FUTURISTIC APPROACHES IN BIOENGINEERING OF CARDIAC SCAFFOLDS FOR THE CUTTING EDGE EXPLORATION IN CARDIOVASCULAR REGENERATIVE MEDICINE

Abstract

Acute myocardial infarction (AMI), which block down blood flow to the myocardium, is one among the serious life-threatening disorder that causes mortality and morbidity. The disadvantages of prevailing cardiac restoration interventions include the lack of heart donor and the use of non-biological active idle materials to substitute destorated tissue. Cardiomyocytes, which are potentially not capable of natural regeneration, require novel therapeutic approaches to stimulate cardiac tissue restoration. This chapter highlights the fundamental three aspects of cardiovascular regenerative medicine (cell, bioactive materials, and scaffolds), with an emphasis on the function of scaffolding. Scaffoldings for heart regenerative medicine are three-dimensional porous constructs that mimic the intercellular cardiac matrix and can stimulate relocation, cell maturation, cellular adhesion, and proliferation. Scaffolds have so far opened up new prospects for potential applications in heart regeneration beyond AMI. For cardiac tissue engineering researchers, it is critical to decide on an appropriate biofabrication of synthetic scaffolding, as well as the best biologically compatible, degradable material for scaffold building, because scaffolds must additionally provide mechanical contractility and electrical conductivity. This chapter focuses on the most advanced shifts of biodegradable hybrid-based scaffolds (hydrogels, nanofibers, patches) for cardiac engineering. In addition, we briefly investigated the future prospects and problems of various types of scaffolds.

Keywords: Acute myocardial infarction (AMI), Cardiomyocytes, Scaffolds, Cell adhesion, Proliferation.

Authors

J. Abhinav
Department of Biotechnology
Prathyusha Engineering College
Tamil Nadu, India.
jabhinavmedico@gmail.com

Dr. S. Shobana
Assistant Professor
Department of Biotechnology
Vel Tech Rangarajan Dr.Sagunthala R & D Institute of Science and Technology
Avadi, Chennai, Tamilnadu, India.
swtshoba@gmail.com

I. INTRODUCTION

A heart is an unprecedented complex organ, a wonder of "engineering by living system", where they contribute together to perform significant role in myocardial contractility, electrical conduction, and in cardiovascular systems [1]. Blood flow in circulatory system follows a unidirectional pattern through an array of valves or cusps with a two-sided pumping mechanism. These valves are generated from tissue folds of a heart, where the contraction and relaxation of valves are responsible for maintaining the open and close mechanism. The contractile elements of heart tissue are known as the cardiomyocytes (CM), primarily located to the myocardium. Cardiomyocytes are three-dimensional synctium that influence the mechanical contractions and unidirectional blood flow through the propagation of electrical signals across the intracellular junctions. The extracellular matrix (ECM) contributes a crucial act in establishing a 3D framework that connects cells, enabling the transmission of forces generated by myocytes. Myocytes exhibit a close-knit connection, forming a functional syncytium where these connections are established via 99 gap junctions, specialized structures that facilitate the passage of ionic currents. Gap junctions are vital for the swift propagation of action potentials, the electrical impulses driving cell contractions. Maintaining proper electrical coupling among cells is of utmost importance to prevent irregular rhythms and reentries, ensuring the coordinated spread of contractions like a wave front. In the atria, cardiac myocytes (CM) constitute around 33% of the cells, while in the ventricles; they make up approximately 50% of the cells [2]. Mature cardiac cells, unlike certain other cells in the body, lack the capacity to regenerate spontaneously, which has provided a substantial challenge for scientists working on CM regeneration.

1. **Impaired Self-Degenerative Mechanism in Mammalian Cardiac Cells:** As the heart ages, the structural and functional properties of its blood vessels changes, particularly in the aorta. Although human cardiac cells (CM) halt to divide before birth, some elasticity persists throughout life. The capacity of CM to regenerate declines with age, from 1% annually at the age of twenty-one to 0.8% at the age of seventy. Thus indicates that fewer than half of a person's CM gets replaced over their lifespan, with around 60% remaining from birth [3]. Changes in several types of cells, such that visceral myocytes, Rouget cells, and endothelial cells (EC), cause a reduction in vascular function in elderly people. These changes cause uneven blood flow and the formation of atherosclerotic plaques, which can lead to myocardial ischemia or a reduction in oxygen delivery to the heart muscle. Ischemic damage causes diseased amendment of the heart, resulting in cardiac tissue destoration and formation of cicatrix driven by induction of inflammatory signaling. They finally result in fibrotic scarring and ventricular malfunction, which leads to heart failure. Mammalian hearts, unlike frogs, lack the regeneration capacity to reverse the cicatrix and recover the function of heart [4]. The processes behind those discrepancies are currently unknown. Adult mammalian hearts have little regenerative capability since there is no reserve pool of cardiovascular progenitor cells as well as postnatal differentiated CM has essentially no proliferative activity [5]. A number of non-communicable diseases may harm non-restorative cardiac cells, resulting in cardiac disease.

2. **Coronary Heart Diseases:** Cardiovascular diseases encompass a range of conditions such as heart attacks, high blood pressure, stroke, ischemic heart disease, birth defects, and inflammatory disease on heart [6]. They are responsible for the highest number of

fatalities in both advanced and developing nations. The World Health Organization (WHO) reports that roughly 19.7 million individuals, accounting for 33% of global deaths, succumb to cardiovascular diseases annually, with 87% of these deaths attributed to heart attacks [7]. Acute myocardial infarction (AMI) or heart failure, which occurs when the flow of blood to the heart muscle is diminished or halted, is a primary cause of physical injury and mortality [8]. AMI may result from intra-arterial thrombosis with a plaque. Furthermore, AMI can be caused by an increased need for or a lack of oxygen, thrombosis, and embolism, all of which can put patients at risk of irreversible myocardial damage and infarction. After experiencing a heart attack, a patient may lose up to 50 grams of muscle mass due to a lack of oxygen, which triggers the release of factors that cause cell death and apoptosis. This loss of muscle mass is a result of hypoxia and can impact and replaces injured cardiac muscle tissue by fibrous tissue. Fibrous tissue replaces injured cardiac muscle cells. Maladaptive remodeling occurs when cardiac fibroblasts transform into myofibroblasts, which cause stiffness and fibrosis. This results in a bleak prognosis and cardiac failure. The scar tissue reduces the heart's capacity to pump and receive blood, resulting in progressive worsening of cardiac function, muscle loss, and eventual heart failure.

Traditional myocardial infarction (MI) therapies include surgeries such as coronary artery bypass, reperfusion, and fibrinolytic therapy. These treatments are more concerned with giving rapid relief from acute symptoms than with enabling the regeneration and renewal of the damaged cardiac tissue. Patients with heart failure may, as a last option, get a heart transplant or a left ventricular assist device (LVAD). However, the outlook varies due to the delicate and invasive nature of transplant surgery, as well as the consequent danger of both short and long-term immune rejections. Cardiac bioengineering seeks to bridge the gap between engineering and medicine by integrating problem-solving abilities and design principles with clinical and physiological scientific expertise. This discipline seeks to improve healthcare by enhancing a diagnosis, evaluation, and medication. It has aided in the development of possible therapies for cardiovascular illnesses (CVDs), such as heart regenerating techniques. Treatments for damaged or infracted cardiac tissue produced by ischemic/reperfusion episodes have been developed using a variety of biotechnologies. The notion of employing autologous cells for cardiac therapy has been investigated using patient-specific cardiomyocytes created using tissue engineering. Current treatments for cardiovascular disease, like artificial vascular grafts, help manage symptoms and slow down the negative changes in the heart, but they are unable to restore the lost heart tissue [9]. As a result, new approaches like tissue engineering are being explored as they have the capability to encourage the regeneration of the injured heart muscle [10].

3. **Cell Therapy and Cardiac Tissue Engineering:** When cardiovascular disease treatment is delayed, it can result in the need for more extensive procedures like cusps or valves replacements. Additionally, the damage caused to the heart tissue by these diseases can lead to further deterioration and ultimately heart failure, which may necessitate a complete heart replacement in extreme cases. However, there is a scarcity of available donor hearts and a high demand for them [11]. Furthermore, the risk of organ rejection due to tissue compatibility issues is also a concern. Emerging therapies, such that cytotherapy as well as regenerative medicine, strive to enhance the limited natural regenerative capabilities of the heart by utilizing cells, chemicals, factors, or patches.

These innovative approaches are being developed to treat cardiovascular diseases. The purpose of cardiac cellular transplantation is to rejuvenate cardiomyocytes that has been damaged by a heart repair [12]. This is accomplished by implanting cells onto or into the dysfunctional cardiac muscle utilizing a variety of ways. Figure 1. represents the scheme of 2D material based cardiac tissue engineering. In tissue engineering techniques, a specific cell types have been combined with growth factors and scaffolds where implanted to recover the injured tissues. The materials employed in this context will aid in the support and organization of cells, as well as the protection and guidance of their growth. They will also aid to hold the cells in place and function as a replacement for the natural extracellular matrix throughout the regeneration process for cardiovascular disorders. This book chapter examines the three fundamental aspects of tissue engineering with an emphasis on cardiac applications.

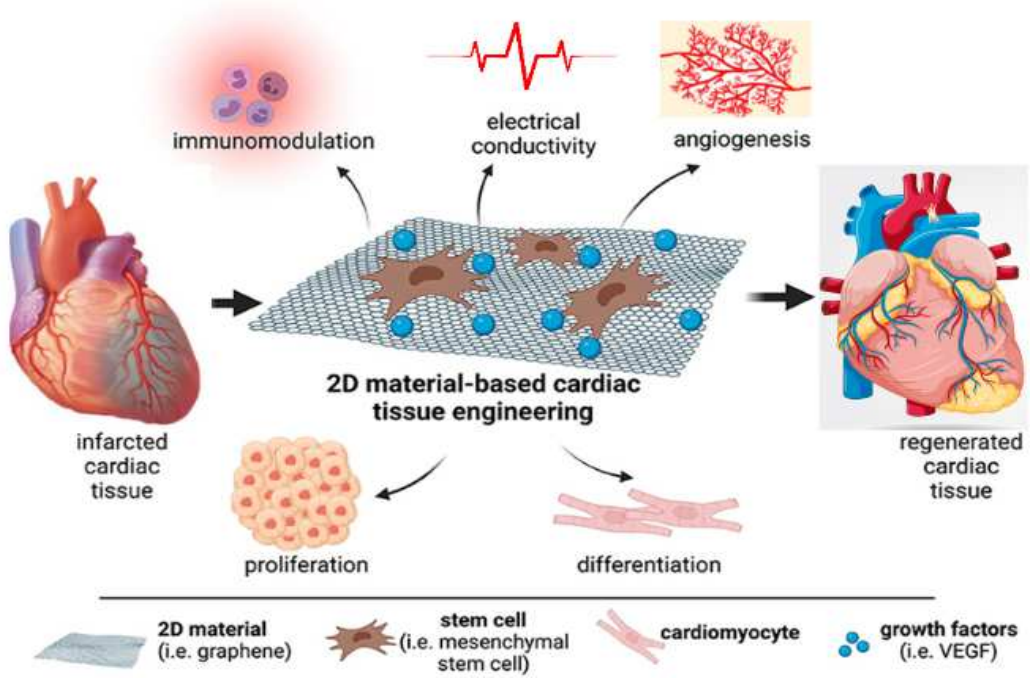

Figure 1: Scheme of 2D material-based heart regenerative medicine. Cardiac bioengineering is the outcome of the combination of three fundamental factors: cells, bioactive materials, and scaffoldings that mimic the intercellular matrices.

II. CELLS AND BIOACTIVE MATERIALS

The heart is made up of many cell kinds, with cardiac myocytes (CM) making up 25-35% of the total. The rest of the cells consist of blood and vascular smooth muscle cells (VSMC), cardiac fibroblasts (CF), cardiac progenitor cells (CPC), lymphatic endothelial cells (EC), Rouget cells, and leukocytes. CM are responsible for contracting and pushing blood, while EC and CF contribute a significant play in enduring tissue function as well as balance. VSMC and Rouget cells regulate blood flow, while CPC have the ability to distinguish into a variety of cell types within the cardiovascular system.

Each type of cell is essential in cardiac biology, which poses a challenge in creating artificial heart muscle. Recent efforts have focused on obtaining human cardiac cells for cardiac injury treatments. Stem cells are encouraged to distinguish into the cardiomyocytes to aid in regenerative medicine [13].

1. **Cell Types:** The heart is made up of several cell types, including cardiomyocytes (CM), which is responsible for around 28-35% of cardiomyocytes. Endothelial cells (EC), cardiac fibroblasts (CF), lymphocytes, cardiovascular progenitor's cells, Rouget cells, as well as vascular smooth muscle cells (VSMC) make up the remaining 70%. Table 1. summarizes the various categories of cells used in the cardiovascular regenerative medicine. The CM is in charge of contracting and pumping blood, whereas the endothelium and cardiac mesenchymal cells are crucial in maintaining cellular repair as well as balance. Vascular ECs enhance the metabolic activity and regulation of blood vessel tone and vasculogenesis, whereas CFs continually regulates the extracellular matrix (ECM). Pericytes and VSMC aid in the regulation of blood circulation within the heart circulatory system. Cardiovascular progenitor's cells are pluripotent cells that display cluster of differentiation markers such as MESP1+, c-Kit, Isl1+, as well as Sca-1, and may differentiate into many cardiovascular cell types [14].

Table 1: Different types of cell types in heart regenerative medicine.

Origin	Cells	Description	Merits	Drawbacks
Embryonic	Fetal cardiac cells	Derived from embryonic cardiovascular system.	Capable for cardiac assimilation and restoration.	Antigenicity, Virulence potential, Ethical issues, Restricted handiness.
Funiculus umbilicalis blood-derived cells	Embryonic stem cells, multipotent cells, hematopoietic progenitor cells (HPC), non-hematopoietic progenitor cells (NHPC).	Cells originated from navel string blood that can distinguish into various kinds.	Minimizing heart tissue damage post-injection into the myocardium.	Antigenicity, Importance of consistent isolation and culturing protocols: senescence and accumulation of mutations during in vitro extension.
Embryonic MSC	Embryonic stem cells derived from embryo's central cellular cluster.	Cells with capacity to develop into cells representing all three primary ectoderm, mesoderm and endoderm layer.	Potential to distinguish into various types of cells.	Linked with cancerous cell development, Ethical concerns.

Somatic stem cells	Adipose tissue-derived stem cells	Derived from body fat, including HSC, MSC, as well as precursor cells of endothelium.	Multi-differential potential, Easy sourcing, Simple harvesting, low expense, and no ethical concerns.	Potential tumorigenicity, Inadequate comprehension of cardiac repair mechanisms.
Autologous vegetal cells	Human-induced pluripotent stem cells (iPSC)	Somatic cells transformed into multipotent cells.	Large-scale production alternatives, Ability to distinguish into various types of heart cells.	Inadequate purity, disparity, and time-consuming/ineffective isolation procedures, Potential for teratoma formation.

Every type of cell is important in circulatory system, and their intricacy makes developing artificial heart muscle difficult. Recent attempts have concentrated on getting human cardiac lineages from multiple cell sources for heart damage applications. Stem cells must be stimulated to develop into cardiac cells, which aids in heart tissue regeneration.

2. **Bioactive Materials (Growth Factors):** Growth factors have a crucial role in facilitating heart tissue regeneration. Using growth factors to convert a subpopulation of undifferentiated cardiac progenitor cells into cardiomyocytes during the neonatal period is one strategy. An alternative method involves transforming cardiac fibroblasts into cells resembling cardiomyocytes through the excessive utterance of specific transcription regulators [15]. Mesp1, a gene that is widely preserved in multicellular organisms, is recognized as a pivotal regulator responsible for initiating the differentiation of mesoderm and endoderm through the blocking or suppressing the Wnt/ß-catenin signal transduction mechanism by DKK1. Following Mesp1's transient expression, transcription regulators including HAND2, MYOCD, FOXH1, MEF2C, GATA4, TBX5, and NKX2-5 are transcription factors that control a cascade of transcriptional events and are required for cardiac development and differentiation [16]. This process is instrumental in driving the development of cardiac tissue. Over expressing GATA, MESP1, and TBX5 has been demonstrated to stimulate the formation of myomere structures within the main connective tissue cells. [17]. Additionally, the presence of HAND2 enhances the effectiveness of converting cells into cardiac progenitor cells (CPC) and improves the performance of damaged hearts after a heart attack [18].

Researchers have investigated pathways such as NOTCH and WNT to govern the maintenance and development of cardiac progenitor cells. NOTCH signaling inhibition has been demonstrated the MEF2C attaching to specific genes to regulate them, and to encouraging heart cell differentiation. Inhibiting BMP and WNT signaling pathways can

also direct cardiac progenitor cells toward a myocardial lineage, as evidenced by the utterance of cardiac-specific indicators such as troponin T and NKX2.5 [19].

MiRNAs can increase cardiomyocyte proliferation and contribute to cardiac tissue regeneration by targeting particular genes involved in cell cycle control. For example, it has been demonstrated that a mixture of microRNAs, such as miR-1, miR-133, miR-208, and miR-499, in various combinations., may stimulate the expression of heart-specific markers in cardiac fibroblasts [20]. These miRNAs can transform fibroblasts into cardiomyocyte-like cells, providing a viable path for cardiac tissue regeneration. Furthermore, miRNAs can be used to block the activity of genes that impede cardiomyocyte growth. MiRNAs such as miR-23b-3p, miR-509-3p, and miR-548c-3p have been discovered by antimitotic gene Meis1 inhibitors. These miRNAs enhance cardiomyocyte proliferation, which is an important part of cardiac tissue regeneration, by targeting Meis1 [21].

The use of miRNAs in cardiac bioengineering provides a precise and focused method of controlling gene expression and cellular function. Using miRNAs' regulatory capacity to promote cardiomyocyte growth is a beneficial approach. Furthermore, employing a cell secretome, which is a collection of chemicals produced by stem cells, has yielded encouraging results in cardiac tissue regeneration. This secretome comprises cytokines, growth factors, and genetic material, all of which promote cardiomyocyte survival, proliferation, differentiation, and neovascularization while minimizing inflammation and fibrosis [22]. Growth factors, in general, contribute a significant aspect of the advancement in cardiac bioengineering and have the potential to revolutionize cardiac tissue regeneration therapy.

III. SCAFFOLDS

Scaffolds are porous structures made from materials that are safe for the body and have biological activity. These structures can promote cell attachment, migration, development, and growth both inside and outside the body [23]. This is especially important in the field of cardiac bioengineering, where scaffolds play a critical role in enhancing heart tissue function and healing.

Scaffolds used in cardiac bioengineering are meant to resemble the extracellular matrix (ECM), in their qualities as possible. The ECM is essential for cell interaction and communication, as well as providing structure and signaling inside tissues. They must have certain properties in the context of cardiac regenerative medicine in order to properly support as well as increase cell growth and function.

1. **Biocompatibility:** The materials selected for creating scaffolds in cardiac bioengineering must be free from toxicity and immune reactions. This criterion also extends to any molecules produced as byproducts during degradation. Scaffolds should be compatible with the body's tissues to prevent any adverse reactions or rejection.

2. **Porosity:** The volume of pore space present in scaffolds is referred to as porosity. Certain mechanical properties, such as the specific mass of the material, may be utilized to determine porosity of the scaffold [24]. Scaffolds must have enough porosity, which is

achieved by well-connected pores, proper pore diameters, and a balanced distribution of pore sizes. The presence of holes allows cells to migrate and aids in the delivery of essential nutrients. Scaffolds should ideally have a permeability of 60-95% to allow liquids, oxygen, and minerals to diffuse, fostering optimal circumstances for heart tissue growth and development [25].

3. **Biodegradability:** Scaffolds must be biodegradable, allowing them to disintegrate slowly as cells build their own extracellular matrix (ECM) and replace the scaffold. Any byproducts of scaffold breakdown must be effectively removed from the body or integrated into various cellular metabolic processes. They should ensure that the scaffold does not hinder the natural healing process.

4. **Surface Characteristics:** In order to enhance the attachment of cells to the scaffolding terrain, it is required to change both the chemical composition and skeletal structure of the scaffoldings. This alteration, also called as functionalization, is accomplished by introducing molecules or atoms on the scaffold's surface. This integration can be accomplished by either chemical or physical means. Coating scaffolds with bioactive compounds throughout their whole surface is a typical method for functionalizing them. Physical functionalization is based on a weak connection between the ligand of the scaffold and the bioactive molecule, which is enhanced by weak electrostatic forces, hydrogen bonds, or hydrophobic interactions. The restricted control over the alignment of functionalized bioactive molecules is a disadvantage of physical functionalization.

5. **Structural Integrity:** Scaffold structural integrity refers to the spatial properties of a 3D scaffold that prevent it from collapsing or distorting. This is significant since several studies, both in vivo as well as in vitro, have shown that cells behave differently depending on the structural integrity of the scaffold. Although the precise processes driving these interactions are unknown, they play an important role. Cell adhesion, migration, and cell shape changes are among the biological responses induced by variable scaffold integrity. Within the context of cardiac tissue engineering, these reactions ultimately influence cell development, differentiation, and proliferation.

6. **Mechanical and Electrical Properties:** Scaffolds mimic the extracellular matrix (ECM) features, providing mechanical support to cells for tissue development and growth. The biomechanical and skeletal features of scaffolds must closely match the parameters of the target tissue to be repaired. The length of the fibers is an important aspect in increasing mechanical strength. The addition of fibers can increase the scaffold's tensile and rigidity. Notably, even at the same mass ratios, the effect of 3 mm fibers outperformed that of 12 mm fibers.

   The electrical activity of scaffolds is important in cardiac applications. The muscle of the heart is an electro active tissue that may transfer electrical signals throughout the organ. Materials are needed to create a comparable bioelectronics interface in cardiac regenerative medicine. The use of conductive materials in scaffold production is favored to assist heart regeneration. Incorporating conductive particles such as titanium dioxide, carbon nanofibers [26], or nanotubes into non-conductive materials can boost their electrical potential.

7. **Bioactivity and Tailorability:** Scaffold surfaces have bioactivity, which means they interact with biological substances, creating a connection among the cellular and the scaffoldings [27]. Furthermore, scaffold proteins can interact with cellular and intercellular spaces as well as with tissues as a whole. It is critical for scaffolds to be versatile in order to meet the unique demands of various cardiac tissues, allowing for flexibility in terms of forms, dimensions, and features.

IV. CELL-DERIVED DECELLULARIZED MATRICES

In cardiac bioengineering, decellularized extracellular matrices provide a natural alternative to artificial scaffolds. Using chemical or physical procedures, cells are entirely removed from tissues, leaving intact the critical structural composites of the extracellular matrix (ECM). This eliminates the potential for negative consequences like inflammation or intolerance. If a three-dimensional structure is not required, the matrices can be dried and crushed for reconstitution into suitable forms or molds.

The acellular biologic ECM scaffolds have shown encouraging results in clinical and preclinical trials in cardiac diseases such as pump malfunction and heart failure. These include reduced fibrotic tissue, increased blood flow to injured cardiac tissue, and structural alterations reversed. A decellularized epicardial cavity taken over by human mesenchymal stem cells were grafted in one case in victim with non-revascularized cardiac blisters, resulting in cicatricial tissue mass reduction after 3 months. Another research from decellularized pulmonic valves and homologous EPCs revealed the possibility of remodeling and growth in juvenile patients with pulmonary valve problems.

However, one drawback of employing isolation of intercellular matrices is the possibility of leftover natural tissue within the scaffold triggering an immunological response in patients [28]. As an alternative, artificial scaffolds can also be considered.

V. MATERIALS EMPLOYED IN CONSTRUCTION OF ARTIFICIAL SCAFFOLDS

1. **Natural Materials for Scaffolds:** Researchers have looked into several extracellular matrix (ECM) components as possible platforms for developing natural biomaterials. Many biomaterials, including collagen, chitosan, silk, alginate, and fibrinogen, are being investigated for use in scaffold construction.

    - **Collagen:** Collagen, an important component of the myocardium ECM, is frequently used in cardiac tissue engineering. Collagen type I, which makes up a large component of the ECM in the heart (70-80%), is chosen because to its minimal antigenicity [29]. Type I collagen is composed of 2 α-1 chains along with a single α-2 chain, resulting in lengthy filaments with density and alignment determining their qualities. This collagen type is divided into fibril forming collagen and non-fibril forming collagen components, with non-fibrillating collagen components capable of constructing connections or interacting with fibrillating collagens or films. Current approaches have concentrated on the application of biological materials made from collagen.

They have focused on the use of biological materials made from collagen to treat illnesses such as heart attack. To promote differentiation and patterning, these materials can supply growth factors or peptides. Initial administration methods include intra-myocardial injection, which needs surgery and risks material leakage. A different strategy is to create "cardiac patches," which have unique qualities including ex vivo cell culture to aid in patch invasion. These patches, which exhibit high engraftment levels, may be implanted into models. Furthermore, a three-dimensional type I collagen matrix paired with autologous hematopoietic lineage cells showed promise in repairing ischemic myocardium. This method enhanced diastolic performance by increasing scar thickness with viable tissue, normalizing heart wall strain in damaged areas, restricting ventricle remodeling, and increasing scar thickness with viable tissue.

- **Alginate:** Alginate is a glycan derived from the cellular matrix of marine algae or other microorganisms. It has biocompatible properties, such as dispersion in modified esters or salts, permeability, biodegradable properties and adjustable rigidity. Alginate is useful in medical fields such as polypeptide delivery, wound repair, and surgical implants, in addition to tissue engineering. However, problems occur in its medical use for cardiac tissue engineering due to low mechanical rigidity and limited bio-integrity, resulting in hampered unstable biological degradability and proliferate. To circumvent these constraints, the focus switches to composite scaffolds made by mixing alginate with different polymers [30].

- **Silk:** There has recently been a surge of interest in investigating silk as a novel biological material for tissue engineering approaches. Silk's promise resides not only in its structural, mechanical, and degradation rate comparability with similar materials like as cold-insoluble globulin, but also in its ability, unlike fibronectin, to avoid contributing to pathological hypertrophy [31]. Silk scaffolds have been shown in animal model trials to have medicinal properties and ability to maintain cardiac progenitor differentiation of cells. It is worth noting how the perspective of silk in cardiac tissue research is critical for demonstrating the preservation and advancement of sarcomeres, notably in increasing titin protein production.

- **Chitosan:** Chitosan is a natural polycarbohydrate that is generated from deacetylated chitin and is present in arthropods such as molluscs, shrimp, oysters, and lobsters. It has a linear structure with glycosidic linkage and residues of d-glucosamine, sometimes with N-acetyl-D-glucosamine groups. It dissolves in mild acidic environments but becomes persistent in water with a pH greater than 6.5. Because of deacetylated chitin to protonate peptide groups in alkaline environment, chitosan has gained appeal in tissue engineering, resulting in biological compatibility, degradability, non-poisonous, an emollient interface, and prevents blood clotting. The scaffolds of deacetylated chitin integrated with stem cells have resulted in excellent results, including improved undifferentiated cell distribution to injured heart tissue, cell retention, and cardiac function preservation. Chitosan scaffolds, on the other hand, have low mechanical strength and disintegrate quickly.

Chitosan-based tissue engineering treatments have yet to be tested in humans. Nonetheless, research is being conducted to investigate the possibility of biological materials derived from chitosan for parenteral therapies delivering precursor cells.

- **Fibrinogen:** Prior investigations have employed gelatin and Factor Ia patches to promote the development of heart cells, replicating the creation of heart muscle. The capacity of these materials to improve specific conductance, especially in proliferating cells alongside various stimuli, is a substantial benefit. These compounds have been shown to be effective at promoting specific maturation qualities, such as the activation of subendocardial branches. Researchers are interested in fibrin, a biomaterial produced spontaneously during thrombosis, both alone and paired with additional supplies. When utilized as a scaffold, fibrinogen has several advantages, including biocompatibility and biodegradability. Fibrin has a three-dimensional structure with random organization and significant interconnectivity, yet its constituent threads are malleable, allowing for bending without breaking [32].

  The mechanical characteristics of fibrin gels are controlled by fibrinogen breakdown, which is governed by the quantity of coagulation factor II utilized and the re-polymerization process. In therapeutic contexts, Factor Ia is derived from blood plasma for homologous uses such as osteoarthrosis therapy. Another useful use that is frequently utilized in surgical operations as an alternative to sutures is fibrin glue. Fibrin has also been used to repair other bodily organs such as the ureters, eye, grudge, pulmonary, hepatic, cardiac valves, and medullary cavities.

2. **Synthetic Polymers for Scaffolds:** Even while not all manmade polymers degrade naturally, not biodegradable biological materials can be altered to do so. Biodegradable polymers have the benefit of being replaceable when mature cells develop their own extracellular matrix (ECM). With the development of polymers that degrade like polycaprolactone (PCL), polyethylene oxide (PEO), polylactic-co-caprolactone, poly(glycerol sebacate), and polylactic-co-glycolic acid, the utilization of synthetic resources in cardiac tissue restoration has recently gained popularity. By incorporating vascular smooth muscle cells (VSMCs), researchers constructed a myocardial patch by porous polycaprolactone to prevent post-myocardial infarction ventricular dilatation. The researchers have developed permeability structures with poly(glycerol sebacate) which direct the alignment in heart tissues and created patches with aniline oligomers and poly(glycerol sebacate) copolymer to increase cardiac cell connection, resulting in an electro active material that greatly improved cell interactions.

Another crucial component of cardiovascular repair is vascular restoration, which scaffolds made of materials like hydropolymer based on polyethylene oxide or PEG. Biomimetic qualities are obtained via polypeptide cross- linkage with polyethylene glycol, resulting in the elongation of the resultant hydrogels. Encapsulated valvular cells aided in the formation of de novo ECM and the breakdown of hydrogels, making these materials interesting candidates for future heart valve development [33]. Another noteworthy method was the use of bioengineered cardiovascular implants (made of PLCL & PLLA) grafted with autologous hematopoietic lineage cells to serve as an extra-cardiac Fontan by cavopulmonary connection in juvenile single ventricle physiology. This procedure produced favorable results, such as the lack of aortic aneurysm development,

allograft infection, disintegration, or accumulation of calcium salts, with some patients reporting asymptomatic graft stenosis.

VI. BIOFABRICATION APPROACHES IN DEVELOPMENT OF ARTIFICIAL SCAFFOLDS

Decellularized biomaterials are used in a variety of approaches to enhance the natural and mechanical characteristics of synthetic scaffolds. The benefit of using artificial biodegradable scaffolds is their versatility for molding and customizing mechanical properties. Synthetic scaffolds that closely resemble the extracellular matrix (ECM) of the cardiac muscle were developed using various techniques [34]. Electrospinning, separation of phases, and 3D printing are examples of these processes and depicted in the Figure 2. Naturally, every approach has its own benefits and drawbacks; this part covers the most generally used ways for building artificial scaffolds and are shown in Table 2.

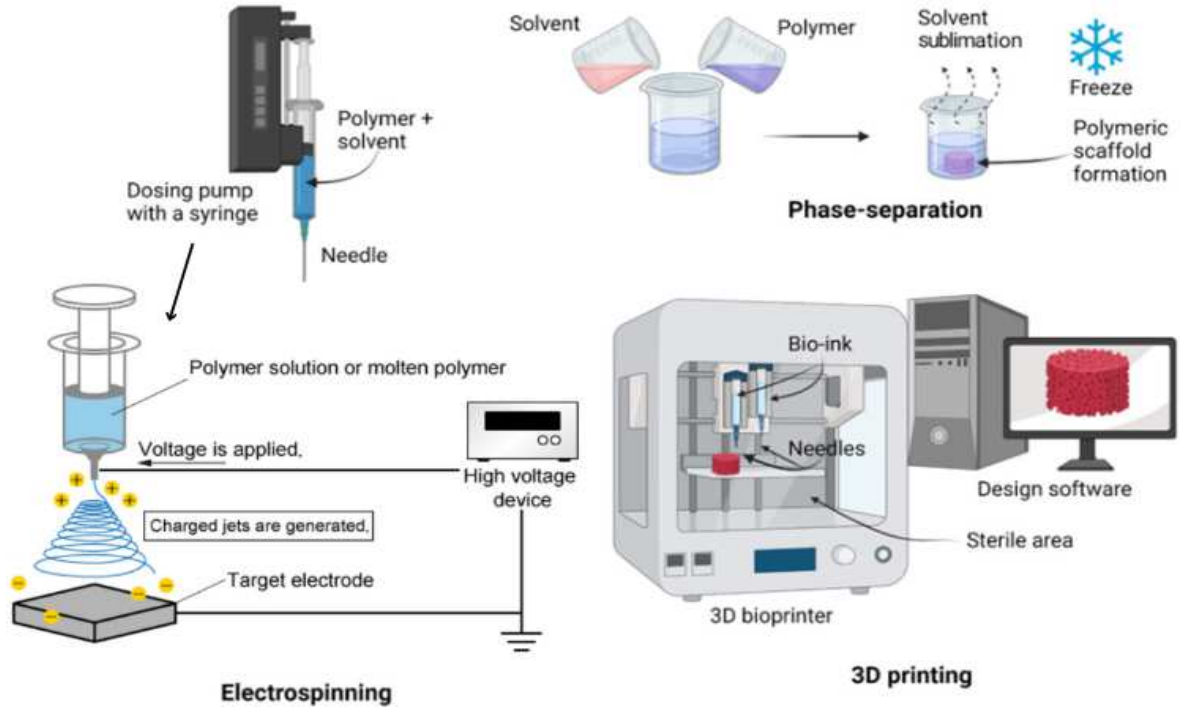

Figure 2: Scheme of biofabrication approaches in development of artificial scaffolds. Three-dimensional printing, phase separation, and electrospray processing are a few examples of such techniques.

1. **Electrospray Processing:** Electrospinning is a process that uses an electro propelled solutions to generate smaller and microscopic fibers from polymerized biological materials. Several important components are used in the technique: an administering pump with a cartridge carrying an acrylic solution, a threaded needle for producing a Taylor cone, a collecting disk varying from a platter to a rotating mandrel, and a heavy voltage electrical supply capable of reaching 25 kV. To summarize, electrospinning is the method by which creating an electrostatic stream of polymeric material driven by a field of higher voltage electricity. The solvent disintegrates as the polyethylene filaments randomly rotate and aggregate on a grounded structure, resulting in hardened polymer fibers.

An investigation showed that lowering the average fiber diameter from 215 to 100 nm within a deacetylchitin and polyvinyl alcohol (PVA) scaffold with multi-walled nanotubes increased specific conductance from 7 10^{-6} S/m to 8 10^{-4} S/m. Polymer molecular weight, voltage, capillary-collector distance, polymeric concentration, cross-linking density, conductance of electrolytic solution, and a volatile deposition of the solvent are the complete factors that influencing fiber properties and qualities. The fiber breadth plays a significant role in cardiovascular tissue restoration. In electrospinning, the breadth of the fibers is critical in producing the conductivity and characteristics required for heart regeneration.

2. **Three Dimensional Bio-printing:** 3D printing is a revolutionary method toward constructing artificial scaffolds in cardiac bioengineering. To create scaffolds, this method employs bio-printers, bio-inks, needles, and designing programs. The procedure begins with the creation of a 3D model in CAD software, which is then transformed into the STL format. This scaffold model is then separated into layers, which are printed and layered consecutively using a layer-by-layer process. The diameter of the needles used determines the resolution of the printed scaffold. By adding HEPA filters and UV lights for sterilization, some contemporary bio-printer machines produce a sterile atmosphere resembling a biosafety cabinet. The sterilizing procedure is visible through the bio-printers' clear windows.

3. **Particulate Leaching:** Solvent casting involves combining a polymer with an organic solvent and then casting the mixture into a three-dimensional mold. Another method is to immerse the mold in a polymer-containing solution. A scaffold is formed when the solvent is removed using processes such as vaporization, suction drying, or lyophilization. The scaffold is then rinsed with water, resulting in the formation of a permeability structure. Salt granules impact and regulate the size of the interior pores. This technique is used in cardiac bioengineering to produce artificial scaffolds using decellularized biomaterials.

4. **Phase Separation:** Thermally induced phase separation, often known as phase separation, is a simple approach utilized towards the fabrication of synthetic scaffolds for cardiac bioengineering. This procedure begins with the creation of a solution containing polymeric biomaterials and solvents, which is then frozen. The solvent is removed without damaging the polymer by frozen-drying once the solution has been developed, resulting in scaffolds with high permeability and interdependence. Certain characteristics of the scaffold's pores may be customized by adjusting the concentration, frigid temperature, and kind of solution.

Table 2: Advantages and disadvantages of each technique used for building artificial scaffolds.

Technique	Advantages	Disadvantages
Electrospinning	- Simple procedure - Low cost of preparation - Consistent sample production - Synthesis of tiny,	- Requires high-voltage equipment - Uses toxic solvents

	coordinated fibers - Improved cell attachment and proliferation - Adjustable porosity and interconnectivity - Unique pore shapes - Strong mechanical properties	
3D Printing	- Precise micro-architecture - Compatibility with various biomaterials - A great degree of control on - structural characteristics, porosity, size of pores, and interdependence.	- The cost of the first investment - The use of hazardous solvents -Mechanical unpredictability
Solvent Cast Technology	- Easy process - Mechanical stability - Minimal equipment requirement	- Challenges in maintaining porosity and salt dispersion - Slow solvent evaporation - Incomplete salt leaching -Inefficient solvent removal
Phase-Separation	- Simple technique - Scaffold preservation - Process at low temperatures	- Lengthy process - Inadequate architecture - Limited size control - Irregular porosity - Unsuitable mechanical properties - Potential toxic solvent residues

VII. MECHANOTRANSDUCTION IN CARDIAC TISSUE ENGINEERING

1. **Mechanical Stimulation:** Mechanical stress has emerged as a critical element in cardiac tissue engineering, regulating proliferation of cells, extracellular matrix (ECM) production, and swelling. When cardiac myocytes from embryonic chicks and newborn rats were combined with collagen and mechanically stimulated, they showed enlargement and enhanced contractile performance. Similar improvements were seen in newborn rat cardiac myocytes combined with collagen I and Matrigel medium and cast in rings before being mechanically stretched. Furthermore, mechanical stress was applied to human cardiac cells cultivated in a gelatin scaffold, which resulted in improved cell dispersion, ECM synthesis, and a structural layout that matched normal myocardium. This is due to the scaffold's stretching, which increases nutrition and oxygen exchange, ultimately improving the cellular microenvironment [35].

2. **Electrical Stimulation:** Electromyostimulation has been demonstrated to promote the differentiation of numerous cell types, including embryonic stem cells (ESC) and hematopoietic stem cells (HSCs), into cardiomyocytes. When these cells are placed on collagen scaffolds, this procedure becomes much more successful. Furthermore, when

paired with Matrigel inside a collagen-based permeable scaffold, ambient electrical fields have been demonstrated to improve the conductive and contractile capabilities of newborn cardiac cells [36]. It is hypothesized that applying electrical fields causes the creation of intracellular reactive oxygen species (ROS), which aids in embryonic stem cells development. The importance of electrode material has been discovered as a result of efforts to optimize electrical stimulation settings. Carbon electrodes have produced the finest results in this area. The intensity and frequency of electrical stimulation have a significant effect on cultured heart tissue. Micropatterned electrodes provide spatial control over the electric field, making them a very useful tool in this context.

While polymeric scaffolds inhibit cardiomyocyte electric communication, limiting synchronized beating of the synthetic tissue, adding gold (Au) nanowires into a porous alginate scaffold has been shown to improve communication. Another method is to impregnate HEMA scaffolds with gold nanoparticles, which provide both elasticity and electrical conductivity. Surprisingly, even without electrical stimulation, these increases in scaffold conductivity resulted in favorable physiological consequences.

VIII. CONCLUSION

Numerous treatment techniques have been explored in recent years to minimize the negative consequences of ischemic tissue spread and ventricular dilatation in cardiac bioengineering. Cellular cardiomyoplasty, which involves implanting several types of cells onto the infracted ventricle, seeks to produce new contractile tissue and enhance heart function. However, challenges persist since the vast majority of cells that were transplanted succumb to mechanical strain throughout the host tissue. Though the paracrine impact is frequently mentioned, the processes underpinning apparent benefits are not entirely understood. To move this therapy further, fundamental problems like effective delivery techniques, appropriate cell types, and administration timing must be addressed. To improve cell survival, new tactics focus on pre-conditioning cells, pre-treating host tissue, or merging cells with other materials.

Encapsulating cells in cell-friendly gelling polymers might be one strategy to localizing cells within sick tissue. These gels, which can comprise bioactive compounds and are minimally invasive, may help to minimize ventricular remodeling. Although combining cells with gels enhances adhesion and residence duration, their mechanical qualities are insufficient to endure heart muscle contractions and control ventricular dilatation. Integrating cells with three-dimensional scaffolds or patches to improve survival, encourage blood vessel creation, and offer mechanical support is an alternative cardiac tissue engineering technique. These scaffolds are versatile in terms of structure and chemistry, frequently outperforming injectable gels. Incorporating growth factors or adhesion motivations improves outcomes even more. However, patch implantation is more intrusive and necessitates vascularization for effective grafting.

While problems persist, new research provides insights into the qualities and methodologies needed for effective cardiac tissue creation. The field's aims continue to include expanding understanding on cell-cell electrical coupling, increasing electro conductive tissue healing, and then eventually constructing mechanical hearts. Despite the

challenges ahead, significant passages have been made, bringing the potential of myocardial tissue engineering closer to fulfillment in medical applications.

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