Bioprinting towards Organ Fabrication: Challenges and Future Trends

Ibrahim Ozbolat and Yin Yu

Abstract—Tissue engineering has been a promising field of research, offering hope for bridging the gap between organ shortage and transplantation needs. However, building three-dimensional (3D) vascularized organs remains the main technological barrier to be overcome. Organ printing, which is defined as computer-aided additive biofabrication of 3D cellular tissue constructs, has shed light on advancing this field into a new era. Organ printing takes advantage of rapid prototyping (RP) technology to print cells, biomaterials, and cell-laden biomaterials individually or in tandem, layer by layer, directly creating 3D tissue-like structures. Here, we overview RP-based bioprinting approaches and discuss the current challenges and trends towards fabricating living organs for transplant in the near future.

Index Terms—Bioadditive manufacturing, bioprinting, organ fabrication, tissue engineering

I. INTRODUCTION

Organ shortage has become more problematic in spite of an increase in willing donors. From July 2000 to July 2001, for example, approximately 80,000 people in the United States awaited an organ transplant, with less than a third receiving it [1]. The solution to this problem, as with the solutions to other grand engineering challenges, requires long-term solutions by building or manufacturing living organs from a person’s own cells. For the past three decades, tissue engineering has emerged as a multidisciplinary field involving scientists, engineers, and physicians, for the purpose of creating biological substitutes mimicking native tissue to replace damaged tissues or restore malfunctioning organs [2]. The traditional tissue engineering strategy is to seed cells onto scaffolds, which can then direct cell proliferation and differentiation into three-dimensional (3D) functioning tissues. Both synthetic and natural polymers have been used to engineer various tissue grafts like skin, cartilage, bone, and bladder [3-6]. To be successfully used for tissue engineering, these materials must be biocompatible and biodegradable, with the mechanical strength to support cell attachment, proliferation, and direct cell differentiation toward certain lineages. Although significant success has been achieved in the past decades both in research and clinical applications [7], it is obvious that complex 3D organs require more precise multicellular structures with vascular network integration, which cannot be fulfilled by traditional methods.

A computer-aided bioadditive manufacturing process has emerged to deposit living cells together with hydrogel-base scaffolds for 3D tissue and organ fabrication. Bioprinting or direct cell printing is an extension of tissue engineering, as it intends to create de novo organs. It uses bioadditive manufacturing technologies, including laser-based writing [9], inkjet-based printing [10] and extrusion-based deposition [11]. Bioprinting offers great precision on spatial placement of the cells themselves, rather than providing scaffold support alone [12]. Although still in its infancy, this technology appears to be more promising for advancing tissue engineering towards organ fabrication, ultimately mitigating organ shortage and saving lives. Figure 1 demonstrates the concept of futuristic 3D direct organ printing technology, where multiple living cells with the supportive media stored in cartridges are printed layer by layer using inkjet printing technology. It offers a controllable fabrication process, which allows precise placement of various biomaterial and/or cell types simultaneously according to the natural compartments of the target tissue or organs. Multiple cell types, including organ-specific cells and blood vessel cells, i.e., smooth muscle and...
endothelial cells (ECs), constitute the entire organ. Although the concept seems to be trivial considering the complexity and functionality of the parts that can be manufactured using contemporary rapid prototyping (RP) technology [13], several challenges impede the evolution of organ printing. This paper discusses the current state of the art in bioprinting technology, its advantages and limitations, and grand challenges and future trends towards fabrication of living organs in both research and clinical scenarios.

II. BIOPRINTING: CURRENT STATE OF THE ART

Bioprinting, where living cells are precisely printed in a certain pattern, has great potential and promise for fabricating engineered living organs. Based on their working principles, bioprinting systems can be primarily classified as: (1) laser-based, (2) inkjet-based, or (3) extrusion-based.

![Bioprinting Techniques]

Fig. 2: Bioprinting techniques including (A) laser-based writing of cells, (B) ink-jet based systems and (C) extrusion-based deposition.

Laser technology has recently been applied in the cell printing process, in which laser energy is used to excite the cells and give patterns to control spatially the cellular environment. A laser-based system was first introduced in 1999 by Odde et al. to process 2D cell patterning [14]. Laser direct-write (LDW) is a biofabrication method capable of rapidly creating precise patterns of viable cells on petri dishes. In LDW, cells suspended in a solution in donor slides are transferred to a collector slide using laser energy. A laser pulse creates a bubble, and shock waves are generated by the bubble formation, which eventually propel cells towards the collector substrate (see Fig. 2(A)). Micro-scale cell patterning can be achieved through optimizing viscosity of biological material (bioink), laser printing speed, laser energy, and pulse frequency [15]. Writing of multiple cell types is also feasible by selectively propelling different cells to the collector substrate. Laser printing technology is also integrated with scaffold printing, where LDW is performed in tandem with photopolymerization of hydrogels. It basically deposits cells in a certain pattern onto a substrate by a laser beam. This is followed by deposition of hydrogel on top of each layer of cells, and the process is repeated for multiple cycles to get a 3D structure. Nahmias et al. successfully performed hepatocytes patterning in collagen and Matrigel™ using laser-guided 3D cell writing [16]. In their study, three layers of cells and hydrogels were alternately deposited on top of each other, forming a 3D cellular structure. Cell viability and proliferation was well-maintained post-deposition.

Inkjet-based bioprinting was introduced in the early 2000s and built a great foundation for future organ printing technologies. In this technique, living cells are printed in the form of droplets through cartridges instead of seeding them on scaffolds (see Fig. 2(B)). It uses a non-contact reporographic technique that takes digital data from a computer representing tissue or organs, and reproduces it onto a substrate using “bioink” made of cells and biomaterials [10]. Boland et al. used a thermal inkjet printer to successfully fabricate 3D cellular assemblies of bovine aortal ECs with thermosensitive gels [10]. Post-incubation, printed structures showed high cell viability and maintained cell phenotype. Cui and his coworkers [17] applied inkjet printing technology to repair human articular cartilage, showing its promising potential for high-efficiency direct tissue regeneration. Huang and his coworkers [18] developed a bipolar wave-based drop-on-demand jetting. In their studies, cell-encapsulated alginate microspheres were jetted and assembled to create vertically oriented, short, tubular structures [19]. Inkjet-based system allows printing single cells or cell aggregates [20, 21] by controlling process parameters such as cell concentration, drop volume, resolution, nozzle diameter and average diameter of printed cells [22]. Weiss and his coworkers [23] developed a multi-head inkjet-based bioprinting platform for fabricating heterogeneous structures with a concentration gradient changing from the bottom up. Multiple growth factors such as fibrinogen and thrombin and cells were printed with spatial precision in a functionally graded manner into rat calvarial defect in-situ [24]. They demonstrated the feasibility of in-situ printing; however, this technique did not seem to be a practical approach for clinicians due to complex nature of the process in their study [24].

Another bioprinting technique has been introduced for printing living cells and is based on the extrusion of continuous filaments made of biomaterials. It is a combination of a fluid-dispensing system and an automated three-axis robotic system for extrusion and printing, respectively [11]. During printing, biomaterial is dispensed by a pressure-assisted system, under the control of “robots,” resulting in precise deposition of cells encapsulated in the cylindrical filaments of desired 3D structures (see Fig. 2(C)). Wang et al. used a 3D syringe-based bioprinting system to deposit different cells with various biocompatible hydrogels [25, 26]. They used hepatocytes and adipose-derived stromal cells (ADSCs) together with gelatin/chitosan hydrogels to engineer an artificial liver. Sun and his coworkers [27-29] built a multi-
nozzle bioprinting system with the capacity to simultaneously deposit cells and multiple biomaterials. Their rheology study and cell viability assay were performed to investigate mechanical-stress-induced cell damage during the printing process [30]. The results showed that cell viability was influenced by material flow rate, material concentration, dispensing pressure, and nozzle geometry. Their findings can serve as a guideline for future studies and optimization of the deposition system. Kachouie et al. proposed a method using hydrogel-encapsulated cells as tissue units to make a construct with geometric patterns specific to target tissue types [31].

Although bioprinting is a promising way as a methodological interface between tissue and engineering, each technique has its own limitations. Laser-based systems have high resolution and enable precise patterning of living cells, where cells can be maintainedregistry within 5.6±2.5µm to the initial parameter [32]. This resolution, certainly, cannot be achieved by other bioprinting techniques that makes laser-based cell writing as a great potential for micro-cellular features in organs and tissues i.e. micro-vascularate. In the authors’ opinion, prolonged fabrication time, laser shock related thermally and mechanically induced cell deformation, interactions of cell components with light, gravitational and random setting of cells in the precursor solution, limitations in printing in third dimension and the need for photo-crosslinkable biomaterials should be overcome for future developments in laser-based systems [15, 33, 34]. In order to improve resolution further and increase throughput, parameters related to laser pulse characteristics (i.e. pulse duration, wavelength, repetition rate, energy and beam focus diameter), precursor solution properties such as viscosity, thickness and surface tension and substrate properties should be optimized [34]. One-directional propulsion of cells toward the collector substrate (top to bottom) certainly limits development of 3D structures with complex heterocellular architectures. In order to expand this technology in the third dimension, a rotating donor-side carousel leveling system with rotational and linear stages can be developed that allows printing multiple cells in different layers for the development of heterogeneous structures. Similar technology has been recently demonstrated with multi-material stereolithography process [35]. In addition, the bioprinting speed can be improved by increasing the laser pulse rate or integrating multiple laser beams [36].

Inkjet-based systems are versatile and affordable that favors cells encapsulation. For instance, Xu et al. [37] developed a fabrication platform with a different working principle as opposed to traditional inkjet printers, where droplets of crosslinker chemical were deposited onto a suspension of cardiomyocytes and alginate for 3D cardiac pseudo tissue fabrication. In addition, the traditional setup allows integrating multiple print heads easily to deposit multiple cell types, which is one of the pivotal steps in fabricating heterogeneous tissues and organs. The other advantage of inkjet printing is that surfaces where the cells are printed and patterned do not have to be flat that favors cell printing in situ [38]. In general, all other techniques require gentle handling of a flat deposition surface that limits their direct applicability in surgery rooms. Despite their great advantages, inkjet printers suffer from drawbacks including significant cell damage and death as well as cell sedimentation and aggregation due to small orifice diameter that restricts printing cells in high densities (<5x10⁶ cell/ml) [22, 39, 40]. In addition, structural integrity of the printed structures is another obstacle, where the droplets do not fuse into each other easily and the shape of droplets cannot be controlled precisely. Adequate structural integrity is crucial to retain the designed and the fabricated shape.

Extrusion-based systems provide relatively better structural integrity due to continuous deposition of cylindrical struts. It is the most convenient technique in rapidly fabricating 3D porous cellular structures [12]. Although this technology lays foundation for cell patterning for scale-up tissue and organ fabrication technologies, it constitutes several limitations such as shear stress induced cell deformation and limited material selection due to need for rapid encapsulation of cells via gelation. Shear stress on nozzle tip wall induces significant drop in the number of living cells when the cell density is high; however, this can be partially alleviated using optimum process parameters such as biomaterial concentration, nozzle pressure (ideally minimum), nozzle diameter and loaded cell density [41]. Restricted biomaterial selection and low resolution and accuracy brings limited applicability of extrusion-based systems [12, 42]. Besides, sufficiently high viscosity is essential for the biomaterial suspension to overcome surface tension-driven droplet formation and be drawn in form of straight filaments. High viscosity, on the other hand, triggers clogging inside the nozzle tip and should be optimized considering the diameter of the nozzle tip.

Considering prolonged fabrication time for printing scale-up tissues and organs, one of the important disadvantages of encapsulating living cells in biomaterials is that cell-biomaterial suspension needs to be stored considerable time in the material reservoir that compromise cell viability and limits their bioactivity. Thus, a more automated way of loading and ejecting cell-biomaterial suspension is required for scale-up tissue and organ fabrication. Recently, Novogen MMX Bioprinter™ [43] developed the technology that the printer head has both suction and ejection capability enabling automatic loading of suspension through the nozzle tip occasionally instead of loading it manually prior to fabrication. Mechanical strength and structural integrity of the fabricated structures is a common drawback among bioprinting techniques, which mainly use hydrogels due to high water content and biocompatibility that allows permeation of nutrients into and cellular products out of the gel [44]. Water content increases their biocompatibility; however, it deteriorates mechanical properties and processability significantly. Although hydrogels possess unique properties, they are intrinsically weak due to high water content and do not withstand mechanical loading during and after gelation process. Gelation of cell encapsulated hydrogels is a
crosslinking reaction initiated by a light, a chemical or thermal transitions [45]. Photo-crosslinking processes compromise cell viability and pose significant limitations on encapsulation of cells within hydrogels [33]. Crosslinking through thermal transitions limit applicability of hydrogels. Thermal transition initiated crosslinking is not easy to handle after the process while temperature changes can result in rapid degradation of the printed thermogel that does not support cell viability in cell media culture [46]. Chemical crosslinking can compromise cell viability if any abrupt pH changes are observed; however, non-acid involved crosslinking, i.e. sodium alginate, occurs gently under mild conditions and at room temperature without producing any toxic components, which has a great potential for tissue engineering [47]. So, new hydrogels should be tailored to enhance mechanical properties and processability for specific bioprinting techniques towards advanced tissue and organ fabrication. In addition, these materials should have the ability to withstand sterilization while sterile conditions certainly needs to be acquired for process safety.

In general, cell encapsulation in biomaterial allows cell patterning that has a great potential for direct organ printing; however, subsequent extracellular matrix (ECM) formation, digestion, and degradation of biomaterial matrix and proliferation of encapsulated cells are not trivial to control. There are still intrinsic limitations for bioprinting due to limited cell proliferation and colonization while cells are immobilized within hydrogels, and do not spread, stretch, and migrate to generate the new tissue. Most recently, a new concept was introduced by Mironov and his coworkers [48] and has great potential in overcoming issues with cell encapsulation within hydrogels. They proposed tissue spheroids as building blocks for organ printing, where tissue spheroids direct self-assembly toward organ fabrication. Although tissue spheroids have been around for a long time in demonstrating cellular fusion and tissue formation, where hang-drop culture is one of the most common techniques [49], bioprinting and assembling them in hydrogel media has brought a significant potential for tissue engineering.

Cell aggregates or cell-laden hydrogels as building blocks at the microscale are selectively deposited to create larger tissues [48, 50, 51]. In this approach, these “minitissues” are considered as a biomaterial with certain measurable and controllable properties and are assembled into tissues with specific features through layer-by-layer stacking [52] or direct assembly [48, 53]. Figure 3 shows hanging drop cultures, where each spheroid contains 20,000 chondrocytes, and fusion between spheroids was observed seven days after harvesting, resulting in a larger-scale cartilage tissue. This shows a great promise in obtaining larger-scale cartilage formation, where researchers have already demonstrated regeneration of cartilage tissue on rabbit knees with comparable properties with that of natural cartilage tissue [42]. Tissue spheroids in that study eliminated or minimized inclusion of biomaterials and hence tissue regeneration achieved without in need of scaffolds. In general, this also reduces complications related with degradation of biomaterials and resulted toxic byproducts. In addition, large scale tissues can be easily obtained by fusion process, where cells in cell/laden hydrogels cannot easily fuse [48]. By taking advantage of tissue spheroids, the time needed for tissue maturation can be significantly reduced, since each tissue spheroid contains a large number of cells that can be printed at once. Moreover, cell viability is enhanced due to the large cell seeding density and less mechanical stress experienced compared with direct cell manipulation [48]. Researchers have been investigating means to reduce the actual size of tissue spheroids which is around 500μm because the current size restricts biomanufacturing of smaller-scale features considering the average size of tissue spheroid as the resolution of the fabrication technology. In addition, more efficient and economical ways of fabricating tissue spheroids are also under investigation. Iwasaki et al. introduced a new technique using micro-patterned tissue culture plates for mass fabrication of spheroids that has the potential for rapid scale-up organ fabrication technology [54].

By using tissue spheroids, Forcaglia and his coworkers at the University of Missouri, Columbia, used RP-based bioprinting together with multicellular spheroids made from smooth muscle cells and fibroblasts, producing scaffold-free vascular constructs [55]. Figure 4 illustrates vascular construct printing, where tissue spheroids are printed sequentially in cylindrical filaments from the bottom up. Upon fusion of tissue spheroids followed by a tissue maturation process of three days post-printing, the support material is pulled away manually to generate the lumen. Multiple cell types, including human umbilical vein smooth muscle cells and human skin fibroblast cells, were printed together to fabricate multicellular...
constructs. The bioprinting platform used in this study, the Novogen MMX Bioprinter™ [43], has been recently commercialized and specializes in developing bioprinting across a broad array of cell types to create functional 3D tissues. When building large-scale organ structures, the mechanical integrity of the printed structures as well as the integration of the vascular network with the rest of the organ seems to be the major challenges to expanding the technology for further applications.

III. ORGAN PRINTING AND ITS CHALLENGES

Organ printing is a computer-aided process in which cells and/or cell-laden biomaterials are placed in the form of aggregates, which then serve as building blocks and are further assembled into a 3D functional organ. It’s an automated approach that offers a pathway for scalable, reproducible mass production of engineered living organs where multiple cell types can be positioned precisely to mimic their natural counterparts. Developing a functional organ requires advances in and integration of three types of technology [56]: (1) cell technology, which addresses the procurement of functional cells at the level needed for clinical applications, (2) biomanufacturing technology, which involves combining the cells with biomaterials in a functional 3D configuration, and (3) technologies for in vivo integration, which addresses the issue of biomanufactured construct immune acceptance, in vivo safety and efficacy, and monitoring of construct integrity and function post-implantation. Success in fabrication of functional organs highly depends on advancements in stem cell technology. Stem cells, which are found in several tissues in the human body [57], can self-renew to produce more stem cells and differentiate into diverse specialized cell types to form various organs [58]. A variety of cell types can be used for this application, such as embryonic stem cells (ESCs) [57], adult stem cells (ASCs) [59], most recently, induced pluripotent stem (iPS) cells [60], and tissue-specific cell lines [61, 62]. Although a patient’s stem cells can be differentiated into organ-specific cells for organ printing, there is still risk of tissue rejection by the receiver [56]. Stem cell behaviors can even change during the bioprinting process. In addition, organ fabrication necessitates various types of organ-specific cells, which is not currently feasible considering the current isolation and differentiation technologies. Although stem cells offer great promise as an unlimited source of cells, a greater understanding of and control over the differentiation process is required in order to generate expandable organ-specific cells in consistent quality with the desired phenotype. In this way, rejection by the recipient side will be minimized post-transplantation. Moreover, imaging modalities such as computed tomography (CT), positron emission tomography (PET), and nuclear magnetic resonance (NMR) imaging should be used to monitor the transplanted organ noninvasively; NMR offers a unique advantage in monitoring organ integrity and function without the need to modify the cells genetically [63]. Although cell and transplantation technology plays a crucial role in organ fabrication, this review focuses on biomanufacturing technology, and the rest of the paper discusses major challenges in the context of bioprinting towards organ fabrication.

Despite the progress in tissue engineering, several challenges must be addressed for organ printing to become a reality. The most critical challenge in organ printing is the integration of a vascular network, which is also a problem that the majority of tissue engineering technologies are facing. Without vascularization, engineered 3D thick tissue or organs cannot get enough nutrients, gas exchange, and waste removal, all of which are needed for maturation during perfusion. This results in low cell viability and malfunction of artificial organs. Systems must be developed to transport nutrients, growth factors, and oxygen to cells while extracting metabolic waste products such as lactic acid, carbon dioxide, and hydrogen ions so the cells can grow and fuse together, forming the organ. Cells in a large 3D organ structure cannot maintain their metabolic functions without vascularization, which is traditionally provided by blood vessels. Bioprinting technology, on the other hand, currently does not allow multiscale tissue fabrication where bifurcated vessels are required to be manufactured with capillaries to mimic natural vascular anatomy. Although several researchers have investigated developing vascular trees using computer models [64], only a few attempts have been made toward fabricating bifurcated or branched channels [55]. Successful maturation towards functionally mechanically integrated bifurcated vessels is still a challenge.

In order to closely mimic natural organs, 3D vascularized organs need to be fabricated using heterocellular aggregates, which was discussed extensively in the literature [48]. In that study, intraorgan vascular branched networks are envisioned to be printed and matured with the rest of the organ. Instead of biomimetically designing and fabricating a vascular tree, which seems to be one of the impediments down the road for organ printing, we alternatively propose printable semipermeable microfluidic channels to mimic a vascular network in perfusing media and facilitating oxygenation for cell viability, coaxing tissue maturation and formation.

Figure 5 illustrates our conceptual model of organ printing through integration of vessel-like micro-fluidic channels with cellular assembly, which has been featured in the literature [1, 65]. The concept allows constructing structures with micro-fluidic channels acting like blood vessels, allowing media perfusion through an extracellular matrix that can support cell viability in 3D. In Fig. 5, vessel-like micro-fluidic channels are printed in a 0°–90° lay-down pattern to develop 3D structures. Oxygenized perfusion media can be pumped into channels for circulation purposes. Round ends are considered for the zigzag deposition pattern to prevent blockage of media flow. After parallel printing of each micro-fluidic channel layer, cellular spheroids, i.e., tissue spheroids or cell encapsulated microspheres, can be deposited between fluidic channels in the form of droplets using another robotic arm; semipermeable fluidic channels allow transport and diffusion of media to the cellular environment. The proposed strategy enables printing semi-permeable micro-fluidic channels in tandem with printing cellular assembly. Concurrent printing has the potential to
reduce the fabrication time, which is crucial for the development of scale-up technologies (see Fig. 7(C)).

Fig. 5: Concept of 3D organ-printing technology where vessel-like microfluidic channels are printed in tandem with tissue spheroids layer by layer. The printed structure can be then connected to a bioreactor for media perfusion (figures are not to scale).

Advantages of tissue spheroids have already been discussed in Section 2; spheroids significantly enhance cell viability of stem-cell-derived organ-specific cells, allowing the development of further organ fabrication techniques in the near future. Printing encapsulated cells in spheroids also greatly reduces shear-stress-induced cell damage compared to printing cells directly loaded within biomaterial media. In general, cells are subjected to shear stress during the printing process, and cell injury and DNA damage should be minimized. The proposed concept can also allow inclusion of multiple cell types in a spatially organized way by integrating another printer unit mounted on the robotic arm to print a secondary type of spheroids precisely.

Fig. 6: Printed cellular microfluidic channels: (A) perfusion of oxygenized cell type media, (B) media flow with intentionally generated air bubbles and (C) the laser confocal image of the mid-plane showing single lumen channel with live/dead staining where CPCs are labeled with calcein AM and ethidium homodimer.

Figure 6 illustrates oxygenized media perfusion through a printed cellular micro-fluidic channel 44cm in length and 1mm wide with a 500µm lumen diameter. The cell media is circulated through the channel without any blockage or swirling, which shows a great potential for developing embedded channels and serving as a vascular network for thick tissue fabrication. Direct printing of these channels allows them to be integrated within a hybrid bioprinting platform and also facilitates patterning them into very complex shapes. Currently, mechanobiological properties of printed channels are under investigation, and electrospun nanofiber reinforcement is performed to match the mechanical properties with that of blood vessels. Elasticity and tensile strength are essential to be biomimetically mediated. Viability of cartilage progenitor cells (CPCs) encapsulated within printed microfluidic channels showed 97.6±1.2% one day post-printing and maintained high cell viability on day 4, with a percentage of 95.8±1.2 where living cells are shown around the lumen on the cut-away view (see Fig. 6 (C)).

It should be ensured that the printed organ functions correctly. For example, a bioprinted pancreatic organ must be able to produce and secrete insulin just like its natural counterpart. Thus, multiple organ-specific cell types are required to be spatially organized to form the complex architecture of an organ. Advancements in bioprinting processes to precisely and selectively place cells, are promising when it comes to achieving heterocellular architectures. Figure 7 illustrates some of the bioprinters that can facilitate printing heterocellular structures, including EnvisionTEC 3D Bioplotter® (where solid filaments are printed in a secondary plotting media), Novogen MMX Bioprinter® (which allows printing heterocellular tissue aggregates and hydrogels as support material), and the Multi-Arm BioPrinter (MABP) (which can print tissue spheroids in tandem with vessel-like microfluidic channels). These technologies can be applied for cellular aggregate biofabrication in such a way as to facilitate self-assembly of the tissue spheroids to mimic the natural process of tissue and organ morphogenesis.

A

Fig. 7: Bioprinters: (A) 3D Bioplotter® (designed by envisionTEC GmbH, Gladbeck, Germany [66]), (B) Novogen MMX Bioprinter™ (designed by Organovo, San Diego, California, USA [43]) and (C) Multi-Arm BioPrinter—“MABP” (designed by the University of Iowa, Iowa City, Iowa, USA).

In order to fabricate scalable organs such as a mouse liver which has 1.3x10⁸ cells per gram [67], the bioprinter needs to run for several hours considering the resolution of the system as in microscale. During prolonged fabrication time, it should deliver biological substances through micro-nozzle or other means without clogging problems or collision issues between the nozzle tip and the printed structure. In addition, sterilization is also crucial during bioprinting and necessitates construction of bioprinters on a small scale to fit in standard biosafety cabinets. In general, commercial bioprinters can cost...
substantially high around $100-200k depending on their unique capabilities, where home-made bioprinters on the other hand roughly cost less than quarter of their commercial counterparts.

Another challenging site for organ printing technology is the rapid or accelerated tissue maturation process, where printed organ constructs should be rapidly fused, remodeled, and maturated toward a solid construct, ensuring mechanical rigidity for transplantation. Collagen and elastin are some of the proteins in the extracellular matrix of human organs, and they are abundant in the connective tissue stromal of parenchymal organs [68, 69]. Thus, production and deposition of these proteins during tissue maturation are essential to enhancing the mechanical properties of the printed organs.

After the organ-printing process, the fabricated structure needs to be transferred to the bioreactor. Bioreactors such as an irrigation dripping perfusion bioreactor can be used to expedite the tissue maturation and organ formation process, providing an optimal environment. The transfer of a printed organ to the bioreactor should be performed gently without inducing any vibration that can degenerate the integrity of fragile gel-based structures. Thus, future bioprinters can be enclosed in a bioreactor system that will allow direct and rapid connection of printed structures to perfusion channels.

IV. PROPOSED ENVISIONED FUTURE

Considering the pathway from isolation of stem cells to transplantation into a human, seamlessly automated protocols and systems are essential for customized functional organ fabrication. This pathway includes (i) blueprint modeling of an organ with its vascular architecture, (ii) generation of a process plan for bioprinting, (iii) isolation of stem cells, (iv) differentiation of stem cells into organ-specific cells, (v) preparation and loading of organ-specific cells and blood vessel cells as well as support medium, and (vi) bioprinting process followed by organogenesis in a bioreactor for transplantation. In order to accelerate the entire process, one can envision a bioreactor enclosing a bioprinter that will immediately facilitate fusion of tissues and keep the printed organ until desired maturation is achieved. In this way, a customized and automated system will facilitate delivering artificial organs to transplant patients in a reasonable amount of time, preferably at earlier stages of their diseases when patients are healthier so that they are better able to withstand surgical interventions.

Miniature organs can be considered a future trend in organ printing and might be a transition towards fully functioning organs. Miniature organs, which can also be called “factory in the human body”, can be built in smaller scale than their natural counterparts and closely perform the most vital function of the associated organ, such as a pancreatic organ that can produce and secrete insulin in substantial amounts to regulate the glucose level to normoglycemia in the human body. The miniature organ can be transplanted and placed in less immune-responsive sites in the human body as an extravascular device or attached to blood vessels as an intravascular device to secrete insulin to the bloodstream. Although the pancreas serves two functions, which are carried out by two different cell groups within the organ, a patient with diabetes will be interested in correcting hyperglycemia through fabrication and transplantation of a pancreatic organ that can restore the function of the endocrine portions of the pancreas, which makes only about 2% of pancreas cells. The endocrine portion is made of approximately a million cell clusters called islets of Langerhans [70]. Four main cell types exist in the islets. α, β, delta and gamma cells secrete glucagon, insulin, somatostatin (regulates/stops α and β cells) and pancreatic polypeptide, respectively. Miniature organs can also be designed and fabricated to bring new functionalities and superiorities in the human body rather than restoring the functionality of their natural counterparts, such as living organs that can continuously generate electricity to eliminate the use of batteries for internal devices such as peace makers.

Another future trend in organ printing is in-situ printing, where living organs can be printed in the human body during operations. Currently, in-situ bioprinting has already been tested for repairing external organs such as skin [38], where the wounded section is filled with multiple cells, including human keratinocytes and fibroblast, with stratified zones throughout the wound bed. A transitional approach seems to be logical to advance the state of the art through printing and repairing partially damaged, diseased, or malfunctioning internal organs that do not have self-repair characteristics, such as the liver. With the recent advancements in robot-assisted surgery, computer-controlled robotic bioprinters will lead the evolution of this technology in the very near future.

V. CONCLUSIONS

This paper discusses the current state of the art in bioprinting with recent trends in organ printing technology towards fabrication of living organs for transplant, where prototype organs can be developed using layer-by-layer technology in the very near future. Although the technology shows a great deal of promise, there is still a long way to go to practically realize this ambitious vision. Overcoming current impediments in cell technology, biomanufacturing technology, and technologies for in-vivo integration is essential for developing seamlessly automated technology from stem cell isolation to transplantation.

ACKNOWLEDGMENT

The authors would like to thank A. Lehman from the Ignacio Ponseti Orthopaedic Cell Biology Lab (The University of Iowa) for the fabrication of chondrocyte spheroids.

REFERENCES


K. W. Binder, "In Situ Bioprinting of the Skin," PhD, Molecular Genetics and Genomics, Wake Forest University, Winston-Salem, North Carolina, 2011.


