Physiological-cell-density 3D bioprinting: Advancing tissue constructs for near-native function

Leyan Xuan,[1,2] Jieting Li,[2] Bingbing Zhan,[2] Jialin Wu,[1,2] Liming Lian,[3] Mingen Xu,[1,*] and Guosheng Tang[2,*]

[1]Zhejiang Key Laboratory of Medical Additive Manufacturing and Information Fusion, School of Automation, Hangzhou Dianzi University, Hangzhou 310018, China
[2]School of Pharmaceutical Sciences, Guangzhou Medical University, Guangzhou 511436, China
[3]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA 30322, USA
*Correspondence: xumingen@hdu.edu.cn (M.X.); guoshengtang@gzhmu.edu.cn (G.T.)

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3D bioprinting is an emerging additive manufacturing technology that enables the fabrication of tissues with defined biological structures and functions through the precise deposition of cell-laden bioinks. These bioinks typically rely on hydrogel matrices to provide structural stability and shape fidelity during printing, thereby enabling the accurate fabrication of complex tissue architectures.

While current bioprinting techniques can create simplified biomimetic tissues, a fundamental challenge remains in replicating the native cell densities of most human solid tissues (~10^7 to ~10^9 cells per cm^3), which can reach billions of cells per cubic centimeter, a crucial feature of organoids' capacity for self-organization and physiological relevance.[1] This ultrahigh cell density and precise spatial organization are essential for structural integrity

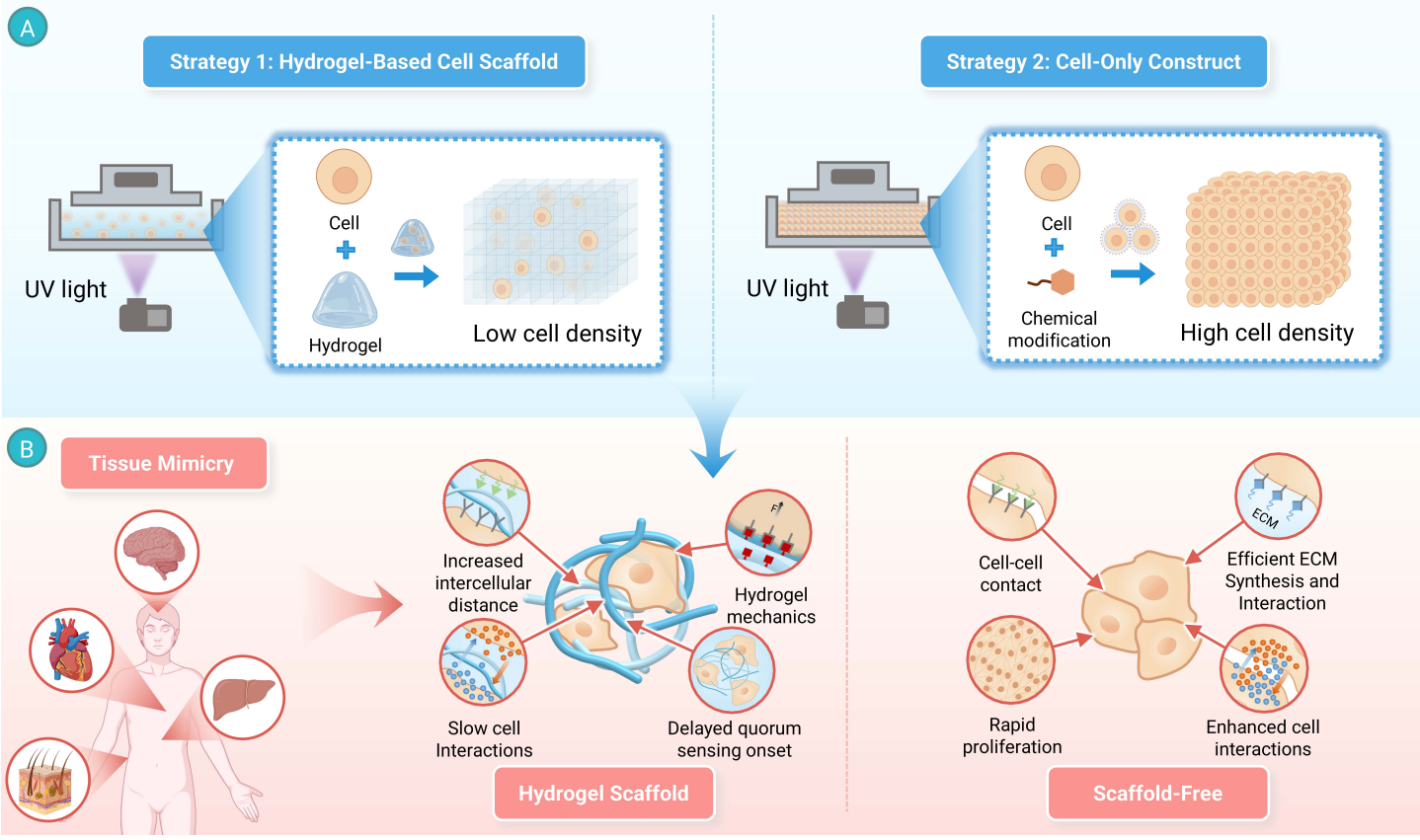

Figure 1. Illustration of the hydrogel-based cell scaffold and cell-only construct. (A) Fabrication process; (B) Niche interactions between cell-hydrogel and cell-cell. Based on icons from BioRender (https://www.biorender.com/).

and complex physiological functions, as exemplified by the cardiomyocyte network in the heart and the neuronal architecture of the cerebral cortex.[2] The lack of direct cell-cell contact in low-density, hydrogel-heavy constructs can prevent the formation of crucial gap junctions for cardiomyocyte synchrony or synaptic connections for neuronal circuits, thereby providing a stronger functional impetus for achieving high cell density. The pursuit of high cell density, however, introduces significant technical conflicts. Excessive hydrogel content, often needed for printability, can impair cell-cell interaction and tissue function. Moreover, high cell density bioinks themselves lead to increased viscosity, pronounced light scattering, and restricted nutrient diffusion, which collectively threaten cell viability and printing precision. Consequently, simultaneously achieving ultrahigh cell density (up to ~10^9 cells mL^{-1}), high cell viability, and high-fidelity represents one of the most persistent challenges in the field.

To address these challenges, various strategies have been proposed for high cell density (up to ~10^8 cells mL^{-1}) bioprinting. Notably, You et al. reported an innovative 3D bioprinting approach that simultaneously achieves high cell density and high resolution.[3] By incorporating the biocompatible supplement iodixanol (IDX) into hydrogel-based bioinks, they tuned the refractive index to match that of the cellular cytoplasm. This reduced light scattering approximately tenfold and significantly improved the printability of high cell density bioinks, achieving a resolution of 50 μm even at concentrations up to 10^8 cells mL^{-1}. The absence of significant adverse effects of IDX on cell viability, proliferation, or phenotype was confirmed by biocompatibility assays, immunofluorescence imaging, and RNA sequencing. Moreover, the printed prevascularized thick tissues exhibited endothelialization and angiogenesis after 14 days of perfusion culture. While this work overcomes key optical and rheological limitations, it does not yet achieve native tissue-level

cell densities, and the constructs remain hydrogel-dependent for structural stability. Nevertheless, it represents a significant advance toward functionally viable, high-density bioprinting. However, the scalability of refractive index matching for different cell types, which may have varying indices, warrants further consideration.

To improve this, Wang et al. developed a transformative strategy that converts living cells directly into a cell-only bioink through cell surface engineering, enabling printed tissues to reach ultrahigh, near-physiological cell densities (up to 10^9 cells mL^{-1}) while minimizing dependence on scaffold materials.[4] Oxidized and methacrylated hyaluronic acid (OMHA) was used as a molecular linker to modify cell membranes via amine-aldehyde coupling reactions, thereby introducing photocrosslinkable methacrylate moieties onto the cell surface. The choice of printing technology is deeply intertwined with the bioink strategy. Using digital light processing (DLP) for layer-by-layer photopolymerization, the authors were able to fabricate complex 2D/3D tissue constructs with hollow chambers, branched channels, and multicellular architectures—achievements that are difficult to replicate with extrusion-based methods. This scaffold-free, ultrahigh cell density bioink eliminates the need for conventional hydrogel scaffolds, enabling cell densities close to physiological levels, supporting direct cell-cell interactions, and enhancing tissue functionality. Four high cell density tissue models were constructed and subjected to systematic functional validation. In a liver model, the printed lobule-like architectures exhibited markedly elevated levels of albumin (ALB), E-cadherin, and cytochrome P1A2 compared with conventional hydrogel-based cultures. Notably, to address the limitations in nutrient and oxygen transport in high–cell-density tissues, constructs with branched channels were printed. 3D liver tissues with perfusable channels enhance cell survival and physiological function, compared to those without channels. Upon transplantation, they effectively integrated with host tissue and promoted angiogenesis, demonstrating robust metabolic function and in vivo integration. Furthermore, the technology was applied to fabricate cortico-motor circuits, which guided axonal growth and established interregional connectivity within just seven days. Functional neuronal circuitry was confirmed using optogenetics and micro-electrode array (MEA) recordings. In a cardiac model, chambered myocardial tissues began to exhibit spontaneous and synchronous rhythmic contractions after only two days of culture, retaining key biological attributes of native heart tissue. Finally, in a skin regeneration model, radially vascularized stem cell/endothelial cell composites significantly accelerated wound closure, enhanced vascularization, and promoted hair follicle regeneration. The molecular mechanism involved the regulation of developmental and inflammation-related gene expression. Collectively, these cross-organ demonstrations establish this approach as a versatile platform for fabricating living tissue constructs that integrate ultrahigh cell density, complex architecture, and essential physiological functions (Figure 1). It is important to note, however, that the potential immunogenicity of introducing new chemical moieties on the cell surface could be a critical consideration for future in vivo applications.

Together, these advances provide powerful technological platforms and conceptual frameworks for constructing 3D tissues with high cell density, structural stability, and functional maturity, yet several critical challenges remain to be addressed. First, under high cell density, the transport of nutrients, oxygen, and metabolic waste remains difficult to control precisely, and issues related to construct size scaling and long-term functional maturation require further investigation. This challenge is particularly relevant for the Wang et al. approach, which achieves near-native density but relies on dense cellular packing that may exacerbate transport limitations. Second, strategies relying on cell surface chemical modifications or hydrogel composition adjustments to support high cell density fabrication often necessitate the introduction of reactive groups, crosslinkers, or functional molecules. The potential impacts on cell membrane integrity, receptor function, signal transduction, and long-term phenotypic stability remain largely unassessed. For instance, the Wang et al. approach's reliance on cell-surface covalent bonding makes long-term assessment of cell membrane integrity critical; conversely, while the You et al. approach is less invasive, it does not solve the fundamental issue of the high-content hydrogel barrier between cells, potentially restricting nutrient diffusion and tissue maturation.

To address these limitations, complementary strategies that address these interconnected challenges are essential. One such avenue is single-cell microgel technology, which presents an approach to achieve scaffold minimization toward near-physiological cell density tissues.[5] By forming an ultrathin hydrogel coating around individual cells, single-cell microgels provide physical protection and tunable interfacial properties without substantially increasing the overall material volume fraction. Achieving minimal material usage with maximal cell content holds promise for simultaneously ensuring high cell density, efficient mass transport, and robust cell-cell interactions. However, translating this promising concept into robust, scalable manufacturing presents distinct hurdles. Key technical challenges include achieving uniform, sub-micron scale coatings encapsulating vast numbers of individual cells in a reproducible manner, and ensuring the mechanical integrity and stability of macroscopic constructs assembled from these discrete microgel units. Future efforts may place greater emphasis on physically driven fabrication strategies for single-cell microgels, enabling gentle cell encapsulation while avoiding complex chemical reactions. By engineering ultrathin (≈1-2 μm) hydrogel layers with functionalization at this interface, such approaches could protect cells from mechanical damage during printing while promoting tight cell-cell contacts and high-density, ordered cellular assembly. Addressing these fabrication and integration challenges is crucial to advance the technology. This direction not only reduces the material fraction in bioprinting systems but also offers new opportunities to construct biomimetic architectures that more closely approximate the cellular density and functional complexity of native tissues.

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AUTHOR CONTRIBUTIONS

Leyan Xuan: Conceptualization, Investigation, Writing—Original Draft; Jieting Li: Investigation, Writing—Original Draft; Bingbing Zhan: Investigation, Writing—Original Draft; Jialin Wu: Visualization, Review & Editing; Liming Lian: Supervision, Resources; Mingen Xu: Supervision, Review & Editing, Funding acquisition; Guosheng Tang: Conceptualization, Supervision, Resources, Writing—Review & Editing, Funding acquisition, Corresponding responsibility. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Guosheng Tang: http://www.guoshengtang-lab.cn/