3D Printing Human Tissue

A Heart for Frankenstein?

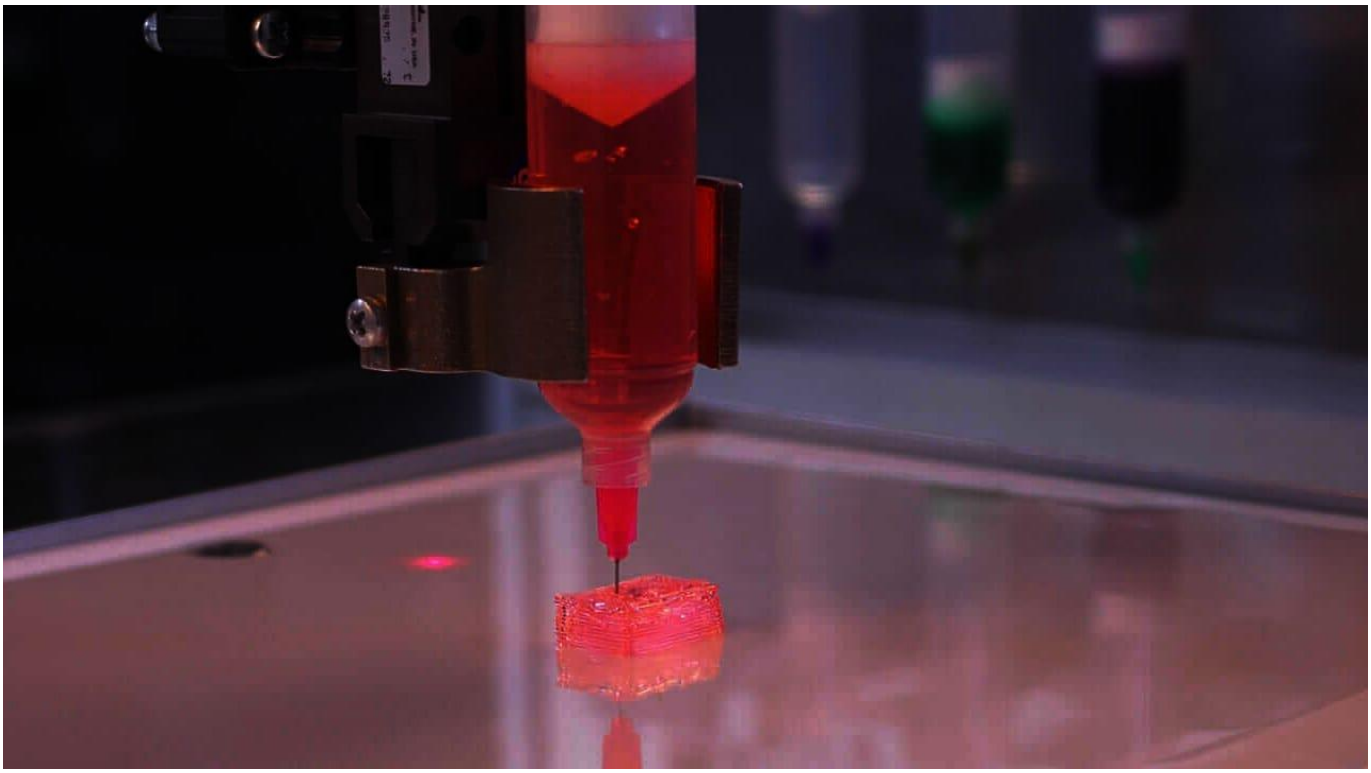

Fig1: 3D bioprinter

A Term Paper

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1. Preface

I find the concept of 3D bioprinting of taking a simple idee and turning it into complex living system compatible with the complex human body fascinating. To have the means to use a technology that evolved from the printing press, to the 2D printer, to the 3D printer and finally into the 3D bioprinter is mind blowing. The benefits to society from mass education, advertisements and prosthetic limbs to potentially printing organs in the future are of great significance in many different aspects of life. It is a good example of the great advances humankind is capable of making in a short period of time. I find the hopeful future this technology has with further development and the advances that have already been made motivating. It's a technology that is still relatively new and thus there is a lot of research yet to be done. There are many ideas on how to use it and phantasies about stretching it to all kinds of complexities, which may or may not be realized. Only time will tell.
Still there are some questions I have that I am convinced the great amount of research that has already been done and extensive experiments that have been carried out will be able to answer. Questions such as what the recent research being done is and what we can realistically expect to see in the future.
How similar 3D bioprinting is to 3D printing with synthetic materials.
What scientific and ethical issues the technology is faced with.
And why it's so groundbreaking.

2. Introduction

As with any biological technology their true roots lie at the beginning of time and have been able to be realized not merely through their inventors but through the knowledge, they based their creations upon, which again are built on the advances of those who went before them. The most mentionable recent breakthroughs in science that have paved the way for the technology we now call 3D bioprinting however are the realization of cells as the building blocks of the human body as well as the technological advancement that has given rise to computers.
It's never hard to find a reason for a technology to be used. 3D printing is no exception with fields such as regenerative medicine, cosmetic surgery and transplantation being interested in improvements to preexisting techniques.
Organ transplants for one are in short supply and thus the thought of being able to grow them in vitro is of great significance. Not only are the waiting list for getting an organ transplant very long and in some cases lethal, but it is also an altogether difficult process considering transportation, preservation and the immune reaction from the host's body.
Being able to fabricate a replacement skin is significant in the beauty industry as wells as the cosmetic industry, working against products getting tested on animals. The Animal testing done, presents us with results that are not of 100% accuracy due

to the physiology of humans and animals differing in parts, leaving room for more efficient technologies.
It is plain to see the great demands 3D-printing is faced with while it still is only at the start of its development, so it is important that our curiosity continues to lead us.

3. History

Charles “Chuck” Hull, an American inventor and engineer, introduced the population of 1983 to stereolithography, the first ever 3D-printing process that works by adding layer upon layer of chemical monomers and oligomers and applying light to create polymers through a photochemical process. Using computer-aided design (CAD) software, necessary information on an object’s desired surface geometry could be sent by file to be expressed via printer. Though groundbreaking, the objects produced with this method were not long-lasting due to the lack of robustness of the first materials used as ink. Thus, this methodology ~~it~~ was solely used for models of objects that would still rely on traditional techniques to make the actual finished product. The scientific world took to this still very alien technology and so the industry grew and research from many different scientists fueled the development of the new ink materials that surfaced in the 1990s. These new, more durable materials revolutionized the function of 3D-printing so much so that the fabrication of finished products became possible. Around this time the desire to apply the basic technology of 3D-printing to the field of biology and medicine started to increase. The notion of potentially being able to print a working human organ being a major driving force behind a significant amount of research into bio inks. The idea was to very simply replace the conventional inks with biological materials capable of imitating the human body. Viable techniques and processes were eventually found and in 1999 the Wake Forrest Institute for Regenerative Medicine were able to use the technology to print a synthetic scaffold of a human bladder. It was later coated with a patient’s own cells marking the first successful growth of a working organ. In 2002 the printing of a miniature kidney followed which, in an animal model, was capable of blood filtration and urine production. 2010 marked the first ever blood vessels made, so it is fair to say that this technology has made some great advancements in the last 60 years and hopes to see many more in the future of tissue engineering for the many fields eager to profit from this technology.

4. The Process of 3D Printing Human Tissue

4.1 Overview

3D Bioprinting, a type of additive manufacturing, uses biomaterial also known as bioink, containing living cells, to create structures that mimics the function of those found in the human body. A dispensing unit extrudes living cells according to a digital blueprint onto a substrate, forming a specific 3D structure by placing the material layer by layer as in conventional 3D printers.
There are 3 basic steps in the process of 3D bioprinting: preprocssessing, processing and post processing.

Preprocessesing entails the preparation for the actual printing. Digital models, a sort of blueprint, of the structure at hand are made using CAD. Computed tomography (CT) and magnetic resonance imaging (MRI) are used to make personalized scans. These are sent to a computer program in a file to analyze its geometry and generate series of thin layers of the structure, which are compressed into a file and then sent to the 3D printer.
The file must not only include information on the movement path according to the geometric structure of each layer but must also give instructions on the control of aspects such as extrusion pressure, frequency and temperature of the bioink.
Once the printer has received the necessary instructions via digital file the next step can commence. The processing in which the structure is physically printed by the 3D printer.
After the printing the structure is typically transferred into a designed chamber (in vitro), comparable to an incubator for a prematurely born child, where cell proliferation, maturing and tissue remodeling occur in the final steps of creating living tissue.

4.2 Bioink

A very significant part of 3D bioprinting concerns the bioink itself. Due to the immense range of different tissues found in the human body, of which there is still limited knowledge, it is unlikely that there will ever be one universal bioink but many individual ones differing in their composition according to their make-up.
The make-up of a bioink is largely decided by a compromise between printability, biocompatibility and mechanical property.
Printability concerns itself with material viscosity, the physical state of the material and other such parameters to understand a materials capability of keeping its structure during and after printing.
Biocompatibility examines the microenvironment of the body in an attempt to mimic its make-up, concerning itself with location of cell proliferation, where the cells differentiate, where they communicate and so on.
The mechanical properties focus on ensuring enough stability to support the cell culture and for the implantation process. A lack thereof could cause degradation of the cell culture or an implant failure.
Ironically these factors seem intrinsically contradictory, a higher viscosity, which facilitates printability, for example simultaneously makes the bioink less suitable for biocompatibility.
This means a very precise compromise between the three factors is necessary for a viable bioink, which comprises a large part of the research connected to this field.
Most bioinks are composed of living cells and an additional carrier material.
Generally, hydrogels are used, which are biopolymers capable of holding water, that play an important role in mechanical stability.
Deposition of the bioink into a temporary support medium is one way to enable the printing of intricate shapes as well as hollow structures, while still meeting the criteria for all factors. Other approaches deal with the “sol-gel” property of gels, meaning their ability to shift between liquid like and solid state depending on the conditions they are in.
There are many different approaches on how to successfully extrude bioink into a 3D shape this also includes a variety of different printing technologies. There are three

main categories of 3D printers used: Droplet-Based Bioprinting, Extrusion-Based Bioprinting and Laser-Based Bioprinting.

4.3 3D Bioprinters

Many parameters influence what a 3D bioprinted structure ends up looking like. Nozzle diameter/method of extrusion/movement speed/extrusion speed and temperature of both environment and bioink are examples of the many things under consideration and careful regulation when bioprinting.
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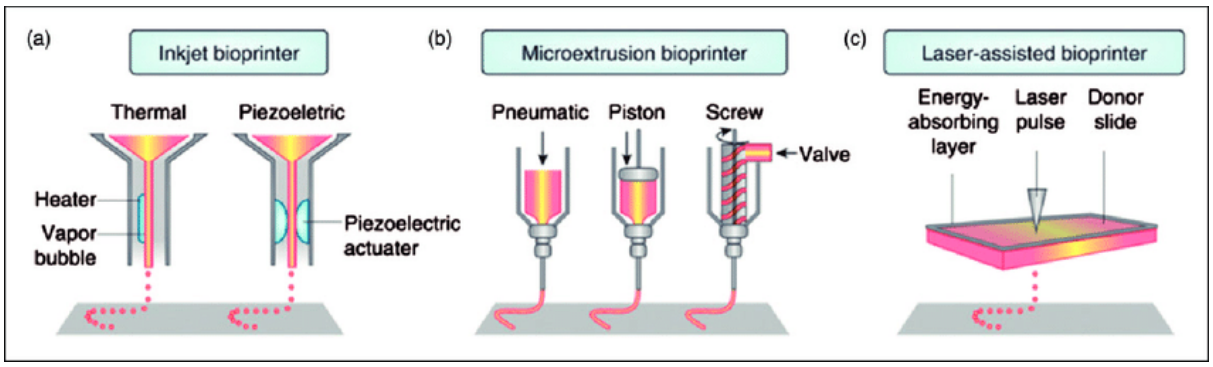

Fig2: Examles of different Bioprinters

4.3.1Extrusion-Based Bioprinting

Extrusion-Based bioprinting is the 3D bioprinting method most commonly used in the field.
It works by extruding cylindrical filaments of biomaterial onto a substrate and layering the filaments on top of each other to form a 3D structure, which is solidified by chemical or physical means. The displacement of bioink is driven pneumatically or mechanically while the biomaterial exits the syringe, analogous to a printer cartridge, through a nozzle.

4.3.2 Doplet-Based Bioprinting

In droplet-based (inkjet bioprinter) bioprinting the displacement of bioink occurs in independent droplets and is regulated by thermal, acoustic or electric energy. The independent droplets contain the living cells. Many closely placed droplets form a layer with additional layers being added vertically to attain the desired 3D structure.

4.3.3Laser-Based Bioprinting

Laser-Based bioprinting includes three main components: a pulsed laser source, a cartridge and a receiving substrate. The cartridge is made up of a glass plate on which a film of ink and a thin layer of a laser absorbing metal

like titanium are located. The pulsing of the laser beam causes the absorbing metal to vaporize leading to the formation of high-pressure bubbles that eject the bioink into the receiving substrate. The amount of biomaterial displaced is controlled by the ejection conditions (e.g., energy and viscosity).

5. Interview

The interview was held in German. This is a rendering of the interview from my notes in English.

Fig3: Dr. Martin Rimann

Dr. Martin Rimann

Dr. Markus Rimann graduated with a degree in Biology in 2005 from the ETH Zurich.
Since 2011 he has been working at the Zurich University of Applied Sciences (ZHAW) in a Tissue Engineering Team.

How is tissue actually printed and is it already fully functional when it is printed?

The printing of tissue is done by means of additive manufacturing processes, where a specific 3D structure is built layer for layer with matrices coming from different nozzles. It is possible to combine different matrices, also known as bio inks, to achieve more realistic 3D structures, that are as similar to those found in the human body as possible.
When the structure is first printed it is not yet considered a tissue. The matrices extruded from the nozzles already contain the cells that will make up the tissue and align them in the according order. When they are first printed however the cells do not yet interact and function as one single organism like they would in the body. The next step occurs in an incubator, where the cells mature. This is where they grow and link together to form what is considered to be a tissue.

What kind of cells are used in the process of printing these tissues and where do you get them from?

Primary cells can be used in the process of recreating tissue, which are obtain for example from cosmetic surgeries, however there are certain limitations that these cells have beside the fact that they are difficult to access. Research is going more and more in the direction of using Stem cells in tissue engineering processes, however though stem cells an ideal base from which the tissue can grow, one of the few sources for these stem cells are embryos, which poses ethical problems. The use of embryonal stem cells means the killing of embryos, which debatably means killing one human being to save another.
There is however a promising alternative method which is able to make induced stem cells by reprogramming fibroblasts by means of a retrovirus. Stem cells are ideal due to their ability to differentiate into many different specific cells that can be

used in the fabrication of tissues. The differentiation of the stem cells is caused by the extra cellular matrix (ECM), that contains growth factors.

What are some problems the technology faces? For example, what is done with overhanging structures?

The biggest challenge 3d printing encounters is finding suitable bio inks. Not only do these materials have to be sturdy enough to be printable and hold their shape but the cells have to be compatible. The relationship of the two factors is difficult to perfect and the main challenge lies in optimizing this ratio. As for overhanging structures, it is possible to approach this issue by printing supporting structures or printing the 3D structure directly into a mould containing a viscous gel, that is later removed when the structure has hardened.

What about printing actual organs, is it realistic?

It is one thing making a tissue or cartilage, but vascularized structures are very different.
They rely on blood vessels to transport oxygen to and waste products from the structures. This blood vessels are approximately 200μm wide which is not anywhere near what can be achieved today with placeholder substances. These structures are very densely vascularized which is necessary for sufficient blood flow but makes it an even greater feat.
The technology for 3D bioprinting functioning complex organs still requires extensive research and I believe it is not realistic anytime in the near future, if at all.

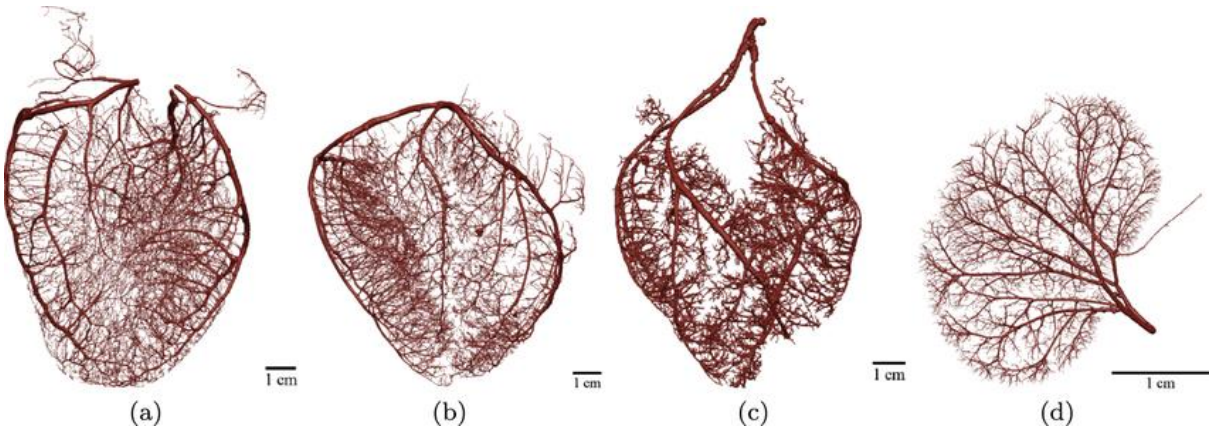

Fig4: The blood vessels of he heart

6. Discussion

6.1 Bioprinter Comparison

	Advantages	Disadvantages	Cost
Extrusion-Based Bioprinting	Is capable of printing high cell densities which is favourable to tissue formation. It is also capable of using slightly viscous bioinks which is favourable both to the printability and mechanical properties. Is capable of printing various different compounds	The high pressure used to eject the bioink can cause the cellular structure to deform which can affect the structures viability and cell compatibility. Lacks in control and resolution. Prone to clogging.	medium
Droplet-Based Bioprinting	Is widely available, prints at a high speed and is not as expensive as the other two options. It is also rather simple and capable of precise control. Compatible with many different biological materials.	Prone to clogging. Biological material needs to be in a more liquid forms than with the other methods.	low
Laser-Based Bioprinting	Is a nozzle free method of printing so there is no issue with possible clogging. Exceeds the other two methods considerably in precision and resolution. Is also capable of creating a structure with great cellular density. High reproducibility.	Laser can induce cytotoxicity. The preparation of the cartridge is laborious. It is also more expensive than the other two methods.	high

6.2 Advantages of 3D bioprinting

3D bioprinting offers a fast and robust method by which tissue can be constructed in vitro. It is capable of printing the necessary microarchitecture for desired

macrotissues while insuring mechanical and structural support are sufficient, a wide range of different bioinks for different types of tissue are available and the opportunity for implanted tissue to remodel is supplied. Further benefits of 3D bioprinting are the minimizing errors alongside the increased precision due it being largely mechanised and not requiring any work by human hands. The fact that the structures can be made with cultured cells from the patient the cell compatibility rises and lessens the risk of rejection. Lastly, it being greatly reproducible, due to the control and named precision, makes it an altogether good method among other technologies in the field of tissue engineering.

6.3 Disadvantages or problems in bioprinting

One of the great challenges bioprinting is faced with is the make-up of the bioinks and trying to make them compatible with the human body(biomimicry) while still ensuring their stability and correct 3D shape. Another disadvantage is the lack of sufficiency when it comes to the scale that the current techniques are capable to make, while the vascularized systems of the body still lay far out of reach. There is also an overall insufficiency in knowledge on the subject that still makes biomimicry a very time-consuming difficult process.

6.4 Ethical Aspect

6.4.1 Ethical issues

The idea of printing 3D organs seems a great solution to the scarcity in donations available to those in need yet who the technology will belong to and how far it will go are both important ethical questions.
It is an issue that a technology as significant as being able to save people's lives should have to belong to somebody, be patented by somebody. Does this not mean that somebody would be able to decide of another person's life or death would that be this ethically correct? It would be contrary to many religious principles, but it would also disturb the idea that all people have the same chances. Perhaps it would become a business which would plummet humanity even deeper into the issue that money has caused.
Another issue questions to which extent humans want to enhance their bodies and what in future we will define as unhealthy or in need of a donation. Is the goal eternal youthfulness?
The perhaps most obvious ethical issue concerns the source of stem cells. Embryonal stem cells acquired through abortion for example still face the issue of whether or not abortion itself is ethically supportable, still faces the question of whether or not an embryo classifies as a human.
On the other hand, the availability of organ donation would solve the issues on who deserves them most, in a situation of scarcity. It would also improve the human life considerably in many cases, giving people an equal chance to life, which is also in alignment with equality and thus in alignment with one of the most widely accepted basic ethical principles. And lastly a move towards a future without animal testing would take away the ethical issues surrounding inequality between species.

6.4.2 Opportunities and Threats

The availability of complex 3D printed human organs would display an opportunity to people in need of an organ donation, people in need of regenerative medicine after damaged skin cause by burns for example and people who were born missing a body part such as an ear. It would give many people a second chance at life or a chance at an improved life which is something to advocate for.
Additionally, the opportunity for a future in which less to no animal testing had to be done would likely benefit the animals themselves.
The Threats the technology could pose would most likely concern the source of the biomaterial. If organ printing does become a reality the issue of where the biomaterial is sourced will emerged. The possibility of human exploitation for cell donations would put innocent humans at risk, thus posing a threat.

6.5 Recent progress

In 2019 researchers at the University of Carnegie Mellon came one step closer to printing a 3D by introducing a new technique called Freeform Reversible Embedding of Suspended Hydrogels (FRESH). Using this technique, the biomaterial is extruded out of the syringe into a support bath of gel where it is built up layer by layer as with the other 3D printing methods. The difference is that the gel allows for a liquid bioink such as collagen to be used, without damaging the cells. The gel can later melt away when it is brought to room temperature leaving the hardened 3D structure unharmed.

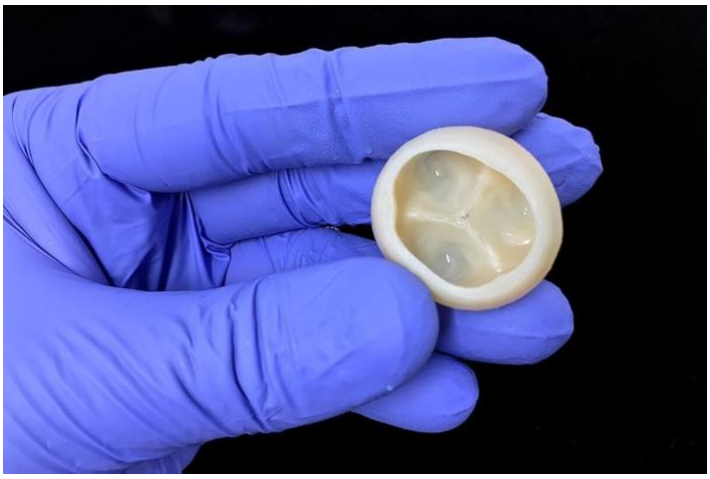

Fig5: Image of functioning heart part from Carnegie Mellon Univeristy

6.6 Human Organs and the future of 3D bioprinting

The actual 3D bioprinting of human vascularized organs such as the liver is a task still greatly regarded as insurmountable due to the sheer complexity they display. They are for example very densely vascularized which poses a problem for stability an issue that 3D bioprinting already has with much larger vessels. In addition, the microstructure of these organs isn't sufficiently documented.
The future of 3D bioprinting hopes to move towards being able to create vascularizes organs that can be used as transplants, for regenerative medicine or even to work towards a future without animal testing. This still requires a great amount of research to be done, effort by scientist and naturally a massive amount of money to be invested in the technology. It requires research on the human body, as it is the inspiration upon which all biomimicry is based, research into bioink and how all vital

factors can be balanced and further advancements in the technology used to print, working on precision and improving cell viability rates.

7. Summary

The paper discusses a rather new topic in the world of tissue engineering. The progress that has been made over the last couple of decades in this field has been immense, going from first very basic attempts to print tissue two dimensionally to being able to use MRI an CT scans to create very accurate layered blueprints for the 3D bioprinter in use today. It is a technology still very much at the beginning of appreciating all the possibilities it has in store. With the rapid developments, improving the bioinks, the 3D printers and the environment in which they are grown, the status of progress is ever changing.

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