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ADVANCEMENT AND FUTURE SCOPE OF THREE DIMENSIONAL (3D) BIOPRINTED HEART

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ABSTRACT

The revolutionization of organ transplantation led to the first three-dimensional (3D) heart with blood vessels. The size of 3D printed organ was reported to be the size of a cherry, according to Israeli professor Tal Dvir of Tek Aviv University in April-2019. Based on activity data for 104 countries in 2008, which represented roughly 90% of the global population, about 100,800 strong organ transplants are performed each year around the world. With 69 400 kidney transplants being the most recent. It is almost 46 percent from living donors and 20,200 liver transplants. There has been a total of 5400 heart transplants, 3400 lung transplants, and 2400 pancreas transplants. After transplantation of organs, such as the kidney, which rejects 25% of the time and the heart, which rejects 40% of the time. Bioprinting organs and tissue patches from a patient's own cells eliminates the need for organ donors and decreases the chance of rejection. This eliminates the issues of organ rejection and long waiting periods for donors.

KEYWORDS: Three Dimensional (3D), Bioprinting, Stem Cells, Differentiation, Bio-Ink, Heart.

1. INTRODUCTION

In this emerging era of technology, a new science fiction story is been made feasible for the printing of an organ. Continuous increase in demand for three dimensional(3D) bioprinting has fueled fresh innovation in the field. Researchers are working to create a future to print a fully functioning heart through the process called 3D Bioprinting. Bioprinting is not as difficult as it looks like, It is also somewhat similar to additive manufacturing methods,

except that it uses the patient's own cells and biomaterials instead of 3D printing, to overcome the problems of organ rejection and long donor waiting lists that plague current surgical procedure techniques such as organ transplantation.[1] One drawback with artificial hearts is that metal and plastic mechanisms may be troublesome to integrate with tissue, or harm the blood due to their unnatural movement vogue. A small team at ETH Zurich/ Swiss Federal Institute of Technology Zurich (German: Eidgenössische Technische Hochschule Zürich), driven by scholarly individual student Nicholas Cohrs, has established what they say is the first soft implant, with its pumping mechanism accomplished by pushing the silicone polymer ventricles to pump like a real heart.[2] The silicone heart that shows a right and a heart ventricle like its real counterpart. however rather than a wall in between the two (2), by an additional chamber they are separated deflated and inflated by pressurised air so as to mimic contractions and pump blood. It's undoubtedly a promising creation that might modification lives, however it's sadly far from ready. When compared to normal heart this silicon printed 3D heart was having more weight, its weight is about 390 grams (approximately 0.86 pound or 13.8 ounces) , based on the series of tests the team conducted, Its latest version is the only one that exists for 3,000 beats, enough to stay somebody alive for thirty to forty-five minutes.[3]

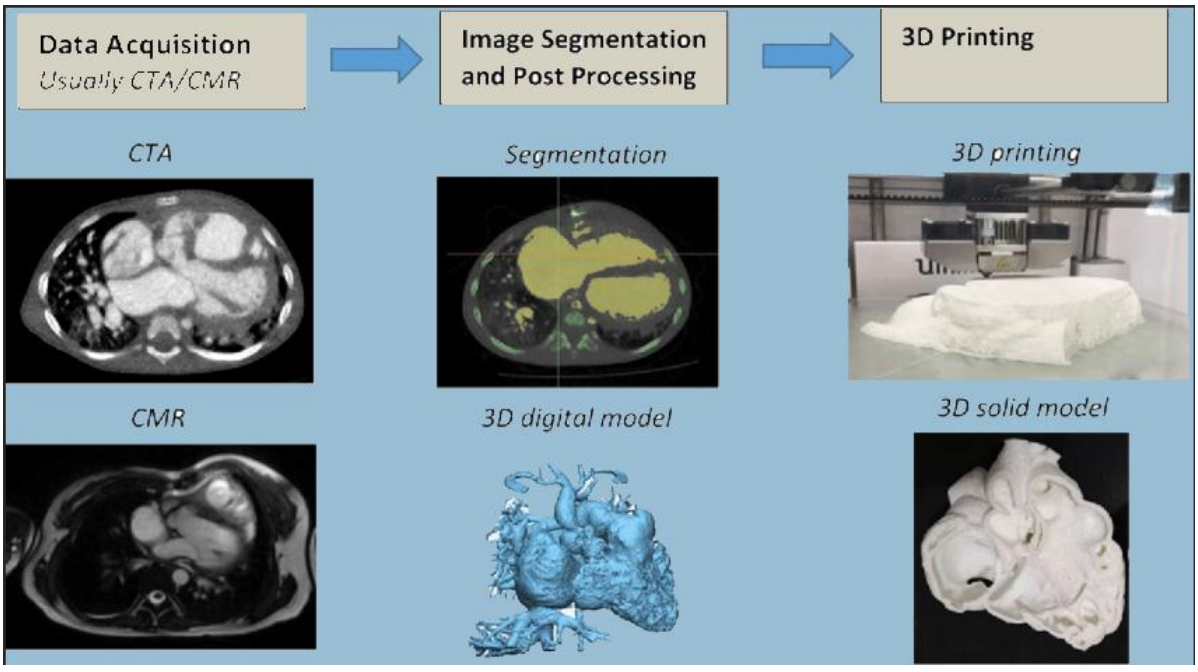

Figure 1*:* Steps include in the 3D printed silicon artificial heart.

Figure 2: ETH Zurich 3D printed heart.

2. History and Background of 3D Bioprinting

3D bioprinting allows for the development of precise 3D cell models and tissue structures, with the development of tissue-like complexity in body-shaped structures. 3D bioprinting, with its high degree of structure and composition, has the potential to address a number of important needs that have yet to be addressed in medical science, Cosmetic testing, treatment availability, recovery, and successful organ implantation are just some of the applications.[4] Stem cells from a patient, such as pluripotent stem cells (iPS cells) or mesenchymal stem cells, can be used to build customized disease models. Various building materials, procedures, and cells may be used to create the desired tissue structure depending on the application.

The Israeli Professor Tal Dvir from Tek Aviv University (April-2019), has revolutionised organ transplantation by creating the world's first 3D printed heart, which is about the size of a cherry and contains blood vessels, collagen, and biological molecules. despite the fact that the cells were able to contract, at the time they failed to have the potential to pump nevertheless. To prepare the heart, scientists used "personalised hydrogel" to create "bio-ink."[5] Charles W. Hull was the first to characterise 3D printing in 1986. with ultraviolet light materials thin layers can be cured which can be sequentially printed as in layers and 3D structure can be formed solid in his process, which he called "stereolithography."[6] 3D printing process is also familiar as additive manufacturing,7 requires layering or even pixel-by-pixel creation of the necessary structure. In 1993, the word "3D printing" was coined to describe work done at MIT(Massachusetts Institute of Technology), which entailed transforming a standard inkjet printer into specialised processing equipment.[7-8] In 3D

bioprinting, process layer-by-layer pinpoint positioning of biochemical, living cells, and biological materials with spatial control of functional components for placement and used to fabricate 3D structures.[9]

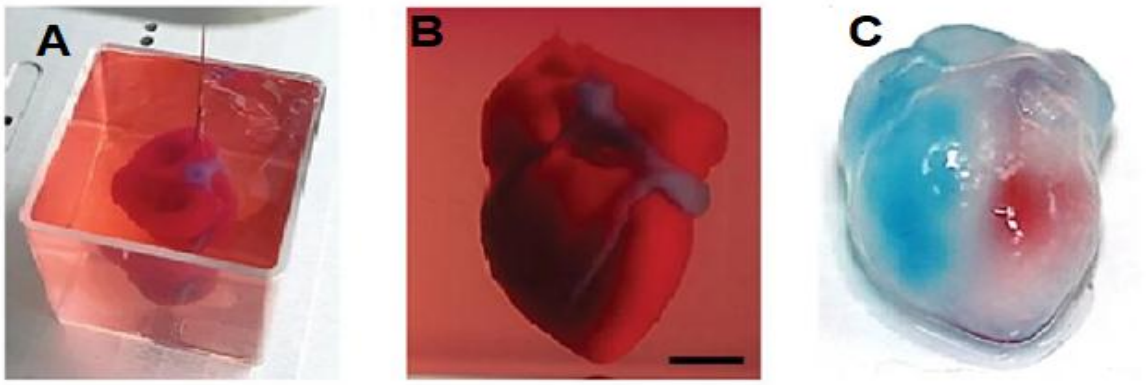

Figure 3: A) printing of 3D bio-heart, B) 3D bio printed heart in the supporting solution, C) completely printed 3D bio- heart by Israel scientist.

In June 2018, the company had managed to bio-print human cardiac tissue, by the start of 2019 it had managed to bio-print varied heart elements, together with valves, ventricles and blood vessels. Steven Morris, chief operating officer at BIOLIFE4D (BioLife**4D** Corporation, Biotechnology company, Chicago, Illinois, United States) adds: "This is an exciting time for BIOLIFE4D, and we are extremely proud of Dr. Birla and the rest of the team for this outstanding achievement. The company's aim is to use bioprinting and mistreatment Using the patient's own cells, researchers were able to build a completely functioning human heart, removing the problems of organ rejection and lengthy donor waiting lists. this can be an enormous milestone for BIOLIFE4D, and one step nearer towards its goal of manufacturing a full-sized human heart feasible for transplant.[10]

3. Brief Details on Bioprinting

Three dimensional (3D) bioprinting allows for the development of precise three-dimensional cell models and tissue structures, with the development of tissue-like complexity in body-shaped structures. 3D bioprinting, with its high degree of structure and composition, has the potential to address a number of important needs that have yet to be addressed in medical science, Cosmetic testing, treatment availability, recovery, and successful organ implantation are just some of the applications.[4] Stem cells from a patient, such as pluripotent stem cells (iPS cells) or mesenchymal stem cells, can be used to build customized disease models. Various building materials, procedures, and cells may be used to create the desired tissue

structure depending on the application. Tissue and tissue 3D bioprinting Cultured cells and a variety of biocompatible materials are combined to make bioinks. For drug testing, modelling, and in vitro transplantation, bioinks can be 3D bioprinted onto active tissue manufacturers is shown in the **figure 4.**

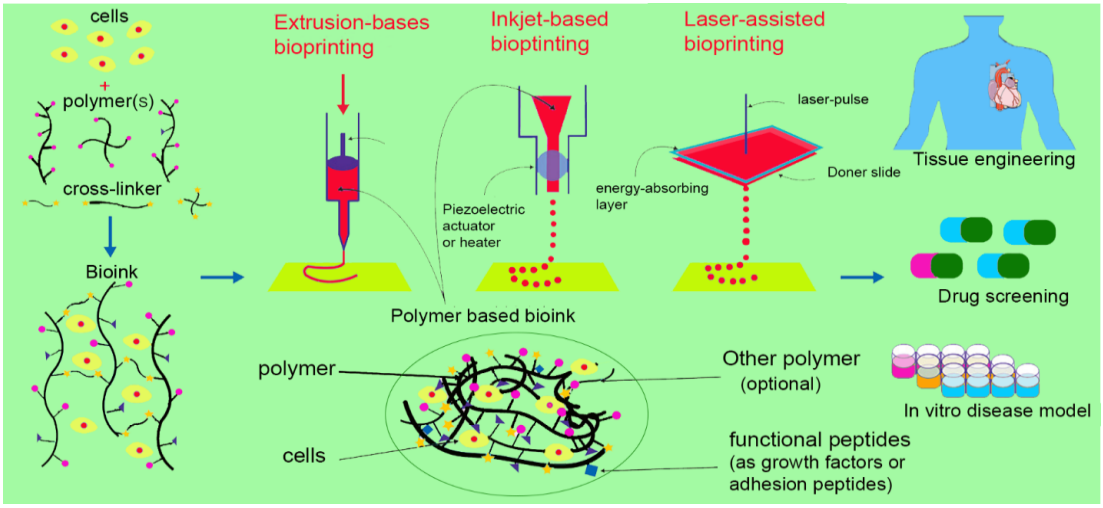

Figure 4: Tissue and tissue 3D bioprinting cultured cells and a variety of biocompatible materials are combined to make bio-inks for drug testing, modelling, and in vitro transplantation.

Preparation, printing, maturation, and implementation are the key steps in the bioprinting process. This can be broken down into three key steps:

- **Pre-bioprinting:** This involves creating the digital model that the printer would use. The techniques used are computed tomography (CT) and magnetic resonance imaging (MRI) scans.
- **Bioprinting** is a printing method in which Bioink is loaded into a printer cartridge and deposition is carried out using a digital model.
- **Post-bioprinting** Post-bioprinting is the mechanical and chemical stimulation of printed parts to create stable structures for biological material.

4. Bio-inks

Bio-inks used in 3D bioprinter with its advantages and disadvantages have been discussed in **Table 1.** To promote cell adhesion, proliferation, and separation after printing, bio-inks include biomaterials and living cells that mimic the external matrix environment. Unlike the materials used in 3D printing, bio-inks should possess following features be:

- Print temperatures not exceeding body temperature

- Low bonding or gelation conditions
- Non-toxic bioactive
- Compounds that can be converted by cells after printing

Table 1: Bioinks used in 3D bioprinter with its advantages and disadvantages.[11]

Bio-ink Material	Advantage	Disadvantage	Overview
Alginate	Crosslinking conditions (Ca2+) are mild. Gelation in a rush Biocompatibility is high.	Degradation kinetics are slow, and cell adhesion is weak.	Brown algae biopolymer derived naturally
Agarose	Crosslinking that isn't toxic High level of stability	It is not biodegradable. Cell adhesion is low.	Polysaccharide extracted from seaweed
Collagen	Biologically significant	soluble in acid	Skin and other connective tissues contain the primary structural protein.
Chitosan	Biocompatibility is high. Properties that battle bacteria	Gelation takes a long time.	Polysaccharide extracted from the shellfish skeleton (e.g., shrimp). Fungal fermentation can be used to make chitosan that isn't derived from animals.
Decellularized ECM	Biologically significant Tissue-specific information a high rate of cell survival	Inconsistent and undefined; lack of native ECM organisation; low stability	A tissue's extracellular matrix when segregated from its native cells
Fibrin/Fibrinogen	Biologically significant Gelation in a rapid	Limited printability	During blood clotting, an insoluble protein is created.
Gelatin	Biocompatibility is high. Solubility in water is high. Gelation that is thermally reversible	Poor shape fidelity; Limited rigidity	Collagen is partly hydrolyzed to create a protein material.
Graphene	Electrically conductive and flexible	Low biological relevance	A carbon-based material that resembles a one-atom-thick graphite layer.
Hydroxyapatite	High rigidity and strength	Low printability; Limited tissue specificity	Calcium apatite is a naturally occurring calcium mineral that is present in teeth and bones.
Hyaluronic Acid (HA)	Cell proliferation is aided by rapid gelation.	Poor stability	Connective, epithelial, and neural tissues all contain large amounts of non-sulfated glycosaminoglycan.
PCL/PLA/PLGA	High rigidity and strength	Cell adhesion and proliferation are poor.	Biodegradable, thermoplastic polymers and/or copolymers
Pluronic® F127	At room temperature, printable Material that has been shear thinning	Long-term cell culture is not suitable.	Block copolymer of poly (ethylene oxide) and poly (propylene oxide) (propylene oxide).

5. Materials used for printing of 3D bioprinted Heart

Cells used in heart printing

- HUVEC/Neonatal Cardiomyocytes
- Mesenchymal Stem Cell/iPSC Derived Neurons

Bio-ink material used

- Alginate/GeIMA
- Graphene/PLGA

6. The stem cell process and stem cells

A) Embryonic Stem Cells: Human Embryonic Stem Cells (hESCs) and, more recently, induced pluripotent stem cells (hiPSCs) produced using 3D bioprinting technology have ushered in a new era of cardiovascular science and therapy possibilities.[12–14,15]

B) Very Small Embryonic-Like Stem Cells (VSELs) are a new type of pluripotent stem cell that has shown promise in recent research.[16]

C) Nuclear Transfer Stem Cells: Somatic cell nuclear transfer (SCNT) is a technique for transporting nuclear material from one cell to another, which was first discovered in 1996, has now advanced to the point that it can generate NTSCs. SCNT begins with the implantation of a donor nucleus (also known as a nucleus donor) into an enucleated oocyte from another fully differentiated somatic cell (such as a fibroblast) (i.e., egg donor or cytoplasmic donor with removed nucleus). The donor nucleus is then genetically reprogrammed by the new host egg cell. In single cell culture Several mitotic divisions result in the development of a blastocyst, which is around 100 cells in an early-stage embryo. The finish result is a clone of the nuclear donor, like original organism it has almost identical DNA. Genotypes and phenotypes regulate the cloning of the nucleus donor, whereas the cytoplasmic or egg donor maintains phenotypes and genotypes in this new entire living organism. This process can be used to make therapeutic as well as reproductive clones. Dolly the cow, the first effective mammalian reproductive clone, was performed in Scotland, United Kingdom, in July 1996.[17,18,19] A total of two dozen other animals have been cloned so far.[20]

D) Reprogrammed Stem Cells: Reprogramming technologies have advanced dramatically since Dr. Shinya Yamanaka and colleagues first developed iPSCs in 2006. This is particularly valid for in vitro and in vivo direct reprogramming methods that use lineage-

specific transcription factors, small molecules or chemicals, and RNA (Ribonucleic acid) signal modifications to generate specific tissue-lineages. These direct approaches avoid the iPSC level, yielding more specific cells like induced neural progenitor cells (iNPCs), which are closer to the target cell lineage, such as subsequent motor neurons and neural cells. Any manual laboratory device for reprogramming the genetic signals of primary cells can be used to make Reprogrammed stem cells (RSCs), but not the SCNT process. iPSCs have emerged as a potential solution to hESCs' ethical and immunogenic problems. Since iPSCs are derived from adult somatic tissues, and hiPSC sources including blood, skin, and urine are abundant, this is the case. Furthermore, since hiPSCs can be acquired from particular immune rejection of the patients, can be prevented when they are autologously transplanted (self-donor). As a result, hiPSCs hold tremendous promise in the field of personalised medicine. iPSCs can be obtained from a variety of places. Almost every mature cell type in the human body, Umbilical cord blood cells, bone marrow cells, peripheral blood cells, fibroblasts, keratinocytes, and even urine cells can all be reprogrammed into iPSCs and then segregated into tissue-specific cells of desired lineages.[21-22]

E) **Adult Stem Cells:** Adult stem cells sparked a lot of buzz when they were first discovered because of their potential for translational applications, but there are still some doubts regarding their clinical usefulness. Adult stem cells derived from particular organs, such as the brain, heart or spinal cord, may be a promising new cell therapy option. The survival, quiescence, and activation of stem cells in adult organs tend to be dependent on specific signals in their microenvironment, according to study.[23]

7. In iPSCs, Pluripotency and Genomic stability are maintained

A wide range of things influences, including the cell's external environment (environmental epigenetics) and genetic structure (genotypes), may have resulted in previously unknown phenotypes. Exogenous epigenetic pathways, such as histone modification and DNA methylation, may be used to produce long-lasting changes in gene expression and thereby affect phenotype.[24]

PSCs' unregulated self-renewal, chromosomal stability, and pluripotency are all dependent on telomere length maintenance. In addition to telomerase, which plays an important role in telomere maintenance, there are many other pathways for telomere lengthening that are

related to epigenetic modifications and genetic recombination. Pluripotent epigenetic reprogramming necessitates telomere repair. It's real.[25]

8. BIOLIFE4D Phase Cell Differentiation

In the BIOLIFE4D's planned bioprinting method, a patient's own reprogrammed stem cells play a healthy, non-controversial, and critical role. Differentiation, a developmental genetic process, assists in the smoothness of this re-programming.[26]

Since every cell in the human body has the same number of genes and DNA, new research has shown that any cell can be "reprogrammed" and converted into nearly any other cell. Previously, stem cell research was restricted to cells originating from human embryos, which presented a moral and ethical quandary for many; however, this is no longer the case. Embryonic stem cells will not be used in the BIOLIFE4D process.

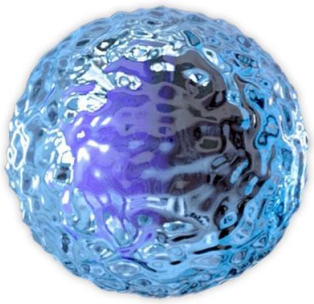

Figure 5: Mature adult specialised cell.

Dr. Shinya Yamanaka, a Nobel Laureate in stem cell research, discovered in his lab in 2006 that injecting a few genes into mature adult specialised cells using a chemical technique produced mature adult specialised cells. For example, blood cells and fat cells can be reprogrammed into iPS cells[12,16] which are adult stem cells that have been converted from advanced adult cells and behave similarly to adult stem cells . This innovation paved the way for medical advancements like BIOLIFE4D's 3D bioprinting processes, which are being developed and optimised.

Regardless of origin, these iPS stem cells have three characteristics: they may divide and rebuild themselves, they are unspecialized, and they may distinguish into tissue or organ-specific cells with unique roles in certain situations. IPS stem cells, in other words, may be reprogrammed to generate various cell types, such as cardiomyocytes (heart cells).[13]

iPS cells will be redirected into organ-specific cells in the BIOLIFE4D phase through a process known as differentiation, which refers to the transformation of one type of cell into several types of specialised cells.[26]

9. 3D Bioprinter manufacturers and their models

Table 2: Different developer, model, type and origin of country for the 3D Bioprinter manufacturers.[27]

S. no	Manufacturer	Model	Type	Country of origin
1	Envision tec	3D-Bioplotter	Extrusion-based	Germany
2	Regen Hu	3DDiscovery	Extrusion-based	Switzerland
3	Poietis	NGB-R, NGB-C	Laser-assisted	France
4	Cellink	Bio X6	Extrusion-based	United States
5	Allevi	Allevi 1, 2, 3	Extrusion-based	Sweden
6	Rokit Healthcare	Dr. Invivo	Extrusion-based	South Korea
7	Inventia Life Science Operations	Rastrum	Ink-jet	Australia
8	Organovo	NovoGen MMX	Extrusion-based	US
9	Aspect Biosystems	RX 1	Extrusion-based	Canada
10	3D Bioprinting Solutions	Fabion	Extrusion-based	Russia

10. Different 3D Bioprinters pictures used in bioprinting

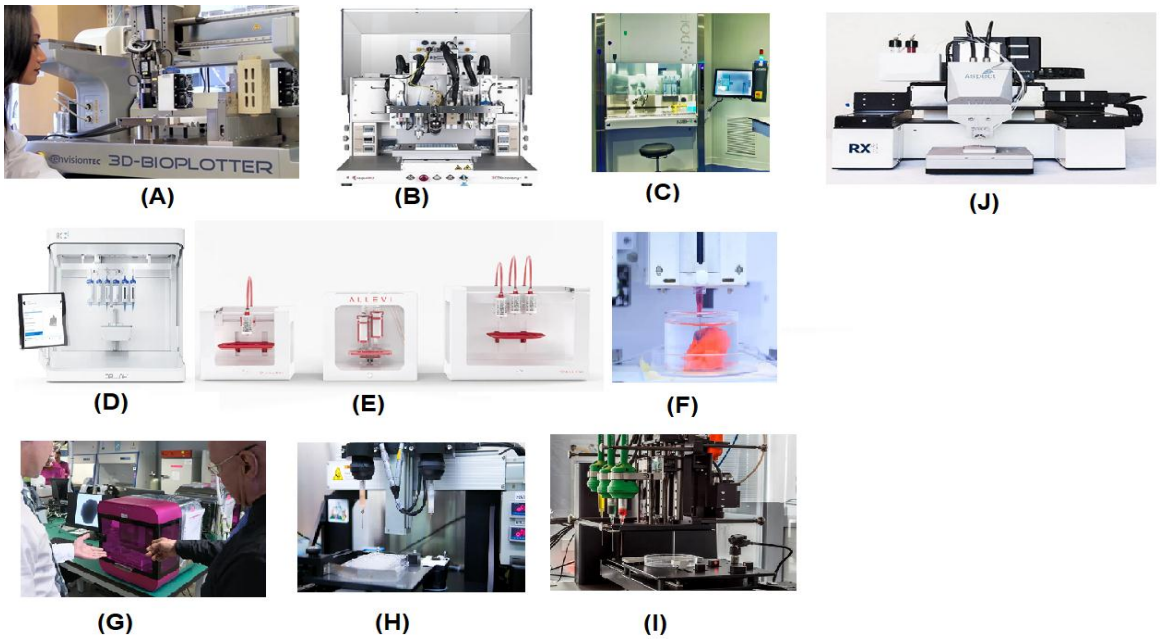

Figure 6: (A) EnvisionTEC bio-Printer, (B) RegenHU bio-printer, (C) Chronicle bio-printer, (D) Cellink bio-printer, (E) Allevi bio-printer, (F) Rokit Healthcare bio-printer, (G) Westpac Wire bio-printer, (H) Bioprintable bio-printer, (I) Bioprintable bio-printer, (J) Aniwaa bio-printer.

11. The BIOLIFE4D approach — A Step-by-Step guide

1. Magnetic Resonance Imaging (MRI) tomography image

An Magnetic Resonance Imaging (MRI) scan will be done on the patient, as well as a blood sample.

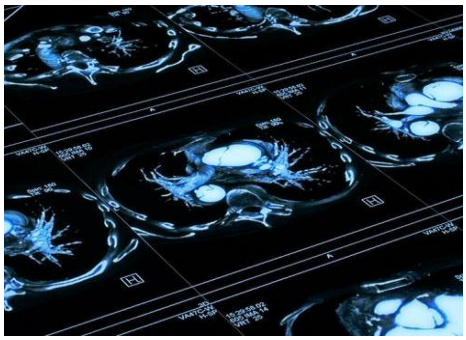

Figure 7: Magnetic Resonance Imaging (MRI).

2. Blood cell

Each human cell can be converted into another since they both have the same number of genes and Deoxyribonucleic Acid (DNA). The blood cells will be transformed into unspecialized adult induced pluripotent stem cells (iPS) in the second phase of the process, which can then be transformed back into the specialised cells of our choosing.[28]

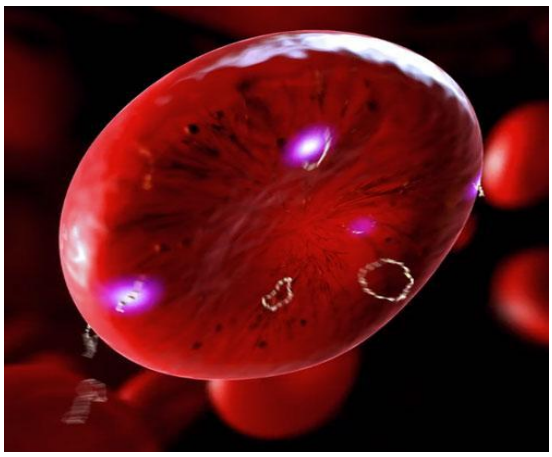

Figure 8: Artist's impression of blood cell.

3. Cell differentiation

iPS cells can be transformed into almost any form of specialised cell in the human body, including cardiomyocytes, through a process known as differentiation. (cells of the heart)

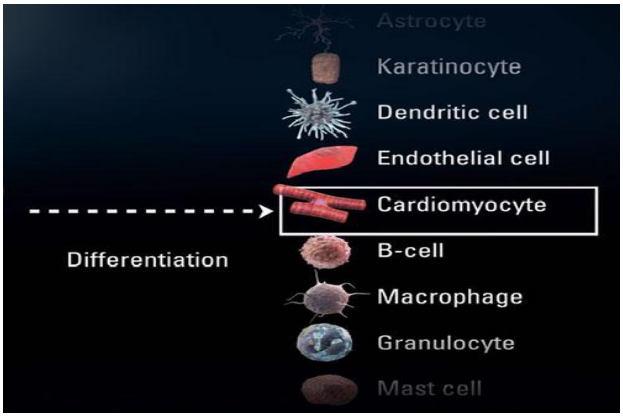

Figure 9: Artist's impression of cell differentiation process.

4. Bio-ink hydrogel

The cells will be mixed with nutrients and other vital factors in a liquid environment to keep them alive and viable in the process (hydrogel). In this aqueous 3D (three-dimensional) environment, the bio-ink of living cells will be preserved.[28]

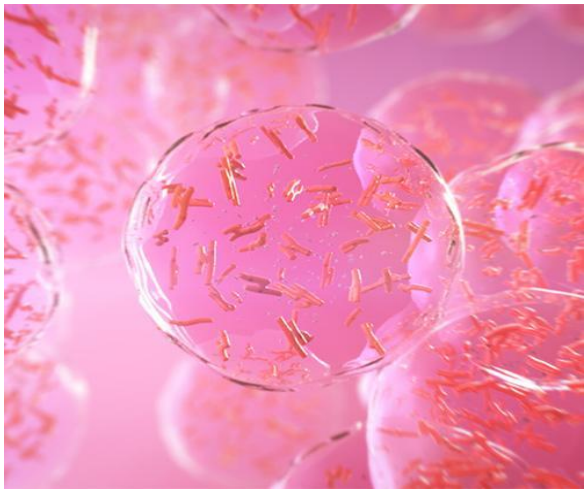

Figure 10: Artist's impression of cells encapsulated within a hydrogel.

5. 3D bioprinter cartridges

After that, the bio-ink will be loaded into a bioprinter, which is a specialised three-dimensional printer that prints while preserving living cells.The bio-ink would then be loaded into a bioprinter, which is a specialised three-dimensional printer that protects living cells when printing.

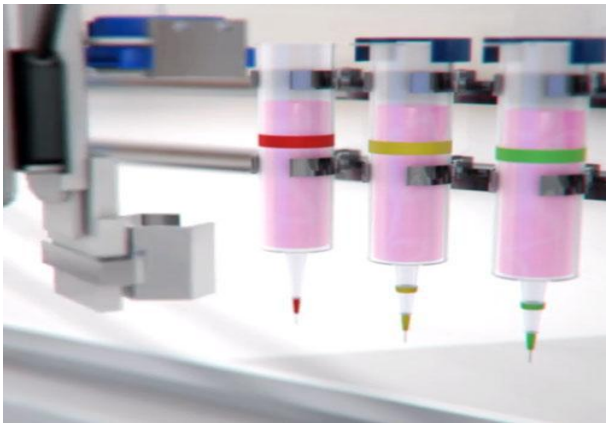

Figure 11: Artist's impression of Bioprinter loaded with Bioink.

6. 3D bioprinter in action

After that, a heart of the proper size will be printed one layer at a time, led by computer software (Auto CAD) and the precise measurements obtained from the MRI (Magnetic resonance imaging). Since the heart cells aren't fused together yet, each layer will need a biocompatible and biodegradable scaffolding to stabilise and hold the cells in place.[28]

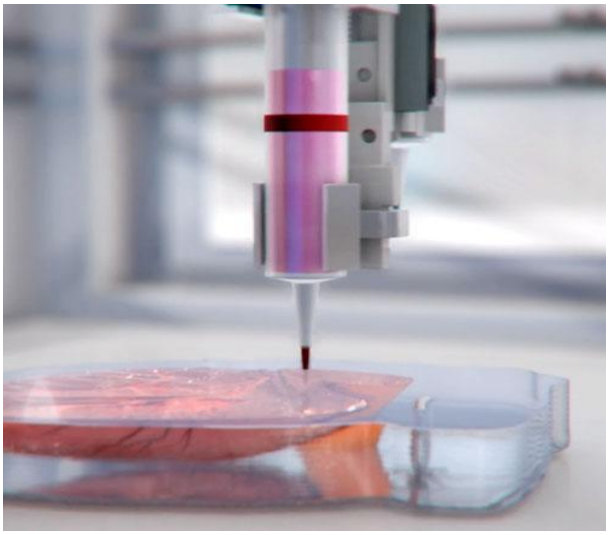

Figure 12: Bioprinting Human Hearts, the BIOLIFE4D Process, as depicted by an artist.

7. Bioreactor with human heart

The heart is then transferred to a bioreactor, which is built to reproduce the nutrient and oxygen-rich conditions found within a human body.

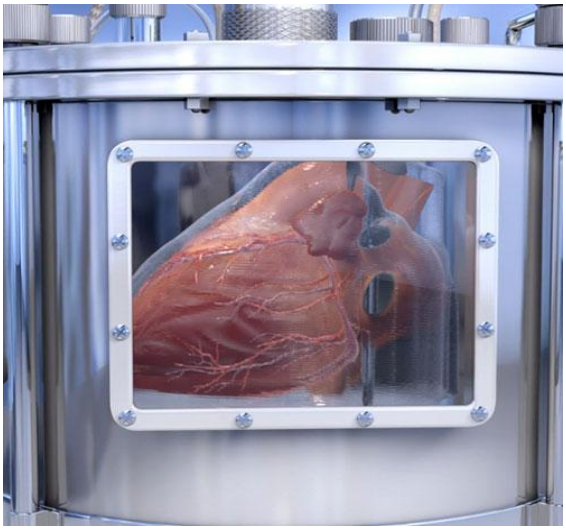

Figure 13: Artist's impression of bioprinted human heart in bioreactor.

8. Self-organization of heart cells

Individual cells would start self-organizing and fusing together to form a network that would eventually connect to form living tissue. The cells are able to beat in synchronisation as well.

Figure14: Artist's impression of self-organization of heart cells.

9. Heart tissue

The scaffolding would be dissolved after the process had progressed far enough, leaving only the completely developed core in the bioreactor until it reached the required degree of strength and maturity.

Figure15: Artist's impression of developed bioprinted heart tissue.

10. Heart in chest cavity

After that, a transplant surgeon can perform a successful patient transplant. The new heart should be a better fit and genetic match for the patient based on the initial MRI and blood sample. – Conventional organ transplant approaches have been hindered by the risk of rejection and the need for immunosuppressive treatment.[28]

Figure 16: Artist's impression of bioprinted heart in chest cavity.

3D Bio-Printed Heart pic from BIOLIFE4D

(BioLife**4D** Corporation, Biotechnology company, Chicago, Illinois, United States)

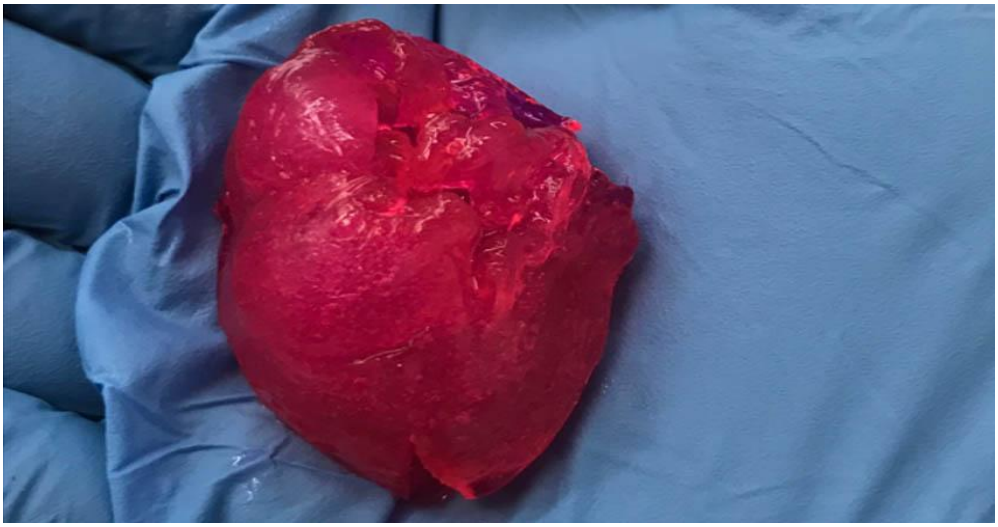

Figure 17: 3D Bioprinted Heart.

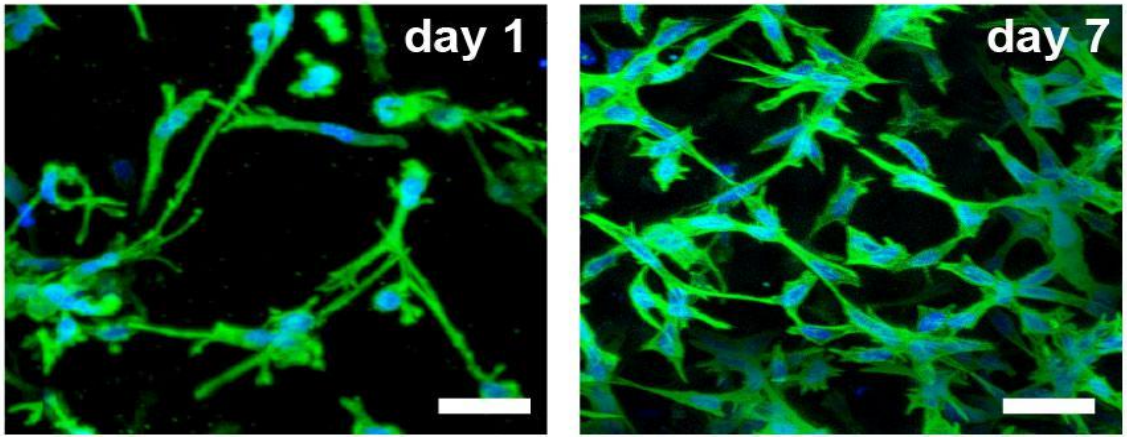

Figure 18: ECM *(*extracellular matrix) and 3D printed cell boards. At 1- and 7-day time points, C2C12(The C2C12 cell line is a subclone of a myoblast line obtained from adult C3H/HeJ mice's thigh muscle.) myoblasts in FRESH printed constructs spread and proliferate in three dimensions, demonstrating that cells spread and proliferate in three dimensions. The scale bars grow to a height of 50 metres. Three-dimensional printing of complex structures, Hinton et al.

CONCLUSION AND FUTURE

- 3D bioprinting is a game-changing technology that can enhance the efficiency, affordability, and personalization of medical care in the future. Researchers can create geometrically well-defined 3D scaffolds seeded with cells in a fast, low-cost, high-throughput manner using this simple technique. The use of bioprinting to build organs and tissue patches from a patient's own cells reduces the need for organ donors while also lowering the risk of rejection. This eliminates the issues of organ rejection and long

waiting periods for donors. BIOLIFE4D aims to develop a full-size human heart that can be transplanted.

- Bioprinting technology is being developed by scientists to print living organs such as livers, kidneys, lungs, and any other organ our bodies need. It has the ability to minimise or eradicate the organ donation crisis, giving everybody a fair chance. In addition, experiments are being conducted to develop the skin, our largest and most delicate organ. This will also make it simpler and quicker for scientists and physicians to treat wounds.
- 3D printing, biomaterials, hybrid inks made of natural and synthetic polymers, and cellular matrices are all being studied.
- Independent 3D printing science, on the other hand, will be inadequate to overcome the vast field of tissue replication. Biomedical engineers, biomaterial specialists, formulation and pharmacological scientists, as well as doctors, would need to cooperate in the future to 3D (three-dimensional) print fully functional organs and tissues.
- Extrusion, laser aids, and bioreactors that maintain cell sterility must all be employed. For better cytokines, cellular networks, and growth factors, 3D printing structures and equipment must support biodegradable co-polymer formulations and a safe climate.
- 3D printed organs and models may be useful in animal research or cadaver training in the future as a result of this achievement. As CAD (Computer-aided design) advances, 3D printing will open previously closed doors for science and the healthcare industry, allowing for more detailed designs of the veins, lungs, valves, ventricles, and sphincters that surround complex body organs and tissues.[14]
- We're thrilled to be able to provide researchers with the tools they'll need to construct the 3D bioprinting of the future. Imagine a world where a heart could be printed in ten days, with less cosmetic operations, a face could be fixed, and medicine could eventually help us live happier and longer lives.

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