Genetically Engineering and Bioprinting to Create Organs

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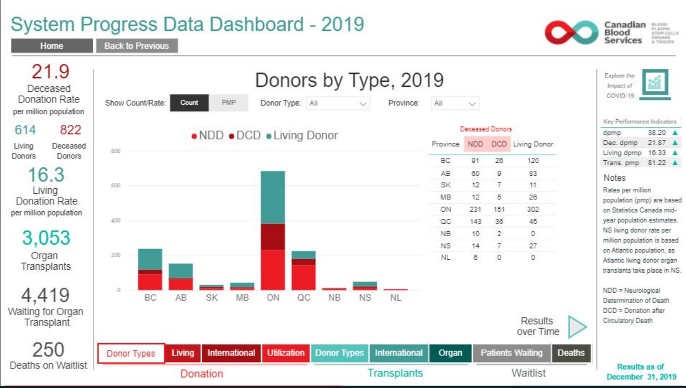
Problem

Many people have dysfunctional organs and they require an organ from a donor. If you look at the chart below you see that many provinces don't have the organs necessary for treatment. This problem increases mortality rates by 42% because there are no willing people that will donate the necessary organs required for treatment meaning that horrible decisions will have to be made for who gets to live from the low amount of organs available. With this new way to create organs we will be able to ensure that everyone waiting for an organ will be able to get the treatment needed for their survival. In 2019 alone 250 people died in Alberta just because there are not enough donors. Although some people strive to help persuade people to become donors we will need a backup plan just in case there are not enough donors in the future.



Method

First we can farm our own stem cells or have a willing donor donate stem cells instead of taking whole organs. Then we will have two choices, to use genetic engineering (CRISPR) or we will use artificial environments to change the state of the stem cell to turn it into the cell that is needed to build the organ. So basically if you use genetic engineering you would use a special protein called Cas-9. Cas-9 is a protein that can be found in many bacteria, what it does is constantly check the bacteria's DNA (Deoxyribonucleic acid) to make sure that it hasn't been infected by an organism such as a bacteriophage. Let's say that it did, what it would then do is cut only that piece of infected DNA (Deoxyribonucleic acid) and replace it with a healthy strand of DNA. This exact method will be applied to changing stem cells. If a stem cell is about to change naturally it would change a piece of DNA and change it so that it slowly changes into a cell such as a tissue cell or an epithelial cell (The lining cell of the lungs and many other organs). Once we have enough stem cells we will be able to create the organ such as a liver or a heart before the previous organ fails.

The Process

Naturally:

- Then if we choose to manipulate the stem cells naturally we would have to make artificial environments
- To create an environment we will have to simulate a similar environment of which the cell would be in if it were to become some type of specialized cell
- But when it is a stem cell it is in it's DNA to keep on replicating itself so that it can create an organ
- Eventually it would become used to these environment changing itself or creating a daughter cell that specializes in something different by becoming a specialized cell

Using Genetic Modification:

- If we choose to use genetic modification then we will have to acquire a Cas9 Protein
- Then we will have to study what makes a stem cell change into the specialized cell that we want
- Once we find the gene that makes a specialized cell such as a brain cell the gene that makes it what it is we will replicate it using a synthetic biological circuit or the CRISPR
- (A synthetic biological circuit is an application of synthetic biology in which the biological parts in a cell are being used to and designed to perform logical functions)
- While we wait to study the gene the stem cell will naturally replicate itself creating many more stem cells and daughter cells
- Once we find the specific gene we will replicate it and put them in Cas9 proteins
- What will then happen is that we will replicate gene that keeps a stem cell what it is so that it can cut that part out to replace it with the gene that will turn into a specialized cell

How can Genetic Engineering be Applied to Organ Creation

Genetic engineering can be used to create organs by taking stem cells or organs from other animals and modifying them. If we are to take an organ from an animal we will have to make changes depending on which animal we take it from, currently scientists say that the best organ to harvest from are pigs. This method of organ transplantation is called xenotransplantation which is when organs, tissues and blood is harvested from a different species. There are many problems with harvesting organs from other animals such as immune defenses size and molecular incompatibility and much more, all of these problems lead to xenograft rejection. But through the advancements in genetic engineering it is now possible to prevent all problems of incompatibility between the domestic pig and a human. Currently there are several techniques to safely transplant the organ of a pig to a person each of the following will be listed: pronuclear and cytoplasmic microinjection , somatic cell nuclear transfer (SCNT) and viral transduction of DNA. The techniques listed above help prevent xenograft rejection and safely transplants the organ from the domestic pig to a human. The Sleeping Beauty microinjection technique is a germline transgenesis (transference of a DNA segment to give the gene to an offspring) but instead it will be used to inject the gene into stem cells to create the desired organ. Another reliable source is the piggyBac microinjection for easy and efficient genomic insertion. The piggyBac system uses the exogenous DNA (DNA originating outside of the organism of study, it is usually partially completed around dead cells) and micro injects it into embryo's which will automatically multiply when DNA is inserted. Cas9 is the protein that we will be using to make specific gene modifications to make small adjustments in the genomes. Currently there are 4 known ways in which the body will reject the organ in xenograft rejection: Hyperacute Rejection (HAR), Delayed Xenograft rejection (DXR), Acute Cellular Rejection (ACR) and Chronic Rejection (CR).

How Can Organs Be 3D Bioprinting

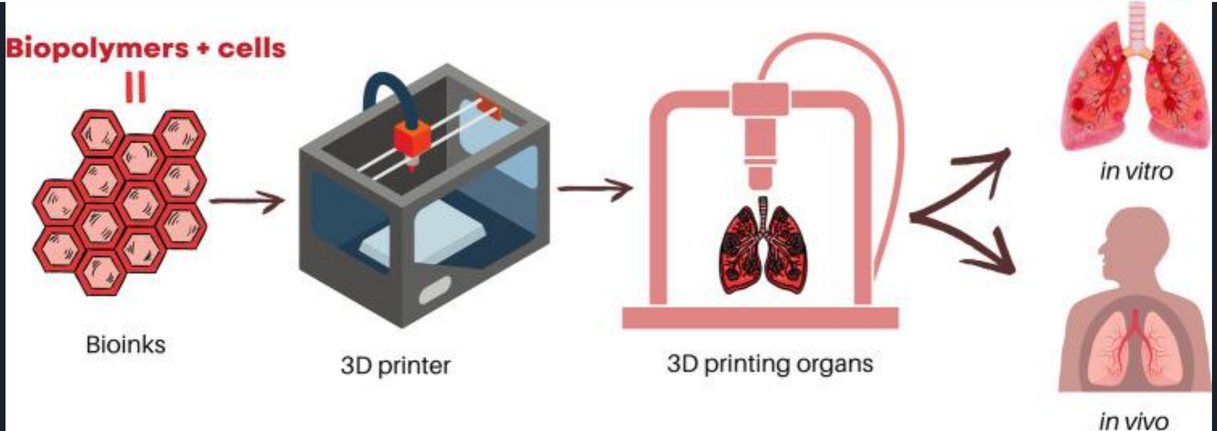
Printing organs is called bioprinting, this is because instead of using plastic the specialized printer is programmed to create an environment in which an organ will be preserved while in the process of creation. This is a very advanced and complex technique that has been improving over the past few years. 3D printing has led to a very big footprint in medical sciences because it could be used for testing and research of diseases on actual tissue. Many other solutions to things such as creating bio materials such as biomolecules. Every little detail has an essential part in how the organ will turn out, the needle of the 3D printer itself is less than 1 millimeter in diameter. Human body's themselves are very regenerative but are restrained by various factors such as growth hormones, different tissue types, the body's resources, physical size and age. If you look at the diagram above you will see that biopolymers are required, biopolymers are substances created by living or biotic organisms. Like many polymers, biopolymers are made by molecules called monomeric units, monomeric units are just chemicals created by molecules that bind together to form a repeated or unique pattern that becomes a biopolymer. A bioink which is shown in the diagram above is a combination of biopolymers and cells that has been chosen for organ creation because of it's excellent biocompatibility. The bioink mostly is composed of cells but the biopolymers are added to make the body think that it is real tissue. In the diagram there are two arrows sprouting to in vitro and in vivo, in vitro means testing outside of a living organism. With in vivo meaning

The process of organ creation and testing for inkjet:

- A biopolymer or synthetic polymer is used and loaded into the 3D printer
- The needle is incredibly thin for high precision
- A piezoelectric or a thermal (the property of certain bodies to be electrically polarized to create an electric field or potential under the action mechanical stress) actuator would be needed to create an evaporated ripple that will let the bioink droplets to flow through rather than having it clogged
- After programming the 3D printer to create a certain organ it will take time for it to be completed
- To guarantee that a organ will work you can use the methods of in vitro meaning that you test the organ functions outside of a living organism or in vivo meaning that you test the organ inside of a living organism

The process of organ creation for extrusion method:

- For the extrusion method the bioink is loaded into the 3D printer for the needle to extrude
- A pneumatic or a screw would be use to slowly extrude the bioink droplets in a certain order to create an organ
- Mainly high viscosity bioinks are used in extrusion bioprinting

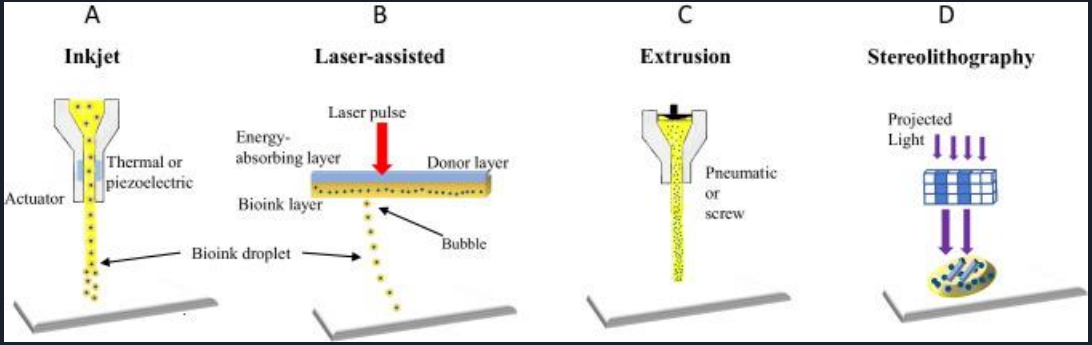


The process of organ creation for laser assisted method:

- A few layers would be needed to protect the bioink and keep maximum precision
- An energy absorbing layer (donor layer) would be needed to absorb the impact of the laser
- A separate bioink layer will be put under the donor layer or energy absorbing layer
- The donor layer only protects the impact and not the heat so the bioink layer slowly bubbles release the bioink but still keeping ultimate precision
- This technique permits high resolution deposition of where the bioink droplets will be in solid or liquid phases
- this photo polymerization method can modulate laser assisted bioprinting of biomaterials as well as a variety of different cells without affecting the cells viability and functionality
- The laser pulse keeps on going until the organ is complete

The process of organ creation for stereolithography method

- Layer by layer a photosensitive polymer layer is applied
- The layers are then solidified by using an ultraviolet light eventually creating a multiplex structure in the end
- The organ format to build off of is made by real organ scans such as Magnetic Resonance Imaging (MRI), Computed axial tomography (CAT scan) and Computed tomography (CT scan)
- For printing different pieces of organs such as alveoli and lungs, this is fixed by using different nozzles to create micro or nano details vital in the alveoli



Using Artificial Environments

This may not be very understandable because it hasn't actually been tested and was just an idea that appeared when thinking of organ creation. Using Artificial environments to create organs has both it's pros and cons, one pro is that it could facilitate more safety in organ creation, a con is that it could completely backfire and that the organ are dysfunctional. How this works is that stem cells are going to be placed in an environment that will synthesize and simulate the environment that the desired cell would be in and experience. After a while the stem cells will evolve into a specialized cell. Some environments such as a high nutrient environment might cause the stem cells to turn into a red blood cells, and high protein environments could create muscle cells. The easiest organ to create is a heart because it is mostly composed of muscle cells and there are barely any chemical reactions. To create a heart the stem cells will have to be shaped to create the 4 chamber and all of the veins and arteries to perfectly align with the recipient's body. The heart can be shaped by using a Da Vinci surgical system, which is an incredibly precise machine that is used for intricate and complex surgeries. With the heart shaped it can now be put into the solution where the stem cells will gradually change and evolve into the specialized muscle cell. With the heart completely finished it can now be used but first for the heart to work it will have to be hooked up to the neurological system so that the brain is able to control the heart and make it beat and spread blood throughout the body. A pro is that this would be quite efficient and would be cheaper than 3D bioprinting and genetic engineering along with xenotransplantation.

The End