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BIOPRINTING THE FUTURE: THE ROLE, TECHNIQUES AND APPLICATIONS OF 3D BIOPRINTING IN CARDIAC TISSUE ENGINEERING

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ABSTRACT

Cardiovascular diseases remain the leading cause of morbidity and mortality worldwide, driving the urgent need for innovative therapeutic strategies. Among emerging technologies, 3D bioprinting has revolutionized cardiac tissue engineering by enabling the fabrication of biomimetic heart tissues with precise spatial organization, cellular composition, and vascularization. This technology leverages bioinks composed of living cells, biomaterials, and signaling factors to construct functional cardiac tissues that closely replicate native myocardium. Recent advances in biomaterials, bioprinting techniques, and stem cell applications have improved tissue viability, mechanical properties, and electrical conductivity are crucial factors for heart tissue regeneration.

Keywords: Cardiac tissue, bioprinting, tissue engineering, bio inks

INTRODUCTION

Over the past decade (2012-2022), the number of kidney, liver, and lung transplants in the United States has increased by more than 50%, with heart transplants increasing by 73%, highlighting the severe shortage of donor organ **[1]** The long-term outcomes of organ transplantation remain uncertain, survival rates for lung and intestine

transplants just exceeding 60%. Additionally, recipients face the lifelong burden of immunosuppressive drug therapy, which not only increases economic costs but also raises the risk of life-threatening infections **[2]** . 3D printing technology as they assisted in resolving a number of manufacturing issues around the world. Chuck Hull is widely regarded as the father of 3D printing because he created stereo lithography (SL), often known as 3D printing, in 1983 **[3]**. Over the past few decades, three-dimensional (3D) printing has gained popularity and is currently being utilized in a number of industrial sectors to quickly and easily fabricate complex materials and structures, surpassing a number of the restrictions of traditional manufacturing methods. By enabling the on-demand "printing" of cells, tissues, and organs, 3D printing, sometimes referred to as additive manufacturing, has also produced impressive advancements in the healthcare industry, particularly in regenerative medicine (3D bioprinting). New scientific disciplines like "tissue engineering" have emerged as a result of these technological advancements **[4]**. Medical modelling is another field where 3D printing is increasingly being applied. In clinical practice, 3D printed models have been shown to be useful for surgical planning and medical education **[5]**.

In its broadest sense, biofabrication is "the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as microtissues, hybrid cell-material constructs through bioprinting or bioassembly" **[6]**. The definition of biofabrication has been further refined to include "bioprinting" and "bioassembly" as complementary parts of the biofabrication process **[7]**. Bioprinting is defined as the positioning of biochemicals, biological materials, and living cells for the generation of bioengineered structures (i.e. additive manufacturing) of biological and biologically relevant materials with the use of computer-aided transfer and build-up processes **[8]**.

Typical 3D printing techniques have further evolved with the creation of sacrificial resin molds for the formation of 3D scaffolds from biological materials. The development of solvent-free, aqueous-based systems have facilitated the printing of biomaterials into 3D scaffolds that can be used for transplantation with or without seeded cells. Generally, 3D bioprinting relies on precisely placing biological components, biochemicals, and living cells layer by layer while controlling the spatial arrangement of functional components of the created 3D structure **[9]**.

Cardiac tissue engineering

Damage to heart muscle, acute or chronic, has long been considered a tipping point for individual health outlook and progression to heart failure. The problem is that adult heart muscle cells, cardiac myocytes, cannot divide to replace injured cells. Thus, despite a limited population of resident cardiac stem cells, the heart cannot repair itself by any native processes **[10]**.

Instead, scar tissue develops over regions of damaged myocardium. Such scar tissue keeps the organ intact but cannot contract. The ideal clinical intervention would either avoid such scar formation, or simply replace formed scar tissue with functioning cardiac muscle tissue. In a first approach to such therapy, investigators have used injections of new cells into damaged areas of cardiac tissue. These studies have met with limited success due to cell death, exit of cells from the heart, and poor cellular integration with the receiving heart tissue **[11]**.

For end-stage cardiovascular disorders, heart transplantation is still the best course of action. Organ shortage is a significant problem in extending the lives of patients with severe cardiovascular disease (CVD), even though some subjects are lucky enough to acquire a donor heart. Many patients pass away while waiting for a donor. Additionally, the number of patients waiting for heart transplants rose by 127% between 2005 and 2016, despite a 57% increase in new heart transplant listings during that same time period, indicating a growing need for donor hearts **[12]**. The acquisition of the donor heart and organ rejection after transplantation are two of the numerous obstacles to a successful heart transplant. Although innovations like ex-vivo heart perfusion have made it feasible to donate hearts to recipients who live far away, it is still difficult to treat organ rejection sustainably **[13]**.

An emerging approach for addressing the growing demand for heart transplantation is 3D bioprinting of cardiac tissue due to its potential to repair cardiac tissue. In addition to restoring the function of infarcted tissue, 3D bio-printed cardiac tissue that is derived from autologous cells is less likely to trigger an immune response. Given these possible benefits, 3D bioprinting has the potential to develop cardiac tissue constructs for the treatment of CVD. Recent approaches to three-dimensional cardiac tissue construction have yielded promising results, indicating its potential for creating alternatives to heart transplantation **[14]**.

Certain combinations of biomaterials and bioprinters are chosen based on the kind of cardiac tissue (myocardium, valves, blood

vessels, connective tissue, etc.) that is being produced. For example, Lee *et al*. used extrusion-based bioprinting in conjunction with collagen/ECM and alginate to create an environment that was favorable to cardiac cell proliferation and crosslinking, two requirements for producing functional cardiac tissue. This resulted in a 3D porous structure with low cytotoxicity **[15]**.

3D Bioprinting techniques

Based on the working principles, 3 major types of 3D bioprinting technologies are:

1. Inkjet-based bioprinting
2. Extrusion-based bioprinting
3. Laser-assisted bioprinting **[16]**.

Inkjet-based bioprinting: Beyond inkjet-based bioprinting, the concept is comparable to that of traditional inkjet printing, which includes desktop inkjet printers. The procedure of printing is non-contact and involves precisely placing tiny droplets of "bioink" onto a hydrogel substrate or culture dish. One This kind of bioprinting can be carried out with piezoelectric or thermal actuators **[17]**.

In piezoelectric inkjet bioprinters, a piezoelectric actuator is used to create acoustic waves that travel through the bioink chamber. Electrostatic inkjet bioprinters use a voltage pulse applied between an electrode and a pressure plate to create droplets. Heat is produced in the bioink chamber during thermal inkjet bioprinting, and this heat causes pressure to be created **[18]**. **(Figure 1)**

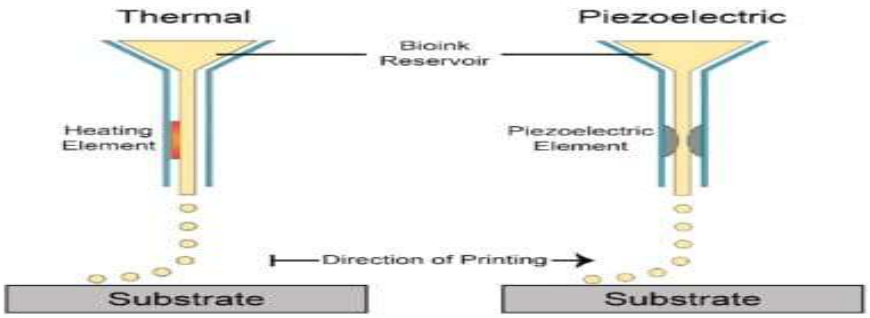

Figure 1: Schematic representation the thermal and piezoelectric inkjet bioprinting

Extrusion-based bioprinting: In recent years, extrusion-based bioprinting, also referred to as direct writing, has become increasingly popular in tissue engineering and biofabrication. Two primary factors underlie the widespread dissemination of the extruded bioinks as a piston-driven deposition setup uses screw-driven systems to manage bioink overflow **(Figure 2)**. When it comes to the deposition of highly viscous biomaterials, this is a significant problem **[19]**.

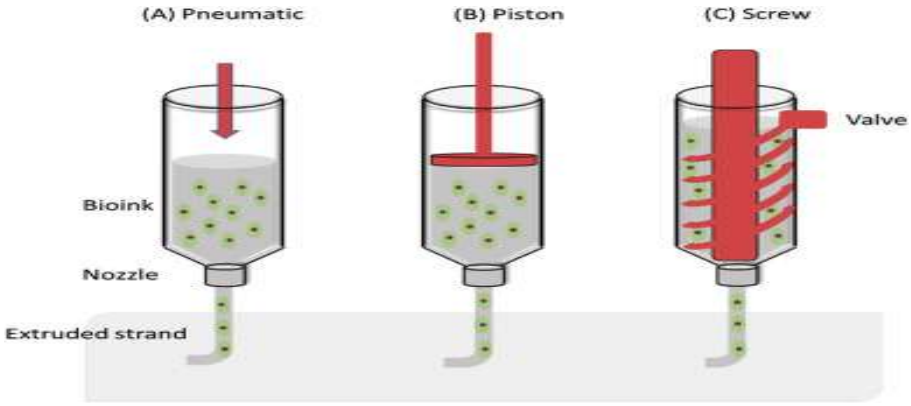

Figure 2: Schematic diagram of extrusion-based bioprinting techniques: pneumatic, piston-driven, and screw-driven method

Laser-assisted bioprinting: The principle behind laser-assisted bioprinting is the use of a laser as an energy source to deposit biomaterials onto a substrate. This bioprinting method, which was initially created for writing metals, has been effectively used to bioprint organ cells or nucleic acids like DNA **[20]**.

In order to print biomaterials with high resolution, the first laser-assisted bioprinter was created in 2004 to transfer biological designs to substrates. The cells in liquid or gel solution that deposit on the metal-film support are known as bioinks. The bioink droplets are subsequently removed as a result of the metal film and incident laser light vaporizing together **[21]**.

In laser-assisted bioprinting, the death of cells due to thermal damage (nano-second laser irritation) is one of the primary problems. To remove this damage, Hopp *et al*. used femto-second lasers **[22]**.

strand:pneumatical force (gas or pressurized air) and mechanical force (screw or piston).

In stage and under the direction of a stage manager, the bioinks are applied to a construction substrate, and the

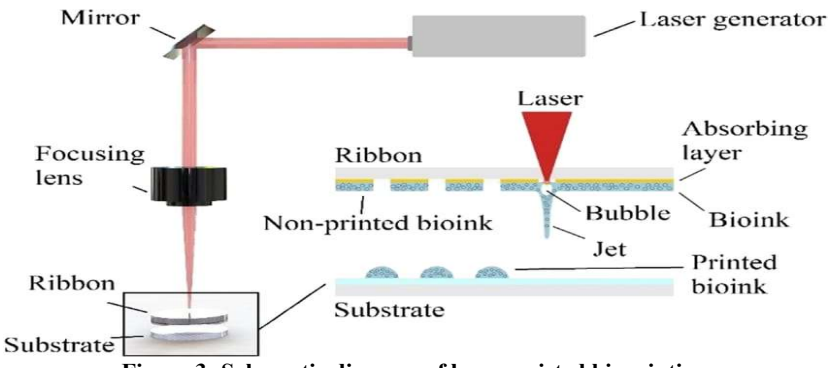

Figure 3: Schematic diagram of laser-assisted bioprinting

Bio inks

The bio-ink defined as any suitable composition of cells along with biologically active components and biomaterials Processed automatically using the fabrication technology **[23]**.

An ideal bioink should have the appropriate mechanical, rheological, chemical, and biological qualities. among other physicochemical attributes.

These properties should lead to:

- The creation of tissue constructs that are sufficiently robust and strong mechanically while maintaining tissue-matching mechanics, ideally in a modifiable way.
- Adaptable stabilization and gelation to support the bioprinting of highly shape-fidelity structures.
- The capacity to simulate the tissues' natural microenvironment through biocompatibility and, if required, biodegradability.
- Adaptability to chemical alterations to satisfy tissue-specific requirements.
- The possibility of producing on a wide scale with little fluctuation from batch to batch **[24]**.

Biomaterials including gelatin, collagen, fibrin/fibrinogen, silk, alginate, gellan gum, dextran, polyethyleneglycol and their use as bioinks will be discussed in the following section.

Gelatin: Collagen is denaturated to make gelatin. It can be produced by hydrolyzing animal bones, tendons, or skins in an acidic or basic manner. It is one among the most popular natural polymers for a variety of biomedical applications because its solution is thermosensitive and can form a hydrogel at lower temperatures in a concentration-dependent way **[25]**.

Biocompatibility, biodegradability, low antigenicity, accessible active groups, lack of hazardous byproducts, ease of processing, and affordability are some of the better benefits of gelatin. It is a material that can be used in tissue engineering and bioprinting because of all of these characteristics, but particularly because of its cellular affinity. Gelatin in a variety of concentrations has been utilized as a bioink material and/or in combination with other polymers for bioprinting applications. Furthermore, its altered forms such as gelatin methacryloyl (GelMA), which may be chemically cross-linked—have also been modified for bioprinting **[26]**.

Collagen: Mammalian cells' extracellular matrix (ECM) contains collagen as its primary structural protein. Since collagen has excellent *in vitro/in vivo* biocompatibility and tissue-matching physicochemical qualities, it

has been used extensively in biomedical applications **[27]**.

In order to create bioprinted multilayer 3D skin tissue structures using LaBP, Koch *et al*. utilized collagen as a bioink formulation with encapsulated keratinocytes and fibroblasts. After bioprinting, they looked at the morphological and viability functions of both cell types. Their results indicated that the produced skin grafts exhibited tissue-specific capabilities and showed the existence of intercellular interactions between various cell types, both of which are encouraging for the creation of intricate multicellular tissue constructs. The same researchers also assessed these creations' in vivo uses and showed how cell proliferation and appropriate differentiation might produce 3D skin-like tissue **[28]**.

Alginate can also be mixed with collagen to create a composite bioink. Additionally, this bioink was used to bioprint 3D porous cellular blocks by encapsulating human adipose tissue-derived stem cells (ASCs) and preosteoblasts. Eleven In this case, the cells were first cultivated on a collagen gel, and alginate was then added to the cell-rich collagen gel. The collagen-alginate bioink's osteogenic potential was higher than that of the alginate-only bioink, according to the data. Furthermore, the ASCs' hepatic lineage differentiation was also accomplished in the bioprinted blocks, suggesting that this novel bioink may find usage in a number of tissue engineering applications. Collagen is also frequently utilized in bioprinting applications as a biopaper **[29]**.

Fibrinogen: A big, fibrous, and soluble glycoprotein called fibrinogen is involved in the development of blood clots. In the presence of Ca2+, thrombin transforms fibrinogen into an insoluble fibrin molecule through intermolecular interactions **[30]**.

Because of their advantageous functions in wound healing, fibrinogen and fibrin are primarily utilized in tissue engineering to create viable tissue constructions for the replacement of injured tissues. They promote cell adhesion, proliferation, and the production of extracellular matrix (ECM) and are biocompatible, biodegradable, and non-immunogenic **[31]**.

There are other composite bioink forms, including fibrin-collagen, which were used in conjunction with electrospinning and inkjet printing to enhance the final construct's mechanical qualities for cartilage tissue engineering **[32]**.

The constructions were made in three stages: first, PCL (Polycaprolactone) was electrospun as a substrate; next, chondrocytes were bioprinted on top of the PCL layer after being

encapsulated in collagen or fibrin; and lastly, another PCL layer was electrospun. They discovered that while the hybrid system had no effect on cell survival rates, it enhanced the construct's mechanical qualities. Complex tissue creation may make use of this novel approach. Gruene *et al*. combined fibrinogen with HA to create a bioink, and then they employed the LaBP technique to assemble multicellular arrays in three dimensions **[33]**.

Silk: Silk is a naturally occurring polymer that has been utilized for ages as a medical suture material. Due to its high elastic content and slow rate of disintegration, it offers many desirable qualities as a biomaterial that are necessary for giving the cells enough support till the new tissue regenerates. Furthermore, it is biocompatible and has a low immunogenicity, making it appropriate for clinical use as well **[34]**. Silk has been used as a scaffolding material for both soft and hard tissue engineering applications for a long time because of these appealing qualities. More recently, it has also been modified for use in bioprinting **[35]**.

Alginate: Alginate, also called aginatic acid, is a naturally occurring anionic polymer that is extracted from brown seaweed and resembles the glycosaminoglycans that are present in the body's natural extracellular matrix. Its biocompatibility, low cytotoxicity, mild gelation procedure, and low cost have made it a popular choice in biomedicine **[36]**. Its ability to gellate quickly under physiological conditions without producing harmful byproducts has made it a popular bioink. By creating bridges between polymer chains, enabling physical cross-linking and solubility, divalent cations like Ca2+ and Ba2+ can easily promote the gelation of alginate **[37]**. It is possible to dissolve the ionically cross-linked alginate gels from a construct by releasing the divaIent ions that cross-link the hydrogel through exchange reactions with monovalent cations found in the surrounding medium **[38]**.

Gellan gum: High molecular weight and hydrophilic, gellan gum is an anionic polymer made by bacteria. When mixed with monovalent or divalent cations, it produces a hydrogel at low temperatures, just like alginate **[39]**. The Food and Drug Administration (FDA) in the United States has authorized it as a direct food additive **[40]**. Gellan gum has been mixed with other polymers in the context of bioprinting to create bioinks with desirable rheological characteristics and to enhance the shape fidelity of the bioprinted structures. When printing pre-defined 3D structures, the viscosity has a significant impact on the resolution of the printed structures **[41]**.

Additionally, gellan gum possesses excellent processability, customizable mechanical qualities, and is reasonably priced to produce all of which are critical for bioprinting procedures **[42]**.

Dextran: Another natural polysaccharide that has been extensively utilized in tissue engineering applications is dextran, which is hydrophilic and nontoxic. Dextran is categorized as a biodegradable substance since dextranase may break it down in mammalian tissues **[43]**. Pescosolido *et al*. created hydrogels with a polysaccharidic semi-interpenetrating polymer network using HA and methacrylated dextran (dexHEMA) **[44]**. In this investigation, equine chondrocytes were encapsulated inside the bioink using a pneumatic dispensing method with a motorized stage. According to the findings, the HA-dexHEMA mixtures exhibited desirable viscoelastic and pseudoplastic properties as bioinks, and they may be applied to a range of bioprinting tasks.

Polyethyleneglycol: PEG is a synthetic polymer made via ethylene oxide polymerization. It can be made with a variety of topologies, including linear or multi-armed architectures, and chain lengths. Because of its mechanical qualities, which are generally strong yet can be tailored, it is a preferred synthetic material for bioprinting and for maintaining the shape of the deposited constructions. Additionally, PEG is non-immunogenic and non-cytotoxic (at higher molecular weights) **[45]**. However, because it is a bioinert substance, cells cannot easily adhere to it; as a result, it must be mixed with other hydrogels that are biologically active. In fact, it has been demonstrated that PEG-based structures degrading characteristics are enhanced by PEG and natural biomaterial composites **[46]**.

APPLICATIONS OF BIOPRINTING CARDIAC TISSUE ENGINEERING

Cardiac Tissue and Patches: The characteristics of cardiac patches make them ideal for clinically significant heart repair. They have been employed especially to replace or repair damaged heart tissues and partially restore cardiac function **[47]**. The use of 3D bioprinting technologies to create tissues has advanced recently. Cui *et al*. (2009) investigated the process of microvascularization by printing fibrin scaffolding and human vascular endothelial cells simultaneously **[48]**. The team discovered the 3D tubular structure in printed patterns, where confluent linings were formed by growth and cell alignment inside channels. This work demonstrated the effects of concurrent scaffold and cell printing on microvasculature and cell growth. Shin *et al*.

created cardiac patches later in 2013 by seeding neonatal rat cardiomyocytes onto carbon nanotubes that included photo-cross-linkable gelatin methacrylate hydrogel **[49]**. The structures exhibited sophisticated electrophysiological activities and excellent mechanical integrity, indicating the potential of incorporating carbon nanotubes into the fabrication of multifunctional cardiac scaffolds. Pre-vascularized and functioning multi-material 3D structures were printed using stem cell-laden dECM bio-inks in a more recent study by Jang *et al*. in 2017 **[50]**. When implanted in hearts in vivo, the stem cell patch was demonstrated to strongly enhance tissue matrix synthesis and vascularization. The patch demonstrated enhanced cardiac functions overall, decreased cardiac fibrosis and hypertrophy, increased cell migration from the cardiac patch to the location of the myocardial infarct, and the development of capillaries and neo-muscle. In 2019, Noor *et al*. most recently used a method that involved printing thick, vascularized, and perfuseable cardiac patches that matched the immunological, cellular, biochemical, and anatomical characteristics of the patients **[51]**.

Cellular sources: For cardiac tissue engineering, cellular sources Bioprinted structures should have the following characteristics: rapid cell division and maturation into the target cell type or types; easy access to autologous cell sources; and non-antigenicity with pathogen immunity. The best possibilities for cardiac tissues include stem cells, such as cardiac stem cells and mesenchymal stem cells produced from bone marrow or cord, progenitor cells, and native cardiomyocytes **[52]**. Flexible levels of self-renewal, the capacity to differentiate into cardiomyocytes, high proliferation, and the potential to lessen thrombogenic effects, graft immunological rejection, and on-demand availability are all provided by stem cells **[53]**. In reference to the use of stem cells in 3D bioprinting, one study that used gelatin-fibrin bio-ink investigated the proportion of co-culturing diverse cardiac cell types, including cardiomyocytes, cardiac fibroblasts, and endothelial cells, and showed how distinct cell types may be hetero-cellularly coupled by bioprinting **[54]**.

Full heart organoid and organ: The long-term objective of cardiac tissue engineering is to create functional heart organs that are similar to natural anatomy. The first application of completely customized, non-supplemented bio-ink materials to print hearts with mechanical characteristics that closely resemble those of decellularized rat hearts was shown by Noor *et al*. in 2019 **[55]**. Later, Lee *et al*. successfully printed five components of

the human heart, including a tri-leaflet heart valve, neonatal-scale collagen heart, and human cardiac ventricle model using the FRESH bioprinting technique **[56]**.

The bioprinted hearts provided a high-resolution, exact representation of the anatomical features unique to each patient. Kupfer *et al*. produced macroscale tissue with geometric structures related to the pump function of the heart muscle in 2020 with reference to bioprinted cardiac organoids. In response to cardiac medications and pacing, the human-chambered muscle pumps demonstrated sustained action potential propagation and beating. Kupfer's research has ramifications for the production of organoids of this kind, which could be used in tissue grafting and cardiac medical devices **[57]**.

CONCLUSION

In general, 3D bioprinting uses a variety of bioprinting methods with varying compositions of bio-ink to produce tissue constructs and functional models. Inkjet based, extrusion-based and laser assisted bioprinting methods all demonstrate various advantages and drawbacks. Biomaterials that are natural, synthetic, or hybrid have been used and studied for their mechanical tunability and cell-supporting qualities. Recent developments in cardiac tissue engineering, from tissue models to whole organs, are important for comprehending clinical research and regenerative medicine. While 3D bioprinting has significant potential for cardiac tissue engineering, there are still several issues that need to be resolved. In clinical medicine, cardiac tissue engineering holds great promise for the replacement, regeneration, and repair of damaged heart tissue and the organ overall. There is hope for interesting applications in clinical translation and regenerative medicine if further innovation is made to overcome existing constraints.

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