ACCSCIENCE PUBLISHING

International Journal of Bioprinting

REVIEW ARTICLE

New frontiers in bioengineering: A perspective on the open challenges in bioprinting

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Abstract

Bioprinting has emerged as a transformative technology in biofabrication, enabling the precise spatial arrangement of biomaterials, living cells, and bioactive factors to generate functional three-dimensional biological constructs. Recent advances are redefining the scope and impact of this field through innovations in both materials and methodologies. Multi-material and multiscale printing strategies are enhancing the ability to replicate the hierarchical architecture and functional gradients of native tissues, while the valorization of waste-derived biomaterials for bioink formulation is introducing sustainable solutions without compromising performance. The integration of bioprinting with organ-on-a-chip systems is providing highly sophisticated *in vitro* models for disease research and drug discovery, and *in situ* bioprinting techniques are opening new possibilities for direct, patient-specific tissue repair. Parallel to these developments, four-dimensional bioprinting introduces the dimension of time, allowing printed constructs to change shape, properties, or function in response to environmental stimuli. The application of artificial intelligence in process monitoring and quality control is improving reproducibility, predictive accuracy, and manufacturing efficiency, thus paving the way for standardized production. Looking ahead, the emerging concept of five-dimensional bioprinting—integrating spatial, temporal, and functional control—suggests a paradigm shift in the design and manufacturing of living systems. Collectively, these advances are broadening the technological capabilities of biofabrication and accelerating the translation of bioprinting from experimental settings toward transformative clinical and industrial applications. This review synthesizes current progress while outlining the opportunities and challenges that will shape the next generation of bioprinting technologies.

Keywords: Multi-material and multiscale bioprinting; Waste biomaterials; Artificial intelligence-enhanced quality control; In situ bioprinting; Organ-on-a-chip; Four-dimensional and five-dimensional bioprinting

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Citation: Chiesa I, Batoni E, Bonatti AF, *et al.* New frontiers in bioengineering: A perspective on the open challenges in bioprinting . *Int J Bioprint.* 2026:12(2):025500517. doi: 10.36922/IJB025500517

Received: December 12, 2025

Revised: January 13, 2026

Accepted: January 28, 2026

Published online: April 17, 2026

1. Introduction

Bioprinting, as defined within the framework of additive manufacturing (AM) processes[1], refers to the layer-by-layer deposition and assembly of biomaterials, with or without living cells, according to a predefined three-dimensional (3D) design.[2] Bioprinting offers several advantages over traditional scaffold fabrication methods used in tissue engineering (TE)[3], such as solvent casting/particulate leaching, gas foaming, phase separation, and freeze drying.[4,5] These benefits include the ability to achieve: (i) the precise placement of diverse materials into 3D structures with defined geometry and internal features (such as pore size, density, and architecture); (ii) the simultaneous processing of various biomaterials, biomolecules, and cells across different scales; (iii) the generation of 3D structures derived from medical imaging data (e.g., computed tomography or magnetic resonance imaging) to tailor them to individual patient requirements; and (iv) the automation of scaffold fabrication, ensuring reproducible constructs that do not depend on operator expertise.[2,6,7] Two different categories of materials can be used in the bioprinting process: (i) biomaterial inks, consisting of inks without a cellular component, and (ii) bioinks, which are inks containing cells. Three categories of bioprinting processes can be used to process bioink/biomaterial inks: extrusion-based bioprinting (EBB), droplet-based bioprinting (DBB), and light-assisted bioprinting (LAB).

Extrusion-based bioprinting is one of the most common techniques for biological and non-biological printing. To create a 3D structure layer by layer, a continuous strand of bioink is extruded onto a substrate using a computer-controlled device. Either a mechanical (piston or screw-driven) or a pneumatic (constant pressure) system can be used to extrude the material from the nozzle.[8] The main advantages of EBB include: (i) the capability to process bioinks across a broad viscosity range (10^{-3} to 10^{4} Pa·s); (ii) the compatibility with high cell density formulations, enabling the fabrication of physiologically relevant constructs within relatively short timeframes; (iii) the tunability of scaffold properties through straightforward modification of biomaterial composition; and (iv) the ability to simultaneously print multiple biomaterials and cell types using multi-nozzle bioprinting systems.[9,10] Conversely, the principal limitations of EBB are: (i) susceptibility to nozzle clogging when processing highly viscous materials; (ii) limited spatial resolution (>100 μm) relative to other bioprinting techniques; (iii) challenges in fabricating geometrically complex structures containing overhangs or voids, which often require support materials; and (iv) potential reduction in cell viability due to shear stresses experienced during extrusion and to gelation or solidification processes occurring post-printing.[11]

Droplet-based bioprinting uses a thermal or piezoelectric mechanism to automatically transport a specific volume of bioink, often containing cells, to specific areas. For the thermal and piezoelectric techniques to release the bioink, a heating pulse from the micro-heater and a piezoelectric actuator, both incorporated into the printhead, are used. In thermal inkjet printers, the heater-generated tiny air bubbles collide, creating pressure pulses that force ink droplets out of the nozzle. Instead, with piezoelectric inkjet printers, each nozzle's actuator is made of polycrystalline piezoelectric ceramic, which creates the minimal pressure needed to eject the ink drops onto the substrate.[12,13] DBB offers significant advantages, including rapid material deposition and high printing resolution (>10 μm), achieved by precisely ejecting low-volume droplets (1–100 pL). However, the technique is limited to low-viscosity bioinks (<20 mPa·s), which can compromise the structural stability of the printed constructs. Additional drawbacks include variability in droplet size, reduced droplet trajectory accuracy, shear-induced cell stress, and potential nozzle clogging.[14]

Light-assisted bioprinting encompasses a group of AM techniques in which a light source enables the deposition or solidification of biomaterials, either through photopolymerization of a monomeric resin (vat-photopolymerization) or via droplet ejection (laser-induced forward transfer [LIFT]). In vat-photopolymerization, the light source initiates the polymerization of a photosensitive resin or monomer within a tank, following digitally predefined trajectories to fabricate 3D constructs. An advanced variant of this approach, two-photon stereolithography (SLA), utilizes the simultaneous focusing of two laser beams at a single point to achieve micro- to nanoscale resolution (<150 nm).[15] Conversely, the LIFT technique employs a high-intensity pulsed laser to transfer droplets of bioink in a non-contact manner. A pulsed laser beam, a ribbon with the bioink to print with, and a receptive substrate make up the three major parts of a LIFT bioprinter. The substrate receives the laser beam after passing through a transparent ribbon. A droplet of bioink, typically containing cells, is discharged onto the substrate when the laser contacts the intermediary layer (often coated with hydrogels that reduce the impact of the previously cited droplets). Compared with other bioprinting methods, LIFT enables the deposition of bioinks with high cell densities (~10^{8} cells/mL) and excellent spatial resolution, while accommodating a wide range of printable material viscosities (1–300 mPa·s). Since the LIFT uses no nozzles, nozzle clogging is avoided,

and as no mechanical stress is placed on the cells during printing, good cell viability can be achieved. However, its application is predominantly limited to two-dimensional (2D) patterning of monolayer structures. The method remains relatively time-consuming and expensive, and it presents challenges when printing with multiple cell types simultaneously.[16,17]

Among LAB biofabrication techniques, volumetric printing (VP) has recently emerged as an innovative approach for the rapid generation of 3D constructs.[18] In this method, a photosensitive hydrogel formulation containing a photoinitiator is loaded into a rotating transparent container and exposed to a series of spatially patterned light projections. These projections are delivered by a coherent light source, such as a laser, coupled with a spatial light modulator (e.g., a digital micromirror device) and computed as filtered tomographic back-projections of the target geometry. Although each exposure is insufficient to induce polymerization, the cumulative superposition of multiple angular projections results in a spatially resolved 3D light dose distribution that locally exceeds the crosslinking threshold, thereby selectively solidifying the material only within the desired volume.[19] VP enables the fabrication of centimeter-scale objects within tens of seconds, representing a drastic reduction in processing time. At the same time, VP achieves good resolution (40–200 μm) while offering high spatial freedom.[18,19]

In addition to the techniques listed so far, electric-field-based methods represent a particularly promising class of biofabrication approaches. Among these, electrospinning (ESP) and melt electrowriting (MEW) have attracted significant attention. ESP is a well-established technique that enables the production of non-woven fibrous meshes with diameters spanning from approximately 2 nm to several micrometers, using polymer solutions derived from either natural or synthetic materials.[20] The process is driven by the application of a high voltage in the range from 10 kV to 50 kV, which induces the stretching of a polymer solution ejected from a metallic needle toward a grounded collector, ultimately leading to fiber deposition. The potential for the generation of ultrafine fibrous structures with tunable porosity and morphology makes ESP particularly appealing for TE, as it allows the fabrication of scaffolds with characteristic dimensions comparable to those of the native extracellular matrix (ECM).[21]

Melt electrowriting is another electric-field-assisted biofabrication technique that operates on molten polymers rather than polymer solutions, enabling their controlled deposition onto a printing substrate.[2] In MEW, polymeric fibers with a diameter ranging from approximately 0.25 to 60 μm can be extruded with high spatial precision.[22] The process is based on the application of an electric field generated by applying a high voltage of up to 10 kV between the nozzle of a translating extruder and a grounded collector, separated by a distance generally in the range of 3 to 7 mm.[23] This configuration enables the fabrication of micro-scale fibrous scaffolds with highly ordered, programmable geometry. In contrast to ESP, which often relies on toxic solvents, MEW directly processes thermoplastic polymers, thereby avoiding solvent-related cytotoxicity issues.

Bioinks are a key element in creating a successful tissue construct, along with the proper manufacturing process. The "supporting biomaterial" utilized in a bioink formulation should, in general, meet requirements relating to both its processability with a particular fabrication method and biological restrictions brought on by the presence of cells.[24] Printability is influenced by several variables, such as the biomaterial's ability to crosslink, its rheological characteristics (viscosity, storage, and loss moduli), printing parameters (extrusion pressure or speed, for example), and its surface tension (i.e., creates chemical bonds between the polymeric chains). In terms of biological qualities, the biomaterial must be biocompatible, non-cytotoxic, and capable of degrading in the human body without the release of any toxic substances.[25]

In the context of TE, bioprinting has become a versatile and powerful approach not only for the fabrication of implantable constructs but also for the development of advanced *in vitro* models for studying physiology, disease mechanisms, and therapeutic responses. As the field matures, however, it is increasingly evident that many of the most critical limitations in bioprinting do not arise from individual technologies used alone, but rather from the difficulty of integrating materials, printing strategies, process control, and biological function into coherent and translatable systems.

This review is therefore guided by the premise that the main open challenges in bioprinting stem from the intrinsic complexity of biological systems. Rather than aiming to be exhaustive, we focus on a selected set of emerging directions that, taken together, capture key bottlenecks and opportunities shaping the next phase of biofabrication, as outlined in Figure 1. The intended readers include researchers and practitioners already familiar with bioprinting fundamentals, for whom the review seeks to provide conceptual clarity and practical insight into how different technological advances jointly contribute to physiological relevance, reproducibility, and clinical potential.

We first examine advances in multi-material and multiscale bioprinting approaches, which are essential for recapitulating the intrinsic complexity, heterogeneity, and

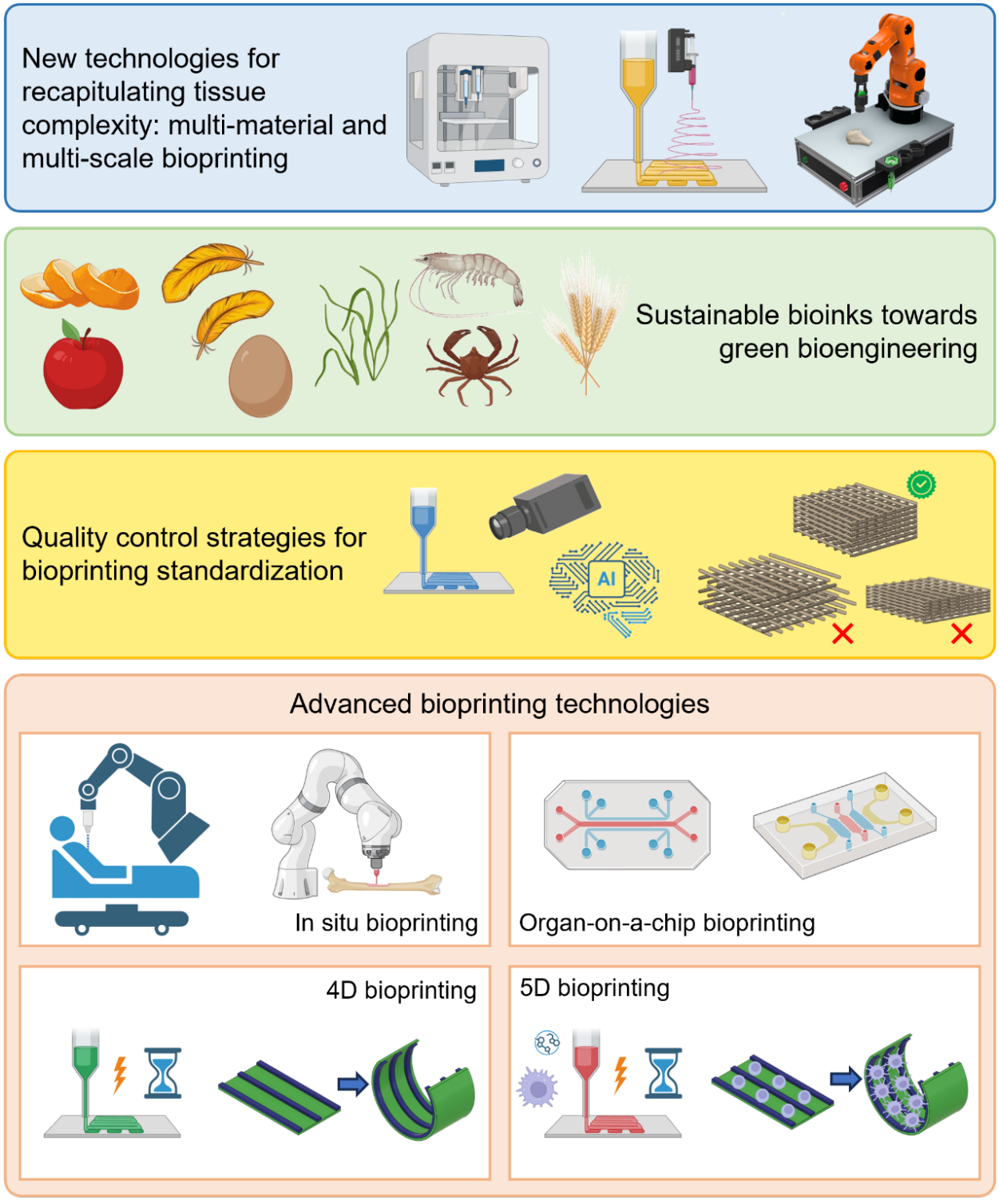

Figure 1. Overview of the emerging trends in bioprinting. Created with MS Power Point.

spatial organization of native tissues. The ability to pattern multiple cell types, biomaterials, and bioactive factors to combine different properties within a single construct is widely recognized as a prerequisite for functional tissue models. Yet, it introduces significant challenges in terms of printing fidelity, material compatibility, and process coordination. Closely linked to this, we analyze recent developments in bioinks and biomaterials, with particular emphasis on sustainable and waste-derived materials. Beyond their environmental relevance, such materials address growing concerns regarding resource efficiency, cost, and scalability, which are increasingly discussed in the literature as critical factors for the long-term viability and industrial translation of bioprinting technologies. The review then addresses quality control (QC) strategies in bioprinting, an area that has emerged as a central requirement for reproducibility, standardization, and regulatory acceptance. *In situ* monitoring, sensor integration, and data-driven approaches are gaining attention as essential tools to bridge the gap between laboratory demonstrations and clinically relevant manufacturing, especially as constructs become more complex and less compliant to post hoc characterization.

Finally, we focus on advanced bioprinting paradigms that directly reflect application-driven needs. *In situ* bioprinting is discussed as a strategy to improve construct integration with host tissues and to better accommodate patient-specific geometries and healing environments. Organ-on-a-chip (OOC) platforms are highlighted for their potential to increase physiological relevance while reducing reliance on animal models, in line with widely recognized ethical and translational imperatives. We further examine four-dimensional (4D) and five-dimensional (5D) bioprinting approaches, which introduce temporal and directional complexity into printed constructs and are increasingly explored as means to model dynamic tissue behaviors, maturation processes, and adaptive responses that more closely resemble native physiology.

By integrating these dimensions into a unified framework, our review aims to elucidate the synergies among material innovation, process development, and functional outcomes, thereby outlining the key technological convergences driving the future of bioprinting.

2. Development of new technologies to better recapitulate biological tissue complexity: Multi-material and multiscale bioprinting

Prior research suggests that biological tissues are highly heterogeneous, exhibiting a bottom-up hierarchical structure closely related to their function. Therefore, the ambition to address the need for biofabricated scaffolds that feature different length scales, with structural and mechanical properties optimal for eliciting specific responses or mimicking those found naturally, is driving biofabrication research toward new strategies. The ability to combine a controlled structure with multiple materials at different scales within a single scaffold is essential for accurately mimicking the complexity of native tissues.[26] In this context, the multi-material and multiscale bioprinting approach has achieved several milestones to recreate the complex 3D microenvironments found in natural tissues and organs, which could help improve the viability and function of bioprinted constructs.[27] Generally, this approach is based on hybrid biofabrication processes, which take advantage of the combination of different bioprinting technologies (e.g., EBB, inkjet bioprinting, laser-assisted bioprinting, ESP) to offer the opportunity of the hierarchical arrangement of biomaterials and heterogeneous cell types to recapitulate the geometry, complexity, and longevity of human tissues. More specifically, multiscale bioprinting refers to the simultaneous use of bioprinting techniques with different effective resolutions to create structures at multiple size scales, including nano-, micro-, and macroscale structures, addressing well-established issues regarding pores (i.e., pore size and shape, interconnectivity, and total porosity) and mechanical properties. Multi-material bioprinting refers to the simultaneous use of different bioinks or biomaterial inks, which may better represent native tissues/organs, within a single bioprinted structure to guide and support new tissue formation over time. Over the years, due to rapid advancements in bioprinting, multi-material and multiscale bioprinting technologies have evolved from cumbersome tools requiring specialized human intervention to well-integrated, automated processes.

This approach to developing advanced TE scaffolds is essential for various applications, including the engineering of biological tissue interfaces, such as enthesis, tendon, and ligament insertion into bone. The inherent gradients in cell type, mechanical strength, structure, and chemical composition of this region render this approach particularly well-suited to reproduce their inherent complexity and heterogeneity with higher fidelity than single-material systems. An example is reported in Criscenti *et al.*[28] and Micalizzi *et al.*[29] where a triphasic scaffold for the regeneration of the bone–ligament interface was fabricated by combining a 3D fiber-deposited poly(ε-caprolactone) (PCL) structure and a poly(lactic-co-glycolic acid) electrospun mesh (Figure 2A). This scaffold exhibited a gradient of physical and mechanical properties, eliciting distinct biological responses in human mesenchymal

stromal cells. Visser *et al.*[30] presented a combination of different extrusion systems to print materials of different properties. More specifically, they employed a screw-driven system to melt and deposit a hard material, PCL, while the piston-driven syringe was used to print a soft hydrogel. Chiesa *et al.*[31] reported on the design, fabrication, and validation of an innovative mixing system for integration into a dual-extruder bioprinter, incorporating an ultrasonic probe within a mixing chamber. Their work addresses a key challenge in interfacial TE: the inherent heterogeneity in both the composition and structure of native interfacial tissues (Figure 2B). Moreover, Cui *et al.*[26] mimicked the hierarchical structure and functionality of bone tissue, combining SLA with fused deposition modeling to create a vascularized bone scaffold, using PLA as the mechanically robust matrix and SLA-printed gelatin methacrylate (GelMA) bioink loaded with human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells for the soft vascular network. In another study, Ainsworth *et al.*[32] investigated the potential of multi-material and multiscale strategies for pre-vascularizing a myocardial construct patterned along a precisely defined vascular pathway. By combining high-resolution MEW with EBB, they demonstrated the feasibility of employing mechanically weaker hydrogels reinforced with MEW fibers, while maintaining spatial control over cellular composition, type, and density.

By integrating MEW with VP, Größbacher *et al.*[32] demonstrated an innovative approach for fabricating heterogeneous living constructs with controlled spatial organization (Figure 2B).[33] In particular, cell-laden photocrosslinkable hydrogel bioinks based on GelMA were polymerized within pre-fabricated MEW scaffolds as concentric material layers containing distinct cell populations, including endothelial cells, smooth muscle cells, and fibroblasts, recapitulating the layered architecture of vascular tissues. In addition, HUVECs were seeded within the tubular lumen. This concentric and spatially defined organization enabled the fabrication of biomimetic vascular-like constructs while decoupling mechanical reinforcement from spatial control over cellular composition.

Kang *et al.*[33] employed an EBB strategy to fabricate multiscale, heterogeneous liver tissue constructs by simultaneously processing multiple cell-laden bioinks.[34] Using a customized cartridge, they generated spatially organized hepatic lobules (around 1 mm) composed of hepatocytes and endothelial cells, featuring an endothelialized lumen and interconnected vascular structures. This hierarchical organization resulted in enhanced hepatic functionality and enabled the assembly of highly vascularized constructs spanning from the micro- to the macroscale. Another example of multi-material EBB is provided by Puistola *et al.*[34], who engineered hierarchically organized, heterogeneous corneal stroma constructs.[35] Human adipose tissue-derived stem cells were embedded in a soft hyaluronic acid (HA)-based bioink and co-printed with an acellular, stiffer HA formulation as alternating filaments arranged in orthogonally oriented layers, enabling spatial guidance of cell alignment while ensuring mechanical stability. The resulting constructs recapitulated key microstructural features of native corneal stroma, demonstrating the suitability of EBB for the fabrication of multiscale heterogeneous tissues. Extending the concept of spatially organized multicellular assembly to a different bioprinting modality, Wan *et al.*[35] employed 3D inkjet bioprinting to fabricate modular artificial lung tissue structures with alveolar-like organization.[36] Alginate hydrogel microspheres were generated and surface-functionalized with type I collagen and polydopamine to promote cell adhesion, followed by the assembly of human bronchial epithelial cells (BEAS-2B), HUVECs, and lung fibroblasts (MRC5), with the optional incorporation of macrophages. This modular, multicellular strategy enabled the recapitulation of key features of the alveolar microenvironment and supported *in vitro* modeling of inflammatory lung injury. Building on these advantages, multi-material and multiscale bioprinting plays a pivotal role in both enabling and challenging the development of advanced bioprinting applications, including OOC platforms. On the one hand, the ability to spatially integrate multiple biomaterials with distinct mechanical, biochemical, and transport properties enables the recreation of key tissue interfaces central to OOC functionality. Similarly, multiscale architectures facilitate the concurrent incorporation of micro- and nanoscale features that govern cell behavior, alongside macroscale structures for handling, perfusion, and system integration.[37,38] On the other hand, this increased material and structural complexity introduces significant challenges in process control, reproducibility, and quality assurance, as each additional bioink or length scale introduces new variables that affect print fidelity, cell viability, and long-term stability. As a result, multi-material and multiscale bioprinting emerges not only as an enabling technology for physiologically relevant OOC models but also as a key driver of improved standardization and QC strategies. These needs naturally extend toward more dynamic and adaptive biofabrication approaches, including 4D and 5D bioprinting, where temporal and directional changes further amplify the importance of integrated process monitoring and control.

Considering hardware solutions, the prevailing

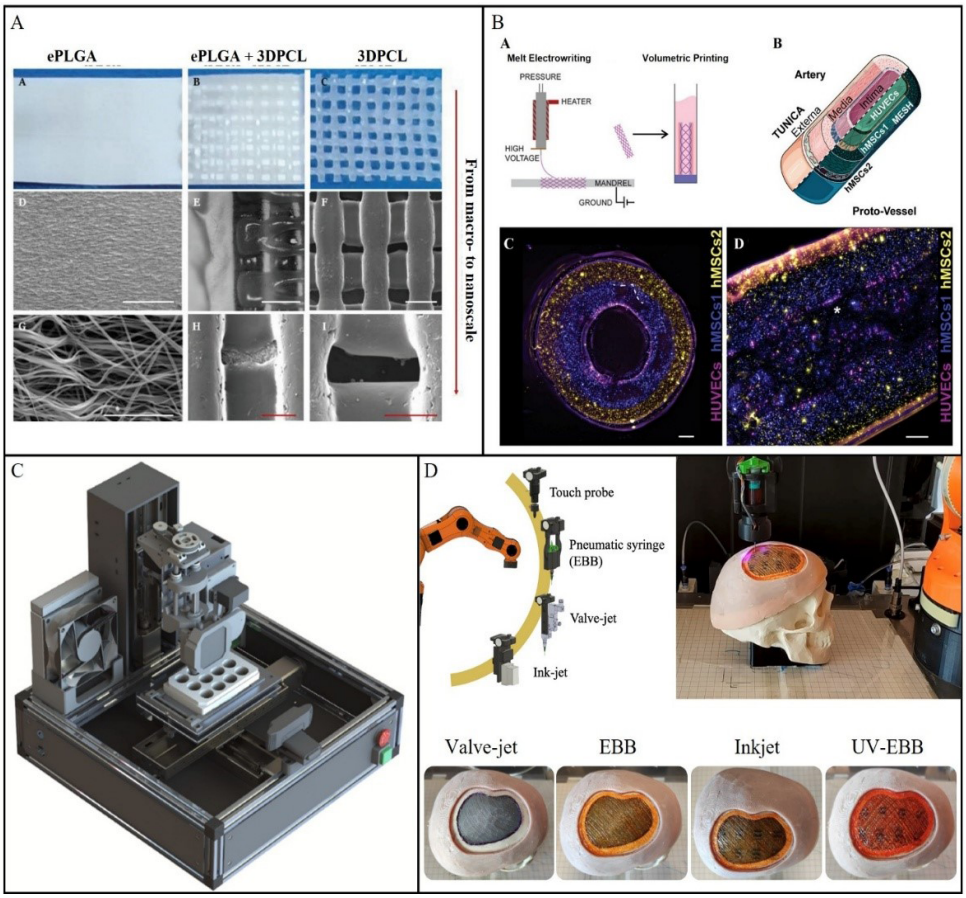

Figure 2. Multi-material and multi-scale bioprinting. (A) Enthesis scaffold from macro- to nanoscale. (First row) Full-size scaffold regions. (Second row) SEM and optical microscope images highlighting the microscopic structure. Scale bar: 500 µm; magnification: 20×. (Third row) SEM images of the scaffold at the micro- and nanoscale. Scale bars: (H, I) 200 µm and (G) 20 µm. Reprinted from Micalizzi *et al.*[28] (B) Sequential MEW and volumetric printing of cell-laden, multi-material, and multi-layer tubular constructs. (First row) Schematic illustration of the sequential fabrication process combining MEW and volumetric printing and diagram of the native vessel structures compared to the proto-vessels, highlighting the multilayered organization and the spatial arrangement of cell types. (Second row) Perpendicular and longitudinal cross-sectional fluorescence images of a three-layer tubular construct consisting of volumetric printed human mesenchymal stem cells (blue and yellow) embedded in the gel layer encapsulating the MEW mesh, and a human umbilical vein endothelial cells-seeded lumen (magenta). Scale bar: 500 microns. Reprinted from Größbacher *et al.*[32] (C) 3D bioprinter equipped with EBB and inkjet printheads for multiscale and multi-material bioprinting. Reprinted from Bonatti *et al.*[38] (D) Robotic platform equipped with EBB, valve-jet, and inkjet printing tools. Reprinted from Guerra *et al.*[39]

Abbreviations: 3D: Three-dimensional; EBB: Extrusion-based bioprinting; MEW: Melt electrowriting; SEM: Scanning electron microscopy.

paradigm to date involves robotic 3D bioprinters equipped with interchangeable tools and printheads mounted to the end of the arms.[39,40] These tools can be individually employed to biofabricate 3D cellular constructs using different printing techniques and biomaterials, thereby supporting the translation of multi-material and multiscale bioprinting into hospital settings and practical applications (Figure 2C,D). Although in its infancy, multi-material and multiscale bioprinting can become a new frontier in TE and regenerative medicine as well as a new market segment that is expected to drive the global bioprinting market in the coming years, as evidenced by the number of small and medium companies that have emerged in this market niche (e.g., REGENHU, Bio3DPrinting, CELLBRICKS) in recent years. However, as material and architectural complexity increase, so do the demands placed on bioink design, process coordination, and reproducibility, highlighting the need for advanced biomaterials and integrated control strategies capable of supporting such complexity.

3. Toward green bioengineering: Development of novel bioinks using sustainable biomaterials

The selection of the biomaterial used as a bioink is a key aspect of 3D bioprinting. Ink suitability depends on the biomaterial formulation, cell source, and presence of growth factors, as well as its processability for the bioprinting

technologies. Regarding ink printability, physicochemical properties, such as rheological behavior and the crosslinking mechanisms, must be fully characterized. The most important rheological parameter is the viscosity, which depends on the polymer type, the concentration, and the molecular weight.[41,42] Although printing fidelity increases with viscosity, cell viability reduces as higher stresses are applied to the suspended cells. In fact, shear-thinning materials are preferred as they exhibit a lower viscosity at high shear rate generated during extrusion, while viscosity increases after extrusion, leading to a high printing fidelity and cell viability.[43] To improve the mechanical and physical stability of 3D-printed constructs, bioinks are often chemically, physically, or enzymatically crosslinked. At the same time, the ink must be biocompatible, biodegradable, and able to support cell adhesion and proliferation during the maturation of the target tissue.[42] Regarding bioinks, these can be mainly divided into two different categories based on their primary function: (i) support/structural bioinks, whose primary role is to act as artificial matrices that promote cell survival during bioprinting while preserving construct shape fidelity (passive role), and (ii) cell-instructive bioinks, which yield physicochemical cues directing cell behavior and guiding differentiation post-bioprinting (active role).[44–46] While the first ones mainly include natural hydrogels (e.g., alginate, decellularized ECM), the second ones comprise functionalized and/or composite biomaterials incorporating signaling (e.g., growth factors, cytokines) or bioactive molecules (e.g., nanoparticles).[47,48] Therefore, these bioinks can replicate the native ECM microenvironment, integrating multiple cues specific to the target tissue. Overall, to satisfy all the requirements for bioprinting, more research is needed to develop novel natural or synthetic material formulations that can be processed into biomaterial inks or bioinks.

In recent years, more attention has been focused on the development of sustainable materials and their application in the bioprinting field.[49] The reason lies in the fact that the use of naturally derived biomaterials could promote a circular economy by recycling and remanufacturing biological or waste products, as well as reducing the material costs.[50] In fact, these are non-toxic materials, usually derived from natural sources (e.g., plants) or from waste products (e.g., livestock or industrial). Examples reported in the literature are pectin and cellulose derived from plants, or keratin and hydroxyapatite from animal wastes, as well as alginate or chitosan from marine wastes.[51–54] In the next section, recent ink formulations are reported, focusing on those derived from natural or waste sources.[55]

3.1. Plant-derived biomaterials

Plant-derived biomaterials are gaining attention as natural sources with properties similar to those of target human tissues. Among them, the most important are pectin- and cellulose-based biomaterials, which are reported in the following sections .[55,56]

3.1.1. Pectin-based biomaterials

Pectin is a natural component of plant cell walls and can be industrially derived from natural sources such as juice, apples, and cider through acidic and thermal extraction. Due to its high molecular weight and hydrophilicity, this hydrogel is an optimal choice for 3D bioprinting. In addition, its viscoelastic and rheological properties can be tuned by adjusting the concentrations of the polymer and/or crosslinker. However, the main limitations of pectin are its reduced cell adhesion and non-homogeneous ionic crosslinking, which restrict its printability and structural fidelity. To deal with these issues, chemical modifications (e.g., methacrylation, silanization with (3-glycidyloxypropyl)trimethoxysilane [GPTMS]) and blending strategies with bioactive polymers (e.g., gelatin, collagen) are often employed.[57–60] These modifications not only enhance cell adhesion but also improve mechanical stability and rheological properties, which are crucial for EBB. Recently, GPTMS has been used as a pectin cross-linking agent to address this challenge.[53] 3D mesoporous wood pile and complex anatomically shaped scaffolds were successfully fabricated through EBB combined with freeze-drying (Figure 3A). In a separate study, pectin was employed as a rheology modifier for gelatin–GPTMS crosslinked biomaterial inks. Rheological analyses and bioprinting experiments demonstrated that pectin plays a crucial role in enhancing viscosity and yield stress, enabling temperature-independent EBB of otherwise low-viscosity gelatin solutions. Such rheological modifications are particularly suitable for extrusion-based techniques that require shear-thinning behavior and rapid shape recovery after deposition, while they may be less compatible with DBB and LAB, where lower viscosity and photocrosslinkable formulations are preferred.[61,62] In this regard, pectin methacrylate, combined with GelMA, has recently emerged as a photocrosslinkable biomaterial with tunable mechanical strength, improved gelation kinetics, and high cytocompatibility.[59,60] These advancements broaden the application of pectin-based biomaterials to LAB as well.

3.1.2. Cellulose-based biomaterials

Cellulose is the most abundant natural polymer in the world and is usually obtained from the biosynthesis of plants such as leaves, peels, silk, and wood.[54] Cellulose-based nanofibers are promising sustainable materials for hydrogels, which exhibit excellent biocompatibility, mechanical performance, adequate compressive strength, and elasticity. However, native cellulose has limited solubility and processability; thus, chemical modifications such as oxidation, carboxymethylation, and methacrylation are commonly introduced to improve the dispersibility, crosslinking capability, and, therefore, its suitability for 3D bioprinting.[63–65] An example of a novel cellulose-based bioink is reported by Sawkin *et al.*[66], where carboxymethyl cellulose was used as a rheology modifier of a poly(lactic-co-glycolic acid) ink to improve its printability and the final shape fidelity of the scaffolds. The cellulosic component optimized ink printability, enabling the incorporation of cells and therapeutic proteins during fabrication. The obtained scaffolds exhibited good mechanical properties, comparable to those of the cancellous bone, making the bioink formulation a potential candidate for EBB in bone TE. Another example is reported by Henriksson *et al.*[67], who formulated a new bioink composed of nanocellulose and HA for encapsulating adipocytes. Cell-laden constructs were successfully fabricated via EBB, demonstrating appropriate shape retention and biological relevance for adipose tissue *in vitro* models. Beyond EBB, photocrosslinkable cellulose formulations, such as cellulose methacrylate, have recently been developed and successfully used in digital light processing bioprinting, yielding hydrogels with improved mechanical integrity and cell viability.[65] Therefore, these studies indicated that chemical modifications of cellulose derivatives can extend the usability of cellulose-based biomaterials across multiple bioprinting techniques.

3.2. Marine waste-derived biomaterials

Marine waste comprises various types of organisms, such as crustacean (cockle shells), fish bones, as well as marine plants, from which many chemical elements like alginate, hydroxyapatite (HAP), and chitin can be extracted.[55]

3.2.1. Alginate-based biomaterials

Alginate is a biocompatible natural polymer derived from marine plants, specifically, brown algae. It is relatively low-cost and has similar properties to those of the ECM, making it a good candidate for biomaterial ink and bioink formulations. However, native alginate displays low viscosity and weak mechanical stability, limiting its ability to retain its shape after printing and to provide sufficient support for cell proliferation. This limitation can be overcome by chemical modifications (e.g., oxidation, methacrylation) and by blending with other polymers to improve rheological behavior and crosslinking control.[49,56,68–70] For instance, Müller *et al.*[70] demonstrated that the addition of nanocellulose to alginate sulfate solution improved the rheological and mechanical properties, thereby improving the printability of alginate-based bioinks for EBB.[71] Similarly, Markstedt *et al.*[71] reported the use of alginate combined with cellulose nanofibrils to improve its rheological properties (e.g., shear thinning and recovery behavior) to incorporate living cells, specifically, human chondrocytes, for the regeneration of cartilage tissues.[72] More recently, methacrylated alginate has been developed as a photocrosslinkable formulation suitable for LAB, offering enhanced structural integrity, tubular stiffness, and improved cell viability[69,70], thus expanding the use of alginate-based biomaterials across other bioprinting techniques.

3.2.2. Chitosan-based biomaterials

Chitosan is a derivative of chitin (the second most abundant natural polysaccharide after cellulose), primarily found in the crustacean shells (e.g., crabs, shrimps, lobsters).[55] It has recently attracted attention due to its intrinsic antibacterial properties, its ability to promote angiogenesis, and its ability to accelerate wound healing.[49] However, native chitosan exhibits limited solubility at physiological pH, weak mechanical properties, and poor crosslinking homogeneity, restricting its direct use in bioprinting. To address these drawbacks, chemical modifications (e.g., carboxymethylation, methacrylation) and composite formulations with other inorganic or organic components are commonly employed to improve solubility, mechanical performance, and printability.[73–75] Coskun *et al.*[76] overcame these limitations by developing a novel bioink comprising chitosan and HAP that can be gelled under physiological conditions upon the addition of glycerol phosphate and sodium hydrogen carbonate to the polymer network. Different formulations were tested and rheologically analyzed, demonstrating, in all cases, shear-thinning behavior compatible with the EBB. Furthermore, *in vitro* tests on the printed scaffolds showed high cell viability, demonstrating their applicability in the fabrication of 3D bone scaffolds.[59] Similarly, He *et al.*[77] improved the mechanical properties of a chitosan-based bioink for bioprinting chondrocyte-laden scaffolds. Specifically, they introduced ethylenediaminetetraacetic acid in a chitosan solution to increase the number of carboxyl groups and increase the hydrogel's mechanical properties when physically crosslinked with calcium. The printability of this novel bioink was assessed by printing 3D scaffolds, showing high printing fidelity and stability compared to

the same formulation without ethylenediaminetetraacetic acid. Furthermore, *in vitro* tests with chondrocytes demonstrated its biocompatibility, cell proliferation, and retention of the chondrogenic phenotype (Figure 3B). Thus, this bioink formulation has the potential to be adopted for the bioprinting of scaffolds for cartilage TE. More recently, methacrylated chitosan and glycol chitosan derivatives have been introduced as photocrosslinkable bioinks suitable for LAB, offering tunable stiffness, high transparency, and enhanced cell viability[75,78,79], therefore, broadening their suitability to different bioprinting techniques.

3.3. Livestock waste-derived biomaterials

Various materials can be extracted from livestock resources, including HAP, keratin, HA, collagen, and calcium phosphate. The common wastes are eggshells, bovine bones, feathers, and slaughterhouse wastes; however, the animal bone remains the most commonly used due to its excellent biocompatibility and bioactivity properties.[50,55]

3.3.1. Hydroxyapatite-based biomaterials

In the literature, numerous studies reported the ability to synthesize HAP from fish bones, promoting its conversion into materials for bone scaffold fabrication. For instance, Yamamura *et al.*[80] demonstrated the feasibility of extracting HAP powder from white mouth croaker and its biocompatibility through cytotoxicity and genotoxicity tests. Another HAP source is eggshell wastes, as recently reported in numerous studies.[52,80–82] The reutilization of this raw material can promote a circular economy and sustainable development, as several tons of eggshell waste are generated each day. An example is reported by Trakoolwannachai *et al.*[52], who synthesized HAP from eggshell to prepare a novel composite biomaterial formulation comprising HAP and PCL in different weight ratios. The HAP/PCL composites were analyzed *in vitro* using Saos-2 cells, showing no cytotoxicity and high viability. However, to be suitable for bioprinting, HAP powders typically require size reduction (nanoscale control), surface functionalization, and blending with biopolymers (e.g., alginate, gelatin, chitosan) to improve printability and homogeneity within the ink.[83–85] Such composite and chemically modified formulations are particularly suitable for EBB, where high viscosity and shear-thinning behavior are required. More recently, photocrosslinkable HAP composites, including GelMA–HAP and methacrylated alginate–HAP, have been developed for LAB, achieving improved osteogenic differentiation and structural fidelity.[86–88]

3.3.2. Keratin-based biomaterials

Keratin is a fibrous structural protein characterized by high tensile strength and mechanical rigidity, making keratin-based materials suitable for many applications. It is usually extracted from chicken feathers[89], but the extraction methods are based on highly toxic and harmful reagents. In addition, native keratin typically exhibits poor solubility and limited mechanical stability, requiring chemical modifications (e.g., methacrylation, thiolation) and blending with polymers (e.g., gelatin, silk fibroin), making these formulations well-suited for EBB.[90,91] To overcome these limitations, Pulidori *et al.*[51] developed a one-pot microwave-assisted process to extract keratin from poultry feathers in an eco-friendly manner. Additionally, they showed that keratin recovered from waste can be used to produce biodegradable, biocompatible bioplastics via ESP. This was achieved by first blending the keratin with gelatin and employing GPTMS as a crosslinking agent (Figure 3C). Such bioplastics hold potential for applications in areas such as bio-packaging and filtration and purification membranes.

An example of keratin-based bioink is reported by Navarro *et al.*[92], who developed a photocrosslinkable ink by using a riboflavin sodium persulfate–hydroquinone photosensitive solution. Scaffolds were sequentially fabricated via ultraviolet crosslinking in a lithography-based 3D printer and used to deliver small molecules, specifically the contracture-inhibiting halofuginone, to heal dermal burn wounds *in vivo*. *In vivo* results showed that halofuginone-loaded printed keratin-based scaffolds are promising, comparable to other similar therapeutic delivery approaches reported in the literature. Moreover, a photocrosslinkable methacrylated keratin has recently been introduced, expanding the keratin's versatility across both EBB and LAB.[93] In the study by Bedir *et al.*[94], 3D patches were fabricated by digital light processing using a GelMA–methacrylated keratin hydrogel, demonstrating their potential for addressing tympanic membrane perforations.

3.4. Industrial waste-derived biomaterials

Recently, efforts have been made to convert industrial waste, such as food, beverage, and textile waste, into various biomaterials that can be used for biomedical applications. However, waste-derived materials often require purification, compositional homogenization, and surface functionalization (e.g., silanization, polymer coating) to improve biocompatibility, printability, and crosslinking efficiency.[95,96] Such modified waste-based formulations can be blended with natural polymers (e.g., alginate, gelatin) to

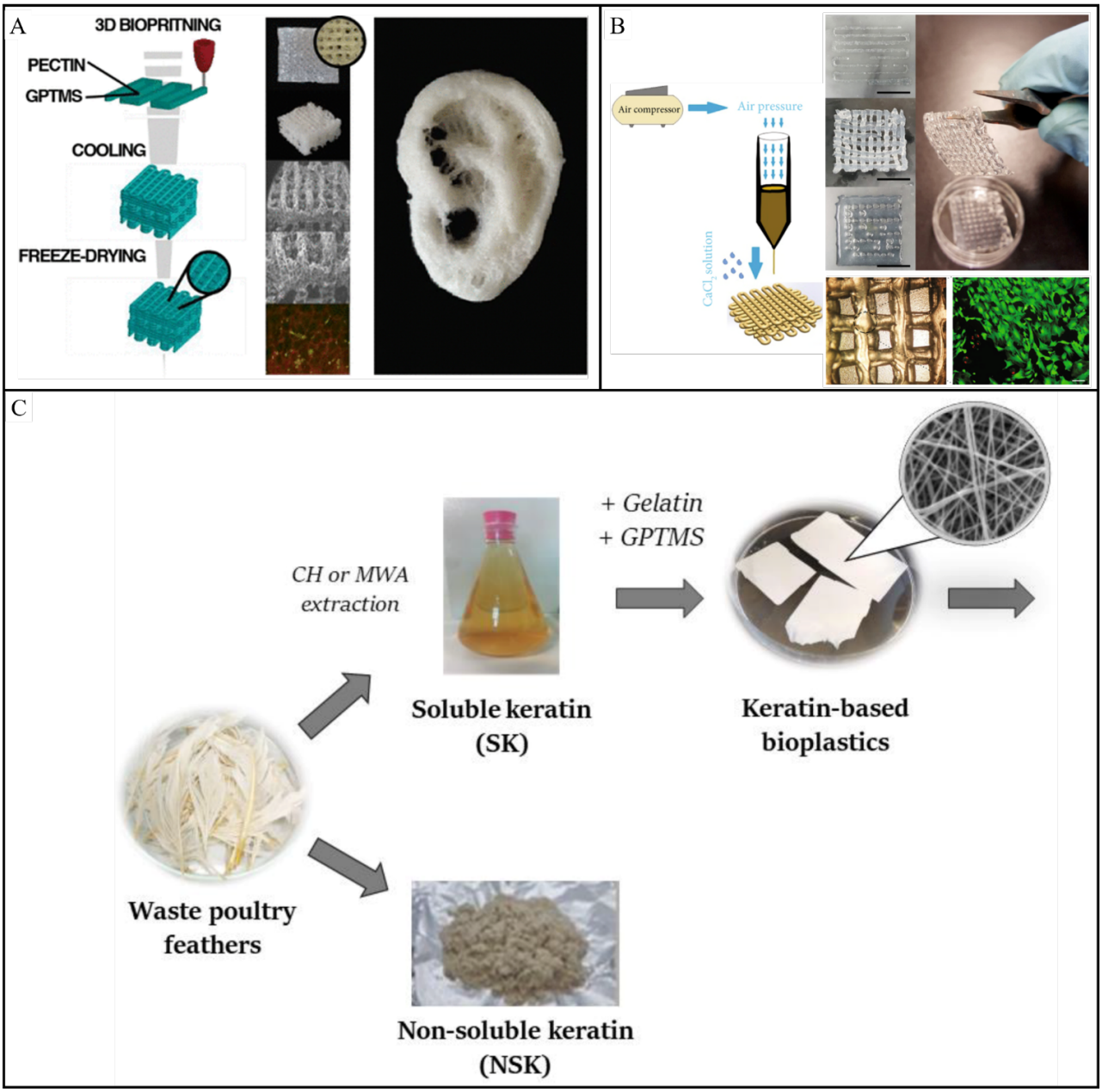

Figure 3. Examples of novel waste-derived ink formulations. (A) Pectin–GPTMS-based biomaterial ink: workflow of the fabrication of woodpile scaffolds, with an image of a freeze-dried printed ear. Reprinted with permission from Lapomarda *et al.*[52] Copyright © 2019, American Chemical Society. (B) Chitosan–EDTA-based bioink: schematic diagram of the bioprinting and subsequent crosslinking with calcium. On the right, printed samples with different chitosan formulations are reported. The best formulation (bottom) was used to evaluate the chondrocyte viability, indicating low cytotoxicity of the hydrogel. Reprinted from He *et al.*[76] (C) A schematic of the keratin extraction procedure proposed by Pulidori *et al.*[50], which was used to prepare keratin-based bioplastic material via electrospinning. Reprinted from Pulidori *et al.*[50]
Abbreviations: EDTA: Ethylenediaminetetraacetic acid; GPTMS: (3-glycidyloxypropyl)trimethoxysilane.

achieve extrusion-compliant rheological behavior.[43,97]

For instance, Yates *et al.*[98] reported the use of beer production wastes as a biomaterial for the scaffold fabrication, after being transformed into tailored biocompatible and non-toxic materials. In fact, beer bagasse, obtained from spent grain waste produced during the beer brewing process, could be used to derive materials rich in calcium, phosphorus, silicon, and magnesium, inorganic components mainly present in bone. In the study, beer bagasse was processed to remove the organic fraction, leaving only the inorganic components, and then pelletized to fabricate 3D scaffolds. *In vitro* and *in vivo* testing were performed to select the best formulation, which demonstrated promising results and the potential of beer production wastes for bone scaffold production. An additional example is provided by Harrison *et al.*[99], who transformed brewer's spent yeast, the by-product of beer production, into bacterial cellulose scaffolds that supported fibroblasts' adhesion and proliferation *in vitro*, illustrating further potential of beverage waste for biomedical scaffold fabrication.

3.5. Comparative analysis and ranking of sustainable biomaterials

To provide a comparative perspective on the sustainable biomaterials discussed in this review, Table 1 summarizes their key features, including cost, ease of chemical modification, printability, biocompatibility, and suitability for bioprinting. Based on that, alginate-based biomaterials have the greatest potential for bioprinting due to their low cost, ease of chemical modification, and tunable rheology, which are suitable for both EBB and LAB. Pectin- and cellulose-based biomaterials also exhibit high printability, with methacrylated forms extending their use to light-based approaches. Chitosan and keratin require additional modifications and/or polymer blending, while HAP and waste-derived materials are promising for composite inks but require modifications to achieve optimal printability.

The use of sustainable, waste-derived biomaterials for bioink development is a key enabler of green bioengineering, offering environmentally responsible alternatives without compromising biological performance. Interest in these materials extends beyond ecological concerns and intersects directly with broader challenges in bioprinting, including integration, scalability, and reproducibility. While sustainable bioinks offer clear advantages in terms of resource efficiency and cost reduction, their inherent variability and complex compositions can affect printability, batch-to-batch consistency, and biological outcomes, making their implementation challenging in practical biofabrication workflows. These challenges highlight the importance of coupling sustainable material development with advanced characterization, *in situ* monitoring, and multi-material printing strategies. By combining these approaches, it becomes possible to compensate for material-specific limitations and ensure that sustainable bioinks meet the technical and regulatory requirements for reproducible, reliable bioprinting. In

Table 1. Comparative analysis of different sustainable materials

Material	Cost	Ease of modification	Printability	Bio-compatibility	Suitable bioprinting techniques	Ref.
Alginate	Low	Easy (blending, oxidation, methacrylation)	High	High	EBB, LAB (AlgMA)	49,56,68–70
Cellulose	Low	Moderate (blending, carboxymethylation, methacrylation)	High	High	EBB, LAB	63–65
Chitosan	Low–medium	Moderate (blending, methacrylation)	Moderate	High	EBB, LAB (ChiMA)	73–75
Pectin	Medium	Moderate (blending, methacrylation)	High	Moderate	EBB, LAB (PecMA)	57–60
Keratin	Medium	Moderate–hard (methacrylation, blending)	Moderate	High	EBB, LAB (KerMA)	90,91,93
Hydroxyapatite	Low–medium	Hard (surface functionalization, blending)	Low–moderate	High	EBB, LAB	83–85
Waste-derived	Low	Moderate–hard (purification, blending)	Moderate	High	EBB	43,95–99

Abbreviations: AlgMA: Methacrylated alginate; ChiMA: Methacrylated chitosan; EBB: Extrusion-based bioprinting; KerMA: Methacrylated keratin; LAB: Light-assisted bioprinting; PecMA: Methacrylated pectin.

this sense, sustainability-driven bioink research serves not only to reduce environmental impact but also as a practical test case for the field's ability to integrate material innovation, process control, and functional design. Beyond environmental and technical considerations, sustainable bioinks also provide new opportunities to tune composition and functionality, which is increasingly important for QC, construct performance, and long-term stability. Their integration is particularly relevant for advanced applications, such as OOC platforms, where precise material behavior is essential to recapitulate tissue interfaces, and for dynamic biofabrication approaches, including 4D and emerging 5D bioprinting, where material responsiveness, adaptability, and mechanical robustness directly influence the success of complex and physiologically relevant constructs.

In summary, sustainable bioinks not only support the broader goal of environmentally conscious bioprinting but also act as a unifying element that connects material innovation, multi-material printing, QC, and advanced paradigms. They exemplify how technical, functional, and ecological objectives must be addressed in parallel to achieve reproducible, scalable, and clinically or experimentally relevant outcomes in biofabrication.

3.6. Challenges in bioink design and clinical translation

Biomaterial and bioink preparation are complex processes that must merge printability performance with biological requirements and, ultimately, clinical applicability. From a physical standpoint, achieving appropriate viscosity and optimal printability is essential and requires fine-tuning of the ink rheological properties by adjusting formulation parameters (e.g., in terms of biomaterial and crosslinker concentration and selection).[100,101] A sterilization procedure can significantly alter ink properties; a comprehensive rheological characterization of sterilized inks should be performed as a prerequisite for clinical translation. In the case of bioinks, rheological optimization must additionally ensure minimal damage and preserve cell viability and function during bioprinting.[100,101]

Beyond rheology, crosslinking presents a key aspect of ink design, as it governs structural integrity during handling, culture, and, possibly, implantation. Rather than being limited to a single post-printing step, crosslinking has recently been implemented as a multiphase process, occurring before, during, and/or after bioprinting, to ensure the ink printability, shape fidelity, and long-term stability.[47,100] To better recapitulate the dynamic nature of the native ECM, recent advances in crosslinking strategies have focused on incorporating reversible, dynamic molecular networks to mimic the cell–ECM interaction and ECM remodeling. In this context, biomaterial functionalization plays a key role in incorporating desirable properties and covering the limitations of the raw biomaterials.[102] However, these advanced crosslinking and functionalization strategies must also meet requirements in terms of reproducibility and safety, avoiding the generation of cytotoxic and clinically unacceptable by-products.[102,103]

The aforementioned bioink design constraints directly reflect challenges in the clinical translation of 3D bioprinting technologies, limiting the number of commercialized natural-based biomaterial inks and bioinks.[104] One of the major challenges is compliance with stringent regulatory and ISO standards. In fact, clinically acceptable bioinks must demonstrate not only printability but also robust performance and reproducibility, ease of use, biocompatibility, and suitability for preclinical screening and clinical applications.[100,104] Due to their natural origin, batch-to-batch variability (e.g., differences in molecular weight, purity, and functional groups) represents a critical issue that must be reduced to align with the Good Manufacturing Practice-compliant manufacturing requirements. Moreover, process-induced variations (e.g., functionalization) may affect the final chemical composition and properties of the inks. Sterilization also poses a key challenge, as commonly used techniques may alter the properties of natural biomaterials, requiring careful selection. In addition, scaling bioinks is a key requirement to enable the fabrication of large functional constructs.[100,105]

Despite these challenges, several commercially available inks composed of naturally derived and, in some cases, waste-derived biomaterials are currently available. Bioinks based on marine- and plant-derived biomaterials such as alginate (e.g., PhotoAlginate® from Advanced BioMatrix), chitosan (e.g., methacrylated chitosan bioink from Adbioink), and alginate/cellulose (e.g., CELLINK Bioink from CELLINK) are commercially available.[106–110] In contrast, inks based on plant-, livestock-, and industrial-waste-derived biomaterials remain largely confined to exploratory and proof-of-concept studies, mainly due to batch reproducibility and the aforementioned challenges.[109,111] Consequently, while waste-derived natural biomaterials offer strong suitability advantages, only marine-derived ones currently exhibit realistic near-term clinical translatability.

4. Quality control strategies in bioprinting

Although bioprinting has raised great interest in recent years as a promising solution to fabricate tissue-engineered constructs for implantation[112,113], very few examples are

currently available for translating bioprinted products into clinical practice. This limitation is mainly due to technological challenges (e.g., the need for new inks, advancements in current fabrication technologies, and the incorporation of vasculature into the construct) and regulatory barriers. As a matter of fact, no clear definition is available regarding the classification of the bioprinted product, as well as the relevant standards that it should comply with for its commercialization.[114–116]

A critical step toward the clinical translation of bioprinted products is establishing QC procedures to ensure that both product specifications and end-user requirements are met. Within international regulatory frameworks, QC standards are essential for the commercialization of medical devices (e.g., ISO 13485) as well as Advanced Therapeutic Medicinal Products, which must comply with good manufacturing practices outlined in the European Directive 2003/94/EC.[117] These regulations are primarily intended to guarantee the safety and efficacy of bioprinted tissue constructs by requiring consistent, reproducible manufacturing with minimal batch-to-batch variation, while maintaining sterility and preventing contamination throughout the process. In the literature, QC approaches are generally discussed in relation to three stages of production: (i) pre-process optimization, (ii) in-process monitoring, and (iii) post-process quality assessment.

Pre-process QC refers to all strategies that aim to optimize the printing process before the production of the final construct. Robust pre-process optimization solutions promise to speed up the development of novel bioprinting inks by reducing the number of trial-and-error experiments, which are often needed to obtain high-quality print results. In recent literature, qualitative and quantitative assays have been proposed to experimentally evaluate ink printability by considering separate aspects of the bioprinting process.[118] For instance, Paxton *et al.*[118] introduced a two-step approach to assess material printability in EBB. They first screened materials based on their ability to form fibers, followed by evaluating whether the biomaterial ink could produce a stable printed construct (Figure 4A).[119] Building on this, Ribeiro *et al.*[119] conducted a quantitative analysis of the behavior of EBB-printed materials after deposition, examining both filament collapse over overhanging structures and the fusion between adjacent printed strands.[120] Another common solution for pre-process QC is process mapping, which involves directly evaluating the bioprinting result for a set of process variables to understand the printing system's behavior and select an optimal final parameter combination. In a recent example, Armstrong *et al.*[120] developed a process map by sweeping extrusion pressure and speed and measuring the resulting line width. The process map was then used to select the optimal parameter combinations for printing scaffolds with spatial gradients in porosity.[121] A major limitation of this approach is the requirement for extensive prior experimentation to build the process map. Taking a step forward, Zanderigo *et al.*[121] showed that a smaller set of experiments can be performed and that the results, combined with a probabilistic model (based on logistic regression), can be used to generate process maps for different quality attributes in EBB.[122] Finally, finite elements and mathematical models have been proposed as tools to optimize the bioprinting process *a priori* without the need for experimentation.[123] For example, Comminal *et al.*[123] developed a computational fluid dynamics model to investigate deposition around the corners in extrusion printing, optimize *a priori* the trajectory profile of the printer, and reduce over- or under-extrusion phenomena. The numerical simulation results from the model highlighted that there was an optimal amount of printing path smoothing (i.e., rounding of sharp corners) that could significantly improve the corner quality.[124]

In-process monitoring can be achieved by adding sensors to the printer, for example, with cameras and other sensors (e.g., temperature, humidity, pressure), and measuring important quality features like strand diameter and spacing in EBB or droplet size and satellite droplet formation in inkjet.[125–127] Recently, Strauß *et al.*[127] proposed an automated method for measuring line width in EBB. The authors developed an image processing approach that captured the printed line from a top view, then binarized and segmented the resulting image. A quantitative analysis was performed on the binarized image by measuring the number of pixels corresponding to the line width and tracking its evolution over time.[128] In another work by the same group, the authors investigated the use of a flow sensor for in-process QC of pneumatic extrusion. In particular, the flow rate is a key parameter to monitor, since variations may lead to under- or over-extrusion and, consequently, decreased shape fidelity of the final construct. The results highlighted that the flow sensor is a valid tool for EBB and can be incorporated into a closed-loop control system to adjust printing pressure in real time.[129,130] Apart from features related to the shape fidelity of the construct (e.g., pore size and morphology, overall dimensions), it is also important to track cell vitality during printing as another key quality feature. Very few examples are currently available in the literature. Recently, Narayanan *et al.*[130] proposed the use of dielectric responses of bioprinted cell-laden constructs as a tool for in-process QC. In particular, the main idea behind their work is that viable cells give a

significantly different dielectric response than non-viable cells when the construct is subjected to an alternating electrical field. The results showed that the technique can be successfully applied as a non-contact, non-destructive in-process monitoring method for cell-laden bioprinted constructs.[131]

Finally, the printed constructs can be analyzed after printing to assess their shape fidelity when compared to the computer design.[28] An optimal post-printing analysis method should be non-destructive and use non-toxic or non-radioactive agents to avoid damaging cells or changing the scaffold functionality. Furthermore, the imaging speed should be fast to avoid changes (i.e., degradation, dehydration) to the printed scaffold. The use of conventional optical microscopy solutions is limited for three main reasons: (i) transparent and reflective hydrogels are commonly used as the main biomaterials; (ii) these techniques are limited to imaging the outer surface of the scaffold, and hence, cannot capture the internal porosity; and (iii) the techniques possess a long analysis time to image the whole scaffold due to small field of view may lead to structural changes In contrast, 3D imaging methods such as micro-computed tomography (CT) and magnetic resonance imaging (MRI) provide deep measurement capabilities and can visualize optically transparent samples without requiring toxic contrast agents.[132–134] More recently, optical coherence tomography (OCT) has emerged as an alternative to these conventional 3D imaging techniques. OCT offers real-time, detailed information on the 3D structure and composition of biological tissues by using back-scattered or back-reflected light, achieving image resolutions of around 1–15 μm at a depth of approximately 3 mm.[135] OCT depends on the sample scattering properties rather than fluorescent or ionizing radiation, which poses a low risk of altering or changing the materials and cells.[136] Wang *et al.*[136] used OCT to quantitatively assess the shape fidelity of EBB-printed scaffolds fabricated with a biomaterial ink containing a mixture of gelatin and alginate. The authors imaged the scaffolds and extracted key quantitative measures like pore size, shape, and interconnectivity, strut size, and surface area, which were then compared to the designed ones as a post-print quality assessment.[137]

As QC is gradually integrated into the bioprinting workflow and the volume of monitoring data increases, new techniques for data integration and automated analysis are needed (Figure 4B).[138] In recent years, machine learning algorithms—a subset of artificial intelligence techniques that enable automatic learning from data to make predictions or decisions without explicit programming—have been explored to enhance the control and automation of the bioprinting process (Figure 4C).[139–142] For example, Fu *et al.*[142] optimized EBB parameters using a support vector machine model. They first defined a set of adjustable printing settings, including cartridge temperature, layer height, and needle gauge for a Pluronic F127-based biomaterial ink. Three-layer grid-like designs were printed using various combinations of these parameters, and print fidelity was assessed by measuring line width and comparing it with the theoretical values. The support vector machine model then generated a 3D map highlighting the parameter combinations likely to produce higher-quality prints within a specified probability threshold.[143] Similarly, Ruberu *et al.*[143] proposed an iterative Bayesian optimization method for refining EBB parameters, such as ink composition, reservoir and bed temperatures, extrusion pressure, and printing speed. The optimization began by printing lattice structures with randomly selected parameters. Each print was visually evaluated to assign a quality index, which was then used to construct a probabilistic model based on a Gaussian process. The model suggested a new set of parameters, which were used to produce the next batch of prints and corresponding quality scores. This iterative process continued until convergence.[144]

In our recent work[144], we applied deep learning techniques for QC in EBB. We created a comprehensive dataset capturing multiple printing scenarios by recording the process from a front view using a high-resolution webcam. Each video corresponded to a print with a distinct combination of parameters, including layer height, flow rate, extrusion setup (pneumatic or piston-actuated), and material color. Two primary types of printing errors were deliberately introduced: under-extrusion (insufficient material) and over-extrusion (excess material). This dataset was used to train a custom convolutional neural network optimized for high classification accuracy and fast response time. The model enabled real-time monitoring and automatically stopped the print if an error occurred, thereby saving time and reducing material waste. Additionally, an automatic parameter optimization system was implemented, using consecutive prints with varying settings to adjust parameters without manual intervention or material characterization.[145]

Despite significant progress, the clinical translation of bioprinting remains limited by both technological and regulatory challenges. Key obstacles include the lack of standardized bioinks with consistent mechanical and biological performance, difficulties in integrating vascular networks within printed constructs, and the absence of clear regulatory definitions and quality standards governing bioprinted products. In the case of sustainable

biomaterials, additional challenges arise from the intrinsic variability of biomaterial sources, which can affect their rheological behavior, batch-to-batch reproducibility, and printing performance (Section 3.6). Additionally, ensuring reproducibility and sterility throughout the printing process remains a significant hurdle.

As bioprinting systems evolve toward greater material diversity, structural complexity, and application specificity, QC emerges as a central integrative element rather than a standalone technical requirement. Effective monitoring and data-driven control strategies are essential to manage the uncertainties introduced by multi-material constructs, novel bioinks, and dynamic printing conditions. Importantly, QC is a key enabler for translating advanced bioprinting paradigms, where post-print characterization alone is often insufficient or impractical. By linking material selection, process parameters, and functional outcomes, QC frameworks provide the connective infrastructure needed to support reproducibility, comparability, and eventual clinical and industrial adoption.

The implementation of robust QC strategies, including pre-process optimization, in-process monitoring, and post-process assessment, is therefore critical not only for compliance but also for advancing bioprinting toward new modalities. Emerging approaches, such as sensor integration, advanced imaging, and artificial intelligence-based data analysis, offer powerful tools for real-time feedback, automated error correction, and process standardization. These strategies are particularly crucial in *in situ* bioprinting, where QC ensures precise deposition in a dynamic physiological environment, and OOC bioprinting, where reproducibility at micro-scale resolution is required to maintain functional tissue models. Similarly, in 4D bioprinting, QC is essential for controlling

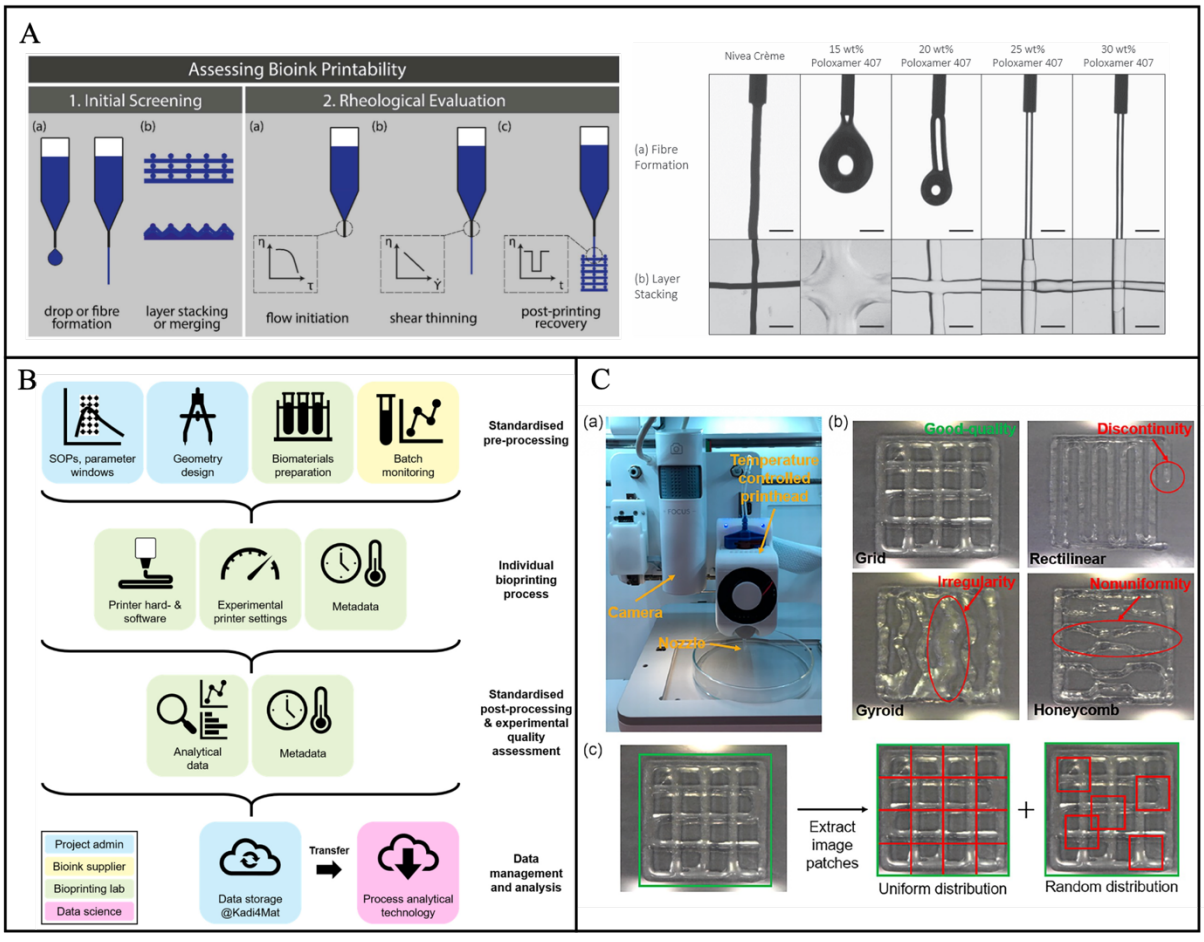

Figure 4. Quality control strategies in bioprinting. (A) Example of pre-process quality control by identifying the EBB printability through simple tests (i.e., layer stacking, fiber formation). Scale bar: 1 mm. Reprinted from Paxton *et al.*[118] (B) Schematic workflow of a bioprinting process with its related data. Reprinted from Schmieg *et al.*[137] (C) Example of an experimental setup for error detection in a bioprinting system where machine learning is used to analyze raw images and monitor printing anomalies. Reprinted with permission from Jin *et al.*[141] Copyright © 2021, American Chemical Society.

the spatial and temporal assembly of responsive materials to achieve desired shape transformations, while in 5D bioprinting, QC becomes critical for coordinating complex geometries with biological functionality.

Critically, QC in these advanced paradigms (described in detail in the following sections) goes beyond conventional post-print inspection. It requires predictive and adaptive frameworks that link material properties, printing dynamics, and biological outcomes, enabling proactive correction and standardization across multiple scales and conditions. Without such integrative QC approaches, the potential of advanced bioprinting modalities, such as *in situ* tissue repair, personalized organ models, and dynamic functional constructs, cannot be fully realized, limiting both clinical translation and industrial scalability.

5. Changing the paradigm of classical bioprinting approaches

The continuous evolution of bioprinting technologies has created a need for innovative strategies that go beyond traditional layer-by-layer fabrication. In this context, *in situ* bioprinting, OOC bioprinting, and the advent of 4D and 5D bioprinting are redefining the way living tissues and complex biological systems are engineered. These approaches collectively aim to overcome the limitations of classical methods by introducing greater precision, adaptability, and biomimicry into the biofabrication process. *In situ* bioprinting enables direct tissue repair and regeneration within the native biological environment, while OOC bioprinting bridges the gap between engineered tissues and functional physiological models for drug testing and disease modeling. Meanwhile, 4D and 5D bioprinting introduce the dimensions of time, responsiveness, and dynamic structural evolution, allowing constructs to change their form or function in response to environmental stimuli.

Advanced bioprinting paradigms make the interdependence between materials, fabrication strategies, and QC particularly evident. These approaches are inherently application-driven and impose stringent requirements on bioink performance, process reliability, and real-time adaptability. For example, *in situ* bioprinting demands tight integration between printing systems and patient-specific environments, while OOC platforms and 4D/5D constructs rely on precisely controlled material responses and dynamic behavior over time. Consequently, these paradigms not only benefit from prior advances in multi-material printing, sustainable bioinks, and monitoring technologies, but also actively expose their current limitations, reinforcing the need for co-development rather than sequential optimization across the bioprinting pipeline.

Together, these innovations represent a paradigm shift toward more dynamic, functional, and personalized biofabrication strategies, ultimately advancing the translation of bioprinted constructs from the laboratory to clinical and industrial applications. At the same time, they include all previously discussed challenges, such as material selection, multi-material processing, sustainability, and QC, and highlight that future progress in bioprinting will depend on the coordinated, co-evolving development of these elements rather than on isolated technological advances.

5.1. *In situ* bioprinting

As previously stated, the introduction of bioprinting has brought numerous improvements to traditional TE methodologies for producing scaffolds for organ/tissue regeneration. However, in its clinical application, this technology—also known as *in vitro* bioprinting—has several limitations. The main drawbacks can be summarized as follows:

(i) The need for a prolonged bioreactor culture period to allow the cellularized scaffold to mature and differentiate to create a functional unit outside the human body; only after this post-bioprinting phase can the structure be implanted to restore the damaged tissue/organ.[146,147]

(ii) Difficulty in handling, transportation, and implantation in the human body of the fragile 3D bioprinted structure can lead to disruption of micro- and/or macro-scaffold architecture[148,149], risk of contamination caused by transportation and manual implantation[150], and the need for a highly sterile environment throughout the process.[151]

(iii) Difference between the actual defect site and the shape/morphology of the fabricated construct due to inaccurate design inputs caused by medical imaging resolution limits: this can result in an extended surgical time to adapt the bioprinted structure to the geometry of the damaged site, and also in compromising the mechanical anchoring to the native tissue.[146,147]

(iv) Difficulty in obtaining an optimal vascularization: 3D bioprinted structures require a complex internal vascular network to supply nutrients and oxygen to all cells seeded on the scaffold, and this is not always easy to reproduce with in vitro bioprinting technologies.[152]

In situ bioprinting (also known as intraoperative bioprinting) is a promising solution to overcome the limitations of bioprinted scaffold-based TE. It directly dispenses bioinks onto and into the damaged site, following pre-planned printing paths. By directly fabricating pro-

healing structures at the defect site, the specific shape and architecture of the tissue/organ to be restored can be easily reproduced. Moreover, directly working in the operating room, a sterile environment is always guaranteed. Finally, this innovative approach will enhance the maturation and differentiation of the bioprinted constructs, as the patient's body itself serves as a bioreactor. This will not only reduce operating time but will also promote vascularization since native progenitor cells may tend to migrate into the *in situ* bioprinted porous scaffold, improving the sprouting of capillaries from the endogenous tissue.[153–157]

At present, two primary strategies for *in situ* bioprinting are being explored: handheld and robotic approaches. The handheld method employs a portable device equipped with a bioprinting unit, enabling the direct deposition of biomaterials. Compared with robot-assisted 3D printers, this technique offers several advantages: (i) as a manual tool, it allows surgeons to shape the defect intraoperatively and achieve the desired structure; (ii) it provides greater surgical dexterity, facilitating deposition within fissures or beneath protrusions of native tissue; (iii) the device is compact, lightweight, and easily maneuverable; and (iv) it is simpler to sterilize and maintain under sterile conditions. This flexibility enables surgeons to construct replacement tissue freehand, tailoring multiple layers to specific tissue requirements without the need for computer-aided path planning. Although still based on layer-by-layer deposition, the "printing pattern" is guided manually by the surgeon.[2,158] A typical handheld bioprinter includes an ergonomic grip, one or more bioink cartridges, extrusion nozzles, a light-curing unit (if required), and a piston-driven or pneumatic extrusion system. The suitability of this approach depends largely on the anatomical site and structural complexity of the target tissue. While relatively simple constructs—such as skin repair sheets or fillers for cartilage and bone defects—can be readily fabricated[158–160], more complex, multi-material tissues with hierarchical organization require advanced mechanisms.[161,162]

In contrast, the robotic approach relies on a manipulator with three or more degrees of freedom, which delivers bioinks to the target site via a bioprinting unit mounted as an end-effector (Figure 5A).[163,164] Although this method reduces human involvement compared with the handheld technique, surgical oversight remains essential to address intraoperative complications or to adjust pre-planned procedures. A standard robotic *in situ* bioprinting system consists of a robotic arm and an interchangeable bioprinting end-effector, whose design depends on the chosen bioprinting technology. Compared to the handheld approach, the robotic systems presents several advantages: (i) access to the printing site through minimally invasive pathways; (ii) use of computer-aided design models or medical imaging (e.g., CT, MRI) to predefine construct geometry and tissue microarchitecture, enabling precise spatial control over the deposition of cells, biomaterials, and growth factors[165]; (iii) execution of complex geometrical tasks (e.g., operating inside a 3D bone cavity, following non-planar toolpaths[166]), force control at very fine scales (<0.1 N), high-resolution and high-precision movements (~0.1 mm), and synchronization with the motion of dynamic organs[167,168]; and (iv) integration of single or multiple bioprinting units within the robotic end-effector. This approach has already demonstrated potential in applications such as skin, cartilage, and bone regeneration[169–171], offering greater capacity to reconstruct large, structurally complex defects compared with handheld systems.

Considering robotic-based *in situ* bioprinting, proven to be the most promising approach, a standardized workflow can be schematized in four main steps (Figure 5B)[172,173]:

(i) Surface scanning and reconstruction: In this step, a 3D model of the defect targeted for bioprinting is generated. Scanning can be performed using various techniques, such as touch probes, optical scanners, or by directly segmenting the defect surface from preoperative volumetric imaging (e.g., MRI, CT).
(ii) Printing path planning: Based on the acquired geometry, the printing pattern to restore the defect is planned.[164]
(iii) Registration of the printing pattern in the robot workspace: The transformation matrix between the reference frame of the patient (robot workspace) and the planning software is computed using anatomical references and/or fiducial artificial markers.[174]
(iv) *In situ* bioprinting: The process is carried out by depositing the biomaterial directly on the damaged area of the patient.

An important concern of *in situ* bioprinting is the deposition onto moving surfaces. When printing onto an organ, it should, in fact, be immobilized, but this is not always possible. For this reason, it is necessary to compensate for the motion of the bioprinting unit during the deposition phase to account for physiological or unexpected movements. To achieve this objective, a vision system or a distance sensor can be used to correct the position of the extruder in real time, thus having a dynamic deposition, for example, during the respiration phase.[175–177]

One more concern is the bulkiness of bioprinting units and robotic bioprinting systems, which have limited their application to superficial tissues and organs. Nevertheless, in the last few years, minimally invasive approaches have

been explored to enable *in situ* bioprinting within the human body via an endoscopic approach. A miniaturized delta robot was designed and installed as the end-effector of an endoscope and tested for *in situ* bioprinting applications within a stomach model. The device was able to extrude a bioink inside the model, ensuring good cell viability and enough precision in deposition, but still needs to be improved, as it is too big, considering that commercially available endoscopes are around 20 mm in diameter.[178] Another example concerns the design of a flexible *in situ* bioprinter. In this case, a novel bioprinting unit was designed for installation on a flexible colonoscope. The actuation system was based on hydraulically actuated soft microtubule artificial muscles, which enable device positioning control. This system was successfully tested on a porcine kidney and inside a colon phantom. Nevertheless, its actuation system was characterized by high hysteresis, which does not allow a high printing resolution, and has a complex miniaturization.[179] Finally, a minimally invasive robotic system was designed to access the human body through a small orifice and be controlled in position via magnetic actuation with external electromagnets. The device was also equipped with a vision system used both to scan the anatomical defect to be filled and to perform QC during printing movements. In this case, tissue movement was not taken into consideration, and the deposition precision is very limited due to the magnetic actuation that does not allow a high printing resolution.[180]

Despite its remarkable potential, *in situ* bioprinting still faces several technological and biological limitations that must be addressed before widespread clinical adoption. Major challenges include restricted printing accuracy on dynamic or irregular anatomical surfaces, limited accessibility to deep or confined surgical sites, and difficulties in achieving precise, real-time control of deposition in the presence of tissue motion. Moreover, miniaturization of printing units, optimization of bioink formulations to ensure both printability and rapid integration with host tissue, and standardization of sterilization and intraoperative workflows remain critical hurdles. To mitigate these issues, current research focuses on integrating advanced imaging and sensing systems for real-time feedback and motion compensation, developing soft, flexible robotic platforms for minimally invasive access, and engineering multifunctional bioinks with enhanced mechanical stability and biological responsiveness. The convergence of these innovations, supported by artificial intelligence-driven path planning and closed-loop control, is expected to significantly improve printing precision, reproducibility, and safety, ultimately bringing *in situ* bioprinting closer to clinical translation.

5.2. Organ-on-a-chip bioprinting

In 1991, the use of cell culture for disease studies was established[181] and, given their simplicity, static 2D cell culture phantoms began to spread in this research area.[182] Although they are still commonly used and often serve as effective tools, 2D cell cultures have many limitations in sufficiently mimicking *in vivo* conditions, mainly due to an overall inability to control the cell culture's structure, mimic cell–cell interactions and biochemical signaling pathways, and model the 3D dynamic microenvironments of real tissues.[182–184] Thus, to overcome these issues, the focus has shifted to 3D cell cultures, which enable a more realistic representation of the complex 3D architecture of human tissues by allowing cell expansion, clustering, and migration.[182,185,186] Particularly, accumulating evidence has highlighted the central role that OOC platforms have gained in this research field. They represent microengineered tissues cultured within 3D microfluidic devices that serve as bioreactors to mimic and study human organs.[182,187–189] Leveraging recent advances in microfluidics, biomaterials, bioengineering, biotechnology, medicine, and biofabrication, OOCs aim to recapitulate the typical signaling pathways, architecture, dynamic microenvironmental conditions, functions, and responses to external stimuli of human organs.[182,186,187,189–191] Compared to conventional 2D and 3D cell culture systems, the main advantages of OOCs include: (i) a continuous perfusion which increases cell viability and proliferation; (ii) gas permeability control, which allows the co-culture with non-oxygen-requiring species (i.e., microbiota's anaerobic microorganisms); (iii) transparency, which enables *in situ* microscopic imaging; (iv) integrability with novel miniaturized sensors and biosensors, allowing the real-time monitoring of relevant environmental parameters during culture; (v) more accurate control over concentration profiles of specific substances (e.g., nutrients, target analytes, reagents) by modulating the laminar flow in the microchannels; (vi) reduced usability cost as a result of the lower amount of culture media and reagents due to the microscale dimensions; and (vii) reproduction of tissue-specific environmental cues, such as specific analytes' gradients (e.g., oxygen), the mechanical stimuli of peristalsis and respiration, and/or the cardiovascular cycle.[182,187,192,193]

Accordingly, OOCs emerge as powerful tools for gaining insights into the functionality of human organs, their physiological signaling pathways, and their responses to the administration of specific substances (e.g., drugs, probiotics, prebiotics, diet). Thus, they pave the way for a faster, more accurate, and patient-specific prediction of the efficacy and potential side effects of novel drugs or

therapeutic solutions compared to the use of traditional *in vitro* or *in vivo* models[187,188,194,195], thus fostering advances in personalized medicine research.

One of the crucial features of OOCs that requires particular attention for optimal mimicking of human organ functions is the reproduction of the complex tissue(s) architecture typical of the investigated organ, which is achievable by employing suitable fabrication techniques and processes. Among OOCs' fabrication techniques, photolithography, soft lithography, replica molding, microcontact printing, and injection molding are the current gold standard.[182,187,196] Despite their widespread use, they face throughput and reproducibility issues as they usually require cleanrooms, high-level microfabrication expertise, and multiple production steps; moreover, secondary facilities for the cell seeding process are needed, therefore increasing the already high expenses in terms of time and production costs.[182,187,190,197,198]

Regarding the issue, 3D bioprinting has the potential to be a game-changer, addressing the limitations of traditional OOC fabrication technologies through its versatility. Indeed, compared to the conventional fabrication techniques, 3D bioprinting requires minimal microfabrication expertise and allows the simultaneous/consecutive bioprinting of multiple bioinks (a formulation of cells suitable for processing by an automated biofabrication technology that may also contain biologically active components and biomaterials[44]) and biomaterial ink (biomaterials used for printing and cell-contact occurs post-fabrication)[44] types with good spatial resolution and reproducibility.[187,198,199] Due to these features, in recent years, this research field has seen an increasing trend in the number of OOC publications involving 3D bioprinting (Figure 5C), which consolidated such fabrication technology as an optimal candidate to automatically fabricate finely tuned, customized, complex, heterogeneous, and functional 3D biomimetic constructs.[182,187,190]

5.2.1. Selection of the most appropriate fabrication technology for organ-on-a-chip three-dimensional bioprinting

Selecting the most appropriate 3D bioprinting technology for OOC applications depends on the organ/tissue to model *in vitro*. Indeed, the target application constrains the main materials to employ, the architecture and characteristic dimensions of its structural and morphological features, and the OOC chip type.[200]

The material choice has to be tailored to the target organ or tissue to model, since, as already discussed in Section 3, certain physical and biological specifications, such as cytocompatibility, biomimicry (i.e., the ability to mimic the tissue's biochemical microenvironment and architecture), shear-thinning rheological behavior (to enhance structural fidelity and cell viability), and mechanical and physical stability, need to be satisfied to obtain the desired outcome. The unique properties of hydrogels such as gelatin, collagen, alginate, or decellularized ECM make them ideal candidates as bioinks or biomaterial inks for OOCs' 3D bioprinting, especially with EBB techniques. Other solidifiable materials (both natural and synthetic), such as temperature-sensitive polymers (e.g., PCL in EBB) and photopolymers (e.g., polyethylene glycol diacrylate or GelMA in LAB), are also employed.[187,201] Another crucial aspect to consider is the characteristic dimension of the OOC's structure. It constrains the printing resolution of the 3D bioprinting technology, which has to be compatible with the target features to fabricate.[202]

As already discussed in Section 1, different printing technologies present different resolutions. Specifically, EBB and DBB (resolutions > 100 μm and > 50 μm respectively) could be suitable for macro-scale components and features with higher dimensions, such as the chip's overall structure (~10 to 50 mm)[203], the chip's compartments and bigger microchannels, or particular scaffold architectures for the *in vitro* culture (~100 μm to 1 mm).[203] Conversely, due to their higher printing resolutions, DBB and LAB techniques could be used for specific micro-scale features such as small microchannels or vascular channels, specific scaffolds' architectural details (~50 to 200 μm), or semipermeable membranes (<100 μm).[191,202,203] When the OOC presents multi-scale features, a hybrid approach is generally the most indicated option.[190] Additionally, the OOC chip type is another factor in selecting the bioprinting technology. In some cases, the biological tissue or sample must be bioprinted directly inside a pre-assembled, sealed commercial chip. In this case, LAB technologies such as SLA and two-photon polymerization can bioprint structures directly within sealed OOCs, following a thorough optical property optimization phase to ensure high-resolution *in situ* bioprinting.[200] On the other hand, a modular approach is used for open prefabricated chips with sealable halves, for which EBB and DBB techniques can be employed to bioprint the tissue construct before a post-printing sealing process.[200] Lastly, recent cutting-edge approaches envision the fabrication of both the OOC's structure and tissue constructs in a single printing process, thus avoiding potential damage introduced by post-printing manipulation.[204,205] Detailed reviews of the commonly used technologies and the relative bioinks or

biomaterial inks in the field of OOCs 3D bioprinting can be found in other studies.[182,187,190,200,206]

5.2.2. Three-dimensional bioprinted organ-on-a-chip: applications, challenges, and future perspectives

Literature findings assess the use of 3D bioprinted OOC platforms to model organs such as the liver (Figure 5D), kidneys, heart, lungs, gut (Figure 5E), bone, vessels, and even tumors. Some examples of the most recent biomedical applications of 3D bioprinted OOCs, along with the bioprinting technique used, dimensional requirements, and materials used, are presented in Table 2.

Although bioprinting has been extensively explored in literature and has been increasingly employed for the fabrication of OOC platforms with multiple potential benefits (i.e., faster drug screening processes, reduction of the expenses connected to drug development), the field is still in its infancy.[187,190] None of the available 3D bioprinting techniques is optimal: LAB methods have high resolutions and can produce complex 3D structures, but the high cost, limited material selection, photo-induced cell damage, and scalability are still challenging; on the other hand, although cost-effective, EBB and DBB have lower resolution and exert higher shear stresses on cells, which lead to lower cell viability.[182,190]

Future perspectives may include developing novel 3D

Table 2. Examples of organ-on-a-chip systems fabricated through three-dimensional bioprinting

OOC system	Feature size	Bioprinting technique	Used materials	Results	Ref.
Liver	~400 μm	EBB	PCL Collagen + HepG2 Gelatin + HUVEC	Better liver function than static culture models	198
Kidney	15–250 μm	EBB	Gelatin Fibrin Pluronic	Selective reabsorption and vectorial transport of solutes	204
Heart	~150 μm	EBB	Thermoplastic polyurethane (TPU) Carbon black nanoparticles-filled TPU PDMS Silver particle-filled polyamide Polylactic acid	Models drug toxicity Mimics heartbeat	205
Lung	~200 μm	EBB	Polycaprolactone Tracheal mucosa-derived dECM (tmdECM) + endothelial cells (EC) tmdECM + lung fibroblasts tmdECM + microvascular EC	Mimics disease response	207
Gut	150–500 μm	EBB	Collagen solution + Caco-2 Collagen solution + HUVECs	Successful cellular proliferation of both types of cells Establishment of cell–cell interactions	208
Bone	~200 μm	DBB	PLGA + Hydroxyapatite + Tricalcium phosphate	Disease modeling Drug testing	209
Vessel	>30 μm	EBB	GelMA + fibroblasts Pluronic	Mimics blood flow Thrombosis modeling Drug screening	210
Blood–brain barrier (BBB)	<10 μm	LAB	Parylene-C-coated VeroClear photopolymer Silicone Polycarbonate	Mimics *in vivo* BBB integrity and compound permeability Drug screening	211,212
Tumor	10–500 μm	EBB	GelMA PEGDA PEGOA GelMA + MCF-7 breast cancer cells	Disease modeling Drug testing	186

Abbreviations: DBB: Droplet-based bioprinting; dECM: Decellularized extracellular matrix; EBB: Extrusion-based bioprinting; EC: Endothelial cells; GelMA: Gelatin methacrylate; HUVEC: Human umbilical vein endothelial cells; LAB: Light-assisted bioprinting; OOC: Organ-on-a-chip; PCL: Polycaprolactone; PDMS: Polydimethylsiloxane; PEGDA: Poly(ethylene glycol) diacrylate; PEGOA: Poly(ethylene glycol) octaacrylate; PLGA: Poly(lactic-co-glycolic acid); TPU: Thermoplastic polyurethane.

bioprinting methods or combining existing techniques to address the field's challenges.[213] While most fabrication strategies employ 3D bioprinting to generate 3D-engineered biomimetic tissues in OOC bioreactors, it may also be possible to bioprint the microfluidic device.[188,195] By combining these options, the single-step 3D bioprinting approach for OOCs has the potential to enable faster design iterations and shorter turnaround times, making OOCs far more accessible and cost-effective.[182,188] In this perspective, hybrid advanced bioprinting platforms can be purposely designed, for example, incorporating SLA for chip fabrication and EBB for simultaneous microtissue fabrication, to overcome the limitations of single-printing approaches.[190] Some single-step 3D bioprinted OOCs are described in.[204,205] Beyond 3D bioprinting, 4D bioprinted OOCs represent a promising future research topic, as they enable the fabrication of bioconstructs and devices that can change shape or functionality in response to external stimuli after bioprinting.[214,215] This will foster not only the generation of more complex 3D structures that conventional 3D bioprinters hardly achieve, but will also enhance OOCs' biomimetic ability to more naturally recapitulate *in vivo*-like responses to stimuli (e.g., pH changes after drug administration).[213]

In summary, with further research regarding the improvement of resolution, vasculature fabrication, process optimization, process standardization, and bioink and biomaterial ink's formulations[187,190], 3D bioprinted, highly efficient, automated, and integrated OOC systems are expected to be broadly used for advanced human organ-level physiology investigations, disease modeling, drug development, and drug screening.

5.3. Four-dimensional bioprinting

In 2013, Dr. Skyler Tibbits[215] coined the term "4D printing" to describe AM processes used to create structures capable of changing shape over time in response to a predefined stimulus.[216] Today, 4D-printed structures are often associated with characteristics, such as shape-changing, self-repairing, and self-assembly, emphasizing that these objects are no longer static but programmable, active systems that perform their functions through changes in physical and/or chemical properties over time.[217–219] The essential elements of 4D printing include AM technologies, active or multi-material systems, and external stimuli, such as temperature, humidity, electric fields, pH, or light.[220,221] Within this framework, AM serves as a key enabling technology, enabling the precise deposition of one or more materials at exact positions without limitations on geometric complexity. Many 4D printing applications rely on active materials—also referred to as responsive or smart materials—that undergo predictable, reproducible, and macroscopically useful physical or chemical changes in response to environmental triggers.[222,223]

Since its introduction in 2013, 4D printing has rapidly expanded across multiple fields, including biomedical applications.[218,224] This growth is driven by advantages over traditional static AM technologies, such as the ability to fabricate complex 3D structures more easily with reduced spatial constraints—since 4D constructs are often initially printed in flat forms—and the use of safer, wireless alternatives to electrical energy, such as chemical potential or thermal energy, enabling their application in challenging environments like the human body.

In biomedical research, 4D printing has been applied to bioactuators, TE, drug delivery systems, and medical device fabrication. For example, in our previous studies, we leveraged the temperature responsiveness of regenerated silk to create two devices for intestinal surgery. Both devices used the same combination of active (regenerated silk) and passive (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) materials, and the same stimulus (temperature increase from room temperature to body temperature). However, the differing functional requirements—intestinal anastomosis versus intestinal distraction enterogenesis—were achieved by varying the spatial arrangement of the materials, forming bilayer tubes (Figure 5F)[225] and core–shell coiled structures[226], respectively. This level of design freedom was enabled by a customized EBB bioprinter (Figure 5G). Finite element models were employed in both studies to predict temperature-triggered contraction of the devices based on the regenerated silk content, followed by experimental validation. The clips for sutureless anastomosis, tested *ex vivo* on porcine intestines, withstood bursting pressures 140% higher than conventional sutured samples (Figure 5H). The core–shell coils for distraction enterogenesis, tested on a porcine phantom, successfully pulled the phantom flaps closer in response to temperature increase.

Although 4D printing has shown promising biomedical applications, several challenges still limit its clinical translation. The main issues include limited availability of biocompatible smart materials with predictable, controllable responses, the complexity of accurately modeling shape transformations in physiological environments, and difficulties in ensuring stable integration of 4D-printed constructs with native tissues. Future efforts should focus on developing new bioactive materials, improving computational models to predict dynamic behavior, and integrating real-time sensing and control strategies during fabrication. Through these advances, 4D printing could evolve into a reliable platform for creating adaptive, patient-specific medical devices and

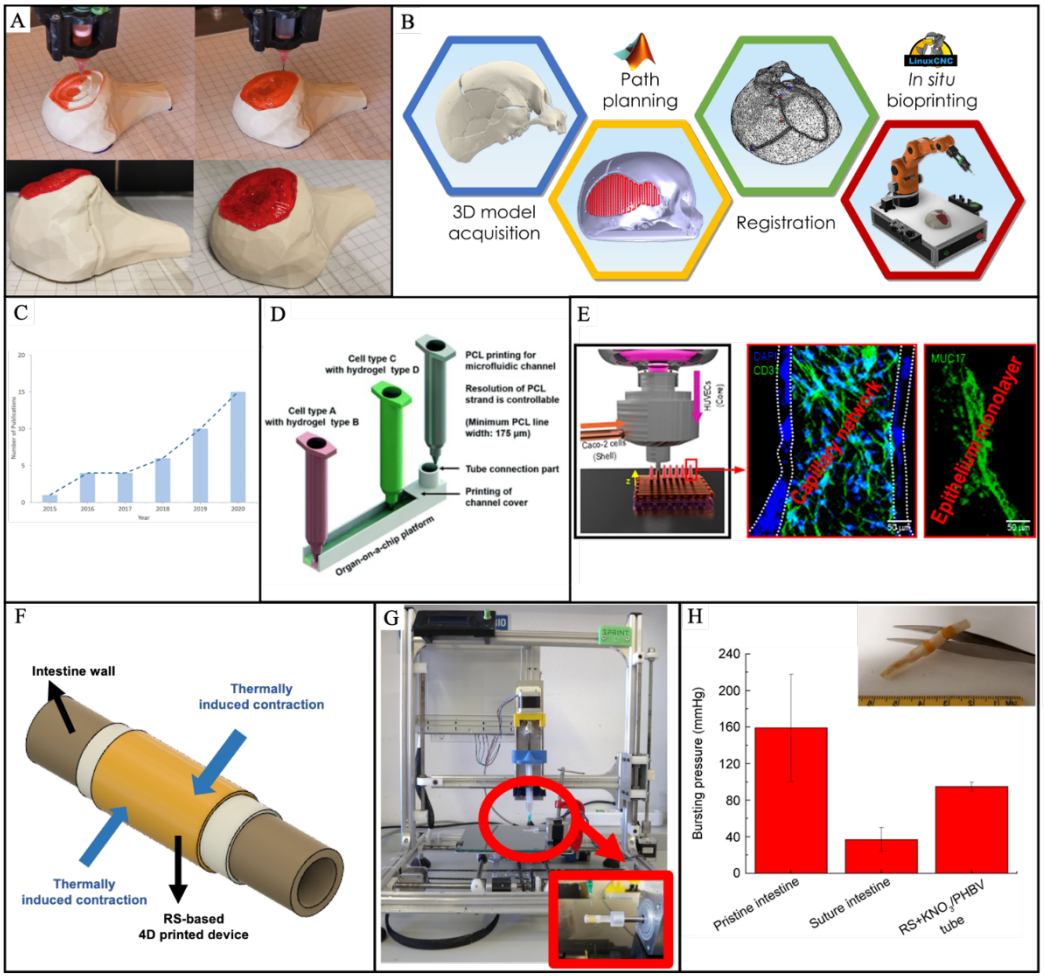

Figure 5. Advanced bioprinting approaches. (A) Robotic-based in situ bioprinting using IMAGObot for the regeneration of a bone defect. Reprinted from Fortunato *et al.*[163] (B) Workflow of the robotic-based in situ bioprinting.[172–174] Created using MS Power Point. (C) Trend of the number of organ-on-chip (OOC) publications involving three-dimensional (3D) bioprinting in recent years. Reprinted from Thakare *et al.*[186] (D & E) Examples of single-step 3D bioprinted OOCs: (D) liver-on-a-chip; reprinted from Lee and Cho[197], and (E) gut-on-a-chip; reprinted with permission from Kim and Kim.[207] Copyright © 2018, American Chemical Society. Scale bar: 50 μm. (F) Cylindrical bilayered structure for intestinal anastomosis. (G) Customized 3D bioprinter equipped with a mandrel for the fabrication of cylindrical structures. (H) Integration of the device in a porcine intestine (top right) and measured values of bursting pressure for the pristine intestine, sutured intestine, and sutureless anastomosis. Reprinted from Bittolo Bon *et al.*[223]

regenerative therapies.

In summary, although 4D printing is still in its early stages and challenges remain—such as the need for biocompatible active materials and reliable mathematical models—it represents a breakthrough technology. Thanks to ongoing advances in materials science, AM, and biology, 4D printing is emerging as a powerful tool for addressing unmet clinical needs in TE and medical device development.

5.4. Five-dimensional bioprinting

In recent years, 5D printing has been gaining considerable attention. This is an evolution of 3D and 4D printing, and for most researchers, it adds a fifth dimension (such as embedded bioactive molecules) to the spatial (x, y, z) and temporal domains. In TE, this novel approach is linked to the development of active structures capable of dynamic shape morphing and controlled in situ signal release.[227]

This concept was formalized in 2020 by Li *et al.*[227], who proposed 5D printing in the context of fabricating biological functional tissues, such as myocardium scaffolds and neuron-like constructs, as integration of advanced manufacturing and life sciences.[228] In 2023, a minireview described the theoretical framework of a 5D approach, defining 5D-printed biopolymeric constructs embedding cell information, such as growth factors or cell-signals,

that are released *in situ* to drive cell environment or cell responses, transforming biofabricated structures from passive to active.[227]

It is important to understand the differences between 3D, 4D, and 5D printing. 3D bioprinting, or 3D biofabrication, is a research field focused on the layer-by-layer fabrication of cell-laden structures to replicate tissue topology; however, these structures remain static over time.[2,229] The 4D printing approach, enabled by the use of smart materials, introduces time-responsive shape changes in printed structures; however, it still lacks active interaction with the surrounding environment.[220]

Five-dimensional printing builds upon 4D structures by incorporating biomolecules that can be released *in situ* to actively interact with the external environment. An example is a bilayer scaffold laden with mesenchymal stem cells that transforms from a planar to a tubular shape at body temperature while releasing growth factors to promote cell proliferation and differentiation.[227] This approach, applied in TE, has demonstrated that such scaffolds can induce morphogenesis and functional development *in vitro*, with potential for *in vivo* applications. Although not explicitly labeled as "5D printing," Sexton *et al.*[229] introduced an advanced pipeline for designing synthetic vascular structures at the organ level. Their approach integrates computational hemodynamics with rapid printing of perfusable tubular networks, offering a scalable biofabrication solution. By combining geometry, function, and embedded design intelligence, their work aligns with the principles underlying 5D printing.[230]

In recent years, novel high-speed platforms such as dynamic interface printing[231] and adaptive VP[232] have been developed to enable faster fabrication of complex structures, including vascular-like geometries surrounding live bioinks. These technologies support real-time adaptation and overprinting, enabling the incorporation of cell signaling biomolecules to create responsive constructs—hallmarks of a 5D system. A *Nature* commentary[232] highlights that modern biofabrication must integrate biological viability, structural fidelity, and scalability, citing dynamic interface printing and high-resolution organoid bioprinting as leading examples. While not explicitly labeled as "5D printing," such systems exemplify the paradigm of embedded information and active function that 5D printing aspires to realize.[233]

Several significant challenges remain. Research and experimental demonstrations of 5D bioprinting applications—either *in vitro* or *in vivo*—are still limited. Biomaterials capable of embedding cell-instructive biomolecules and responding to specific stimuli with both shape morphing and signal release are scarce.[227] Moreover, this responsive release is often mistaken for traditional controlled or drug release; however, in 5D printing, it refers to a smart, stimulus-driven release that actively interacts with and adapts to the surrounding environment, based on the needs of embedded cells and the external tissue. To enable broader adoption, standardization and industrial scalability are essential for ensuring reproducibility across laboratories and facilitating eventual commercialization. Recent research priorities emphasize artificial intelligence-driven manufacturing, dynamic bioinks, and high-speed printing platforms as key components for advancing the field of biofabrication.[234]

From 2020 to 2025, 5D printing has evolved from a conceptual proposal[228] to the development of shape-morphing, information-embedded scaffolds, with increasing integration into advanced bioprinting platforms, such as dynamic interface printing and adaptive VP.

However, based on these considerations, what exactly is 5D printing? For some researchers, it represents an evolution of the 4D printing approach, adding a fifth dimension: the controlled release of cell-signaling biomolecules in response to environmental stimuli. In this view, 5D printing enables the creation of constructs that not only change shape over time but also actively interact with their biological surroundings through smart biochemical responses. For others, 5D printing is understood as a 4D approach enhanced by the integration of advanced biofabrication platforms and artificial intelligence-driven design. Here, the fifth dimension refers to the computational and algorithmic intelligence that guides the fabrication of complex 3D architectures—such as scaffolds with embedded vascular networks—that can later undergo shape transformation upon external stimulus. In this interpretation, the "fifth dimension" lies in the intelligent design and real-time adaptation enabled by external digital systems, particularly artificial intelligence.

For us, 5D printing is the combination of smart materials, living cells, bioactive molecules, and advanced 3D biofabrication techniques to create multi-material, multiscale structures that replicate the topological, biochemical, and mechanical characteristics of neotissues. These constructs are capable of dynamically adapting their properties in response to external stimuli and of actively interacting with and modifying their environment—just as native tissues do during tissue genesis. In this framework, the fifth dimension can be defined as the emergence of living, adaptive functionality, where printed constructs behave as evolving biological systems rather than static (3D) or pre-programmed (4D) structures. In this sense, 5D printing can also be described as "evolutionary biofabrication," where artificial intelligence algorithms

serve as tools to simultaneously manage and optimize the numerous parameters involved in the complex process of tissue formation.

It is important to note that the concept of 5D bioprinting is not yet defined by a unified or standardized framework and is currently used in the literature as a conceptual extension of 4D bioprinting to describe biologically adaptive and environment-responsive biofabricated systems. In this review, our intention is not to define 5D bioprinting as a standardized term, but rather to present and compare the different interpretations proposed by various researchers and to share our own perspective on this emerging concept. By doing so, we aim to encourage discussion and critical evaluation of how such multidimensional approaches could shape the future evolution of bioprinting technologies.

From this perspective, concepts associated with 5D bioprinting should be viewed as an extension of ongoing developments discussed throughout this review, rather than as a distinct or fully established technological paradigm. In particular, the integration of multi-material and multiscale bioprinting strategies, the use of sustainable and smart biomaterials, and the implementation of robust QC approaches are essential prerequisites for the realization of adaptive and biologically responsive constructs. Similarly, advances in *in situ* bioprinting, OOC platforms, and 4D bioprinting provide both the technological and conceptual foundations for exploring such dynamic biofabrication frameworks. Within this context, 5D bioprinting can be interpreted as a unifying, forward-looking concept that builds on existing methodologies to describe increasingly interactive, environment-responsive bioprinted systems.

6. Conclusion

Recent advances in bioprinting are rapidly expanding the achievable complexity and functionality of biofabricated systems; however, their translation remains constrained by several unresolved interdisciplinary challenges. While multi-material and multiscale printing strategies enable increasingly faithful replication of tissue architecture, their effective implementation depends on the development of well-characterized, reproducible bioinks—an issue that becomes particularly critical when incorporating sustainable and waste-derived biomaterials. In this context, robust QC frameworks and standardized validation protocols remain major bottlenecks, especially as bioprinted constructs transition from static designs to dynamic, adaptive systems.

The integration of bioprinting with OOC platforms, *in situ* fabrication, and 4D bioprinting further highlights the need for tighter coupling between material design, process control, and biological performance assessment. As temporal responsiveness and environmental interaction become central design parameters, data-driven approaches, including artificial intelligence, are likely to play an essential role in managing process complexity and ensuring reproducibility. Within this landscape, 5D bioprinting should be regarded not as a mature technology but as a conceptual framework that synthesizes spatial, temporal, and functional dimensions into a unified biofabrication perspective.

Progress toward clinically and industrially relevant bioprinting will depend on coordinated advances in sustainable biomaterials, dynamic biofabrication strategies, and rigorous QC, alongside early consideration of scalability and regulatory constraints. Addressing these challenges collectively will be key to transforming bioprinting from an experimental tool into a reliable and sustainable platform approach for clinical translation.

Acknowledgments

None.

Funding

This work is supported by the European Union's Horizon Europe research and innovation program (grant no. 101191747, TENTACLE; grant no. 101191804, LUMINATE; grant no. 101178568, DAEDALUS) and from the European Union–Next Generation EU, Mission 4-Component 1 (CUP I53D23002200006), through the Prin2022 Prometheus project, "4D printing self-deploying bio-enabled polymer scaffolds for the non-invasive treatment of bleeding intestinal ulcers" (grant no.: 2022BZLTTK). Additional funding was provided by the European Union–Next Generation EU, Mission 4-Component 2, Investment 1.5 (CUP I53C22000780001) under the Tuscany Health Ecosystem, Spoke 4: Nanotechnologies for diagnosis and therapy. This work was also partially supported by the Italian Ministry of Education and Research (MUR; In the Departments of Excellence program) within the framework of the FoReLab and CrossLab projects (Departments of Excellence).

Conflict of interest

Carmelo De Maria, Giovanni Vozzi, and Gabriele Maria Fortunato serve as the Editorial Board Member of the journal, but did not in any way involve in the editorial and peer-review process conducted for this paper, directly or indirectly. Other authors declare they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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