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REVIEW ARTICLE

3D bioprinting technologies: Current applications and emerging trends

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Abstract

Three-dimensional bioprinting has rapidly advanced as a key technology in tissue engineering and regenerative medicine. While many reviews provide broad overviews of bioprinting techniques and materials, this work offers a focused analysis of the interface between bioprinting technologies and bioink chemistry. It examines how deposition mechanisms—inkjet, laser-assisted, vat-polymerization, and extrusion-based systems—impose constraints on bioink viscosity, cross-linking strategies, and mechanical properties, ultimately influencing printing resolution, construct scale, and cell viability. This review is organized around three fundamental trade-offs: resolution versus construct size, printability versus biological functionality, and scalability versus cell viability. Natural, synthetic, and hybrid bioinks are evaluated not only by composition but also by their physicochemical properties and their role in directing cell fate in neural, vascular, and musculoskeletal applications. Key translational challenges are highlighted, including immunogenicity, foreign body responses, batch-to-batch variability, and the lack of reproducible, Good Manufacturing Practice–compliant manufacturing workflows. Finally, the review identifies priorities for advancing the field, including stimuli-responsive bioinks, real-time monitoring integration, artificial intelligence–assisted design, and multi-material bioprinting. These directions are essential for bridging the gap between laboratory-scale innovation and clinically viable tissue constructs.

Keywords: Bioinks; Tissue engineering; Regenerative medicine; Extrusion bioprinting; Biomedical materials

1. Introduction

The roots of 3D bioprinting lie in the early progress of tissue engineering in the late 20th century. A key milestone was Eugene Bell's 1981 work[1] on *in vitro* living tissue, which demonstrated the feasibility of creating functional tissue substitutes and laid the groundwork for modern regenerative approaches. In parallel, the invention of stereolithography (SLA) by Charles Hull[2] in 1984 introduced 3D printing technology, later adapted for biomedical applications. Soon after, in 1988, Robert Klebe[3] pioneered "cytoscribing," the first demonstration of printing living cells with precision—an early step toward bioprinting. The 1990s and 2000s saw transformative contributions from Robert Langer, who advanced biomaterials and drug delivery,[4] and Anthony Atala, who developed lab-grown tissues and organs.[5] Their work accelerated the integration of tissue engineering with emerging printing technologies. As the field matured, regulatory frameworks also evolved, supporting innovation and ensuring the safe translation of bioprinting research into clinical and industrial applications. Together, these scientific and regulatory milestones shaped the foundation of today's 3D bioprinting.

Regulatory change has reinforced this trajectory. The 2022 United States (US) Food and Drug Administration (FDA) Modernization Act 2.0 removed the mandatory requirement for animal testing in drug development, encouraging the use of cell-based assays, organ-on-chip systems, and computational models as alternatives.[6,7] This creates a direct opening for 3D bioprinting: bioprinted tissue and disease models that mimic human architecture and cellular composition can complement or replace animal data in preclinical testing, particularly when combined with induced pluripotent stem cells (iPSCs) to preserve patient specificity without the ethical constraints associated with embryonic stem cells.[8,9] Given that failures in translating preclinical findings to the clinic are estimated to cost the pharmaceutical industry $28 billion annually[10] and that the global bioprinting market is projected to grow from $1.3 billion in 2022 to $3.3 billion by 2027[11], the convergence of regulatory reform and market demand has turned bioprinting from a research curiosity into an active translational pipeline.[12-15]

These scientific and regulatory milestones, combined with sustained interdisciplinary collaboration across engineering, biology, and medicine, underlie the bioprinting technologies discussed below and the field's transition from proof-of-concept demonstrations toward the industrial and clinical adoption analyzed throughout this review.

One of the fundamental components of the 3D bioprinting process is the bioink, a material crucial to the success of bioprinted constructs. The selection of suitable bioinks is a complex process that requires careful consideration of the application's specific requirements, as different bioinks are classified based on their composition, mechanical properties, and biocompatibility.[16] The importance of selecting the right bioink cannot be overstated, as it directly influences the outcome of the bioprinting process, whether for tissue engineering, drug development, or disease modeling. Recent advancements in the synthesis and formulation of bioinks, particularly those developed in the last five years, have expanded the possibilities for creating more sophisticated and functional tissues. However, a gap remains between the bioinks used in research and those that meet industrial and clinical demands, an issue this review will address.

Three-dimensional bioprinting has already demonstrated significant potential in well-established applications, such as producing skin[17-19], bone[20-22], cartilage[23-25], and vascular tissues.[26-28] However, the technology is also being explored in novel areas that extend beyond traditional biomedical applications, promising to reshape industries such as food production, pharmaceutical development, and cosmetics. In this review, we examine these emerging trends, providing a comprehensive overview of the current state of 3D bioprinting technologies. Additionally, we delve into the biomaterials used in 3D bioprinting, dissecting their classifications, functionalities, and the challenges they present. Bioinks are categorized into three main types: natural, synthetic, and semi-natural, with a focus on their strengths and limitations in creating complex tissue and disease models. By exploring these diverse applications and material considerations, this review aims to highlight both the immense potential of 3D bioprinting and the challenges that must be addressed to fully realize its impact across various industries.

Several recent reviews have already cataloged the breadth of 3D bioprinting technologies, bioinks, and applications in comprehensive detail.[29-32] Rather than providing another broad overview of bioprinting techniques and biomaterials, this review focuses on the critical interface between bioprinting technologies and bioink chemistry. Specifically, it examines how the deposition mechanisms of inkjet, laser-assisted, vat-polymerization, and extrusion-based bioprinting dictate the viscosity, cross-linking strategies, and mechanical properties of compatible bioinks, and how these constraints subsequently determine printing resolution, construct size, and cell viability. Consistent with the definition adopted throughout this review, bioprinting refers to the fabrication of tissue-relevant constructs through the deposition of living cells within a biomaterial matrix. Consequently, cell-free thermoplastic extrusion

techniques, such as conventional fused deposition modeling of polycaprolactone (PCL) or poly(lactic-co-glycolic acid) (PLGA), are excluded unless integrated with cell-laden bioinks or used in hybrid bioprinting approaches. Within this framework, the review is organized around three key trade-offs that govern technology and bioink selection: resolution versus construct size, printability versus biological functionality, and scalability versus cell viability. Particular emphasis is placed on translational challenges—including immunogenicity, foreign body responses, batch-to-batch variability, scalable manufacturing, and compliance with Good Manufacturing Practice (GMP) standards—which remain significant barriers to clinical translation. Addressing these challenges is likely to have a greater impact on the clinical and industrial adoption of bioprinting than the continued development of new printing modalities alone.

2. Primary techniques in bioprinting

In 3D bioprinting, several key techniques have been developed, each offering unique benefits and facing specific challenges. The most explored methods include inkjet-based, laser-assisted, and extrusion-based bioprinting, each playing a critical role in advancing tissue engineering, drug development, and regenerative medicine. Across all of these platforms, a 3D construct is assembled not by a single deposition event but by repeating that event according to a computer-aided design (CAD) or medical-imaging-derived digital model: the print head, laser spot, or projected light pattern traces a 2D path within the X–Y plane to complete one layer, the build stage is then indexed by a defined increment along the Z-axis, and this raster-and-index cycle repeats until the full 3D geometry has been assembled layer-by-layer.

2.1. Inkjet-based bioprinting

Inkjet-based bioprinting, also known as drop-on-demand bioprinting, is one of the earliest adapted techniques for precisely depositing controlled volumes of bioink at specified locations.[33] Originally adapted from conventional 2D inkjet printers, where the ink was replaced with biomaterials and the paper with an adjustable platform, the technology has since advanced to offer greater resolution, precision, and speed in handling biomaterials. In recent developments, researchers have repurposed commercially available HP printers for bioprinting. For example, a method was devised to interface an HP26 inkjet cartridge with a computer, allowing for customized applications of inkjet printing in bioprinting research. This involved capturing the drive characteristics of the HP26 cartridge and creating a driver board that permits precise control over nozzle firing timing and properties, ensuring accurate bioink deposition.[34] Moreover, there have been efforts to enhance print resolution by integrating screw-based servo stages. These innovations led to the development of an on-demand printing mode, which enabled the construction of more intricate 3D structures through layer-by-layer printing based on complex image data.[35] This advancement confirmed the effectiveness of on-demand printing in fabricating sophisticated 3D tissue structures, highlighting the potential of inkjet 3D biofabrication in creating complex biofunctional tissues.

Thermal inkjet printers operate by electrically heating the print head, which creates pulses that force droplets out of the nozzle. Localized heating, reaching 200–300 °C for about 2 μs, has minimal impact on cell viability. Their advantages include high print speed, low cost, and wide availability. However, their challenges include the risk of thermal and mechanical stress on cells, as well as printability challenges, such as low droplet directionality, non-uniform droplet size, nozzle clogging, and unreliable cell encapsulation.[36-40]

2.2. Acoustic inkjet printing

Acoustic inkjet printing uses acoustic waves to form droplets. Piezoelectric inkjet printers apply voltage to a piezoelectric material, causing shape changes that eject droplets. Alternatively, acoustic radiation forces can be used for droplet formation at the air–liquid interface. These printers offer uniform droplet size and directionality while avoiding heat and pressure stress on cells. Open-pool, nozzle-less systems further reduce cell viability loss and prevent nozzle clogging. However, there are concerns about potential cell damage from the 15–25 kHz frequencies used, and material viscosity must be kept low to ensure proper droplet ejection.[41-45]

Inkjet bioprinting is cost-effective, partly due to the ease of modifying 2D printers for 3D use. It provides high resolution (up to 50 μm) and speed, supporting rapid advancements. The technique is compatible with a wide range of biological materials and maintains high cell viability. It also allows precise positioning of cells, with the ability to achieve single-cell-per-droplet resolution. Additionally, inkjet bioprinting can create concentration gradients of cells, materials, or growth factors by varying drop density, enabling the printing of more complex tissue constructs.

More recently, work on inkjet-compatible polymer bioinks has continued to refine the trade-off between printability and post-printing cell proliferation; for example, polyvinylpyrrolidone-based bioinks have been shown to modulate droplet formation and downstream cell proliferation independently of the core cell–encapsulating

hydrogel, illustrating that carrier polymer chemistry, not only cell density, is an active design variable in inkjet bioprinting.[32]

There have been significant advancements in recent years, with researchers exploring its potential for creating functional tissues and organs. Notable applications include the regeneration of functional skin and cartilage *in situ*, where inkjet bioprinting facilitated the deposition of primary or stem cells with uniform density throughout a lesion. These studies demonstrated that high cell viability and functionality could be maintained post-printing, marking a significant step forward in tissue engineering. For instance, research published in 2012 successfully used this approach to regenerate functional skin, highlighting its potential in wound healing and reconstructive surgery.[46] Similarly, in 2011, researchers demonstrated the ability to regenerate cartilage[47], providing a promising solution for repairing joint damage and osteoarthritis.

In addition to soft tissues, inkjet bioprinting has been applied to the creation of more complex structures, such as bone-like tissues. A 2014 study demonstrated the successful 3D printing of bone-like tissue, offering a potential method for repairing or replacing damaged bone.[48] Furthermore, research into neural tissue fabrication has shown excellent cell viability and the potential for creating functional neural networks, as evidenced by a 2016 study that explored the development of neural tissues using inkjet bioprinting.[49] Another significant achievement was the creation of beating cardiac tissue, first demonstrated in 2009, which underscored the capability of inkjet bioprinting to produce tissues that not only mimic the structure but also the function of native tissues.[50] Additionally, advancements in 2017 led to the fabrication of full-thickness skin models with pigmentation, further expanding the applications of inkjet bioprinting in dermatological research and therapy.[51]

Despite these successes, inkjet bioprinting faces several challenges. One common drawback is that the biomaterials used must initially be in liquid form to facilitate droplet formation, but they must also be able to solidify into a 3D structure with the necessary structural organization and functionality.[52,53] This requirement has led to the exploration of materials that can be cross-linked after deposition using chemical, pH, or UV mechanisms. However, some cross-linking methods can slow the bioprinting process or introduce toxic byproducts, reducing cell viability and functionality. Moreover, achieving the appropriate cell density remains a challenge; the concentrations typically used for droplet formation are often lower than needed, as higher cell densities can inhibit some hydrogel cross-linking mechanisms.[54]

Other technical issues include low droplet directionality, unreliable cell encapsulation due to low ink concentration, and limitations related to vertical printing and restricted material viscosities. These challenges suggest that future developments in inkjet bioprinting may involve combining it with other printing techniques to overcome these limitations and improve the precision and reliability of bioprinted constructs.[29]

2.3. Laser-assisted bioprinting

Laser-assisted bioprinting (LAB) is a highly precise technique originally developed for depositing metals onto substrates, but it has since been adapted for biological applications. This method was notably advanced by Odde and Renn[55], who successfully used it to print viable embryonic chick spinal cord cells. In their study, Odde and Renn demonstrated the use of laser-guided direct writing to pattern living cells with micrometer-scale precision. By employing optical forces, embryonic chick spinal cord cells were successfully deposited onto a glass surface, forming small cell clusters. The technique also enabled long-range transport of cells through hollow optical fibers while maintaining cell viability, as evidenced by normal cell adhesion and neurite outgrowth after exposure.

The process of LAB consists of three main components: a donor-slide, a laser pulse, and a receiver-slide. The donor-slide is composed of three layers: a transparent glass substrate, a metal layer, and a bioink layer. When a laser pulse strikes the donor-slide, it vaporizes the hydrogel beneath the metal layer, generating a force that propels the bioink onto the receiver-slide, allowing for precise deposition of cells and biomaterials (Figure 1).

One of the major advantages of LAB is its scaffold-free nature, which minimizes stress on cells and results in very high cell viability, often exceeding 95%.[56] LAB also offers superior resolution, typically ranging from 10 to 50 μm, making it more accurate than many other bioprinting techniques. A 2017 study demonstrated that LAB can accurately deposit single cells per droplet, highlighting its high precision and suitability for controlled cell patterning.[57] Furthermore, LAB's ability to accurately position multiple cell types with high precision makes it particularly promising for complex tissue engineering applications, and it shows great potential when combined with other bioprinting techniques.[58]

However, LAB presents its own set of challenges. The process is expensive, which can limit its accessibility for broader research and clinical applications. Additionally, LAB faces issues related to stability and scalability, making it less suitable for large-scale production compared to other bioprinting methods.[59] These limitations suggest that future advancements in LAB may focus on reducing

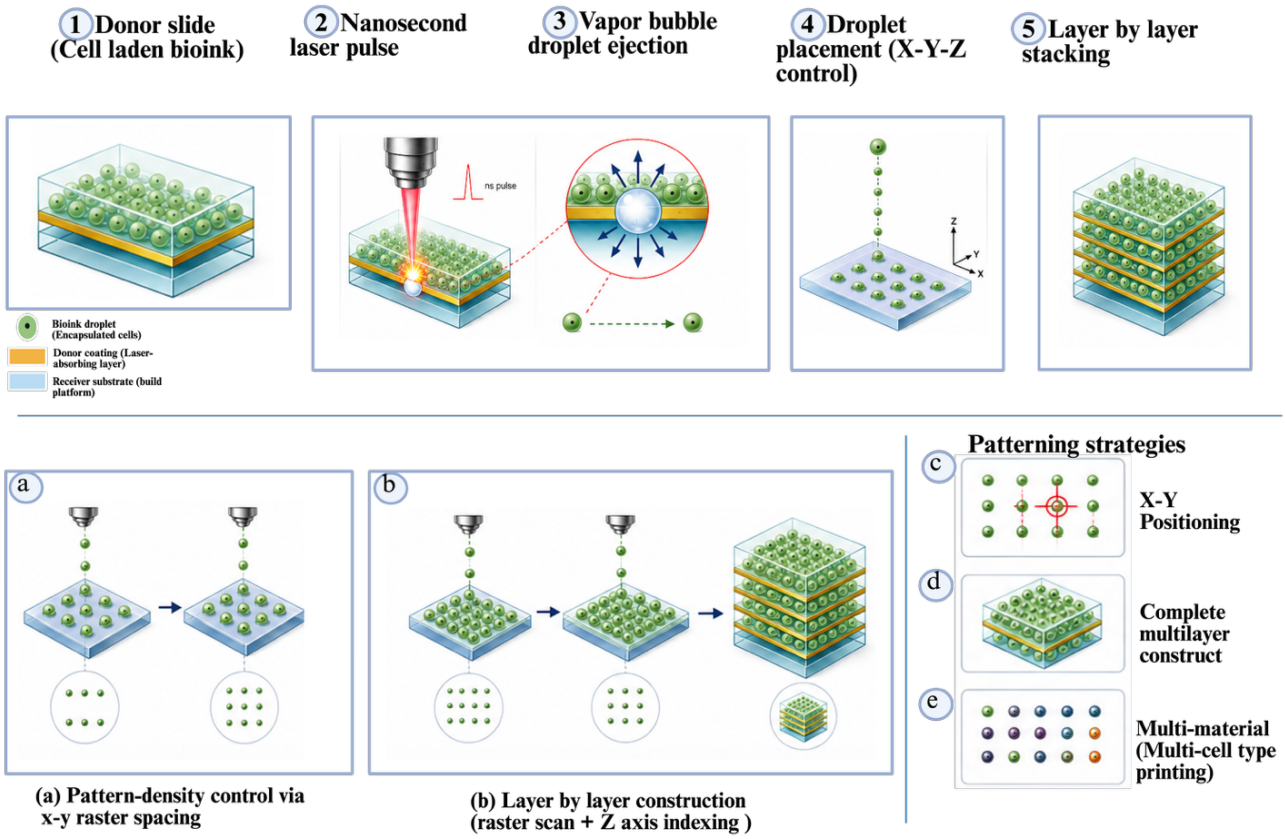

Figure 1. Overview of the laser-assisted bioprinting (LAB) process, from single-droplet transfer to a completed 3D construct. Legend: Green sphere: Encapsulated cell (bioink droplet); Orange bar: Donor coating/laser-absorbing layer; Blue bar: Receiver substrate. Steps 1-5 (top row): (1) the donor slide is coated with a laser-absorbing layer and a layer of cell-laden bioink; (2) a focused nanosecond laser pulse irradiates the absorbing layer through the donor slide; (3) local vaporization of the absorbing layer generates an expanding vapor bubble that ejects a single bioink droplet toward the receiver slide; (4) the droplet lands at a computer-controlled X–Y–Z coordinate on the receiver slide; (5) this single-droplet event is repeated to build a multilayer construct. Bottom panels: (a) Increasing the density of the X–Y raster pattern increases the number of droplets deposited per layer, illustrating pattern-density control. (b) The layer-by-layer construction sequence: a patterned layer is completed on the receiver slide, the build stage is indexed downward by one layer thickness along the Z-axis, and the raster-and-index cycle is repeated until the full multilayer geometry encoded in the source digital model has been assembled. (c) Computer-controlled targeting addresses a specific X–Y coordinate for droplet placement within the array. (d) A completed multilayer construct. (e) Droplets of distinct bioinks or cell types, represented by the differently colored spheres, can be deposited within the same array, illustrating the capacity of LAB for multi-material and multi-cell-type patterning.

costs and improving the scalability of the technique while maintaining its high precision and cell viability.[60]

Recent research has continued to explore the potential of LAB in creating complex and functional biological structures. The technique's high resolution and accuracy make it particularly useful in applications that require the precise placement of cells and biomaterials. A study in 2017 by Keriquel *et al.*[57] explored the use of LAB for *in situ* printing of mesenchymal stromal cells (MSCs) to enhance bone regeneration in a mouse calvaria defect model. The researchers tested different cell printing geometries, demonstrating that a disk-shaped arrangement of MSCs, combined with a collagen and nano-hydroxyapatite matrix, significantly improved bone formation compared to other configurations. The study highlights the potential of LAB to control cell distribution and enhance tissue regeneration by optimizing the geometry of cell deposition. Another study, published in 2013 by Michael *et al.*[61], investigated the use of LAB to create a multi-layered, fully cellularized skin substitute for treating burn wounds. The printed skin constructs, composed of 20 layers of fibroblasts and keratinocytes, were implanted into full-thickness skin wounds in mice and successfully integrated with the surrounding tissue, forming skin-like structures. The

constructs demonstrated good cell survival, proliferation, and initial signs of tissue differentiation, including the formation of blood vessels.[62-66]

More recent work has extended LAB from single-cell-type deposition toward spatially organized multicellular patterning relevant to vascularization: in situ laser-assisted printing of endothelial cells in defined geometric patterns within a mouse calvarial defect produced organized microvascular networks and substantially increased both vascularization and bone regeneration rates relative to randomly seeded controls, directly demonstrating that the spatial precision unique to LAB translates into a measurable, quantifiable *in vivo* functional benefit rather than only a structural one.[67]

2.4. Stereolithography-based bioprinting

Stereolithography-based bioprinting has emerged as a key technique for fabricating complex tissue scaffolds, particularly those requiring high resolution and precise control over their structural properties. This method constructs 3D tissue scaffolds in a layer-by-layer manner, directly utilizing biomedical imaging data to create anatomically accurate models. A crucial element of SLA-based bioprinting is the illumination source, which largely determines the resolution, accuracy, and mechanical properties of the printed constructs. The bioinks and biomaterials used must be photocrosslinkable, meaning they are cross-linked upon exposure to the illumination source.

In the SLA process, bioink is prepared by mixing cells with a photosensitive prepolymer solution. The 3D model is first converted into a stack of slice images, which are then used as input for the bioprinting system. The bioink is dispensed into a petri dish to achieve the desired layer thickness. The slice images from the model are projected onto the dispensed bioink, serving as a mask for cross-linking the material. The uncross-linked portions are subsequently removed, leaving behind a 3D-printed scaffold with encapsulated cells.

This technology offers several advantages: it provides multiscale capability and high resolution, enabling the rapid printing of highly complex scaffolds. This method also allows for the fabrication of implantable scaffolds with anatomically accurate geometry, precisely controlled surface characteristics, and tunable physical and chemical properties. SLA-based bioprinting can be classified based on the type of illumination source used:

(i) Digital light processing (DLP) projection SLA 3D bioprinting: Also known as dynamic projection or maskless projection systems, this method utilizes a digital micromirror device to project patterned light as a dynamic mask.
(ii) Directed laser writing bioprinting: This technique employs two-photon polymerization, allowing direct writing within the volume of a photocrosslinkable hydrogel. It utilizes a pulsed laser source and optical components to focus the laser beam, resulting in highly localized polymerization within the hydrogel.

This technology has proven highly effective in fabricating implantable scaffolds for bone and cartilage regeneration. By using precise masks derived from computed tomography images, SLA has been employed to bioprint bone structures with exceptional accuracy.[68] This technique has enabled the creation of hydroxyapatite implants, where a controlled scaffold architecture supports successful bone tissue regeneration.[69] Additionally, SLA has been used to fabricate 3D cartilage scaffolds that exhibit strong chondrocyte adhesion, with scaffold geometry guiding cellular behavior.[70] The integration of vascularization networks in scaffolds using UV-based microscale SLA has also shown promise for peripheral nerve repair, further expanding SLA's applications in regenerative medicine.[71]

Beyond implantable scaffolds, SLA-based bioprinting has been utilized to develop models for studying cell behavior in 3D microenvironments. Ma *et al.*[72] fabricated liver-module structures in 2016, incorporating human iPSCs and hepatic progenitor cells, offering potential for drug-screening applications. SLA has also been used to investigate the interactions and progression of postmetastatic cancer cells with breast cancer and bone stromal cells, using bioprinted scaffolds on a table-top SLA system.[73] Furthermore, SLA has helped identify factors guiding cell differentiation, as demonstrated by scaffolds promoting neural stem cell adhesion and neurite extension when combined with electrospinning techniques.[74]

2.5. Extrusion-based bioprinting

Extrusion-based bioprinting is a pressure-driven technique where bioink is extruded through a nozzle using pneumatic or mechanical pressure to create predesigned structures or scaffolds. This method is particularly well-suited for printing high-density cell constructs and larger tissue structures, such as those required for hard tissues over 10 mm in size. Hydrogels, commonly used as bioinks, are typically non-Newtonian fluids, meaning their viscosity changes with shear rate. The higher the viscosity of the bioink, the greater the shear stress exerted during extrusion, which can lead to increased apoptotic activity in printed cells. Proper control of viscosity is crucial, as shear-thinning can affect printing quality, leading to poor definition if viscosities are too low.

A key advantage of extrusion-based bioprinting is its ability to print with very high cell densities, making it ideal for creating large, organized constructs within clinically relevant timeframes. Self-healing hydrogels, which maintain their shape due to non-covalent reversible bonds, are often used to improve the structural integrity of printed constructs. Additionally, interpenetrating polymer networks represent an advanced structure for hydrogels, offering enhanced mechanical properties. One of the strengths of extrusion-based bioprinting is that it does not intrinsically require chemical curing during the printing process; however, post-printing cross-linking is commonly applied depending on the bioink. This feature allows detailed investigation of printing parameters such as bioink viscosity, nozzle diameter, and shear stress prior to printing, which is critical for optimizing cell survival and function.

However, extrusion-based bioprinting has some limitations. The resolution is generally over 100 μm, which limits its application for certain soft tissues requiring finer detail. The pressure required for extrusion can potentially alter cell morphology and function, and the associated shear stress may negatively affect cell survivability, metabolic activity, and overall function. Despite these challenges, extrusion-based bioprinting remains a versatile and widely used method, particularly in applications involving hard tissues and large-scale constructs. Careful selection of biomaterials and bioink concentrations is essential to ensure the survival and functionality of the printed cells.

Extrusion-based bioprinting has been successfully applied to the regeneration of complex tissue structures, including the formation of an ear composed of auricular cartilage and fat tissue.[75] This application highlights the technique's ability to create large, anatomically accurate structures with the necessary mechanical properties and cell densities for functional tissue regeneration. By precisely depositing bioinks in predesigned patterns, extrusion-based bioprinting enables the creation of hybrid tissues that combine different cell types and materials, mimicking the intricate composition of natural tissues.

In cartilage tissue engineering, extrusion-based bioprinting has been utilized to bioprint scaffolds composed of PCL, alginate, and chondrocytes. This combination of materials supports the formation of cartilage with enhanced structural integrity and cell viability.[76] Additionally, Pati *et al.*[77] investigated, in 2014, hybrid scaffolds that combine PCL with decellularized extracellular matrix (dECM), providing a supportive environment that closely resembles native tissue.

2.6. Microextrusion bioprinting

Microextrusion bioprinting is a technique widely adopted from nonbiological 3D printing and is increasingly utilized in tissue engineering. These bioprinters typically consist of temperature-controlled material-handling systems, a dispensing system, and a movable stage that operates along the x, y, and z axes. Additional components include a fiberoptic light source for illuminating the deposition area or activating photoinitiators, a video camera for axial command control, and a piezoelectric humidifier. Some advanced systems also feature multiple print heads, allowing for the serial dispensing of different materials without the need for retooling.[78]

Microextrusion bioprinting operates by robotic-controlled extrusion of materials, depositing continuous beads onto a substrate rather than liquid droplets. These beads are laid down in two dimensions, with the stage or print head moving along the z-axis to build up layers that form the final 3D structure. This method is compatible with a wide range of materials, including hydrogels, biocompatible copolymers, and cell spheroids. Common dispensing systems used in microextrusion include pneumatic systems, which are suited for high-viscosity materials and offer simpler drive mechanisms, and mechanical systems, which provide more direct and spatial control over material flow, making them ideal for dispensing high-viscosity hydrogels. Mechanical systems, often piston- or screw-based, are particularly beneficial for scaffold-less tissue spheroid bioprinting, though they typically have reduced maximum force capabilities due to their smaller, more complex components.[79]

Microextrusion bioprinting has several advantages. It is compatible with a wide range of fluid properties and biocompatible materials, from high-viscosity materials that provide structural support to lower-viscosity materials that create a suitable environment for cell viability. The technique's high resolution allows for the fabrication of complex structures designed with CAD software and facilitates the precise patterning of multiple cell types. A key benefit of microextrusion bioprinting is its ability to deposit very high cell densities.[78,80,81]

However, microextrusion bioprinting also has some drawbacks. Cell viability is generally lower than in inkjet-based bioprinting, with survival rates ranging from 40% to 86%, primarily due to the shear stresses imposed on cells in viscous fluids. Additionally, reducing pressure to mitigate shear stress often results in a loss of resolution and print speed, posing challenges in achieving high-resolution and rapid fabrication simultaneously.[81]

Microextrusion bioprinting has been successfully used to fabricate complex tissues such as aortic valves. In 2013, Duan *et al.*[82] bioprinted valve conduits from alginate/gelatin hydrogels, replicating the mechanical and biological properties of native valves. This precise layering of materials demonstrates the versatility of microextrusion in creating anatomically accurate, functional implants for cardiovascular applications.

Beyond tissue fabrication, microextrusion bioprinting is also advancing the development of *in vitro* models for drug testing and disease research. For example, direct cell writing of 3D micro-organs has been employed to create pharmacokinetic models, enabling more accurate predictions of drug absorption, distribution, metabolism, and excretion.[83] This application is particularly valuable for screening potential drug candidates and optimizing dosage forms. Additionally, this technology has been utilized to develop 3D *in vitro* cancer models, such as an ovarian cancer co-culture model.[84] This high-throughput cell patterning platform allows researchers to study tumor growth, cell interactions, and treatment responses in a controlled environment, providing insights that are crucial for developing new cancer therapies.

2.7. Vat-polymerization bioprinting

Vat-polymerization bioprinting is a precision technique that uses laser-induced photopolymerization to fabricate 3D structures, utilizing methods such as SLA.[85] This approach is known for its ability to produce highly complex and intricate scaffolds with smooth surfaces and high resolution, making it ideal for applications requiring fine detail, such as customized implants and tissue models. Vat-polymerization bioprinting enables rapid and precise 3D printing, but it is limited by the need for photopolymer materials, which restricts material choices and can be costly.[86] Additionally, there are challenges related to the potential cytotoxicity of photoinitiators, light scattering limitations for thicker constructs, and maintaining cell viability due to exposure to light during the process. Despite these challenges, vat-polymerization bioprinting remains a valuable technique in tissue engineering and regenerative medicine due to its capacity to handle complex geometries and produce detailed, high-resolution structures.[31] A considerable amount of research has been carried out on vat-polymerization bioprinting for tissue regeneration, covering a variety of tissues such as bone[87], skin[88], liver[89], heart[89], and blood vessels.[90]

Mechanistically, vat-polymerization bioprinting relies on light-triggered free-radical (or, less commonly, cationic) chain polymerization: a photoinitiator dissolved in the resin absorbs photons at a specific wavelength and cleaves to generate reactive radicals, which propagate through the reactive end-groups of the prepolymer (e.g., the methacrylate groups of gelatin methacryloyl [GelMA] or poly(ethylene glycol) diacrylate [PEGDA]) to form a cross-linked network within the illuminated volume. Because polymerization only occurs where the light dose exceeds a material-specific gelation threshold, spatial resolution is governed by how tightly that light can be focused and confined—the pixel size of the digital micromirror device in DLP systems, the raster/point-scanning laser spot in laser-based SLA, or the diffraction-limited focal volume of the femtosecond pulse in two-photon polymerization—rather than by nozzle or needle dimensions as in extrusion or inkjet systems. This is what allows vat-polymerization bioprinting to reach the highest resolutions among the bioprinting modalities discussed here, but it also means resolution degrades with construct depth as scattering and absorption reduce light penetration, a key limitation for thick, cell-dense constructs.[77-79]

Read together, these five modalities illustrate a set of recurring trade-offs rather than a simple hierarchy of "better" and "worse" techniques. Resolution and printable construct size trade off directly against one another: vat-polymerization and LAB reach single-cell to tens-of-micron precision but are impractical for centimeter-scale, high-throughput constructs, whereas extrusion-based and microextrusion systems scale readily to clinically relevant volumes at the cost of an order-of-magnitude loss in resolution. Printability and biological functionality trade off similarly: bioinks formulated for high shape fidelity (higher viscosity, higher polymer/cross-linker content) generally impose greater shear or light-dose stress on encapsulated cells, while formulations optimized for cell viability tend to print with lower structural fidelity. Finally, scalability and cell viability rarely improve together—nozzle-based methods that increase throughput by using larger nozzles or higher pressures typically do so at the expense of shear-induced cell damage. Table 1 summarizes representative quantitative ranges for these parameters across techniques to make this trade-off structure explicit.

2.8. FRESH bioprinting

Freeform reversible embedding of suspended hydrogels (FRESH) bioprinting is a technique that uses a thermoreversible support gel to provide temporary support

for soft, delicate structures during the printing process.[91,92] This support gel stabilizes the printed structure and can be melted away afterward, leaving the final construct intact. This technology is implemented on an extrusion-based bioprinter that has been modified with a custom syringe designed for precise hydrogel deposition. This method is particularly advantageous for creating intricate, soft tissue structures that require support during printing but must remain flexible and functional after the support material is removed.[93] It allows for the creation of complex and delicate geometries that would be difficult to achieve with other methods, making it a powerful tool in tissue engineering and regenerative medicine.[94] FRESH bioprinting has revolutionized the field, enabling high-resolution printing of complex structures, such as whole organs, as evidenced by Lee *et al.*[95], who enhanced this method to FRESH 2.0, allowing the construction of a detailed vascular tree from a CAD model and functional cardiac structures demonstrating contractions and native-like calcium handling. Similarly, Noor *et al.*[96] advanced the technology by bioprinting an entire heart model from decellularized omentum and patient-derived iPSCs, pushing toward fully personalized organ fabrication. Each of these bioprinting technologies presents strengths that make them suitable for specific applications, but they also come with inherent limitations that must be considered when selecting the appropriate method. As 3D bioprinting evolves, ongoing research aims to overcome these limitations, enhancing the precision, efficiency, and applicability of each technique. **Section 3** explores recent advancements and challenges associated with these bioprinting methods, providing a comprehensive understanding of their roles in the current landscape of bioprinting technology. The above literature highlights different 3D bioprinting technologies and is summarized in Table 2.

3. Bioinks

Advancing 3D bioprinting technologies hinges on the development of bioinks that closely mimic the extracellular matrix (ECM) of natural tissues. Bioinks, the essential materials that replace the ECM in bioprinting, are crucial for enabling the precise deposition of living cells alongside biocompatible materials in a layer-by-layer fashion. These bioinks form the scaffold that integrates living cells with supportive materials, facilitating the creation of complex tissue structures.

The success of bioinks largely depends on their ability to replicate the physical, mechanical, and biological

Table 1. Comparative quantitative performance metrics of major 3D bioprinting technologies

Technology	Typical resolution	Typical print speed/ throughput	Bioink viscosity range	Reported cell viability	Representative scale/ Notes	Representative post-print structural/ mechanical performance
Inkjet/Droplet-based	10–80 µm[29,32]	Fast, drop-on-demand (up to thousands of drops/s)[32]	Low viscosity, <10 mPa·s (acoustic)[41-45]	74–90%[29,97]	Best for thin, low-viscosity constructs; single-cell to few-cell droplets	Weak, low-viscosity hydrogels; requires post-deposition cross-linking for any shape retention; poor unsupported vertical build
Laser-assisted (LAB)	5–50 µm; single-cell precision[32,56,67]	Slow-moderate; limited by pulse rate and stage travel[59]	High viscosity tolerated (nozzle-free)[56]	>90–95%[56,67]	Best for precise, small-area cell patterning (e.g., *in situ* prevascularization)[67]	Scaffold-free transfer preserves fine spatial patterning; final construct strength is set entirely by the receiving bioink/matrix (e.g., collagen–hydroxyapatite) rather than by the print step itself
Vat-polymerization (SLA/DLP/2PP)	2–50 µm[85,97]	0.6–840 mm^3/s (DLP); slower for point-scanning 2PP[97]	Low-viscosity photocrosslinkable resins only[31,85,86]	75–95%[97]	Best for anatomically accurate, high-resolution scaffolds; limited build depth	High shape fidelity from covalent, photocrosslinked networks; mechanically tunable via resin chemistry, but thick-section strength is limited by light penetration with depth

(*Cont'd...*)

Table 1. (*Continued*)

Technology	Typical resolution	Typical print speed/ throughput	Bioink viscosity range	Reported cell viability	Representative scale/ Notes	Representative post-print structural/ mechanical performance
Extrusion-based	≥100 μm, commonly 200–500 μm[29,85]	0.008–63 mm^3/s depending on nozzle/ pressure[97]	High viscosity, shear-thinning hydrogels[85]	40–90%[29,97]	Best for large, high-cell-density constructs and hard tissues	Shear-thinning, high-polymer-content bioinks give good shape fidelity; self-healing hydrogels and interpenetrating polymer networks further improve post-print structural integrity, at the cost of higher shear stress on cells
Microextrusion	100–500 μm[78,85]	Moderate; trade-off between resolution and speed[81]	Wide range, including spheroids and pastes[78-81]	40–86%[81]	Best for multicellular spheroid/organoid assembly	Structural integrity is more dispensing-system-dependent than standard extrusion (pneumatic vs. piston/screw); resolution-fidelity trade-off is more pronounced
FRESH (embedded/ support-bath)	100–200 μm (extrusion-limited)[91,92]	Moderate; support-bath preparation adds time[92,93]	Compatible with very low–viscosity bioinks (e.g., collagen) owing to support bath[91]	>90%[91,95]	Best for soft, low-viscosity, free-form geometries (e.g., vascular trees, whole-organ scale models)	Thermoreversible support bath enables shape retention for very low–viscosity, mechanically weak bioinks (e.g., collagen, fibrin) that cannot self-support unaided; final mechanical strength depends on the cross-linking chemistry applied after support removal

Note: Ranges are drawn from representative primary studies and quantitative reviews cited in the text and are intended as indicative bands rather than fixed values, as exact figures vary with bioink formulation, cell type, and printer hardware.
Abbreviations: DLP: Digital light processing; FRESH: Freeform reversible embedding of suspended hydrogels; LAB: Laser-assisted bioprinting; SLA: Stereolithography; 2PP: Two-photon polymerization.

Table 2. Summary of 3D bioprinting technologies

Technology	Description	Advantages	Disadvantages	Applications
Inkjet/ Droplet-based bioprinting[29,48]	Used for both biological and non-biological applications to deliver controlled volumes of liquid. Modified 2D ink-based printers were first used in bioprinting. Includes thermal inkjet and acoustic inkjet printers.	• Low cost • High resolution (up to 50 μm) • High speed • High cell viability • Compatibility with many biological materials • Precision positioning of cells • Concentration gradients of cells/ materials/growth factors • Wide availability	• Material must be in liquid form • Post-deposition cross-linking required • Low droplet directionality • Vertical printing and restricted viscosities • Risk of thermal/mechanical stress (thermal) • Nonuniform droplet size (thermal) • Frequent clogging of nozzle (thermal) • Viscosity limitations (<10 centipoise) (acoustic)	• Regeneration of functional skin[29] and cartilage[47] • 3D printed bone-like tissue[48] • High potential for neural network creation[49] • Beating cardiac pseudo tissue[50] • Full thickness skin models with pigmentation[98]

(*Cont'd...*)

Table 2. (*Continued*)

Technology	Description	Advantages	Disadvantages	Applications
Laser-assisted bioprinting (LAB)[30]	Developed for depositing metals, adapted for embryonic chick spinal cord cells. Consists of donor-slide, laser pulse, and receiver-slide.	• Scaffold-free • High cell viability (>95%) • High resolution (10–50 μm) • Accurate positioning of multiple cell types	• Expensive process • Low stability and scalability • Loss of unused donor-slide bioink/ cells not transferred during transfer (light-based cell wastage)	• Printing of mesenchymal stem cells for bone regeneration[30] • Skin tissue regeneration using keratinocytes and fibroblasts[61]
SLA-based bioprinting[30,99]	Fabricates 3D structures in a layer-by-layer manner using controlled illumination sources. Uses photosensitive prepolymer solution mixed with cells.	• Multi-scale capability • High resolution • Rapid printing • Anatomically accurate scaffolds • Tunable properties based on illumination source	• Photocrosslinkable materials required • Potential UV damage to cells • Slow process for large constructs • High equipment cost • Unpolymerized bioink and non-incorporated cells discarded during post-processing (light-based cell wastage)	• Rapid bioprinting of bone structures[100] • Cartilage scaffold fabrication.[70] • Scaffolds with vascularization for nerve repair[71] • Liver structure for drug screening[72] • Cancer cell interaction studies[73] • Neural stem cell scaffolds[74]
Extrusion-based bioprinting[30]	Uses pneumatic or mechanical pressure to extrude bioink through a nozzle for scaffold fabrication.	• High cell densities • Applicable for larger hard tissues • Organized constructs at clinically relevant sizes • Detailed viscosity, nozzle diameter, and shear stress studies before printing	• Limited resolution (>100 μm) • Extrusion pressure may alter cell morphology and function • Shear stress may affect cell morphology and activity	• Ear regeneration with auricular cartilage[75] • PCL–alginate–chondrocyte scaffold for cartilage[76] • Hybrid scaffolds (PCL & decellularized extracellular matrix)[77] • Fabrication of cell-laden hydrogels with optimized mechanical strength and high shape fidelity for tissue engineering applications[101-104]
Microextrusion bioprinting[30]	Uses temperature-controlled material handling and robotic extrusion of bioink onto a substrate. Often involves pneumatic or mechanical dispensing systems.	• Wide range of compatible fluids • High cell densities • Deposition of multicellular spheroids • Compatible with various hydrogels and biocompatible materials	• Lower cell viability compared to inkjet • Shear stresses in viscous fluids • Challenges in increasing resolution and print speed	• Aortic valves[82] • Branched vascular trees[105,106] • *In vitro* pharmacokinetic[83] • Tumor models[84]

(*Cont'd...*)

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Table 2. (*Continued*)

Technology	Description	Advantages	Disadvantages	Applications
Vat-polymerization bioprinting[86]	Uses laser-induced photopolymerization for 3D structure fabrication. Includes SLA, digital light processing (DLP), continuous liquid interface production (CLIP), and computed axial lithography (CAL) methods.	• Enables rapid and precise 3D printing • Handles highly complex scaffolds	• Limited material choices due to the need for photopolymers • High cost of photopolymer resins • Potential cytotoxicity of photoinitiators • Light scattering limitations for thick constructs • Uncross-linked resin/cell suspension outside the build volume is discarded, wasting non-incorporated cells	• Implantable scaffolds with controlled architecture
FRESH bioprinitng[92]	Uses a thermoreversible support gel that can support soft, delicate structures during printing and can be later melted away. It is implemented on an extrusion-based bioprinter modified with a custom syringe designed for precision hydrogel deposition.	• Built on open-source hardware and software • Highly adaptable • Cost effective • Ability to 3D print a range of hydrogels • High cell viability (generally >90%)[93,95]	• Time-consuming support preparation • Limited to thermally sensitive bioinks	• Human femur from computed tomography data[92] • Full-size model of the human heart[94] • Cardiac tissue for *in vitro* pharmacology[107]

Abbreviations: FRESH: Freeform reversible embedding of suspended hydrogels; PCL: Polycaprolactone; SLA: Stereolithography.

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properties of the target tissue.[108] Natural bioinks, derived from sources such as collagen, gelatin, and Matrigel, are highly valued for their inherent similarity to the natural ECM. Their non-toxic, biocompatible, and biodegradable properties make them well-suited for various tissue engineering applications. However, a significant limitation of natural bioinks is their low mechanical strength, which can result in poor structural fidelity during printing.[109] Conversely, synthetic bioinks offer greater control over mechanical properties and enhanced structural integrity; however, they often lack inherent bioactivity, such as cell-adhesive motifs, unless they are functionalized, which can limit effective tissue integration.[110] Semi-natural bioinks, which are hybrids of natural and synthetic materials, aim to combine the best of both worlds by offering tunable mechanical properties while retaining the advantageous biocompatibility and bioactivity of natural inks.

Initially, bioinks were primarily composed of "natural" materials obtained from sources such as collagen derived from animal and human tissues.[111] While these natural biomaterials offer desirable properties for tissue scaffolds, their use is often limited by immunogenicity.[112,113] Understanding the immunogenicity and antigenicity of these materials is critical for their successful application. Immunogenicity refers to the ability of a material to trigger an immune response, while antigenicity relates to its recognition by specific antibodies. When introduced into the body, natural biomaterials are often perceived as foreign by the immune system, leading to potential cellular and humoral immune reactions. Factors such as the species source, processing methods, and structural characteristics of these materials influence their antigenicity.

To address these challenges, synthetic biomaterials have been developed to offer more control over composition and properties, minimizing immunogenic potential. These materials can be engineered with precise chemical structures and surface characteristics to reduce immune recognition. For example, biocompatible polymers such as poly(ethylene glycol) (PEG) can be used to decrease the likelihood of an immune response, while PLGA provides tunable degradation rates and mechanical properties with low immunogenicity.[114] Similarly, PCL is known for its excellent biocompatibility. The choice of bioink is crucial, as it must align with the specific requirements of the intended application, balancing factors such as mechanical strength, biocompatibility, rheological properties, and compatibility with the chosen printing method.[115]

Natural biomaterials, such as collagen, gelatin, alginate, fibrin, hyaluronic acid, chitosan, agarose, and silk fibroin, are frequently used in bioinks due to their biocompatibility and resemblance to the natural ECM.[116-119] These materials are often employed in extrusion-based bioprinting due to their shear-thinning properties, which allow for smooth extrusion through the nozzle. Collagen, the primary structural protein in mammalian tissues, is widely utilized for its ability to support cell adhesion and tissue regeneration.[52] It is particularly effective in SLA-based bioprinting where its gelation properties under light exposure are advantageous. Gelatin, a derivative of collagen, is favored for its biodegradability and low antigenicity.[120] Modified forms such as GelMA[120] are used in SLA and DLP due to their photocrosslinkable properties, allowing for the creation of detailed, layered structures. Alginate[117], derived from brown seaweed, is commonly used in extrusion-based and inkjet-based bioprinting due to its rapid gelation under physiological conditions. However, its lack of cell-adhesive properties necessitates modifications, such as the addition of RGD peptides, to enhance bioactivity.[121] Fibrin, which plays a key role in wound healing and tissue regeneration, is often used in LAB due to its ability to form stable, cell-laden constructs.[122] Other natural materials such as hyaluronic acid[123], chitosan, agarose, and silk fibroin offer unique properties such as hydration, antimicrobial effects, and mechanical strength, making them versatile options for various printing methods and tissue engineering applications.[124,125]

Synthetic bioinks address the limitations of natural materials by offering greater control over mechanical properties and degradation rates. These bioinks are commonly used in extrusion-based and SLA-based bioprinting methods, where precise control over material flow and solidification is required. PEG is widely used for its ability to form hydrogels with tunable characteristics, making it suitable for SLA-based bioprinting.[126] PLGA provides a balance of mechanical strength and biodegradability, making it ideal for extrusion-based methods where structural integrity is critical. PCL is known for its excellent biocompatibility and mechanical properties, particularly in applications requiring long-term structural support, such as bone and cartilage regeneration[127], which are typically fabricated using extrusion-based bioprinting. Poly(vinyl alcohol) (PVA) and poly(hydroxyethyl methacrylate) (pHEMA) are also used in extrusion-based and SLA-based bioprinting, valued for their hydrophilic properties and ability to form hydrogels with good mechanical strength.[128,129]

It is worth noting that several of these synthetic polymers—particularly PCL and PLGA—are also widely used as cell-free thermoplastic scaffold materials deposited by conventional fused deposition modeling, a process that, by itself, does not qualify as bioprinting under the definition used in this review because it does not co-deposit

living cells. We include PCL, PLGA, and related synthetic polymers here specifically in their capacity as components of cell-laden or hybrid bioinks (e.g., PCL–alginate–chondrocyte or PCL–dECM composites, discussed in Section 3), not as a general endorsement of cell-free thermoplastic scaffolding as a bioprinting method.

Hybrid and composite bioinks, which combine natural and synthetic materials, are emerging as promising solutions to the limitations of using either type alone. These bioinks are compatible with a variety of printing methods, including extrusion-based and SLA-based bioprinting, depending on their composition. For example, GelMA blended with hyaluronic acid enhances viscosity and mechanical properties, making it suitable for SLA-based methods.[130,131] Alginate–polyacrylamide hydrogels and silk fibroin–PEG hydrogels are used in extrusion-based bioprinting to leverage the strengths of both natural and synthetic components, providing hybrid materials that support structural integrity and cell viability.[132,133] Composite bioinks, incorporating elements such as nanoparticles or growth factors, expand the capabilities of bioprinting by enabling the creation of more complex and functional tissue models. These bioinks are often employed in laser-assisted and SLA-based bioprinting to create structures with enhanced biological activity and mechanical properties.[134]

The development of bioinks remains a critical and evolving area of research in 3D bioprinting, with significant opportunities for further exploration. While current advancements have leveraged the unique properties of natural, synthetic, and hybrid materials, aligning these with appropriate bioprinting technologies continues to present challenges and opportunities. The quest to perfect bioinks that can faithfully mimic the ECM, support cell viability, and maintain structural integrity in complex tissue constructs is far from complete. Ongoing research is essential to address existing limitations, including enhancing mechanical properties, improving biocompatibility, and expanding the range of printable tissues.

Beyond this compositional classification, bioink chemistry and mechanics act directly on cell fate through mechanotransduction and biochemical signaling, and the optimal formulation is tissue-specific rather than universal. For neural applications, very soft substrates (storage modulus in the low hundreds of Pa to a few kPa) are generally required to support neurite outgrowth and prevent glial overactivation, favoring peptide- or hyaluronic acid–based bioinks with minimal stiffness rather than mechanically robust synthetic networks. For vascular constructs, bioinks must instead balance sufficient stiffness and burst pressure resistance to withstand physiological flow with a degradation profile slow enough to allow endothelial lumen formation and remodeling, which is why GelMA–hyaluronic acid and collagen–fibrin blends are favored over stiffer, slowly degrading synthetic hydrogels in this application. For musculoskeletal tissues (bone and cartilage), higher stiffness and mineral- or growth-factor-loaded composites (e.g., hydroxyapatite–alginate–gelatin systems) are needed to direct osteogenic or chondrogenic differentiation and to bear mechanical load during maturation. Considered this way, the natural/synthetic/hybrid classification used above describes what a bioink is made of, but it is this function-oriented mapping between mechanical/chemical cues and cell fate that determines whether a given material choice will actually support the intended tissue outcome, and it is this mapping, rather than material class alone, that should guide bioink selection in practice.[135-142]

3.1. Chemistry of bioinks

The development of bioinks for 3D bioprinting is a dynamic and ongoing area of research, with a strong focus on the chemical processes that enable these materials to form stable, functional structures. Central to this process is the method of cross-linking, which solidifies the bioink and provides the mechanical integrity necessary for maintaining the printed construct's shape and functionality. Cross-linking methods include chemical cross-linking, photocrosslinking, enzymatic cross-linking, and self-assembly, each offering distinct advantages depending on the application.

Cross-linking strategies in bioink chemistry are generally divided into covalent (chemical) and physical (ionic, thermal, or supramolecular) mechanisms, and the two are not interchangeable in either bond strength or reversibility. Chemical cross-linking employs agents such as glutaraldehyde or carbodiimide chemistries to form irreversible covalent bonds between biomaterial molecules; this approach is particularly effective for protein-based materials such as collagen, which benefit from the robust, stable networks formed through covalent bonding.[143,144] Alginate, by contrast, is not chemically cross-linked at all under standard bioprinting conditions: it gels through ionotropic (physical) cross-linking, in which divalent cations such as Ca^{2+} bridge adjacent guluronate blocks to form reversible, non-covalent "egg-box" junctions.[145] This distinction has practical consequences. Calcium-mediated alginate gelation is rapid and cytocompatible, making it attractive for extrusion and inkjet bioprinting, but because the cross-links are ionic rather than covalent, the resulting scaffolds are comparatively weak, prone to ion exchange with physiological fluids, and susceptible to

gradual de-cross-linking and mechanical softening *in vivo*, the opposite of the resilient, long-term mechanical stability that covalent chemical cross-linking can provide.[145]

Photocrosslinking is another widely used method, utilizing light-sensitive compounds within the bioink that react to UV or visible light, triggering rapid gelation.[146] This method is particularly advantageous for creating intricate structures with high precision. For example, hyaluronic acid–based bioinks and GelMA are often used in SLA[147] and DLP bioprinting, where controlled light exposure induces cross-linking, allowing for the fabrication of detailed, layer-by-layer constructs.[148]

Enzymatic cross-linking is an innovative approach that uses enzymes to promote the formation of cross-links within the bioink. This method is ideal for sensitive, cell-laden bioinks where maintaining cell viability is crucial. Enzymes such as horseradish peroxidase (HRP) and transglutaminase are commonly used to catalyze cross-linking reactions in bioinks composed of materials such as chitosan, gelatin, and hyaluronic acid.[149] The advantages of enzymatic cross-linking include high biocompatibility and tunable gelation kinetics, which make it particularly useful for applications such as injectable hydrogels and complex 3D bioprinting.[150,151] For example, dextran–tyramine hydrogels cross-linked with HRP/H_2O_2 have shown promise in cartilage regeneration, offering suitable mechanical properties and biocompatibility.

Self-assembly is another critical process in bioink formation, particularly for peptide-based bioinks.[152] Peptides such as diphenylalanine (FF) and Fmoc-diphenylalanine (Fmoc-FF) can organize themselves into desired structures based on their inherent sequences, forming networks without the need for additional cross-linking agents.[153] This self-assembly is driven by mechanisms such as electrostatic interactions, hydrogen bonding, and hydrophobic interactions, which allow the peptides to form stable nanostructures like nanotubes and hydrogels. Self-assembling bioinks are particularly advantageous for applications where maintaining a biocompatible environment is essential, as they eliminate the need for potentially toxic cross-linking agents.[101,104]

The structural arrangement of the network in bioinks significantly influences their performance in 3D bioprinting. Different polymer architectures, including linear, branched, cross-linked, star, and dendrimer polymers, each contribute unique properties to the bioink, affecting factors such as viscosity, mechanical strength, and degradation rates.

Linear polymers, such as PEG and PVA, are characterized by their chain-like structure, where monomers are linked end to end and can be blended to modulate the structural and mechanical properties of the resulting polymer networks.[154-156] These polymers are commonly used in bioinks for their simplicity and ease of modification.

Branched polymers, such as hyperbranched polyglycerol, consist of linear chains with additional branches, influencing properties such as density and stiffness. In bioinks, branched polymers enhance the viscosity and shear-thinning behavior, which is vital for maintaining print resolution and ensuring that the bioink flows easily through the printer nozzle while quickly solidifying to hold the intended structure. These properties are particularly useful in applications where precise control over the bioink's flow and deposition is required.[157,158]

Cross-linked polymers form a 3D network through covalent bonds between linear chains, resulting in enhanced rigidity and mechanical strength.[159] Examples include alginate and GelMA, which are widely used in applications such as cartilage and bone tissue engineering, where structural integrity is critical.[160] The cross-linked structure provides the necessary mechanical strength and stability, allowing the printed constructs to retain their shape under mechanical stress.

Star polymers, such as star-shaped PEG, feature multiple linear chains extending from a central core, leading to bioinks with highly cross-linked networks. These polymers improve the mechanical properties and control the degradation rates of hydrogels, making them ideal for creating constructs that need to maintain their structural integrity over extended periods. Star polymers are particularly useful in tissue engineering applications where the bioink must support the long-term function of the printed tissue.[161]

Dendrimers are highly branched, spherical polymers that surround a central core with repeating units. Dendrimers such as polyamidoamine (PAMAM) are employed in bioinks for their high functionality and ability to form dense, uniform networks. This structure allows for precise control over the bioink's mechanical properties and the release of bioactive agents, making dendrimers suitable for drug delivery systems and therapeutic applications. The dendritic architecture enhances the precision of drug delivery, providing controlled release of bioactive molecules, which is particularly valuable in creating bioinks for regenerative medicine.[162,163]

The chemistry of bioinks, including the various cross-linking methods and polymer structures, plays a pivotal role in determining the success of 3D bioprinting applications. Each cross-linking technique and polymer architecture offers distinct advantages that can be tailored

to meet specific requirements, whether it be for soft tissue engineering, bone regeneration, or drug delivery systems.

4. Translational and manufacturing barriers

Chemical sophistication does not by itself guarantee clinical or industrial translatability. Natural and hybrid bioinks sourced from animal or human tissue (collagen, gelatin, dECM) retain a degree of immunogenicity and can provoke a foreign body reaction once implanted, and the magnitude of this response depends on species source, processing method, and residual cellular debris, factors that are difficult to standardize across batches and suppliers.[112,113] Batch-to-batch variability is a related and often underestimated barrier: naturally derived polymers vary in molecular weight, degree of substitution (e.g., methacrylation in GelMA), and endotoxin content between lots, which directly changes rheology, cross-linking kinetics, and printability, and is a major reason why protocols optimized in one laboratory frequently fail to reproduce in another.[109,110,164,165]

These material-level barriers compound with process-level ones. Few bioprinting workflows currently operate under, or are designed for, GMP-compatible conditions: closed-system bioink handling, in-line quality control (e.g., real-time rheological or optical monitoring of extrusion), and validated sterilization routes remain largely absent from published bioprinting workflows.[164,166] Reproducibility across printers, operators, and sites is similarly under-addressed—print parameters (nozzle geometry, pressure, temperature, light dose) are rarely reported with sufficient detail to allow independent replication, and few studies benchmark inter-batch or inter-printer variability quantitatively. Closing this gap will require standardized reporting of bioink and process parameters, industry–academic collaboration on GMP-compatible bioprinting platforms, and regulatory frameworks specific to bioprinted products, none of which are yet mature enough to support routine clinical translation.[164,166,167]

5. Novel applications of 3D bioprinting

5.1. Beyond biomedicine: A brief outlook on adjacent applications

Three-dimensional bioprinting techniques and bioinks developed for tissue engineering have also been adapted, in modified form, to two non-biomedical domains: extrusion-based food printing and biomimetic coral-restoration substrates. We mention these only briefly and outside the review's core scope, since neither involves the deposition of living cells together with a biomaterial carrier in the sense used elsewhere in this article, and both are reviewed in depth elsewhere.[168-176,177-181] Food printing repurposes extrusion, hot-melt, and hydrogel-forming print heads—hardware directly descended from extrusion

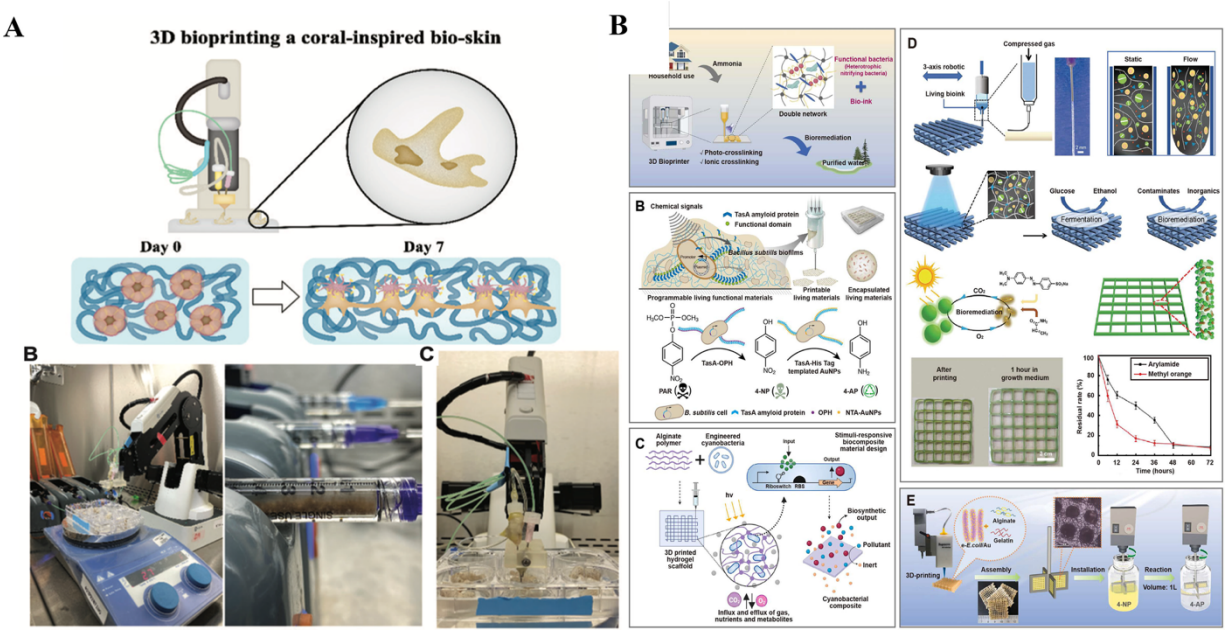

Figure 2. 3D bioprinting of biofunctional living materials for biological and environmental applications. (A) 3D bioprinting of coral-polyp bio-skin using ultrashort and biofunctionalized peptide bioinks for transplantation on coral skeletons. Reprinted from Valle-Pérez *et al.*[182] (B) 3D bioprinting of microbial-based living materials for advanced energy and environmental applications. Reprinted from Pu *et al.*[183]

bioprinting—to deposit edible hydrocolloids, starches, and protein isolates for personalized nutrition, but it remains constrained by scale-up, regulatory, and consumer-acceptance barriers unrelated to cell biology.[168-176] Coral restoration adapts biomimetic, calcium carbonate–based 3D printing and peptide-functionalized bioinks (Figure 2) to fabricate settlement substrates that promote coral larval attachment; several of these substrates do incorporate living coral tissue or peptide bioinks developed in parallel with this group's biomedical work, which is the main point of methodological overlap with the rest of this review.[177-181] Collectively, these examples illustrate the growing translation of bioprinting hardware and bioink technologies from biomedical research into adjacent manufacturing sectors. Nevertheless, a comprehensive evaluation of the technological advances and associated challenges in these fields falls outside the scope of the present review.

5.2. Tissue engineering

Three-dimensional bioprinting is quickly becoming one of the most powerful tools in tissue engineering, allowing scientists to build complex, personalized tissue structures with a high level of precision. This technology works by carefully layering bioinks, mixtures that usually contain hydrogels, living cells, and sometimes additional biological molecules or nanoparticles, to form scaffolds that closely resemble the body's natural tissues.[29,102,184] Over the past few years, major progress has been made in developing more advanced bioinks. For example, some bioinks now incorporate components such as dECM, exosomes, graphene, and peptide hydrogels, all of which help improve cell survival, tissue development, and how well the printed tissue works in the body.[52,184,185] Thanks to these advances, modern bioprinters are now capable of printing vascularized tissues that are substantially thicker than what was possible in the past, a breakthrough made possible by cutting-edge vascularization methods from research hubs such as the Wyss Institute.[186] These strides bring us closer to the long-term goal of printing fully functional, transplantable organs.

5.2.1. Key applications

The technology and bioink choices reported for each tissue type below follow directly from the mechanical and biochemical design requirements introduced in **Section 3** (soft, low-modulus formulations for neural applications; burst-resistant, slowly degrading networks for vascular constructs; and stiff, mineral- or growth-factor-loaded composites for bone and cartilage). The following subsections therefore evaluate not only what has been demonstrated for each tissue system, but also how closely each demonstration approaches the mechanical, vascularization, and long-term functional benchmarks required for clinical translation, a question revisited critically in the synthesis that closes this section and **Section 5.2.2**.

(a) Bone tissue engineering

Three-dimensional bioprinting has ushered in a transformative era in bone tissue engineering, allowing the fabrication of patient-specific scaffolds that exhibit optimal porosity, mechanical robustness, and high bioactivity. A recent work by Maresca *et al.*[187] highlights the development of 3D-bioprinted bone scaffolds using bioinks composed of hydrogels and bone-forming cells; these structures closely mimic the micro- and nano-architecture of the natural ECM, fostering osteogenic differentiation and functional tissue formation. Similarly, Lv *et al.*[188] advanced bone regeneration by incorporating sodium alginate, hydroxyapatite, and gelatin hydrogels loaded with osteoblast and osteoclast precursor cells, producing scaffolds that accurately replicate in vivo bone remodeling processes. Khalaf *et al.*[189] reviewed the success of various bioprinted constructs, especially those integrating stem cells and calcium phosphate–based materials, in achieving robust bone tissue formation and supporting *in vivo* repair. Another comprehensive review by Kang *et al.*[190] examines the application of not only traditional 3D bioprinting methods but also 4D approaches using stimulus-responsive hydrogels to generate dynamic, vascularized bone environments, signifying a shift toward actively adaptive, customizable implants. These approaches are complemented by reviews on bioinks and state-of-the-art scaffold architectures, emphasizing advances in commercial and lab-made biomaterials tailored for bone regeneration and the clinical translation of these platforms.[191] The field continues to advance, with ongoing research into bioreactor integration, *in situ* scaffold vascularization, and strategies for tuning degradation kinetics to match natural bone healing.[192,193]

(b) Cartilage tissue engineering

Cartilage regeneration faces unique hurdles due to the tissue's avascular nature and mechanical complexity. 3D bioprinting offers critical solutions by allowing intricate control over scaffold microstructure and cell distribution. Daly *et al.*[194] demonstrated the fabrication of biomimetic, multichannel hydrogel systems capable of supporting chondrogenic differentiation and the generation of osteochondral (bone-cartilage interface) tissues, recapitulating the native zonal architecture needed for functional repair. Moreover, *in situ* bioprinting applied directly to cartilage defects has been demonstrated in animal models to restore joint function and enhance repair.

These efforts collectively highlight a new era of precisely engineered, mechanically robust, and biologically faithful cartilage substitutes enabled by bioprinting.[195]

(c) Neuronal tissue engineering

Three-dimensional bioprinting is rapidly emerging as a transformative technology in neuronal tissue engineering, offering precise spatial control over multiple neural cell types and enabling the fabrication of complex, biomimetic brain and nerve tissue constructs. By using bioinks tailored with neural stem cells, iPSC-derived neurons, glial cells, and ECM mimics, researchers have successfully engineered 3D scaffolds that support cell differentiation, neurite extension, and synaptic network formation *in vitro*.[135,136] For example, bioprinted neural tissues have shown functional neural network activity and high viability when using advanced hydrogel systems and microfluidic patterning for spatial guidance.[135,137] Recent approaches also incorporate conductive polymers and nanomaterials to better mimic the electrophysiological environment of the nervous system, further enhancing neuronal maturation and signal transduction.[138,139]

Beyond basic models, 3D bioprinting is being applied to create human cortex-mimetic architectures with layered organization to study neural circuit formation and to develop individualized disease models, such as for glioblastoma and neuroblastoma, allowing for high-throughput drug screening and investigation of tumor microenvironments.[135,139,140] A recent study developed a 3D-bioprinted Parkinson's disease model using dopaminergic neurons encapsulated in biomimetic

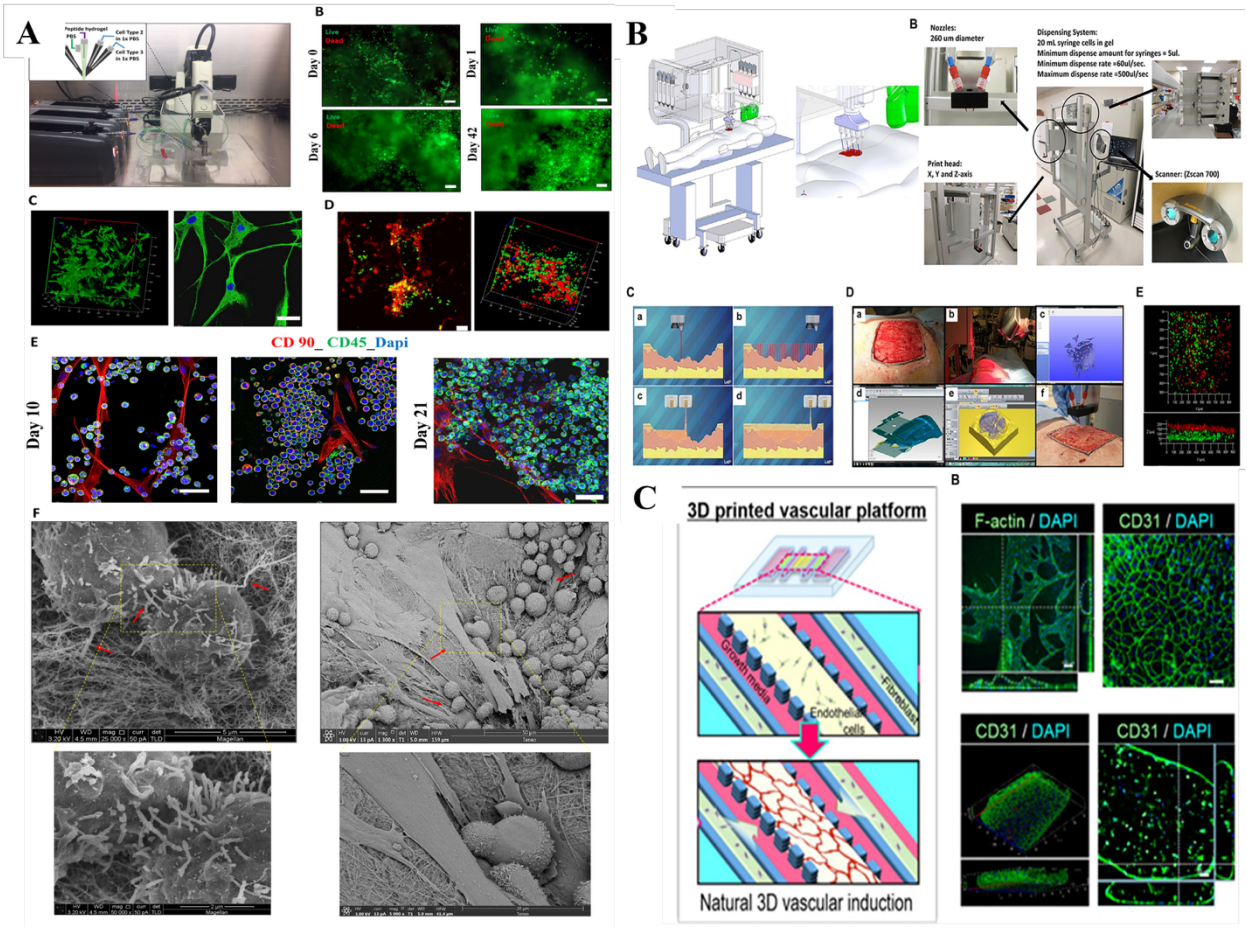

Figure 3. Representative biomedical applications of 3D bioprinting for tissue modeling, regeneration, and vascularization. (A) Automated fabrication of a 3D bone marrow niche-like acute myeloid leukemia model by robotic 3D bioprinting. Reprinted from Alhattab *et al.*[184] (B) Skin bioprinter prototype and *in situ* bioprinting concept. Schematic design and main components of a mobile skin bioprinter equipped with multiple dispensing nozzles and a 3D wound scanner. The system enables layer-by-layer deposition of cell-laden bioinks directly onto full-thickness skin wounds. (C) Vascular network formation on the 3D cell-printed vascular platform. Formation of perfusable vascular networks within a 3D cell-printed vascular platform, demonstrating the ability to generate integrated vasculature essential for tissue survival and function. (B) and (C) reprinted from Chen *et al.*[166]

ultrashort self-assembling peptide scaffolds. This model supports both mouse and human dopaminergic neurons, exhibiting sustained neuronal activity and responsiveness to neurotoxins like 6-hydroxydopamine. Vascularization via co-culture with endothelial cells enhanced neurite growth and network density. The system employs a hybrid extrusion-based robotic bioprinter guided by a mouse-brain-shaped mold to improve print fidelity and anatomical relevance. This platform offers a promising tool for studying Parkinson's disease pathogenesis and testing therapies.[102] Furthermore, researchers are increasingly utilizing multi-material printing to recreate neuron–glia interactions and brain barrier functions, which are critical for accurate models of neurodegeneration, neurodevelopmental processes, and injury repair.[136,139] These advances position 3D bioprinting as a robust, customizable platform for neuroregeneration, modeling complex brain diseases, and testing targeted therapeutics.[140,141]

(d) Additional applications

Three-dimensional bioprinting is also at the forefront of engineering a wide array of other tissues. In the vascular domain, Skylar-Scott *et al.*[186] achieved the creation of thick, vascularized tissues with embedded channel networks capable of sustained perfusion, a foundational advance for the survival and maturation of multi-layered constructs and the eventual printing of whole organs. Noor *et al.*[96] printed perfusable, cell-laden cardiac patches with high structural fidelity and demonstrated their integration and contractility in experimental systems, pointing toward future applications in heart repair. In the skin and wound-healing field, Yanez *et al.*[196] manufactured bioprinted skin model patches comprising multiple cellular layers, successfully supporting re-epithelialization and accelerated wound closure in animal models. A recent study has developed advanced liver bioinks by combining dECM with gelatin to create composite bioinks (dECM gBioink) that significantly enhance printability, mechanical strength, and cell compatibility, enabling the fabrication of stable, layered liver lobule-mimetic structures exhibiting excellent hepatic function and responsiveness to hepatotoxic drugs.[197] Finally, organ-on-a-chip technologies are increasingly integrating 3D-printed, sensor-enabled tissues that provide physiologically relevant drug screening and disease modeling capabilities. Representative examples of these diverse applications are shown in Figure 3.

Collectively, these application areas illustrate a consistent pattern rather than an unrelated set of case studies: in every tissue system, printing technique and bioink chemistry are selected according to the mechanical and biochemical requirements, with extrusion- and microextrusion-based systems favored for larger, mechanically loaded bone and cartilage constructs, and higher-resolution inkjet, laser-assisted, or vat-polymerization approaches favored where fine spatial patterning of soft neural or vascular networks is required. At the same time, most of the demonstrations cited above remain preclinical proof-of-concept studies in small-animal models or *in vitro* platforms, and few report the quantitative mechanical, vascularization, or long-term functional benchmarks needed to compare results across studies or to predict clinical performance. This gap between structural feasibility and standardized, quantitative functional validation, rather than any single remaining technological limitation, is the central translational obstacle examined in **Section 5.2.2**.

5.2.2. Challenges

Despite these technological advances, significant challenges persist. Bioink properties such as printability, biocompatibility, and the ability to support long-term cell function remain suboptimal for many complex tissue types, and the simultaneous achievement of mechanical stability and cellular viability is a central hurdle.[164,165] In addition, the lack of scalable stem cell sources, incomplete knowledge of optimal *in vitro* culture conditions, and, especially, the absence of mature vascular networks within thick engineered tissues continue to impede the fabrication of fully functional, clinically translatable organs.[142,164,166] Regulatory and ethical considerations, as well as inconsistent or undeveloped manufacturing standards, further complicate clinical translation and widespread adoption.[164,166] Current research is focused on improving bioink formulations, including the use of advanced polymers, nanoparticles, and hybrid materials, along with innovative vascularization techniques, such as the direct printing of vascular scaffolds and the incorporation of angiogenic factors.[164,166,198] Smart sensors and biosensors are increasingly being embedded within constructs for real-time monitoring of tissue health and function during and post-printing.[199,200]

Manufacturability, reproducibility, and quality control deserve particular emphasis here, since they are frequently under-addressed relative to the biological challenges above. Batch-to-batch variability in natural and hybrid bioinks, incomplete reporting of print parameters, and the near-absence of GMP-compatible, closed-system bioprinting workflows currently limit the reproducibility of published results across laboratories and are, in practice, as significant a barrier to clinical translation as the biological hurdles of vascularization and innervation discussed above.[109,110,164-166]

Meanwhile, the integration of multi-material and

multi-cell printing strategies, enhanced by automated and open-source bioprinting platforms, is yielding increasingly complex and physiologically relevant tissue models and promoting global collaboration in the field.[105,201] As regulatory frameworks mature and manufacturing processes are standardized, bioprinting is poised to drive forward personalized medicine, enabling the fabrication of patient-specific tissues for transplantation and regenerative therapies.[164,166,167]

6. Future perspective

One of the most critical areas of development lies in the integration of vascular and neural networks within 3D-printed tissues.[202] Current challenges include achieving precise spatial localization of cells and overcoming issues related to cell density and sedimentation due to gravitational forces. To address these challenges, future research will likely focus on improving the micro-architecture of ECM components to better replicate biological processes.[203] This could involve refining bioprinting techniques to allow for the co-printing of multiple cell types and materials, ensuring that printed tissues more closely mimic their natural counterparts.[204]

The development of smart or programmable tissue architectures, capable of responding to external stimuli such as pH, temperature, and magnetic fields, represents a significant leap forward. These innovations will likely enhance the interaction between cells and printed structures, moving bioprinting from a focus on structural similarity to one that prioritizes functional performance.[205] As these technologies evolve, the goal of complete organ regeneration, with fully integrated vascular and neural networks, becomes increasingly attainable. This will not only transform the landscape of regenerative medicine but also address the critical shortage of donor organs, offering patient-specific, autologous solutions that reduce the risk of graft rejection.

The advancements in the scalability and reproducibility of printed tissues are also an area of research.[206] As bioprinting techniques are refined, there will be greater emphasis on creating larger, more complex tissues and organs that can be used in clinical settings. This will involve not only improvements in the bioprinting processes themselves but also the development of new bioinks and biomaterials that can support the long-term viability and function of printed tissues. The convergence of bioprinting with other technologies, such as machine learning and artificial intelligence, is also expected to play a crucial role in optimizing these processes, leading to more precise and reliable outcomes in tissue engineering.

7. Conclusion

Three-dimensional bioprinting has evolved from conceptual beginnings into a set of practical, complementary technologies for tissue engineering and regenerative medicine, but the field is best understood, as this review has argued, through the trade-offs that link deposition mechanism, bioink chemistry, and biological outcome rather than through a simple inventory of methods and materials. Resolution still trades against printable construct size, printability against biological functionality, and scalability against cell viability, and no single technique or bioink class resolves all three simultaneously; technology and material selection therefore remain application-specific decisions rather than a search for one universally superior approach. Several unresolved challenges continue to gate clinical translation, specifically: achieving mature, perfusable vascularization and innervation within thick constructs; sustaining long-term functional maturation of printed tissue rather than short-term structural mimicry; and closing the gap between laboratory-scale reproducibility and GMP-compatible, quality-controlled manufacturing. Addressing these will depend less on new bioprinting hardware alone and more on parallel advances in smart, stimuli-responsive bioinks; embedded real-time monitoring of construct health during and after printing; AI-assisted design and process control to predict printability and optimize parameters ahead of trial-and-error experimentation; and multi-material, multi-cell printing platforms capable of recapitulating the compositional complexity of native tissue. Sustained collaboration between bioengineers, materials chemists, cell biologists, and regulatory scientists will be required to move bioprinted constructs from proof-of-concept demonstrations toward standardized, clinically, and industrially deployable products.

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Conflict of interest

Prof. Dr. Charlotte A. E. Hauser is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. The authors declare no conflict of interest.

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