

The Science Journal of the Lander College of Arts and Sciences

Volume 10
Number 1 *Tenth Anniversary Edition: Fall 2016*

Article 12

2016

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Recommended Citation

Sirota, C. (2016). 3D Organ Printing. *The Science Journal of the Lander College of Arts and Sciences*, 10 (1). Retrieved from

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3D Organ Printing

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Abstract

The global shortage of donor organs is a cause for countless fatalities across the world. Although, diseases can be treated through organ transplantation it can come along with many complications. Not only is there a high demand for donor organs, there is also the risk of the body's rejection of the newly implanted organ. Through the method of 3D printing organs, many lives could be saved as well as reducing the need for donor organs. Finding materials to create a suitable scaffold is the focus of many experiments. Materials that are used in organ printing are made from soft materials, therefore, suspended hydrogel techniques are utilized for printing organs and for creating vascularization systems in the printed organs. The vascularization level of 3D printed organs is the most complicated because of its vast and detailed preciseness. Detailed magnetic resonance imaging is taken to generate the 3D image of the structure and consequently print the image layer by layer as opposed to the older method of manually applying cells onto scaffolds. While many research teams have made progress with 3D organ printing, complex organs such as the heart and kidney are currently undergoing research to be implemented into clinical settings.

Introduction

Transplanting new organs is a way of trying to deal with life threatening diseases. Instead of the chronic maintenance of diseased organs medical professionals would be able to replace them, thus curing the condition. One leading major health crisis is the shortage of organs available for transplants. Although medicine's advancements help people live longer, organs tend to fail more often as a result of longer lifespans. For instance, in the United States, a name is added to the Transplant List on an average of every ten minutes. Additionally, the number of patients needing organs have doubled while the amount of transplant procedures has barely increased (OPTN, 2016). 3D organ printing technology is a promising step to help solve the organ transplantation crisis.

The field of regenerative medicine deals with replacing, engineering, or regenerating human cells, tissues, or organs in order to help achieve normal function. Regenerative medicine is not a new field; Alexis Carrel, a Nobel Prize winner, writes in his book, "The Culture of Organs" (Carrel, Lindbergh, 1938) about technologies that are still used today for blood vessel grafting.

Thomas Boland, self-described as the "grandfather of bioprinting," took an old Lexmark computer, emptied the ink cartridge and filled it with collagen instead. He then glued a thin, black silicon sheet onto blank paper and inserted it into the printer. He opened a Word document on his PC, typed his initials, and hit print. The paper came out with "TB" clearly presented in off-white proteins. By 2000, Boland and his team had reconfigured a Hewlett-Packard DeskJet 550C to print with E. coli bacteria. Then they expanded to larger mammalian cells obtained from Chinese hamsters and lab rats. Once printed, 90 percent of the cells remained viable, which meant the product was useful, not only art. Boland introduced 3D printing for cellular construct when he patented the process for using the ink-jet printer for printing cells. Boland's process used a system where cells were deposited into 3D matrices placed on a substrate. Instead of using ink, 3D printers use cells in a cartridge where the cells are

being layered in order to create a three-dimensional structure that can lead to functional organs and tissues (Wilson, Boland, 2003). This paper will review the current progress in organ printing technology with its promises and challenges and evaluate whether functional organs can be produced.

Methods

The information in this paper was obtained by the analysis of scientific articles and research papers obtained from the Touro College Online Library, specifically, the Health Science related databases such as Pubmed, Proquest Medical Library, and EBSCO multisearch. Additionally, websites like NIH.gov, Webmd.com, MayoClinic.com and OPTN.com were used to gain general knowledge and information about the subject. Keywords such as "3D organ printing" and "regenerative medicine" were used to search for scientific articles.

Discussion: The Bladder

At first, researchers were pipetting cells into petri dishes by hand without the use of 3D printers. To do so, researchers would seed the cells from the petri dishes onto artificial scaffolds; a suitable base for the organs. The scaffolds were made from biodegradable polymers or collagen which provided a temporary matrix for the cells to cling to until they were able to stand on their own. Dr. Anthony Atala, the lead researcher at Wake Forest Institute for Regenerative Medicine, implanted the first lab grown bladder organs into seven patients at Boston Children's Hospital between 1999 and 2001 (Atala et. al., 2006).

There are a variety of injuries that can lead to damage or loss of the bladder, requiring eventual replacement or repair of the organ. Children with congenital anomalies such as: bladder exstrophy, myelomeningocele, or posterior urethral valves, can develop high-pressure and hypertonic low compliant bladders. Often times, these patients require cystoplasty when drug treatment fails. Gastrointestinal segments are frequently used as donor tissues for cystoplasty. However, when such tissues are incorporated into the urinary tract, several complications

can ensue; such as metabolic disturbances, urolithiasis, increased mucous production, and malignant disease (Emedicine, 2016).

Atala, along with his research team, explored an alternative approach using autologous engineered bladder tissue for reconstruction. Seven patients with myelomeningocele, aged 4-19 years old, with poorly compliant bladders were identified as candidates for cystoplasty. Urethral and muscle cells from the patients were grown in a culture, and seeded on a biodegradable bladder shaped scaffold made of collagen, or a composite of collagen and polyglycolic acid. About seven weeks after biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted into patients. Tests such as serial urodynamics, cytograms, ultrasounds, bladder biopsies and serum analyses were performed. The bowel function of the patients returned to normal promptly after surgery with no metabolic consequences and renal function was preserved (Atala et. al., 2006).

Researchers soon adopted 3-D printers to make scaffolds more precisely because manually placing the cells onto scaffold remained a time-consuming and arduous process. Engineered bladders were made possible because they can be made with just two cell types however, an organ such as a kidney consists of thirty cell types. When attempting to engineer more complex tissues, there is no way to manually place different cell types into different locations that can replicate the native tissue structures. Manual placement is not the optimal method for delivering cells (Murphy, Atala, 2014).

The Heart

The human heart is a complex biological machine. It pumps blood to all parts of the body, begins to beat three weeks after conception and does not stop until the day of death. It is remarkable how the heart can last that long and function with the same redundancy and efficacy. The heart operates for billions of cycles, it is chemically powered and electrically synchronized. The heart is composed of over a hundred billion cells including cardiomyocytes, conduction system, fibroblasts, endothelial cells, smooth muscle cells and neurons. The main reason researchers are trying to 3D print the heart is because the heart is unable to regenerate itself and for this reason heart disease is the leading cause of death in first world countries (Mayo Clinic, 2016).

A heart transplant is a lifesaving procedure which removes a damaged or diseased heart and replaces it with a new one. Heart failures come as a result of different conditions such as coronary heart disease, damaged heart muscle or valves, congenital heart defects, or viral infections of the heart. Once the human heart is damaged, there is only a limited amount of regeneration that can occur and may require a heart transplant. A heart transplant comes with many risks and complications

such as infection or the recipient's body attempting to reject the newly placed heart. To prevent rejection, the patients must immediately be given immunosuppressants as a lifelong treatment (Mayo clinic, 2016). There are currently 4,138 candidates who are on the UNOS list for a heart transplant (OPTN, 2016). The high number of candidates results in an agonizing wait for a new heart which usually does not have a positive ending. This long waiting list can be reduced through the possibility of 3D printing of the human heart.

The hierarchical structure and function in the heart has levels that scales from the nanometer level up to the macroscopic level. Every heart beat is powered by molecular motors, the actin and myosin, which are only a few nanometers in size and generate pico newtons amount of force. The actin and myosin are built into larger structures called the sarcomeres, the contractile unit of a muscle cell. Then, these are built into myofibrils which form tissue and surrounds the ventricles of the heart. At this present day, technology is unable to engineer something this complex starting from the nanometer scale and up. Yet, scientists are able to figure out where they are able to interface with the system, by providing information into the biology that can guide the living system to do the work of producing the components of the microstructures. Once the cells are formed, they themselves manufacture the microstructures necessary and the 3D bioprinting scaffolds, the suitable base, are used to build larger structures up to the size of a whole organ.

The embryo is a perfect example of biological manufacturing, it starts off with one cell and those cells divide and build an extracellular matrix around themselves until there is a full functioning organism. The extracellular matrix is a nanofiber network of protein and other molecules. It mechanically integrates cells into tissues, acts as an insoluble signaling network and functions as a scaffold that fills the space between the cells and tissues. This matrix acts as information because it provides cells with instructive cues on how to organize and form tissue. It signals between the cells using physics, chemistry and mechanics and is integral to tissue formation and function (Rozario, Desimone, 2010). A technique called Bottom Up Engineering of the Extracellular Matrix (ECM) was an attempt to build the ECM in the same way cells do; from single molecules into a complex 3D fiber with tissue specific structure and function according to the organ that is being engineered. This technique thereby accelerates the system of tissue formation (Szymanski et. al., 2014).

The idea of using the extracellular matrix is utilized in the following technique for 3D bioprinting organs and other structures. Soft materials such as collagen and fibrin are difficult for scientists to work with because they collapse under their own weight and do not have the mechanical strength to be printed

in air. To overcome this problem, a gel in gel technique dubbed Freeform reversible embedding of suspended hydrogels is used. This approach involves soft materials such as fibrin, collagen and the polysaccharide alginate. The materials are inside a thick slurry of gelatin microparticles and water in a petri dish. The semiliquid mixture of gelatin microparticles is able to flow around the printing needle as it operates. At the same time, the soft biomaterials being released are firmly implanted during the layer by layer printing technique. Once the printing of the scaffolds is completed, the dish is warmed up to 37 degrees Celsius thereby removing the gelatin and revealing the scaffold replica of an organ or tissue (Hinton et. al., 2015, Figure 1).

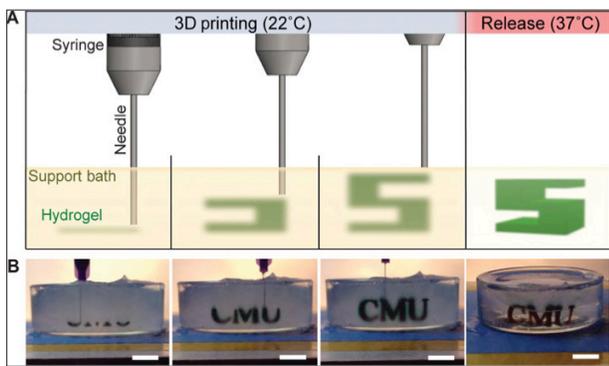


Figure 1 (Hinton et. al., 2015)
Freeform reversible embedding of suspended hydrogels technique

One application of this technique is using magnetic resonance imaging (MRI) to print patient specific scaffolds to engineer tissues. Through magnetic imaging the right coronary arterial tree is 3D printed. The right coronary artery along with the left coronary artery supplies blood to the heart. The right coronary artery is specifically responsible to supply blood to the right atrium, right ventricle, bottom portion of the left ventricle and the back of the septum. Additionally, the coronary arteries become blocked during a heart attack. The 3D printed artery was made of ECM materials such as fibrin, hyaluronic acid and alginate with solid walls and an open lumen. On a makerbot 3D printer the artery took about one hour to print which is the timeframe within the viability of cells. The wall thickness, lumen diameter and density can be tailored based on patient anatomy. To evaluate whether the 3D printed arterial tree was manifold it was set up in a custom made 3D printed perfusion fixture. A solution of CaCl_2 and 0.1% black food coloring was injected into the root of the tree. Perfusion was successful and captured on camera. The printed coronary artery has not been used for transplants such as a coronary artery bypass, yet this technique is a step up from the current printing of just straight tubes. This technique creates more complex architecture like the real coronary artery tree that can hold a better potential graft (Hinton et. al., 2015, Figure 2).

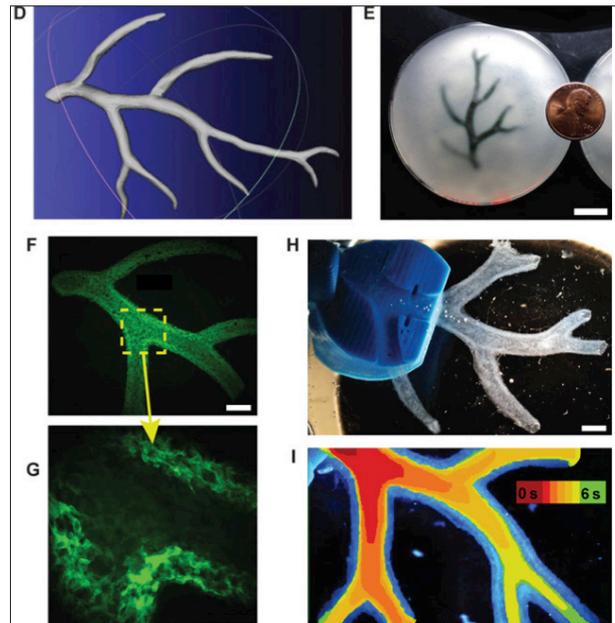


Figure 2 (Hinton et. al., 2015)

(D) A model of a section of a human right coronary arterial tree from 3D MRI is processed at full scale into machine code for FRESH printing. (E) An example of the arterial tree printed in alginate (black) and embedded in the gelatin slurry support bath. (F) A section of the arterial trees printed in fluorescent alginate (green) and imaged in 3D to show the hollow lumen and multiple bifurcations. (G) A zoomed-in view of the arterial tree shows the defined vessel wall that is <1 mm thick and the well-formed lumen. (H) A dark-field image of the arterial tree mounted in a perfusion fixture to position a syringe in the root of the tree. (I) A time-lapse image of black dye perfused through the arterial tree false-colored at time points of 0 to 6 s to show flow through the lumen and not through the vessel wall. Scale bars, 4 mm (B), 10 mm (E), 2.5 mm (F), 1 mm (G), and 2.5 mm (H and I). (Hinton et. al., 2015).

Another application of the Freeform reversible embedding of suspended hydrogels technique is used to build the embryonic heart. The 3D model of the embryonic chick heart was generated from 3D optical imaging data of a fluorescently labeled 5-day-old heart. The embryonic stage is the time when the heart muscle forms. It is the only time when the human body has the potential to form new heart muscle. In order to accurately 3D print an organ, there needs to be accurate data input to the system. The first step is the 3D imaging of the embryonic heart to identify the ECM macrostructure and microstructure associated with myogenesis and vascularization. 3D imaging goes through the embryonic heart and images the entire heart, ventricles, and trabeculated tissue together with all the exquisite details. A 3D computer model of the trabeculated embryonic heart is generated based on confocal imaging of cells and the extracellular matrix. Then, based on the computer model the 3D heart is printed and compared with the computer generated image (Hinton et. al., 2015, Figure 3).

One step further in 3D cardiac printing is the ability to print cardiac cells called organoids. This research called “Body on a Chip” is currently ongoing at Wake Forest Baptist Medical Center. The research converted human skin cells into a network of functioning heart cells, and also fused them with lab grown liver cells using a 3D printer. The heart organoid is able to beat because it contains specialized cardiac cells and the cells receive correct environmental cues such as keeping them at

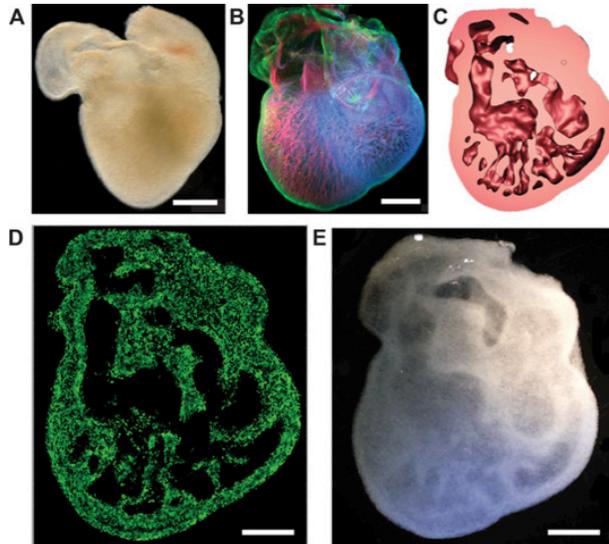


Figure 3
Printed embryonic heart scaffolds with complex internal and external architectures based on 3D imaging data from whole organs

the same temperature as the human body. The organoid heart cells are made by genetically modifying adult human skin cells into induced pluripotent stem cells. Then, these cells are reprogrammed to produce the organoids which have a diameter of 0.25 millimeters. The organoids grow and form balls which then are 3D printed into different forms and sizes. These cells will create tiny organ-like structures that mimic the function of the real organ. The organ structures are then placed on a chip to provide for an online monitoring. The ultimate goal is to create mini organs to test them with different biological and chemical

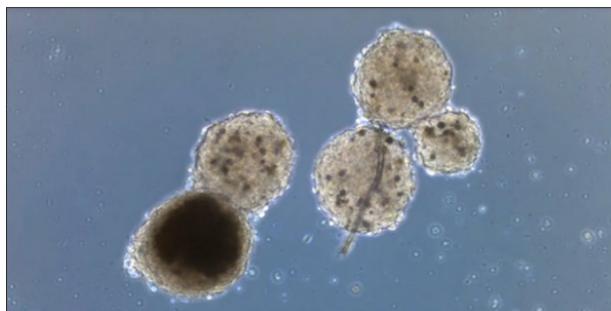


Figure 4 (Wakehealth.edu)
3D printed beating, cardiac cells called “organoids”

agents and the effectiveness of various treatments against diseases (Wakehealth.edu 2016, Figure 4).

The Kidney

In addition to the great need of heart transplants there is even a greater number of patients waiting for a kidney transplant. Currently, there are over a hundred thousand people anxiously waiting for a lifesaving kidney (OPTN, 2016). The kidneys are responsible for removing excess fluid and waste from the blood. When the kidneys lose their filtering ability, there is a dangerously high level of fluid and waste accumulating in the body. This condition is known as kidney failure or end stage kidney disease. A kidney transplant procedure is often the best way to treat a kidney failure. Only one kidney is necessary to replace two failed kidneys, thus making a living donor kidney transplantation possible (Kidney.org, 2014).

A kidney transplant can pose risks to both the donor and the recipient. There can be possible surgical complications such as pain, infection, blood loss, blood clots, allergic reactions to anesthesia, pneumonia, injury to surrounding tissue and organs, or even death. There are also long term risks for donating a kidney such as hypertension, large amounts of protein in the urine, hernia, organ impairment or failure that can lead to the need for dialysis or transplantation and can even cause death. Additionally, there is the problem of the recipient’s body rejecting the kidney as a foreign invader. Just as in a heart transplant, the patient will need to remain on immunosuppressants every day to prevent rejection of the new kidney. These anti-rejection medications have a large number of possible side effects because the body’s immune system is suppressed (kidney.org, 2014).

Dr. Anthony Atala demonstrated an early stage experiment that could solve the kidney organ donor problem. The experiment uses a 3D printer that contains live cells and kidney shaped structure of collagen to output a transplantable kidney. A blueprint is created through a CT scan that goes layer by layer using computerized morphometric imaging analysis and 3D reconstruction which leads to actually imaging the kidneys. The image can even do a full 360-degree rotation to analyze the kidney in its full volumetric characteristics. This information is then taken and scanned into a printing computerized form. The printer is then guided by the computer imaging and drips the cells layer by layer over the scaffold causing the inert mold to come to life. Overtime, the millions of cells begin to communicate and function as one organ. It took the research team about seven hours to print the 3D kidney structure (Murphy, Atala, 2014, Figure 5, Xu et al., 2013).

Vascularization

The kidney represents a challenge more than other organs due to the kidney’s detailed, tiny structures that allow the organ

to perform its sophisticated filtration job by removing waste chemicals from blood and turning the waste into urine. The function of removing waste is processed by roughly one million nephrons which are tiny vessels made up of even smaller urine collecting structures called tubules. The kidneys are also responsible to measure chemicals such as sodium and potassium that are then released back into the bloodstream in healthy dosag-



Figure 5 (Murphy, Atala, 2014) (TED.com 2011)
Dr. Atala holding his printed kidney during a TED talk called “On Printing a Human Kidney,” in March 2011.

es. The kidney’s complex vascular structure especially poses a challenge. The vascular tree of the kidney can come down to the size of a capillary about eight microns in diameter. Due to the kidney’s complexity, it has not been printed for transplant in humans at this present time, however researchers are avidly looking for ways to make it a reality and help people suffering from dialysis treatments while being on a multiple year waiting list for a kidney (Murphy, Atala, 2014).

For a transplant tissue to thrive, a complex level of vascularization must be achieved. To some extent, capillaries can branch out from already existing blood vessels and into transplanted tissue on their own. Researchers have seen signs of spontaneous vascularization in small areas of engineered tissue such as in healed rat bone defects around three millimeters in diameter (Fielding, Bose, 2013). Rather than letting blood vessels spontaneously branch off and expand to engineered tissue, researchers are crafting templates for more orderly vascular growth. The idea is to create hierarchical microvascular networks that will guide the endothelial cells that line blood vessels to form tubes along predetermined courses. However, capillaries are small, measuring just a few microns in diameter. Therefore, even with high resolution printers, such small vascular structures would most likely collapse especially when printed into soft, biocompatible gel.

Jennifer Lewis researched the vascularization problem using a customized, high resolution 3D printer that can form microchannels in biocompatible gels. Lewis’s research group is able to print hydrogel materials down at the micron length

scale. The smallest microvascular channel the group was able to print was about ten microns in diameter. To solve the problem of collapsing channels, she prints them in fugitive ink which is a substance designed to melt away forming the channels pattern. The fugitive ink used is called Pluronic 4127, a gel that is often used in eyeglass lense cleanser and cosmetics. Pluronic 4127 is made up of three parts, the two poles of the molecule are hydrophilic while the middle segment is hydrophobic. The ink also liquefies when it is cooled down as opposed to most materials that solidify when they are cooled down (Wu et. al., 2011).

Lewis also used Pluronic F127 as the matrix for the printed channels, but the matrix molecules were modified so that they polymerize, and thus solidify, in the presence of UV light. This allows the matrix to become firm before cooling the gel so that the fugitive ink melts away. Taking advantage of the printer’s fine-tipped nozzle, the research team printed a capillary network of fluorescently labeled fugitive ink into the Jello-like matrix. Through this technique, a way to pattern hydrogels with vascular channels was accomplished (Wu et. al., 2011). The next step is to take advantage of the self-organizing quality of endothelial cells in the 3-D–printed constructs, seeding the printed vascular structures with these blood vessel–lining cells. The self organizing quality is the tendency of the finest capillaries to grow spontaneously out of larger microvascular structures which is the work of biology when the cells are given a reasonable environment (Perryn et. al., 2008).

Inspired by some of Lewis’s lab work with “fugitive inks,” Jordan Miller, at Rice University, created a technique for 3D printing of vasculature-mimicking channels. Using a simple open-source 3D printer, Miller and his team constructed a carbohydrate lattice made from a combination of simple and complex sugars. There is a major difference between Miller’s experiments and those done by Jennifer Lewis. Lewis uses machines that are very high-end and have incredible precision, but they are not easily duplicated by others. Miller saw potential in a cheaper printer called the Frostruder, a printer originally used to extrude sugar frosting for printing fancy designs onto edible treats. Miller adapted a printer to incorporate elements of the Frostruder printer’s design and was soon able to print dissolvable lattices of carbohydrate filaments (Miller et. al., 2012). The experiment used a process called “3-D sacrificial molding” that is similar to the lost-wax method used by sculptors. His printer deposits filaments of carbohydrate on top of each other in sequence so they are self-supporting. Then the entire lattice structure is covered in a protective layer of a biodegradable polymer. After pouring and cross linking a cell-filled gel over the carbohydrate lattice, the lattice is dissolved with an aqueous solution (Miller et. al., 2012).

Miller's channels are not as small as Lewis' channels. His channels range from 150 microns to around a millimeter in diameter. However, when he and colleagues seeded the channels with endothelial cells, they lined the interiors of the channels and even began to penetrate the surrounding cell-gel mixture. By guiding blood cells into the larger channels, he can set the stage for endothelial cells to spontaneously form their own capillary networks. Miller has successfully pumped human blood through his constructs in vitro, and the research team plans to cooperate with a surgeon to connect one of his printed tissues to the vascular system of a rat to see how long he can get blood to flow through his channels (Miller et al., 2012).

Vascularization procedures done by Miller and team take a short amount of time to complete which benefits the large scale tissues, like liver cells. Large scale tissue cannot survive the several hours it takes in the extruder nozzle long enough to build something the size of the human liver. Additionally, quickly pouring the cells and gels over the 3-D-printed lattice is easier on fragile cells than the arduous process of printing. The one disadvantage is that the researchers cannot control the exact placement of the cells. Therefore, this may not be the optimal method for experiments involving multiple cell types. In the versions of the constructs printed with rat liver cells or with human embryonic kidney cells, the cells near the channels survive longer than the cells deeper in the gel, suggesting that the experiment is leading in the right direction (Miller et al., 2012).

Conclusion

At this present time, a 3D printed organ is unable to be inserted into a patient with the same efficacy as a normal organ. Many more years of research need to be conducted. There are constantly many advances being made by countless numbers of research teams who are devoted to help suffering patients and increase the availability of organs for transplant. However, even though 3D printed organs cannot be inserted into patients, they can be used to assist doctors and surgeons in surgery. Advanced models of organs that are 3D printed are able to prepare surgeons on what they might encounter in the operating room. Simply by looking at models, doctors are able to avoid potential complications that could have been unforeseeable without the physical model. 3D printed organs can also help train medical students and professionals. Researchers and Doctors are able to replicate the part of the patient with the complicated problem so students can learn how to go about saving lives. It also allows healthcare professionals to better explain situations to the patient. The healthcare provider can physically hold the model and explain what is wrong and the different procedures available for the patient. Currently, the main use of the 3D printed organs has been for screening new medications and modeling various diseases.

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