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3D bioprinting of iPSC-derived tissues: From structural fabrication to functional maturation and clinical translation

Running title: iPSC-based 3D bioprinting

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Abstract

Three-dimensional (3D) bioprinting combined with induced pluripotent stem cells (iPSCs) offers a practical route to building human tissue models with improved structural and functional relevance. By controlling cell placement and local microenvironments, these systems better reproduce tissue organization and multicellular interactions than conventional culture methods. This review provides a comprehensive analysis of recent advances in iPSC-based bioprinting, with a focus on how biofabrication strategies shape tissue organization and function. Recent work has shifted the field away from simply achieving structural fidelity toward maintaining stable and reproducible function. Progress in bioink design, vascularization strategies, and multi-material printing has enabled the generation of cardiac tissues with perfusable networks, neural constructs with coordinated activity, and metabolic tissues with sustained functional output. These advances have strengthened the use of bioprinted tissues in disease modeling and drug evaluation. Evidence from early clinical studies suggests that translation is currently driven by modular and well-defined products rather than fully printed organs. Cardiac patches, dopaminergic progenitor cell therapies, stem cell–derived islets, and retinal implants illustrate how simpler, function-oriented constructs can meet clinical and manufacturing requirements. The review further discusses key challenges for clinical translation, including tissue maturation, manufacturing scalability, and regulatory standardization. By connecting technological advances with emerging clinical evidence, this review establishes a conceptual framework for translating iPSC-based bioprinting into practical therapeutic applications.

Keywords: iPSCs; 3D bioprinting; Biofabrication; Tissue maturation; Clinical translation

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1. Introduction

The advent of induced pluripotent stem cell (iPSC) technology has revolutionized regenerative medicine and human disease modeling by providing an unlimited source of patient-specific, pluripotent-derived cells[1-3]. However, translating iPSC technology into reliable therapeutic and screening platforms remains hindered, not only by the commonly recognized challenge of generating specific lineages, but more critically by a set of iPSC-specific biological vulnerabilities that persist even after differentiation. Specifically, iPSC-derived cells are often fragile and highly sensitive to mechanical and biochemical perturbations; they tend to maintain an immature, fetal-like phenotype unless provided with precisely orchestrated microenvironmental cues; and their fate and function remain exquisitely dependent on dynamic, tissue-specific signaling and mechanical inputs[4-6]. These characteristics make conventional two-dimensional (2D) culture systems largely inadequate: iPSC-derived cells in 2D commonly exhibit immature metabolic profiles, insufficient structural and functional maturation, and poor reproducibility in response to external stimuli.

Although three-dimensional (3D) self-organizing models, such as organoids, have improved cell–cell and cell–matrix interactions to some extent and provided new tools for studying human development and disease[7-9], they fall short in addressing the unique demands of iPSC-derived cells. Organoid formation relies on stochastic self-organization, offering limited control over tissue architecture and cellular composition, and suffers from significant batch-to-batch variability[10]. Furthermore, most organoid models lack engineered vascular networks, restricting oxygen and nutrient delivery and thereby limiting long-term culture and scale-up capabilities[11]. These limitations are particularly problematic for iPSC-derived cells, which require precise, reproducible, and scalable control over their microenvironment to achieve stable functional maturation—a prerequisite for drug screening and translational research [12].

It is precisely at this nexus that 3D bioprinting emerges as a critical enabling technology tailored to the specific challenges of iPSC-based applications[13-15]. Unlike stochastic self-organization, 3D bioprinting follows a "design–fabrication–validation" engineering paradigm, allowing fine control

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over tissue geometry, spatial cell distribution, and microenvironmental parameters across both macro- and microscales[16,17]. This capability directly addresses the fragility, differentiation sensitivity, and maturation dependency of iPSC-derived cells by enabling the construction of protective, physiologically relevant niches with reproducible architecture and dynamic cue presentation. By integrating iPSC biology with programmable manufacturing technologies, 3D bioprinting is driving a paradigm shift from "letting cells self-organize into tissues" toward "on-demand manufacturing of human tissue systems with predictable structure and function"[18]. To provide an overview of the workflow and key steps involved in iPSC-based bioprinting, a schematic illustration is presented in Figure 1.

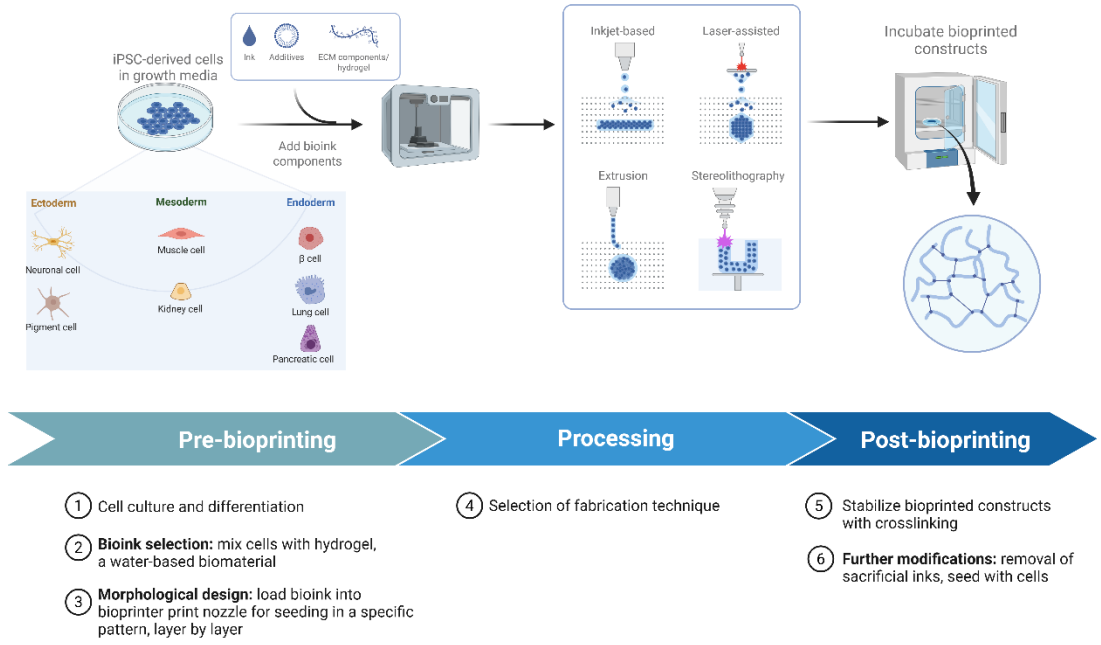


Figure 1. Workflow and conceptual framework of iPSC-based 3D bioprinting. Created with BioRender.com.

In recent years, with the maturation of key technologies including embedded bioprinting, sacrificial ink-based vascularization strategies, multi-material printing, and modular organoid assembly, the research focus in 3D bioprinting has undergone a significant transition. The emphasis has shifted from early-stage concerns about "maintaining cell viability during the printing process" toward questions of "whether functional, batch-consistent, and scalable human tissues can be constructed"[19-21]. This trend aligns closely with the biotechnology industry's

pressing demand for standardized in vitro models and manufacturable cell therapy products, positioning 3D bioprinting as an increasingly important bridge connecting iPSC basic research with real-world application scenarios[21,22].

From an application- and translation-oriented perspective, this review systematically synthesizes key advances in 3D bioprinting for the construction of iPSC-derived tissues, with a focus on identifying which technological pathways have already demonstrated realistic potential to support drug development, disease modeling, and cell therapy-rather than merely cataloging methodological innovations.

2. Application-oriented 3D bioprinting strategies for iPSCs

From an application-oriented perspective, not all 3D bioprinting strategies are equally suitable for iPSC-derived cells. iPSC-derived cells typically exhibit distinctive biological characteristics, including high mechanosensitivity, incomplete maturation, and stringent dependence on microenvironmental cues, rendering them particularly vulnerable to the selection of materials and structural design during both the fabrication process and subsequent culture. Consequently, a paradigm shift has emerged in recent years toward an "application-driven technology selection" approach, wherein the optimal printing strategy is dictated by the intended application scenario—such as drug screening, disease modeling, or cell therapy—rather than pursuing a one-size-fits-all solution. To contextualize the specific considerations required for iPSC-based bioprinting, Table 1 provides a concise comparison between iPSC-derived cells and other commonly printed cell types (e.g., primary cells, immortalized cell lines, mesenchymal stem cells) across key parameters that influence biofabrication outcomes, including printing modality compatibility, material requirements, shear sensitivity, phototoxicity risk, and post-printing maturation demands.

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Table 1. Comparison of iPSC-derived cells with other commonly printed cell types in bioprinting applications

Parameter	iPSC-Derived Cells	Primary Cells	Immortalized Cell Lines	Mesenchymal Stem Cells (MSCs)	Ref
Printing modality	Extrusion, inkjet, DLP, embedded (preferred)	Extrusion, inkjet, DLP (cell-type dependent)	All modalities (high tolerance)	Extrusion, inkjet, embedded	10-12
Material compatibility	Require soft, ECM-mimetic hydrogels, sensitive to stiffness	Variable; tissue-dependent	Tolerate broad stiffness range	Prefer intermediate stiffness	5,7,9
Shear sensitivity	High; shear stress >10^2 Pa compromises viability and pluripotency; may induce genomic instability	Moderate; varies by tissue origin	Low; tolerant of high shear (up to 10^5 Pa)	Moderate; shear may affect differentiation potential	6,23,24
Phototoxicity risk	High; prolonged UV/blue light exposure during DLP or 2PP can cause DNA damage and alter differentiation trajectories	Moderate	Low; established photoresistant lines available	Moderate; light-sensitive differentiation pathways exist	25-27
Post-printing maturation requirement	Essential; requires weeks of culture with dynamic cues (electrical, mechanical, biochemical) to achieve adult-like function	Minimal to moderate; often retain native phenotype	None; proliferative but lack physiological relevance	Moderate; requires lineage-specific induction post-printing	4,15,16,28
Batch-to-batch variability	High; differentiation protocols and maturation states vary significantly	Moderate; donor-dependent	Low; highly consistent	Low to moderate; donor/ passage dependent	7,9,12
Key advantage for bioprinting	Patient-specific, unlimited source, human-relevant functional readouts	Native phenotype, immediate functionality	Easy expansion, high reproducibility, low cost	Multipotent, immunomodulatory, well-characterized	10,11,27
Key limitation for bioprinting	Fragile, immature, long maturation time, high sensitivity to microenvironment	Limited expansion, donor variability, availability constraints	Poor physiological relevance, tumorigenic potential	Age-related decline, limited differentiation capacity	4,5,12,23,27

This comparison underscores that iPSC-derived cells are among the most demanding cell types for bioprinting, requiring careful optimization of printing parameters, bioink chemistry, and post-

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printing culture conditions to achieve stable and functionally mature tissue constructs.

2.1 Direct bioprinting: tissue construction pathway for rapid application validation

Direct bioprinting encompasses a family of additive manufacturing techniques in which cell-laden bioinks are deposited layer-by-layer onto a receiving substrate to generate predefined 3D constructs[29,30](Figure 2A). The most commonly employed modalities include extrusion-based bioprinting, which utilizes pneumatic or mechanical dispensing of viscous bioinks through a nozzle; inkjet-based bioprinting, which relies on thermal or piezoelectric generation of droplets; and laser-assisted bioprinting, which employs laser-induced forward transfer for high-precision cell deposition[11,31]. In this approach, iPSC-derived cells are directly encapsulated within bioinks and processed through extrusion or jetting-based printing, representing one of the most streamlined strategies with high compatibility with automated platforms. This method is particularly well-suited for application scenarios that demand robust functional readouts but have relatively low requirements for tissue scale and complex vascular architecture, such as drug screening and toxicity assessment.

For iPSC-based applications, direct bioprinting offers several distinct advantages. First, its compatibility with multi-well plate formats enables high-throughput fabrication of standardized tissue constructs suitable for drug screening and toxicity testing[32]. Faulkner-Jones *et al*. demonstrated the bioprinting of human iPSC-derived hepatic progenitors in 96-well plate formats, achieving high post-printing viability ($\geqslant$ 89%) and subsequent differentiation into hepatocyte-like cells with functional albumin secretion[33]. Concurrently, Gu *et al*. reported the first example of 3D bioprinting of human iPSCs using a clinically amenable polysaccharide-based bioink[34]. This pioneering work demonstrated that bioprinted human iPSCs could maintain pluripotency and viability post-printing, undergo in situ proliferation, and be successively differentiated into lineage-committed cell types, establishing a foundational platform for patient-specific tissue engineering. Second, the simplicity and speed of direct bioprinting facilitate rapid iteration of bioink formulations and culture conditions, making it particularly valuable for early-stage process development and proof-of-concept studies[35]. Third, the ability to precisely control cell density and

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spatial organization enables the construction of defined microenvironments for investigating cell-cell and cell-matrix interactions[36].

In cardiac tissue studies, direct bioprinting of iPSC-derived cardiomyocytes has been demonstrated to construct 3D tissues exhibiting synchronous contraction, electrophysiological activity, and drug responsiveness. Compared with 2D models, such tissues display enhanced consistency in chronic drug stimulation protocols and multi-batch replicate experiments, positioning them as a potential upgrade to conventional 2D cell models within the pharmaceutical industry[28]. This consistency is particularly valuable for applications requiring reproducible functional readouts across multiple experimental runs.

However, the application of direct bioprinting to iPSC-derived cells is constrained by several inherent limitations. Extrusion-based approaches inevitably expose cells to mechanical shear stresses ranging from 10^2 to 10^5 Pa, which can compromise membrane integrity, induce apoptotic signaling, and alter gene expression profiles in mechanosensitive iPSC-derived cells[6,23]. Blaeser *et al*. systematically characterized the relationship between printing parameters and cell damage, demonstrating that nozzle geometry, printing pressure, and bioink viscosity collectively determine the extent of shear-induced injury[24]. Moreover, the open architecture of directly printed constructs often lacks sufficient mechanical support for extended culture periods, limiting their utility for applications requiring long-term functional maintenance or extensive tissue maturation[37]. Consequently, this strategy typically requires integration with maturation-inducing modalities—such as electrical stimulation or perfusion culture—to achieve sustained functionality when applied to cardiac or other excitable tissues.

2.2 Embedded bioprinting and sacrificial ink strategies: toward long-term functional maintenance

Embedded bioprinting, also known as freeform reversible embedding of suspended hydrogels (FRESH), involves the deposition of bioinks within a thermos-reversible support bath that provides mechanical stability during printing[38] (Figure 2B). This approach circumvents the gravitational constraints of direct printing, enabling fabrication of complex, overhanging

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geometries that would otherwise collapse during layer-by-layer assembly. The support bath—typically composed of gelatin, Pluronic F127, or other shear-yielding materials—can be subsequently removed by temperature modulation or dissolution, yielding freestanding constructs with high structural fidelity[39]. For iPSC-derived cells, embedded printing offers the critical advantage of shielding cells from excessive shear stress while maintaining precise spatial control, as the support bath dissipates printing-induced forces and provides a protective environment during fabrication[39].

Sacrificial ink strategies represent a complementary paradigm wherein fugitive materials are co-printed alongside cell-laden bioinks and subsequently removed to generate perfusable channels, void spaces, or hierarchical vascular networks[40]. The foundational work by Miller *et al*. demonstrated the use of carbohydrate glass as a sacrificial template to create interconnected vascular channels within 3D tissue constructs, enabling endothelialization and perfusion[41]. Subsequent advances by Kolesky *et al*. established the co-printing of Pluronic F127 sacrificial ink with cell-laden gelatin-fibrinogen bioinks to fabricate thick, vascularized tissues containing endothelialized channels capable of supporting nutrient diffusion over millimeter-scale distances[42].

Recent innovations in sacrificial writing have substantially expanded the complexity and functionality of vascularized iPSC-derived tissues. Stankey *et al*. developed coaxial sacrificial writing (co-SWIFT), a technique that enables the fabrication of hierarchically branched, multilayer vascular networks within dense cellular matrices[19]. When applied to iPSC-derived cardiac tissue constructs, co-SWIFT-generated vascular networks supported endothelialization, facilitated perfusion-mediated nutrient delivery, and enabled synchronized beating responses to cardioactive drugs over extended culture periods. Similarly, Skylar-Scott *et al*. demonstrated the biomanufacturing of organ-specific tissues with high cellular density (up to 2×10^8 cells/mL) using an integrated approach combining sacrificial writing with cell aggregate assembly[14].

From an application perspective, embedded bioprinting and sacrificial strategies significantly enhance the stability of iPSC-derived tissues during long-term culture and complex functional assessments. In embedded-printed cardiac or hepatic tissues, cellular metabolism gradually shifts

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from glycolysis toward oxidative phosphorylation, resulting in more stable functional readouts over extended time scales. This positions these strategies as important tools for disease modeling and pre-clinical studies in cell therapy. The convergence of embedded printing and sacrificial strategies has emerged as a powerful platform for constructing thick, vascularized tissues that maintain long-term functionality—a critical requirement for iPSC-derived tissues intended for cell therapy applications[15]. By enabling the integration of perfusable vasculature with parenchymal components, these approaches address the fundamental diffusion limitation that historically constrained the size and viability of engineered tissues. However, the inherent complexity of the fabrication process and the stringent requirements imposed on material systems present new challenges for scalable manufacturing.

2.3 High-resolution structural printing and multi-material systems: serving human-specific disease modeling

For tissue types that critically depend on spatial hierarchy and cellular alignment, such as the nervous system, droplet-based and multi-material bioprinting offer unparalleled precision control. By accurately positioning neural progenitor cells at distinct developmental stages or with region-specific fates, researchers can construct neural tissue models featuring cortex-like stratified architectures.

High-resolution structural printing techniques enable the fabrication of microscale features that recapitulate the intricate architecture of native tissues. Among these, digital light processing (DLP) utilizes projected light patterns to photopolymerize entire layers simultaneously, achieving lateral resolutions of 10–50 μm and fabrication speeds orders of magnitude faster than extrusion-based methods[25,43]. Two-photon polymerization (2PP) extends resolution to the sub-micrometer scale by exploiting nonlinear absorption, enabling the creation of precisely defined topographical features that mimic the extracellular matrix (ECM) architecture of specialized niches such as the basement membrane or neural synaptic clefts[26].

For human-specific disease modeling, the integration of high-resolution printing with multi-material capabilities is particularly transformative. Multi-material systems enable the spatial

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patterning of distinct cell types, growth factors, and ECM components within a single construct, allowing recapitulation of the cellular and structural heterogeneity characteristic of diseased tissues[44,45] (Figure 2C). This level of control is essential for modeling complex pathological processes that involve multiple cell types and compartmentalized microenvironments.

Using a programmable multi-nozzle system, Skylar-Scott *et al*. printed constructs containing alternating layers of human iPSC-derived cardiomyocytes and fibroblasts, recapitulating the anisotropic structure of native myocardium[14]. The printed tissues exhibited synchronized contraction and responded appropriately to cardioactive drugs, demonstrating the value of multi-material spatial control for functional tissue modeling.

In the context of neural tissue engineering, Mello *et al*. recently demonstrated the utility of DLP-printed microwell arrays for generating size-controlled motor neuron precursor spheroids from human iPSCs[46]. The study revealed that spheroid size, dictated by microwell geometry, significantly influenced differentiation outcomes, with specific dimensions optimizing the expression of motor neuron markers MNX1 and ISL1. This approach enabled scalable production of uniform neuronal spheroids with high reproducibility, facilitating mechanistic studies of motor neuron development and disease. Such precision in controlling the size and geometry of neural constructs is particularly valuable for modeling neurodevelopmental disorders where cellular organization is disrupted.

The greatest application value of such strategies lies in their capacity to model human-specific disease mechanisms. Compared with animal models, 3D-printed human neural tissues more closely recapitulate human developmental trajectories and pathological features, thereby providing a novel experimental platform for investigating neurodegenerative diseases, epilepsy, and brain tumor invasion. The combination of high-resolution printing and multi-material systems has enabled the construction of compartmentalized models of complex tissue interfaces. Takebe *et al*. highlighted the synergy between organoid technology and microengineering approaches, demonstrating that controlled spatial organization of multiple iPSC-derived cell types within defined architectural frameworks enhances functional maturation and enables modeling of tissue-

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tissue interactions that are inaccessible in conventional culture systems[47].

2.4 Modular bioprinting: toward manufacturable complex tissue systems

In recent years, a modular bioprinting strategy based on organoids or cell aggregates as "organoid building blocks" has gradually emerged. This approach emphasizes not single-cell precision but rather the spatial relationships and interfacial interactions between tissue modules, thereby preserving biological complexity while enhancing manufacturability. Modular bioprinting adopts a "bottom-up" assembly strategy wherein prefabricated tissue modules—such as spheroids, organoids, or microtissue units—are assembled into larger, functional constructs[48] (Figure 2D). This approach decouples the fabrication of individual modules from their assembly into complex tissue systems, offering several strategic advantages for iPSC-based applications.

Firstly, modular strategies enable independent optimization of each module's composition, differentiation status, and maturation trajectory before assembly[20]. For iPSC-derived cells, which often require extended culture periods to achieve functional maturity, this decoupling allows for quality control at the module level, ensuring that only fully characterized and functionally validated components are incorporated into final constructs. This quality-by-design approach is particularly valuable for applications requiring stringent reproducibility, such as drug screening and cell therapy manufacturing.

Secondly, modular assembly facilitates controlled integration of vascular and parenchymal components. Stevens *et al*. developed a modular approach for constructing vascularized liver tissues wherein endothelialized vascular modules were assembled with iPSC-derived hepatocyte modules, enabling the formation of perfusable vascular networks that supported hepatocyte function and survival over extended periods[49]. This modular strategy mimics the natural developmental process of tissue vascularization, wherein vascular structures form in parallel with parenchymal differentiation, thereby recapitulating the spatiotemporal coordination observed in native organogenesis.

Thirdly, the modular paradigm aligns well with scalable manufacturing principles. Daly *et al*. established a platform for 3D bioprinting of high cell-density heterogeneous tissue models through

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spheroid fusion within self-healing hydrogels[50]. In this approach, spheroids are deposited layer-by-layer into a self-healing support bath that provides mechanical stability during printing while shielding cells from shear stress. Following deposition, the spheroids undergo controlled fusion to form continuous, high-density tissues with preserved spatial organization. The authors demonstrated the fabrication of heterogeneous constructs containing up to 10^8 cells/mL—a cell density approaching that of native tissues—with spatially patterned cell types and maintained viability over extended culture periods. This approach leverages the inherent capacity of tissue modules to fuse and reorganize, reducing the technical complexity associated with direct printing of large constructs while enabling the production of highly cellularized, functional tissues. In a seminal study, Lawlor *et al*. introduced a high-throughput bioprinting platform for spheroid-based tissue fabrication, demonstrating unprecedented scalability while maintaining high cell viability (>90%). The platform achieved fabrication rates an order of magnitude faster than existing techniques and was successfully applied to generate centimeter-scale cartilage constructs containing approximately 600 chondrogenic spheroids in under 40 minutes, as well as calvarial bone regeneration in a rat model with near-complete defect closure. This work represents a major advance toward scalable manufacturing of iPSC-derived tissue constructs for translational applications[51].

From an industrial and translational perspective, modular printing offers a more pragmatic pathway toward scalable manufacturing. Through standardized tissue building blocks and automated assembly processes, complex tissue models can achieve batch-to-batch consistency and cost control, laying a foundation for the application of iPSC technology in drug development and cell therapy. Therefore, modular bioprinting represents a promising direction for transitioning iPSC-based tissue engineering from laboratory-scale fabrication toward industrial-scale manufacturing, addressing key challenges in reproducibility, scalability, and quality control that must be resolved for clinical translation[52].

Taken together, 3D bioprinting strategies for iPSC-derived cells are undergoing a transition from "technology exploration" toward "application alignment." Different printing modalities exhibit clear trade-offs among maturity, complexity, and manufacturability, and understanding these trade-

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offs is critical for advancing iPSC bioprinting toward real-world applications.

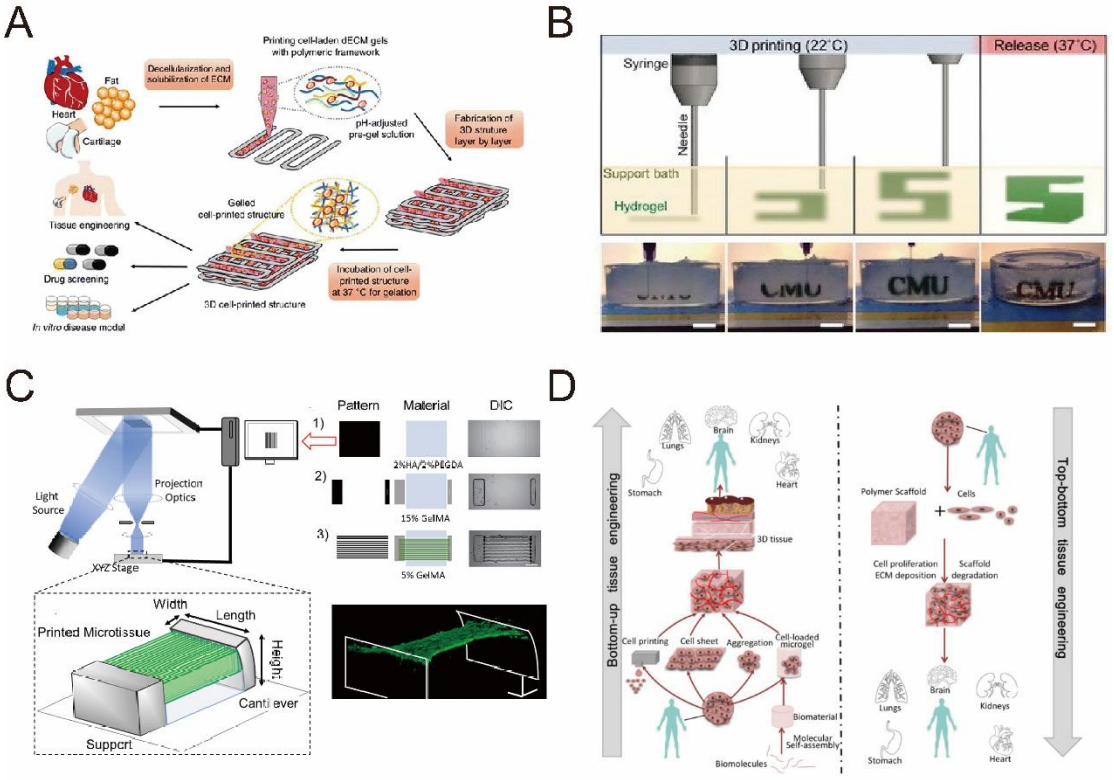


Figure 2. Application-oriented 3D bioprinting strategies for iPSC-based tissue engineering. (A) Direct bioprinting of decellularized extracellular matrix (dECM)-based bioinks enables rapid fabrication of cell-laden constructs, supporting fast validation in tissue engineering, disease modeling, and drug screening applications[30].(B) Embedded bioprinting and sacrificial ink strategies (e.g., FRESH) allow the generation of complex, soft, and perfusable structures, facilitating long-term structural stability and functional maintenance of engineered tissues[38]. (C) High-resolution and multi-material bioprinting enables precise spatial patterning and controlled deposition of multiple bioinks, generating tissue architectures with defined interfaces and microenvironmental gradients for human-specific disease modeling[44]. (D) Modular bioprinting strategies integrate bottom-up assembly of cellular building blocks and top-down scaffold-based approaches, enabling scalable fabrication of complex and manufacturable tissue systems[48]. Panel A adapted from Zhang et al.[30] under the terms of the Creative Commons CC BY 4.0 license. Panel B adapted from Hinton et al.[38] under the terms of the Creative Commons CC BY license. Copyright © 2015 The Authors, published by AAAS. Panel C adapted with permission from Liu et al.[44] Copyright © 2020 Elsevier. Panel D adapted from Wang et al.[48] under the

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Table 2 Summary of printing strategies, bioinks, representative applications, advantages, and limitations in iPSC-based bioprinting

Printing Strategy	Typical Bioinks	Representative Applications	Advantages	Limitations	Ref
Direct extrusion bioprinting	GelMA, alginate, collagen, dECM	Drug screening, toxicity testing, simple tissue models	High cell density, low cost, multi-well compatible	Shear stress, limited resolution, weak long-term support	23,33
Inkjet bioprinting	Low-viscosity hydrogels, collagen, fibrin	High-throughput array, cell patterning	Fast, low cost, non-contact deposition, good viability	Limited viscosity range, nozzle clogging, uneven droplet size	11,43
DLP / Stereolithography	,GelMA, PEGDA, HAMA	High-resolution structures, vascular networks, neural scaffolds	High resolution, fast printing speed, complex geometries	Phototoxicity, limited bioink options, light exposure	25,26
Embedded bioprinting (FRESH)	Soft hydrogels with support bath	Soft tissue, vascularized tissues, cardiac patches	Protects fragile cells enables overhanging structures	Support bath removal, slower process	38,39
Sacrificial ink printing	Pluronic F127, gelatin, carbohydrate glass	Vascular channels, perfusable networks, thick tissues	Enables endothelialization and perfusion	Extra processing, residue risk	14,41
Multi-material bioprinting	Sequential or simultaneous multiple bioinks	Heterogeneous tissues (e.g., cardiac with fibroblasts, neural with glia)	Spatial cell patterning; tissue interfaces	Complex setup, cross-contamination risk, alignment challenges	14,44
Modular / spheroid-based bioprinting	Spheroids, organoids, cell aggregates assembled in support hydrogel	Tissue modules, organ bud assembly, high-density constructs	High cellular density, preserves cell–cell contacts, quality control at module level	Fusion time required, variability in spheroid size and maturation state	46,50

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2.5 Printing-induced cellular damage: shear stress and phototoxicity

Beyond the choice of printing strategy, the process of bioprinting imposes physical and chemical stresses on encapsulated iPSC-derived cells that can compromise their survival, genomic integrity, pluripotency, and lineage specification. Two major damage modalities—shear stress during extrusion-based printing and phototoxicity during light-based printing—require particular

attention for iPSCs due to their heightened sensitivity compared with immortalized cell lines or primary cells.

2.5.1 Shear stress

During extrusion-based bioprinting, cells suspended in viscous bioinks experience mechanical shear forces as they pass through nozzles or microfluidic channels[23]. These forces arise from the velocity gradient within the flowing fluid and scale with printing pressure, nozzle geometry (diameter, length, taper angle), bioink viscosity, and flow rate. Typical shear stress ranges in extrusion bioprinting span from 10^2 to 10^5 Pa, which can exceed the physiological shear levels experienced by most cell types[24]. Blaeser *et al*. systematically characterized the relationship between printing parameters and cell damage, demonstrating that nozzle diameters below 200 μm and printing pressures above 300 kPa significantly reduce cell viability, with shear-induced injury exhibiting both immediate (membrane rupture) and delayed (apoptosis) components[24].

For iPSCs, shear stress poses unique risks beyond acute viability loss. Compared with differentiated iPSC-derived cardiomyocytes, undifferentiated iPSCs exhibit lower elastic modulus, with reported values of approximately 3 ± 2.3 kPa for iPSCs and 14.1 ± 4 kPa for iPSC-CMs, suggesting that changes in cytoskeletal organization during differentiation are associated with increased cellular stiffness. Therefore, iPSCs may be particularly sensitive to mechanical perturbations during extrusion-based bioprinting[38,53]. Limraksasin *et al*. demonstrated that continuous shear stress at 0.15, 0.5, and 1.5 Pa influenced the proliferation, pluripotency marker expression, and osteogenic differentiation of mouse iPSCs in vitro[54]. Their study showed that shear loading reduced cell number under osteogenic induction conditions, with the strongest reduction observed at 1.5 Pa, which may reflect cell detachment or cell loss. In addition, shear stress altered the expression of pluripotency-related markers: Oct3/4, Sox2, and Nanog mRNA levels slightly increased after shear exposure, whereas Klf4 expression decreased in a force-dependent manner. These findings indicate that even relatively low levels of mechanical stimulation can modulate iPSC proliferation status and pluripotency-associated transcriptional programs, thereby affecting subsequent lineage. Additionally, shear-exposed iPSCs showed

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changes in proliferation status and lineage differentiation potential, underscoring the importance of carefully controlling shear conditions during iPSC bioprinting[54].

Importantly, shear-induced damage in iPSCs is not limited to immediate cell death. Sublethal shear stress can induce sustained mechanotransduction signaling through Rho/ROCK and YAP/TAZ pathways, leading to prolonged alterations in gene expression profiles[6]. This mechanomemory effect has been shown to bias subsequent differentiation decisions, potentially compromising the reproducibility and predictability of iPSC-derived tissues for drug screening or therapeutic applications[6]. Although direct evidence linking extrusion-associated shear stress to DNA damage in iPSCs remains limited, mechanical and oxidative stress can affect pluripotent stem-cell differentiation and genome integrity. Therefore, shear exposure should be carefully controlled during iPSC bioprinting to minimize potential effects on pluripotency maintenance and downstream lineage specification[55,56].

To mitigate shear-induced damage, several strategies have been developed. The use of shear-thinning bioinks that reduce viscosity under extrusion while rapidly recovering post-printing protects cells by lowering peak shear forces[57]. Embedded bioprinting (FRESH) further shields cells by depositing bioinks into a low-shear support bath that dissipates extrusion forces[38]. Additionally, the incorporation of cytoprotective additives such as Ficoll, pluronic F68, or recombinant albumin has been shown to reduce shear-mediated membrane damage[24]. Optimizing printing parameters—using larger nozzle diameters (>250 μm), lower printing pressures (<50 kPa), and slower extrusion speeds—remains a practical first-line approach, although trade-offs with resolution must be balanced[24,57].

2.5.2 Light-induced phototoxicity

Light-based bioprinting modalities, including digital light processing (DLP), stereolithography, and two-photon polymerization (2PP), offer superior resolution (10–50 μm for DLP; sub-micrometer for 2PP) and faster fabrication speeds compared to extrusion methods [39,40]. However, these techniques expose cells to potentially damaging ultraviolet (UV) or blue light in the presence of photoinitiators that generate reactive species upon irradiation[58].

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iPSCs may be particularly sensitive to phototoxic stress because pluripotent stem cells require strict genomic surveillance and exhibit DNA damage-response profiles distinct from differentiated cells[59,60]. UV exposure can induce bulky DNA photolesions, including cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), which are mainly repaired through nucleotide excision repair[61]. Although human pluripotent stem cells may have active DNA repair capacity, low-dose UVC can trigger pronounced apoptotic responses, and iPSC lines may show heterogeneous repair capacity[59]. Dannenmann *et al.* further showed that human iPSCs maintain genomic integrity through enhanced apoptosis induction and antioxidant defense; after UVC irradiation, they accumulated fewer nuclear and mitochondrial DNA lesions than parental fibroblasts but displayed higher sensitivity to stress-induced apoptosis[62]. Therefore, light-based bioprinting should minimize UV/blue-light and photoinitiator-induced stress to reduce potential effects on DNA damage responses, pluripotency maintenance, and lineage specification[59,62].

Beyond DNA damage, light exposure may also perturb signaling pathways and differentiation programs. In hiPSCs, optogenetic control of TGF-β signaling has shown that light-regulated pathway activation can direct mesenchymal lineage differentiation, indicating that light inputs can actively reshape cell-fate decisions. Therefore, in light-based bioprinting, light dose, wavelength, exposure duration, and photoinitiator chemistry should be carefully optimized to minimize unwanted effects on cell viability, signaling dynamics, and lineage specification[63,64].

To mitigate phototoxicity, several strategies have been adopted, including the use of cytocompatible photoinitiators, longer-wavelength illumination, reduced exposure time, and bioink formulations that minimize reactive oxygen species generation[58,64]. First, visible-light-based projection stereolithography using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) has been shown to enable live cell-scaffold fabrication while avoiding the stronger DNA-damaging effects associated with ultraviolet irradiation[64]. Second, operating at longer wavelengths, such as 405 nm rather than 365 nm, can reduce photon energy and lower the risk of UV-associated DNA damage, although light dose and exposure duration still require careful optimization[58,64]. Third, because photoinitiator activation can generate reactive species, antioxidant-based strategies

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may help reduce oxidative stress during or after light-based fabrication, but their effectiveness should be validated for each iPSC-derived cell type and bioink system[65]. Finally, recent volumetric bioprinting approaches, including xolography and computed axial lithography, offer rapid layer-free fabrication and may reduce cumulative light exposure compared with conventional layer-by-layer photopolymerization[25,66].

2.5.3 Implications for iPSC-based bioprinting

The susceptibility of iPSCs to both shear- and light-induced damage has profound implications for the design and interpretation of bioprinting studies[16]. Routine viability assessments using membrane integrity assays, such as live/dead staining, may underestimate printing-induced damage, because sublethal effects on pluripotency, genomic stability, and differentiation potential often require longer-term functional assays to detect[59,62]. Batch-to-batch variability in printed tissue performance may partly reflect uncontrolled differences in printing parameters that influence shear stress and light exposure. Therefore, standardizing nozzle geometry, extrusion pressure, bioink viscosity, printing temperature, wavelength, light dose, and exposure duration is essential for achieving reproducible tissue function[23]. The choice of printing modality should also be guided by the intended application. Extrusion-based printing may be preferable for applications requiring high cell density and broad bioink compatibility, whereas light-based methods provide higher spatial resolution but require careful optimization of photoinitiator chemistry and optical exposure to minimize phototoxic side effects[16,23].

Acknowledging these damage mechanisms, recent efforts have focused on developing “cell-friendly” bioprinting workflows[16]. Sacrificial and embedded printing strategies can partially shield iPSC-derived cells from direct mechanical stress by depositing bioinks into supportive or shear-yielding matrices, thereby improving the fabrication of soft and fragile constructs[38,57]. Shear-thinning and jammed microgel bioinks provide another strategy, as they reduce extrusion-associated stress during printing while rapidly recovering structural stability after deposition[57]. For light-based bioprinting, the use of cytocompatible photoinitiators, visible-light-based polymerization, and optimized optical exposure can help reduce phototoxicity while maintaining

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printing resolution[64]. Real-time monitoring and improved control of printing parameters, including nozzle geometry, extrusion pressure, bioink viscosity, temperature, wavelength, and light dose, may further enhance reproducibility and reduce printing-induced cellular damage[43]. Ultimately, understanding and mitigating this damage mechanisms will be critical for transitioning iPSC bioprinting from proof-of-concept studies toward reproducible, scalable, and clinically translatable tissue manufacturing[16,43].

3. Bioinks and supportive systems: from “printable materials” to “application-level functional carriers”

In iPSC-based bioprinting systems, bioinks and support materials do not merely serve a structural scaffolding role; rather, they directly determine whether the tissue model can sustain long-term functional output, achieve batch-to-batch consistency, and be suitable for scalable manufacturing. Therefore, from an application-oriented perspective, the central objective of bioink design has shifted from "enabling printability" to "supporting predictable functional performance." This evolution reflects a fundamental shift in perspective: from materials that are merely "printable" to those that actively support and direct tissue function[67]. This transition is particularly critical for iPSC-derived cells, whose functional outcomes are intimately linked to the physicochemical properties of their surrounding matrix[31,68]. In this section, we systematically examine the key design parameters that govern bioink performance in iPSC-based applications, emphasizing how each parameter contributes to the transition from structural fidelity to functional relevance.

3.1 Rheological properties: the engineering foundation for ensuring consistency

For application-oriented iPSC-based bioprinting systems, bioinks must first and foremost possess a stable and reproducible printing window. Rheological properties govern the printability and structural fidelity of bioinks, serving as the engineering foundation upon which reproducible construct fabrication depends[37]. For iPSC-based applications, maintaining consistent rheological behavior across batches is essential for translating laboratory-scale successes into manufacturable products. Key rheological parameters include viscosity, shear-thinning behavior, yield stress, and recovery kinetics[57].

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Shear-thinning bioinks reduce viscosity under shear stress during extrusion, protecting cells from mechanical damage while enabling smooth flow through printing nozzles. Upon deposition, rapid recovery of viscosity ensures shape fidelity and prevents structural collapse[69]. Shear-thinning behavior, self-healing properties, and rapid solidification capacity not only influence printing resolution but also directly determine the consistency of cellular mechanical forces experienced during the printing process. Studies have shown that even minor variations in shear stress during printing can lead to significant functional fluctuations in iPSC-derived cells during subsequent culture—a challenge that is particularly critical in applications demanding high reproducibility, such as drug screening. The rheological profile must be carefully balanced: insufficient viscosity leads to filament spreading and loss of resolution, while excessive viscosity increases shear stress exposure and compromises cell viability.

Recent advances in rheological characterization have enabled the development of bioinks with tailored flow properties. Beyond printability, the polymer backbone structure, crosslinking chemistry, and network density jointly determine hydrogel viscosity, pore architecture, stiffness, stress relaxation, degradation behavior, and charge distribution, all of which can directly influence iPSC-derived cell survival, lineage specification, and functional maturation[68,70-72]. Hydrogel systems based on dynamic physical crosslinking or reversible covalent bonds have been widely adopted for iPSC bioprinting, as they ensure structural stability during fabrication while avoiding the inhibitory effects of excessive stiffness on cell maturation. Their advantage lies in ensuring structural stability during fabrication while avoiding the inhibitory effects of excessive stiffness on cell maturation. For iPSC-derived cells, an appropriate crosslinking density is particularly important: overly dense networks may restrict cell spreading, nutrient diffusion, endogenous ECM deposition, and tissue remodeling, whereas insufficient crosslinking may lead to poor shape fidelity and heterogeneous mechanical cues after printing[70,71]. Matrix stiffness and viscoelasticity also regulate mechanotransduction pathways, including integrin-mediated adhesion, cytoskeletal tension, and YAP/TAZ-related signaling, thereby affecting differentiation trajectory and maturation state[68,72].

Amorim *et al*. systematically investigated the rheological properties of GelMA/GelMA-AEMA

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hydrogel blends for iPSC-derived chondrocyte encapsulation, demonstrating that the optimized blend exhibited superior flowability, strong shear-thinning behavior, and yield-stress fluid characteristics, making it highly suitable for extrusion-based bioprinting while maintaining matrix formation capacity over 21 days of culture[73](Figure 3A). The study highlighted the importance of systematic rheological screening in identifying formulations that balance printability with cytocompatibility. In addition, the introduction of charged functional groups, such as amine- or carboxyl-containing moieties, can alter hydrogel swelling, ionic interactions, growth factor retention, and cell–matrix adhesion, thereby indirectly modulating differentiation and maturation cues within printed iPSC-derived tissues[68,71].

Microgel-based bioinks represent a significant advancement in rheological design. Pal *et al*. developed interparticle-crosslinked, self-supporting microgels that eliminate the need for filler hydrogels while preserving essential microscale void spaces[74]. These microgels exhibited excellent shear-thinning and self-healing properties, enabling high print fidelity across multiple bioprinting modalities, including extrusion-based, embedded, and aspiration-assisted approaches. The self-healing behavior—wherein microgels rapidly reassemble after shear-induced disruption—is particularly advantageous for iPSC-derived cells, which are sensitive to prolonged mechanical disturbance[27]. Mechanistically, the granular architecture of microgel bioinks creates a locally permissive and porous microenvironment that facilitates nutrient transport, cell migration, and endogenous matrix deposition[71,74]. Such properties are especially relevant for iPSC-derived tissues, which often require prolonged culture and progressive matrix remodeling to acquire stable tissue-specific functions[27,75].

From an application perspective, such materials provide an engineering foundation for achieving consistent printing outcomes across batches and operators, representing a prerequisite for transitioning toward standardized manufacturing. The continued development of rheologically optimized bioinks with tailored shear-thinning and self-healing properties will be critical for advancing iPSC bioprinting from laboratory-scale exploration toward industrial-scale production.

3.2 Biocompatibility and degradation behavior: supporting long-term function beyond

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short-term survival

In most application scenarios, the value of a tissue model does not depend on initial viability but rather on whether it can sustain stable function over weeks or even longer periods. Overemphasizing initial viability while neglecting long-term functionality was a common limitation in early iPSC bioprinting studies. Traditional biocompatibility assessments often focus on acute cytotoxicity and short-term cell viability. However, for iPSC-derived tissues intended for long-term applications—such as cell therapy or disease modeling—degradation behavior plays an equally critical role[75]. The degradation kinetics of bioinks must be carefully matched to the tissue regeneration or maturation timeline: too rapid degradation compromises structural integrity and may expose cells to uncontrolled mechanical environments, while too slow degradation may impede tissue remodeling, ECM deposition, and functional maturation[70].

Application-oriented research has increasingly recognized that controlled degradation behavior is a critical parameter in bioink design. For iPSC-derived cells, which often require prolonged culture periods to achieve functional maturity, bioinks with tunable degradation profiles are essential. Ideally, the degradation of the bioink should proceed in parallel with the deposition of endogenous ECM by iPSC-derived cells, resulting in a seamless transition from synthetic scaffold to native tissue[71]. This "dynamic reciprocity" between scaffold degradation and matrix deposition enables the progressive establishment of a cell-derived microenvironment that better supports long-term function. By gradually yielding to the ECM secreted by cells themselves during culture, materials can facilitate a transition from a "material-dominated state" to a "cell-dominated state," thereby substantially enhancing the stability of functional readouts. Thus, degradation rate is not merely a structural parameter but a functional regulator: fast degradation may destabilize tissue geometry and mechanical loading, whereas delayed degradation may prevent cell spreading, multicellular organization, and maturation-associated ECM remodeling[70-72].

Recent advances in degradable hydrogel systems have demonstrated the feasibility of this approach. Lifwergren *et al.* recently developed an elastic, protease-responsive hydrogel system incorporating protease-degradable crosslinkers[76]. This system enables the filaments to function as

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sacrificial templates for perfusable tubular structures while supporting high cell viability across multiple cell types. The protease-responsive design allows cell-mediated degradation that matches the kinetics of tissue remodeling, addressing a key limitation of conventional bioinks with fixed degradation rates. This strategy has been demonstrated to prolong functional maintenance in cardiac, hepatic, and islet-like tissue models. Protease-responsive crosslinkers are particularly relevant for iPSC-derived tissues because they allow cells to locally remodel their surrounding matrix, thereby coupling material degradation with tissue maturation rather than imposing a fixed degradation schedule[70,76].

The integration of elastomeric components into bioink formulations offers another strategy for achieving mechanical stability while supporting long-term function. Landau *et al.* developed composite bioinks incorporating poly(octamethylene maleate (anhydride) citrate) (POMaC) elastomeric microparticles into biologically derived hydrogels. This approach synergistically combined the cell compatibility of natural hydrogels with the mechanical stability of elastomeric polymers, effectively mitigating tissue compaction and swelling over extended culture periods. The composite bioink supported enhanced functional assembly of human iPSC-derived cardiac tissues and primary vasculature, with improved resilience against host tissue stress upon *in vivo* implantation[77] (Figure 3B). This example illustrates that mechanical reinforcement should not simply aim to increase stiffness; rather, it should preserve tissue geometry while maintaining a mechanically permissive environment for contraction, vascular assembly, and long-term remodeling[72,77].

Together, these advances underscore a fundamental shift in biocompatibility assessment: from evaluating short-term survival to ensuring long-term functional stability. For iPSC-based applications, bioink performance should therefore be evaluated through an integrated material–cell framework, in which polymer structure, crosslinking density, stiffness, charge, degradation kinetics, and ECM-like biochemical signals collectively regulate cell viability, lineage commitment, tissue remodeling, and durable functional maturation[68,70-72]. For iPSC-based applications, the ability to engineer controlled degradation profiles that synchronize with tissue maturation represents a critical enabler for translating laboratory-scale constructs into clinically

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relevant tissue products.

3.3 ECM mimicry and tissue-specific signaling: enhancing functional relevance

From an application perspective, the value of ECM signals within bioinks lies not in "highly recapitulating *in vivo* complexity" but rather in their ability to reduce functional heterogeneity and enhance model predictive capacity. ECM provides not only structural support but also biochemical and biophysical cues that regulate cell behavior[78]. For iPSC-derived cells, which often exhibit immature phenotypes in standard culture conditions, the incorporation of ECM-mimetic signals into bioinks can significantly enhance functional relevance. The native ECM comprises a complex assemblage of collagens, laminins, fibronectin, proteoglycans, and glycosaminoglycans (GAGs) that collectively orchestrate cell adhesion, migration, proliferation, and differentiation[79]. Mechanistically, ECM-like signals influence iPSC fate through several interconnected routes: integrin-mediated adhesion regulates cytoskeletal organization and mechanotransduction; GAG-rich domains retain and present growth factors; and tissue-specific matrix proteins provide lineage-relevant biochemical cues that support polarization, maturation, and tissue-specific function[70-72,78,79].

Native ECM components and their derived peptides have been widely employed to improve cell adhesion and polarization. In recent years, tissue-specific ECM components can be incorporated into bioinks to recapitulate the native microenvironment. Decellularized ECM (dECM) derived from target organs has emerged as a particularly promising bioink component because it preserves native tissue architecture and biochemical cues while minimizing immunogenicity. As reviewed by Jin *et al.*, dECM scaffolds retain growth factors, cytokines, and matrix proteins that coordinate cellular adhesion, proliferation, and differentiation, with tissue-specific biochemical and biomechanical characteristics that make them ideally suited for targeted applications[80].

In iPSC-derived cardiac, neural, and islet cell models, dECM-based bioinks have been demonstrated to accelerate functional maturation and reduce batch-to-batch functional variability. This characteristic holds significant importance for quality control in drug screening and cell therapy product manufacturing. Yan *et al.* demonstrated that decellularized ECM derived from

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pluripotent stem cell aggregates exhibited distinct protein expression profiles and differentially influenced stem cell proliferation and neural differentiation[81](Figure 3C). ECM from neural progenitor cell aggregates accelerated the expression of β-tubulin III and Nestin, highlighting the potential of PSC-derived ECMs as functional bioink components that can direct cell fate.

However, despite these compelling advantages, the translational application of dECM bioinks faces several critical limitations. Batch-to-batch variability arises from donor age, tissue source, and processing conditions, complicating reproducible manufacturing. The undefined composition of dECM, while biologically potent, creates a "black box" that hinders mechanistic understanding and quality control. Potential immunogenicity persists due to incomplete removal of DNA and xenoantigens, which may trigger foreign body responses *in vivo*. Furthermore, the lack of standardized protocols for decellularization, sterilization, and quality assessment impedes clinical translation. Addressing these challenges will require developing defined ECM-mimetic platforms or identifying minimal functional component mixtures that retain the benefits of dECM while enabling reproducible production[82].

The presentation of immobilized growth factors, adhesion ligands, and mechanical cues at physiologically relevant densities and spatial distributions can direct iPSC-derived cells toward mature, functional phenotypes[72]. Wang *et al*. recently demonstrated the construction of biomimetic vascularized iPSC-hepatocyte spheroids using microfluidic microcapsules with hybrid hydrogel shells composed of sodium alginate and hyaluronic acid methacryloyl (HAMA)[83]. By selectively degrading the alginate component, the resulting porous HAMA shells facilitated the attachment of human umbilical vein endothelial cells (HUVECs) and the formation of vascularized networks around the iPSC-hepatocyte cores. This spatially organized architecture promoted hepatic functions—including albumin secretion, urea synthesis, and cytochrome P450 activity—while providing immune protection. Upon orthotopic transplantation in a mouse model of acute liver failure, the vascularized spheroids demonstrated superior therapeutic efficacy compared to non-vascularized controls, highlighting the critical role of vascularized ECM-mimetic microenvironments in supporting iPSC-derived cell function[83].

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Thus, these advances underscore that the strategic use of ECM-mimetic signals—particularly tissue-specific dECM and defined ECM-inspired components—represents a powerful approach for enhancing functional maturation, reducing variability, and improving predictive capacity in iPSC-based tissue models. As the field moves toward translational applications, the ability to engineer ECM-mimetic microenvironments that balance biological complexity with manufacturing consistency will be essential for realizing the full potential of iPSC-derived tissues.

3.4 Functionalized and responsive materials: targeting functional output rather than structural complexity

The frontier of bioink development lies in the design of functionalized and responsive materials that actively participate in tissue function[58]. Unlike traditional bioinks that serve primarily as passive scaffolds, these next-generation materials incorporate dynamic features that enable temporal control over the cellular microenvironment. The introduction of conductive and bio-responsive bioinks reflects a paradigm shift in the field from "structural biomimicry" toward "functional biomimicry." This paradigm shift from "structural complexity" to "functional output" is particularly relevant for iPSC-based applications, where the goal is not merely to fabricate a structure but to generate a tissue that performs predictable, physiologically relevant functions.

Stimuli-responsive materials enable constructs to undergo programmed shape or property changes in response to environmental cues such as temperature, light, pH, and magnetic fields, offering a dynamic approach to mimic the morphogenetic processes observed during native tissue development[84]. Beyond the widely studied PNIPAM-based systems, recent advances have yielded thermoresponsive and light-responsive platforms with potential relevance to iPSC-based applications. Pylostomou *et al*. developed EXPECT, a thermosensitive embedded bioprinting platform based on pNIPAAm-CS, Carbopol 940, and gelatin, which used cyclic temperature stimulation to maintain directional cell migration and spatial organization during long-term culture[85]. Liang *et al*. further engineered a defined thermoresponsive hydrogel microniche for iPSC expansion and controlled release, in which temperature-mediated softening enabled gentle, non-enzymatic cell recovery while maintaining iPSC viability and pluripotency[86]. Light-

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responsive platforms provide another strategy for dynamic regulation of matrix mechanics. Ansari *et al.* developed a visible-light-responsive hydrogel that enables reversible stiffness modulation under blue and green light, providing a tool to study how dynamic tissue stiffness regulates stem-cell mechanosensing while avoiding UV-associated cytotoxicity[87]. In the context of iPSC-derived tissue maturation, Pramanick *et al*. introduced a 4D bioprinting platform in which cell-generated forces drive programmable shape morphing of printed iPSC-derived heart tissues, thereby promoting cell and extracellular matrix alignment and enhancing tissue-level function[88]. Together, these studies suggest that stimuli-responsive materials should be viewed not merely as shape-changing systems, but as dynamic microenvironmental regulators that can support iPSC expansion, spatial organization, tissue remodeling, and functional maturation.

Dynamic crosslinking mechanisms enable temporal changes in matrix stiffness and viscoelasticity to mimic the dynamic mechanical microenvironments encountered during tissue development, homeostasis, and repair. These systems allow initial mechanical properties optimized for printability, followed by programmed stiffening or softening that guides cell behavior over time[89]. As reviewed by Mathur *et al*., 4D printing integrates additive manufacturing with smart materials to create dynamic structures capable of controlled shape and functional changes in response to external stimuli such as temperature, pH, light, or magnetic fields[89]. This transformative approach has opened new avenues for tissue engineering and personalized medicine, enabling the fabrication of adaptive scaffolds that can actively participate in tissue morphogenesis and regeneration[89](Figure 3D). Rosales and Anseth reviewed the design of reversible hydrogels that capture ECM dynamics, highlighting how dynamic covalent chemistry enables materials that respond to cellular traction forces or enzymatic activity[90]. Recent advances have extended dynamic crosslinking concepts into stem-cell and iPSC-related tissue engineering. House *et al*. developed a dynamically stiffening hydrogel platform integrated with strain-responsive surface patterns to study hiPSC-derived cardiomyocyte maturation. Their results showed that physiologically relevant matrix stiffness improved sarcomere organization, connexin-43 expression, and beating behavior, whereas premature matrix stiffening impaired cardiomyocyte function, highlighting the importance of temporally regulated mechanical cues for iPSC-derived

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tissue maturation[91]. Reversible supramolecular systems provide another approach for on-demand mechanical regulation. Linke *et al*. fabricated host–guest crosslinked hydrogels in which stiffness could be reversibly switched by adding competing guest molecules, enabling dynamic control of stem-cell mechanosensing, traction force generation, YAP/TAZ signaling, and proliferation[92]. Dynamic covalent chemistry has also been used to create adaptive 4D-printed scaffolds. Liu *et al*. developed amphiphilic dynamic thermoset polyurethanes with thermally reversible covalent bonds, enabling body-temperature-triggered shape memory, water-triggered programmable deformation, and swelling-stiffening behavior for minimally invasive implantation[93]. In addition, dynamic imine/Diels–Alder networks have been used to fabricate gelatin-based hydrogels with self-healing, shape-memory, cytocompatible, and 3D-printable properties[94]. Together, these studies indicate that dynamic crosslinking should be viewed not merely as a material innovation, but as a strategy for reproducing the time-varying mechanical environments that influence stem-cell mechanotransduction, tissue remodeling, and functional maturation.

Conductive components facilitate electrical integration in excitable tissues such as cardiac or neural constructs. Shin *et al*. demonstrated carbon-nanotube-embedded hydrogel sheets that enhanced cardiac construct function by improving electrical signal propagation and synchronized contraction[95]. For iPSC-derived cardiomyocytes, such conductive bioinks can support the development of mature electrophysiological phenotypes essential for drug screening and disease modeling.

Cell-driven morphogenetic materials represent an emerging paradigm wherein programmed shape changes are driven by cell-generated forces rather than external stimuli. Ding *et al*. developed a 4D printed degradable hydrogel scaffold platform that leverages cell contractile forces (CCFs) to drive tissue morphogenesis[96]. In this system, hydrogels initially provide mechanical support to maintain structural integrity, followed by rapid degradation that amplifies CCFs through enhanced cell-cell interactions and increased local cell density, thereby inducing programmed shape transformations. By modulating initial print geometries, complex tissue constructs can be generated via controlled global shape changes. This approach not only enables scaffold-free construct formation but also supports 4D tissue engineering by coupling tissue

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differentiation with dynamic shape evolution—representing a paradigm shift from externally triggered to cell-driven morphogenesis[96].

Immunomodulatory properties are increasingly recognized as critical for cell therapy applications where allogeneic transplantation requires mitigation of host immune responses. Vegas *et al*. developed a combinatorial hydrogel library that identified materials capable of mitigating foreign body responses in non-human primates[97]. This approach has profound implications for iPSC-derived cell therapies, where encapsulation materials must not only support cell function but also prevent immune-mediated rejection.

Notably, from an application-oriented perspective, the incorporation of functional materials should aim to enhance model stability and predictability rather than merely increase system complexity. This principle is increasingly becoming a consensus in high-impact translational research. For iPSC-based applications, functionalized bioinks can actively guide iPSC-derived cells toward desired behaviors—such as synchronized contraction in cardiomyocytes, glucose-responsive insulin secretion in β cells, or synaptic activity in neurons—thereby transforming the bioink from a passive carrier into an active participant in tissue function[28,98]. The integration of responsive and functionalized materials represents a critical step toward the clinical translation of iPSC-derived tissue constructs, enabling dynamic interactions between engineered tissues and their biological environment that were previously unattainable with conventional biomaterials.

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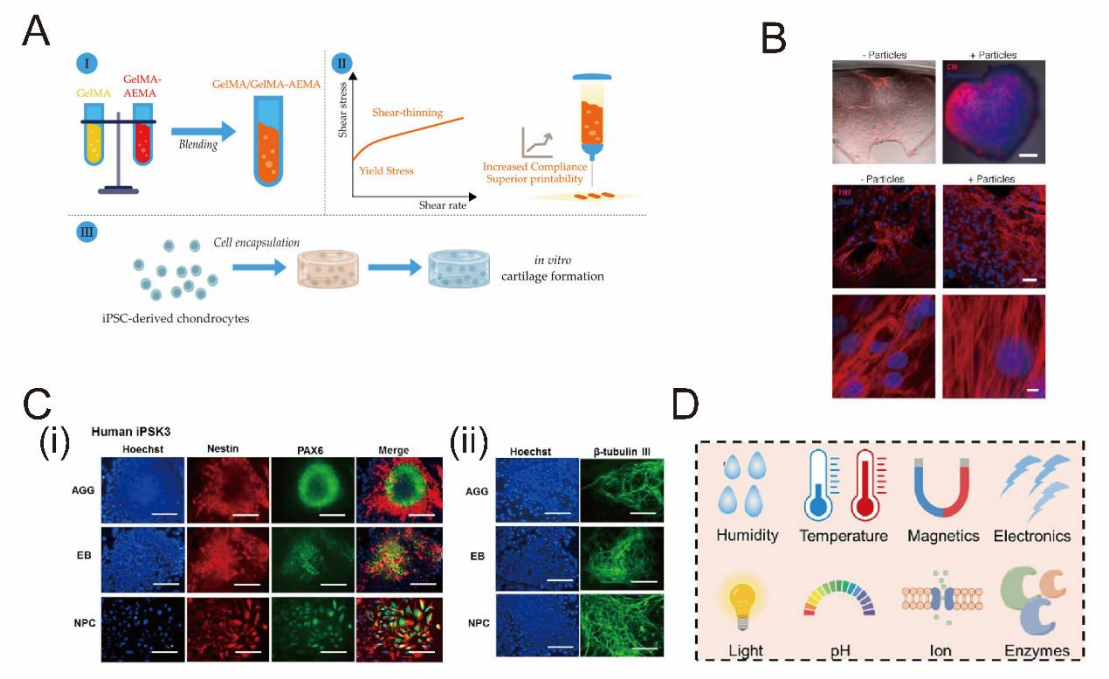


Figure 3. Bioink design principles governing printability, cellular behavior, and functional tissue formation. (A) Engineering of GelMA-based composite hydrogels with tunable rheological properties, enabling shear-thinning behavior, improved print fidelity, and support for iPSC-derived chondrocyte encapsulation and cartilage formation[73]. (B) Bioink composition regulates structural stability and functional maturation, as particle-reinforced systems improve cardiomyocyte organization, electrophysiological responsiveness, and synchronized contractility in engineered cardiac tissues[77]. (C) Extracellular matrix (ECM) context directs lineage specification and maturation, as demonstrated by stage-dependent neural differentiation of human iPSCs on distinct scaffold environments[81]. (D) Functionalized and responsive bioinks enable dynamic regulation of printed constructs, where external stimuli such as temperature, light, pH, and electromagnetic cues can modulate structural and biological properties in 4D bioprinting systems[89]. Panel A adapted from Amorim et al.[73] under the terms of the Creative Commons CC BY 4.0 license. Copyright © 2025 The Authors, published by MDPI. Panel B adapted from Landau et al.[77] under the terms of the Creative Commons CC BY 4.0 license. Copyright © 2025 The Authors, published by KeAi/Elsevier. Panel C adapted with permission from Yan et al.[81] Copyright © 2015 Elsevier. Panel D adapted from Mathur et al.89 under the terms of the Creative Commons CC BY 4.0 license. Copyright © 2025 The Authors, published by Springer Nature.

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4. Tissue maturation and functional stabilization of bioprinted iPSC tissues

3D bioprinting places iPSCs and their derivatives in a more physiological three-dimensional environment, but structural fidelity does not ensure functional competence[16,27]. To summarize the major engineering factors required for the maturation and functional stabilization of bioprinted iPSC-derived tissues, a conceptual framework is shown in Figure 4. The main bottleneck is no longer print resolution or immediate post-printing survival. It is the limited long-term maturation, unstable functional output, and poor batch reproducibility of printed tissues[27,99]. In hiPSC-derived cardiac tissues, this appears as immature electromechanical coupling and variable contractile synchrony[44,99]. In liver models, metabolic and secretory functions often decline during extended culture[100] In neural constructs, network formation remains slow and phenotypic drift is common[44]. In islet and β-like cell systems, translational value still depends on the amplitude, kinetics, and durability of glucose-stimulated insulin secretion[100,101]. These findings indicate that the field is moving from cell-preserving bioprinting to function-preserving bioprinting[27,99,102,103]. Maturation in bioprinted iPSC tissues is controlled by several factors, including cell state, bioink composition, printing-induced stress, construct geometry, and post-printing culture conditions [27,99]. A 3D hydrogel alone rarely produces mature tissue behavior[16,27]. Stable function usually requires appropriate cell density, spatial organization, tissue-specific extracellular matrix factors, and sustained nutrient exchange[99,100]. This is why current evaluation is shifting away from viability and morphology alone[16,27]. More attention should be given to tissue-level readouts over time, such as contractile performance in cardiac models, network activity in neural models, hepatic metabolic function, and glucose responsiveness in islet systems[44,99-103]. In this context, maturation should be defined by reproducible and durable functional output rather than by a small set of lineage markers[99,101,103].

These findings underscore that the bottleneck has shifted from structural fidelity to functional maturity. Therefore, "maturation" in bioprinted iPSC-derived tissues must be redefined by stable, physiologically relevant functional output rather than lineage marker expression alone. Tissue-specific functional benchmarks should include: cardiac – synchronous contractility, contraction force (>10 μN), calcium handling kinetics, and drug chronotropy[104]; liver – albumin secretion

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(>20 pg/cell/day), urea synthesis, and inducible cytochrome P450 activity[100,105]; neural – spontaneous synchronous firing, action potential amplitude, and neurotransmitter responsiveness[106]; islet – glucose-stimulated insulin secretion (stimulation index ⩾3–5) with biphasic kinetics[98,101]. Quantitative, function-centered criteria across tissue types will enable cross-study comparison and accelerate translation.

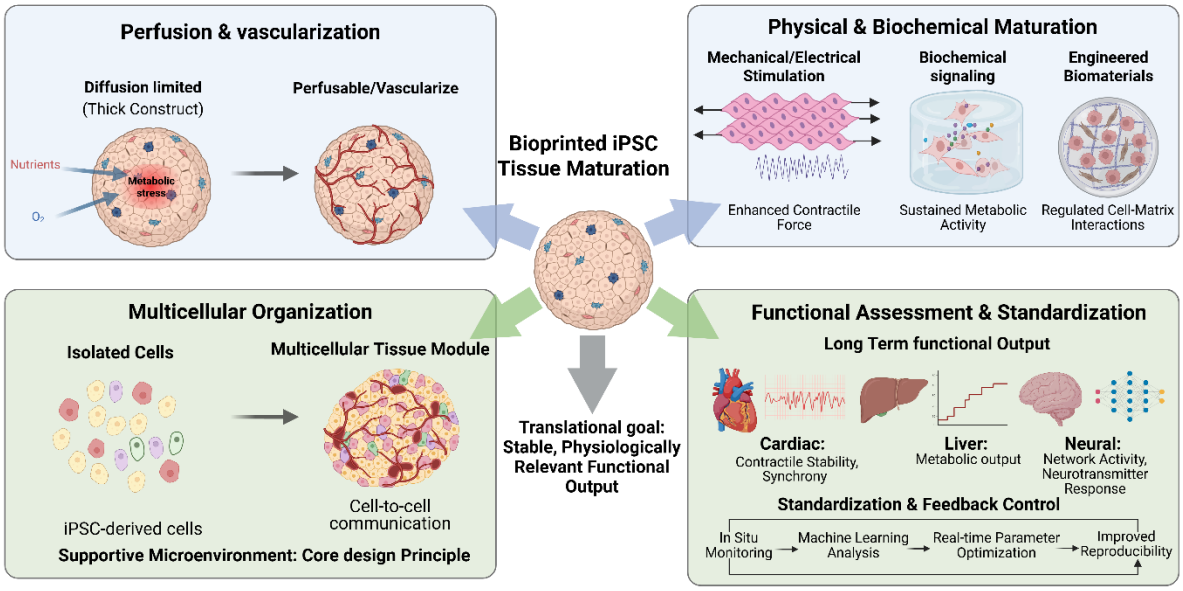


Figure 4. Engineering framework for tissue maturation and functional stabilization of bioprinted iPSC-derived tissues. Perfusion and vascularization, physical/biochemical stimulation, engineered biomaterials, and multicellular organization jointly support the maturation of bioprinted iPSC tissues. Functional assessment and standardization further enable evaluation of tissue-specific outputs, including cardiac contractility, liver metabolic activity, and neural network function, ultimately supporting stable and physiologically relevant tissue performance. Created with BioRender.com.

4.1 Perfusion and vascularization

Perfusion is a key determinant of the stability of bioprinted tissues[14,15]. In thick constructs, diffusion alone is insufficient to maintain oxygen and nutrient supply. This limitation often leads to metabolic stress and functional decline during long-term culture[14]. The issue is particularly evident in cardiac, hepatic, and pancreatic tissues, which have relatively high metabolic demand[15,40]. For this reason, recent studies increasingly focus on perfusable architectures and

vascularized constructs rather than geometric complexity alone[14,15,40]. Skylar-Scott *et al*. developed a vascularized tissue fabrication strategy using sacrificial bioinks to create perfusable channels within dense cell constructs, enabling improved nutrient transport and long-term tissue viability[14] Noor *et al*. reported a personalized hydrogel-based 3D bioprinting approach capable of generating vascularized cardiac patches derived from patient-specific iPSCs[15]. More recently, Grigoryan *et al*. demonstrated that stereolithographic printing of vascular networks could support rapid perfusion and improve tissue viability in thick engineered constructs, further highlighting the importance of vascular integration in bioprinted tissues[107].

Vascularization also reduces spatial heterogeneity within printed tissues[14]. When nutrient transport is limited, central regions of the construct frequently display reduced viability and impaired function[15]. Introducing vascular channels or perfusion systems can improve mass transport and support tissue maturation[14,40]. For example, vascularized iPSC-derived islet constructs generated by 3D printing have demonstrated improved glucose responsiveness and graft survival in experimental models[103]. These observations indicate that perfusion should be considered a fundamental requirement for functional tissue engineering rather than a supplementary design feature.

4.2 Physical and biochemical maturation

Three-dimensional culture alone is usually insufficient to drive full maturation of iPSC-derived tissues[104]. The phenotype of printed constructs is strongly influenced by mechanical factors, biochemical signaling, and electrical stimulation[104,108]. Ronaldson-Bouchard *et al.* showed that progressive electrical stimulation significantly improved sarcomere organization and electrophysiological maturation in engineered human cardiac tissues derived from pluripotent stem cells[104]. Zhang *et al.* demonstrated that mechanical conditioning enhanced contractile force and structural alignment in engineered cardiac constructs, highlighting the importance of electromechanical training for cardiac maturation[108]. In hepatic models, maturation also depends on biochemical factors and tissue-like cellular organization[100,105]. Ma *et al*. reported that 3D bioprinted hepatic constructs containing iPSC-derived hepatocytes exhibited sustained albumin

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secretion and metabolic activity when cultured in a supportive hydrogel microenvironment[105]. In neural systems, bioink composition plays a critical role in regulating neuronal differentiation and network formation. For instance, Benwood *et al*. developed a smart bioink that supported the generation of patient-derived iPSC models of Alzheimer's disease and promoted neuronal network development in printed constructs[102]. Recent studies further suggest that engineered biomaterials with dynamic mechanical properties can enhance stem-cell-derived tissue maturation by regulating cell-matrix interactions and mechano-transduction pathways[109]. Together, these studies demonstrate that post-printing maturation relies on coordinated mechanical, biochemical, and microenvironmental regulation rather than on three-dimensional structure alone.

4.3 Multicellular organization and microenvironment engineering

Multicellular organization is a central requirement for functional bioprinted iPSC tissues. Many iPSC-derived cells do not maintain stable function in isolation. Their phenotype depends on local interactions with endothelial cells, stromal cells, supporting glia, or tissue-specific matrix components[106,110]. This is particularly important in cardiac and neural systems, where cell-cell communication directly affects synchronization, signal propagation, and long-term stability[106,111]. Recent work has therefore shifted from printing single-cell populations toward assembling multicellular tissue modules with defined spatial relationships[110]. In practice, the goal is not to maximize complexity, but to introduce the minimum cellular and matrix components needed to preserve tissue-level function. Jo *et al*. proposed bioprinting-assisted tissue assembly as a scalable strategy to organize microtissue building blocks into larger constructs while preserving structural and functional hierarchy[110]. In the neural field, Yan *et al*. showed that 3D bioprinting could generate human neural tissues with functional connectivity, indicating that controlled spatial assembly can support network-level organization rather than simple cell survival[106]. In cardiac microtissues, Cao *et al*. further demonstrated that the local tissue microenvironment shapes the state of hiPSC-derived endothelial cells, showing that endothelial identity itself is influenced by multicellular context[111]. Together, these studies suggest that microenvironment engineering should be treated as a core design principle rather than a downstream refinement step.

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4.4 Functional assessment and standardization of bioprinted tissues

As bioprinted iPSC tissues move closer to translational use, functional assessment must extend beyond viability, morphology, and marker expression. These measures remain necessary, but they do not adequately capture tissue performance over time. More relevant endpoints include contractile stability, electrophysiological behavior, metabolic output, network activity, and stimulus-response dynamics, depending on the tissue type[112,113]. Equally important is the consistency of these readouts across batches and culture periods. A construct that performs well at one time point but shows marked drift during prolonged culture has limited value for drug screening or preclinical testing.

Standardization is therefore becoming a major issue in the field. Recent studies have started to combine in situ monitoring, machine learning, and feedback control to reduce print-to-print variation and improve quality assessment[112,113]. Bonatti *et al.* developed a deep learning-based quality control loop for extrusion bioprinting, showing that automated image-based analysis can support parameter optimization during fabrication[112]. Yang *et al.* further integrated optical coherence tomography with feedback control to detect structural defects during printing and improve fidelity in real time[113]. At a broader level, Han *et al.* emphasized that tissue-engineered medical products require clearer standards for manufacturing and supervision if they are to enter clinical translation[114]. Taken together, these studies show that the next stage of iPSC bioprinting will depend not only on better tissue design, but also on better functional benchmarks and more standardized production workflows.

5. Representative applications of iPSC-based bioprinting

The combination of induced pluripotent stem cell (iPSC) technology and three-dimensional bioprinting has enabled the generation of physiologically relevant human tissue models. Compared with conventional two-dimensional systems, bioprinted constructs preserve spatial architecture, multicellular interactions, and microenvironmental factors. These features improve the predictive value of in vitro platforms for disease modeling and drug evaluation. Several representative applications have emerged, including cardiovascular tissues, neural systems, liver

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and kidney constructs, pancreatic islet models, and tumor microenvironment systems.

5.1 Cardiac and cardiovascular models

Cardiac tissue engineering represents one of the most mature areas of iPSC-based bioprinting. Cardiomyocyte function can be evaluated using well-established readouts including contractile force, calcium handling, and electrophysiological activity. These quantitative measurements allow reproducible evaluation of engineered cardiac tissues. A landmark study by Ronaldson-Bouchard *et al*. engineered human cardiac tissues from pluripotent stem cell-derived cardiomyocytes embedded within a fibrin-based matrix. The tissues were cultured under progressive electrical stimulation for several weeks. This conditioning significantly improved sarcomere alignment, mitochondrial maturation, and electrophysiological properties. The engineered constructs generated contractile forces approaching those observed in adult myocardium, demonstrating that engineered tissues can achieve advanced functional maturation in vitro[104]. Bioprinting approaches have extended this concept by enabling spatially organized tissue fabrication. Noor *et al*. reported a personalized 3D bioprinting platform that used patient-derived cells and extracellular matrix components extracted from omental tissue to fabricate vascularized cardiac patches. The printed constructs contained organized myocardial fibers and perfusable vascular channels, demonstrating the feasibility of patient-specific cardiac tissue engineering[15] (Figure 5A). Engineered cardiac tissues have also been used in pharmacological screening. Mills *et al*. developed cardiac organoids composed of iPSC-derived cardiomyocytes and stromal cells. These organoids reproduced metabolic pathways associated with drug-induced cardiotoxicity. When exposed to cardiotoxic compounds such as doxorubicin, the constructs displayed contractile dysfunction and metabolic changes that closely resembled clinical cardiotoxic responses[115]. Together, these studies demonstrate that bioprinted cardiac tissues can reproduce essential structural and functional properties of the human heart, making them valuable tools for drug development and disease modeling.

5.2 Neural system models

Bioprinted neural tissues provide powerful platforms for studying brain development and

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neurological diseases. Conventional cell culture models cannot reproduce the complex spatial organization of neural networks. Yan *et al*. demonstrated that 3D bioprinting can generate neural tissues with functional connectivity. Using neural progenitor cells derived from human pluripotent stem cells, the researchers printed layered neural constructs that supported neuronal differentiation and synaptic formation. Electrophysiological recordings confirmed spontaneous neural activity and network connectivity, indicating the formation of functional neural circuits[106] (Figure 5B). Bioprinted neural constructs have also been applied to neurodegenerative disease modeling. Benwood *et al*. developed a "smart bioink" composed of gelatin-based materials and extracellular matrix components that supported the printing of patient-derived iPSC neural cells. The printed constructs exhibited neuronal maturation and Alzheimer's-related pathological features, including amyloid aggregation and altered neuronal morphology. This platform enabled investigation of disease mechanisms in a three-dimensional environment[102]. In cancer research, neural bioprinting systems have been used to study tumor invasion. Truong *et al.* developed a microfluidic three-dimensional brain-like model that allowed glioma stem cells to invade neural tissue structures. The model revealed that tumor cell invasion dynamics depend strongly on extracellular matrix composition and tissue architecture[116].

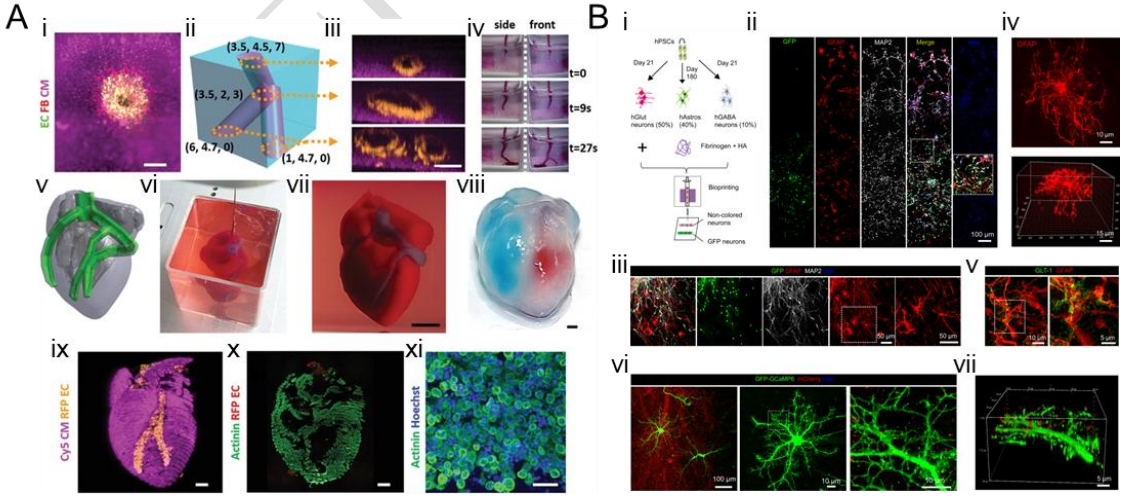


Figure 5. Bioprinted cardiac and neural tissues with vascularization and functional maturation. (A) Bioprinting of thick, vascularized cardiac tissues with embedded perfusable lumens enables effective mass transport and supports tissue viability. Complex architectures, including organ-

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scale human heart models generated from CAD-guided designs, demonstrate structural fidelity, multicellular organization, and chamber formation, highlighting the potential for large-scale cardiac tissue engineering[15]. (B) Bioprinted neural tissues incorporating neurons and astrocytes enable controlled multicellular organization and progressive maturation. Immunofluorescence analysis shows the presence of neuronal (MAP2) and astrocytic (GFAP, GLT-1) markers, while calcium imaging and three-dimensional reconstruction reveal synchronized neuronal activity and interconnected network formation, indicating functional neural tissue development[106]. Panel A adapted from Noor et al.[15] under the terms of the Creative Commons CC BY 4.0 license. Copyright © 2019 The Authors, published by Wiley-VCH. Panel B adapted with permission from Yan et al.[106] Copyright © 2024 Elsevier.

5.3 Liver and kidney

Liver and kidney tissues perform highly complex metabolic and transport functions that require organized tissue architecture. Conventional cell culture models often fail to maintain these functions over extended periods. Ma *et al*. developed a patterned 3D bioprinting technique that assembled human iPSC-derived hepatocytes into biomimetic hepatic tissues. The printed constructs displayed stable albumin secretion and maintained cytochrome P450 enzyme activity for extended culture periods. The system was subsequently used to evaluate hepatotoxic drug responses[105]. Similarly, He *et al*. reported a hepatic tissue model generated through extrusion-based bioprinting using iPSC-derived hepatocytes embedded within hydrogel matrices. The printed tissues exhibited enhanced metabolic function and long-term stability compared with traditional culture systems, demonstrating their utility in drug metabolism studies[100]. For renal tissues, Homan *et al*. showed that fluid flow significantly enhances kidney organoid maturation. In their study, kidney organoids were cultured within a microfluidic system that provided continuous perfusion. Flow stimulation promoted vascularization and improved structural organization, illustrating the importance of microenvironmental factors in renal tissue engineering[117] (Figure 6A).

5.4 Pancreatic islet

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Pancreatic islet engineering is widely regarded as one of the most promising applications of iPSC-based tissue engineering for clinical translation. Diabetes results from the loss or dysfunction of insulin-producing β cells. Balboa *et al.* demonstrated that human stem cell-derived pancreatic islets can achieve metabolic and functional maturation in vitro, exhibiting glucose-responsive insulin secretion and metabolic profiles comparable to primary human islets[101]. Bioprinting technologies further enhance this approach by providing structural organization and immune protection. Chen *et al*. developed a 3D-printed microdevice containing vascularized islet constructs composed of stem-cell-derived β-like cells and microvascular fragments. The device-maintained glucose-responsive insulin secretion and improved graft survival following transplantation in experimental models[103] (Figure 6B).

5.5 Tumor models

Three-dimensional tumor models represent another rapidly expanding application of bioprinted iPSC systems. Compared with two-dimensional cultures, bioprinted tumor constructs better reproduce tumor heterogeneity and interactions with the tumor microenvironment. Neal *et al.* demonstrated that three-dimensional tumor organoid cultures preserve immune cell infiltration and cytokine signaling patterns characteristic of the tumor microenvironment. These models enable investigation of tumor-immune interactions and therapeutic responses[118]. Reid *et al*. developed a controllable 3D bioprinting platform that enables the reproducible generation of large-scale mammary epithelial organoid structures. By precisely regulating initial cell number and spatial distribution, the printed constructs exhibited consistent morphology and organization, thereby providing a robust platform for investigating epithelial morphogenesis, tumor growth dynamics, and cell-matrix interactions[119] (Figure 6C).

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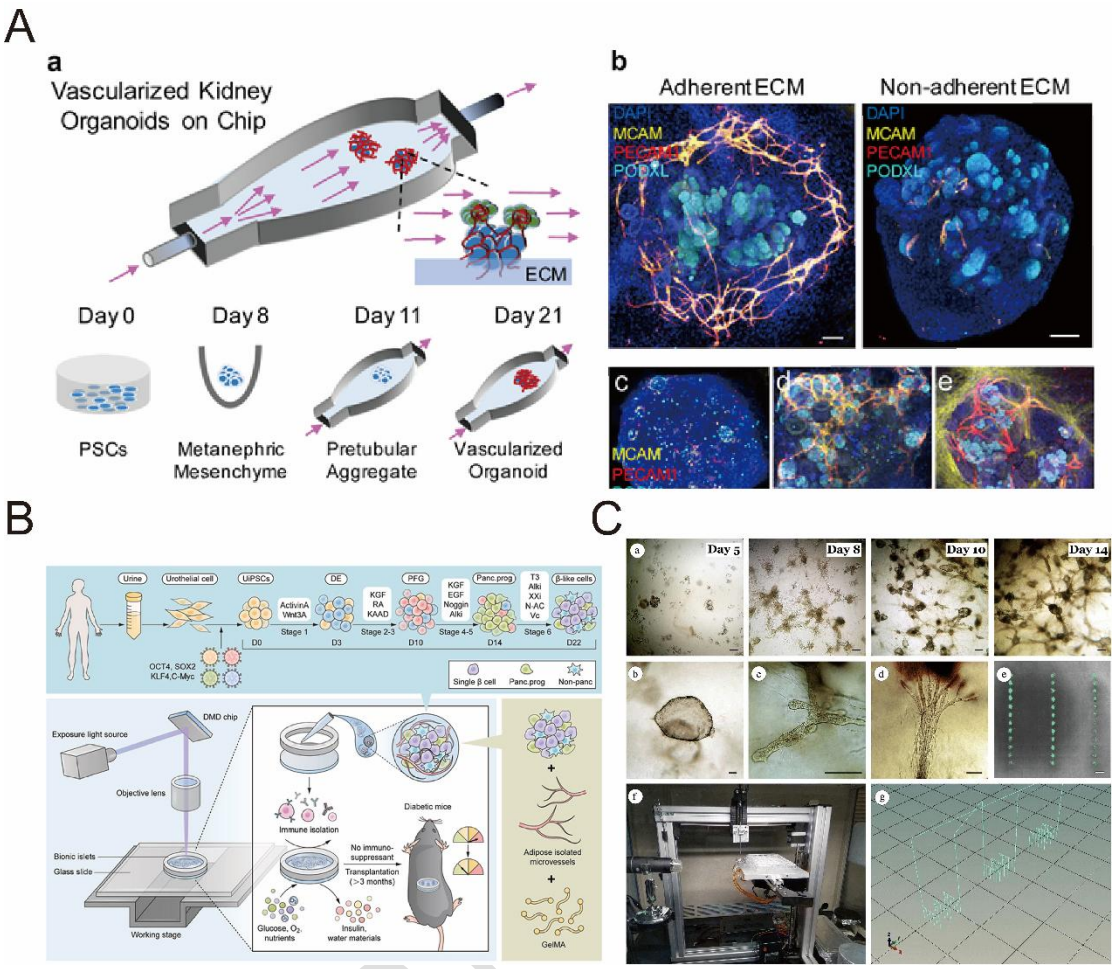


Figure 6. Bioprinting-enabled models of kidney, pancreatic islet, and tumor systems (A) Perfusion-based engineering of renal organoids, where developing iPSC-derived organoids are cultured on engineered extracellular matrices within millifluidic systems under controlled fluidic shear stress, promoting vascularization and tissue maturation (organoids not to scale). Enhanced peripheral vascular network formation is observed on adherent compared with non-adherent ECM substrates[117]. (B) A 3D-printed microdevice incorporating vascularized islet constructs composed of iPSC-derived β-like cells and microvascular fragments, enabling structural organization and functional support for type 1 diabetes therapy[103]. (C) Comparison of manual matrix embedding and 3D bioprinting in organoid formation. Conventional embedding results in heterogeneous, randomly distributed organoids with diverse morphologies, whereas bioprinting enables controlled spatial organization, reproducible growth patterns, and programmable construct architecture[119]. Panel A adapted with permission from Homan et al.[117] Copyright © 2019 Springer Nature. Panel B adapted from Chen et al.[103] under the terms of the

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5.6 Clinical translation and industrialization

From a clinical translation perspective, the most relevant question for iPSC-based bioprinting is not whether fully printed organs have already been implanted in humans. The more important issue is which product formats have demonstrated clinical acceptability. Current evidence indicates that the first translational breakthroughs rarely involve the most complex constructs. Instead, they typically emerge from tissue modules or cellular devices with clearly defined functional endpoints, well-defined product boundaries, and relatively controllable manufacturing pathways. A representative example in cardiac regeneration is engineered heart muscle (EHM) allografts. A study published in Nature in 2025 reported that engineered myocardial patches derived from iPSC-derived cardiomyocytes and stromal cells achieved remuscularization in non-human primate models of chronic heart failure and were subsequently applied in a first-in-human implantation. The authors further emphasized that these data provide critical preclinical and translational evidence supporting the first clinical trial of engineered heart tissue for cardiac repair [120] A phase I/II study demonstrated that allogeneic iPSC-derived dopaminergic progenitor cells transplanted into patients with Parkinson's disease could survive *in vivo*, produce dopamine, and show no evidence of tumor formation, indicating promising safety and potential therapeutic benefit[121]. Almost simultaneously, Cell published phase I/IIa clinical results using hESC-derived A9 dopaminergic progenitor cells, further demonstrating that pluripotent stem cell-derived dopaminergic products have entered a genuine stage of clinical validation[122]. For the bioprinting field, these findings are highly significant because they confirm that stem-cell-derived neural therapies can achieve clinical feasibility even within the highly sensitive environment of the human brain.

Diabetes represents one of the clearest pathways toward industrialization. A 2025 New England Journal of Medicine report on zimislecel (VX-880) demonstrated that fully differentiated stem

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cell-derived islets can restore physiological insulin production in patients with type 1 diabetes and support further clinical development[123]. At the same time, VX-264, an encapsulated stem cell-derived islet device, entered clinical trials, indicating that the “cell + device” strategy has already been incorporated into regulatory frameworks. Although VX-264 is not strictly a 3D bioprinted product, its product logic closely resembles future printed islet devices. The key challenge is no longer whether β-like cells can be generated, but how to achieve an engineering balance between immune protection and nutrient exchange. In addition, a recent study reported transplantation of autologous chemically induced pluripotent stem cell-derived islets, demonstrating long-term restoration of pancreatic function in a patient[124]. These findings further reinforce the conclusion that pluripotent stem cell-derived islet therapy has entered a clinically feasible stage.

Ophthalmology is one of the earliest fields to establish clinical precedents for pluripotent stem cell-derived tissue products. As early as 2017, the New England Journal of Medicine reported the feasibility of autologous iPSC-derived retinal pigment epithelium (RPE) sheets for the treatment of wet age-related macular degeneration[125]. In recent years, more mature engineering strategies have emerged. A long-term follow-up study published in Ophthalmology in 2024 reported that hESC-derived bioengineered RPE implants used for geographic atrophy were generally safe and well tolerated during a median follow-up of approximately three years, while also showing early signs of efficacy[126]. In 2025, Cell Stem Cell reported the safety and preliminary visual improvement associated with RPESC-RPE in a low-dose cohort of patients with dry age-related macular degeneration[127]. For the fields of bioprinting and biofabrication, these cases demonstrate that scaffold-based or monolayer cell products are particularly well suited to clinical translation in anatomical systems with clearly defined spaces and delivery routes, such as the eye. Taken together, these studies across cardiac, neural, metabolic, and ophthalmic systems outline the current landscape of clinical translation, which is further summarized in Table 3.

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Table 3. Clinical progress of iPSC-based therapies: cell products, delivery routes, and therapeutic outcomes

Disease	Cell product	Delivery method	Clinical stage	Key finding	Ref
Heart failure	Engineered heart muscle	Patch implantation	First-in-human	Remuscularization	[120]
Parkinson's disease	iPSC dopaminergic progenitors	Stereotactic injection	Phase I/II	Dopamine restoration	[122]
Diabetes	Stem cell-derived islets	Portal infusion	Clinical	Insulin independence	[123]
AMD	RPE sheet	Subretinal implant	Clinical	Vision stabilization	[125,126]

A study published in Journal of Neurosurgery: Spine in 2022 reported a 10-year safety follow-up of hPSC-derived oligodendrocyte progenitor cells (LCTOPC1/AST-OPC1) transplanted in patients with acute thoracic spinal cord injury. The results supported the long-term tolerability of pluripotent stem cell-derived neural products[128]. Although these therapies do not yet involve printed tissues, they provide critical references for safety evaluation, long-term monitoring, and regulatory considerations relevant to future bioprinted neural constructs.

From an industrial perspective, these clinical examples provide several important lessons. First, the products that enter clinical trials earliest are usually tissue modules rather than complete organs. Second, products with clearly defined structures—such as patches, monolayer scaffolds, encapsulated devices, or stereotactically delivered cell suspensions—are easier to standardize and regulate. Third, the speed of clinical translation is determined not only by biological feasibility but also by factors such as cell source stability, scalable manufacturing processes, established delivery techniques, and clearly defined quality attributes. These considerations explain why cardiac patches, islet devices, RPE implants, and dopaminergic progenitor cells have reached clinical validation earlier than fully printed organs.

When these clinical cases are considered in the context of iPSC bioprinting, a clearer conclusion emerges. The first bioprinted products to reach the clinic will likely not be the most structurally

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complex constructs. Instead, they will be modular tissues with well-defined functional readouts, clear local therapeutic targets, and scalable manufacturing strategies. In the context of this review, the applications that most closely fit this paradigm include myocardial patches, encapsulated pancreatic islet devices, RPE or retinal scaffold implants, and certain neural tissue modules. Therefore, the purpose of this section is not merely to list clinical examples but to demonstrate that pluripotent stem cell-derived tissue products can already be manufactured, delivered, and regulated in clinical settings. The next step for iPSC bioprinting is to build upon this established translational pathway by further improving spatial control, functional stability, and manufacturing scalability.

6. Future iPSC-based bioprinting research directions

Despite rapid progress in iPSC-based bioprinting, several scientific and engineering challenges remain before these systems can achieve widespread biomedical and clinical applications. Future research is expected to focus on improving tissue maturation, vascularization, multicellular organization, manufacturing scalability, and regulatory standardization.

6.1 Improving maturation of iPSC-derived tissues

One major limitation of current bioprinted tissues is the immature phenotype of many iPSC-derived cell types. Cardiomyocytes, hepatocytes, neurons, and β-like cells often resemble fetal rather than adult cells. Improving maturation therefore remains a central research priority. Electrical stimulation has emerged as an effective strategy for promoting cardiomyocyte maturation. Ronaldson-Bouchard *et al*. demonstrated that progressive electrical pacing dramatically improves the structural and functional maturation of engineered cardiac tissues derived from human pluripotent stem cells. The stimulated tissues exhibited improved sarcomere organization, mitochondrial development, and contractile force generation approaching adult myocardium levels[104]. Metabolic conditioning is another promising approach. Yang *et al*. showed that switching culture conditions from glycolytic to oxidative metabolism enhances mitochondrial maturation and electrophysiological stability in human iPSC-derived cardiomyocytes. This metabolic transition promotes adult-like cardiac phenotypes and improves the reliability of

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engineered cardiac models[4]. These studies suggest that combining biofabrication with biochemical and biophysical conditioning strategies will be essential for producing mature functional tissues.

6.2 Vascularization and perfusion engineering

The absence of vascular networks remains one of the most critical bottlenecks in the fabrication of thick tissues. Without adequate nutrient and oxygen transport, large constructs often suffer from central necrosis and functional decline. Several strategies have been developed to address this challenge. Skylar-Scott *et al*. introduced a bioprinting method known as sacrificial writing into functional tissue (SWIFT). In this system, dense cellular matrices containing pluripotent stem cell-derived organoids are printed with sacrificial channels that can later be perfused with culture medium. The resulting constructs support long-term cell viability and improved tissue function [14]. Another important advance is the development of vascularized organoids. Wimmer *et al*. generated blood vessel organoids from human pluripotent stem cells that closely resemble human microvasculature. When transplanted into mice, these vascular organoids integrated with host circulation and formed functional vascular networks[129]. These findings indicate that integrating vascular networks with bioprinted tissues will be essential for generating clinically relevant tissue constructs.

6.3 Engineering multicellular tissue architecture

Native tissues are composed of multiple interacting cell types organized in precise spatial arrangements. Replicating this complexity remains a major challenge for tissue bioprinting. A representative example is the development of cardiac organoids containing cardiomyocytes, fibroblasts, and endothelial cells. Lewis-Israeli *et al*. demonstrated that such multicellular cardiac organoids reproduce key features of early human heart development and can model congenital heart disease phenotypes in vitro[130]. Similarly, Takebe *et al*. generated liver organoids by co-culturing human iPSC-derived hepatic endoderm with endothelial and mesenchymal cells. These multicellular constructs formed vascularized liver buds capable of functional integration after transplantation in animal models[131]. These studies highlight the importance of recreating multicellular microenvironments for accurate tissue modeling.

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6.4 Scaling up manufacturing and bioprocess development

For iPSC-based bioprinting to move toward clinical and industrial applications, manufacturing processes must become scalable and reproducible. Large-scale expansion of pluripotent stem cells is a key requirement. Olmer *et al*. demonstrated that human iPSCs can be expanded efficiently in suspension bioreactors while maintaining pluripotency and genomic stability. This scalable culture system enables the production of large quantities of stem cells suitable for downstream tissue engineering applications[132]. In addition, automated bioprinting technologies are improving the reproducibility of tissue fabrication. Murphy and Atala reviewed emerging biofabrication systems capable of producing complex tissue structures with high spatial resolution and standardized manufacturing protocols[10].

6.5 Establishing regulatory and quality control frameworks

The translation of bioprinted tissues into clinical therapies requires clear regulatory pathways and standardized quality control metrics. Compared with traditional pharmaceuticals, bioprinted tissues present unique challenges due to their structural complexity and cellular heterogeneity. Recent regulatory discussions emphasize the importance of defining critical quality attributes (CQAs) such as cell identity, purity, functional activity, and structural integrity. Marks *et al*. highlighted that the clinical development of pluripotent stem cell therapies requires standardized assays for safety evaluation, including genomic stability, tumorigenicity testing, and functional characterization[133]. Furthermore, emerging regulatory frameworks for advanced therapy medicinal products (ATMPs), together with broader perspectives on stem cell therapy safety and efficacy, emphasize the need for harmonized standards in manufacturing and quality control[134]. Developing such regulatory and quality assurance systems will be essential for translating bioprinted tissues into safe and effective clinical therapies.

6.6 Development of advanced bioinks for iPSC bioprinting

Bioinks play a central role in determining the structural fidelity, cell viability, and functional maturation of bioprinted tissues. For iPSC-based bioprinting, bioinks materials should not only support cell survival during the printing process but also maintain cell pluripotency or guide

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lineage-specific differentiation. Therefore, the next generation of bioinks is expected to incorporate biomimetic extracellular matrix components, dynamic mechanical properties, and programmable biochemical signals.

One promising direction is the development of extracellular matrix (ECM)-derived bioinks. Decellularized ECM materials retain many biochemical factors present in native tissues, including growth factors and structural proteins. Pati *et al*. developed tissue-specific decellularized ECM bioinks derived from cardiac, cartilage, and adipose tissues. When used for bioprinting, these ECM bioinks promoted lineage-specific differentiation and improved tissue-specific gene expression compared with conventional hydrogel matrices[135].

Another important strategy is the design of dynamic or responsive bioinks that change their properties over time. Native tissues continuously remodel their extracellular matrix during development and regeneration. To mimic this process, Ouyang *et al*. engineered shear-thinning and self-healing hydrogel bioinks based on hyaluronic acid. These materials allowed high cell viability during printing while providing dynamic mechanical environments that supported long-term cell culture and tissue remodeling[136].

Smart bioinks capable of regulating stem cell fate have also attracted increasing attention. Ribeiro *et al*. developed tunable gelatin methacrylate (GelMA)-based bioinks with adjustable stiffness and biochemical signals. By modulating the mechanical properties of the hydrogel, the researchers demonstrated that stem cell differentiation and tissue organization could be controlled within printed constructs[137].

Recent studies have also explored multi-component bioinks that combine natural and synthetic biomaterials. Such hybrid bioinks can simultaneously provide biological signals and mechanical stability. For example, Chimene *et al*. introduced nanocomposite bioinks containing nanosilicate particles that improved printability and enhanced cell–matrix interactions. These materials supported the formation of structurally stable constructs while promoting osteogenic differentiation of stem cells[138].

In future, bioink development is expected to integrate emerging technologies such as synthetic

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biology, programmable biomaterials, and spatially controlled biochemical gradients. These innovations may allow bioinks to actively regulate tissue maturation, vascularization, and multicellular organization after printing. Ultimately, advanced bioinks will be essential for transforming iPSC bioprinting from a structural fabrication technology into a platform capable of generating stable and functional human tissues.

6.7 AI-assisted bioprinting

Although significant progress has been achieved in bioprinting technologies, current systems still face limitations in printing precision, parameter optimization, and tissue reproducibility. The fabrication of complex living tissues requires precise coordination of multiple variables, including bioink rheology, nozzle geometry, printing speed, extrusion pressure, and cell density. Traditionally, these parameters are optimized through trial-and-error experimentation, which is time-consuming and difficult to standardize. As a result, increasing attention has been directed toward AI-assisted bioprinting, where machine learning algorithms are used to predict printing outcomes, optimize printing parameters, and guide tissue design.

One of the earliest demonstrations of AI-assisted printing optimization was reported by An *et al.*, who developed a machine learning framework capable of predicting print fidelity in extrusion-based bioprinting. By integrating rheological parameters and printing conditions into a predictive model, the algorithm was able to identify optimal printing conditions for hydrogel bioinks. This approach significantly reduced experimental iterations and improved structural accuracy of printed constructs[139].

Artificial intelligence has also been applied to real-time print monitoring. Jiang *et al*. developed a computer-vision system combined with deep learning algorithms to monitor filament formation during bioprinting. The system could automatically detect printing defects and adjust printing parameters in real time, thereby improving printing stability and construct reproducibility[140]. Such adaptive control systems represent an important step toward autonomous biofabrication.

Another emerging direction is AI-guided tissue design. Instead of simply printing predefined structures, machine learning algorithms can generate optimized tissue architectures that improve

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nutrient diffusion and mechanical stability. Ma *et al*. developed a deep learning-based framework that predicts optimal scaffold architectures for tissue engineering. Their model analyzed thousands of scaffold designs and identified structural patterns that enhanced mass transport and mechanical performance in printed tissues[141].

AI technologies are also beginning to influence multi-material bioprinting. In complex tissue fabrication, different bioinks must be precisely deposited to recreate heterogeneous tissue structures. Recent studies have applied machine learning approaches to optimize the spatial distribution and deposition sequence of multiple biomaterials, improving structural fidelity and reducing printing errors in multi-material constructs[142].

Beyond printing optimization, AI is expected to play an important role in digital biofabrication workflows. Future systems may integrate spatial transcriptomics, imaging data, and computational modeling to generate digital templates of tissues prior to printing. Machine learning models could then translate these biological datasets into printable tissue architectures. Such integrated platforms may enable the development of digital twins of tissues, where computational models continuously guide and refine the printing process.

Overall, AI-assisted bioprinting represents a promising strategy for overcoming several key limitations of current biofabrication technologies. By enabling automated parameter optimization, adaptive printing control, and data-driven tissue design, artificial intelligence may significantly improve the precision, reproducibility, and scalability of iPSC-based bioprinting. Continued integration of machine learning, robotics, and biofabrication technologies will likely accelerate the transition from experimental tissue models toward clinically relevant engineered tissues.

7. Conclusion

The integration of induced pluripotent stem cell (iPSC) technology with three-dimensional bioprinting has created new opportunities for building human tissue models with improved physiological relevance. Compared with conventional cell culture systems, iPSC-based bioprinting enables the spatial organization of multiple cell types within biomimetic microenvironments. This capability allows researchers to better reproduce tissue architecture, cell-

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cell interactions, and functional properties that are difficult to achieve in two-dimensional systems. As a result, bioprinted tissues have emerged as powerful platforms for disease modeling, drug screening, and regenerative medicine research.

Over the past decade, significant progress has been made in several key areas. Advances in stem cell differentiation protocols have improved the availability of functional cell types for biofabrication. At the same time, innovations in bioink design, vascularization strategies, and printing technologies have enabled the construction of increasingly complex tissue structures. Bioprinted cardiac tissues, neural constructs, hepatic models, pancreatic islets, and tumor microenvironment platforms have already demonstrated promising capabilities in both basic research and translational studies.

Despite these advances, important challenges remain before iPSC bioprinting can achieve widespread biomedical and clinical applications. Many iPSC-derived cells still exhibit immature phenotypes compared with adult tissues, and large tissue constructs often lack sufficient vascularization. In addition, reproducible manufacturing, quality control, and regulatory frameworks remain underdeveloped for complex living products. Addressing these issues will require interdisciplinary collaboration among stem cell biologists, biomaterials scientists, engineers, and clinicians.

Looking forward, the next stage of development will likely involve the integration of several emerging technologies. Advanced bioinks that mimic tissue-specific extracellular matrices may provide more supportive microenvironments for stem cell differentiation and tissue maturation. Vascularized printing strategies will be essential for generating thick and functional tissues. In parallel, artificial intelligence and computational design tools may enable automated optimization of printing parameters and tissue architectures, improving reproducibility and scalability.

Ultimately, the future success of iPSC-based bioprinting will depend on its ability to move beyond proof-of-concept demonstrations toward reliable and standardized biofabrication platforms. With continued advances in stem cell biology, biomaterials engineering, and digital manufacturing technologies, iPSC bioprinting has the potential to become a transformative approach for

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constructing human tissues and developing next-generation regenerative therapies.

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Conflict of interest

The authors declare no conflict of interest.

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Consent for publication

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Availability of data

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