3Drenal offers an alternative and a solution to the challenges of costly kidney transplants. Using a 3D bio-printer, 3Drenal replicates the functionality of the kidney through mechanisms of cell fusion and organogenesis. Unlike other 3D printers, which layer material to form solid objects, bio-printers construct organic structures by combining polymers and cells. 3Drenal uses a structured scaffold to establish the architecture of the kidney and then layers this structure using print heads containing proteins, organic fillers, and stem cells. These stem cells, which are taken from the patient to ensure kidney acceptance, differentiate into cells of various renal structures. Differentiation is achieved through use of natural signaling proteins to stimulate the stem cells. The goal of 3Drenal is to increase personalization of the kidney—and eventually other organs—while limiting costs and greatly increasing production efficiency.
PRESENT TECHNOLOGY

3D printing has been around for a fairly brief period of time. Three-dimensional printing was developed in the 1990s by Emanuel Sachs from the Massachusetts Institute of Technology. This method first created a 3D computer model of a desired object. These blueprints would then be sent to the printer to be constructed. Other types of 3D printing processes have been developed over the years including multi-jet modeling, selective laser sintering, and fused-deposition modeling.¹

In recent years, biological printing has attempted to solve the large deficiency in organs for transplant. It has also addressed the issue of rejection due to incompatible blood types by using the patient’s own cells. Medical researchers have realized the feasibility of printing out genuine tissues and cells with bio-printers and are extending their vision to a future of mass-produced printed organs. The main goal of many researchers is to someday be able to supply surgeons with various types of tissues that they can have on demand, and even take the cells of patients and print fully functional and implantable organs with them.²

Scientists have printed individual cells as well as blood vessels and cardiac tissue, but have not yet been able to print an entire organ. In 2003, Makoto Nakamura became one of the first researchers to create a 3D structure with living cells using inkjet printer technology. He successfully built a tube of living cells about 1 millimeter in diameter which contained double walls of two different types of cells. Gabor Forgacs of the University of Missouri in Columbia printed blood vessels and sheets of cardiac cells that fused into tissue after 70 hours. After just 90

¹ Barnatt. 3D Printing Explaining the Future.
hours, these fused cells began to beat like regular heart tissue. Forgacs and his team are currently hoping to strengthen the muscles of printed blood vessel tubes so that they are resilient enough to be grafted onto natural blood vessels, specifically ones that are narrower than 6 millimeters.³

Forgacs’ extensive work was first made possible in December 2009 when Invetech, an innovator in the biomedical, industrial, and consumer markets, delivered the world’s first production model 3D bio-printer to Organovo, a large company which had made Invetech its technology development partner in May 2009. As of 2010, and continuing into 2011, Invetech has delivered a number of 3D printers to Organovo, who have supplied researchers around the world with printers so they can explore tissue repair and organ replacement through bio-printing.⁴ The immensity of this advancement was recognized by Time magazine when it named Invetech’s bio-printer as one of the “50 Best Inventions of 2010.”⁵

The basic bio-printer has two print heads. One prints bio-paper, which is the sheet of material upon which the printing will occur (usually collagen, gelatin, or hyaluronic acid). The other contains bio-ink, which is composed of thousands of different cells that are dispensed in spheroids. Spheroids are used because of their liquid-like nature, which allows them to easily fuse together and form solid, rigid structures using processes witnessed in natural organogenesis.

Recent studies have linked bone marrow stem cells to the development of renal structures. Studies conducted in 2001 by Imasawa et al. have shown the ability of hematopoietic and mesenchymal stem cells to repair glomeruli and tubules in damaged kidneys. A 2002 study by Iwano et al. exposed damaged mice kidneys to bone marrow stem cells, mixed with a cocktail of fibroblast-specific proteins (proteins that play a role in tissue regeneration). They showed that

³ Marris. How to Print Out a Blood Vessel.
⁵ Kluger. 3D Bio-printer-50 Best Inventions.
these proteins work in tandem with stem cells to stimulate differentiation into renal structures, remodeling damaged kidneys.\textsuperscript{6}

HISTORY

Organogenesis is the process by which cells form organs. In humans, organogenesis usually begins around the fifth week of the fetus's development. It requires the natural affinity of cells to fuse together and reassemble in predictable relationships, a phenomenon discovered in 1955 by Johannes Holtfreter. This discovery was further supported by Malcolm Steinberg’s discovery in 1996 of differential adhesion, describing the natural grouping of different types of cells into patterns.

Organogenesis makes use of stem cells, which differentiate into specialized cells of the body's organs. A study conducted in 1960 by Ernest A. McCulloch and James E. Till confirmed the existence of two major divisions of stem cells: adult and embryonic. Most adult stem cells are limited by the types of cells they can differentiate into. However, researchers in the 1950s discovered that the adult stem cells in bone marrow can be further classified into hematopoietic stem cells and bone marrow stromal stem cells (mesenchymal stem cells), each of which differentiates into different types of cells in the body. This allows for a wider range of specialization when using bone marrow stem cells as opposed to various other types of adult stem cells.\textsuperscript{7} In 1999 and 2000, through experiments on mouse tissue, scientists found that manipulation of tissues could lead to different cell types; that is, bone marrow could be altered to produce nerve or even liver cells.\textsuperscript{8}

There are currently only two treatments for kidney disease: dialysis and transplantation.

\textsuperscript{6} El Nahas. \textit{Renal Remodelling}.
\textsuperscript{7} NIH. \textit{Stem Cell Basics}.
\textsuperscript{8} UK Stem Cell Research. \textit{History of Stem Cell Research}.
The process of dialysis uses a machine known as a dialyzer that performs the kidney’s filtration function, clearing dirty blood of wastes. Dr. Willem Kolff is thought of as the father of dialysis because he constructed the first artificial kidney (dialyzer) in 1943. In the 1960s, Dr. Belding Scribner created a small, portable dialysis machine for those suffering from chronic kidney disease. This revolutionized the medical field, but dialysis still currently remains a time-consuming and unpleasant process.

A second method for treatment of kidney disease is organ transplantation. Kidney transplants were first performed on animals, the first of which was completed by Alexis Carrel on a chicken. Joseph Murray conducted further transplant research using dogs and eventually discovered a technique for kidney transplantation in humans, the first of which was performed in 1954. Although transplantation is a more permanent and sustainable option for treating kidney disease, it is costly and the list of donors is much shorter than that of the patients.

FUTURE TECHNOLOGY

3Drenal is a 3D bio-printer that will utilize biological applications of 3D printing, as well as methods of tissue construction, organogenesis, and manipulation of adult stem cells and signaling proteins in order to construct a transplantable kidney that will be compatible to the individual. In addition, 3Drenal will incorporate a self-sustaining mechanism to check for error in kidney construction by mimicking renal pathways.

The fundamental infrastructure of 3Drenal involves use of the bio-printer in conjunction with layering and scaffolding to accurately replicate the architecture of the kidney. Since the kidney is structurally composed of many branches and tubules, the use of a hollow, cylindrical scaffold would yield a functioning kidney. The two print heads of the 3D bio-printer and the process by which they build the organ are integral to the formation of the kidney. 3Drenal
employs a method that builds the organ from the bottom-up, layer by layer. By alternately placing layers of bio-paper and spheroids of bio-ink on top of one another, the cylindrical shape of the kidney tubes can be outlined, and the next step of the process can then begin.\(^9\)

The process of organ printing using 3Drenal involves use of the natural tendency of cells to fuse together. After the initial shape of the tubule has been formed, each individual spheroid undergoes internal changes and reorganizes itself in accordance to cell adhesion. Following this, the newly organized spheroids fuse together to form the structure of a kidney tube. Once multiple tubes, branches, and tissues have been formed using the 3D printer, it is necessary that they then be fused together to construct a functional kidney. It is here that the natural kidney development process can be utilized.

Adult bone marrow stem cells, extracted from the kidney recipient, will be placed within the scaffold to differentiate into the constituents of the kidney. Their flexibility allows a more readily available and more versatile supply of stem cells for differentiation. This would also ensure that the blood types of the individual and the printed organ would be identical and there would be no immune reaction to the new organ. In order to stimulate the development of the stem cells, various proteins will be dispensed from print heads. The proteins interact with the stem cells and promote their development into specialized renal cells.

The two most important tissues in the formation of a kidney are the metanephrogenic mesenchyme and the ureteric buds. The metanephrogenic mesenchyme and the ureteric buds reciprocally interact with one another to create the kidney. When the buds emerge from nephric ducts, they enter the mesenchyme. This in turn causes loose mesenchymal cells to create epithelial aggregates, which will eventually differentiate into the nephrons of the kidney. At the same time, as the metanephrogenic mesenchyme differentiates, it induces the ureteric buds to

\(^9\) The Engineer. *Building Body Parts with 3D Printing*
Elongate and branch out. These branched buds will eventually form renal collecting ducts and the ureters that carry urine from the kidney to the bladder.

These reciprocal processes require the presence of many different signaling factors that instruct certain tissues to perform specific jobs. The metanephrogenic mesenchyme tissue, for example, can only further differentiate into nephron tissue. The factor that is thought to regulate this behavior is known as WT1, and any cells without this factor that are uninduced will die. Additional factors that are crucial to the proper development of the kidney are fibroblast growth factor 2 (FGF2) and bone morphogenetic protein 7 (BMP7). These factors are secreted from the ureteric bud and are necessary to prevent the mesenchyme cells from undergoing apoptosis (programmed cell death) by converting them into stem cells. These stem cells then have the ability to either form new nephrons or produce stromal cells (which produce factors that allow continued growth of the ureteric bud and enable further differentiation of the nephron).\(^\text{10}\)

Once 3Drenal has printed out various kidney cells, the cells will fuse naturally to form these fundamental renal tissues. The tissues will be able to synthesize the necessary signal factors and undergo the natural developmental processes of the kidney. This will ensure that the resultant kidney is both functional and long-lasting as a transplanted organ.

Simultaneous to kidney organogenesis, 3Drenal will be supplemented with a process of checking for erred construction. 3Drenal will expose the kidney to a cocktail of substances which will be stored in the print heads and released periodically throughout the kidney's development. These substances will be chemicals the kidney deals with to complete its bodily functions. In order for a kidney to function, it must interact specifically with solutes in the plasma, filtering wastes, reabsorbing nutrients, and secreting leftover waste from the plasma. All of these wastes are excreted as urine, which moves from the kidney to the urinary bladder. The plasma then must

leave the kidney through the renal vein. By validating that all of these molecular interactions occur as they do in a natural kidney, 3Drenal maps out any faults in the kidney's synthesis.

A plasma similar to blood will be poured where the renal artery would be situated after transplantation. In accordance with the process undertaken in the actual organ, the plasma will contain nitrogenous substances, dissolved carbon dioxide, water, glucose, and salt. In order for the kidney to function, it needs to separate these solutes from the plasma through filtration, uptake nutrients through reabsorption, and secrete wastes into the filtrate. After building a portion of the kidney's architecture, 3Drenal will take a micro-sample of the urine produced as the plasma is exposed to the nephrons of that section of the kidney. This sample will be tested to detect any irregularities, such as too much glucose in the urine. The test mechanism will ensure that the kidney is able to carry out its intended excretory functions. By detecting which substances are expelled as waste and which are kept in the plasma, 3Drenal is able to assure maximum competency.

BREAKTHROUGHS

In order for 3Drenal to construct the kidney, and ensure its functionality and efficiency, several technological breakthroughs are necessary: the identification and implementation of stem cell-signaling proteins, and the ability to maintain stability of the constructed kidney, while remodeling poorly constructed parts that are detected through the checking mechanism.

The adult marrow stem cells that will be used for 3Drenal require specific signaling proteins to initiate their function. Through the system for kidney genesis, the stem cells will interact with these proteins in order to differentiate into the desired tissue. However, due to the specificity of these proteins, it is necessary to isolate them and carefully distinguish between

their different functions in order to produce a more controlled environment for stem cell differentiation. In addition, these proteins need to be isolated from their natural environments through protein purification and stored in the printer's print head, a process that is complicated by the sheer number of specific proteins that are required for proper stem cell specialization. Furthermore, many of these proteins have yet to be pinpointed and their exact functions remain unknown. This would necessitate further exploration into the numerous types of proteins in existence, research that could take scientists many years.

Another breakthrough necessary to the success of 3Drenal to construct a viable kidney for transplant is the complete maintenance of functions corresponding with the built renal structures. Due to the complexity in their construction, some structures may fail to carry out their proper functions. Though the process for checking the kidney for errors detects these issues, it is unable to destroy the damaged area and rebuild the dysfunctional part. Without the ability to fix any defective structures, the whole process would need to be repeated, which is both time-consuming and costly.

To prevent 3Drenal from having to restart its entire kidney construction, a mechanism to accurately and efficiently rebuild would have to be installed. It would have to be able to destroy a small portion of the kidney, and subsequently dispense more appropriate stem cells and the corresponding proteins to re-develop that region. This would require the ability of 3Drenal to both locate the deficient site in the kidney and then proceed to destroy that part alone. It would then have to repeat the printing process for just that area, an innovation that would involve intelligent programming of the bio-printer to recognize the site of repair, as well as a method of narrowing down the area of layering and printing.

DESIGN PROCESS
Various approaches were considered for the optimization of 3Drenal’s functionality and effectiveness. Although there were merits to many of these ideas, there were ultimately either flaws in the processes or existing alternatives that would be much more beneficial for the purposes of our designed plans.

Germ Layers of Gestation/Embryonic Development

Using the differentiation of different germ layers to create myriad various organs was considered for 3Drenal to be able to accurately construct organs based on the system of embryonic development. Since the germ layers form during embryonic growth and play a crucial role in the development of all the organs of the body, the organs could be formed more naturally, with optimal structure and function. This posed problems, however, due to the excess of organs it would create, as 3Drenal would develop the constituents of an entire organism. In addition, simulating exact embryonic conditions and the germ layers of the embryo would be unfeasible, as well as highly complex, within a 3D printer. Instead of making matters simpler using the bio-printer, this simulation would only complicate the organogenesis and present further risks and difficulties.

Pancreas Transplant vs. Kidney Transplant

The pancreas, relative to the kidney, is one of the simpler organs in the body because it is limited to endocrine and exocrine functions. It is also an extremely important organ. The pancreas produces insulin and glucagon and it secretes pancreatic juices which help with digestion in the small intestine. These functions are crucial to the workings of humans. The pancreas was considered as a potential organ of production for 3Drenal because of the numerous difficulties in treating pancreatic cancer. Patients who have undergone surgery are likely to survive no more than five years and the cancer frequently reappears after surgery. An implanted,
healthy pancreas would be a great stepping stone in extending the life span of people diagnosed with pancreatic cancer.

The kidney, on the other hand, while more complex, could potentially lead to greater breakthroughs in medicine and benefit more people suffering from kidney-related diseases, such as chronic kidney disorder (CKD). Scientists have already been able to print blood vessels and because the nephron of the kidney is tube-shaped like blood vessels, it proved to be a more viable and applicable option for a printing process still in its early stages of development. Due to their similarities in shape, the advances made in production of blood vessels could also be then applied to the nephron. For the most part, the kidney is a more practical, resourceful, and rewarding choice to focus on for organ printing and transplantation. Successful kidney printing could go a long way in increasing the quality of life for tens of thousands of patients everywhere.

*Embryonic Stem Cells vs. Adult Marrow Stem Cells*

Embryonic stem cells were first considered as a way of initiating the process of organogenesis, but, ultimately, adult marrow stem cells seemed to be a better choice. Embryonic stem cells are able to differentiate into a large range of cell types, whereas adult marrow stem cells are limited and can only transform into a few types of cells. However, with the use of proteins to guide the development of marrow stem cells into specific structures, previous limitations on differentiation are no longer an issue.

Additionally, there is controversy over the use of embryonic stem cells. Adult marrow stem cells are more readily available, and thus would be a better option in the long run for a process that in the future will ideally take place rapidly and efficiently. The adult stem cells can be taken straight from the individual requiring the transplant, ensuring that there will always be some sort of supply of them. This also eliminates the risk of rejection of the kidney after
transplantation.

CONSEQUENCES

3Drenal allows patients who are suffering from kidney disease to more easily and rapidly receive transplants for damaged kidneys, just by giving a sample of their bone marrow. This would be much faster than waiting for a donor. When a donor is necessary, a person in need is put on a list and must wait for an indefinite period of time to receive an organ. Then, there is the complication of finding a perfect match, without which the body will reject the new organ. If hospitals can print organs, many more patients will be healed with organs made from their own DNA on time.

3Drenal also frees many patients, especially children, from the burden of painful and tedious dialysis, and also allows them to lead more productive and longer lives, with fewer limitations on diet and fluid intake. Kidney failure strikes about 11.5 percent of adults ages 20 or older and has a number of causes, including diabetic or congenital kidney disease and glomerular disease.\footnote{National Kidney Info Clearinghouse. \textit{The Kidneys and How They Work.}} With 3Drenal printing kidneys in a fast, efficient process, more kidneys would become available to the population. This would mean less time and money wasted on dialysis treatments and greater mobility and energy for those affected by kidney disorder.

3Drenal opens the doors to other types of artificial organ construction. By applying the same mechanism with different materials, virtually any of the body's organs can be built from scratch. This allows for a revolutionized medicinal process for transplants. Patients with disorders that affect a single organ can be treated, as well as patients with afflictions affecting whole organ systems. Though this would require many advances in the development of the bio-printer and stem cell research, it would be able to personalize the process of organ
transplantation, all the while allowing for the eventual mass-production of organs.

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