Bio-Design and Manufacturing (2022) 5:433–436
https://doi.org/10.1007/s42242-022-00203-5

EDITORIAL

Simultaneous multimaterial multimethod bioprinting

Hongzhao Zhou[1,2] · Peng Liu[1,2] · Ziqi Gao[1,2] · Qi Li[1,2] · Weikang Lv[1,2] · Jun Yin[1,2] · Bin Zhang[1,2] · Huayong Yang[1,2] · Liang Ma[1,2]

Received: 10 June 2022 / Accepted: 12 June 2022 / Published online: 28 June 2022

Bioprinting: a powerful tool to fabricate biomimetic tissues and organs

Bioprinting is an important technology in the field of biofabrication that aims to create functional biomimetic structures. The flexibility, versatility and functionality of bioprinting enable the fabrication of intricate biological structures by combining cells, biomaterials or growth factors as printing bioinks [1, 2]. Conventional bioprinting technologies now face challenges, including the contradiction between the overall mechanical stability and the biological microenvironment of the construct and the vascularization of the printed tissues/organs. People have come to realize that it is unrealistic to build fully functional tissues/organs with just a single material. The complex composition and diversity of native tissues/organs require more than what a single-material bioprinting technique can possibly deliver [3].

Multimaterial bioprinting

Multimaterial bioprinting technology, therefore as a solution to the present challenges, enables different cells and biomaterials to construct heterogeneous structures, which may be a better representative of native tissues/organs. Some attempts at multimaterial printing have been made for the most common bioprinting methods, including extrusion, inkjet and light-assisted printing, which have been well categorized and discussed [4, 5]. We can conclude that multimaterial extrusion and inkjet printing have the advantages of a simple printing method and reasonably fast material switching speed, but they are limited to bioink rheological properties. Light-assisted technology is high in resolution, but material switching requires a complex mechanism and costs relatively long time, and the applicable bioinks need to be crosslinked with a photoinitiator that may have cell cytotoxins. The integration of bioinks with different properties in the same heterogeneous structure remains a significant challenge for bioprinting to create fully functional tissues/organs and requires more advanced technologies.

Combination of printing techniques

Multimaterial printing within single fabrication techniques cannot overcome the limitation of the material properties of the bioink. To fully explore and make use of the diverse properties of different biomaterials, it would be helpful to combine various three-dimensional (3D) bioprinting techniques in a single platform. Very few cases can be found for the combination of different processing techniques, including a case for the digital light process (DLP) and extrusion bioprinting [6] and another case for the combination of extrusion and electrospinning [7].

The need for printing with multiple materials simultaneously

A challenge that most multimaterial printing technologies face is the construction of complex heterogeneous tissues/organs in a reasonable amount of time. To simplify the printing process as well as the computer-aided design (CAD) work, even when equipped with multiple nozzles, the present approach can either print repeating structures at the same time or print in a sequential manner. For the manufacturing of heterogeneous tissue structures, it is desired that different cells or biomaterials in the same layer are deposited simultaneously. This may significantly increase the printing efficiency, thereby avoiding the reduction in the viability of the cells in

Hongzhao Zhou and Peng Liu have contributed equally to this work.

✉ Liang Ma
liangma@zju.edu.cn

1 State Key Laboratory of Fluid Power and Mechatronic Systems, Zhejiang University, Hangzhou 310058, China

2 School of Mechanical Engineering, Zhejiang University, Hangzhou 310058, China

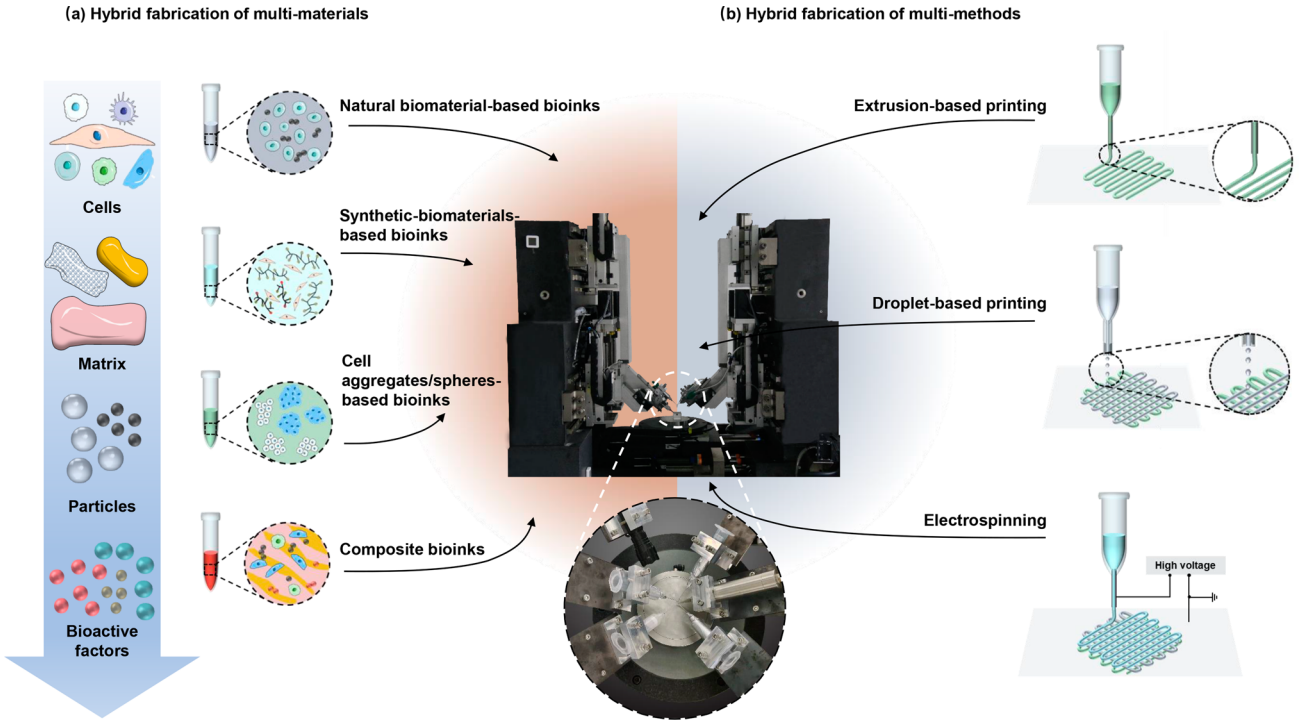

Fig. 1 Schematic illustration of the hybrid fabrication platform for multimaterial (**a**) and multimethod (**b**) bioprinting

the bioink due to prolonged exposure to the environment. Additionally, the adhesion between different materials can be enhanced, which may also affect the overall mechanical stability of the construct.

Modular designed platform for simultaneous printing

To improve the printing efficiency of constructing multilayered slicing structures, some researchers have developed a multiaxis Cartesian bioprinter with two individually controlled nozzles [8] or used multiple six-degree-of-freedom (6-DOF) robots that can print in parallel [9]. Although these methods have the ability to realize simultaneous bioprinting with two nozzles, they lack the ability to perform the bioprinting simultaneously with more printing methods.

In this report, a manufacturing method of a bioprinter based on the independent control of multiple nozzles is designed, which can support the simultaneous printing of multiple nozzles. The modular design increases the flexibility of the combined printing method, which can be tailored upon requirements for manufacturing precision or the chosen biomaterials, as shown in Fig. 1. The multimaterial biofabrication method proposed in this paper is very flexible and suitable for bioinks based on natural biomaterials, synthetic biomaterials, cell aggregates or composites and can accurately reproduce the complex composition of heterogeneous tissues. Due to the modular design approach, coaxial printing and embedded bioprinting technology can be easily applied to realize the construction of more complex human tissues/organs. In addition, other nozzle-based printing methods, such as droplet-based extrusion, electrospinning and other technologies, can be introduced through modular design to achieve composite manufacturing of process fusion.

Printing demonstrations

With the aforementioned powerful hybrid bioprinting platform, the construction of some complex heterogeneous tissues/organs that cannot be printed with a single method in one printing platform can be achieved.

As a demonstration, a full-thickness vascularized skin patch model and a three-layer tubular vessel model are printed using the proposed multinozzle-based multimaterial printing method. As shown in Fig. 2a, the designed skin patch model can be fabricated by the combination of conventional extrusion, coaxial extrusion, inkjet and stereolithography techniques. Figures 2a$_{i}$–2a$_{iv}$ represent the construction of the dermis, subcutaneous blood vessels, microvascular layer, and epidermal layer of the skin patch model. It can be found that the biomaterials needed to construct the full-thickness vascularized skin patch model are diverse from layer to layer,

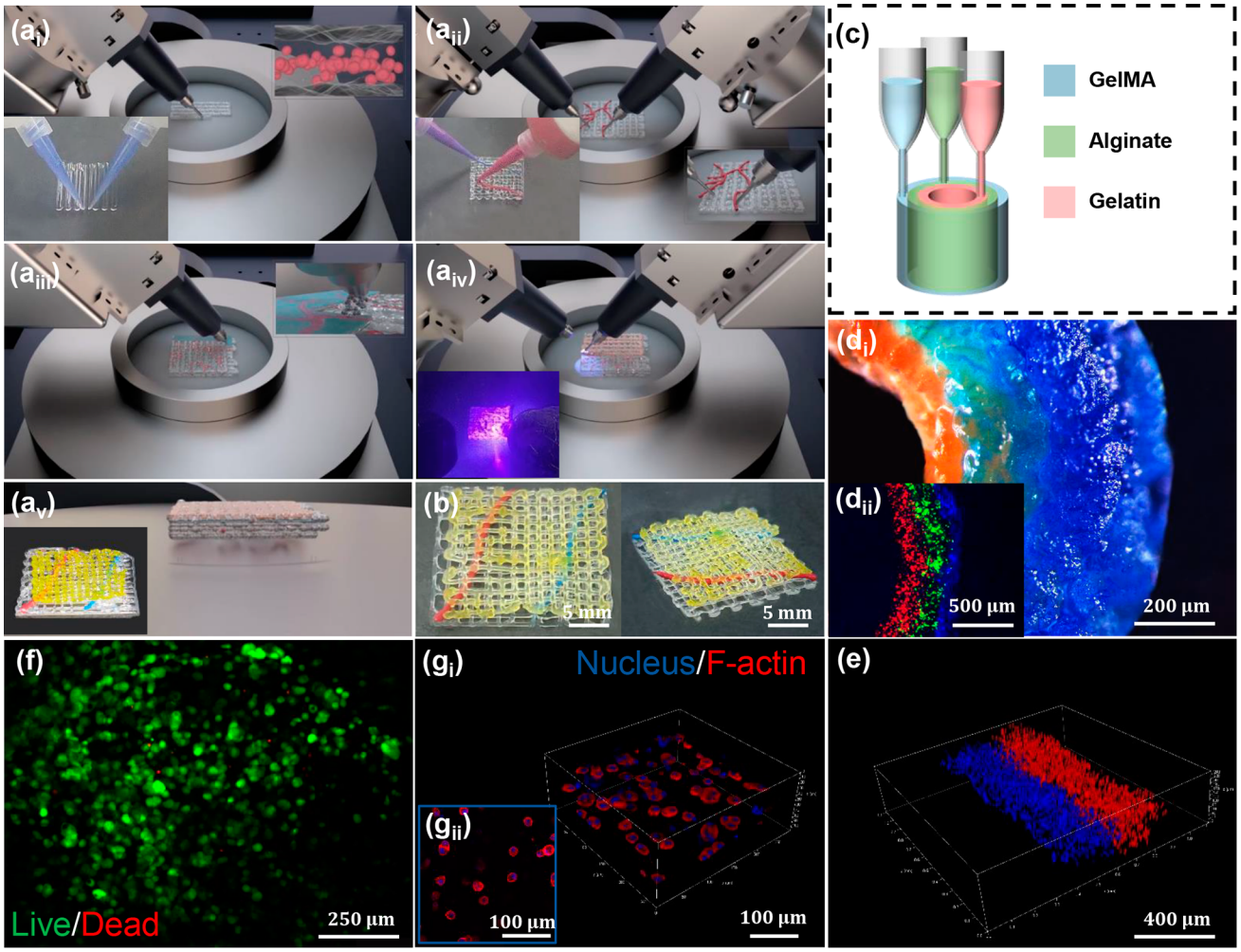

Fig. 2 Construction of multimaterial heterogeneous biological tissues: **a** the printing process of the full-thickness vascularized skin patch model constructed based on the multinozzle technology; **b** model of the skin patch generated by printing; **c** schematic diagram of the three-layer tubular model and biomaterials distribution; **d** the three-layer tubular model under microscope observation ($\mathbf{d_i}$), the fluorescent particle distribution of the three-layer tubular model ($\mathbf{d_{ii}}$); **e** the distribution of fluorescent particles in the inner and outer layers of the tubular model observed by confocal microscope; **f** the activity of smooth muscle cells MOVAS on the third day/dead staining; **g** 3D confocal image of a partial vessel model with endothelial MOVAS ($\mathbf{g_i}$), MOVAS observed under high magnification ($\mathbf{g_{ii}}$)

and the multimaterial bioprinting technology in this paper has the ability to accurately reproduce the actual skin tissue model in a reasonably short time.

To better verify the advantages of the method proposed in this paper in the construction of multilayer tubular biological tissue, a three-layer hollow tube model with a diameter around 800 μm was designed, as shown in Fig. 2c. Three different bioinks are used to construct the inner, middle and outer layers. Figure 2d shows that the three-layer tubular model constructed by the multiextrusion-based multimaterial bioprinting technology fits closely, and the inner and outer vessels are well fitted and maintain a good morphology under the confocal microscope view (Fig. 2e).

In addition, the cell culture of the tubular model was performed in this paper, and it could be observed that smooth muscle cells MOVAS remain highly variable after four days of culture in the constructed model (Fig. 2f). Confocal microscopy showed that MOVAS could be evenly distributed on the surface of the tubular model. Under high magnification, these cells were tightly connected to each other to form a single muscle layer (Fig. 2g), indicating that our simultaneous multimaterial bioprinting method has the potential to create a multilayer complex structure with high-activity cells.

Future perspectives

Multimaterial bioprinting technology is constantly changing with the development of new printing technologies. This work reports a multimaterial bioprinting method with a modular printing execution unit. Based on the existing bioprinting methods, through redesign, combined with multifunctional devices assembled into new functions, and with the change in printing technology, the printing unit can also be replaced upon requirements. At present, there are already two combinations of manufacturing technologies for human tissue printing, composite manufacturing methods based on extrusion and inkjet technology for skin printing and composite manufacturing technology based on photocuring and extrusion to construct cell-containing hydrogel structures. It is foreseeable that in the future multimaterial bioprinting field, composite manufacturing of multiple processes, such as microextrusion, inkjet, stereolithography and microfluidic-based bioprinting, will be one of the major trends.

In addition to composite manufacturing in the process, multimaterial bioprinting can be further expanded by using advanced material concepts, using self-assembled materials to achieve microstructure or biospecific expression. Studies have shown that self-healing suspensions can be used and freer fabrication of complex structure in liquid can be achieved. In addition, multimaterial bioprinting can improve the current single-material design by sacrificing ink materials, realizing the construction of multilayer tubular blood vessels, and introducing stimuli-responsive materials as bioinks to realize the integration of four-dimensional (4D) printing in biomanufacturing.

We believe that with such sophisticated simultaneous multimaterial multimethod bioprinting, fully functional heterogeneous tissues/organs with different cells and delicate microstructures can be finally printed with relatively high printing efficiency for tissue/organ transplantation.

Acknowledgements The authors would like to thank the National Key Research and Development Program of China (No. 2018YFA0703000) and the National Natural Science Foundation of China (Nos. 51875518 and 52105310) for funding.

Author contributions HZZ and PL did most of the writing for the manuscript and the experimental validation. ZQG, QL, and WKL contributed to methodology and investigation. JY and BZ provided resources. HYY, LM, and HZZ contributed to funding acquisition. LM and HZZ conceived and supervised the study, and contributed to writing—review and editing.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Zhang B, Gao L, Ma L et al (2019) 3D bioprinting: a novel avenue for manufacturing tissues and organs. Engineering 5(4):777–794. https://doi.org/10.1016/j.eng.2019.03.009
2. Zhang B, Luo YC, Ma L et al (2018) 3D bioprinting: an emerging technology full of opportunities and challenges. Bio-Des Manuf 1(1):2–13. https://doi.org/10.1007/s42242-018-0004-3
3. Dalton PD, Woodfield TBF, Mironov V et al (2020) Advances in hybrid fabrication toward hierarchical tissue constructs. Adv Sci 7(11):1902953. https://doi.org/10.1002/advs.201902953
4. Tavafoghi M, Darabi MA, Mahmoodi M et al (2021) Multimaterial bioprinting and combination of processing techniques towards the fabrication of biomimetic tissues and organs. Biofabrication 13(4):042002. https://doi.org/10.1088/1758-5090/ac0b9a
5. Ravanbakhsh H, Karamzadeh V, Bao GY et al (2021) Emerging technologies in multi-material bioprinting. Adv Mater 33(49):2104730. https://doi.org/10.1002/adma.202104730
6. Beh CW, Yew DS, Chai RJ et al (2021) A fluid-supported 3D hydrogel bioprinting method. Biomaterials 276:121034. https://doi.org/10.1016/j.biomaterials.2021.121034
7. Yoon Y, Kim CH, Lee JE et al (2019) 3D bioprinted complex constructs reinforced by hybrid multilayers of electrospun nanofiber sheets. Biofabrication 11(2):025015. https://doi.org/10.1088/1758-5090/ab08c2
8. Ozbolat IT, Chen H, Yu Y (2014) Development of 'Multi-arm Bioprinter' for hybrid biofabrication of tissue engineering constructs. Robot Comput-Integr Manuf 30(3):295–304. https://doi.org/10.1016/j.rcim.2013.10.005
9. Zhang Z, Wu C, Dai C et al (2022) A multi-axis robot-based bioprinting system supporting natural cell function preservation and cardiac tissue fabrication. Bioact Mater 18:138–150. https://doi.org/10.1016/j.bioactmat.2022.02.009