International Journal of Multidisciplinary and Current Educational Research (IJMCER)
ISSN: 2581-7027 ||Volume|| 5 ||Issue|| 4 ||Pages 158-174 ||2023||

Organ or cell transplantation to save lives of patients suffering from organ failure disorders

Ava Movahed Abtahi[1]
[1] *Department of Biology and Chemistry, Washington Adventist University, Takoma Park, Maryland, USA*

HIGHLIGHTS

- This review aims to detail the various aspects of the human body's cardiovascular system, toward technological advancements such as CRISPER, 3D-Bioprinting, Xenotransplantation, and Organoids for End-stage organ failure.
- Advancements in 3D-bioprinting technology have allowed scientists to create cardiac tissue that can be used for treating various cardiovascular diseases.
- The development of new bio-inks and 3D-printers that are designed for high-quality construction has allowed researchers to create more effective and efficient tissue treatments.
- CRISPR gene editing improves the effectiveness of animal/human chimerism by preventing particular organ creation in the host zygote that may be filled by human cells.
- The improved immunosuppressive regimes and genetic alteration of a donor have led to a significant increase in the survival rate of heterotopic cardiac graft animals.

ABSTRACT : End-stage organ failure that is refractory to medical treatment can be a lethal disease. Organ or cell transplantation may save or lengthen the lives of many people struggling with illnesses related to organ dysfunction. The shortage of transplantable organs to meet current demand is a significant problem in the field of solid organ transplantation. Consequently, the number of patients on transplant waitlists increases every day, as do the number of those dying while waiting for an organ. Bio-generative engineering has lately produced technological improvements that enable the regeneration of cells, and, in some instances, the development of new tissues and organs [21]. Significant breakthroughs and advances in tissue engineering and regenerative drugs are being made in this setting, with 3D-bioprinting of organs and tissues having a substantial impact on the scientific community[21]. Aside from that, organ decellularization and its use as a framework for the recellularization process to generate new organs have shown promising outcomes. Several pre-clinical and animal studies have shown the safety and efficacy of employing different cells, i.e., stem cells or T regulatory cells, following transplantation for tissue repair, immunosuppression, or tolerance induction. However, in modest clinical studies, cell therapy has made tremendous advances in solid organ transplantation. Recent findings have shown, the use of cell therapy in solid organ transplantation seems to be advancing [16] which will be leading to many feasible advancements. There are, however, further challenges to address: 1. infusion dosage and 2. timing. End-stage organ disease is best treated through organ transplantation. However, a worldwide lack of donor organs has hampered the advancement of organ replacement. Human-animal chimeras use autologous stem tissues, similar to induced pluripotent stem cells, to produce immune-matched and patient-specific human tissue in host animals. Interspecies chimeras have gained much attention because of their potential applications in fundamental and translational research. This review focuses on the cardiovascular system and other areas in the human body, outlines recent technical advancements, and highlights the most promising xenogeneic cell treatment possibilities.

I. INTRODUCTION

Worldwide, it is projected that half a million people are on the waitlist for organ transplantation, and since organs are in short supply. Death rates, because of the failure of a critical organ, are rising [10]. A living or deceased donor may provide up to twenty-five different tissues or organs, saving several lives. Typically, multiple organs can be donated from a single individual, such as the heart, lungs, pancreas, liver, kidney, bone marrow, etc. Organ or cell transplantation to save the lives of patients suffering from organ failure disorders is evolving, and it is worth researching. This review will focus on organ or cell transplantation and current interventions within heart transplantation. A lot is happening in the twenty-first century in the field of organ transplantation, including xenotransplantation and 3D-bioprinting options. Because of the low regeneration ability of affected cardiomyocytes, cardiovascular disorders are a significant health problem globally. There remain few treatment options for ischemic injury. Innovative cell replacement techniques may make regenerative medicine more efficient.

Despite massive efforts to produce human cells in vitro, existing scientific restrictions and human donors' scarcity have limited their usage in clinical settings [16]. Xenotransplantation can deliver an unending supply of organs and tissues for organ and cell replacements, by giving the ability to close the gap between awaiting patients and donor availability. Because of their physical and physiological similarities to humans, pigs are regarded as the best prospects for xenogeneic cells and tissue. In stem cell treatment and regenerative medicine, porcine cells are being explored intensively. With an emphasis on the cardiovascular system, this review summarizes current advances and emphasizes the most promising options in xenogeneic cell therapy.Recent advancements in tissue engineering and regenerative drugs offer the potential to replace and regenerate organs and tissues, while repairing specific congenital abnormalities through lab-grown tissues and organs, xenogeneic organs, or bio-artificial organs. Because cells involved in tissue engineering and regenerative medicine come from the same patient (autologous), tissue or organ rejection by the patient's immune system could be avoided with these types of transplants. Contrasted to cells/organs from another person might provoke an immunological response, limiting their usage [20]. Immunosuppressive medications may suppress the immune response, but they come with a slew of inevitable adverse effects. The most significant side effect of immunosuppressant drugs is an increased risk of infection. Other, less serious side effects can include loss of appetite, nausea, vomiting, increased hair growth, and hand trembling. These effects typically subside as the body adjusts to the immunosuppressant drugs. Xenogeneic cells, which are derived from other species, may also be employed in regenerative medicine.

Xenotransplantation may play two roles in the field of transplant medicine: first by replacing the need for human organ transplantation or secondly as a complement to human organ transplantation; or as a "bridge" organ until a "destination" organ is identified [21]. Bioprinting (3D-bioprinting) uses biomaterials with 3D-printing to create components that mimic natural tissues, blood vessels, and bones in the body. It is primarily employed in drug development; as it enables facile fabrication of spatially-patterned co-culture models as well as reduces the probability of cross-contamination caused by handling of different cell types owing to constraining of the physical space [25] . In this quest, due to their ability to mimic the spatial and chemical attributes of native tissues, three-dimensional (3D) tissue models have now proven to provide better results for drug screening compared to traditional two-dimensional (2D) models [22]. More recently, as cell scaffolds have been researched to aid in regenerating injured ligaments and joints; while Since roughly 2007, bioprinting has been employed in medicine to help research or replicate practically every tissue, organ, and cartilage in the body.
The spike in the number of people suffering from chronic ailments and severe organ damage has mirrored the increase in human life expectancy. In managing end-stage organ dysfunction, an organ transplant is currently a viable option for improving survival and quality of life of patients. On the other hand, the imbalance between demand and supply for human organs is severe. However, with advancements in the use of CRISPR/Cas technology, genetic editing has sparked a surge of interest in xenotransplantation [13]. As This technology has played a critical role in xenotransplantation, and it can help in coming up with inadequate organs, opening new opportunities for patients in need of a transplant.

Physicians treating patients with heart failure may use a range of medical devices to help sustain the patients and, at the very least, delay the cardiac failure progression. Allograft transplantation is the definitive biological treatment for cardiac failure, which replaces the damaged heart with a healthy one. Advanced regenerative medicinal techniques are now being researched with the aim of 3D-bioprinting and building a whole heart from cellular/ biologic antecedents. Once constructed, the heart would be both an artificial and a biologic construct (biofabricated), describing the integrated biologic replacement components. Bioprinting is an additive manufacturing technique. With the advancement of computers and polymers, additive manufacturing has quickly become a viable method of producing a wide range of products. Computer-Aided Design (CAD) software is used to transmit instructions to computer-aided manufacturing (CAM) machines, which employ a layer-by-layer additive engineering technique to produce the object. When doctors need anatomical models to help them plan intricate procedures, 3D-printing creates them. This is a watershed moment in additive manufacturing (AM) as these advances can be a light to many medical challenges. For example, conjoined individuals may benefit from 3D-printed models of their vasculature, and patients with congenital cardiac malformations may benefit from 3D-printed representations of their hearts to help design tissue repair. Implanted medical devices are being manufactured in an increasingly large number of clinical settings, thanks to additive manufacturing. Patients' detailed data (e.g., MRI or CT imaging) is used to create these 3D-printed implants, and the dimensionality of the printed implants is identical to the dimensions of the tissue to be replaced.3D replacements are used in various procedures, including tracheal, complex jaw, sternum, and cranial replacements. All of this has resulted from software advancements that allow for the rapid transformation of large image datasets into a programming language that the 3D-printing can process.

Also, the increased use of reasonably cheap 3D printers with rapid, high-resolution printing is becoming more widely used in many industries. 3D-bioprinting medical equipment has the potential to transform medical treatment in the future, enabling equipment to be manufactured at a cheap cost, in a short period, and at the time of service. With the changeover from three-Dimensional printing to 3D-bioprinting, it is now acknowledged that tissue is a 3D entity composed of a complex structure of specialized cells that lives and acts in three dimensions (ECM).

II. CRISPR TECHNOLOGY

CRISPR technology has seen a wide range of uses in the scientific world. CRISPR is a bacteria's adaptive immune system, and using it to generate cells and organs for donation could be risky for the receiving hosts, as the full potentials are still in trials. CRISPR/Cas9 is the standard gene-editing method for modifying the genome of many cell types (primary cells, cell lines) and producing transgenic animals in the shortest period because of its simplicity of use and better editing effectiveness [13]. These development indicators continue to motivate scientists to work harder to identify new services for the system in several sectors. Because the CRISPR system uses an RNA-based editing process, it can scale or target many genes at once. CRISPR screens have emerged as a powerful platform for genomic studies, enabling researchers to test the impact of different techniques at the genomic level in the existence of a switchable phenotype or output by creating a genomic sequence CRISPR library with almost 100k gRNA which targets the complete genome.

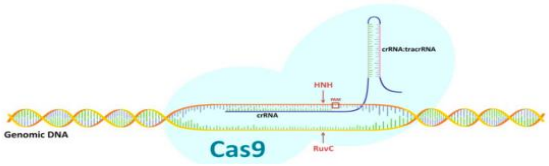


Figure 1. Diagram of CRISPR/Cas9 system. Description of a figure in the text. [28]

In the sgRNA sequence, gRNA is distinguished as an element corresponding to the bacterial crRNA fragment that complements the target locus in DNA. The gRNA molecule is an element designed by the researcher [15] . Many variants of the CRISPR/Cas9 system have been developed in which the engineered gRNA recognizes a sequence from 18 to 24 nucleotides in length and Cas9 recognizes different PAM sequences from two to eight nucleotides in length. In genetic engineering, the system most commonly used is that found in ***Streptococcus pyogenes***. It requires a 20-nucleotide gRNA molecule linked to a Cas9 nuclease and a three-nucleotide-long PAM sequence (specifically NGG, where N is an arbitrary base nucleotide followed by two guanosine nucleotides) [12].There are fewer uses of CRISPR in transplantation than there are in other medical domains like cancer, neurology, or developmental biology. However, exciting advances, notably in xenotransplantation, are constantly being made [18]. As the demand for organ donors grows, scientists and physicians must consider other choices (i.e., 3D-bioprinting, organoid, xenotransplantation). These alternate ways use advances in CRISPR technology to reduce the time it takes to get applications from the bench to the bedside [13]. CRISPR gene editing improves the effectiveness of animal/human chimerism by preventing particular organ creation in the host zygote that may be filled by human cells. In future chimeric organ development investigations, researchers will use sophisticated CRISPR-mediated transgenic sheep or pigs as zygote and surrogate species.

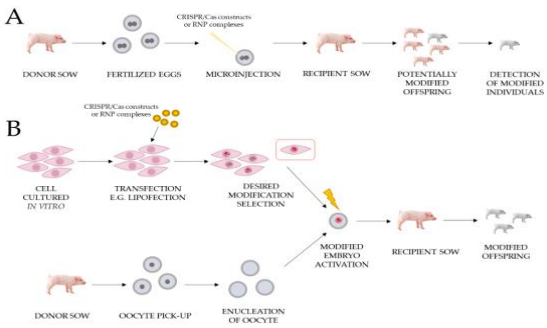


Figure 2. Workflows for obtaining gene edited pigs using the CRISPR/Cas system for xenotransplantation purposes. The figure shows two methods: (A) Microinjection of CRISPR/Cas modified constructs or RNPs complexes into porcine zygotes. (B) Somatic cell nuclear transfer (SCNT) [28]

Strategies to Help Overcome Immune Reactivity in Xenotransplantation : Not all animals are ideal for producing organs for human transplantation. This can be for many reasons, such as differences in size, life expectancy, body temperature, immunological tissue rejection, and infection risk [10]. Pigs have been the most common source of cells/organs so far because they have many advantages for xenotransplantation, including their similar size to human organs and ease of cloning and genetic modification. Further, pigs have many progeny. Rejection, in which the recipient's immune system identifies and attacks the new organ as foreign, is the most significant practical impediment to xenotransplantation. Drugs like cyclosporine have long been used in organ transplantation to suppress recipients' immune systems, enabling donated organs to function without being attacked and rejected as alien [10]. A more severe reaction termed "hyperacute rejection" emerges when organs are transplanted into humans. In a matter of minutes, a person's immune system attacks the transplanted organ and kills it.

In addition, scientists have now found a groundbreaking development in stem cell and gene editing (CRISPR/Cas9) technology, bringing them closer to generating human organs in non-human animals [5]. Essentially, human stem cells would be implanted into a pig blastocyst or embryo, developing into the vital organ. Before this may become a viable supplier of human organs, at least four fundamental problems must be addressed. Master gene regulators of organ development in pigs must be discovered first to inhibit the formation of the organ that will be generated from human cells. Second, researchers must optimize the reprogramming of patient-derived human pluripotent stem cells. These stem cells will subsequently be implanted into the genetically engineered pig embryo to fill the vacant organ niche created by gene deletion and develop into particular organ cell lineages. Gene editing is required to remove galactosyl moieties from the exterior of pig cells to guarantee that the patient's immune system does not cause hyperacute rejections to the pig xenografts. Finally, gene editing would be required to remove hundreds of oncogenic swine retroviruses that might cause cancer or zoonotic diseases in transplant recipients [5]. It is now possible to develop patient-specific transplantable organs in pigs from the intersection and integration of these new technologies, known as blastocyst complementation.

One method being tested to avoid organ rejection is creating transgenic pigs. The pigs are genetically engineered to generate human proteins, making it hard for the immune system to differentiate between porcine and non-porcine organs. A transgenic pig is made by putting a small amount of DNA into a viable pig egg, which is then inserted into a sow who then gives birth to transgenic piglets. [16] In recent research, this method has been shown to prevent hyperacute rejections in non-human primates who received organs from transgenic pigs. By removing the pig gene-products that induce hyperacute rejection, new cloning methods may further improve pig organs' immune compatibility. In principle, these advancements should allow animal organs to be treated similarly to human organs after transplantation, using regular immunosuppressive regimens.Biomedical technology progresses in lockstep with advances in genetic engineering. The development of genetically altered pigs was easier and faster with the advent of CRISPR/cas9 gene editing. Using homologous recombination, it used to take three years to create genetically modified pigs. Pigs may now be produced in 5 months using CRISPR/cas9 technology, a more efficient and cost-effective method. Although recellularization and decellularization treatments for organs hold a promising future of transplantation, there is no single agreement on a thorough decellularization strategy. Often, these treatments do not accomplish full decellularization, or if they do, they may be significantly detrimental to the extracellular matrix. Reichart et al used small animal models from closely related species, like mice and rats. Reichart et al. recently used an Non-Human Primate (NHP) models to study the host immunological response to entirely decellularized lung obtained from genetically modified and wild-type pigs by implanting decellularized pig scaffolds sections beneath the skin. Surprisingly, the decellularized genetically designed platform elicited a host of immunological responses.

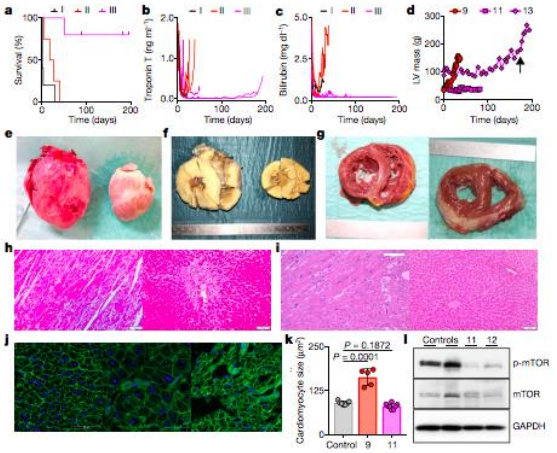
Animals and organs with chimeric DNA have also been investigated. Wang et al. found that in pig endothelial cells, substituting porcine CD31 with human CD31 decreased xenogeneic neutrophil-mediated cytotoxicity by inhibiting NETosis. However, the research was carried out in a 2D growing environment in vitro. Even though the study is intriguing, it was conducted in phenotypically similar animals, and its applicability in a human-to-pig paradigm must be examined. Xenotransplantation also brings up several ethical, cultural, and spiritual issues that must be addressed. Many Islamic cultures prohibit swine organs, like heart valves, to substitute human organs that have failed [17]. However, “the opinions of certain Islamic authorities have transitioned to authorizing organ transplants from pigs since the victims would die without them. Last year, the Fiqh Council of North America issued a FATAAWAH or FATWA addressing organ donation and transplantation, where it considered organ donation and transplantation to be Islamically permissible in principle. As a result, the ban against utilizing a swine organ for transplantation is overridden by the need to save human lives [17].

Porcine products cannot be eaten by Jewish patients, although they may be utilized as donor organs. Furthermore, Judaism encourages xenotransplantation to extend or save the lives of sick or dying from organ failure. Xenotransplantation is also supported by Catholic ethicists in Canada and the United States, as long as the method protects the human identity by keeping the patient identity anonymous and does not employ terminated embryos as a reservoir of human stem cells. These ethicists underline the need to use pluripotent stem cells rather than embryonic stem cells in these techniques. On the other hand, many religious ethicists argue that these procedures should not result in the mixing of animal and human brains or hormonal cells, which are considered to be distinctive to human identity. The ethical principle of autonomy, which states that doctors must tell all patients of any faith about the origin of the organs to be transplanted, underpins these tolerant views about xenotransplantation.

Possible Innovative ways a Heart Can Be Xenotransplanted : Cardiac xenotransplantation (CXTx) is still a viable treatment option for end-stage heart failure. Heterotopic cardiac xenograft survival has increased from minutes to over eight months as a result of genetic alteration of the donor and improved immunosuppressive regimes. A genetically modified pig heart was recently transplanted into a 57-year-old Baltimore man by Dr. Bartley Griffith and his team at Maryland School University [7]. The patient had advanced heart failure and ventricular fibrillation. However, the patient was deemed not suitable for a human heart transplant or left ventricular support device (LVAD) , and the decision was made to move forward with a xenotransplantation. Therefore, in order to move forward with the xenotransplantation ten genetic changes were made to the pig heart, including removing four pig genes and the inclusion of six human genes. The surgical team expects that the transplant's sustained success will give a new option to assist individuals on the waiting list for organs. However, other doctors are skeptical about the transplant's ethical implications.

Surgeons at NYU Langone Health accomplished a proof-of-concept experiment in October 2021, connecting a pig kidney to a brain-dead human corpse [7]. For two days after therapy, the organ continued to function normally. Revivicor, a regenerative medicine business in Virginia, supplied the kidney. Revivicor had acquired a regulatory license a year before 2022 to modify pigs for food and medicinal purposes genetically. However, pigs create a chemical in their cells that triggers an immunological reaction in humans after organ transplantation. Revivicor generated organs that might evade the immune response by removing that sugar moiety on the cell surface via genetic engineering.With the successful transfer of a gene-edited pig heart into a live human on January 7, 2022, xenotransplantation made another giant step forward. This time Revivicor delivered a heart from a donor pig that had been genetically altered in ten ways. They deleted three genes in the pig's genome that cause organ rejection and replaced them with six human genes to assist the patient's body in accepting the new heart [7]. To prevent the pig heart from becoming too big, they removed one more gene. Because the operation was not part of a formal clinical study and the patient was using new immunosuppressive medicines, scientists are cautious about drawing inferences from this technique; further study of the patient and further experimentation are needed. Companies like eGenesis and Qihan Biotech view this as a positive step forward and advance in their xenotransplantation research.

Because of breakthroughs in pharmaceutical development, cardiac xenotransplantation is now possible. Transplant recipients must use immunosuppressive medications to prevent organ rejection [18]. However, other studies of animal-to-animal transplants, such as when a pig organ was implanted in a baboon, showed such medications are ineffective, implying that traditional immune suppression might also be ineffective in humans. Monoclonal antibodies that inhibit "co-stimulatory" molecules like CD40 and CD154 have been created to overcome this issue. These methods for immune modulation are also the basis behind preventing the transmission of the human immunodeficiency virus (HIV) Monoclonal antibodies are far more effective in stopping human immune cells from targeting pig organs, as opposed to standard immunosuppressants, but their role in development of cancer in recipients remains high.. Galactosyltransferase (GTKO) is a carbohydrate antigen that is absent in pigs with a mutation in the 1,3-galacturonic acid transtransferase gene (GTKO) (Gal) [1]. In this particular instance, the participation of anti-Gal antibodies in GTKO cardiac xenograft rejection has been significantly reduced or eliminated. Nonhuman primates with heterotopic GTKO cardiac xenografts had a greater survival rate than humans. GTKO graft rejection is defined by the buildup of vascular antibodies and the accumulation of dynamic complements. Antibodies have been found against porcine antigens linked to complement, hemostatic, and inflammation control, as well as novel carbohydrate antigens [1]. Their role in rejection is still being researched. Early cardiac xenograft dysfunction limits orthotopic CXTx perioperative cardiac xenograft dysfunction (PCXD). Hearts afflicted by PCXD, on the other hand, regain whole cardiac function, and orthotopic survival of more than two months has been documented without being rejected.



In **figure 3**. above the survival, laboratory parameters, necropsy and histology after orthotopic xenotransplantation. By a research conducted by Längin et. al as the figures indicated: a, Kaplan–Meier curve of survival of groups I (black; n = 5 animals), II (red; n = 4 animals) and III (magenta; n = 5 animals). Two-sided log-rank test, P = 0.0007. b, c, Serum concentrations of cardiac troponin T (b) and bilirubin (c). d, Left ventricular (LV) masses of xenografted hearts from animals 9 (group II), 11 and 13 (both group III); note increased graft growth after discontinuation of temsirolimus (arrow). e–g, Front view of the porcine donor heart and own heart of baboon 3 (e, left and right, respectively; group I) and transverse cuts of the porcine donor hearts (left) and the baboons' own hearts (right) of animals 3 (f) and 11 (g). Note the extensive left ventricular hypertrophy and reduction of left ventricular cavity of the donor organ of baboon 3 in contrast to animal 11. h, i, Haematoxylin and eosin staining of the left ventricular myocardium of the donor (left) and the liver of the recipient (right). Scale bars, 100 μm. h, The myocardium of animal 9 showed multifocal cell necrosis with hypereosinophilia, small vessel thrombosis, moderate interstitial infiltration of lymphocytes, neutrophils and macrophages. The liver of this animal had multifocal centrilobular cell vacuolization and necrosis as well as multifocal intralesional hemorrhages. i, The myocardium of baboon 11 had sporadic infiltrations of lymphocytes, multifocal minor interstitial oedema whereas the liver had small vacuolar degeneration of hepatocytes (lipid type). j, Wheat germ agglutinin-stained myocardial sections of a sham-operated porcine heart (left), and the hearts transplanted into animals 9 (center) and 11 (right). Scale bar, 50 μm. e–j, n = 4, groups I/II; n = 3, group III; n = 1, control; one representative biological sample for each group is shown for group I/II, group III and control (j). k, Quantitative analysis of cardiomyocyte cross sectional areas. Data are mean ± s.d., P values are indicated, one-way analysis of variance (ANOVA) with Holm–Sidak's multiple comparisons test (n = 3 biologically independent samples with 5–8 measurements each). l, Western blot analysis of myocardium from transplanted hearts of animals 11 and 12 showed reduced mTOR phosphorylation (p-mTOR) compared to age matched control samples. n = 2, group III; n = 2, controls [14]

III. 3D-BIOPRINTING :

Advanced heart failure is a comprehensive categorization that refers to illnesses that affect the myocardium, vasculature, or heart valves in the body. It is the leading cause of morbidity and death in most industrialized nations, including the United States. Advanced cardiovascular disease (CVD) is typically the result of the degradation of one or more of the heart's structures and cells, which will eventually need to be replaced to improve the prognosis of people afflicted. End-stage heart diseased patients who are refractory to medical management are candidates for heart transplantation [21]. At the same time, specific valve replacement procedures are treated using xenografts of bovine or pig heart valves, which are harvested from animals. CVD may also be treated using artificial valves and vascular grafts. Although each kind of transplant has the potential to cure one of the diseases associated with CVD, they all have drawbacks, such as a scarcity of easily accessible donor organs, anticoagulant medication, immunological rejection, and limited durability. As a result, additional therapy options that are both easily accessible and suitable are needed.Cardiovascular tissue engineering aims to restore cardiac muscle, blood arteries, and heart valves that have been injured or rendered inefficient. The stem cells' differentiation into operational and mature tissue on biomaterials that help in the tissue's development and growth, for example, is one of the current approaches for achieving this aim. Hydrogels and decellularized matrices, whether synthetic or natural, are often used as biomaterials because they form a porous, interconnected polymeric system that allows cells to proliferate, move and get essential nutrients. Allogeneic and autologous stem cells, in particular, are a popular subject in cardiac bioengineering because of their capacity to reduce immunological rejections of grafts, reduce thrombogenic effects, and potentially make tissues accessible on

demand. It is necessary to integrate cardiomyocytes, endothelial cells, and cardiac fibroblasts produced from a variety of stem cell sources to construct functional heart architecture.

3D-bioprinting for Tissue and Organ Regeneration : 3D-bioprinting can be used to build various organ models for faster and less expensive testing. 3D-bioprinting may be done in multiple ways, i.e., laser-assisted, extrusion, and inkjet bioprinting [16]. However, the addition of scaffold biomaterials to cell-based complexes, i.e., bio-ink or exogenously derived matrix proteins, as well as the difficulty of perfusing these constructions in a bioreactor, restrict what may be accomplished with bio-printed tissues.

	Inkjet	Laser-assisted	Extrusion
Cost	Low	High	Moderate
Cell viability	>85%	>95%	40–80%
Print speed	Fast	Medium	Slow
Supported viscosities	3.5–12 mPa/s	1–300 mPa/s	30 mPa/s to above 6×10^7 mPa/s
Resolution	High	High	Moderate
Quality of vertical structure	Poor	Fair	Good
Cell density	Low $<10^6$ cells/mL	Medium $<10^8$ cells/mL	High (cell spheroids)
Representative hydrogel materials for bioinks	• Alginate • PEGDMA • Collagen	• Collagen • Matrigel	• Alginate • GelMA • Collagen
Reported applications in tissue engineering	• Blood vessel • Bone • Cartilage • Neuron	• blood vessel • bone • skin • adipose	• Blood vessel • Bone • Cartilage • Neuron • Muscle • Tumor • Controlled release of biomacromolecules • Organ-on-a-chip

Table 1. Comparison of the different techniques employed for the fabrication of 3D bioprinted cell-laden hydrogel constructs for tissue regeneration [19].

Tissue injury and degeneration are typical of humans; nevertheless, the human body's regenerative capacities are inadequate when coping with this stress. Traditional treatments for these disorders rely on organ or tissue transplant, which is contingent on donor availability, which is increasingly scarce and has the risk of graft rejection due to an immunological reaction [21]. Regenerative medicine and tissue engineering are being developed to address these problems. One of the most sophisticated methods used in tissue engineering is additive manufacturing. It combines material science and biology to create organ and tissue frameworks. Its primary aim is to repair injured cells or organs by replicating the intrinsic biological tissue complexity (cellular niche) to aid tissue regeneration and cell differentiation. Developing interphase between the cell, the scaffold, and the growth factors are required for this method. Scaffolds may function as a foundation for cells to grow in response to growth stimuli. However, This approach is quite haphazard and does not allow for a customized 3D distribution of cells, besides being time-consuming and difficult. Consequently, they are inappropriate for therapeutic usage from a logistical and financial standpoint [21]. In this regard, additive manufacturing is being studied for tissue engineering because it employs a top-down approach to the layer-by-layer construction of complex tissue. Hence, accurate and consistent geometric shapes are crucial, because of the controlled essence of matter deposition, and anatomical 3D models of the cells generated by computer graphics.

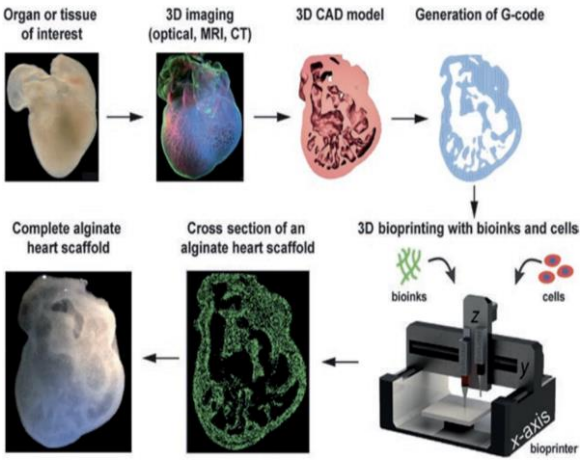


Figure 4. Three-dimensional bioprinting: spatial arrangement of cells, molecules, and growth factors within a confined 3D structure based on an alginate hydrogel bioink, using a computer-aided design for the production of tissues and whole organs. [29] 3D-bioprinting is a more sophisticated use of additive manufacturing (AM) technology that involves the layer-by-layer creation of a tissue or organ from the bottom up. By depositing materials and cells in a precise pattern that replicates natural cellular architecture,

3D-bioprinting focuses on restoring complex tissues' typical structure and function. In 3D-bioprinting, Biomolecules or cells are printed in a specified way directly onto a substrate so that the cells may stick together to build the needed 3D construct. Bioprinting involves living organisms such as cells, tissues, and organs, therefore it must consider the modalities associated with living tissues, such as material biocompatibility, cell sensitivity to printing processes, growth factor administration, and perfusion. Because the whole procedure is mechanized, accura

te cell patterning and ECM organization may be achieved. The linked holes in the bioprinted tissues are suitable for gas and nutrient perfusion and intra- and inter-cellular communications because of their layer-by-layer design [5]. These bioprinted tissues, which have better intercellular communication, may serve as a good model for in vivo physiology. Animal models are not suited to anticipate human pathophysiological reactions, such a finding might add to the data gained during pre-clinical studies.

The application of 3D-bioprinting for the construction of functioning tissues, organs, and medicines has garnered a lot of interest in recent years. Biomolecules, biomaterials, and cells may be controlled layer by layer in a 3D tissue construct with geometrical complexity using 3D-bioprinting. According to contemporary tissue engineering research, biomaterial inks and bio-inks are two types of materials employed in 3D-bioprinting of cells, organs, tissues, and medicinal items, depending on whether tissues/cells are encapsulated. The frameworks for biomaterial inks should be able to physically assist 3D in vitro models while also retaining the different morphologies of each batch [23]. Besides, biomaterial ink used to print the framework must print without clogging and be versatile enough to be used in a variety of printing conditions. Based on their biocompatibility, polycaprolactone (PCL), polydimethylsiloxane (PDMS), and its derivatives may offer mechanical and physical support to in vitro models while having no impact on the cellular activities.Light-assisted, inkjet-based, and extrusion-based bioprinting technologies are only some techniques that have been established and used to generate highly purposeful 3D structures [23]. Also, via computer-aided manufacturing and computer-aided design, these technologies often build 3D Systems with excellent realism. Every bioprinting technology has a variable resolution and size for printing every layer. With digital light processing, plane-by-plane bioprinting was achieved in September of 2020 (DLP), while Line-by-line bioprinting was done in the instance of extrusion-based bioprinting. While conventional light-assisted printing methods are time-consuming because they create 3D cell structures in units of layers or points, the newly invented volumetric printing technique is far quicker, as it takes seconds to rely on the results. The volumetric printing approach produces a 3D tissue structure by creating a holographic pattern with three overlapping laser beams and selectively curing photocurable bio-ink in the bath. The time it takes to fix the bio-ink is substantially shortened because of the employment of three laser beams simultaneously, surpassing the constraints of the traditional one-layer stacking production approach.

IV. THE 3D-BIOPRINTING PROCESS :

Bioprinting is a fundamental process that operates like that of low-cost inkjet printers; the primary difference is that bioprinters deposit elements as strands, whereas inkjet printers deposit materials as droplets. It is based on using isolated cells mixed with a bio-ink that are placed in a syringe and then propelled through the syringe tip with pneumatic pressure. Extrusion-based bioprinting technique is mainly employed in current bioprinters, with extrusion-based systems accounting for most commercially available bioprinters. Extrusion-based bioprinting is used to create 3D objects. Additionally, other technologies such as Inkjet and Laser-Induced Forward Transfer (LIFT) might be needed for higher-resolution bioprinting. The precision of LIFT and Inkjet bioprinting is the most significant advantage of these technologies. It is almost certain that extrusion-based will be merged with more excellent resolution methods for the transplantation of minor elements, such as the nerves and microvasculature, in whole-heart bioprinting to implant these structures successfully.

Although an extensive range of biomaterials may be used in tissue engineering, only a tiny proportion of these elements are appropriate for bioprinting applications. Some of the elements that can be utilized are soft hydrogels such as gelatin, pluronic, collagen, and fibrin. The concentration and viability of the early cell suspension employed are critical, just as they are in other tissue engineering applications. In addition, the printing speed, pneumatic pressure, cell-laden bio-ink viscosity, and tip diameter are all important printing characteristics. Bio-inks with smaller tip sizes and a high viscosity need a more significant printing pressure. The diameter of the fibers is affected by the printing speed, with faster rates corresponding to thin strands. Each bioprinting application is unique, necessitating meticulous optimization of bioprinting factors. For example another technique would be angiogenesis bioprinting which refers to the formation of new vessels from preexisting vascular networks. In this technique cells are able to travel through the blood circulation and reach the desired location to proliferate and differentiate into mature endothelial cells (ECs). Hence, they come

together to form a new vascular network to supply blood [26]. The fine-tuning of bioprinting constraints is essential for each bioprinting request, and the needs vary considerably between tissue bioprinting and organ bioprinting applications.

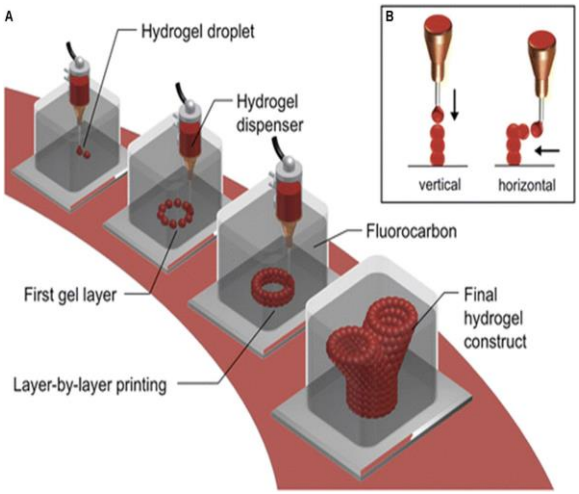


Figure 5. In this figure by Blaeser et. al the figure shows Submerged bioprinting. (A) Bioprinting cell-laden hydrogels (single drops), layer by layer as per a predefined and constructed model. The bioprinting is conducted, by submerging the nozzle, in high-density perfluorocarbons, which are chemical inert and immiscible in both water and oil. Perfluorocarbons are also known to display excellent oxygen and carbon dioxide transport capability, which provides an ideal environment for submerged cells. (B) The hydrogel drops can be printed either in a vertical or lateral dimension. With perfluorocarbon providing a buoyant support, printing can be conducted to produce branching structures, without solid support [4]

3D-Bioprinted Constructs Prefabrication : The construction of a patient-specific 3D model, which may start with experimental imagery or computer-aided design models, is required before 3D-bioprinting of sick or injured heart tissue. Anatomically precise models may be created using medical imaging data. Current imaging approaches for building physiologically appropriate 3D models include cardiac magnetic resonance imaging (MRI), volumetric 3D-echocardiography, and electrocardiography-gated computer tomography (CT) since the imaging dataset must be volumetric (CMR). Three-Dimensional echocardiography is a particularly attractive data source because of its abundance, cheap cost, and absence of ionizing radiation. A preferable alternative is a 3D transesophageal echocardiography source of data to construct observed cardiac model features as valve leaflets or ventricular chambers. However, since ultrasound imaging has artifacts and limits that might cause anatomic data loss inside CMR, CT and an ultrasound shadow are the most workable imaging modalities for 3D printing. CT scans have superior spatial resolution and can image patients with metal implants and pacemakers, which is impossible with CMR scanning. CMR may also produce high-resolution pictures without exposing patients to radiation and analyze tissue composition without using iodinated contrast material. CMR scans are often used to make 3D printed models of fetal heart chambers and vasculature and reconstruct intracardiac cancers.

The 3D anatomical information received from 3D echocardiography dimensional imagery databases is transformed into a 3D patient-specific digital form of the cardiac tissues via image segmentation. Further, the advancement of segmentation approaches may be traced to a growing interest in anatomical modeling and urgent demand for tailored structural cardiac therapies. Previously, only CT scans were used for segmentation, but newer research has utilized CMR images to simulate congenital heart and systemic vascular diseases. Researchers have also shown that 3D transesophageal echocardiography pictures and echocardiographic data gathered from many viewpoints and echocardiographic data paired with CT data may be used to rebuild mitral leaflets and annulus. The target anatomical geometry is recognized and divided based on the threshold intensities of pixels in gray-scale two-dimensional projections, sagittal, axial, and coronal. The computer then creates segmentation masks, in which pixels in the same intensity range are clustered together and allocated to be reproduced with a single material. The shows are then turned into patient-specific three-dimensional digital cardiovascular models and stored as Standard Tessellation Language files in computer-aided programs. They can be updated and changed before being transferred for bioprinting.

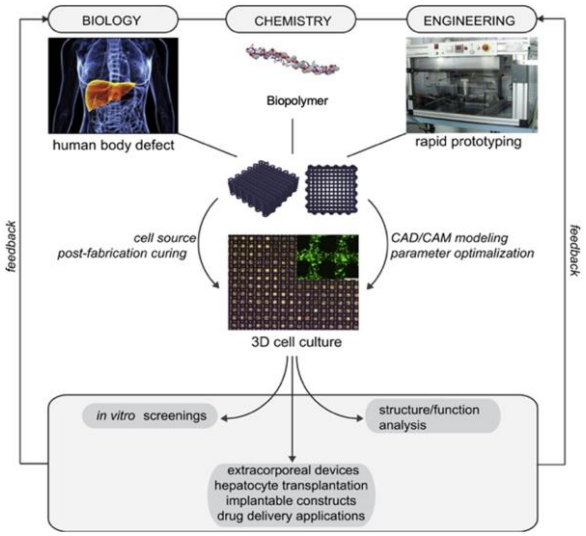


Figure 6. Diagrammatic representation of the sequential process for the fabrication of patient specific structures. [30]

Post-Fabrication : After 3D printing cardiac tissue, post-fabrication procedures are needed to acclimate the items to their new functionalities. These approaches are chosen based on the different features required by cardiac tissue for maximum function, such as contraction, blood receipt and distribution, and electrical communication. Mechanical stimulation may be achieved by continuously extending and contracting the material, similar to a cardiac patch. This guarantees that the material is able to contract and relax for an extended period while preserving its original shape. Limitations in oxygen delivery, deep cell seeding in a scaffold, and tissue regeneration have all hampered the development of heart tissue. Perfusion methods were employed to supply nutrients across the bioprinted scaffold as well as seed cardiac cells to overcome these challenges. Perfusion cell seeding causes cardiac cells to be evenly distributed all over the scaffold, allowing for better proliferation and cell survival.

Besides mechanical stimulation and perfusion, electrical conditioning is required for the onset of muscle contraction. Electrical pulse activation of bioprinted cardiac tissues increases cardiac cell conductivity and elicits a contraction rate equivalent to pacemaker cells. To coordinate ventricular and atrial shape and create a synchronized beating of all cardiomyocytes, electrical stimulation is employed since cardiomyocyte contraction must occur concurrently [6]. Mechanical perfusion and electrical stimulation have been used to get desired features of a working heart, implying that post-fabrication is required to produce completely functional 3D-bioprinted cardiac tissue. Several in vivo studies have used 3D printing to develop decellularized ECM scaffolds to treat myocardial infarction and restore heart function. Alonzo et al. used stem cell-laden decellularized extracellular matrix (ECM) bio-inks to 3D print pre-vascularized and functional metamaterial constructions (67). These patches promoted solid vascular permeability and tissue matrix formation when applied to infarcted rat hearts in vivo. Besides improved cardiac function, the patterned patch showed reduced cardiac hypertrophy and fibrosis, enhanced flow of migrants from patch to infarct site, modernity and blood capillaries growth, and neo-muscle and microvascular creation. More complex bio-inks, such as the complete decellularized ECM, have also improved myocyte development significantly. When combined with a polymer-based bio-ink for 3D printing, decellularized ECM may build biological scaffolds and provide biochemical cues more proficiently. Nevertheless, further clinical experiments with decellularized ECM are needed to better understand its ability to treat myocardial infarction successfully and convert those findings into human clinical trials.

Organoids : Organoid technology emerges as a self-contained research instrument. Organoids are three-dimensional (3D) constructs generated in a 3D culture environment from somatic stem cells (SCs), cancer cells, embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) [8]. Undifferentiated cells have the potential to self-renew and repair a variety of organs and tissues. Based on the developmental phase in which they are discovered, stem cells are divided into embryonic cells and adult stem cells. Embryonic cells are a type of cell that can multiply endlessly, differentiate, and self-renew in various ways. They are derived from early embryos [8]. Adult stem cells include progenitor cells, which are immature multipotent or pluripotent stem cells.

Mature progenitor cells may be found in various tissues and are crucial for tissue repair and regeneration after injury.

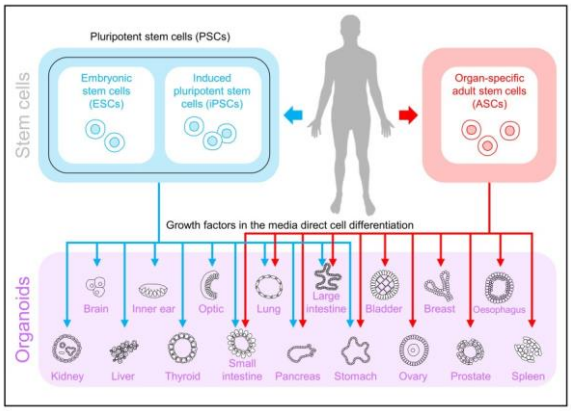


Figure 7. A variety of organoids can be generated from two main sources of stem cells: organ-specific adult stem cells (ASCs) and pluripotent stem cells (PSCs). These stem cells are then differentiated with the addition of growth factors to the media. [31]

Three-dimensional (3D) cell culture is a method of growing cells in an artificially constructed environment where they may interact in three dimensions. 2D culture models may not adequately represent the reaction of a tissue to a specific molecule, making 3D cell culture methods fundamental in disciplines like drug development [11]. Because of the cellular microenvironment's capacity to affect drug response, several promising medications that passed the 2D monolayer screening approach may have failed. Furthermore, tissues are made up of various cell types from multiple origins that are organized temporally and spatially and the extracellular matrix (ECM). In addition, several 2D culture systems use different cell types with ECM that are often produced by the cells themselves or provided externally. As a result, 3D cell culture methods are more representative of in vivo cell biology and may be used to investigate the physiological aspects of human disorders. Organoid culture is one of the three-dimensional cell culture techniques employed.

Organoids were created to functionally and morphologically mimic human organs made up of several cell types. In recent years, human organoids have been successfully used to diagnose pathologies after bacterial or viral infection. Human proximal respiratory organoids have been shown to distinguish between the human-infective influenza virus (H7N9) and viruses that do not effectively infect humans, e.g., swine-infective influenza virus (H1N1) and avian-infective influenza virus (H7N2) [11]. Human baby lung organoids were generated to imitate a common respiratory viral infection in babies and newborns. Human organoids, in particular, have been promptly applied to establishing a COVID-19 therapy. Further studies have implicated that SARS-CoV-2 infection in human organoids of the kidneys, intestines, liver, lungs, eyes, and brain SARS-CoV-2 causes organ damage in various ways.Furthermore, models of stomach cancer have been created using organoid technology. In terms of precise manipulation, there are several minor discrepancies across research. Organoids from gastric cancer grew substantially faster in vitro than normal controls, and tumor development of organoid engrafts in vivo paralleled the matching organoid expansion rates in vitro. The patterns and the expression of common stomach cancer indicators, including carcinoembryonic antigens and periodic acid Schiff reaction, could efficiently duplicate essential aspects of the parent tumors [11]. These organoids included a variety of mutations that were common in stomach cancer and may be found in primary tumors.

3D-bioprinting for Heart Valves and Cardiac Muscle : Valves constructed of mammalian cells are being designed and printed with the help of 3D-bioprinting technology. These researchers used CAD/CAM techniques to create anatomically accurate valve constructs of bio-ink biomaterials and valve-derived cells that provide the construct with its initial strength. [3] Again, More work must be done to determine the circumstances that allow the valves to mature appropriately and accomplish biological and biomechanical functions similar to those of the human valve. Continuous research into endothelial cell linings for vascular conduits will help develop a cellular coating to create an anti-thrombogenic lining on its surfaces, which will be beneficial in the future. When the bio-fabricated heart is completed, the valves will be robotically inserted into their anatomical positions, which will be accomplished by bioprinting them independently from each of the various chambers of the heart before the final assembly of the whole bio-fabricated heart.

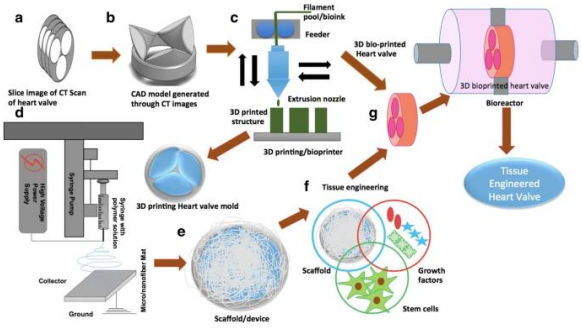


Figure 8. Schematic representation of the proposed process for the generation of 3D heart valves through combining either bioprinting or a combination of 3D printing and electrospinning with a bioreactor to arrive at functional tissue-engineered heart valves. [27]

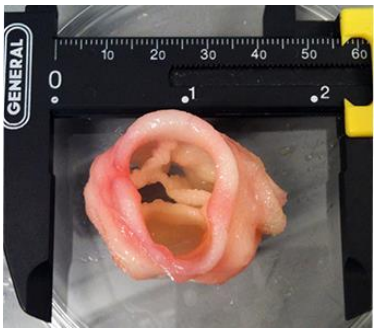


Figure 9. A 3-D print of an artificial heart valve; Image: Jonathan T. Butcher, Cornell University [32]

The myocardium, which plays a critical function in the filling and contraction of the heart chambers, is another constituent of the whole bio-fabricated heart. The search for cardiomyocytes' autologous source is still ongoing, and new knowledge of cell differentiation will undoubtedly assist in this endeavor. Autologous cardiomyocytes from a patients' progenitor cells are possible due to induced pluripotent stem cell technology. Stem cell-generated cardiomyocytes produced from individuals with a range of cardiomyopathies are being used to construct 3D micro-physiologic systems.

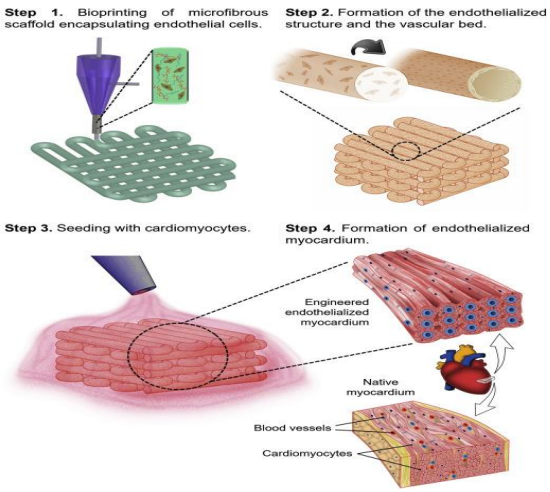


Figure 10. Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium [33]

Pediatric patients, particularly those with hereditary abnormalities resulting in inadequate tissue to allow heart reconstruction, might be studied using 3D and 4D bioprinting. It's yet unclear how the sophisticated 3D-bioprinted and transplant heart cells will develop and respond to the physiologic cues that control average organ growth and maturity. It will take longer than anticipated to investigate how all of the constituents of a

whole bio-manufactured heart develop and operate after implantation, due to restrictions of currently available technologies. Before being implemented, new technologies, such as 3D printing for clinical uses and 3D tissue engineering for therapeutic purposes, must be thoroughly examined. Printing heart models to aid in disease assessment and intervention has finally become a reality, as the research about this topic has been growing substantially. Still, this new capacity raises new considerations about how rapidly this technology should be employed in medical treatment. From image data to final print accuracy, every aspect of the 3D-printing process will be evaluated. 3D-bioprinting will need much more scrutiny since the result must match the tissue being replaced in terms of effectiveness and durability. All of these obstacles open the possibility that, in the future, a 3D-bioprinter may be programmed to generate a heart segment, or the whole bio-fabricated heart, depending on designs that repair the flaws that cause cardiac failure in humans.

The Future of Cardiac Tissue Engineering : In its broadest sense, tissue engineering encompasses all the tissues and cells in the body. Cardiac tissue engineering aims to develop actual hearts for clinical transplants or sections of the heart, each with its own set of therapeutic applications. Bioengineering 3D heart muscle, ventricles, biological pumps, blood arteries, valves, and full bioartificial hearts are the focus of cardiovascular tissue engineering, with significant progress being achieved on all fronts. 3D cardiovascular systems are planar tissue constructions modeled after the physiological and morphological properties of human cardiac muscle tissue. In acute myocardial infarction, 3D cardiac patches may be employed to enhance contractile performance by using genetically engineered heart muscle tissue. Biological pumps are hollow chambered pulsing structures composed of tubular grafts surrounded by contractile cardiomyocytes. They have the potential to be used as a biological left ventricular assist device.

Bioengineered ventricles should resemble mammalian left ventricles' structural and morphological properties. In congenital cardiac surgery, they can be used to surgically correct hypoplastic left heart syndrome, where newborn babies are born with poorly developed left ventricles and other heart conditions. Replacement vascular grafts and valves made from tissue engineering materials are intended to be employed in coronary bypass surgery and valve replacement treatments, among other applications. The fabrication of full bio-manufactured hearts for clinical transplant, which is the focus of this study, is the proverbial "holy grail" of cardiac tissue-engineered research and development. Individuals with heart ailments may benefit from genetically manipulating heart components or a whole bioartificial heart, which might alter the standard of care. Based on the intensity of the client's illness, a cardiac patch might be adequate to restore the lost contractile role, but ultimately, a tissue-engineered heart may be required in case of chronic heart failure.Most of the studies investigating the complete fabrication of bioartificial heart employed a cellular scaffold that was populated with neonatal induced pluripotent stem (iPS) or ventricular rat myocyte (NVRMs) produced cardiomyocytes. In instances, functional performance has been established by monitoring left ventricular diastolic pressure, which is 1 mm Hg in most cases. Recent research demonstrated some recent achievements in bioprinting hearts with omental tissue as bio-ink and cells obtained from induced pluripotent stem cells, even though no functional performance data was provided. [3] Almost all the work done on the subject of bioprinting hearts has contributed to demonstrating the field's feasibility and has unquestionably moved it from science fantasy to scientific realism. This explains the potential of bioprinting human hearts and the accessibility of critical technology to enable it. Shortly, bioprinted hearts will be accessible for clinical use thanks to the solid scientific basis that has been established. However, there are a few technological and scientific challenges to be solved before this vision can be accomplished; as a result, it is difficult to determine when this will occur in the future. According to the latest advancements in whole heart bioengineering, a human heart will be available for clinical transplantation within the next few years.

V. CONCLUSION

In summary, xenotransplantation of pig organs or tissues to humans is still in its early phases of development. This innovative technique may offer unlimited supply of donor organs for transplant candidates, thus will significantly reduce the demand for human organ donors. However, Genetic engineering of animal organs can raise some issues, as use of pigs for human tissue transplants can be a controversial practice concerning ethical views. This in turn creates a climate for proliferation of religious and other non-rational concerns, such as the claim that xenotransplantation violates God's will. [24] Further, there are various risks associated with the use of such organs, with the possibility of immunological rejection and endogenous infections. While it is best that all xenotransplantation ethical debates should be accompanied by further physiological, bioethical, and psychological evaluations of stem cells and CRISPR-based biotech in organ transplants to determine the impact of transplanting human embryonic stem cells into pigs' hearts. Therefore, It is the role of society to keep its moral conscience and evaluate the scientific foundations of these ethical problems using the best possible

experimental procedures. While, ethical considerations are paramount but should not hinder medical advances in xenotransplantation for human organ transplantation. Xenotransplantation has entered an exciting new age after decades of study. While debates about clinical xenotransplantation studies continue, attempts to find the best transgenic pig as a single or multiple organ donor also continues. It is unknown if a single genetically modified pig would suffice as a supplier for several organs (lungs, liver, heart, and kidneys) or whether organ-specific genetically altered pigs will be required. Technological innovations, such as 3D-bioprinting, may aid in acquiring essential information more quickly and at a lower cost. Therefore, ways for comprehending the remaining physiological and pathobiological hurdles require further studies.

Producing therapeutically appropriate tissues and integrating heterogeneous cell types holds considerable potential with 3D cardiovascular bioprinting constructs. The engineering of remote working tissue units is limited by current methods, technology, and materials. Essential biological procedures, improved technology, and material science advances must be clarified before entire organs can be developed. Bioreactor systems are vital components in tissue maturation after printing because they offer tissue-specific physiology in vitro settings. Due to bioreactor technology, several connective tissue schemes may be cultured in vitro in dynamic settings as perfusion systems and spinner flasks. In addition, perfusion bioreactor system flow might be enhanced further by utilizing computational fluid dynamics. Computational analysis might be used in this manner to get a better understanding of the environment and physical stimuli, which are critical for tissue functionality establishment.

In essence, the advancement of 3D-bioprinting technologies has helped cardiac tissue engineering advance toward clinically significant cardiovascular disease therapeutics. 3D-bioprinting holds promise for duplicating structures like the myocardial and cardiac valves, and blood arteries identical to those observed in the natural heart, although the science and technology are still in their early phases. The emergence of new bio-inks and printers designed for high construction would enhance research and aid in the eventual goal of creating complete organs. Thanks to advancements in stem cell biology and elevated heart cell synthesis, organs can be produced on-demand in the future. However, further research is needed on implanting and integrating synthetic structures. Regardless, 3D-bioprinting is a practical approach for delivering modified or genetically modified cardiovascular cells from the lab to the patient's bedside.

3D-bioprinting has significant potential in patient-tailored cardiovascular care and clinical tool development, and may also aid patient and physician education. Tissue-engineered live valve replacement can benefit from 3D-bioprinted cell-enclosed valve conduits. 3D printing is being used as a manufacturing approach for tissue engineering of heart valves, and several issues that arise during traditional scaffold construction are being addressed. A procedure for fabricating durable heart valves utilizing a 3D printing approach has been revealed. As advancements occur in the near future 4D bioprinting and xenotransplantation will continue to also garner interest. The capacity to construct sophisticated and stimuli-responsive structures are greatly enhanced by 4D printing technology. When exposed to UV light, electricity, heat, or other energy sources, or when subjected to a predefined stimulus, such as osmotic pressure and exposure, the organ's 3D structure undergoes instantaneous self-transformation after being printed. With 4D bioprinting technology, the homogeneous tissue generated by 3D-printers may be transformed into a fourth dimension, allowing for more precise medical applications. As four-dimensional printed structures would unlock the ability to fabricate tissue structures that can undergo morphological changes. In this case, Stimuli-responsive materials and cell traction forces can be used for the development of bioinks for 4D bioprinting. Where with further advancements of 4D bioprinting is a promising technology for the development of biological structures that can mimic the structure of native cellular structures in the near future.

KEYWORDS
Xenotransplantation
Organ Transplant
3D-Bioprinting
Heart Transplant
Organoids
CRISPR-Cas

ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
4D	Four-dimensional
AM	Additive manufacturing
CAD	Computer-Aided Design
CAM	Computer-Aided Manufacturing
CT	Electrocardiography-gated computer tomography
CVD	Cardiovascular disease
CXTx	Cardiac xenotransplantation
ECs	Endothelial cells
ECM	Extracellular matrix
ESCs	Embryonic stem cells
GTKO	Galactosyltransferase
H1N1	Swine-infective influenza virus
H7N2	Avian-infectious influenza virus
H7N9	Human-infective influenza virus
HIV	Human immunodeficiency virus
iPS	Induced pluripotent stem
iPSCs	Induced pluripotent stem cells
LIFT	Laser-Induced Forward Transfer
LVAD	Left ventricular support device
MRI	Magnetic resonance imaging
NHP	Non-Human Primate
PCL	Polycaprolactone
PCXD	Perioperative cardiac xenograft dysfunction
PDMS	Polydimethylsiloxane
SCs	Stem cells
NVRMs	Ventricular rat myocyte

VI. ACKNOWLEDGEMENTS

I would like to express my most profound appreciation to the individuals who supported me throughout my years of learning at WAU. I want to start by thanking the reviewers of my manuscript: Anna-Karla Carreño, for reading the first draft! Further, the experts who accepted and reviewed my review during their busy schedules, and set a time to advocate my work. I would also like to thank Mr. Sam Dey for helping me spread the word of my need for reviewers. Overall, I cannot thank each of my reviewers enough, Dr.Afshin Abedi, Dr.Ivan Chavez, Dr.Si Pham, Dr.Rohan Goswami, and Dr.Bimal Francis, for responding to this last-minute request. Especially I appreciate the fact that although most of the reviewers of this document have never met me personally in person, they accepted my offer with open arms within a couple of emails. This greatly shows their fantastic personalities and mentorship for the younger generation. Further to my advisors, Dr.Melinda Ekkens-Villanueva and Dr.Samuel Perez; They have been my most incredible support system both educationally and personally throughout all aspects of my journey as a Biology student, as I lack words to express my gratitude towards them. Lastly, I would like to extend my deepest gratitude to Dr. Perez, the chair of the Department of Biology and Chemistry. I'm incredibly grateful for having a great mentor and advisor like him, who sparked and supported my passion in every crazy little idea I had. My journey would not have been possible without his support, nurturing, and leadership in our department and toward his students.

FORMATTING OF FUNDING : This research manuscript did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Alonzo, Matthew, et al. "3D-- bioprinting of Cardiac Tissue and Cardiac Stem Cell Therapy." Translational Research, vol. 211, no. 1, Sept. 2019, pp. 64–83, https://doi.org/10.1016/j.trsl.2019.04.004.

[2] Ashammakhi, N., Ahadian, S., Zengjie, F., Suthiwanich, K., Lorestani, F., Orive, G., Ostrovidov, S., & Khademhosseini, A. (2018). Advances and Future Perspectives in 4D Bioprinting. Biotechnology journal, 13(12), e1800148. https://doi.org/10.1002/biot.201800148

[3] Birla, R. K., & Williams, S. K. (2020). 3D bioprinting and its potential impact on cardiac failure treatment: An industry perspective. APL bioengineering, 4(1), 010903. https://doi.org/10.1063/1.5128371

[4] Blaeser, A., Duarte Campos, D. F., Weber, M., Neuss, S., Theek, B., Fischer, H., et al. (2013). Biofabrication under fluorocarbon: a novel freeform fabrication technique to generate high aspect ratio tissue-engineered constructs. Bioresearch Open Access 2, 374–384. Doi:10.1089/biores.2013.0031

[5] Cai, Songjie, and Anil Chandraker. "Cell Therapy in Solid Organ Transplantation." Current Gene Therapy, vol. 19, no. 2, Aug. 2019, pp. 71–80, https://doi.org/10.2174/1566523219666190603103840.

[6] Cui, Haitao, et al. "3D-- bioprinting for Organ Regeneration." Advanced Healthcare Materials, vol. 6, no. 1, Dec. 2016, p. 1601118,https://doi.org/10.1002/adhm.201601118.

[7] Doxzen, Kevin. "Gene-Edited Pig Heart Transplanted into a Human Patient." World Economic Forum, 19 Jan. 2022, www.weforum.org/agenda/2022/01/gene-edited-pig-heart-transplanted-into-human-patient/.

[8] Driehuis, Else, and Hans Clevers. "CRISPR/Cas 9 Genome Editing and Its Applications in Organoids." American Journal of Physiology-Gastrointestinal and Liver Physiology, vol. 312, no. 3, Mar. 2017, pp. G257–65, https://doi.org/10.1152/ajpgi.00410.2016.

[9] Galow, Anne-Marie, et al. "Xenogeneic and Stem Cell-Based Therapy for Cardiovascular Dis eases: Genetic Engineering of Porcine Cells and Their Applications in Heart Regeneration." International Journal of Molecular Sciences, vol. 21, no. 24, Jan. 2020, p. 9686, https://doi.org/10.3390/ijms21249686.

[10] Goerlich, Corbin E., et al. "Blood Cardioplegia Induction, Perfusion Storage and Graft Dysfunction in Cardiac Xenotransplantation." Frontiers in Immunology, vol. 12, June 2021, https://doi.org/10.3389/fimmu.2021.667093.

[11] Hendriks, Delilah, et al. "CRISPR-Cas Tools and Their Application in Genetic Engineering of Human Stem Cells and Organoids." Cell Stem Cell, vol. 27, no. 5, Nov. 2020, pp. 705–31, https://doi.org/10.1016/j.stem.2020.10.014.

[12] Hsu, P.D.; Scott, D.A.; Weinstein, J.A.; Ran, F.A.; Konermann, S.; Agarwala, V.; Li, Y.; Fine, E.J.; Wu, X.; Shalem, O.; et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat. Biotechnol. 2013, 31, 827–832.

[13] Kuscu, Cem, et al. "Applications of CRISPR Technologies in Transplantation." American Journal of Transplantation, vol. 20, no. 12, July 2020, pp. 3285–93, https://doi.org/10.1111/ajt.16095.

[14] Längin, M., Mayr, T., Reichart, B. et al. Consistent success in life-supporting porcine

cardiac xenotransplantation. Nature 564, 430–433 (2018). https://doi.org/10.1038/s41586-018-0765-z

[15] Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science 2012, 337, 816–821.

[16] Li, Ping, et al. "The Potential Role of 3D-bioprinting in Xenotransplantation." Current Opinion in Organ Transplantation, vol. 24, no. 5, Oct. 2019, pp. 547–54, https://doi.org/10.1097/mot.0000000000000684.

[17] Loike, John D., and Alan Kadish. "Ethical Rejections of Xenotransplantation?" EMBO Reports, vol. 19, no. 8, July 2018, https://doi.org/10.15252/embr.201846337.

[18] Lu, Tianyu, et al. "Xenotransplantation: Current Status in Preclinical Research." Frontiers in Immunology, vol. 10, no. 2, Jan. 2020, https://doi.org/10.3389/fimmu.2019.03060.

[19] Mandrycky, C., Wang, Z., Kim, K., and Kim, D.-H. (2016). 3D bioprinting for engineering complex tissues. Biotechnol. Adv. 34, 422–434. doi: 10.1016/j.biotechadv.2015.12.011

[20] Mohiuddin, Muhammad M., et al. "Current Status of Pig Heart Xenotransplantation." International Journal of Surgery, vol. 23, no. 1, Nov. 2015, pp. 234–39, https://doi.org/10.1016/j.ijsu.2015.08.038.

[21] Parihar, Arpana, et al. "3D-- Printing: Advancement in Biogenerative Engineering to Combat Shortage of Organs and Bioapplicable Materials." Regenerative Engineering and Translational Medicine, July 2021, https://doi.org/10.1007/s40883-021-00219-w.

[22] Peng, W., Datta, P., Ayan, B., Ozbolat, V., Sosnoski, D., & Ozbolat, I. T. (2017). 3D bioprinting for drug discovery and development in pharmaceutics. Acta biomaterialia, 57, 26–46. https://doi.org/10.1016/j.actbio.2017.05.025

[23] Reichart, Bruno, et al. "Pig-To-Non-Human Primate Heart Transplantation: The Final Step toward Clinical Xenotransplantation?" The Journal of Heart and Lung Transplantation, vol. 39, no. 8, Aug. 2020, pp. 751–57, https://doi.org/10.1016/j.healun.2020.05.004.

[24] Rollin B. E. (2020). Ethical and Societal Issues Occasioned by Xenotransplantation. Animals : an open access journal from MDPI, 10(9), 1695. https://doi.org/10.3390/ani10091695

[25] Satpathy, A., Datta, P., Wu, Y., Ayan, B., Bayram, E., & Ozbolat, I. T. (2018). Developments with 3D bioprinting for novel drug discovery. Expert opinion on drug discovery, 13(12), 1115–1129. https://doi.org/10.1080/17460441.2018.1542427

[26] Tomasina, C., Bodet, T., Mota, C., Moroni, L., & Camarero-Espinosa, S. (2019). Bioprinting Vasculature: Materials, Cells and Emergent Techniques. Materials (Basel, Switzerland), 12(17), 2701. https://doi.org/10.3390/ma12172701

[27] [Fig 8] Vashistha, R., Kumar, P., Dangi, A.K. et al. Quest for cardiovascular interventions: precise modeling and 3D printing of heart valves. J Biol Eng 13, 12 (2019). https://doi.org/10.1186/s13036-018-0132-5

[28] [Fig 1,2] Ryczek N, Hryhorowicz M, Zeyland J, Lipiński D, Słomski R. CRISPR/Cas Technology in Pig-to-Human Xenotransplantation Research. Int J Mol Sci. 2021 Mar 21;22(6):3196. doi: 10.3390/ijms22063196. PMID: 33801123; PMCID: PMC8004187.

[29] [Fig 4] Feinberg, A. W., and Miller, J. S. (2017). Progress in three-dimensional bioprinting. MRS Bull. 42,
557–562. doi: 10.1557/mrs.2017.166

[30] [Fig 6] Billiet, T., Vandenhaute, M., Schelfhout, J., Van Vlierberghe, S., and Dubruel, P. (2012). A review of
trends and limitations in hydrogel-rapid prototyping for tissue engineering. Biomaterials 33, 6020–6041. doi: 10.1016/j.biomaterials.2012.04.050

[31] [Fig 7] Organoids: definition, culturing methods, and clinical applications https://cytosmart.com/resources/resources/organoids-definition-culturing-methods-and-clinical-applications

[32] [Fig 9] Creating Valve Tissue Using 3D Bioprinting - ASME. https://www.asme.org/topics-resources/content/creating-valve-tissue-using-3d-bioprinting

[33] [Fig 10] Zhang YS, Arneri A, Bersini S, Shin SR, Zhu K, Goli-Malekabadi Z, Aleman J, Colosi C, Busignani F, Dell'Erba V, Bishop C, Shupe T, Demarchi D, Moretti M, Rasponi M, Dokmeci MR, Atala A, Khademhosseini A. Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials. 2016 Dec;110:45-59. doi: 10.1016/j.biomaterials.2016.09.003. Epub 2016 Sep 5. PMID: 27710832; PMCID: PMC5198581.