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3D Printing of Organs for Transplantation: Where Are We and Where Are We Heading?

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Abstract In the field of transplantation, the demand for organs continues to increase and has far outpaced the supply. This ever-growing unmet need for organs calls for innovative solutions in order to save more lives. The development of new technologies in the field of biomedical engineering might be able to provide some solutions. With the advent of 3D bioprinting, the potential development of tissues or organ grafts from autologous cells might be within the reach in the near future. Based on the technology and platform used for regular 3D printing, 3D bioprinters have the ability to create biologically functional tissues by dispensing layer after layer of bioink and biogel that if left to mature with the proper environment will produce a functional tissue copy with normal metabolic activity. In the present day, 3D-bioprinted bladders, tracheal grafts, bone, and cartilage have proven to be functional after development and implantation in animal models and humans. Promising ongoing projects in different institutions around the world are focused on the development of 3D-bioprinted organs such as the livers and kidneys with integrated vasculature, in order for the tissue to be able to thrive once it has been transplanted. This review focuses on the background, the present, and the future of 3D bioprinting and its potential role in transplantation.

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Introduction

Since the dawn of the era of transplantation more than five decades ago, the demand for organs has been rapidly increasing and has outpaced the supply. This subsequently has dramatically increased the waitlist for transplantation. There are many factors that cause this increased wait time, from the inadequate number of donors, the allocation parameters for organs based on geographical locations, to the quality of available organs given the projections of very modest growth in donor numbers, and increasing donor age and comorbidities. The latter point remains a challenge as mortality in the waitlist continues to increase [1, 2].

In the year 2015, more than 120,000 patients alone in the USA are on the waiting list for organ transplantation. However, only ~ 18,000 patients have received a transplant in the first 6 months of the year, this represents only 12.5 % of the patients on the original donor list. Many others who did not receive a transplant during this time have died of organ failure while waiting [1].

However, the development of new technologies and the fast growth of biomedical engineering have helped to develop many potential new options that might provide solutions for the transplant crisis. This new field has the anachronisms of either organ engineering and/or regenerative medicine. Within organ engineering, there have been a lot of advances in the last decade, among them, is three-dimensional bioprinting (3D bioprinting).

3D bioprinting can be defined as the use of a technology or technique for the purpose of precise positioning of layers of cells and biological materials to support them in a three-dimensional fashion in order to resemble or replicate a functional tissue or

organ [3••]. The goal of 3D bioprinting is to reproduce a functioning tissue or organ with its natural microenvironment and architecture that can mimic the original organ and eventually can be used to replace or assist the organ. At present, a variety of different 3D bioprinting concepts have been developed, among the best known are: *Bioink 3D printing*, *Biomimicry*, and *Autonomous self-assembly* [3••]. For the purpose of the present article, we will focus on Bioink 3D printing.

In the field of transplantation, the use of 3D bioprinting promises great opportunities for the development of different biological structures, from the creation of small vessels that can be used for vascular replacement grafts to the potential creation of implantable functional organs created from the cells of the recipient, thus eliminating the risk of organ rejection. These printed organs can initially act as assist organs to increase the quality of life of the recipient and eventually over time could become viable replacements.

In the present article, we will review both current and potential new novel 3D bioprinting applications in the field of medicine and will address the present challenges associated with the implementation of the technology along with future challenges in the field that will need to be overcome prior to regular use of this technology.

Background

3D printing was born in the 1980s, when Charles Hull invented stereolithography [4]. Stereolithography is a type of printing (Fig. 1) where a laser is used to solidify a polymer material extruded from a needle to form a solid 3D structure.

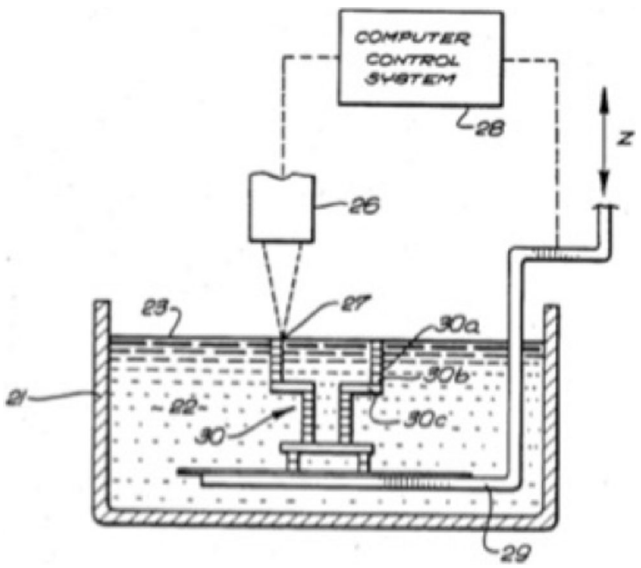

Fig. 1 Hull's stereolithography process. Illustration from the original patent demonstrating the block diagram, schematic, and elevational sectional view for practicing the invention. (From Hull [4])

The instructions for the design came from computer software where a preset 3D shape order was sent to the printer [4].

In the following decade, biomedical scientists began to realize the potential for 3D printing applications in the biosciences. In 1999, the group lead by Atala and colleagues at Wake Forest Institute for Regenerative Medicine was able to devise a 3D printer with the purpose of creating a synthetic biodegradable 3D scaffold of a human bladder with seeded autologous urothelial and muscle cells [5]. This is one of the most characteristic examples of how 3D bioprinting started to gain shape and had a direct translational application.

In the present day, with the development of imaging technologies, the ability to reproduce an organ in a 3D digital format with CT, MRI, or ultrasound leads to the idea of creation of synthetic organs such as 3D-printed hepatic models with the primary purpose of reproducing the internal (vascular and biliary) anatomy of the liver and evaluate the volumes of the liver (Fig. 2). This innovative idea emerged to address the need for an ideal pre-operative evaluation of patients that are candidates for living donor liver transplantation (LDLT) [6]. The basic idea was to transfer the digitized information of the CT or MRI imaging to a standard 3D printer and print a liver that would resemble that of the donor graft. These models have already provided assistance for a global evaluation of the liver and minimize intraoperative complications [6]. Collaborators from the Yale School of Medicine and the Center for Engineering Innovation and Design (CEID) at Yale have developed 3D plastic 1:1 scale models of the knee and other articular structures with the goal to aid the orthopedic surgeons to prepare for complicated surgeries in order to have a better planning of the procedures [7]. Now, in our institution, as well as others around the world, the next step is being taken, from printing 3D anatomical models (Fig. 3) to developing 3D functional tissues and organs based on the same principles of 3D printing.

3D Bioprinting Fundamentals

3D bioprinting consists of a set of techniques that transfer biologically active materials onto a substrate [8]. First of all, it is important to mention the basic components of a 3D bioprinter. (1) The printer head mount consists of a metal plate where the print heads are attached and remotely controlled by a motor or series of motors along the x, y, and z axes. (Fig. 4) (2) The print heads (either glass capillary or syringe shaped) are where the (3) bioink (biomaterial composed of living cells intended to create the 3D structure) or (4) biogel (extracellular matrix fluid/gel to support the cells) is contained and released. (5) The printing platform provides a flat surface where the petri dish or biogel container is placed to support the new structure and will be positioned in the center of the platform by motors in the x and y axes in order for the print heads to extrude the

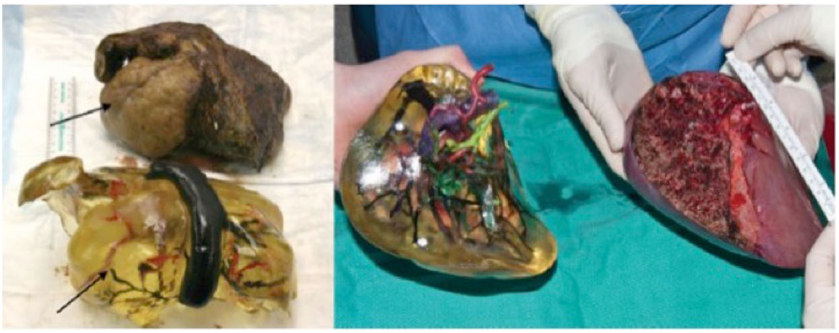

Fig. 2 Preoperatively 3D printer liver and actually explanted liver of a recipient (*left*). Preoperatively 3D-printed right lobe and actual right lobe of a donor. (From Zein, N.N., et al. with permission from Wiley) [6]

bioink or biogel for 3D printing. (6) The reservoir is where the bioink or biogel is contained inside the printer heads. Some printers instead use individual cartridges or needles that are loaded onto the printer heads to inject/dispense the bioink or biogel into the petri dish or container [3••, 9].

The 3D bioprinting process can be achieved by three different printer modalities based on the current technologies, know as *micro-extrusion bioprinting*, *inkjet bioprinting*, or *laser bioprinting* as described in one of the most well-known manuscripts on 3D bioprinting by Murphy and Atala [3••]. They can vary in the biological materials used for printing, the way the material is dispensed, and the resolution and detail of the structure [3••]. Each of these technologies is described in detail in the next section based on Murphy's descriptions.

- *Micro-extrusion Bioprinting*—This type of 3D bioprinting is characterized by a temperature-controlled biomaterial dispensing system. It is based on the use of a standard 3D printing set-up with printer heads capable of moving in the x, y, and z axes, a fiber-optic light-illuminated deposition area for photo-initiator activator and a piezoelectric humidifier. This system generates continuous beads of biomaterial that is deposited in two dimensions, with subsequent layers placed by moving on the 3 axis sequentially then move higher in y axis with a final product that resembles the original as a three-dimensional structure. The process is guided by computational software for exact positioning of each of the heads to guide the extrusion of the printing material. Micro-extrusion printers have proven valuable for the creation of aortic valves and vascular structures. The materials used are cell spheroids (small capsules where cells can grow), high viscosity hydrogels, and biocompatible co-polymers [3••, 10].
- *Inkjet bioprinting*—Inkjet printers work by either thermal or acoustic forces that are able to eject the liquid-containing cells onto the scaffold or a biogel base where the structure will be created. *Thermal inkjet printers* use electricity to heat the print head to produce pulses of pressure that stimulate the droplets from the nozzle of the print head to fall into the biogel. The advantages of thermal inkjet printers are high print speed, low cost, and wide availability. The *acoustic inkjet bioprinters* contain a piezo-electric crystal that creates an acoustic wave inside the print head that will further stimulate the disposition of

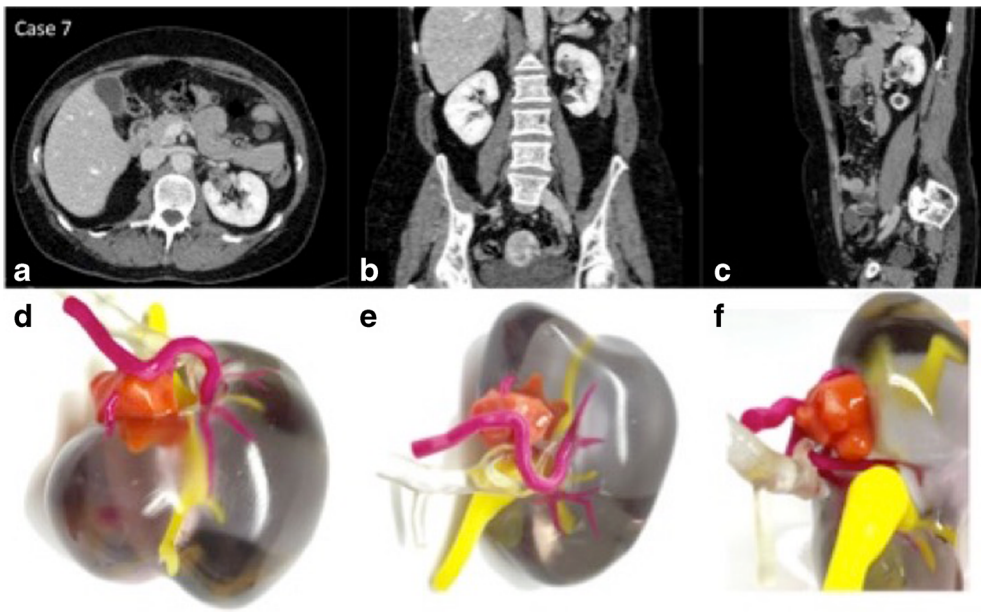

Fig. 3 3D-printed model for a tumor kidney case. (With permission from Springer Science + Business Media: Bernhard JC, Isotani S, Matsugasumi T, et al.: Personalized 3D-printed model of kidney and tumor anatomy: a useful tool for patient education. World Journal Urol 2015. DOI 10.1007/s00345-015-1632-2) [33]

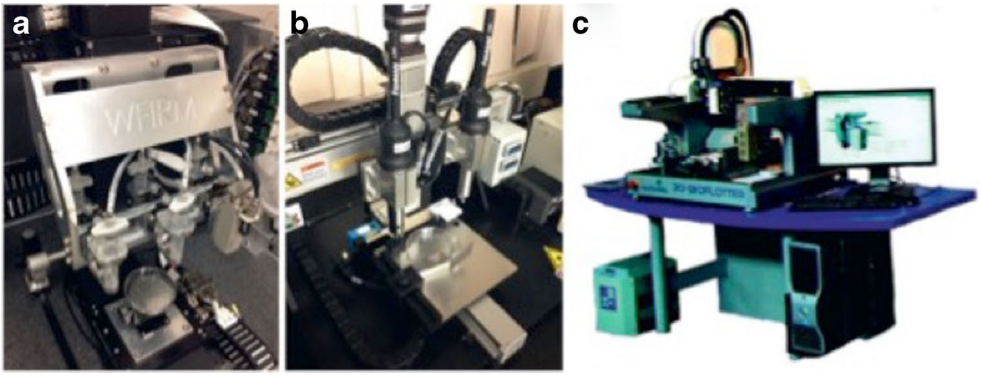

Fig. 4 3D bioprinters around the world. **a** 3D Organ Printer (Designed by Wake Forest Institute for Regenerative Medicine). **b** Commercialized Novogen MMX bioprinter TM (Designed by Organovo Inc). **c** 3D Bioplotter (Designed by Envisiontec, Germany). (From Seol YJ, Kang HW, Lee SJ, et al.) [34]

the cells into the scaffold or biogel base [3••]. The advantages of acoustic inkjet printers include the ability to create and control a uniform droplet size and high droplet directionality. However, damage can be induced to the cell membrane. Inkjet bioprinting is limited to low cell densities to avoid nozzle clogging and reduce shear stress. Inkjet bioprinters have proven value for the regeneration of functional skin and cartilage in situ [10, 11].

- *Laser-assisted Bioprinting*—A Laser-assisted bioprinter (LAB) usually consists of a pulsed laser beam, a focusing system, a ribbon that has donor transport support that is commonly made from glass covered with a laser-energy-absorbing layer (gold or titanium), and a layer of biomaterial containing cells/hydrogel and a receiving substrate facing the ribbon. In LAB, the laser pulse will focus on the absorbing layer to create high-pressure bubbles, which will propel cells toward the receiving substrate plate. LAB is compatible with wide range of biomaterial viscosities. LAB can produce high-resolution 3D structures with high cell viability. High cost of a LAB device has prevented further basic research in the field [3••].

The 3D Bioprinting Process

The layer-by-layer basic fabrication of 3D printing is as follows:

1. *Pre-Processing*—Generation of a computerized design of the structure—blue print
2. *Processing*
   (a) *A layer of hydrogel* (extracellular matrix) is printed or preset in a petri dish or container, which will function as the foundation base for the printed tissue.
   (b) *Bioink*—usually made out of tissue spheroids or cultured cells. This bioink is loaded into the printer heads and disposed on the layer of hydrogel.
   (c) Dispensing bioink or hydrogel repeatedly until many layers are formed.
   (d) As layers are built, the deposited bioink will fuse together forming a 3D structure containing cells and hydrogel.
3. Maturation—Printed structure is then placed in an incubator and left to mature for 24 hours—1 week (depending on the materials used) [12]

The printed tissue can then be used in pharmaceutical drug testing or implantation in animal or in vitro models. In the future, hopefully, these printed tissues can be use for human transplantation.

Present Success in 3D-Printed Tissues and Organs

As of the present day, this technology has been studied in academia, research institutions, and by biotechnology firms (e.g., Organovo Holdings Inc., San Diego, CA, USA) for possible use in tissue engineering applications, where tissues, organs, and body parts are built using 3D bioprinting techniques with the purpose of basic experimentation or the eventual development of implantable organs and tissues.

The use of 3D bioprinting has already resulted in the successful printing of blood vessels and vascular networks [13], the bones [14], cartilage [15], ears [16], and tracheal grafts [17]. In 2014, Bon Verweij and collaborators at the Utrecht Medical Center were able to replace a complete skull and successfully implant a 3D-printed synthetic component into a patient, with no adverse effects. Although this skull graft was synthetic, it demonstrates the feasibility for implants to be custom-tailored for the patient in need, with the potential of the eventual creation of a biological skull graft [18]. The use of 3D bioprinting in ophthalmology is currently limited, but the potential is clear for the generation of ocular tissues (e.g., conjunctiva, sclera, and corneas) using 3D-bioprinting technologies in the future.

One of the greatest success stories written so far in the field of bioengineering and the creation of three-dimensional

organs was done at the Wake Forest Institute for Regenerative Medicine led by Atala et al. [5]. The idea was to provide patients with non-functional bladders an autologous bladder instead of the traditional cystoplasty with gastrointestinal tissue. The team was able to engineer a human bladder by isolating autologous bladder urothelial and muscle cells, expanding the cells in vitro and seeding them to a bladder biodegradable three-dimensional scaffold. Then, the tissue is anastomosed to the native bladder and covered with fibrin glue as well as omentum [5]. They demonstrated that the implantation of bioengineered 3D organs is feasible after running a clinical trial on seven patients in need of a cystoplasty. [5].

Manoor et al. were able to merge the field of cybernetics and tissue engineering together in order to create a 3D-printed bionic ear as proof of concept. They printed a cell-seeded hydrogel matrix in the anatomic geometry of human ear along with intertwined conducting polymer consisting of infused silver nanoparticles. This allowed in vitro culturing of cartilage tissue around an inductive coil antenna in the ear, which subsequently enables detecting sound waves, thus exhibiting auditory sensing [16].

Although the whole idea behind 3D bioprinting is to create tissues and organs by assembling biological materials, the use of an aid, such as already created biological scaffolds, is also being explored. The use of 3D extracellular matrix scaffolds, obtained by decellularization (which involves the use of a detergents perfused through the vasculature to remove cellular elements but allows ECM to remain intact) of allogenic or xenogeneic organs or tissues, is a promising approach for the replacement of complex tissues and whole organs, since it provides a template with extracellular matrix that can support cells and can contain the vascular network needed to deliver nutrients and oxygen for survival. Recent studies have shown that decellularization of organs such as the livers, lungs, and heart is feasible. Song et al. recently incorporated endothelial and epithelial cells into a decellularized kidney ECM scaffold in which the cells were integrated into the interstitial space and the vascular networks [19]. Yagi et al. described a successful decellularization technique in adult ischemic porcine livers [20]. The aim of this technique is to obtain a liver matrix suitable for supporting functional hepatocytes as well as to maintain the functional vascular network of the original organ including the biliary network.

The source of the organs for decellularization can be an issue. Use of porcine organs would vastly simplify logistics, enable precise quality control for obtaining decellularized grafts, avoid spread of human viruses, and indeed appears to be the chosen approach for some tissues [21•] (Soto-Gutierrez 2012). Even though any xenogeneic material creates the potential for immune complications, few have been observed thus far. Unrecovered human donor organs could also provide a large quantity of potential scaffolds. Adult human organs, however, are rarely pristine, and their recovery introduces many ethical questions. Still, it is fortunate that alternate options exist, and determination of the best approach can be done when investigating clinical applications.

Current Developments by Tissue Type/Organ

In the following section, we will briefly describe outstanding work presently being developed at different institutions focused on particular tissues or organs that could offer potential applications for the field of transplantation.

Vascular Structures

Integral vascular structures are fundamental for a successful organ transplant. Autologous vascular conduits or the vascular structures from deceased donor are frequently the first choice for anastomosing the new organ to the recipient when necessary. They are sometimes not usable due to their length, diameter, or integrity. With the development of bioengineering, synthetic vascular grafts were successfully used for large diameter and high-flow grafts. However, these often come with complications such as vascular thrombosis and infection, leading to longer post-operative hospital stays and increased treatment costs [22]. Tissue-engineered vascular grafts (TEVGs) offer an attractive theoretical alternative. Kurobe et al. described their experience with biodegradable vascular scaffolds with seeded autologous cells that were implanted for congenital heart surgery with TEVG used as an extra-cardiac cavopulmonary connection and demonstrated that their grafts functioned without aneurismal change or graft rupture [23]. The creation of 3D vascular structures like these would signify a great success. Actually, the creation of vascular conduits without scaffold is actually in progress. Norotte et al. described a layer-by-layer printing technique with the use of multicellular spheroids containing smooth muscle cells and fibroblasts along with agarose rods, resulting in single- and double-layered small diameter vascular tubes [24]. In the field of liver surgery and transplantation, these TEVG conduits could be very useful for the treatment of portal vein thrombosis and the restoration of the intrahepatic portal flow, performing a meso-rex bypass from the superior mesenteric vein to donor left portal vein. The TEVGs have also been discussed in the LDLT where often we need extension grafts for the drainage of segment V and VIII branches to the middle hepatic vein or vena cava to avoid outflow issues in right lobe grafts as well as portal vein reconstructions.

Furthermore, the investigators of 3D bioprinting field are studying out how to print branched vascular structures. Although it is still a complex task to achieve, some authors like Visconti et al. have been able to design the adequate blueprints in order to guide the 3D bioprinters to create optimal branched vascular trees [25]. At our institution, ongoing

efforts to develop these branched structures with 3D printing are already giving good results, and animal models are a priority to evaluate their functionality. (Fig. 5). The other structure that could have a potential benefit in the field is the engineering or 3D printing of bile ducts that could be used for repair, bypasses, or as network for potential 3D livers.

Liver

An even more daring approach is the 3D printing of functional liver tissue. Although to the present date there are no reports of successfully printed perfusable hepatic structures, early experiments have demonstrated promising data. For example, Robbins et al. with the use of the NovoGen MMX Bioprinter ™ (Organovo Holdings, Inc., San Diego, CA, USA) demonstrated the feasibility of printing metabolically functional 3D hepatic structures and proving that the tissue was capable of cell-cell interaction, protein production, and enzymatic activity. Also, they were able to enhance the complexity of the tissue by adding to the three-dimensional structure hepatic stellate cells and endothelial cells [26]. The group at Drexel led by Chang et al. developed a three-dimensional liver micro-organ that consists of a microscale in vitro device housing a chamber of 3D liver cell-encapsuated hydrogel-based tissue that resembles the natural microenvironment of the hepatocyte in order to achieve biological functionality. A great enhancement of this system is that it included a dynamic perfusion in order to assess the cell metabolic function by perfusion of drugs [27]. Their model was developed to provide NASA with a liver tissue analog to assess drug pharmacokinetic profiles in planetary environments. Although these models are intended for drug or disease experimentation rather than for tissue transplantation, they set the basis to further understand the microenvironment of the liver in order to determine what interactions are needed to establish a fully functional structure that can be transplanted into an animal or human. Further integration of all the 3D techniques currently in practice are needed to achieve the creation of a fully functional bioprinted liver that could be transplanted. One of the most important elements of a successful model is the presence of a vascular network to support the liver cells. Miller et al. published their experience with creating perfusable vascular networks by printing 3D filament networks of carbohydrate glass and used them as a template in engineered tissues containing living cells to generate cylindrical networks that could be lined with endothelial cells and perfused with blood. They were able to prove in a rat hepatocyte model that the perfused vascular channels were able to maintain the metabolic function of the cells [13].

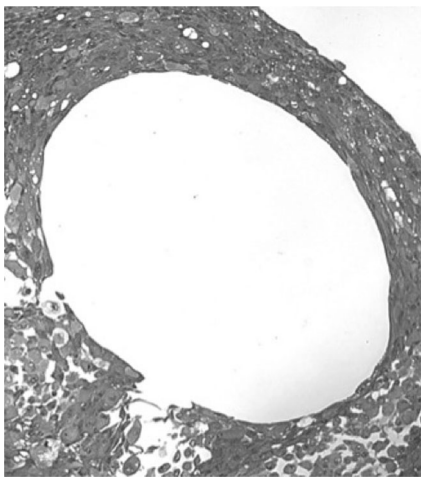

Fig. 5 Pathology slide of a printed vessel in the 3D Bioprinting lab at Yale School of Medicine. (Courtesy of Dr. John Geibel)

Kidney

Although not much data has been published in kidney 3D printing, King et al. from Organovo recently presented their in vitro model of a multicellular, three-dimensional tissue model of human kidney proximal tubule. In their printed model, they were able to observe the interface between tubular epithelium and renal interstitial cells. Extensive endothelial networks were also observed [28]. Promising data such as this can be helpful to the field of transplantation, in order to assess immunological models for rejection in the laboratory with 3D renal tissue. The use of this model for that purpose has the potential to modify the current immunosuppressant therapies. In terms of three-dimensional scaffolds for renal tissue engineering, many authors have demonstrated that the adequate use of decellularization agents can preserve critical structural and functional properties necessary for use of these three-dimensional scaffolds for promoting cellular repopulation or even establish the adequate blue prints to instruct a 3D printer to design a brand new kidney [19, 29].

Future Horizons and Conclusion

The next step for tissue engineering and 3D printing is to continue these extraordinary, mostly in vitro [30, 31] (Ott 2008, Ott 2010), initial efforts by assessing in vivo organ viability and functionality in animal models. Development of such models with any attendant techniques and technologies will likely introduce further challenges and complications [32] (Lysaght 2004). Knowledge gleaned from current in vitro pharmacology and disease models will likely contribute to these efforts.

Better understanding of intercellular communication and tissue microenvironments will provide the blueprint for constructing bio-artificial organs with native-like architecture and functionality. Although common themes emerge, ultimate printing protocols will almost certainly be highly *tissue*- or *organ*-specific for 3D printers to assemble physiologically

appropriate microstructures. As we have seen, decellularized organ scaffolds provide an excellent starting point.

Many issues remain unsolved for the majority of protocols; yet, it is encouraging to note the examples of already successful implantation (bladder and trachea). While complete ontogenic replication is a lofty goal, this should also not impede consideration of bioengineered tissue for another application—namely, as bridge therapy while awaiting organ transplantation. Whether bioengineered tissue serves as organ *supplement* or organ *replacement* may ultimately hinge on those age-old constraints of patient disease and organ availability.

Compliance With Ethical Standards

Conflict of Interest Armando Salim Munoz-Abraham, Manuel I. Rodriguez-Davalos, Alessandra Bertacco, Brian Wengerter, John P. Geibel, and David C. Mulligan declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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