# Futuristic Approaches in Bioengineering of Cardiac Scaffolds for the Cutting Edge Exploration in Cardiovascular Regenerative Medicine

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**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 drawbacks of current cardiac repair interventions include the scarcity of heart transplant candidates and the use of non-bioactive inert materials to replace damaged tissue. Cardiomyocytes, which are potentially not capable of natural regeneration, require new treatment strategies to stimulate heart tissue regeneration. This chapter highlights the fundamental three aspects of cardiac tissue engineering (cell, growth factors, and scaffolds), with a focus on the role of scaffolds. Scaffolds for cardiac tissue engineering are three-dimensional porous constructs that mimic the extracellular heart matrix and can stimulate cell adhesion, migration, differentiation, 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 artificial scaffold biofabrication technique, as well as the best biocompatible biodegradable biomaterial for scaffold construction, because scaffolds must additionally provide mechanical contractility and electrical conductivity. This chapter focuses on the most recent advancements in the development 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.

1. **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) plays a crucial role 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.

**1.1 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, decreasing from 1% each year at age 21 to 0.8% at age 70. This 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 cell types, such as visceral muscle cells, 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 pathological remodeling of the heart, resulting in CM loss and scar formation driven by activation inflammatory signaling. This eventually leads to fibrotic scarring and ventricular malfunction, which leads to heart failure. Mammalian hearts, unlike frogs, lack the regeneration capacity to reverse the fibrotic scar and recover CM and cardiac function [4]. The processes behind these discrepancies are currently unknown. Adult mammalian hearts have little regenerative capability since there is no reserve pool of cardiac progenitor stem cells and postnatal differentiated CM has essentially no proliferative activity [5]. A number of chronic disorders can harm non-regenerable cardiac tissues, resulting in cardiovascular disease.

**1.2 Coronary Heart Diseases**

Cardiovascular diseases encompass a range of conditions such as heart attacks, high blood pressure, stroke, coronary artery disease, birth defects, and rheumatic heart disease [6]. They are responsible for the highest number of fatalities in both advanced and developing nations. The World Health Organization (WHO) reports that approximately 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 attack, which happens when blood supply to the heart muscle is reduced or stopped, 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 potential to encourage the regeneration of the damaged heart tissue [10].

**1.3 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 as cell therapy and tissue engineering, 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 cell therapy is to repair heart tissue that has been damaged by a heart attack [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 cardiac tissue engineering. Cardiac bioengineering is the result of combining three basic elements: cells, bioactive materials, and scaffolds that imitate the extracellular matrices.

**2. Cells and Bioactive Materials**

The heart is composed of various types of cells, with cardiac myocytes (CM) making up 25-35% of the total. The rest of the cells consist of blood and lymphatic endothelial cells (EC), cardiac fibroblasts (CF), cardiac progenitor cells (CPC), vascular smooth muscle cells (VSMC), Rouget cells, and leukocytes. CM are responsible for contracting and pushing blood, while EC and CF play important roles in maintaining tissue function and balance. VSMC and Rouget cells regulate blood flow, while CPC have the potential to differentiate into various 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 differentiate into cardiac cells to aid in tissue regeneration [13].

**2.1. Cell Types**

The heart is made up of several cell types, including cardiomyocytes (CM), which account for around 30-35% of heart cells. Endothelial cells (EC), cardiac fibroblasts (CF), vascular smooth muscle cells (VSMC), cardiac progenitor cells (CPC), pericytes, and immune cells make up the remaining 70%. Table 1. summarizes the different types of cells used in the cardiac tissue engineering. The CM is in charge of contracting and pumping blood, whereas the EC and CF are crucial in maintaining tissue function and balance. Vascular ECs are metabolically active and regulate blood vessel tone and angiogenesis, whereas CFs constantly regulates the extracellular matrix (ECM). Pericytes and VSMC aid in the regulation of blood flow within the heart vasculature. CPC are multipotent cells that display surface markers such as c-Kit, Sca-1, MESP1+, and Isl1+ and may differentiate into many cardiovascular cell types [14].

**Table 1.** Different types of cell types in cardiac tissue engineering.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source** | **Cells** | **Definition** | **Advantages** | **Disadvantages** |
| Embryonic | Fetal CM | Derived from fetal heart. | Potential for cardiac integration and regeneration. | Immunogenicity, Malignant potential, Ethical questions, Limited availability. |
| Human umbilical cord blood-derived cells | Pluripotent stem cells, mesenchymal stem cells (MSC), hematopoietic stem cells (HSC), non-hematopoietic stem cells (NHSC). | Cells derived from umbilical cord blood that can differentiate into various types. | Reduction of infarct after intramyocardial injection. | Immunogenicity, Need for standardized isolation and culture procedures, Senescence and mutational acquisition during in vitro expansion. |
| Embryonic MSC | Pluripotent stem cells derived from embryo's inner cell mass. | Cells with potential to differentiate into cells from all three embryonic germ layers. | Potential to differentiate into various cell types. | Associated with malignant transformation, Legal issues. |
| Adult stem cells | Adipose stem cells | Derived from adipose tissue, including MSC, HSC, and endothelial progenitor cells (EPC). | Multipotent potential, Easy sourcing, Easy harvesting, Low cost, No ethical issues. | Potential tumorigenicity, Limited understanding of cardiac repair mechanisms. |
| Autologous somatic cells | Human-induced pluripotent stem cells (iPSC) | Somatic cells converted into pluripotent cells. | Large-scale production possibilities, Ability to differentiate into various cardiac cell types. | Poor purity, Heterogeneity, Laborious/inefficient isolation techniques, Potential for teratoma formation. |

Every cell type is important in cardiac biology, 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.

**2.2 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. Another approach is to reprogramme cardiac fibroblasts into cardiomyocyte-like cells by over expression of certain transcription factors [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 inhibition of the Wnt/ß-catenin signaling pathway by DKK1. Following Mesp1's transient expression, transcription factors including as GATA4, HAND2, MYOCD, NKX2-5, FOXH1, MEF2C, and TBX5 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. GATA, MESP1, and TBX5 over expression have been shown to activate the production of sarcomere structures in fibroblasts [17]. Furthermore, the presence of HAND2 increases the efficiency of reprogramming to cardiac progenitor cells (CPC) and the function of wounded hearts following a myocardial infarction [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 to increase MEF2C binding to target genes, encouraging cardiac differentiation. Inhibiting BMP and WNT signaling pathways can also direct cardiac progenitor cells toward a myocardial lineage, as evidenced by the expression of cardiac-specific markers 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 combination of miRNAs, including miR-1, miR-133, miR-208, and miR-499, 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-548c-3p, miR-509-3p, and miR-23b-3p have been discovered as 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, play an important role in the advancement of cardiac bioengineering and have the potential to revolutionize cardiac tissue regeneration therapy.

**3. 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 [23]. They must have certain properties in the context of cardiac tissue engineering in order to properly support and increase cell growth and function.

**3.1. 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.

**3.2. Porosity**

The volume of pore space present in scaffolds is referred to as porosity. Some physical properties, such as the density of the material, can be used to calculate the 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].

**3.3. 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.

**3.4. Surface Characteristics**

In order to enhance the attachment of cells to the scaffold surface, it is necessary to modify both the chemical composition and physical structure of the scaffolds. This alteration, also called as functionalization, is accomplished by adding atoms or molecules onto the surface of the scaffold. This functionalization can be accomplished by either physical or chemical 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.

**3.5. 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 vitro and in vivo, have demonstrated 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.

**3.6. Mechanical and Electrical Properties**

Scaffolds mimic the extracellular matrix (ECM) features, providing mechanical support to cells for growth and tissue formation. The mechanical and structural 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 improve the strength and stiffness of the scaffold. 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 tissue engineering. 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.

**3.7. Bioactivity and Tailorability**

Scaffold surfaces have bioactivity, which means they interact with biological substances, resulting in the formation of a link between cells and the scaffold [27]. Furthermore, scaffolds can interact with tissues, including cells and extracellular matrices. 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.

**4. 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 components of the extracellular matrix (ECM). This avoids the possibility of adverse effects such as inflammation or rejection. 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 pericardial matrix colonized with human mesenchymal stem cells was implanted in one case in patients with non-revascularizable cardiac scars, resulting in scar mass reduction after three months. Another study using human decellularized pulmonary heart valves and autologous EPCs revealed the possibility of remodeling and growth in juvenile patients with pulmonary valve problems.

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

**4. Materials Employed in Construction of Artificial Scaffolds**

**4.1. 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.

**4.1.1. 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 low immunogenicity [29]. Collagen type I is composed of two α-1 chains and one α-2 chain, resulting in lengthy fibers with density and alignment determining their qualities. This collagen type is divided into fibrillar and non-fibrillar components, with non-fibrillar components capable of forming networks or interacting with fibrillar collagens or membranes. Recent research has concentrated on the application of collagen-based biomaterials.

Recent research has focused on the use of collagen-based biomaterials to treat illnesses such as myocardial infarction. 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 3D collagen type I matrix paired with autologous bone marrow mononuclear cells showed promise in repairing ischemic myocardium. This method enhanced diastolic performance by increasing scar thickness with viable tissue, normalizing heart wall stress in damaged regions, limiting ventricular remodeling, and increasing scar thickness with viable tissue.

**4.1.2. Alginate:** Alginate is a polysaccharide derived from the cell walls of marine algae or other microorganisms. It has biocompatible properties, such as solubility in modified salts or esters, permeability, biodegradable properties and adjustable viscosity. 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 stability and limited biological stability, resulting in hampered proliferation and biodegradability instability. To circumvent these constraints, the focus switches to composite scaffolds made by mixing alginate with different polymers [30].

**4.1.3. Silk:** There has recently been a surge of interest in investigating silk as a new biomaterial for tissue engineering applications. Silk's promise resides not only in its structural, mechanical, and degradation rate similarities to other materials like as fibronectin, 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 therapeutic effects and the capacity to retain cell differentiation into cardiac lineages. It is worth noting that the orientation of silk in cardiac tissue research is critical for demonstrating the preservation and advancement of sarcomeres, notably in increasing titin protein production.

**4.1.4. Chitosan:** Chitosan is a natural polysaccharide that is generated from chitin by deacetylation and is present in crustaceans such as shrimp, crabs, and lobsters. It has a linear structure with glycosidic bonds and d-glucosamine residues, sometimes with N-acetyl-d-glucosamine (NAG) groups. It dissolves in mild acids but becomes insoluble in aqueous solutions with a pH greater than 6.5. Because of its capacity to protonate amino groups in acidic circumstances, chitosan has gained appeal in tissue engineering, resulting in biocompatibility, non-toxicity, anti-thrombogenic characteristics, biodegradability, and a hydrophilic surface. The combination of chitosan scaffolds with stem cells has resulted in excellent results, including improved stem 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 chitosan-based biomaterials for injectable therapies delivering progenitor cells.

**4.1.5. Fibrinogen:** Previous research has used collagen and fibrin patches to stimulate cardiac cell maturation, replicating the creation of heart muscle. The capacity of these materials to improve electrical conductivity, particularly in differentiating cells and other stimuli, is a substantial benefit. These compounds have been shown to be effective at promoting specific maturation qualities, such as the induction of Purkinje cells. Researchers are interested in fibrin, a naturally occurring biomaterial generated during coagulation, both alone and in combination with other materials. 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 amount of thrombin utilized and the re-polymerization process. In therapeutic contexts, fibrin is derived from plasma for autologous uses such as osteoarthritis treatment. 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 urinary tract, eye, liver, lung, spleen, heart valves, and bone cavities.

**4.2. Artificial Materials for Scaffolds**

While not all synthetic materials are intrinsically biodegradable, non-biodegradable biomaterials can be modified to become biodegradable. Biodegradable materials have the benefit of being replaceable when mature cells develop their own extracellular matrix (ECM). The use of synthetic materials in cardiac tissue engineering has recently gained prominence, particularly in the development of biodegradable polymers such as polycaprolactone (PCL), polyglycerol sebacate (PGS), polyethylene glycol (PEG), polylactic-co-caprolactone (PLCL), and polylactic-co-glycolic acid (PLGA). Researchers developed a porous PCL cardiac patch to inhibit post-myocardial infarction ventricular dilatation by the inclusion of vascular smooth muscle cells (VSMCs). The researchers have developed porous structures with PGS to direct the alignment of heart cells and created patches utilizing PGS copolymerized with aniline trimer to increase cardiac cell connection, resulting in an electro active material that greatly improved cell interactions.

Another critical part of heart repair is valve restoration, which uses materials such as PEG-based hydrogels as scaffolds. Biomimetic qualities are obtained by cross-linking PEG with peptides, 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 Bio-engineered vascular grafts (made of PLLA and PLCL) implanted with autologous bone marrow mononuclear cells to serve as an extracardiac complete cavopulmonary conduit in juvenile univentricular physiology. This procedure produced favorable results, such as the lack of aneurysmal development, graft rupture, graft infection, or calcification, with some patients reporting asymptomatic graft stenosis.

**5. Biofabrication Approaches in Development of Artificial Scaffolds**

Decellularized biomaterials are used in a variety of ways to improve the physical and biological qualities of artificial scaffolds. The benefit of using artificial biodegradable scaffolds is their versatility for molding and customizing mechanical properties. Several ways have been devised to produce artificial scaffolds that closely match the myocardium's extracellular matrix (ECM) [34]. Electrospinning, separation of phases, and 3D printing are examples of these processes and depicted in the Figure 2. Naturally, each technique has advantages and disadvantages, and we will cover the most generally used ways for building artificial scaffolds in this part are shown in Table 2.



**Figure 2.** Scheme of biofabrication approaches in development of artificial scaffolds. These include, but are not limited to, electrospinning, phase separation, and 3D printing.

**5.1. Electrospinning**

Electrospinning is a technique used to create smaller and nanoscale fibers from polymeric biomaterials using an electrically driven solution. Several important components are used in the technique: a dosing pump with a syringe carrying the polymer solution, a needle producing a Taylor cone, a collecting drum that can range from a plate to a revolving mandrel, and a high-voltage power source ranging up to 25 kV. To summarize, electrospinning is the process of creating a charged stream of polymeric biomaterial that is driven by a high-voltage electric field. The solvent evaporates as the polymer fibers 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 chitosan and PVA scaffold with multi-wall carbon nanotubes increased electrical conductivity from 7 10-5 S/m to 8 10-3 S/m. Polymer molecular weight, voltage, capillary-collector distance, polymer concentration, solution conductivity, and solvent volatility are all factors that impact fiber characteristics and qualities. Fiber diameter is an important element in heart tissue engineering. In electrospinning, the diameter of the fibers is critical in producing the conductivity and characteristics required for heart regeneration.

**5.2. 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.

**5.3. 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. Following that, the scaffold is washed with water, resulting in the creation of a porous 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.

**5.4. Phase Separation**

Thermally induced phase separation, often known as phase separation, is a simple approach utilized in the fabrication of artificial scaffolds for cardiac bioengineering. This procedure begins with the creation of a solution containing polymeric biomaterials and solvents, which is then frozen. After the solution has been prepared, the solvent is removed without degrading the polymer by freeze-drying, resulting in scaffolds with good porosity and interconnectivity. Certain characteristics of the scaffold's pores may be customized by adjusting the freezing temperature, concentration, and kind of solvent and solute.

**Table 2.** Advantages, and disadvantages of each techniques used for building artificial scaffolds.

|  |  |  |
| --- | --- | --- |
| **Technique** | **Advantages** | **Disadvantages** |
| **Electrospinning** | - Simple process - Low preparation cost - Uniform sample production - Generation of fine, aligned fibers - Improved cell attachment and proliferation - Adjustable porosity and interconnectivity - Unique pore shapes - Strong mechanical properties | - Requires high-voltage equipment - Uses toxic solvents |
| **3D Printing** | - Precise micro-architecture - Compatibility with various biomaterials - High control over structural properties - Porosity, pore size, interconnectivity | - Initial investment cost - Use of toxic solvents - Mechanical instability |
| **Solvent Casting/Particulate Leaching** | - 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 |

**6. Mechanotransduction in Cardiac Tissue Engineering**

**6.1. 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].

**6.2. Electrical Stimulation**

Electrical stimulation has been shown to promote the differentiation of numerous cell types, including embryonic stem cells (ESC) and bone marrow stem cells (BMSC), 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.

**7. 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, difficulties continue since the majority of transplanted cells succumb to mechanical forces within 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-electrical interactions, increasing electroactive tissue healing, and eventually constructing artificial hearts. Despite the challenges ahead, significant progress has been made, bringing the potential of cardiac tissue engineering closer to fulfillment in medical applications.

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