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Bioprinting scale-up tissue and organ constructs for transplantation

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Bioprinting is an emerging field that is having a revolutionary impact on the medical sciences. It offers great precision for the spatial placement of cells, proteins, genes, drugs, and biologically active particles to better guide tissue generation and formation. This emerging biotechnology appears to be promising for advancing tissue engineering toward functional tissue and organ fabrication for transplantation, drug testing, research investigations, and cancer or disease modeling, and has recently attracted growing interest worldwide among researchers and the general public. In this Opinion, I highlight possibilities for the bioprinting scale-up of functional tissue and organ constructs for transplantation and provide the reader with alternative approaches, their limitations, and promising directions for new research prospects.

Bioprinting: a promising technology to revolutionize medicine

Bioprinting can be defined as the spatial patterning of living cells and other biologics by stacking and assembling them using a computer-aided layer-by-layer deposition approach to develop living tissue and organ analogs for tissue engineering, regenerative medicine, pharmacokinetic, and other biological studies [1]. It uses four approaches to deposit living cells: inkjet [2], extrusion [3], acoustic [4] and laser [5] based. Given its great benefit in spatially arranging multiple cell types to recapitulate tissue biology, bioprinting is a game-changer in the rapid development of tissue constructs and is receiving enormous attention. Although bioprinting of functional 3D whole organs for transplantation remains in the realm of science fiction, the field is moving forward, providing hope that shortages in tissue grafts and organ transplantation will be mitigated to some extent in the future [6]. While current tissue-engineering strategies cannot enable fabrication of fully functional tissues or organs [7], bioprinting enables precise placement of biologics to recapitulate heterocellular tissue biology to some degree. Current technology enables the development of organ or tissue constructs that do not require substantial vascularization, as well as mini-tissue models mimicking the biology of natural counterparts for pharmaceutical testing or cancer studies [8].

Here I present recent approaches to the bioprinting scale-up of functional tissue and organ constructs for transplantation, including bioprinting vascularized tissue and organ constructs *in vitro* and *in situ* bioprinting technology to build tissues directly in defect sites. I discuss major roadblocks to this approach and provide potential solutions and future directions.

Bioprinting of vascularized tissue and organ constructs *in vitro*

Organ bioprinting holds great promise for the future, but whole-organ bioprinting has remained elusive due to several limitations associated with biology, bioprinting technology, bioink material, and the post-bioprinting maturation process [9]. The bioprinting of functional tissues is an intermediate stage toward achieving organ-level complexity. *In vitro* fabrication of functional tissues is a sophisticated phenomenon comprising a hierarchical arrangement of multiple cell types, including a multiscale network of vasculature in stroma and parenchyma, along with lymphatic vessels and, occasionally, neural and muscle tissue, depending on the tissue type. *In vitro* engineered tissue models that incorporate all of these components are still far on the horizon. Bioprinting technology offers a great benefit in the hierarchical arrangement of cells or building tissue blocks in a 3D microenvironment, but the bioink and the post-bioprinting maturation phase are as important as the bioprinting process itself. The bioink material is crucial because it should provide the spectrum of biochemical (i.e., chemokines, growth factors, adhesion factors, or signaling proteins) and physical (i.e., interstitial flow, mechanical and structural properties of extracellular matrix) cues to promote an environment for cell survival, motility, and differentiation [10]. In addition, the bioink should exhibit high mechanical integrity and structural stability without dissolving after bioprinting, enable differentiation of autologous stem cells into tissue-specific cell lineages, facilitate engraftment with the endogenous tissue without generating an immune response, demonstrate bioprintability with ease of shear thinning, rapid solidification and formability, and be affordable, abundant, and commercially available with appropriate regulations for clinical use [11]. A variety of bioinks, including naturally and synthetically derived materials, has been used for tissue regeneration, as detailed in the literature [12,13]. The post-bioprinting process is also crucial and necessitates mechanical and chemical stimulation and

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Keywords: bioprinting; bioprinting for transplantation; vascularized-tissue printing; *in situ* bioprinting.

0167-7799/
 http://dx.doi.org/10.1016/j.tibtech.2015.04.005

signaling to regulate tissue remodeling and growth, the development of new bioreactor technologies enabling rapid maturation of tissues, multiscale vascularization for survivability of tissues, and mechanical integrity and innervation for transplantation.

Although several researchers have studied bioprinting of tissue constructs, the fabrication of scale-up tissues with a high volumetric oxygen-consumption rate, such as cardiac, pancreas, or liver tissue, is still a challenge. One major roadblock is associated with the integration of the vascular hierarchical network spanning arteries and veins down to capillaries. To bioprint vascularized thick tissues, highly repeatable and straightforward technologies and protocols should be developed in logical steps, from simple to complex. Since it is difficult to print capillaries at the submicron scale using current technology, an alternative could be to bioprint the macrovasculature and then leave nature to create the capillaries. To this end, two alternative approaches have been considered: (i) indirect bioprinting by utilizing a fugitive ink that is removed by thermally induced decrosslinking, leaving a vascular network behind [14,15]; and (ii) direct bioprinting of a vasculature network in a tubular shape [16–19] (Figure 1).

Several recent attempts have been made to bioprint a fugitive bioink to create vascular channels [15,20–22]. Cell laden hydrogels were used to fabricate the tissue construct, and integration of the vascular network demonstrated increased cell viability inside the construct; regions near channels exhibited significant differences compared with regions away from channels. Although most researchers have attempted to create a vascular network on a macroscale and generate an endothelium lining inside the lumen via gluing endothelial cells through perfusion, Lee *et al.* [21] took one step forward and successfully achieved angiogenesis by sprouting endothelial cells within a fibrin network loaded with other pericyte-like supporting cells (Figure 1A,B). Their study demonstrated that endothelial spouting generated a considerable increase in the permeability of the tissue construct. More advanced angiogenesis and vasculogenesis have already been developed in microfluidic devices; several supporting cells have been attempted and used in cancer metastasis studies [23]. Despite the flexibility in bioprinting channels and the ability to create angiogenesis, this technology still faces major challenges. First, loading cells in hydrogels does not support cell–cell interactions, because those take place *in vivo*, and limited phenotypic stability and activity of cells are observed during prolonged *in vitro* incubation. Second, while fibrin is a suitable environment for angiogenesis because it has a crucial role in blood clotting [24], it is not a convenient environment for tissue-specific cells, such as islets in a pancreas or follicles in a lymph node; a scaffold-free environment should be considered for these. A recent article [25] demonstrated contiguous vascularization of cell aggregates in tumor spheroid models and robust angiogenesis into the fibrin matrix where spheroids were encapsulated, showing the possibility of generating anastomosis of vascular networks of stromal and parenchymal tissues *in vitro*.

The other approach is direct bioprinting of a vascular network via: (i) bioprinting of scaffold-free branched vascular tubes [19] using tissue spheroids as building blocks [26] that are printed inside a mold pattern; and (ii) bioprinting of vasculature using direct extrusion of a tubular network [16–18]. A recent study [3] demonstrated hybrid biofabrication of vasculature in tandem with tissue strands, where fibroblast tissue strands quickly fuse to each other, maturate, and form the tissue around the vasculature (Figure 1C–E). Tissue strands were made scaffold free and used as building blocks to construct the scale-up tissue due to their quick fusion, folding, and maturation capabilities. This approach demonstrated the proof of concept toward larger-scale perfusable tissues; further work is needed to demonstrate a complex vascular network within larger tissues with vascularization on multiple scales that can be envisioned using a Multi-Arm BioPrinter [27]. Although vascularization is important for larger-scale tissue constructs for transplantation, anastomosis to the circulatory system and functionality post-transplantation should also be considered. The vascular network should be designed and bioprinted so that it can be sutured to a vascular network easily, and it should have certain properties, such as enough mechanical properties to satisfy suture retention and burst pressure, sufficient intactness of endothelium to prevent thrombosis, and a high patency rate to support occlusion-free circulation [28]. Compared with indirect bioprinting of a vascular network, the direct bioprinting of vasculature can be more convenient, suturing to the host at the time of implantation.

From *in vitro* to *in situ*: regenerating tissues through direct bioprinting into defect sites

Bioprinting living tissue constructs or cell laden scaffolds *in vitro* has been well studied, and thin tissues or tissues that do not need vascularization, including skin, cartilage, and blood vessels, have been grown [12]. However, *in situ* bioprinting can enable the growth of thick tissues in critical defects with the help of vascularization driven by nature in lesions. Therefore, it is a promising direction for the bioprinting of porous tissue analogs that can engraft with endogenous tissue and regenerate new tissue along with vasculogenesis through the migration of progenitor cells into the tissue construct and sprouting of capillaries from the endogenous tissue.

The idea of *in situ* bioprinting was first proposed by Weiss using inkjet technology [29]; however, translating bioprinters into operating rooms was considered to be challenging due to the perception that surgeons can be considered artists and prefer off-the-shelf solutions, such as using prefabricated tissue constructs and cutting or carving them into a form to be implanted into the defect site. Limited research has been performed on *in situ* bioprinting since Weiss proposed this technology. Inkjet-based bioprinting of skin cells has been tested for burn wounds [30], and laser-assisted bioprinting has been performed to test the feasibility of printing nanohydroxyapatite particles on a mouse model [31]. The idea of bioprinting skin cells (fibroblasts and keratinocytes zonally) has been considered feasible for transitioning the technology to clinical settings, with the hope of repairing major wound defects of soldiers on the battlefield.