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# The Viability of Organ Printing

Estie Schick

## Abstract

Organ printing is an emerging technology that can potentially replace the need for human organ transplants altogether. Organ printing uses bioprinting methods to create three-dimensional biological constructs. Although it has not yet been implemented successfully, with nearly two decades of research devoted to this area, much progress has been made. This article outlines the various aspects of the organ printing process, describes both the accomplishments and challenges of bioprinting, and discusses the feasibility of bioprinting as a viable method for organ replacement.

## Introduction

The cutting-edge principles of organ printing technology have been compared to the age-old properties of Johannes Gutenberg's printing press (Mironov et. al., 2008). The essential elements necessary for printing a book include a printing press, ink, paper, movable type, and a written text to be printed. These very same components can be applied to the up-and-coming field of bioprinting. Bioprinting is literally biological printing and utilizes the technology of a bioprinter to build a three-dimensional biological construct. This incredible feat is performed by the printer placing cells, bioink, layer-by-layer in specific locations onto a biopaper suitable for sustaining cell life. Printing biomaterials is obviously much more complex, but at its most basic levels it is analogous to the printing methods of a simple printing press. The necessary components for bioprinting are a bioprinter, bioink, biopaper, a method for depositing the biomaterials in set locations, and a model of the tissue or organ to be printed.

Organ printing technology has emerged as the topic of much research and discussion because of the shortage of organs for transplantation. There are other options besides for human organ donation such as xenotransplantation as well as artificial or mechanical organs (Boland et. al., 2003). But these options are the source of deleterious side effects, causing many to look to bioprinting as the future method for organ replacement.

That is not to say that there are not many roadblocks in the way of organ printing. The entire idea of 3D printing is somewhat reminiscent of science fiction and that is even before live human organs enter the picture.

Because this is such a new field of study, there has not yet been much success in actually printing an organ. There are important steps and milestones that must be met along the way. In fact, there is no hope of an organ being printed successfully before tissue can be printed flawlessly. And there is certainly a long way to go before organs will be printed on an industrial scale. So it is important to ask: will bioprinting be a viable method for replacing damaged organs?

## Materials and Methods

In order to answer the question proposed above, many journal articles relating to this topic have been read. Touro College's library database was also used to search for relevant studies and reviews. The next step taken was to look for articles that were referenced by those obtained through the Touro College Library search engines that seemed pertinent. All of the articles accumulated through this research have been used in an attempt to conclusively answer the question of the viability of organ printing.

## Results

### The Basics of Bioprinting: Bioprinters

Bioprinting uses computer-aided printing technology to deposit cells layer-by-layer in specific locations and form three-dimensional biological constructs. Many factors play a part in determining the efficacy of the bioprinting method, and a major one is the bioprinter. A decade ago, one of the first studies detailing the transformation of an ordinary commercial inkjet printer into a bioprinter was published. Inkjet printing was chosen specifically because the cells in the bioink were kept more hydrated than could

be obtained using any other printing method. In addition, inkjet printers are a significantly more economical choice than a more complex 3D printer. Inkjet printers are able to deposit tiny ink drops onto a substrate upon demand using thermal inkjet technology (Wilson, Boland, 2003). A small air bubble is heated until it expands and then collapses. When it collapses, that air bubble serves as the pressure pulse that forces a very tiny droplet of bioink out of the nozzle. The temperature can reach as high as 300° C, but the entire process is so instantaneous that pulse lasts only a couple of microseconds. Consequently the heightened temperatures do not permeate the bioink, and living cells can thereby be printed. With the advantage of inkjet based bioprinting, living cells can be printed at the same time as nutrients, drugs and growth factors, as well as gels and scaffolds (Cui, Boland, 2009).

Multiple printers were studied and each one was optimized for a specific application. The Cannon BJ2200 printer was modified so that cells could be printed onto very thin samples- as thin as 1 mm. Temperature controls were also added so that none of the living cell samples could be denatured by a heat above 100° F. The printer software drivers of an HP 550C were adapted so that solutions with different electrical charges and different viscosities could be printed. In order for pH, charge and viscosity of the cell sample not to affect the printing, new software was written that adjusts voltages constantly. Using the designs for the HP 660C printer, a new printer was built with a base that allowed for height adjustment. Consequently, the printed samples could be moved along the x and y planes. That same printer was further modified so that large mammalian cells could be printed. It would be impossible for cells of this size to fit through the nozzle of a regular inkjet printer so modifications had to be made to the print head. The new print head is made of nine individual pumps which can be operated individually, allowing multiple cells types to be printed onto the same sample. The nine pumps can be used simultaneously or a specific pump can be programmed to deposit cells at a given time. New software has been created that allows someone to simply enter the instructions on the computer and then watch the printer carry out those directions (Wilson, Boland, 2003).

In contrast to the inkjet printing method, laser assisted bioprinting has also emerged as a viable bioprinting technique. The Laser Induced Forward Transfer (LIFT) was originally used as a mechanism for transferring metals. It has been applied to bioprinting, resulting in a bioprinter named LaBP, the laser-assisted biological printer. This printer deposits suspended cell material onto a thin metal ribbon which is then hit with a laser pulse. The liquid solution is thereby deposited onto a sample of biopaper. In a recent study, factors such as cell density, viscosity, laser printing speed and laser energy were optimized to result in cell printing with the highest resolution. Rabbit carcinoma cells and human umbilical vein endothelial cells were used as bioink and suspended in liquid form. Different suspensions were prepared and their respective viscosities were measured. A correlation was drawn between high cell viscosity and a small droplet diameter which yields a high printing resolution. Different laser intensities and various laser speeds were tested as well. Decreased laser energy droplets and high laser scanning speeds resulted in high cell printing resolution. This study demonstrated that laser assisted bioprinting could successfully print biological structures, and a high cell-level resolution can be obtained. One advantage that laser-assisted bioprinting holds over inkjet bioprinting is the ability to print a high volume of cells per droplet. This is possible because the LaBP can print cells from a bioink with a concentration as high as 108 cells/ml. Using inkjet printing technology, there is a concern with using high concentration bioink because the printer head can clog. This is not an issue with laser-assisted bioprinting, and as a result high concentrations of cells can be used and cells can be still be printed one by one. The authors suggest further studies that implement a cell recognition scanning technology, which would help ensure that only one cell is being deposited with each laser pulse (Guillotin et. al., 2010).

### The Basics of Bioprinting: Biopaper

Cell printing necessitates the use of biopaper so that the cells can be hydrated after printing. The drying process of the ink will have an effect on cell survival, and therefore the materials used as biopaper are imperative to the bioprinting process (Xu et. al., 2006). Therefore, in an innovative study, hydrogels are well suited to act as biopa-

per. The bioprinter that was used in the study was modified so that multiple types of hydrogel with or without cell suspensions can be printed into patterns. A correlation was drawn between certain printer control settings and the width of the printed hydrogel pattern. For example, the nozzle velocity, nozzle diameter and flow rate all have an effect on the hydrogel patterns. The specific hydrogel used in this study was formed by cross-linking hyaluronic acid with polyethylene glycol. This hydrogel (without cell suspensions) was printed multiple times, each time with varied printer settings. These tests yielded pattern widths ranging from 603.218  $\mu\text{m}$  down to 141.38  $\mu\text{m}$ . In all experiments, the temperature and humidity levels were controlled. Printing speed, nozzle diameter and injection speed were all varied, and a narrow hydrogel pattern width (i.e. 141.38  $\mu\text{m}$ ) was obtained with a fast printing speed, slow flow rate, and most importantly, small needle diameter (Song et. al., 2010).

In a groundbreaking study, a cell printer successfully printed nine cell types into a 3D construct using thermo-reversible gels. These gels are well-suited to become the biopaper in a printed tissue or organ because of their unique qualities. The gels are biodegradable, nontoxic and thermo-reversible, meaning that they are in a gel state at temperatures above 32° C and in a liquid state at temperatures below 20° C. The authors theorized that by dropping a layer of gel onto a heated substrate, printing cell aggregates onto that biopaper and repeating that process, 3D constructs would be formed as the cell aggregates fused together. In order to successfully perform that experiment, the optimal gel thickness and cell aggregate size had to be determined.

Both thermosensitive gels and collagen gels were prepared and their minimal thicknesses were measured. The reasons for using both types of gels in the 3D construct are to provide stability and strength, as well as forming a 'drug-delivery service.' This allows certain growth factors and bioactive agents to be released throughout the construct in a controlled fashion. The specific advantage of thermosensitive gels is that the time it takes for the gel to form directly affects the distribution of cells within the gel. Therefore, using gels that respond to temperature which gel more quickly than gelation controlled by solvent, pH, or

ionic cross linking proves to be beneficial. Cell aggregates were also prepared using bovine aortal endothelial cells. The cells were printed onto series of gel layers. The cells were suspended in a liquid solution and did not spread out once they were printed on the gel layers. In addition, there was little, if any, mingling of the gel layers. But in order for this method to successfully form 3D tissue, fusion needs to occur between the cell aggregates. Although fusion of cell aggregates appeared to be more effective in collagen gels than in thermo-reversible gels, fusion did occur in those gels as well. Furthermore, it was demonstrated that fusion was not limited to the cells in the top layer of gel, but it was equally prevalent within the many layers of gel. In addition, a live/dead assay was performed, and showed that while cells that were not spread throughout the gels underwent cell death, the cells within the gels were remained alive. According to the authors, the adhesiveness of gels for cells is a property that can be modified, so the lower rate of diffusion through thermosensitive gels is not so worrisome. The aspect of the experiment that is slightly problematic is the small amount of apoptosis and necrosis that occurred as a result of cells being printed. The authors suggest further studies using aggregates modified with additional survival factors or genetic antiapoptotic modifications. Though there can be many changes made that will improve the results of this study, it demonstrated that by using thermo-reversible gels as biopaper, 3D organ printing is feasible (Boland et. al., 2003).

### **The Basics of Bioprinting: Bioink**

The physical properties of sodium alginate hydrogel cross-linked with calcium chloride were examined in a study that found it well-suited to behave as bioink. Sodium alginate hydrogel has the unique property of fast gelation at room temperature because it solidifies upon contact with calcium chloride, making it a prime candidate for bioprinting. Because of the ionic cross-link controlled gelation, and because cells and growth factors can easily be suspended within the sodium alginate hydrogel, it makes an effective bioink. A multinozzle printing system was used, allowing the speed of gel injection to be controlled. The bioprinter used in this study was modified with a multinozzle injection syringes, as well as the ability to control stage and syringe motion in the x, y, and z axes. A 3D algi-

nate structure was printed using a four-nozzle system. Each gel layer was composed of a sodium alginate solution followed by a calcium chloride solution printed in the pattern of a lattice structure.

A 'layered pattern accumulation test' was performed to determine whether the sodium alginate-calcium chloride gel could be used to create 3D tissue constructs. The gels were printed using the multinozzle printer into a lattice pattern. The pattern held successfully, although it acquired a sideways slant due to the viscosity of sodium alginate hydrogel. This caused each layer to drag on the layer immediately below it. In future studies that problem should be rectified by using creating a system that will control the gel hardness and solidification time. Despite this setback, the feasibility of using cells suspended in sodium alginate hydrogel to print 3D biological constructs was clearly demonstrated by this study (Song et. al., 2011).

### Bioprinting vs. Scaffold-Based Tissue Engineering

Tissue engineering is a field that combines biology, chemistry, physics and engineering in order to create or repair biological tissue. Because of the complexities in the structure and mechanics of biological tissue, there are obviously many challenges in the creation of tissue that performs and functions exactly the way it should. Nonetheless, for years tissue engineering has been incredibly successful. Popular uses of tissue engineering are to repair or replace body tissue including skin, muscle, bone, and blood vessels.

Typically, living cells are used as the primary engineering material in tissue engineering. These cells are placed or 'seeded' into a 3D artificial rigid structure- a scaffold. Scaffolding allows many of the challenges of tissue engineering to be overcome. For example, by using a solid structure like a scaffold, the implanted cells can attach onto its surfaces and eventually are able to form into three-dimensional tissues. One difficulty in creating scaffolds is that they need to be structured in a way that encourages optimal tissue formation to occur. This dictates what material the scaffold will be constructed from (i.e. how porous the material is.) This is important so that the nutrients can diffuse easily through the scaffold and reach the cells as necessary (Chan, Leong, 2008). The scaffolding

material should also be biodegradable so that the scaffold will not have to be removed surgically. Instead, the scaffold needs to provide support to the cells while they are still forming their own structures, and then become absorbed by them when the three-dimensional tissue is fully formed. Additional factors that are important in scaffold-based tissue engineering are immunogenicity, the toxicity of the scaffolding material, and inflammatory response by the host (Norotte et. al., 2009).

There are cases when the use of scaffolds in tissue engineering proves detrimental to the newly forming tissue. Scaffolds can reduce the amount of connection between cells and can cause the misalignment of extracellular matrix. Additionally, it is difficult to place the many different types of cells usually found in an organ in specific locations in a solid scaffold. Another major problem with scaffolding is that it is not yet possible for vascular tissue to be formed, resulting in the absence of vascularization in any scaffold-based engineered tissue (Boland et. al. 2003). For these reasons and more, other scaffold-free tissue engineering options have been explored.

Bioprinting uses 3D printing technology to print cells layer-by-layer and create biological materials, and is an example of scaffold-free tissue engineering. One of the largest roadblocks in the success of scaffold-based tissue engineering was the inability to create vascular structures. In 2009, vascular tissue was successfully engineered using scaffold-free bioprinting. In this study, a rapid prototyping technology was developed which instructed a bioprinter to deposit bioink onto biopaper. More specifically, multicellular tissue spheroids of Chinese hamster ovary cells, human skin fibroblast cells, and human umbilical vein smooth muscle cells were used as bioink. In addition, agarose rods were used to build a template for the tubular vascular structure. These materials were placed layer by layer onto a biopaper made of collagen gel using a bioprinter that was designed with two printing heads. This allowed the simultaneous placement of the multicellular spheroids as well as the agarose rods. The use of the agarose rods as a template allowed the diameter of the tubular structure, the wall thickness, and the branching pattern of the vasculature to be accurately controlled. Once the spheroids were all deposited in the correct locations, their fusion was

monitored. It took 5-7 days for fusion to be complete and the tubular vasculature to be fully formed. The fused products were placed in a bioreactor for further maturation. Maturation is important before implantation because there are many cellular mechanical properties that need to be developed.

Once it was determined that the bioprinting was successful, the study was repeated with some variations. In the first, multicellular cylinders are used instead of spheroids. The bioprinter attachments had to be adjusted, but the printing of cylindrical units allowed for computer automation. In another, double-layered vascular tubes were created using both HUVSMC and HCF cylinders in specific patterns. Spheroids of different sizes were tested, and various bioprinter attachments were experimented with.

This study proved the effectiveness of scaffold-free tissue engineering using bioprinting. High cell density was achieved because the engineering materials only consisted of cells. In addition, when multicellular cylinders were used, fusion occurred within 2-4 days and uniform tubes were formed with minimal cell damage. There are some limitations though with methods and materials used in the study. For example, the thickness of the vascular wall prevents all cells from access to the diffused nutrients and oxygen. Therefore, apoptotic cells were observed in no apparent pattern throughout the final construct. In order to avoid this issue, microvasculature is necessary, but even with the advances that have been made, there is no visible solution as of yet. If it were possible to print thinner vascular walls, the cells would avoid apoptosis and cell viability would be increased. But the wall thickness and tube diameter of the vascular tissue is limited by micropipette size and resolution restrictions. Another issue that arose was the removal of the agarose rods. In the current study, the rods are removed manually. But this limits the geometry of the vascular branch necessitating open ends, and becomes more difficult to accomplish with more complex geometric constructs. The authors suggest thermosensitive or photo-sensitive gels as an alternative to agarose in order to eliminate this problem. This study demonstrated the advantages of scaffold-free tissue engineering over scaffolding, but in the process came up with a host of limitations specific to the methods used (Norotte et. al., 2009).

## **Successful Bioprinting of Mammalian Cells**

One of the major hurdles to overcome in the study of tissue engineering is the complete interaction of the many cell types needed to fabricate complex tissue or organs. These cells need to be placed in very specific locations and fuse together forming a functional biological construct. A study demonstrated that mammalian cells can be successfully printed using a modified HP inkjet thermal printer and retain their functionality. Although bacterial cells had previously been printed successfully, the heat and pressure that are part and parcel of thermal printing had the potential to damage mammalian cells which are more sensitive than their bacterial counterparts. With the use of a modified HP 550C as bioprinter, soy agar and collagen hydrogels as biopaper and Chinese Hamster Ovary cells and embryonic rat motoneurons as bioink, viable mammalian cells were printed.

Suspended cells were printed in circular patterns onto the hydrogel-coated coverslips. Over the next few days the cells were studied under epifluorescent microscopes to determine whether or not the thermal printing process proved lethal. Green fluorescent light was observed, leading to the conclusion that the cells survived the stresses of printing. In addition to monitoring cell growth with advanced microscopy, an assay was performed to measure the percentage of lysed cells. When a cell undergoes lysis, an enzyme called LDH is released. By determining the amount of LDH present, the percentage of cell lysis was measured to be less than 10% in all cases, and  $3.3\% \pm 3.7\%$  on average. The reason the cells were not damaged and killed by the temperatures near  $300^{\circ}\text{C}$  is because the heat does not have time to spread through the cells suspended in liquid. The droplets of bioink are printed so quickly that most of the cells do not experience a substantial rise in temperature (Xu et. al., 2005).

## **Inkjet Printing of Neurons Results in Viable Cell Structures**

A lot of research is being devoted to the generation of nervous tissue because most neuronal cells have very low rates of regeneration. In the previously recounted study it was demonstrated that over 90% of cells can go through an inkjet printer and avoid lysis. The physiological



properties of those printed cells were examined in an innovative study. Although cell viability has previously been proven, this study aimed to determine whether cells that have been printed can retain their function. The cells involved in this study were rat primary hippocampal and cortical neurons. Although the temperature and other stresses of bioprinting largely do not affect printed cells, they may affect the electrophysiological properties of neurons. An example of a neural property that might be affected is the ability to fire action potentials.

A modified HP 550 inkjet printer was used to deposit the bioink in a circular pattern onto a collagen gel based biopaper. Axon and dendrite regeneration were demonstrated using immunostaining using MAP-2 as a dendritic marker and NF150 as an axonal filament marker. Immunostaining showed that the hippocampal and cortical neurons had regenerated all axonal and dendritic processes. This had been a concern- that the neurons would lose their neuronal phenotypes through the printing process. That would be very worrisome because the neurons could turn into other cells types like glial cells or cancer cells after losing their own cell phenotypes. After two weeks, the patch-clamp method was used to measure various electrophysiological properties including firing thresholds, repetitive firing, and after-hyperpolarization. This is an electrophysiological technique that studies multiple ion channels in excitable cells such as neurons and records their voltage currents. Results showed that the membranes of the cortical neurons contained mature voltage-gated potassium and sodium channels. In addition, no significant differences in electrophysiological activity were observed between regular hippocampal neurons and those that had been printed. Both cortical and hippocampal neurons were found capable of initiating action potentials. As is the case with mammalian cells, the retention of functionality after printing is due to the incredibly fast timeframe exhibited in thermal inkjet printing. The neurons were also not vulnerable to the shear and pressure of the inkjet printing because they cells had been trypsinized. That meant that the printed cells had no internal architecture and were not damaged by the shear stresses. Had the cells been affected they would have experienced either apoptosis or heat shock. Because the cells retained their

function, it can be inferred that neither effect took place. Once these tests were administered on the single-layer neuron structures, 3D structures were printed. Fibrin gel was formed by printing thrombin droplets over layers of fibrinogen. NT2 neurons were printed layer-by-layer with the fibrin gel. High resolution SEM was used to examine the fibrin scaffold, and determined that it was well suited to serve as a scaffold for neurons because of its porous microstructure, allowing nutrients and oxygen to be delivered easily to the neurons within the scaffold. Another advantage that fibrin has over other hydrogel is the strong affinity of neurons for fibrin. Because the neurons attach strongly onto the fibrin scaffold, cell signaling is kept intact and cell functions are carried out. This study examined both 2D and 3D printing of neurons and demonstrated neuron viability and retention of cell phenotype and electrophysiological function (Xu et. al., 2006).

### Laser-Assisted Bioprinting of Skin Substitutes

Once a person experiences an extensive burn injury, there are a limited number of options for their rehabilitation. If the wound is large, skin grafts cannot cover the entire area, due to their finite nature. There are a number of clinically approved skin substitutes like Integra and Matriderm which serve as either permanent or temporary wound coverage. These options leave scarring, discoloring, absence of hair follicles and can lead to other damaging side effects as well. Therefore, tissue engineering of skin substitutes is under high demand. Many challenges stand in the way of fabricating skin, due in part to the many cell types which need to be arranged in a very specific pattern. Furthermore, the functions of the engineered skin are greatly affected by the microenvironment of each cell type. A recent study demonstrated that a skin substitute could be created using laser-assisted bioprinting. The different cells types involved in the engineering process included human osteosarcoma cells, mouth endothelial cells, human osteoprogenitor cells, rodent olfactory ensheathing cells, human endothelial cells and human adipose derived mesenchymal stem cells. The cells were mixed with a collagen hydrogel before printing.

Twenty layers each of fibroblasts and keratinocytes were printed on top of a layer of Matriderm using laser printing technology. The Matriderm layer was im-

portant because it helps keep the printed skin constructs more stable during transplantation. The 3D skin constructs were incubated overnight, and then pieces were punched out and transplanted into the skin fold chambers of 12 mice that exhibited full-thickness wounds. In addition to this in vivo approach, the 3D constructs were cultivated in vitro as a control group.

The mice reacted well to the treatment, showing no discomfort or inflammation as a result of the transplantation procedure. In addition, after 11 days, the transplanted skin substitute and the surrounding mouse skin had fused completely together. There were no sharp lines delineating the border between real and substitute skin, and while the substitute skin was shiny at first it became matt as time passed. The keratinocytes and fibroblasts had been labeled before printing and implantation so that extensive tests could be administered even after transplantation. Results of these assays showed that the keratinocytes formed a stratified layer of tissue on top of the fibroblasts and Matrigel much like an epidermis. Although this epidermis was thinner than the natural mouse epidermis, after 11 days the two completely fused together. The thinner epidermis in the substituted skin might pose a problem because it is less stable than the epidermis of natural skin, but the methods of the study can be modified in the future to amend that flaw. In this study it was also shown that fibroblasts formed a multi-layer sheet of tissue. Some remained above the Matrigel where they had been printed and secreted collagen, while others spread through the Matrigel layer.

A skin construct that is incredibly similar to native skin was successfully printed using LaBP. The cells survived the bioprinting process without their phenotype being impacted in any way. One major advantage of the bioprinted skin substitutes is that blood vessel formation was observed in the skin constructs. Fast vascularization is imperative so that the cells can receive oxygen and eliminate cell waste. Complete vascularization was not achieved, but the authors assume that the issue was due to the time constraints of the study and that complete vascularization of the skin substitutes needs more time to be carried out. Despite this setback, this study demonstrated that blood vessels branched from the wound site and spread through

the skin substitute very quickly, which is of the highest priority among engineered tissue (Michael et. al., 2013).

### **Effective Microvasculature Fabrication Using Inkjet Bioprinting**

In the previously recounted study, a skin substitute was fabricated using laser-assisted bioprinting technology. Despite the fact that skin is a very complex organ, it is one of the few successfully engineered tissue constructs. Because skin is relatively thin, vascular tissue can either grow from the native skin and migrate through the skin construct, or nutrients and wastes can diffuse through the engineered tissue to and from the vasculature of the host's native tissue. Vasculature is one of the main challenges in tissue engineering because cells cannot survive without pathways for nutrients to be delivered and cellular waste to be eliminated. Another study detailed the use of inkjet bioprinters in the creation of human microvasculature. An HP 500 thermal inkjet printer was modified so that human microvascular endothelial cells and fibrin could be printed simultaneously. HMVEC are the only cells with the ability to form capillaries, and also have the unique property of adjusting their number and structure based on their micro-environments. Fibrin can be used in many ways- fibrin can be produced by the blood and plays a part in natural wound healing, fibrin gels are used as adhesives during surgery, fibrin glue can be used as a skin graft, and fibrin has been utilized extensively in tissue engineering. In this study, fibrin gel was used as a biopaper substrate for the HMVEC to be printed onto, and it was polymerized by combining varying concentrations of fibrinogen, thrombin and calcium. After the printed construct was incubated, a scanning electron microscope was used to facilitate the examination of the microstructure of the fibrin. Its mechanical properties were tested as well using an MTS electromechanical testing system. Results showed that the fibrin gel scaffold underwent only minor deformations as a consequence of the bioprinting process. A Live/Dead Viability/Cytotoxicity Kit was used to stain the HMVEC so that the formation of microvasculature could be observed and analyzed. After only 7 days, proliferation of the cells was detected and a confluent lining of cells was formed after



21 days. The microvasculature exhibited tubular structures which is consistent with the channels and tubes usually formed by endothelial cells. This demonstrates that thermal inkjet printers can be used to successfully fabricate human microvasculature which is fully functional (Cui, Bolland, 2009).

### Challenges in the Way of Organ Printing

Before any of the technology and methods proposed above can be implemented, one of the first steps to be done in organ printing is creating an organ blueprint. It is a computer-aided design that uses computer software to create a three-dimensional model. The software program then directs the bioprinter to deposit each biocomponent layer-by-layer. The challenge with organ blueprints is that they need to account for the post-printing processing that the 3D printed construct will undergo as a result of tissue fusion and maturation (Mironov et. al., 2008).

Many studies have experimented with various bioprinters, biopapers, and bioink in order to optimize the bioprinting process. In addition to further improvements in these areas, bioreactors are an important component of the bioprinting process. Bioreactors are commonly used in tissue engineering but there are specific properties that bioprinting necessitates. A bioreactor enables the post-processing step, probably bioprinting's most crucial step, to occur. After a tissue construct or organ is printed, the cells need time to fuse together and assemble a functional 3D construct. The bioreactor needs to be integrated closely enough with the bioprinter that the fragile printed constructs can be placed in its sterile conditions without incurring damage. The bioreactor also needs to allow perfusion of the vasculature in the printed construct. It takes time though before the vascular system is developed so the bioreactor also needs to provide a temporary irrigation system. This can be achieved using porous needles with pressure controlled dripper systems that can provide the wet environment that the tissue needs for its development. When the vasculature is sufficiently developed, the irrigation is terminated and perfusion of the vascular tree commences (Mironov et. al., 2011).

The last step in the bioprinting process is post-processing, and as was previously mentioned, it is probably

the most crucial one. That is why biomonitoring procedures must be created and applied. It is important to monitor the tissue maturation and the kinetics of tissue self-assembly. In addition, maturogens that aid and accelerate post-processing and tissue maturation are necessary. Maturogens are biological, chemical, or physical factors and procedures that effectively ensure that the printed cell constructs become a fully-functional three-dimensional organ (Mironov et. al., 2008).

### The Feasibility of Organ Printing

There are three major phases in the organ printing procedure: preprocessing, processing, and post-processing. Preprocessing involves the development of an organ blueprint or alternate CAD. Processing refers to the actual printing of cells onto a substrate, forming a 3D construct. Post-processing concerns the fusion of the cells, the perfusion of the vasculature and tissue maturation. Many studies have been recounted throughout this paper which address every aspect of the organ printing process. Obviously much advancement must be made in every aspect of bioprinting technology before it can be applied to organ printing, but the feasibility of using bioprinting technologies to print an organ is strongly indicated by the groundbreaking scientific research that has inundated this field in the recent years (Mironov et. al., 2003).

### Conclusion

After reviewing the scientific data related to bioprinting, it is safe to say that there is currently no way to successfully print a fully functional organ. But that is not to say that bioprinting isn't a viable method for organ regeneration. Bioprinting is a science that is less than two decades old and as a result, the technology and mechanisms are not advanced enough at this stage in time. The research that has been reviewed in this paper demonstrates though that every aspect of the organ printing process is being tackled and is a work-in-progress.

So much success has been achieved in so few years and there is definitely a long way to go. Each study brings forth an important piece of the enormous puzzle that is bioprinting. There are obviously many revisions to the experiments and advancements to the technology that must be undergone before organ printing can make the

leap from the lab to industrial-level production. Nevertheless, the viability of organ printing is affirmed by the enormous amount of progress and success in the bioprinting field.

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