Scaffold-Based or Scaffold-Free Bioprinting: Competing or Complementing Approaches?

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Bioprinting is an emerging technology to fabricate artificial tissues and organs through additive manufacturing of living cells in a tissues-specific pattern by stacking them layer by layer. Two major approaches have been proposed in the literature: bioprinting cells in a scaffold matrix to support cell proliferation and growth, and bioprinting cells without using a scaffold structure. Despite great progress, particularly in scaffold-based approaches along with recent significant attempts, printing large-scale tissues and organs is still elusive. This paper demonstrates recent significant attempts in scaffold-based and scaffold-free tissue printing approaches, discusses the advantages and limitations of both approaches, and presents a conceptual framework for bioprinting of scale-up tissue by complementing the benefits of these approaches.
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Introduction

Bioprinting is a promising technology in regenerating tissues and organs and has recently gained enormous attention due to its unique benefits, including precise placement of biologics (i.e., cells, growth factors, and genes) to recapitulate heterocellular tissue biology, rapid fabrication of scalable tissue constructs, precise fabrication of anatomically accurate tissue replacement parts, and generation of high throughput assays for pharmaceutical applications such as drug toxicity, drug discovery, and clinical diagnostics [1–3]. Although the technology has recently progressed a great deal, bioprinting of whole organs has remained elusive due to several limitations, such as the need for a multiscale vascular network, the complexity of tissue biology and spatial arrangement of cells, and the limited long-term functionality and mechanical integrity of the printed constructs [4,5]. There are two major approaches studied in the literature for biofabrication of tissues and organs: scaffold-based [6,7] and scaffold-free [8,9] bioprinting. In the former approach, cells are printed within exogenous biomaterial matrix such as hydrogels to support their growth, proliferation, and interactions in three dimensions (3D). In the latter approach, exogenous biomaterials are not used to house cells to enable a 3D network for their interactions; rather, the cell pellet is confined in a printed mold structure to let them aggregate and secrete extracellular matrix (ECM) components to hold them together. Alternatively, pre-aggregated cells can be bioprinted in 3D printed mold structures for their fusion and maturation toward tissue generation [8]. Both approaches have advantages and disadvantages, and they can complement each other in pursuit of the demanding goal of bioprinting scale-up tissues and organs. This article presents the two approaches and their capabilities, discusses their strengths and limitations, and shares with the reader future possibilities for tissue and organ printing.

Scaffold-Based Bioprinting

Hydrogels are the preferred major class of bioink materials used in tissue engineering. They can be derived naturally or synthetically, and they are widely used to encapsulate cells during the bioprinting process while they mimic the ECM environment, allow cells to grow, possess a degree of flexibility very similar to natural tissue due to their water absorbent characteristics, and are abundant and affordable [6]. Several hydrogels have been engineered to adapt them for bioprinting processes: these include alginate, collagen, gelatin, gelatin methacrylate, hyaluronic acid, Extracel™, fibrin, polyethylene glycol, and Pluronic® F127 [7]. In general, hydrogels need a shear-thinning property in order to be applied in bioprinting processes. In other words, they need to possess pseudoplastic characteristics, where their viscosities need to decrease as shear stress increases. Their viscosities should be high enough to keep the hydrogel in the reservoir without letting it flow through the nozzle, and their viscosities should decrease as the shear stress increases when it flows through the nozzle with a cross-sectional area relatively smaller than the cross-sectional area in the reservoir. In addition, hydrogels need to possess quick gelation characteristics through different crosslinking mechanisms such as physical or chemical crosslinking so that they can generate anatomically correct shapes. Figure 1 shows bioprinting technologies used in scaffold-based bioprinting, where a demonstrative tubular vascular tissue scaffold is presented. In extrusion-based bioprinting (see Fig. 1(*a*)), which is the most commonly used bioprinting technique [10,11], cell-laden scaffolds are fabricated through layer-by layer deposition of extruded filaments, which can be solidified via physical or chemical crosslinking mechanisms. Figure 1(*b*) shows droplet-based bioprinting, where cells loaded in a cartridge reservoir are printed through ejection of the ink in droplet form by means of thermal [12] or piezoelectric energy [13]. The deposited droplets are then fused to the previously deposited construct by means of different crosslinking mechanisms. The crosslinking mechanism may be similar to the crosslinking mechanisms used in extrusion-based bioprinting. Figure 1(*c*) shows the laser-based bioprinting process, where cells loaded in a hydrogel solution coated under quartz support are exposed to a laser beam [14,15]. Due to generated thermal energy, a bubble formation occurs, which propels the bioink solution toward the collecting place, where the tissue scaffold is built. The bioink solution is dispensed in the form of a jet droplet, which turns into a rounded droplet as it flies and fuses to the previously deposited construct. All these mechanisms can be modified to some extent to alter their capabilities; however, scaffold-based

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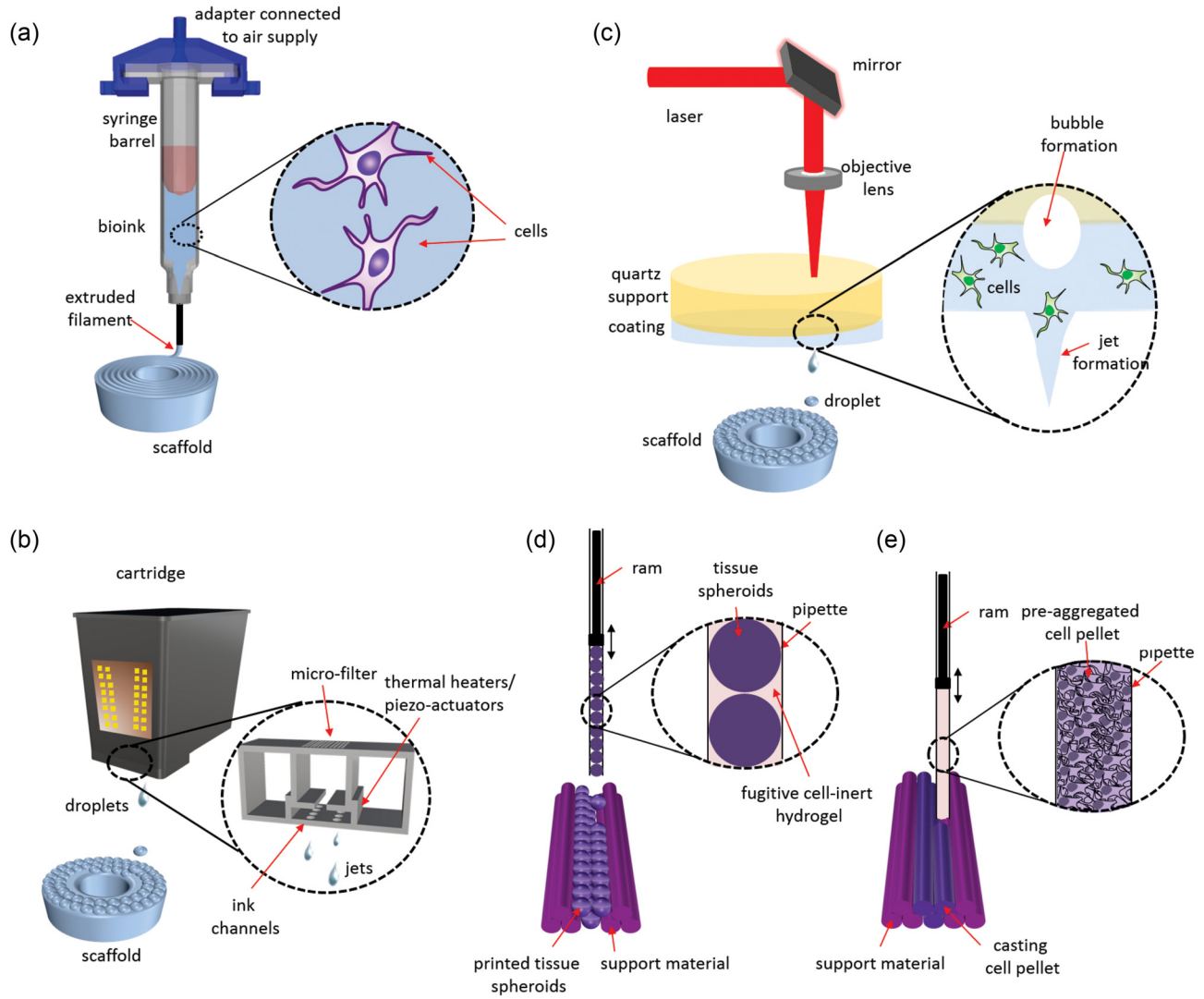

Fig. 1 Scaffold-based and scaffold-free bioprinting technologies: (*a*) extrusion-based bioprinting, (*b*) droplet-based bioprinting, (*c*) laser-based bioprinting, (*d*) bioprinting tissue spheroids, and (*e*) bioprinting cell pellet

bioprinting techniques in general enable deposition ease and practicality for tissue fabrication.

In addition to direct encapsulation of cells in hydrogels during bioprinting processes, microcarriers have been used as reinforcement blocks, where cells can be loaded in microcarriers in different geometries (spherical, in general [16]) with porous architecture. When cells are cultured on them, they allow the cells to quickly proliferate. Cultured cells in microcarriers can then be printed in a delivery medium such as hydrogel. It was demonstrated in a recent study that cells can interact and aggregate better inside the microcarriers than can cells loaded in the hydrogel solution alone [17].

In addition to hydrogels and microcarriers, decellularized matrix components that are derived from nature's own scaffold have been considered as a new bioink source for advanced tissue fabrication. Taylor's groundbreaking work in organ decellularization [18] has attracted numerous researchers in the last five years in regeneration of organs, which later inspired Cho and coworkers [19] to use decellularized matrix components in printing tissue analogs. In their recent study, they decellularized tissues and chopped them into smaller fragments, which were then loaded with cells and printed with a poly-caprolactone frame to support the tissue analogs. The approach seems to have a great benefit toward biomimetic tissue and organ printing, when the decellularized proteins (such as collagen, hyaluronic acid, etc.) can be tuned in a way that they can be printed in solid form directly without need of a hard polymer frame for future studies.

Scaffold-Free Bioprinting

Scaffold-free bioprinting has been considered a promising direction in tissue fabrication because it enables recapitulating native tissues in a shorter period of time than the commonly used cell-laden hydrogel approach. Instead of expecting cells to proliferate in hydrogels, one can start with considerably high cell numbers, that is, close to natural tissues, triggering them to deposit ECM components in a confined space per demand such as cylinder, torus, spheroids, and honeycomb [20]. The hydrogel-free nature of the biomaterials facilitates quick fusion and maturation of building blocks, where the technology has been demonstrated to fabricate cardiac patches [21], blood vessels [8], and nerve tissues [22]. Several biofabrication approaches have been described in the literature for cell aggregates, particularly tissue spheroids. These methods include the hanging drop [23,24], pellet (re-aggregation) culture or conical tube [25], micromolding [26,27], microfluidics (hydrodynamic cell trapping) [28,29], liquid overlay [30], spinner flask [31], and rotating wall vessel techniques [32]. It should be noted that not all of them have been applied in fabricating spheroids for bioprinting purposes, but any of them can be considered as an alternative approach as long as the technique

facilitates efficient and economical generation of spheroids for scale-up tissue printing activities. Not just homocellular but heterocellular examples have been demonstrated as well [33]. Prefabricated tissue spheroids are loaded in pipettes and dispensed using a mechanical ram-driven extrusion-based bioprinting system in a carrier hydrogel that is inert to cell adhesion (see Fig. 1(*d*)). In the meantime, a mold structure is 3D printed, where the bioprinted spheroids are cast inside the mold, letting them fuse and maturate into tissue followed by removal of the mold material. Instead of delivering cells in high density in aggregated mature spheroid form, delivering them directly in pellet form works more efficiently [22,34]. In that case, bioprinting cells into printed micromolds is essential to confine cells inside the molds and trigger them to aggregate in the shape of the molds (see Fig. 1(*e*)). Thus, two materials need to be deposited into the construct, where cell pellet can be printed inside hydrogels that are inert to cell adhesion such as agarose or alginate. There is a controversy among some Scientists about when the applied molding approach should be considered a scaffold. Although the mold itself supports the tissue to grow and maturate, cells do not use mold matrix to proliferate through; thus, the applied mold can be considered as a support structure, which is very common in traditional additive manufacturing technologies [35] used for supporting overhangs. The major hurdle with this approach is the difficulty of making large-scale tissues without using a temporary molding material. Thus, tissue strands can be considered as an alternative approach to tissue printing, where long strands of tissues can be fabricated and printed using a custom-made nozzle apparatus. In this case, the laborious nature of spheroid preparation and loading can be eliminated, and the need for printing an enclosure mold is eliminated for cell pellets. Although this approach provides the unique advantage of printing tissue strands in tandem with vasculature, increasing the size of the tissue strands or the need for neocapillarization within them can be considered as milestones on the way to generating larger-scale tissues and organs in the future [36].

A Comparison: Scaffold-Based or Scaffold-Free Bioprinting?

In general, cell encapsulation in biomaterials allows cell patterning that has a great potential for direct organ printing due to the complex heterocellular composition of native tissues; however, subsequent ECM formation, digestion and degradation of biomaterial matrix, and proliferation of encapsulated cells are not trivial to control. There are intrinsic limitations for scaffold-based bioprinting due to restricted cell proliferation and colonization, while cells are immobilized within hydrogels and do not spread, stretch, and migrate to generate the new tissue successfully. Cell-aggregate-based bioinks, on the other hand, have great advantages in bioprinting. First of all, they better facilitate cellular interactions, including homocellular and heterocellular interactions, while cells are loaded with very high cell densities close to native tissue and not immobilized in a hydrogel network. This enables generation of tissue with close biomimicry and preserves the cell phenotype and functionality for longer times. In addition, tissues can be fabricated in a very short period of time in culture conditions, which cannot be easily achieved using hydrogels. Despite these advantages, they have several limitations. First of all, a very high number of cells is needed to prepare a sufficient amount of aggregates. These numbers can go up to a few hundred million cells depending on the cell size and how quickly they deposit ECM. In general, expanding cells in these numbers is labor-intensive and costly, and some cell types cannot grow quickly, which limits their applicability and availability. In addition, parenchymal cells in highly metabolic organs do not secrete many ECM components, and the resulting cell aggregates are very weak in their mechanical and structural integrity. Therefore, supporting stromal cells should be cocultured to provide enough strength for bioprinting uses. In addition to mechanical properties, the dimensional constraints are another hurdle. In general, the permeability of cell aggregates is lower than that of hydrogels, and the diffusion of media and oxygen is highly limited. Thus, fabrication of tissue spheroids over 500 μm can induce hypoxia, which is hard for highly metabolic cells to survive. Resilient cells (i.e., fibroblast) or cells that like hypoxia (i.e., chondrocyte) can surpass these limitations. Thus, neocapillarization inside the cell aggregates is highly desired for scale-up fabrication of tissues and organs. From a bioprinting standpoint, the bioprinting of cell aggregate is highly trivial when the bioink is loaded in near-pellet form when the bioink can be in a liquid state and printed like a hydrogel-based bioink without the need for any other means. In

Table 1 Comparison of scaffold-based and scaffold-free bioprinting

		Scaffold-based bioprinting	Scaffold-free bioprinting
Process capabilities	Resolution	High	Low
	Accuracy	High	Low
	Bioprinting time	Short	Medium-long
	Processing modes	Extrusion-based, droplet-based, or laser-based bioprinting	Extrusion-based bioprinting
	Cell viability	High	Medium-high
	Control of single-cell printing	Feasible	Not feasible
	Throughput	High	Medium
Bioink consideration	Bioink types	Hydrogels, microcarriers, and decellularized matrix	Cell pellet, tissue spheroids, and tissue strands
	Bioink mode	Liquid, sol-gel, or solid	Liquid or solid
	Bioprintability	Easy	Difficult
	Bioink viscosity	Low to high	Medium to very high
	Multicellular feasibility	Yes	Yes
	Affordability	Low cost to high cost	High cost
	Commercial availability	Available	Available
End product capabilities	Cell interactions	Limited	High
	Mechanical/structural integrity	High	Low
	Tissue regeneration time	Considerably long	Short
	Tissue biomimicry	Low to medium	High
	Applications	Transplantation and drug testing	Transplantation and drug testing
Advantages		Easy to bioprint, economical, scalable, and high resolution	Rapid tissue maturation and generation, cell-friendly
Disadvantages		Toxicity, degradation, and limited cell-to-cell interactions	Need for high cell numbers initially, low scalability, and weak mechanical integrity

that case, a supporting molding structure is needed for cells to aggregate, and that support structure should be printed with minimum mold cavity space; otherwise, cells pellet do not form aggregates, but rather stay as a cell suspension. For scale-up tissue printing missions, the need for molding is thus not ideal. When the aggregates are loaded in a fully or semi-aggregated form such as tissue spheroids or strands, printing is not trivial while the bioink is in a solid state that should be transferred to the printing stage with minimum stress on the cells. Thus, hydrogels or biological oil can be used as a medium to deliver them to the bioprinting stage; however, such a medium brings an issue when it needs to be washed out from the printed construct. Despite their great advantages, tissue spheroids have several challenges during bioprinting processes. First of all, cell aggregates need to be printed before they become fully maturated, such as in the first ten days of cell aggregation; otherwise, maturated cell aggregates lose their ability to fuse. In addition, loading cell aggregates into the nozzle, which is a pipette, in general [8], is quite difficult. Tissue spheroids need a delivering medium to be extruded, in which case the delivering medium will be a fugitive ink such as a thermosensitive hydrogel that is inert to cell adhesion. In addition, tissue spheroids have quick fusion capabilities that trigger their aggregation inside the nozzle tip and make their printability highly challenging. Upon printing, there is also a risk that tissue spheroids may not contact each other tightly enough. This generates gap between spheroids, and the resulting tissue becomes leaky. Last and the most important, fabrication of a huge number of tissue spheroids and bioprinting them in an automated way during long-duration bioprinting missions is another hurdle considering the transition of the technology to scale-up tissue fabrication in the near future. Despite these challenges, bioprinting tissue spheroids is an exemplary means to create tissues in vitro, and further modifications have been made on the technology.

Microcarriers, on the other hand, possess bioprinting limitations similar to those of tissue spheroids and have shortcomings associated with degradation and related end products similar to hydrogels. Although it can be considered as an intermediate stage between cell aggregates and hydrogels, there are challenges that must be overcome in order to deploy microcarriers in bioprinting efficiently. These challenges include the difficulty of ensuring contact between microcarriers; the degradation of the microcarrier biomaterial and associated end products that can be toxic to cells; and the risks of clogging the nozzle tip due to the hard and adhesive nature of the microcarriers that can trigger their aggregation inside the nozzle tip.

Despite the advantages of the scaffold-free bioprinting approach in the fabrication of tissues with better biomimicry in a shorter period time, the majority of the research community prefers hydrogel-based bioink for several reasons. First of all, hydrogels are commercially available and affordable in general, and they are abundant for bioprinting processes. Bioprinting has a steep learning curve for operators, and affordable bioink materials are preferred for ease of use. Due to their abundance and affordability, larger tissue constructs can be manufactured both in volume and quantity, providing a great scalability feature. In addition, hydrogels have great shear-thinning properties, and they can be printed through various means such as crosslinking and bioprinting mechanisms as discussed before. Besides, bioprinting in hydrogels does not require very high cell numbers to start with; cell densities in the ranges of a few millions per milliliter are sufficient to grow into larger numbers in vitro or in vivo; however, larger cell densities better support tissue growth. The scaffold-based approach limits cell growth in long incubation periods in vitro; however, it performs well, when the tissue constructs are implanted while the body can degrade and absorb the scaffold material easily, triggering neovascularization through the migration of endothelial cells into the scaffold construct and the generation of a capillary network within them. Currently, tissue engineering approaches are also being investigated to enable the growth of nerves into transplanted tissues [37]. Table 1 lays out a comparison between scaffold-based and scaffold-free bioprinting considering a wide-array of components and performance metrics of bioprinting.

The Demanding Goal: Future Perspective for Organ Printing

Despite considerable progress in the context of bioprinting in the last decade, there is still much work remaining for whole-organ bioprinting [1]. Scaffold-based or scaffold-free bioprinting approaches alone do not possess the required capability to create scale-up tissues. The major challenge with the scaffold-based bioprinting approach is that parenchymal cells do not preserve their viability, phenotypic stability, or functionality long term in vitro. Therefore, the parenchyma tissue can better grow in the scaffold-free approach. While the scaffold-free approach does not possess sufficient mechanical stiffness, the stromal tissue of the organ can be considered using scaffold-based bioprinting. Although there is a perception that scaffold-based and scaffold-free bioprinting complete with each other, they can complement each other toward the demanding goal of larger-scale tissue and organ bioprinting. One can thus consider a combination of these two approaches, where the vascular network can be bioprinted for both perfusion and mechanical support purposes, and the parenchymal side can

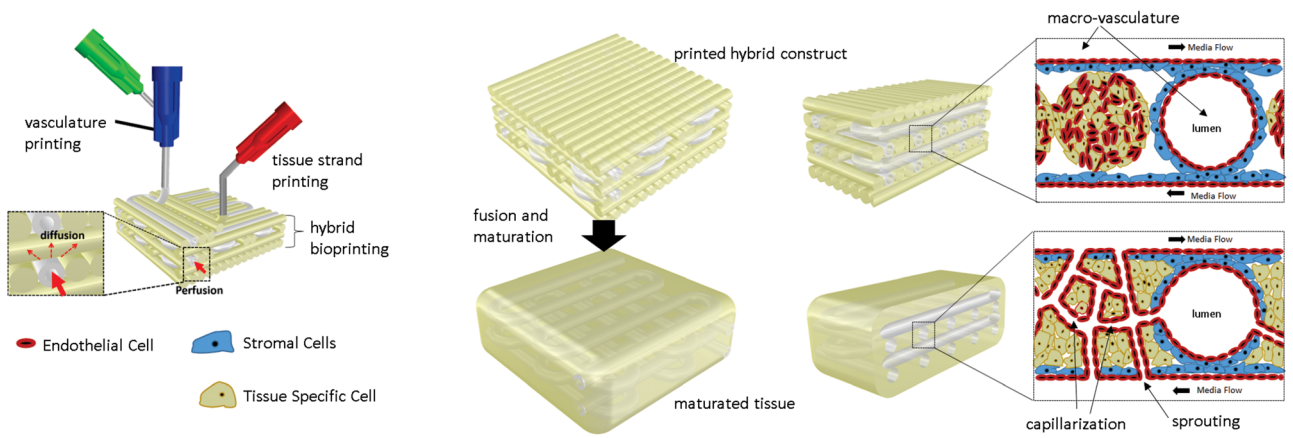

Fig. 2 Hybrid bioprinting of scaffold-based vascular constructs in tandem with scaffold-free parenchyma tissue, where fusion, tissue remodeling, and self-assembly of tissue strands take place and sprouting can take place between the macrovascular network and capillaries in tissue strands. This concept generalizes the tissue used; however, for different tissue types, modifications on the system would be essential.

be integrated via scaffold-free bioprinting. In this case, a multi-scale vascular network needs to be created to complete the scale-up tissue infrastructure. This has been recently performed using manual staking of cell sheet technology, where sprouting of microcapillaries was performed by placing a vascular network. Figure 2 demonstrates the concept of the scale-up tissue printing process, where a vascular network can be printed in tandem with the parenchymal tissue. Upon printing, scaffold-free parenchymal tissue can fuse, maturate, and self-assemble around the vasculature. Further, culturing of the tissue construct while applying ideal perfusion conditions can enable the sprouting of capillaries by bridging the main vascular network with the capillary network forming inside the parenchymal tissue.

Although the scaffold-based and scaffold-free bioprinting approaches have been studied alone, this perspective paper proposes a hybrid bioprinting concept, where they can complement each other toward future organ printing technologies. Studies have been done to construct perfusable cardiac tissues using a 3D cell sheet fabrication technology, in which endothelial cells within a cardiac cell sheet sprouted and connected to the main blood vessel upon perfusion of growth-factor-rich culture media [38]. Other studies have also shown that a prevascularized hepatic bud, when transplanted in vivo, can successfully anastomose to the main blood vessel and survive for a long period of time, carrying out its function [39]. In addition, a recent article [40] demonstrated vascularization of cell aggregates in tumor spheroid models and robust sprouting angiogenesis into the matrix where the aggregates were loaded, showing the great possibility of enabling anastomosis of vascular networks of two aggregates during the fusion process. All of these highlights offer foreseeable potential for the hybrid bioprinting technique to have a similar nature-driven process upon perfusion.

Conclusion

This paper presents scaffold-based and scaffold-free bioprinting techniques and compares them according to various criteria, including bioprintability, biological performance, mechanical and structural integrity, affordability, and practicality. Although both approaches possess intrinsic advantages and disadvantages, they can complement each other toward the demanding goal of larger-scale tissue and organ printing that necessitates the safe delivery of biologics, spatial patterning of cells to recapitulate tissue biology, a mild tissue micro-environment for better cellular interactions, mechanical stiffness and stability to preserve the tissue structure, longer-term preservation of tissue functionality, and, most importantly, a vascular network in multiple scale.

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