PERSPECTIVE

Bioprinting living organs: The next milestone in organ transplantation?

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Organ failure remains a harsh problem, leading to millions of patients waiting on the transplantation list. Tissue engineering (TE) organ grafts hold great potential to cover the huge demand for organ transplantation. Combining with the advances in developmental biology, material science, and engineering, the emerging bioprinting technology enables TE grafts to replicate the complexity of human organs, from cell and extracellular matrix (ECM) composites to architecture features. This perspective provides a glimpse of how quickly and profoundly bioprinting can potentially be applied to address problems in TE organ grafts fabrication.

CONTENT

Whole organ transplantation is the gold standard for the treatment of end-stage organ dysfunction. However, it is exceptionally difficult to obtain histocompatibility, resulting in only few selected patients receiving functional organ transplants. As in many other countries, there are a huge number of patients on the national waitlist for organ transplantation. According to the recently released Report on Organ Transplantation Development in China, from 2015 to 2020, the organ donation rate per million people (PMP) increased from 2.01 to 3.70, with a total donation of up to 29334 cases.[1] While approximately 15-fold more patients are waiting for proper histocompatibility matches.

Organ transplantation attempts can be traced back to the 18th century. In July 1883, Swiss surgeon Theodor Kocher transplanted thyroid tissue into a patient with radical thyroidectomy.[2] As an attempt at curing a complex internal disease by replacing an organ, this operation constituted the first organ transplant in the modern sense and was awarded the Nobel Prize for Physiology or Medicine in 1909. Then, kidney (1954), heart (1967), and lung (1983) transplantation, et al., continually succeeded, owing to the development of surgery techniques (e.g., blood vessel suture) and immune rejection suppression drugs (e.g., ciclosporin). Due to the large gap between organ demand and donation, xenotransplantation was taken into consideration. After one century of unremitting efforts, in January 2022, an exciting milestone was reached: the first porcine to human cardiac xenotransplantation was done.[3,4] The donor pig contained 10 genetic modifications to avoid immune rejection and growth arrest to match the anatomy of human heart size. The heart functioned well for 49 days post-transplantation but unfortunately failed after 60 days. Despite multiple gene modifications, the heterogenous materials (e.g., cellular secretomes) might also trigger the scattered myocyte necrosis, interstitial edema, et al., which were observed from the histologic examination.

Allografts and xenografts represent the natural solutions for organ failure; however, the insufficient supply, lifelong immune rejection, and as-yet-unknown heterogenous-related risks leave organ failure treatment remains intractable. Compared to the natural solutions, the artificial organ stands for the ultimate pursuit, possessing precise design to replace the failed organ, and even with enhanced functions, to regain normal life instead of just being alive. The Si-Fi concept is coming into reality due to progress in TE and three-dimensional (3D) bioprinting. From cell type diversity to irregular structures, human organs exhibit micro-macro complexity, which is unable to replicate with traditional TE methods. While, through anatomical-guided patterns, cellular-ECM simulated multi-inks, and sophistically controlled equipment (based on extrusion, stereolithography, et al.), 3D bioprinting holds enormous potential to construct tissue engineered human organ grafts (Figure 1).

Recently, scientists have made great efforts to expand the frontiers of 3D bioprinting, and several breaking-throughs have been achieved. Heart failure is the leading cause of death, encouraging researchers to fabricate cardiac substitutes. Whereas, the traditional TE constructs are only capable of providing heart shape, difficult to introduce functional cells and blood vessels simultaneously. In 2019, for the first time, Tal Dvir and colleagues printed a mini human heart (height: 20 mm; diameter: 14 mm) containing cardiomyocytes, endothelial cells, and major blood vessels (Figure 1 I-II).[5] To this end, a two-nozzle extrusion- and Alginate-Xanthan gum supporting bath-based printing technique was applied, and decellularized ECM-derived hydrogel was chosen to prepare the cardiac parenchymal tissue printing ink for further clinical translation considerations. However, besides the size, the printed heart lacked small and capillary vessels, and did not show beating behaviors, which is essential for heart function. In another study, Lee et al., using an updated FRESH (freeform reversible embedding of suspended hydrogels) technique, printed a neonatal-sized human heart (height: 55 mm; diameter: 37 mm) with high-fidelity of outer shape and inner ventricle structures (Figure 1 III-IV).[6] By adopting the gelatin microparticle supporting slurry, the FRESH enabled extrusion 3D printing to fabricate large-sized structures using soft hydrogels, i.e., collagen in the study. Besides, owing to the improved printing resolution, the left anterior descending coronary artery was partially fabricated, and this multiscale vascular network demonstrated well-perfusing capacity. At the same time, a complex vascular network replicating the distal lung tissue and the surrounding air space was printed by Grigoryan and colleagues, applying a stereolithography (SLA) printing technique called SLATE (stereolithographic apparatus for tissue engineering) (Figure 1 V-VI).[7] In this study, the authors identified a food dye additive, i.e., tartrazine, as photoabsorber, yields a high resolution of 50 μm, and most importantly tartrazine is biocompatible, and suitable for bioapplications. Indeed, to further demonstrate the potential of the SLATE technique, the authors fabricated an *in vivo* therapeutic hepatic transplantation. The designed anchor structure enabled hepatocyte aggregates entrapment and facilitated remodeling between the graft and host tissue; the microchannel networks containing human umbilical vein endothelial cells improved tissue engraftment.

The outstanding studies contribute a step forward in 3D bioprinting human organ grafts from the aspects of printing resolution, heterostructures, and vascular complexities. However, it has to admit that 3D bioprinting is still in its infancy, facing many challenges before clinical translation (Figure 1 C). For instance: 1) how to formulate the bioinks? Printing bioinks contain biomaterials, cells, and optimally, biomolecular ingredients. Most ideally, the biomaterial should possess biophysical and chemical properties similar to the native ECM, instructing the functionalization of the encapsulated cells. When applied to extrusion-based bioprinting, the shear-shining characteristic is preferred to maintain cell vitality. Human organ contains billions of cells; thus, it is challenging to prepare such a mountain of immune-compatible cells. Another issue could be the cellular differentiation stage. Do tissue-specific cells superior to stem cells, harnessing the development biology to form the organ post-printing? 2) how to deposit different bioinks precisely? As aforementioned, an organ is a highly integrated system containing various components and heterogenicity, e.g., the parenchymal tissue, the vascular and neural network. All these features require different inks to construct. So far, the most

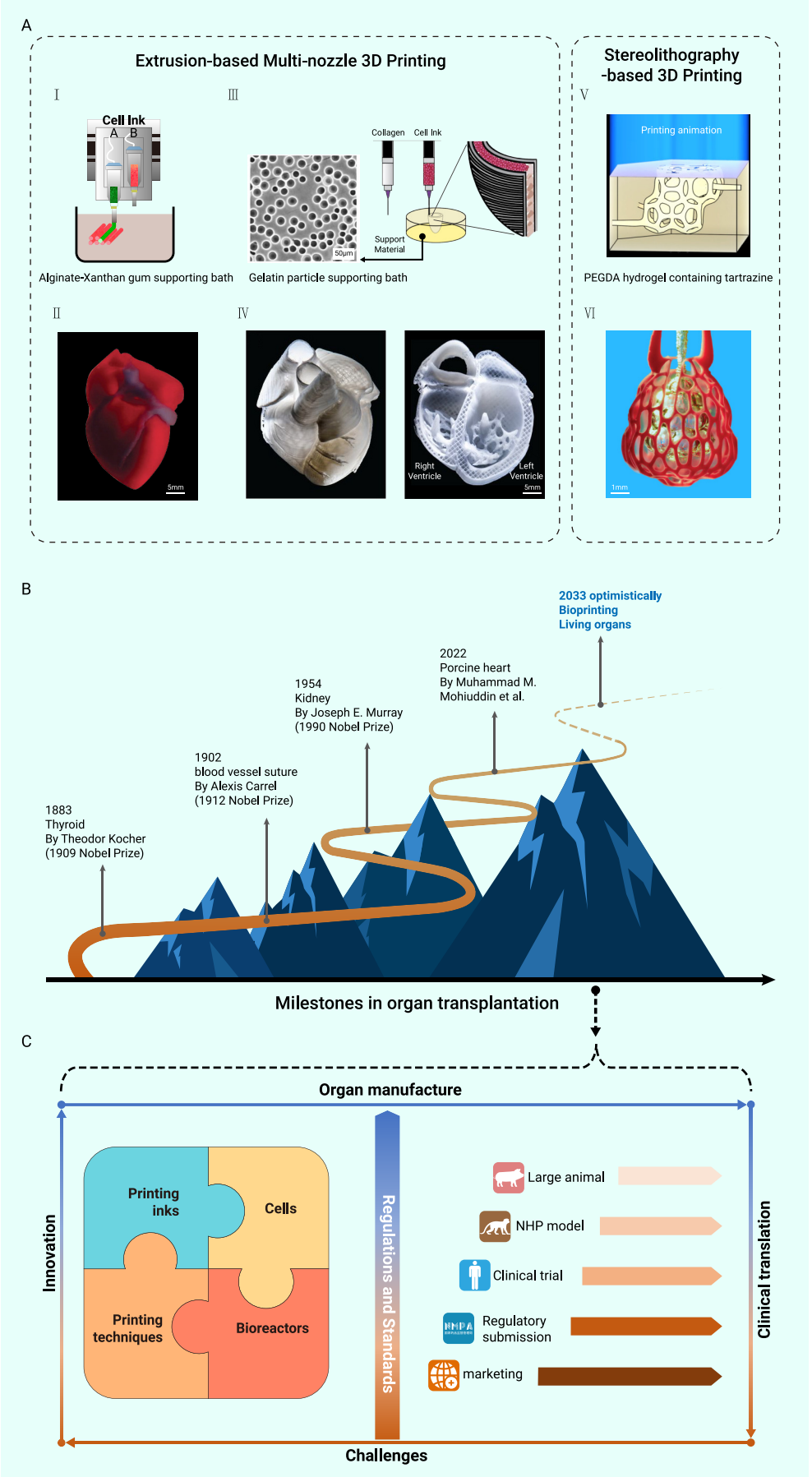

Figure 1. Perspectives of bioprinting living organs (A) Representative progresses of 3D bioprinting organs. Extrusion-based two-channel bioprinting was applied to construct miniaturized (I, II)[5] and neonatal-sized human heart (III, IV)[6] with specialized advancements, respectively; SLA-based bioprinting technique was used to replicate the complex vascular network of distal lung tissue and the surrounding air space (V, VI)[7]. Images are adapted from the indicated references with permission. (B) Do 3D-bioprinted organs be the next milestone in human organ transplantation? (C) The challenges that need to be overcome during organ 3D bioprinting and clinical translation.

commonly used 3D printers are extrusion-based and SLA-based. SLA-based bioprinting can achieve complex structures with high resolution, while, time-consuming. Meanwhile, synergistic printing of cells containing multiple-bioinks with SLA remains unresolved. Vice versa for extrusion-based bioprinting, using nuzzle arrays, it is convenient to pattern different inks, and possibly, dynamic inks from one nuzzle via connecting to several inlets, mimicking the continuous gradient features. Resolution is a hurdle for extrusion bioprinting, as thinning filaments decrease cell vitality. Ideally, a combination of extrusion- and SLA-based bioprinting could advance human organ fabrication. 3) how the organ graft exerts functions? on the one hand, during fabrication, the vascularization and innervation should not be stayed out of focus, which are crucial in maintaining and regulating cellular homeostasis; On the other hand, in order to quickly integrate into the host physiological system, the *in vitro* maturation of the fabricated organ should be needed. 4) how to manage the bioprinted organs' clinical transfer? Owning to its complexity of bio-components and working mechanisms, a whole set of regulatory rules and standards should be established accordingly. Besides, the definition of bioprinted organs, e.g., human organs or devices, should be considered.

Collectively, although "the 3D bioprinting of a fully functional organ is yet to be achieved, scientists now have the ability to build constructs that start to recapitulate the structural, mechanical, and biological properties of native tissues".[6] Taking bone tissue as an example, through two-channel bioprinting, we constructed a bone graft with remarkable mechanical and osteogenic performances, owing to the polyethylene glycol diacrylate/nanoclay frame and the inner hyaluronic acid/osteoblast bioactive network.[8] Further, to replicate the bony hierarchical feature, inspired by a typical fractal structure of Koch snowflake, we recently created a design-to-fabrication workflow by embedding the graded data on the basis of fractal design and then constructed a bone structure containing a controllable porous gradient in the radial direction via extrusion 3D printing, representing the cortical to cancellous bone features.[9] Thus, in the near future, the clinical breakthroughs of 3D bioprinting are expected to play a leading role in less complex tissues and organs, such as bone, cartilage, skin, and bladder, among which tissue-engineered bladder has been translated into the clinic.[10]

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DECLARATION OF INTERESTS

The authors declare no competing interests.