assemblies can be manipulated by altering the magnetic template's shape, offering further versatility in microtissue assembly [122]. The advantages of magnetic bioprinting are manifold. Its speed and ease of handling are particularly noteworthy. This technique yields cultures within a remarkably short timeframe, ranging from 15 minutes to a few hours, and allows for easy manipulation and transfer of cells using magnetic forces [123]. Moreover, this method is highly adaptable to mobile device-based imaging, which significantly enhances accuracy and throughput. Furthermore, in the context of hyperthermia-based cancer treatments, magnetic bioprinting plays a pivotal role. The inclusion of magnetic particles in a hydrogel matrix enables precise control over the delivery of therapeutic agents, allowing for targeted treatment of cancer cells. This approach capitalizes on the ability of magnetic particles to generate heat in the presence of an alternating magnetic field, selectively damaging or destroying cancer cells [124].

Coaxial bioprinting

Coaxial bioprinting, an extrusion-based 3D bioprinting technology, has emerged as a promising method in tissue engineering and regenerative medicine [125]. This technique involves the simultaneous extrusion of multiple bioinks through coaxial nozzles to create strands with distinct compartments, including an inner core and an outer shell. It offers several advantages in creating complex hierarchical tubular structures with tunable biological and mechanical properties [126]. The core and shell materials used in coaxial bioprinting must meet specific criteria [127-128]. Firstly, they should exhibit low viscosity to minimize shear force-induced damage to cells during the printing process, thus facilitating the printing of biologically suitable tissues. Secondly, these materials should demonstrate degradation behavior that can be controlled in both short-term and long-term functions. The resulting degradation products should be non-toxic and harmless, ensuring the normal function of the tissue without sudden collapse at the graft site [129]. Additionally, they should possess certain mechanical properties to meet the stretching and compression requirements of the implant site [130]. Coaxial bioprinting excels in generating hierarchical tubular structures, a crucial aspect in tissue engineering. By utilizing sacrificial materials in the core, it enables the creation of hollow or tubular structures that mimic natural vascular networks [131]. This is pivotal in ensuring the viability of cells within the construct. Moreover, coaxial bioprinting allows for the combination of different biomaterials, each serving a specific function. For instance, a softer, biocompatible

material can be used for the core to support cell viability, while a stronger material forms the shell for mechanical support [132]. This versatility is essential for success in tissue engineering applications. Despite its advantages, coaxial bioprinting is not without its challenges. Alginate, a commonly used material in coaxial bioprinting, may have limitations in supporting normal cell growth and development due to its poor biological performance [126]. Achieving optimal printability and shape fidelity, especially with different properties of biomaterials in the core and shell, may require careful parameter optimization.

FRESH bioprinting

Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting addresses a critical challenge in 3D printing soft structures, which is their tendency to collapse during the printing process [133]. This prompted the development of gel-based support baths, breaking the link between gelation time and mechanics. These baths physically support the extrusion printing of soft hydrogels, enabling the creation of stiffer scaffolds that can self-support post-support bath removal [134]. While natural polymers like alginate, collagen, and GelMA have been commonly used in FRESH bioprinting, there is potential for the utilization of synthetic polymer-based bioinks to gain control over interfacial, mechanical, and degradation properties of the printed scaffolds [135-136]. However, there has been limited success in 3D printing such bioinks using an embedded printing strategy [137]. Notable exceptions include the work by Hull et al. with their FRESH bioprinting of UNIversal Orthogonal Network (UNION) bioinks, based on Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC) [138]. It's worth noting that SPAAC click chemistry, while effective, is inherently nonreversible under physiological conditions, which may present challenges in the long-term degradation of synthetic polymer-based bioinks [139-140]. A significant advancement in FRESH v2.0 lies in the preparation of the support bath. Instead of pulverizing large gelatin blocks in a consumer-grade blender, support bath particles are now generated through a coacervation approach. This results in smaller, more spherical, and more uniform microparticles, enhancing print resolution. With FRESH v2.0, the authors have achieved impressive results, printing individual collagen filaments as thin as 20 μm in diameter [141]. The FRESH technique has made substantial contributions to the field of bioprinting, allowing for the creation of complex 3D tissue and organ models with a wide range of biocompatible hydrogel and cell-laden bioinks. This method's unique aspects include the viscoplastic behavior of the support bath, customizable aqueous phase compatible with various gelation mechanisms, and support bath liquification for nondestructive print release under biologically compatible conditions [142]. It has found applications in printing functional and cellularized tissue constructs, medical devices, and even organs at the scale of ventricle-like heart chambers [143-144].

Microfluidic bioprinting

Microfluidic bioprinting represents a significant advancement at the intersection of engineering, physics, chemistry, and biotechnology. This technology leverages the precise manipulation of minute volumes of fluids, cells, and molecules within microchannels, ranging from nanometers to hundreds of micrometers [145]. Microfluidic systems have found applications in various fields, including disease diagnostics, drug delivery, and biosensing [146-147]. When applied to tissue engineering, the integration of microfluidic techniques with bioprinting has led to notable progress in the fabrication of complex tissue constructs [148]. Microfluidic chips, characterized by intricate networks of microchannels, enable the creation of zonally heterogeneous tissue constructs, a crucial feature for accurately replicating the complexity and functionality of native tissues, which rarely consist of homogenous cell populations [149-150]. These chips have been effectively incorporated into various bioprinting techniques, including Digital Light Processing (DLP) bioprinting and Extrusion-Based Bioprinting (EBB) [151-152]. DLP bioprinting, for example, involves a closed chamber microfluidic chip that facilitates rapid switching between different bioinks, enabling the creation of multi-layered structures and multi-material tissue patterns [153]. Additionally, EBB bioprinting, traditionally involving layer-by-layer extrusion of bioink, was enhanced by integrating a microfluidic chip to achieve laminar flow and facilitate smooth transitions between different biomaterials [154]. Furthermore, the unification of microfluidic systems with bioprinting techniques has yielded innovative technologies for generating 3D tissue models, organ-on-a-chip devices, and lab-on-a-chip platforms [155-156]. Amir et al. demonstrated a stereolithography-based bioprinting platform employing a microfluidic device to fabricate multicomponent hydrogel constructs, thereby enabling precise control over the deposition of different bioinks [157]. Ghorbanian et al. developed a microfluidic direct writer capable of delivering two different alginate gel solutions alternatively, allowing for the fabrication of complex 3D hydrogel constructs [158]. Hardin et al. designed a microfluidic printhead that facilitated the printing of multiple viscoelastic inks, providing a versatile approach for creating heterogeneous tissue structures [159].

Despite these promising advancements, challenges persist in the field of microfluidic-enhanced bioprinting. Issues such as scaling up tissue structures, handling fragile hydrogel fibers, and achieving desired geometrical outcomes remain areas of active research [160]. Integration of microfluidic systems within AM-assisted extrusion bioprinters is a recent trend aimed at addressing these challenges, enabling layered scale-up of precise tissue constructs [161-162]. Additionally, ongoing research focuses on improving the speed, versatility, and material compatibility of microfluidic-enhanced bioprinting technologies [163].

Volumetric bioprinting

Volumetric bioprinting is a pioneering approach empowers the swift creation of 3D constructs, spanning diverse sizes and intricate architectures, within an astonishingly compressed temporal window, ranging from mere seconds to tens of seconds [164]. At its fundamental core, volumetric additive manufacturing hinges on the projection of an array of 2D patterned optical light fields within a volume of a photosensitive polymer. These 2D light patterns accumulate cumulatively to yield an optical 3D dose distribution, instigating the polymerization of the irradiated material into the desired object [165]. In its initial conceptualization, this method gave rise to rudimentary objects by irradiating a reservoir of photosensitive polymer with a superposition of multiple beams from fixed, predetermined orientations [166]. Subsequent strides in volumetric printing, inspired by Computed Tomography (CT), have incorporated dynamic 2D light fields, enabling the production of more intricate and complex objects [167]. Notable headway has been made with technical photopolymers like acrylates and elastomeric resins, showcasing the capacity to resolve features as fine as 80 µm [168]. Volumetric bioprinting holds immense promise in advancing medicine by facilitating the rapid creation of large-scale structures. Integrating it with other printing methods such as 4D bioprinting may leverage their individual strengths, offering a more comprehensive approach to tissue engineering and biofabrication [169].

4D bioprinting

4D bioprinting is an advancement in fabrication technology, building upon the principles of 3D printing by introducing an additional dimension, referred to as time [170]. This innovation was pioneered by Tibbits at the Massachusetts Institute of Technology, who demonstrated its potential by printing prototypes that could self-transform in response to external stimuli. In this scope, a printed strand initially straightened itself into the letters "MIT" when immersed in water [171]. Qi and other research groups at the Georgia Institute of Technology have also contributed to the development of 4D printing [172]. Since its inception, 4D printing has garnered significant attention, leading to a proliferation of published research papers. This technique has transcended research boundaries and found applications in diverse fields such as education, art, industry, and biomedicine [173]. Recently, 4D printing has evolved into 4D bioprinting, a groundbreaking concept that involves printing biocompatible materials or living cells into complex constructs [174]. This extension of 4D printing is characterized by the ability of the printed bioconstruct to undergo changes in size, shape, and functionality over time. This transformation can be either spontaneous or triggered by an external stimulus [175]. Unlike traditional 3D bioprinting, 4D bioprinting offers solutions to some of its limitations. For instance, 3D bioprinting struggles with fabricating complex structures like blood vessels, due to the risk of collapse [176]. 4D bioprinting, on the other hand, provides a pathway to overcome this challenge by printing initially flat biological structures and then inducing the transformation into functional tubular structures, like blood vessels, through external stimuli [177]. The applications of 4D bioprinting are vast and promising, particularly in fields such as tissue engineering, drug delivery, and wound repair [178-180]. One noteworthy application is in vessel fabrication, where 4D bioprinting enables the creation of intricate structures that would be challenging or impossible to achieve using conventional methods [181]. To achieve these transformations, 4D bioprinting relies on stimulus-responsive or "smart" materials. These materials react to various stimuli, including physical (e.g., water, temperature, light, electric field, and magnetic field), chemical (e.g., pH value and ion concentration), or biological (e.g., glucose and enzymes) signals [182]. Therefore, water-responsive materials can induce swelling, twisting, folding, and other deformations when exposed to moisture [183]. Temperature-responsive materials, like shape memory alloys, enable reversible deformations, expanding the possibilities for 4D bioprinting [184]. Electric, light, and magnetic fields are also utilized as stimuli, influencing the behavior of polyelectrolytes and composites of magnetic nanoparticles [185]. Additionally, chemical and biological stimuli, such as pH value, ion concentration, glucose, and enzymes, play a vital role in triggering transformations in printed structures [186]. Various printing techniques, including extrusion-based, inkjet-based, and laser-based methods, can also be employed for 4D bioprinting [187-188]. In terms of material composition, 4D bioprinted constructs can be categorized into single-material structures and multiple-material structures. Single-material structures involve using a single material that deforms due to either spatially

nonuniform designs or nonuniform external stimuli [189]. Conversely, multiple-material structures employ a combination of materials with varying responses to external stimuli, enabling programmable deformations [190]. Recent advances in 4D bioprinting have demonstrated its potential in biomedical applications. Smart stents, responsive to stimuli like temperature and pH changes, offer promising solutions for treating vascular stenosis and other endoluminal body structures [190]. Moreover, 4D bioprinting shows great promise in drug delivery systems, enabling precise control over drug release in response to physiological changes [191]. 4D bioprinting has been also applied to develop shape-changing scaffolds for bone repair and tissue engineering, as well as adaptive conduits for nerve regeneration [192-193].

Electrospinning bioprinting

Electrospinning is a highly versatile technique employed in tissue engineering, allowing for the creation of micro-/nanofibers from polymer solutions or melts through the manipulation of electrostatic forces [194]. This process necessitates a setup comprising a syringe containing the polymer solution connected to a metallic needle, a syringe pump for flow rate regulation, a high voltage power supply, and a metallic collector. When voltage is applied between the syringe and collector, the solution extruded through the needle forms an electrically charged jet, which is subsequently drawn toward the collector. As the solvent evaporates during its travel, the jet's diameter undergoes significant reduction, culminating in the deposition of a mass of fibers on the metallic collector [195]. This methodology has found application in diverse areas such as vascular grafts, osteochondral tissue engineering, bone substitutes, and wound healing strategies [196-198]. Notably, electrospun fibers integrated within 3D-printed structures have demonstrated the controlled delivery of bioactive agents [199-200]. Noteworthy combinations include gelatin and collagen electrospun fibers with 3D-bioprinted scaffolds, as well as urinary bladder matrix electrospun meshes integrated with 3D-bioprinted structures for specialized culture devices [201]. The combination of 3D bioprinting and electrospinning offers a promising strategy for fabricating scaffolds with controlled pore structures and nano-scale features, which closely mimic the natural ECM morphology and size [202]. Recently, there has been a concerted effort to understand and optimize the interactions between cells and scaffolds for effective tissue regeneration, particularly in contexts like skeletal muscle [203]. Factors such as scaffold thickness, hydrophilicity, porosity, roughness, stiffness, surface charges, and incorporation of cell attachment moieties (RGD) play pivotal roles in directing cellular behaviors critical for tissue regeneration [204]. Microgrooves in scaffolds have been demonstrated to promote cellular alignment and myofiber formation, while mechanical and electroconductive properties are crucial for mimicking native muscle tissue [205]. To enhance the mechanical properties and structural complexity of scaffolds for skeletal muscle regeneration, a combination of electrospinning and 3D printing has shown great promise [206]. This approach offers constructs with a large surface-area-to-volume ratio, improved mechanical properties, better interconnectivity, high porosity, enhanced cell attachment, unidirectional cell alignment, and fibrous tissue formation. Additionally, the integration of melt electrospun meshes with 3D bioprinted hydrogel constructs holds potential for achieving heterogeneous mechanical characteristics akin to native articular cartilage [207].

Recent Bioprinter Technologies

In recent years, the field of bioprinting methods and bioprinter systems have witnessed remarkable advancements, revolutionizing the way biological tissues and organs are fabricated for research, pharmaceutical testing, and potentially clinical applications [208] (Supplement 1). Low-cost bioprinters employing microextrusion-based technology have gained traction due to their affordability and accessibility [148]. Moreover, the integration of microfluidic components offers unparalleled control over the bio-printing process, opening new avenues for tissue fabrication. Support-free multiaxial printing, offering increased geometric complexity without the need for support structures, represents another promising trend. While currently prevalent in high-end systems, this technology holds immense potential for in vivo applications. High-resolution printing using focused light, a departure from traditional extrusion-based methods, offers substantial improvements in speed, resolution, and cell viability. As new developments like Computed Axial Lithography (CAL) enter the bioprinting arena, existing technologies are poised to become more affordable and integrated into low-cost systems [167,209]. The future of bioprinting is poised for significant evolution, with several key trends shaping the landscape. Modular print heads are emerging as a transformative feature, allowing users to interchange printing tools for different bio-inks and technologies [210]. This modularity extends the capabilities of bioprinters, enhancing their versatility at a relatively minor additional investment. Furthermore, microfluidic technologies, currently present in high-end systems, are expected to become more accessible as costs decrease, revolutionizing

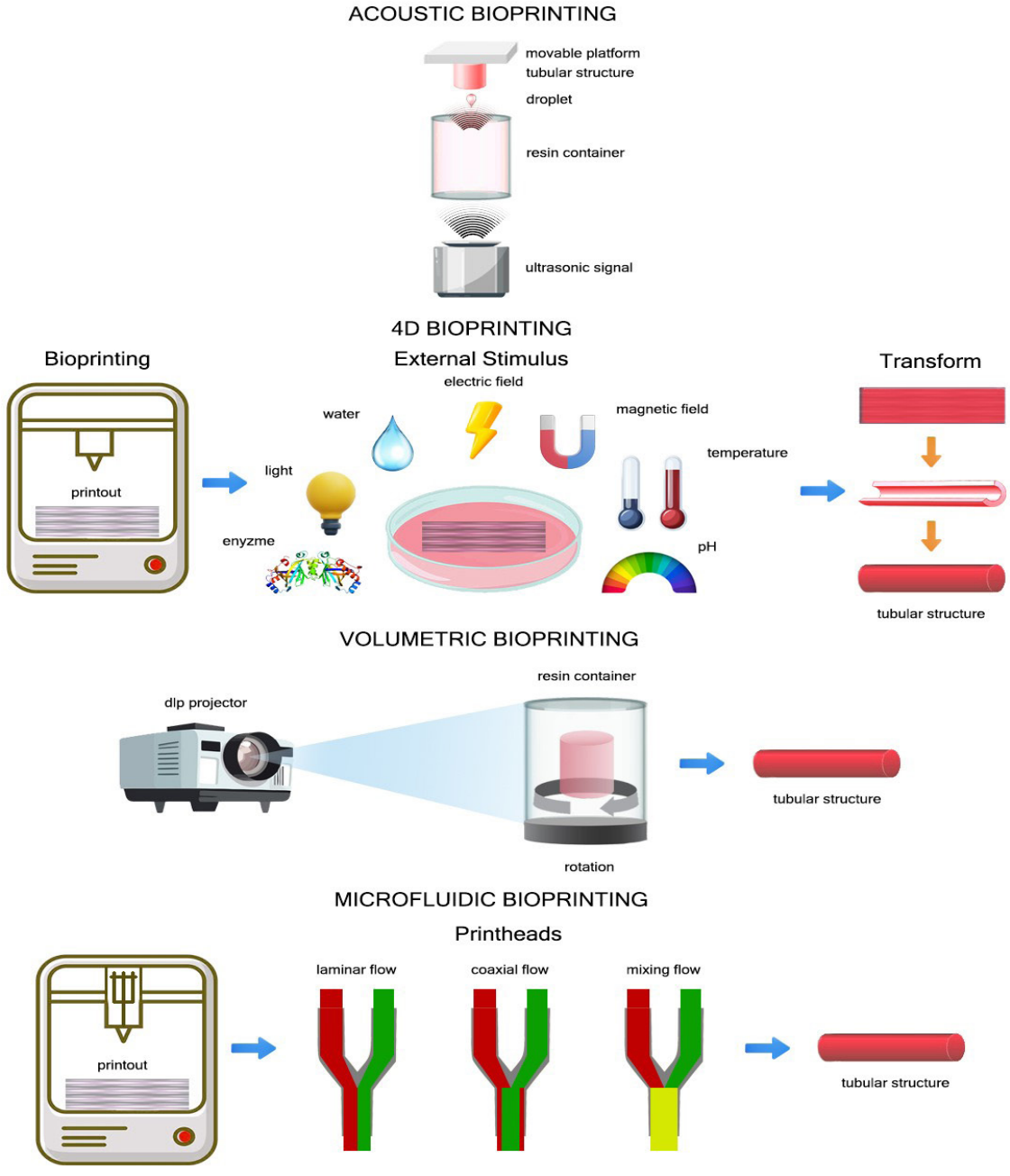

Figure 1: *Recent bioprinter technologies.*

low-cost bioprinting [211]. Figure 1 illustrates the recent bioprinter technologies and working principle.

Clinical Applications of Current Methods

3D bioprinting holds immense promise in reshaping the landscape of clinical applications. The trajectory of its advancement is driven by a confluence of critical medical needs, including the demands of aging populations, the scarcity of organ donors, and the imperative to reduce reliance on animal testing in therapeutic development. As bioprinting technology matures, it stands at the forefront of regenerative medicine, offering solutions to repair, replace, or regenerate a spectrum of tissues. These encompass vital structures like cartilage, bone, skin, and periodontal tissues, alongside the intricate vascular and cardiovascular systems [212]. This capability transcends the boundaries of traditional medicine, heralding a new era where patient-specific drug screening and disease modeling can be conducted on bioprinted tissues [213]. The potential to customize drug responses based on individual patient profiles is poised to revolutionize pharmaceutical interventions. Furthermore, bioprinting is poised to play a pivotal role in transplantation

medicine. The ability to fabricate full or partial organs addresses a longstanding global challenge - the shortage of viable organ donors. This breakthrough, if realized, could significantly reduce transplant waiting lists and alleviate the suffering of countless patients. Additionally, the integration of 3D bioprinting with other advanced techniques, such as microfluidics and computed axial lithography, is expanding the horizons of what can be achieved. This convergence of technologies is particularly promising in the fabrication of zonally heterogeneous tissue constructs and the creation of support-free, volumetric geometries. In parallel, the emergence of 3D bioprinting for medications stands as a testament to the technology's potential to individualize treatment. Personalized dosages, quick-dissolving pills, and the elimination of intolerant fillers represent a paradigm shift in pharmaceutical manufacturing. Medications like levetiracetam exemplify the transformative potential of 3D-printed drugs, offering improved therapeutic outcomes for patients [214]. In the realm of clinical education, 3D bioprinting is proving to be an invaluable tool. Anatomical models created through bioprinting serve as powerful educational aids, enhancing the training of clinicians across diverse specialties. From neurosurgery to vascular surgery, these models provide a tangible platform for practicing complex procedures and visualizing intricate anatomical structures. Such advancements not only benefit trainee clinicians but also serve as valuable resources for experienced practitioners preparing for intricate interventions [215]. Moreover, these technologies extend their impact to patient education [216-217]. Complex medical conditions, like congenital heart disease or liver cancer, can be visually and tactically explained to patients through custom-printed anatomical models. This not only fosters a deeper understanding of their condition but also facilitates more informed consent processes. Patients can now actively engage in their healthcare decisions, empowered by a clearer comprehension of their own anatomy and the proposed medical interventions. Beyond these transformative applications, 3D bioprinting is revolutionizing imaging technologies [218]. Specially designed objects, known as phantoms, are crafted to precisely mimic human tissues. These phantoms serve as essential tools for testing and calibrating medical imaging systems, ensuring their accuracy and reliability in clinical settings. The clinical applications of 3D bioprinting are poised to redefine the landscape of healthcare. From tissue regeneration to drug personalization, from transplantation breakthroughs to enhanced medical education, the potential impact of this technology is boundless. As research continues to push boundaries and technology advances, we stand on the brink of a new era in medicine, one where individualized, precision healthcare is not just an aspiration, but a tangible reality.

Limitations of Current Systems

Each bioprinting technique offers distinct advantages and encounters specific challenges. Coaxial bioprinting, known for its proficiency in creating intricate tubular structures, excels in combining diverse biomaterials for specialized functions. This method is particularly adept at emulating natural vascular networks, a critical factor for sustaining cell viability within constructs. However, it grapples with potential limitations, particularly with commonly utilized materials like alginate, which may pose constraints on supporting optimal cell growth. In contrast, FRESH bioprinting addresses a prevalent issue in 3D printing-the susceptibility of soft structures to collapse during the printing process. By employing gel-based support baths, it enables the fabrication of stiffer scaffolds, obviating the need for additional support structures. This innovative feature significantly streamlines the printing process. However, challenges persist in the 3D printing of synthetic polymer-based bioinks using an embedded strategy. This method demands continued research to refine its application. Microfluidic bioprinting, situated at the convergence of engineering, physics, chemistry, and biotechnology, allows for the meticulous manipulation of fluids and molecules within microchannels. This integration has propelled significant advancements in fabricating tissue constructs with accurately replicated complexity and functionality. The intricate networks of microchannels within microfluidic chips play a pivotal role in creating tissues with finely tuned properties. Nonetheless, ongoing research is essential to enhance the speed, versatility, and material compatibility of this bioprinting technology. Volumetric bioprinting marks a transformative leap in additive manufacturing, enabling the rapid fabrication of cell-laden constructs with diverse sizes and intricate architectures. This method holds immense promise, particularly in the creation of large-scale functional organs. However, its full potential hinges on seamless integration with other techniques and further development of stimulus-responsive materials. 4D bioprinting, introducing the dimension of time, has emerged as a solution to the challenges faced by traditional 3D bioprinting, especially in fabricating complex structures like blood vessels. Its potential applications span tissue engineering, drug delivery, and wound repair, but its efficacy relies on the availability of stimulus-responsive materials. Furthermore, the integration of electrospinning with 3D bioprinting has shown remarkable promise in enhancing scaffold properties for applications in skeletal muscle tissue engineering. The combination of electrospun meshes with

3D bioprinted hydrogel constructs yields structures with improved mechanical properties, cell attachment, and tissue formation. However, precise parameter tuning remains critical for optimal results. While each bioprinting method presents promising avenues in tissue engineering and regenerative medicine, ongoing research and innovation are imperative to surmount their respective challenges and fully harness their potential.

Future Perspectives

The evaluation covered a diverse range of bioprinter models, each with unique printing technologies, print head configurations, and XYZ positional accuracy. Build volume specifications were examined to understand the bioprinters' capacity for intricate structures. Temperature control features for both the print bed and head were assessed for accommodating various biomaterials. The provision of a sterile environment was considered crucial for construct integrity and viability. User-friendly interfaces and display options were evaluated for ease of operation. Photocuring capabilities were examined for compatibility with light-curable materials. The inclusion of a camera system for real-time monitoring and quality control was also assessed. Calibration methods were investigated to determine precision and accuracy. For precise and up-to-date details, researchers are advised to consult official company documentation. Refer to Table 1 for a comprehensive view of bioprinters according to supplier datasheets.

Table 1: A comprehensive view of commercially available bioprinters.

Company	Model	Technology	Print Heads	XYZ Accuracy (µm)	Build Volume (mm)	Bed Temp. (°C)	Head Temp. (°C)	Sterilization	On-board Display	Photocuring (nm)	On-board Camera	Calibration
CELLINK	Inkredible+	Extrusion-Based (Pneumatic)	2	10/10/100	130x80x100	up to 65	Up to 130	Air: HEPA 13 UV: None	Yes	365, 405	No	Manual
	BIO X	Extrusion-Based (Pneumatic)	3	1/1/2001	130x90x70	4 to 65	4 to 250	Air: HEPA 14 UV: UV-C (275nm), 20mW	Integrated Display, DNA Studio	365, 405, 485, 520	No	Manual and Automatic
	BIO X6	Extrusion-Based (Pneumatic)	6	1/1/2001	128×90×90	4 to 65	4 to 250	Air: 2xHEPA 14 UV: UV-C (275nm), 30mW	Tablet or Computer	365, 405, 485, 520	No	Automatic
EnvisionTEC 3D	3D-Bioplotter Developer Series	Modular Tool Changer	3	1/1/2001	150x150x150	-10 to 80	Low: 0 to 70 High: 30 to 250	Particle filter only	No	365	Yes	Automatic
	3D-Bioplotter Manufacturer Series	Modular Tool Changer	5	1/1/2001	150x150x150	-10 to 80	Low: 0 to 70 High: 30 to 250	Particle and sterile filter	No	365	Standard	Automatic
RegenHU	R-GEN 100	Modular Print Heads	5	0.5/0.5/0.5	130x90x65	5 to 80	Modular: 4 to 80 Melt: up to 240	No	No	365, 405	Yes	Automatic
	R-GEN 200	Modular Print Heads	5	0.5/0.5/0.5	130x90x65	5 to 80	Modular: 4 to 80 Melt: up to 240	Class II BSC	Yes	365, 405	Yes	Automatic

Advanced Solution	BioAssembly-Bot 200	Modular Print Heads (4-axis Robotic Arm)	5	Unavailable	27.9x17.8x6.9	10 to 60	-4 to 150	Air: HEPA filter	Touch Pad	365, 405	Yes	Automatic
	BioAssembly-Bot 400	Modular Print Heads (6-axis Robotic Arm)	8	Unavailable	30.5x25.4x17.8	10 to 60	-4 to 150	Air: 99.97% of particles to 0.3 µ (w/ HEPA)	Touch Pad	365, 405	Yes	Automatic
Regemat 3D	BIO V1	Modular Print Heads	4	150x150x0.4	150x160x110	-20 to 100	-20 to 100, Thermo-plastic extruder: up to 250	No	No	365, 385, 405	No	Automatic
	REG4LIFE	Modular Print Heads	7	150x150x0.4	120x120x100	-20 to 100	-20 to 100, Thermo-plastic extruder: up to 250	No	No	365, 385, 405	No	Automatic
Allevi	Allevi 1	Extru-sion-Based (Pneumatic)	1	7.5x7.5x1	90x60x130	No	4 to 160	No	No	365, 405	No	Automatic
	Allevi 2	Extru-sion-Based (Pneumatic)	2	5x5x1	90x60x130	No	E1: Up to 160 E2: Up to 70	No	No	365, 405	No	Automatic
	Allevi 3	Extru-sion-Based (Pneumatic)	3	1x1x1	90x60x130	Up to 60	4 to 160	No	No	365, 405	No	Automatic
Aspect Bio-systems	RX1	Modular Print Heads	6	0.5x0.5x0.5	90x150x70	No	No	No	No	No	Yes	Automatic
Axolotl Biosystems	Axo-A3	Modular Print Heads	3	1x1x1	130x90x80	Down to -10	3 to 265	Air: HEPA filter UV: UV-C	No	365, 395, 405	Op-tional	Automatic
	Axo-A6	Modular Print Heads	6	1x1x1	130x90x80	Down to -10	3 to 265	Air: HEPA filter UV: UV-C	No	365, 395, 405	Op-tional	Automatic
Brinter	Brinter CORE	Modular Print Heads	4	3x3x3	150x110x40	Up to 100	4 to 250	UV: UV-C (265nm)	No	365, 405, 450	Yes	Automatic
	Brinter ONE	Modular Tool Changer	4	3x3x3	304x174x80	4 to 100	4 to 250	UV: UV-C (265nm)	No	365, 405, 450	Yes	Automatic
GeSim	BioScaffolder	Extru-sion-Based Bioprinter	3	2x2x10	124.4x345.4x40.6	Optional	4 to 80, Up to 190, Up to 250	No	No	Optional	Yes	Automatic

Fluicell	Biopixlar	Robot Arm	3	2x2x2	35/50 standard dish holders	Unavailable	Unavailable	Unavailable	No	370 to 680	Inverted Microscope	Automatic
Poietis	NGB-R Bioprinter	Modular Print Heads (6-axis robotic arm)	3	10x10x10	6-well and 12-well plates	Optional	30 to 50	Class II BSC	Yes	365, 405	Optional	Automatic
MicroFab	Jetlab 4	Modular Print Heads	4	30x30x30	200x150x50	Up to 100	Optional	Optional	No	Optional	Yes	Automatic
	Jetlab II	Modular Print Heads	4	4x4x4	300x300x40	Up to 100	Optional	Optional	No	Optional	Yes	Automatic

Bioprinting, a revolutionary technology in tissue engineering and regenerative medicine, encompasses several cutting-edge methods, each with its unique strengths and challenges. Among these, the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting technique addresses a critical challenge in 3D printing soft structures. By utilizing gel-based support baths, it enables the creation of stiffer scaffolds and allows for post-support bath removal. This breakthrough has paved the way for the fabrication of complex 3D tissue and organ models. Natural polymers like alginate, collagen, and GelMA have been commonly used in FRESH bioprinting, but there is a growing potential for the utilization of synthetic polymer-based bioinks. However, challenges persist in 3D printing such bioinks using an embedded strategy, with notable exceptions like the work by Hull et al. using strain-promoted azide-alkyne cycloaddition (SPAAC) chemistry [138]. Although SPAAC click chemistry is effective, it is inherently nonreversible under physiological conditions, which may pose challenges in long-term degradation [139-140]. Microfluidic bioprinting, at the crossroads of engineering, physics, chemistry, and biotechnology, allows for precise manipulation of fluids, cells, and molecules within microchannels. This integration has led to significant progress in fabricating zonally heterogeneous tissue constructs [149-150]. The intricate networks of microchannels in microfluidic chips are pivotal in creating tissues with accurately replicated complexity and functionality. Techniques like DLP bioprinting and EBB have been enhanced with the integration of microfluidic chips, enabling the creation of multi-layered structures and facilitating smooth transitions between different biomaterials. Ongoing research aims to improve the speed, versatility, and material compatibility of microfluidic-enhanced bioprinting technologies [163]. Additionally, integration of microfluidic systems within AM-assisted extrusion bioprinters is a promising trend to address challenges like scaling up tissue structures and achieving desired geometrical outcomes. Volumetric printing marks a transformative leap in additive manufacturing, allowing for the rapid fabrication with diverse sizes and intricate architectures. The future of volumetric bioprinting in medicine is promising especially in the creation of large-scale functional organs. However, integration with other methods and further development of stimulus-responsive materials may be more useful in fully realizing its potential. Similarly, 4D bioprinting overcomes some of the limitations faced by traditional 3D bioprinting, particularly in fabricating complex structures like blood vessels [175]. The potential applications of 4D bioprinting in tissue engineering, drug delivery, and wound repair are extensive and promising. Furthermore, electrospinning integration with 3D bioprinting has shown promise in enhancing scaffold properties for applications in skeletal muscle tissue engineering [205]. The combination of electrospun meshes with 3D bioprinted hydrogel constructs offers constructs with improved mechanical properties, cell attachment, and tissue formation.

Recent advancements in bioprinter technologies have led to a range of low-cost models to high-end systems. Low-cost bioprinters with microextrusion-based technology provide accessible options with suitable resolution for various applications [148]. High-end droplet-based bioprinters excel in precision and performance, offering larger build spaces for complex tissue production. Emerging trends, such as multi-material printing, in situ bioprinting, and AI integration, are poised to further enhance the capabilities of

bioprinting technologies [14]. Each bioprinting technique brings unique advantages and challenges, necessitating ongoing research and innovation. On the other hand, bioprinters have witnessed an extraordinary evolution, offering a diverse range of precision instruments tailored to specific research needs (Supplement 1, Data Sources). CELLINK's Inkredible+ bioprinter redefines the boundaries of accuracy with an XYZ precision of 10x10x100 µm. This exceptional precision grants researchers the power to construct tissue models with unparalleled intricacy and accuracy. EnvisionTEC's 3D-Bioplotter Developer Series ascends to even greater heights, boasting a precision of 1x1x1 µm. This staggering precision paves the way for the creation of highly detailed and intricately structured tissue constructs, setting a new standard for bioprinting precision. RegenHU's R-GEN 100, a paragon of versatility, melds precision with adaptability. Featuring a precision of 0.5x0.5x0.5 µm and a spacious build volume of 130x90x65 mm, this bioprinter embodies a remarkable capacity to cater to an extensive spectrum of tissue types. Its versatility positions it as an invaluable instrument for researchers navigating the diverse landscape of tissue engineering. Taking a departure from convention, Advanced Solution's BioAssemblyBot 200 harnesses the formidable capabilities of a 4-axis robotic arm, introducing a new dimension of motion control precision. This innovative feature empowers the fabrication of intricate tissue structures within its 27.9x17.8x6.9 mm build volume. The system's robotic arm introduces an unparalleled level of flexibility and precision, proving particularly advantageous for intricate tissue engineering applications. Regemat 3D's BIO V1 assumes a prominent role with its impressive XYZ precision of 150x150x0.4 µm. This high degree of precision enables the creation of intricate and detailed tissue constructs within its substantial build volume of 150x160x110 mm. The BIO V1 stands as an invaluable tool for researchers seeking to replicate the intricate architecture of native tissues. Although compact in size, Allevi's Allevi 1 upholds impressive precision, boasting a precision of 7.5x7.5x1 µm. This precision ensures accuracy in the fabrication of smaller tissue models within its build volume of 90x60x130 mm. Despite its smaller footprint, the Allevi 1 proves to be a valuable asset for researchers focused on creating precise and detailed tissue constructs. Beyond precision, the sterilization capabilities offered by these bioprinting systems are paramount. Poietis's NGB-R Bioprinter, equipped with Class II BSC sterilization, ensures aseptic conditions that are indispensable for sensitive applications in tissue engineering and regenerative medicine. Brinter CORE and Brinter ONE leverage UV-C photocuring at 265nm, a wavelength known for its effectiveness in initiating the crosslinking of certain bio-inks. This feature augments the structural integrity of the printed tissues, ensuring they maintain their form and function over time. Additionally, several systems offer automated calibration features, streamlining the setup process and ensuring consistent and reliable printing results. The CELLINK BIO X6 distinguishes itself with support for up to six different bio-inks, a capability of immense value for researchers aiming to create complex, multi-material tissue constructs that closely emulate the heterogeneity found in native tissues. In terms of build volume, EnvisionTEC's 3D-Bioplotter, RegenHU's R-GEN 100, and RegenHU's R-GEN 200 offer expansive workspaces. These ample build volumes provide abundant room for fabricating large and intricate tissue structures, opening up new horizons for tissue engineering applications. Furthermore, precise temperature control proves to be a critical feature when working with temperature-sensitive bio-inks and materials. This feature is offered by several systems, such as advanced solution's BioAssemblyBot 200 and 400, ensuring that the printing process is meticulously optimized for the specific materials being used.

CONCLUSION

The review provides valuable insights into the diverse range of bioprinting methods, systems and their collective potential to advance tissue engineering and regenerative medicine. It highlights the potential for integration with other printing methods and the development of biomimetic materials to further enhance the capabilities of bioprinting technologies. This collective array of capabilities is propelling the field forward, ushering in a new era of possibilities for creating functional and lifelike biological constructs.

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