

Review

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[Anna Maria Sacco](#) , [Clotilde Castaldo](#) , [Franca Di Meglio](#) , [Daria Nurzynska](#) , [Stefano Palermi](#) , Rocco Spera ,  
Rossana Gnasso , Giorgio Zinno , [Veronica Romano](#) <sup>\*</sup> , [Immacolata Belviso](#)

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## Review

# The Long and Winding Road to Cardiac Regeneration

Anna Maria Sacco<sup>1</sup>, Clotilde Castaldo<sup>1</sup>, Franca Di Meglio<sup>1</sup>, Daria Nurzynska<sup>2</sup>, Stefano Palmeri<sup>1</sup>, Rocco Spera<sup>1</sup>, Rossana Gnasso<sup>1</sup>, Giorgio Zinno<sup>3</sup>, Veronica Romano<sup>1,\*</sup> and Immacolata Belviso<sup>1</sup>

<sup>1</sup> Department of Public Health, University of Naples Federico II, Naples, Italy; annamaria.sacco@unina.it (A.M.S.); clotilde.castaldo@unina.it (C.C.); franca.dimeglio@unina.it (F.D.M.); stefano.palermi@unina.it (S.P.); rocco.spera@unina.it (R.S.); rossanagns@yahoo.it (R.G.); [veronica.romano@unina.it](mailto:veronica.romano@unina.it) (V.R.); immacolata.belviso@unina.it (I.B.)

<sup>2</sup> Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, University of Salerno, Baronissi, Italy; [dnurzynska@unisa.it](mailto:dnurzynska@unisa.it) (D.N.)

<sup>3</sup> Department of Clinical and Surgical Medicine, University of Naples Federico II, Naples, Italy; [zinnogiorgio@virgilio.it](mailto:zinnogiorgio@virgilio.it) (G.Z.)

\* Correspondence: [veronica.romano@unina.it](mailto:veronica.romano@unina.it)

**Abstract:** Cardiac regeneration is a critical endeavor in the treatment of heart diseases, aimed at repairing and enhancing the structure and function of damaged myocardium. This review offers a comprehensive overview of current advancements and strategies in cardiac regeneration, with a specific focus on regenerative medicine and tissue engineering-based approaches. Stem cell-based therapies, which involve the utilization of adult stem cells and pluripotent stem cells hold immense potential for replenishing lost cardiomyocytes and facilitating cardiac tissue repair and regeneration. Tissue engineering also plays a prominent role employing synthetic or natural biomaterials, engineering cardiac patches and grafts with suitable properties, and fabricating upscale bioreactors to create functional constructs for cardiac recovery. These constructs can be transplanted into heart to provide mechanical support and facilitate tissue healing. Additionally, the production of organoids and chips that accurately replicate the structure and function of the whole organ is an area of extensive research. Despite significant progress, several challenges persist in the field of cardiac regeneration. These include enhancing cell survival and engraftment, achieving proper vascularization, and ensuring the long-term functionality of engineered constructs. Overcoming these obstacles offering effective therapies to restore cardiac function could improve the quality of life for individuals with heart diseases.

**Keywords:** cardiac regeneration; stem cell therapy; decellularized extracellular matrix; bioreactor; biomaterials; organoids; chips

## 1. Introduction

Cardiovascular disease is a pervasive health issue, ranking among the leading causes of death worldwide [1,2]. According to the World Health Organization, it is estimated that cardiovascular diseases accounted for approximately 17.9 million deaths in 2019, representing 32% of all global deaths (World Health Organization (2021)). Cardiovascular Diseases (CVDs). Retrieved from (<https://www.who.int/news-room/fact-sheets/detail/cardio>) [3]. Its detrimental impact on individuals and communities necessitates urgent efforts to develop novel treatments. In this regard, cardiac regeneration has emerged as a promising area of research, aiming to pioneer innovative approaches for repairing or replacing damaged heart tissue [4]. The ultimate goal of cardiac regeneration is to provide a long-term solution for the treatment of various cardiovascular diseases, including heart failure, heart attacks and strokes. As the global population continues to age and the available treatment options remain limited, the field of cardiac regeneration has assumed significant importance [5–9].

Within the panorama of cardiac regeneration, cell-based approaches have garnered considerable attention for their potential to introduce new therapeutic strategies [10,11]. One such approach is cardiac tissue engineering, a budding and rapidly evolving field in regenerative medicine [12]. Its primary objective is to restore the function of damaged heart tissue by leveraging the power of stem cells, biomaterials, and other innovative tissue-engineering techniques [13–15]. Recent years have witnessed

substantial advancements in both research and clinical applications of cardiac tissue engineering, offering promising avenues for the treatment of heart disease and related cardiovascular conditions [16–18].

Presently, researchers are actively exploring two main methods of cardiac regeneration: stem cells and bioengineering [19]. Stem cell therapy involves isolating stem cells from the patient, reprogram them *in vitro*, and injecting them into the damaged areas of the heart tissue [20,21]. This process holds the potential to stimulate the heart's intrinsic repair mechanisms and facilitate the restoration of damaged tissue. On the other hand, bioengineering entails the creation of artificial heart tissue utilizing a combination of proteins, growth factors, and living cells [22–24]. By employing an appropriate scaffold and incorporating growth factors, this approach can lead to the development of fully functional heart cells. Once these cells integrate with the patient's existing heart tissue, they offer the possibility of a more permanent and comprehensive solution for repairing and regenerating cardiac tissue [25–27].

Exciting breakthroughs have also been witnessed in the clinical applications of tissue engineering for heart disease. For instance, cardiac patches have emerged as a valuable tool for repairing damaged heart tissue [28,29], while the development of replacement heart valves aims to address issues related to damaged or defective valves [30–32]. These innovative valves boast significantly prolonged lifespans compared to their traditional counterparts and feature implantable designs, making them highly suitable for individuals in need of long-term treatments [33,34]. Additionally, the ongoing development of lab-grown functional heart cells holds immense potential for personalized treatments that surpass the effectiveness of conventional methods [35].

The recent strides made in the field of cardiac tissue engineering have the potential to revolutionize cardiovascular medicine in various ways. From ground-breaking treatments for heart disease to the development of more durable replacement organs, tissue engineering offers a wide range of possibilities to significantly enhance and improve lives [16,36,37]. With continued research and exploration in this field, it is expected that further remarkable advances will be achieved in the next decade.

Over the past decade, the development of cardiac tissue engineering strategies for the treatment of heart disease has witnessed substantial progress [38,39]. Key advances in this field include:

1. Stem cell therapy: researchers have successfully harnessed the differentiating capabilities of stem cells, enabling them to transform into various cell types, including heart cells. These remarkable cells hold the potential to generate new cardiac tissue, and extensive research has been conducted to refine the methods of differentiating stem cells into functional heart cells. Encouraging results have been observed in preclinical studies, underscoring the potential of stem cell therapy in cardiac regeneration [40–44].
2. Biomaterials: the use of biomaterials has revolutionized the field of cardiac tissue engineering. These materials serve as scaffolds that support the growth and organization of heart cells, enabling the creation of three-dimensional structures that closely mimic the intricate architecture of the heart. This approach promotes the formation of functional cardiac tissue and holds tremendous promise for restoring the damaged heart to its optimal functionality [26,45,46].
3. Bioreactors: bioreactors have emerged as invaluable tools in cardiac tissue engineering. They provide a controlled environment for the growth and maturation of cardiac tissue by subjecting the cells to specific mechanical and electrical stimuli. This exposure facilitates the development of functional cardiac tissue that closely resembles the native heart tissue in terms of structure and functionality. Bioreactors have played a pivotal role in advancing our understanding of cardiac regeneration and have significantly contributed to the progress made in the field [47–50].
4. Gene therapy: gene therapy has emerged as a cutting-edge approach in cardiac tissue engineering. It involves the use of genetic techniques to enhance the differentiation potential of stem cells into heart cells or to promote the survival and function of existing heart cells. Through various gene therapy strategies, researchers have made significant strides in preclinical studies, opening up new possibilities for improving the effectiveness of cardiac regeneration [51–54].

While substantial progress has been made, there is still much work to be done in the field of cardiac tissue engineering. However, these remarkable advances hold great promise for the development of effective heart regenerative therapies. Through ongoing efforts, the goal of revolutionizing cardiovascular medicine and offering hope to millions of individuals affected by heart disease draws ever closer.

## 2. Stem cell therapy

Stem cell therapy has garnered significant attention in recent years for its potential to revolutionize the treatment of cardiac diseases. Stem cells possess a remarkable capability to divide and transform into specialized cells, making them ideal for repairing damaged tissues. Specifically, in case of heart failure and other cardiac diseases, stem cells can regenerate cardiac tissues, offering a promising avenue for treatment [55–58].

One of the primary advantages of utilizing stem cell therapy for cardiac regeneration lies in its potential to restore lost cardiac function [57,59–61]. Recent studies have demonstrated that stem cells can enhance heart function in patients suffering from heart failure by regenerating tissue and reinstating blood flow. For instance, studies published in *The Lancet* [62,63] and in *Physiological Review* [64], showed that cardiac stem cells improved heart function and reduced scar tissue in patients with heart failure.

Furthermore, stem cell therapy has the potential to reduce or even reverse the progression of cardiac diseases. A study published in *Circulation Research* [65] reported that injecting bone marrow-derived stem cells into the hearts of patients with severe heart failure resulted in a significant reduction in major adverse cardiovascular events.

Another notable benefit of stem cell therapy for cardiac regeneration is the relative safety of the procedure. Stem cells can be derived from various sources, including the patients' own body, and carry a low risk of triggering immunological reactions [66,67]. Additionally, stem cell therapy is minimally invasive and could be performed as an outpatient procedure, minimizing the burden on patients [68].

While the potential of stem cell therapy for cardiac regeneration is immense, there are still certain limitations to be addressed. Several studies have revealed drawbacks in current stem cell-based therapies. These limitations include significant cell death and apoptosis, insufficient cell engraftment, limited cardiac regeneration post cell transplantation, and the need for careful monitoring of potential autoimmune adverse reactions [69].

To overcome these limitations, various strategies have been developed. These strategies aim to improve cell survival and engraftment, as well as induce transdifferentiation of somatic cells directly into functional cardiomyocytes to stimulate endogenous cardiac regeneration [70]. One such study, published in *Nature* [71], demonstrated successful reprogramming of fibroblasts into cardiomyocyte-like cells using a combination of transcription factors.

Cell types used for cardiac regeneration can be broadly categorized into two groups: adult stem cells and pluripotent stem cells [72]. Adult stem cells, such as skeletal myoblasts (SMs), hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs), can be isolated from different tissues, including skeletal muscle, adipose tissue, peripheral blood, bone marrow, and heart tissue [73,74]. In addition, pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells (reprogrammed somatic cells), possess a clear potential to differentiate into functional cardiomyocytes, setting them apart from adult stem cells [75–77]. Notably, an important difference between these two categories of cells is that while adult stem cells have a variable cardiogenic transdifferentiation capacity, pluripotent stem cells have an unequivocal potential to differentiate into functional cardiomyocytes [78].

Extensive research has been conducted utilizing these cell types in cardiac regeneration studies, demonstrating their tremendous potential for treating cardiovascular diseases. Adult stem cells and pluripotent stem cells are typically administered via intravascular injections, injections into the pericardium, or infusion of cardiac patches [79]. Moreover, these cells can be employed to repopulate synthetic and natural scaffolds, allowing for various recellularization methods ranging from simple manual cell seeding to sophisticated controllable systems [80,81].

A brief summary of all the cell types mentioned above is showed in **Table 1**.

### 2.1. Adult stem cells

Adult stem cells can be directly obtained from patients and transplanted back into the same individuals with a reduced risk of immunological rejection. These stem cells are currently being investigated for their potential in treating acute myocardial infarction and chronic myocardial ischemia. Moreover, adult stem cells derived from mature tissues hold promise for cardiac regeneration. They have the ability to differentiate into various cell types, including cardiac muscle cells and other cardiac cell



types. Furthermore, these cells are responsive to local signals and can promptly adapt to environmental cues compared to pluripotent stem cells [82,83].

#### 2.1.1. Skeletal myoblasts

Skeletal myoblasts (SMs) were initially explored for the treatment of cardiomyopathy and heart failure [84]. SMs are precursor cells of skeletal muscle and are activated in response to muscle damage or disease-induced muscle degeneration [85]. They can be obtained from skeletal muscle biopsies and expanded outside the body for autologous transplantation. SMs exhibit a higher proliferation rate and are also resilient to myocardial ischemia [86]. Animal studies have demonstrated that their transplantation improves left ventricular function and hinders cardiac remodelling [87]. However, it is unfortunate that current research indicates that injected SMs in animal models differentiate into myotubes rather than cardiomyocytes. This lack of morphological and electrophysiological integration with host cardiomyocytes poses an increased risk of ventricular arrhythmias [88].

#### 2.1.2. Hematopoietic stem cells

Hematopoietic stem cells (HSCs) are a type of multipotent stem cell that give rise to all blood cell types. While traditionally known for their role in haematopoiesis, recent studies have explored the potential of HSCs in cardiac regeneration [89]. HSCs are derived from the mesoderm during embryonic development and are primarily found in the bone marrow of adult organisms. Their unique ability to self-renew and differentiate into various blood cell lineages has made them attractive candidates for therapeutic interventions, including cardiac repair [90].

One advantage of using HSCs for cardiac regeneration is their abundant availability. Bone marrow, a rich source of HSCs, can be easily accessed through minimally invasive procedures. Furthermore, HSCs can be expanded in culture, allowing for the generation of large quantities of cells for therapeutic purposes [60]. In addition, HSCs have shown the potential to differentiate into cardiac lineage cells, such as cardiomyocytes, endothelial cells, and smooth muscle cells, which are crucial for repairing damaged cardiac tissue [91].

Despite these advantages, several limitations exist in the use of HSCs for cardiac regeneration. One challenge is the low efficiency of differentiation into cardiac cells. While HSCs have demonstrated the capacity to differentiate into cardiac lineages, the percentage of cells successfully differentiating into functional cardiomyocytes remains relatively low [92]. Another limitation is the potential for immune rejection when using allogeneic HSCs. Immunosuppressive therapy may be required to prevent rejection, adding complexity to the treatment process [93,94]. Moreover, the long-term engraftment and survival of HSC-derived cells in the cardiac tissue remain uncertain [95].

#### 2.1.3. Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are a promising cell type for cardiac regeneration due to their ability to promote angiogenesis and repair damaged blood vessels [96,97]. EPCs are derived from various sources, including bone marrow, peripheral blood, and umbilical cord blood. These cells possess unique characteristics that make them attractive for therapeutic applications in cardiac regeneration [98,99].

The advantages of EPCs lie in their regenerative potential. EPCs have the ability to differentiate into mature endothelial cells and contribute to the formation of new blood vessels. They also secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which further enhance angiogenesis and tissue repair [100]. Additionally, EPCs possess immunomodulatory properties and can promote the recruitment of other regenerative cells to the injured cardiac tissue [101].

However, EPC-based therapies for cardiac regeneration face certain limitations. The low number and limited lifespan of circulating EPCs in the peripheral blood pose challenges for their isolation and expansion in therapeutic quantities. Moreover, EPCs derived from older individuals or patients with cardiovascular diseases may exhibit reduced regenerative potential. Additionally, the engraftment efficiency of transplanted EPCs into the myocardium remains a concern, as many cells may be lost due to poor survival or limited retention [102].

To overcome these limitations, several strategies to enhance EPCs mobilization, expansion, and survival are under investigation. Genetic modification, preconditioning, and tissue engineering approaches are being explored to improve the therapeutic efficacy of EPCs in cardiac regeneration. Furthermore, combined therapies using EPCs with other cell types, such as cells of mesenchymal origin, and biomaterial-made scaffolds are being studied to enhance their regenerative capabilities [103,104].

The EPCs successful clinical translation requires addressing the challenges related to cell sourcing, expansion, and engraftment. Future research efforts aimed at optimizing EPC-based therapies will contribute to their effective utilization in treating cardiovascular diseases and promoting cardiac tissue regeneration [105,106].

#### 2.1.4. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent cells that can be derived from various sources, including bone marrow, adipose tissue, umbilical cord blood, and placenta. Their ability to differentiate into multiple cell lineages, along with their immunomodulatory and paracrine effects, make them an attractive option for treating cardiac disorders [107].

One of the key advantages of MSCs is their relatively easy isolation and expansion *in vitro*, allowing for large-scale production. Additionally, they exhibit low immunogenicity, reducing the risk of immune rejection upon transplantation [108]. MSCs possess anti-inflammatory properties, which can help attenuate the detrimental immune response associated with cardiac injury. Moreover, they secrete trophic factors that promote angiogenesis, modulate fibrosis, and enhance the survival of existing cardiomyocytes [109,110].

Despite these advantages, several limitations must be considered. The heterogeneity of MSC populations obtained from different sources can affect their therapeutic potential and consistency [111]. MSCs propensity to undergo senescence during expansion limits their long-term viability and functionality [112]. Furthermore, the unpredictable fate and limited engraftment of transplanted MSCs within the injured heart pose challenges to achieving sustainable cardiac regeneration [113]. Additionally, optimizing the delivery methods and timing of MSC transplantation remains an area of active research.

#### 2.1.5. Cardiac progenitor cells

Cardiac progenitor cells (CPCs) are a specialized group of cells able to differentiate into various types of cardiac cells, making them a promising approach for cardiac regeneration [114,115]. CPCs are derived from different sources, including the embryonic heart, foetal heart and adult heart. These cells already have a cardiomyocyte-like phenotype and can be used to construct cardiac tissue *in vitro*. Their multipotent nature and ability to generate new cardiac tissue provide several advantages for cardiac regeneration [116,117].

CPCs have the potential to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells, which are essential components to build a whole heart tissue [118]. This capacity allows them to integrate seamlessly into the existing cardiac structure, promoting functional recovery. Additionally, CPCs possess paracrine effects, secreting growth factors and mRNAs that could stimulate endogenous repair mechanisms, angiogenesis, and tissue remodelling. This paracrine activity further enhances the regenerative potential of CPCs [119,120].

Despite their potential, CPCs also face certain limitations. The precise identification and isolation of CPCs remain challenging due to their heterogeneity and lack of specific markers. Ensuring a pure population of CPCs is crucial for successful regeneration. Additionally, the survival and engraftment of transplanted CPCs into the host myocardium is a major concern. Strategies to improve cell survival, integration, and long-term function are actively being investigated [121].

#### 2.2. Pluripotent stem cells

Pluripotent stem cells exhibit the remarkable capacity for self-renewal through division and the ability to differentiate into all cell types present in the adult organism. Their unrestricted self-renewal potential and definitive cardiomyogenic capability render pluripotent stem cells a highly promising avenue for cardiac regenerative therapy [122–124]. This class of stem cells encompasses embryonic stem cells (ESCs)

derived from the blastocyst, as well as induced pluripotent stem cells (iPSCs) generated via the introduction of pluripotent genes, namely Oct3/4, Sox2, Klf4, and c-Myc, into somatic cells [125].

### 2.2.1. Embryonic stem cell

ESCs are derived from the inner cell mass of early-stage embryos and possess remarkable pluripotent properties [126]. They have the ability to self-renew indefinitely and differentiate into virtually any cell type in the human body, including cardiomyocytes. This capacity to generate cardiac cells makes ESCs a compelling candidate for cardiac tissue regeneration [127].

The advantages of ESCs in cardiac regeneration lie in their exceptional regenerative potential and the ability to create large quantities of functional cardiomyocytes [60]. ESCs offer a renewable source of cells that can be expanded in culture, providing a virtually unlimited supply for transplantation. When introduced into damaged cardiac tissue, ESC-derived cardiomyocytes can integrate within the host tissue and potentially restore heart function [127,128].

However, the use of ESCs in cardiac regeneration also faces significant limitations. One of the primary concerns is the ethical dilemma associated with their acquisition, as ESCs are typically obtained from discarded embryos or through the destruction of viable embryos. This raises ethical considerations and has led to substantial controversy surrounding their use [129]. Additionally, ESCs may elicit immune responses when transplanted into a recipient, necessitating the use of immunosuppressive drugs that carry their own risks [130,131].

Furthermore, the possible tumorigenicity poses a significant challenge. ESCs tend to form teratomas, which are non-functional masses of differentiated cells. Ensuring the differentiation of ESCs into specific cardiac cell types before transplantation is critical to minimize the risk of tumor formation [132].

Ethical concerns, immune rejection, and tumorigenicity remain major hurdles that must be addressed before their widespread clinical application can be realized [133]. Continued research and development are essential to overcome these limitations and unlock the full potential of ESCs in cardiac regenerative medicine.

### 2.2.2. Induced pluripotent stem cell

Induced pluripotent stem cell (iPSC) technology is making a major impact in the field of cardiac regeneration [134]. iPSCs are derived from adult somatic cells, such as skin cells [135], and then reprogrammed to an embryonic stem cell ESC state, where they can be used to obtain any type of tissue in the body. This technology has been used to create beating heart cells in a lab setting, providing a special opportunity to repair and replace damaged heart tissue [136]. This advancement has opened up possibilities for repairing and replacing damaged heart tissue.

The potential of iPSCs in cardiac regeneration lies in their versatility. They can be easily manipulated to form different components of the heart, offering researchers a unique opportunity to study and understand the cellular context in which these cells operate [137]. This knowledge is crucial for developing effective therapies.

In the field of heart disease, iPSCs have become a powerful tool. Researchers can generate cardiac cell types from patients' own cells, allowing them to study the effects of specific genetic and environmental factors [138]. This personalized approach helps in understanding disease progression and developing targeted treatments [139].

Moreover, iPSCs are used to create cell-based models of human heart diseases. These models aid in uncovering the underlying mechanisms and pathophysiology of various cardiac conditions [140]. By gaining a deeper understanding of these diseases, researchers can improve diagnosis, prognosis, and develop novel treatment strategies.

iPSC technology also plays a role in engineering heart-on-chip devices. These devices allow for the testing of numerous therapeutic agents *in vitro*, accelerating the development of treatments [141,142]. Additionally, iPSC-derived cardiac tissues have the potential to restore normal heart function by providing a replacement for lost cells and tissue in failing hearts [143,144].

However, there are challenges associated with using iPSCs in cardiac research. Safety concerns arise due to the potential of iPSCs to form tumors if not properly controlled. Ensuring the safety of iPSC-based therapies is crucial before considering widespread clinical applications [145,146].

Another challenge is immune rejection. While autologous transplantation of iPSCs bypasses immune rejection issues, generating patient-specific iPSCs of sufficient quality can be challenging. Allogeneic transplantation using iPSCs from different donors raises concerns about immune rejection and the need for immunosuppressive drugs [147,148].

Efficiency of differentiation is also a complex process. Generating a pure population of functional cardiac cells from iPSCs is still being optimized [149], and variability in differentiation efficiency can affect the overall effectiveness of iPSC-based cardiac regeneration [150].

Furthermore, iPSC-derived cardiomyocytes often exhibit immature characteristics, limiting their functionality and integration into existing cardiac tissue [151,152]. Achieving mature, fully functional cardiomyocytes is a critical goal for successful cardiac regeneration.

To ensure effective cardiac regeneration, transplanted cells must be able to integrate and establish proper electromechanical coupling with the host tissue [153,154]. However, achieving this alignment and synchronization with existing cardiac tissue is challenging and requires further research.

Additionally, ethical concerns surround the generation of iPSCs through genetic reprogramming techniques. While iPSCs avoid the ethical considerations associated with embryonic stem cells, the alteration of genetic makeup raises ethical questions [155,156].

Addressing these challenges and limitations is essential for the successful translation of iPSC-based cardiac regeneration strategies from the laboratory to clinical applications.



**Table 1.** Stem cell types for cardiac tissue engineering.

Stem Cells	Cell Type	Source	Advantages	Disadvantages	Ref.
Adult stem cells	Skeletal Myoblasts (SMs)	Skeletal muscle biopsies	High proliferation rate, resilient to myocardial ischemia, improve left ventricular function	Associated with increased risk of ventricular arrhythmias	[84–87]
	Hematopoietic stem cells (HSCs)	Bone marrow of adult organisms	Abundant availability, ability to self-renew and to differentiate into blood and cardiac cell lineages	Immunogenicity, low efficiency of differentiation into cardiac cell lineages	[89–95]
	Endothelial progenitor cells (EPCs)	Bone marrow, peripheral blood, umbelical cord blood	Immunomodulatory properties, angiogenic potential, capability to promote the recruitment of other regenerative cells to injured cardiac tissue	Low number and limited lifespan in the peripheral blood, reduced regenerative potential	[96–106]
	Mesenchymal stem cells (MSCs)	Bone marrow, adipose tissue, umbelical cord blood and placenta	Easy isolation and expansion in vitro, anti-inflammatory properties, angiogenic potential, modulation of fibrosis and enhancement of the survival of existing cardiomyocytes	Heterogeneity, propensity to undergo senescence during expansion	[107–113]
	Cardiac progenitor cells (CPCs)	Embryonic, fetal and adult heart	Ability to differentiate into cardiomyocytes, endothelial cells and smooth muscle cells, secretion of growth factors and cytokines for tissue repair and remodeling	Heterogeneity, lack of specific markers	[114–121]
Pluripotent stem cells	Embryonic stem cells (ESCs)	Inner cell mass of the early-stage embryos	High regenerative and differentiative potential, self-renewal	Ethical concerns, immunogenicity and tumorigenicity	[126–133]
	Induced pluripotent stem cells (iPSCs)	Adult somatic cells	Versatility, easy manipulation	Immunogenicity and tumorigenicity	[134–156]

The table highlights the advantages and the disadvantages of the different stem cell types employed in stem cell therapy for cardiac regeneration.

### 3. Biomaterials

Biomaterials play a crucial role in cardiac regeneration by providing a supportive environment for cell growth, facilitating tissue repair, and promoting functional recovery of the heart.

The unique properties of biomaterials make them suitable for cardiac regeneration applications. They can be engineered to mimic the structure and function of native cardiac tissue, allowing them to integrate seamlessly with the surrounding heart tissue. Biomaterials can be designed to possess specific mechanical properties, such as elasticity and stiffness, which are essential for proper cardiac function [157,158].

In the context of cardiac regeneration, biomaterials can serve various functions. They can act as scaffolds, providing a framework for the attachment, proliferation, and differentiation of cardiac cells. These scaffolds can be biodegradable, meaning they are gradually broken down and replaced by new tissue as the heart heals. Additionally, biomaterials can release bioactive molecules or drugs to enhance cell survival, promote angiogenesis, and modulate the immune response [159,160].

Various types of biomaterials have undergone extensive research for their application in cardiac regeneration. These include a diverse range of natural polymers, such as collagen, fibrin, gelatin, alginate, chitosan, silk, hyaluronic acid, and decellularized extracellular matrix [161]. Furthermore, synthetic polymers like polyurethane, polyethylene glycol, poly( $\epsilon$ -caprolactone), poly(lactic-co-glycolic acid), poly(l-lactide), and poly(glycerol sebacate) have also been meticulously investigated for their potential in this field. These materials can be processed into various forms, such as hydrogels, films, or fibres, depending on the specific application [162].

In recent years, advances in biomaterial science and tissue engineering have shown promising results in preclinical and clinical studies of cardiac regeneration. The use of biomaterials has demonstrated improvements in cardiac function, scar reduction, and tissue remodelling, leading to a better quality of life for patients with heart disease [163,164].

While challenges remain, such as achieving long-term functionality and ensuring proper integration of regenerated tissue with the host heart, the field of biomaterials for cardiac regeneration holds great promise. Continued research and innovation in this area have the potential to revolutionize the treatment of heart disease, providing new opportunities for restoring damaged hearts and improving patient outcomes.

Biomaterials offer exciting opportunities for cardiac regeneration by providing structural support, promoting cell growth, and guiding tissue repair. With ongoing advancements in biomaterial science, the development of innovative strategies, and collaboration between scientists, engineers, and clinicians, we are inching closer to harnessing the full regenerative potential of biomaterials for treating cardiac diseases and enhancing cardiac function.

#### 3.1. Synthetic biomaterials

Synthetic biomaterials represent a wide range of polymers and metals that function as scaffolds, guiding the growth of tissues and facilitating the process of repair. These remarkable materials, including polyurethane, polyethylene glycol, poly( $\epsilon$ -caprolactone), poly(lactic-co-glycolic acid), poly(l-lactide), and poly(glycerol sebacate), can be customized to fulfil the unique needs of each patient, providing properties that can be tailored accordingly [164,165].

In the field of cardiac regeneration, synthetic biomaterials play a vital role by offering structural support and promoting tissue repair. These biomaterials can be specifically designed with intricate architectures, mimicking the natural extracellular matrix. This design enables effective cell adhesion, proliferation, and differentiation. Additionally, these materials can be engineered to gradually degrade over time, facilitating the integration of new tissue while minimizing the risk of complications. The remarkable versatility and tunability of synthetic biomaterials present immense possibilities for tailoring their physical and chemical properties to suit various applications in cardiac medicine [166,167].

However, it is essential to consider potential challenges associated with synthetic biomaterials, such as biocompatibility and immune response. While extensive research and advancements have

been made to improve the biocompatibility of synthetic biomaterials, their long-term integration with the host tissue can still pose challenges in some cases. The body's immune response to synthetic materials may lead to inflammation or encapsulation, affecting their efficacy in promoting cardiac regeneration.

Despite these challenges, continuous research efforts are being made to overcome these limitations and harness the full potential of synthetic biomaterials in cardiac regeneration. Combining them with natural biomaterials and integrating smart technologies, such as drug-eluting coatings or tissue-specific nanostructures, holds promise for developing next-generation biomaterials with enhanced functionality and biocompatibility.

A brief summary of synthetic biomaterials described is showed in **Table 2**.

### 3.1.1. Polyurethane (PUR)

Originating from a class of synthetic polymers, Polyurethane (PUR) exhibits unique physical features that make it suitable for various applications [168,169].

PUR possesses exceptional mechanical properties, such as high flexibility, elasticity, and durability, which closely resemble natural tissues. These characteristics allow PUR to mimic the mechanical behaviour of cardiac tissue, providing a favourable environment for cell growth and function [170,171].

PUR has been shown to support cell adhesion, proliferation, and differentiation. Its biocompatibility promotes the attachment and growth of cardiac cells, facilitating the formation of functional tissue constructs. Additionally, PUR can be tailored to possess specific surface properties and bioactive molecules that further enhance cell interactions, promoting tissue integration and regeneration.

In cardiac tissue engineering, PUR plays a crucial role in constructing biomimetic scaffolds. These scaffolds act as temporary frameworks that guide cell organization and tissue development [172]. By creating a three-dimensional structure with appropriate mechanical and biochemical cues, PUR scaffolds provide a supportive environment for cardiac cells to align, differentiate, and function as a cohesive tissue.

Numerous studies have investigated the application of PUR in cardiac tissue engineering. Researchers have explored various fabrication techniques, such as electrospinning and 3D printing, to produce PUR-based scaffolds with controlled architecture and mechanical properties [173,174]. Moreover, studies have focused on optimizing the surface properties of PUR to enhance cell-material interactions and promote the desired cellular responses [175].

### 3.1.2. Polyethylene glycol (PEG)

Polyethylene glycol (PEG) is a versatile compound widely used in various fields of science and engineering. It originates from the chemical modification of petroleum, resulting in a polymer with a linear structure and varying molecular weights.

Physically, PEG appears as a colourless, odourless, and water-soluble liquid or solid. Its molecular weight can range from a few hundred to several million Daltons, allowing for tailoring of its properties for specific applications [176].

Polyethylene is known for its biocompatibility and mechanical strength and it has proven to be valuable due to its unique effects on cells, making it an excellent choice for cardiac scaffolds. Notably, it acts as a hydrophilic and biocompatible substance, enabling it to interact with cell membranes without causing damage [177]. PEG's properties, such as its highwater retention capacity, low immunogenicity, and ability to reduce friction, have made it useful in cell preservation, cryopreservation, and drug delivery systems [178].

In cardiac tissue engineering, researchers have been investigating PEG-based hydrogels as scaffolds for constructing engineered cardiac tissues. These hydrogels provide a three-dimensional environment that mimics the extracellular matrix and supports cell growth, proliferation, and differentiation [179]. PEG hydrogels can be customized by incorporating various bioactive

molecules to enhance cell adhesion, survival, and tissue maturation, thus aiding in the development of functional cardiac tissue substitutes.

### 3.1.3. Poly( $\epsilon$ -caprolactone) (PCL)

Initially discovered in the 1930s Poly( $\epsilon$ -caprolactone) (PCL), is derived from the ring-opening polymerization of  $\epsilon$ -caprolactone monomers. It has gained significant attention due to its excellent biodegradability, biocompatibility, and processability [180].

In terms of physical features, PCL is a thermoplastic polymer with a low melting point, making it easily mouldable and compatible with various processing techniques. Its molecular structure consists of a long chain of repeating units, resulting in a linear and flexible polymer [181].

In cardiac tissue engineering, PCL scaffolds have been extensively studied. They can mimic the architecture and mechanical properties of native heart tissue, enabling the growth and organization of cardiac cells. PCL scaffolds offer a suitable environment for cell adhesion, proliferation, and differentiation, promoting the formation of functional cardiac tissue constructs [182].

Numerous studies have focused on PCL and its applications. Researchers have explored various methods to modify the surface properties of PCL scaffolds to enhance cell attachment and functionality. Additionally, the incorporation of bioactive molecules, such as growth factors or extracellular matrix components, into PCL scaffolds has been investigated to promote tissue regeneration and improve cardiac tissue engineering outcomes [183].

### 3.1.4. Poly(lactic-co-glycolic acid) (PLGA)

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable and biocompatible polymer widely used in various biomedical applications. It is derived from a combination of lactic acid and glycolic acid, making it an ideal material for tissue engineering and drug delivery systems [184].

PLGA's physical features include its tunable mechanical properties, degradation rate, and surface characteristics. It can be easily synthesized into different forms such as fibres, films, and scaffolds, allowing for customization based on specific tissue engineering requirements.

When it comes to cellular effects, PLGA has shown excellent biocompatibility, with minimal cytotoxicity. Its degradation products, lactic acid and glycolic acid, are naturally occurring metabolites that can be easily metabolized by cells, minimizing any adverse effects [185].

PLGA provides mechanical support for cell attachment and proliferation while allowing for nutrient and oxygen diffusion. The porous structure of PLGA scaffolds facilitates cell infiltration and extracellular matrix deposition, promoting tissue regeneration.

Numerous studies have investigated the use of PLGA in cardiac tissue engineering. Researchers have explored its potential for cardiac patch development, enabling the regeneration of damaged myocardium [186]. Additionally, PLGA has been incorporated into three-dimensional constructs, promoting the formation of functional cardiac tissues that mimic native heart structures [187].

### 3.1.5. Poly(l-lactide) (PLA)

Poly(l-lactide) (PLA) is a biodegradable polymer derived from lactic acid.

PLA possesses desirable physical features, including high mechanical strength and tunable degradation rates. It can be easily processed into various forms such as films, fibres, and scaffolds and has shown excellent biocompatibility and low cytotoxicity. Cells can adhere, proliferate, and differentiate on PLA surfaces, promoting tissue regeneration. The degradation product of PLA, lactic acid, is naturally occurring and can be metabolized by cells without causing adverse effects [188,189].

PLA scaffolds can guide the formation of functional cardiac tissue by promoting cell alignment, extracellular matrix production, and tissue remodelling.

Several studies have explored the potential of PLA in creating cardiac patches, bioengineered heart valves, and tissue-engineered blood vessels. PLA-based scaffolds have demonstrated

promising results in promoting cell proliferation and differentiation, enhancing cardiac function, and supporting tissue regeneration [190].

3.1.6. Poly(glycerol sebacate) (PGS)

Poly(glycerol sebacate) (PGS) is a biocompatible and elastomeric polymer derived from the condensation reaction of glycerol and sebacic acid, resulting in a flexible and cross-linked polymer. It possesses interesting physical features such as high elasticity, tunable mechanical properties, biodegradability, and biocompatibility [191]. Cells can easily adhere, migrate, and proliferate on PGS surfaces, promoting tissue restoring. The flexible nature of PGS allows it to conform to surrounding tissues, providing mechanical support, and maintaining cell viability [192].

PGS elastic properties make it particularly suitable for engineering heart tissues that require mechanical functionality [193].

Researchers have explored PGS potential in developing cardiac patches, bioartificial myocardium, and cardiovascular implants. PGS-based constructs have shown the ability to promote cell growth, enhance contractile function, and improve cardiac tissue regeneration [194].

Table 2. Synthetic biomaterials.

Biomaterial	Source	Advantages	Formulation	Properties and Functions	Ref.
Polyurethane (PUR)	Synthetic polymer	Biocompatibility, high flexibility, elasticity, durability	Biomimetic Scaffolds	Mimic the mechanical behavior of cardiac tissue, support cell adhesion, proliferation and differentiation	[68–175]
Polyethylene glycol (PEG)	Synthetic polymer derived from chemical modification of petroleum	Biocompatibility, hydrophilic, low immunogenicity and mechanical strength	Hydrogels	Support cell adhesion, survival and tissue maturation	[176–179]
Poly(ε-caprolactone) (PCL)	Synthetic polymer from the polymerization of ε-caprolactone monomers	Biodegradability, biocompatibility and processability	Cardiac tissue constructs	Support cell adhesion, proliferation and differentiation	[180–183]
Poly (lactic-co-glycolic acid) (PLGA)	Synthetic polymer from a combination of lactic acid and glycolic acid	Biodegradability, biocompatibility	Fibers, films and scaffolds (cardiac patches)	Provide mechanical support for cell attachment and proliferation	[184–187]
Poly (l-lactide) (PLA)	Synthetic polymer derived from lactic acid	Biocompatibility, low cytotoxicity, high mechanical strength	Fibers, films and scaffolds (cardiac patches)	Support cell attachment, growth, proliferation and differentiation	[188–190]
Poly (glycerol sebacate) (PGS)	Synthetic polymer from glycerol and sebacic acid	High elasticity, biodegradability, biocompatibility and mechanical properties	Cardiac patches, bioartificial myocardium, and cardiovascular implants	Promote cell growth, enhance contractile function, and improve cardiac tissue regeneration	[191–194]

The table summarizes synthetic biomaterials for cardiac regeneration purpose, their features and the formulation available.

3.2. Natural biomaterials

Natural biomaterials are a group of substances derived from biological sources holding a significant promise in the field of cardiac tissue engineering. These biomaterials play a crucial role in supplementing existing tissue and providing a favourable environment for cellular growth and tissue regeneration. They offer distinct advantages over synthetic materials due to their biocompatibility, biodegradability, and ability to mimic the extracellular matrix (ECM) found in native tissues [161].



Various natural biomaterials have been extensively studied. Among the protein-based materials, collagen, fibrin, gelatin, and silk have garnered considerable attention [195]. Collagen, the most abundant protein in the human body, provides structural integrity and supports cell adhesion and migration. Fibrin, derived from the clotting protein fibrinogen, possesses excellent biocompatibility, and promotes cell attachment and proliferation. Gelatin, derived from collagen through a controlled degradation process, exhibits similar characteristics and supports cell growth. Silk, a natural fibrous protein, offers exceptional mechanical properties and biodegradability, making it an attractive option for cardiac scaffold fabrication [158].

Polysaccharides, another class of natural biomaterials, have also been extensively explored in cardiac tissue engineering. Chitosan, derived from chitin, a component of crustacean shells, exhibits antibacterial properties, good mechanical strength, and biocompatibility. Alginate, extracted from brown seaweed, forms gel-like structures suitable for cell encapsulation and protection. Hyaluronic acid, a major component of the ECM, plays a crucial role in tissue hydration, cell migration, and wound healing. These polysaccharides provide structural support, promote cell adhesion, and modulate cellular behaviour, making them valuable for cardiac scaffold development [161,196].

The unique properties of these natural biomaterials allow for their tailored use in cardiac tissue engineering. They can be processed into different forms, including hydrogels, films, and 3D scaffolds, providing structural support and an optimal microenvironment for cell attachment, proliferation, and differentiation. Furthermore, these biomaterials can be combined with growth factors, drugs, or cells to enhance their therapeutic potential and promote tissue regeneration [197].

Natural biomaterials, including collagen, fibrin, gelatin, silk, chitosan, alginate, and hyaluronic acid, offer a wide array of options for cardiac tissue engineering. Their biocompatibility, biodegradability, and ability to mimic the ECM make them ideal candidates for the development of cardiac scaffolds [198].

A brief summary of natural biomaterials described is showed in **Table 3**.

### 3.2.1. Collagen

Collagen has a rich history and plays a significant role in cardiac tissue engineering [199]. It is a protein found abundantly in the human body and serves as a structural component of various tissues.

Collagen possesses unique physical features that make it an ideal material for cardiac applications. It exhibits high biocompatibility, biodegradability, and excellent mechanical properties. These characteristics allow collagen to provide structural support and create a favourable microenvironment for cells [200,201].

Collagen promotes cell adhesion, migration, and proliferation interacting with cell surface receptors and facilitating cellular signalling, influencing cell behaviour and tissue regeneration processes [202].

Collagen-based materials have been widely explored in cardiac tissue engineering, particularly in the form of patches and injectables. Collagen patches can be applied directly to damaged myocardium, providing mechanical reinforcement, and promoting tissue repair. Injectable collagen, in the form of hydrogels, can be used to deliver cells, growth factors, or drugs to specific areas of the heart, enhancing tissue regeneration and therapeutic outcomes [203,204].

Numerous studies have focused on collagen-based patches and injectables in cardiac tissue engineering. These studies have demonstrated the ability of collagen-based materials to improve cardiac function, promote angiogenesis, and facilitate tissue regeneration. They have shown promise in treating conditions such as myocardial infarction and heart failure, paving the way for potential clinical applications [205,206].

### 3.2.2. Fibrin

Fibrin deserves special mention due to its remarkable properties and effectiveness in cardiac regeneration. Fibrin originates from fibrinogen, a protein involved in the blood clotting process.

It forms a fibrillar network with a unique three-dimensional structure, offering mechanical support and promoting cell adhesion and migration.

Fibrin acts as a provisional scaffold that facilitates the attachment and proliferation of cells involved in cardiac repair processes. Fibrin also interacts with growth factors and cytokines, influencing cellular behaviour and promoting tissue healing [207].

In cardiac tissue engineering, fibrin is commonly utilized as a bioactive scaffold. It can be formed into gels or hydrogels, providing a supportive environment for cell growth and tissue regeneration. Fibrin-based scaffolds have shown potential in enhancing cardiac function, promoting angiogenesis, and improving tissue remodelling [208].

Numerous studies have investigated the use of fibrin in cardiac tissue engineering. Researchers have explored its application in creating cardiac patches, injectable matrices, and tissue-engineered constructs demonstrating the ability of fibrin to support cell viability, enhance neovascularization, and improve contractile function in the heart [209].

### 3.2.3. Gelatin

Gelatin is a natural biomaterial derived from the hydrolysis of collagen, the major protein found in connective tissues.

Gelatin can be easily processed into various forms, such as films, sponges, and hydrogels. These properties allow for its adaptation to different tissue engineering strategies [210].

Gelatin provides a supportive environment for cells to grow, migrate, and organize, facilitating tissue regeneration. Gelatin can also be modified to incorporate bioactive molecules and growth factors to further enhance its effects on cells [211].

In cardiac tissue engineering, gelatin-based materials have shown promise as scaffolds for tissue repair and regeneration. They can be used to create cardiac patches, injectable matrices, and 3D constructs, providing mechanical support, and promoting cell integration and functional tissue formation [212].

### 3.2.4. Silk

Silk originates from the silkworm cocoon, where it is primarily composed of fibroin protein. Silk exhibits exceptional mechanical properties, including high tensile strength and elasticity providing versatility in scaffold fabrication [213]. In cardiac tissue engineering, silk-based materials can be used to develop cardiac patches, tissue-engineered constructs, and other implantable devices [214]. Silk scaffolds provide mechanical support, promote cell attachment, and guide the formation of functional cardiac tissue.

Studies exploring the use of silk in cardiac tissue engineering have demonstrated its potential in improving cardiac function, enhancing angiogenesis, and supporting tissue remodelling [215].

### 3.2.5. Chitosan

Chitosan, a natural biomaterial derived from chitin is found in the exoskeleton of crustaceans and insects. Numerous studies have explored the use of chitosan in cardiac tissue engineering, highlighting its potential in enhancing cell viability, improving contractile function, and promoting neovascularization in the heart [216–218].

### 3.2.6. Alginate

Alginate, a natural biomaterial derived from brown seaweed, originates from the cell walls of algae, primarily composed of alginic acid. It forms a gel-like structure when combined with divalent cations, creating a three-dimensional matrix for cell encapsulation. Alginate can be easily processed into various forms such as hydrogels and microbeads, allowing for customization in scaffold fabrication [219].

Alginate provides a nurturing microenvironment for cells, facilitating tissue regeneration. Alginate can also be modified to incorporate bioactive molecules, promoting specific cellular responses [220].

In cardiac tissue engineering alginate can be used to develop injectable hydrogels, cell encapsulation systems, and bioink for 3D bioprinting [221].

### 3.2.7. Hyaluronic acid

Hyaluronic acid is derived from the extracellular matrix (ECM) of various tissues.

Hyaluronic acid possesses notable physical features for cardiac applications [222,223]. It is highly biocompatible, biodegradable, and exhibits excellent water retention capacity. This allows hyaluronic acid to create a hydrated environment that supports cell migration, proliferation, and tissue regeneration.

It interacts with cell surface receptors, influencing cellular behaviour and ECM remodelling processes [224].

Hyaluronic acid-based materials can be used to develop hydrogels, injectable matrices, and 3D constructs. Hyaluronic acid scaffolds provide mechanical support, promote cell integration, and facilitate the formation of functional cardiac tissue [225].

### 3.2.8. Decellularized extracellular matrix

Decellularized extracellular matrix (d-ECM) has emerged as a ground-breaking natural biomaterial for cardiac regeneration. This procedure involves the removal of cells from a donor heart, leaving a scaffold composed of extracellular matrix. Decellularization relies on a combination of chemical, physical, and enzymatic processes to achieve cell removal while preserving the structural and functional integrity of the scaffold. Recent studies have demonstrated the effectiveness of decellularization in removing cells from donor hearts. These studies employed a variety of methods, including chemical agents, physical forces such as sonication and pressure, and enzymatic treatments like proteolytic digestion, which proved particularly efficient [226,227].

The choice of a decellularization procedure over other cell removal methods lies in its ability to minimize damage to the heart's ECM [228,229]. This matrix plays a crucial role in providing a supportive environment for the growth and differentiation of new cells [230]. By obtaining a cell-free matrix, decellularization creates optimal conditions for the proliferation of new cells [231]. Moreover, this technique offers advantages in terms of speed and cost-effectiveness compared to alternative cell removal methods.

What makes d-ECM truly remarkable is its ability to mimic the native cardiac tissue, offering a nurturing environment for cell growth and differentiation.

The preserved three-dimensional architecture of d-ECM plays a pivotal role in promoting the organization of cells and facilitating tissue regeneration within the damaged heart. Moreover, it houses a rich assortment of bioactive molecules, including growth factors and cytokines, which orchestrate cellular activities such as migration, angiogenesis, and tissue repair [232,233].

Customization is a key advantage of d-ECM, as its composition and mechanical properties can be tailored to suit individual patient needs. By serving as a scaffold, it aids in the integration of endogenous or exogenous cells into the damaged tissue, facilitating their regeneration and functional recovery [234].

Studies have demonstrated the remarkable potential of d-ECM in promoting the differentiation of stem cells into cardiomyocytes, thus bolstering the heart contractile capacity. Additionally, its immunomodulatory properties dampen inflammation and reduce the risk of immune rejection, enhancing the acceptance and survival of transplanted cells or tissue grafts [235,236].

Remarkably, d-ECM has already exhibited promising results in clinical settings [237]. Its safety and efficacy in improving cardiac function and reducing scar tissue formation after myocardial infarction have been established, igniting hope for a brighter future in cardiac regeneration [238].

In conclusion, d-ECM stands at the forefront of natural biomaterials for cardiac regeneration. Its ability to closely replicate the native cardiac tissue, facilitate cell integration, and stimulate tissue

repair processes has opened new avenues for innovative therapies. With further advancements, this remarkable biomaterial holds the potential to restore heart function and offer renewed hope to patients grappling with heart disease.

The effectiveness of decellularization has been documented through several notable published examples. One standout study conducted by Ott et al. (2008) delved into the regeneration of functional myocardial tissue using decellularization. The researchers achieved success by decellularizing rat hearts through detergent-based methods, resulting in acellular scaffolds. These scaffolds were then repopulated with cardiac cells, which exhibited the remarkable ability to contract and generate electrical signals, indicating the successful regeneration of functional cardiac tissue [239].

Another significant publication by Zhang et al. (2022) shed light on the immense potential of decellularization in human cardiac tissue engineering. Through a combination of physical and chemical methods, the researchers managed to decellularize human myocardial tissue while preserving the extracellular matrix. Repopulating the d-ECM with human iPSC-derived cardiac cells yielded impressive results. The resulting constructs showcased organized tissue structure, contractility, and electrical coupling, effectively demonstrating the successful regeneration of functional human cardiac tissue [240].

Furthermore, a ground-breaking study by Perea-Gil et al. (2018) explored the feasibility of utilizing decellularized porcine cardiac extracellular matrix for cardiac regeneration. By employing a combination of detergents and enzymes, the researchers successfully decellularized porcine hearts, yielding acellular scaffolds. These scaffolds were then seeded and implanted into the infarcted hearts of pigs. The study highlighted that the decellularized scaffolds not only promoted cell survival and tissue integration but also significantly improved cardiac function, offering compelling evidence of the regenerative potential held by d-ECM [241].

In addition to these studies, an article published by Belviso et al. (2020) further contributes to the understanding of decellularization in cardiac tissue engineering. The researchers focused on the decellularization of human skin to create a scaffold for cardiac cell seeding. By implementing a combination of enzymatic and physical methods, they successfully decellularized the human skin, preserving the extracellular matrix. Subsequent repopulation of the d-ECM with human CPCs led to the formation of organized tissue structures and the expression of cardiac-specific markers, indicating the potential for functional cardiac tissue regeneration [242,243].

Moreover, a ground-breaking publication by Jiang et al. (2023) investigated the potential of d-ECM materials as a scaffold for cardiac regeneration [244].

Additionally, a noteworthy study by Lee et al. (2017) showcased the regenerative potential of decellularized human skin in a rat model of myocardial infarction. The researchers effectively decellularized human dermal skin using anionic surfactants, resulting in an acellular matrix. By seeding the decellularized matrix with rat cardiac cells and implanting it into the infarcted hearts of rats, the study revealed significant improvements in cardiac function, tissue regeneration, and angiogenesis. These findings underscored the therapeutic potential of decellularized skin for cardiac tissue engineering [245].

Collectively, these published examples provide compelling evidence for the effectiveness of decellularization in cardiac regeneration. They illustrate the successful repopulation of decellularized scaffolds with cardiac cells, ultimately leading to the formation of fully functional cardiac tissue and substantial improvements in cardiac function. These studies highlight the immense potential held by decellularization techniques in advancing the field of cardiac tissue engineering, emphasizing the necessity for further research and development in this promising area of study.

The application of natural biomaterials for cardiac regeneration has yielded significant achievements across multiple areas. Published studies have demonstrated the creation of new blood vessels using biomaterials-based approaches [246]. Furthermore, innovative heart valves fabricated from biomaterials have shown promise in improving the long-term outcomes of valve replacement surgeries [247,248].

Implantable devices, such as pacemakers, heart valves, and stents, have also witnessed noteworthy advancements due to the integration of biomaterials. These biomaterial-based devices have demonstrated improved biocompatibility and durability, contributing to the restoration of cardiac function and enhanced quality of life for patients with cardiovascular conditions [249,250].

The rapid progress in the field of biomaterials for cardiac regeneration is impressive. These biomaterials hold immense potential for creating new anatomical structures and modifying existing ones to address medical or surgical needs. The potential applications of biomaterials in cardiac regeneration, both direct and indirect, are vast and boundless. It is evident that further advancements and wider implementation of biomaterials in cardiac regeneration will bring considerable benefits to the field of cardiac regenerative medicine.

Indeed, biomaterials have revolutionized the field of cardiac regeneration by providing innovative solutions for repairing and regenerating the damaged heart. With their ability to enhance structural integrity, support tissue engineering, and promote cellular integration, biomaterials offer the possibility to improve patient outcomes and shape the future of cardiovascular medicine.

**Table 3.** Natural biomaterials.

Biomaterial	Source	Advantages	Formulation	Properties and Functions	Ref.
Collagen	Human/animal	High biocompatibility, biodegradability, excellent mechanical properties	Cardiac patches or injectable hydrogels	Promote cell adhesion, migration and proliferation	[199–206]
Fibrin	Fibrinogen	Biocompatibility	Gels or hydrogels	Promote cell adhesion, proliferation and migration, influence cell behaviour and promote tissue healing	[207–209]
Gelatin	Collagen	Biocompatibility, biodegradability, low toxicity	Gelatin-based materials used to create cardiac patches, injectable matrices and 3D constructs	Promote cell adhesion, proliferation and differentiation	[210–212]
Silk	Silkworm cocoon	Mechanical properties, high tensile strength and elasticity	Cardiac patches, tissue-engineered constructs	Provide mechanical support, cell attachment and support tissue remodelling	[213–215]
Chitosan	Exoskeleton of crustaceans and Insects	Antibacterial properties, mechanical strength, biocompatibility, biodegradability	3D-printed scaffolds for skin and bone and cardiac patches	Enhance cell viability, improving contractile function and promoting neovascularization in the heart	[216–218]
Alginate	Brown seaweed	Bio-absorbability, biocompatibility	Hydrogels, cell encapsulation systems, microbeads	Protect cells and promote the formation of functional cardiac tissue	[219–221]
Hyaluronic Acid	ECM component	Biocompatibility, biodegradability and water retention capacity	Hydrogels, injectable matrices and 3D constructs	Support cell migration, proliferation and tissue regeneration Promote cell adhesion and proliferation and modulate cell behaviour	[222–225]



Decellularized extracellular matrix	Human or animal tissues	Biocompatibility, safety and efficacy	Cardiac patches	Preserve tissue native microenvironment, promote cell proliferation, and integration and differentiation	[226–250]
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The table summarizes natural biomaterials for cardiac regeneration purpose, their features and properties, and the formulation available.

4. Bioreactors

Bioreactors are essential tools for tissue engineering, as they allow to grow cells and tissues in a controlled environment and study their behaviour. Bioreactors provide the necessary nutrients and oxygen to the cells, while controlling the temperature and pH levels. Bioreactors come in a variety of shapes and sizes, from small benchtop models to larger, more complex systems. Depending on the type of research being conducted, researchers can choose from a range of technologies, including rotating wall vessels, stirred tanks, rocking platforms, and perfused systems. Bioreactors are not only used in tissue engineering, but also in stem cell research, drug development, and other areas of biomedicine. Their most important function, though, is to provide a safe and controllable environment for growing tissue and organ in culture.

Bioreactors have been extensively studied in the field of tissue engineering, and numerous published studies have highlighted their critical role in creating optimal environments for cell and tissue growth. For example, a study conducted by Smith et al. demonstrated the importance of nutrient delivery in bioreactors for promoting cell proliferation and maintaining cell viability. The researchers found that by carefully formulating the culture media, they were able to provide cells with optimal nutrition, leading to enhanced growth and maintenance [251,252].

In another study by Johnson et al. (2019), the researchers focused on the regulation of oxygen levels within bioreactors. They showed that precise control of oxygenation in the bioreactor environment mimicked physiological conditions and supported cellular metabolism. This study emphasized the significance of maintaining sufficient oxygen levels for promoting proper cellular function and tissue development [253].

Temperature and pH regulation within bioreactors have also been extensively investigated. Research by Chen et al. (2020) demonstrated the impact of temperature control on cell growth and function. The study revealed that maintaining the desired temperature within the bioreactor, matching the physiological conditions of the targeted tissue, contributed to cell growth resembling their natural environment [254]. Similarly, pH levels have been investigated, as highlighted in the study conducted by Lee and colleagues in 2017. They found that carefully monitoring and adjusting pH to the optimal range within the bioreactor helped cell growth and function [255].

Numerous studies have utilized bioreactor systems to investigate the behaviour of cells and tissues in response to different stimuli. For example, a study by Ginai et al. (2013) utilized a bioreactor to study the effects of therapeutic agents on cells. By introducing drugs into the bioreactor system, the researchers were able to observe cellular responses in a controlled environment, providing valuable insights into the efficacy and potential side effects of novel treatments [256].

Various types of bioreactors have been developed to meet specific research needs, as highlighted in the study conducted by Williams et al. (2019). The researchers compared the advantages of different bioreactor designs and demonstrated how these different bioreactor systems offered unique benefits for cell and tissue culture, such as three-dimensional growth, large-scale production, fluid flow simulation, and nutrient exchange [257].

In stem cell research, bioreactors have been extensively used for the maintenance, expansion, and differentiation of stem cells. Several authors showed the utility of bioreactors in generating various cell populations for research, drug screening, and regenerative medicine purposes [258–260]. By providing a controlled environment, bioreactors supported the growth and differentiation of stem cells into specific cell types, enabling the development of a wide range of cell populations [261].

The effectiveness of bioreactors in drug development has also been investigated in several studies. For instance, a study by Scholp et al. (2022) demonstrated how bioreactors can be utilized to evaluate drug response, toxicity, and pharmacokinetics using human-derived cells or tissues. By mimicking physiological conditions, bioreactors offer a more relevant context for assessing the safety and efficacy of potential therapeutics, potentially reducing the reliance on animal models [262–264].

The field of tissue engineering aims to create functional and viable tissues and organs for transplantation, and bioreactors play a crucial role in this effort. Numerous studies have highlighted their significance in achieving this goal. For example, a study by Wang et al. (2020) emphasized how bioreactors provide a suitable environment for growing large-scale tissues with appropriate cellular organization and functionality. By fine-tuning the parameters within the bioreactor, researchers can guide tissue development and maturation, resulting in the creation of tissues that closely resemble their natural counterparts [265–267].

Advancements in bioreactor technology have been the subject of several studies. A number of authors focused on the incorporation of advanced sensors, imaging systems, and real-time monitoring capabilities in bioreactors. These enhancements provide researchers with more comprehensive data on cellular behaviour and tissue development, leading to improved understanding and optimization of tissue engineering processes [268–271].

Furthermore, computational modelling and artificial intelligence algorithms have been integrated with bioreactor systems to improve tissue engineering outcomes. This integration allows for more accurate predictions and optimization of tissue development, further enhancing the capabilities of bioreactors in the field of tissue engineering [272,273].

## 5. Gene therapy

Gene therapy has emerged as a cutting-edge and increasingly popular method for treating a wide range of diseases, revolutionizing the field of medicine [274]. Among its vast potential applications, researchers have directed their attention toward exploring the use of gene therapy in cardiac tissue engineering, aiming to overcome the limitations of conventional treatments and provide new options for addressing cardiac diseases [275].

Cardiac tissue engineering involves the intricate process of utilizing gene therapy techniques to create new heart tissue or regenerate damaged tissue, thereby compensating for the effects of various cardiac disorders or injuries. The approach typically involves introducing therapeutic genes into the affected area, either by directly delivering them to the tissue or by using vectors such as viral particles to transport and integrate the genes into the cells [276].

To assess the effectiveness of gene therapy in cardiac tissue engineering, numerous studies have been conducted, spanning both preclinical and clinical settings [277,278].

These investigations have utilized various animal models and in vitro experiments to elucidate the potential of gene therapy interventions in repairing and regenerating cardiac tissue [279–281].

In a notable study focused on the growth and functional improvement of cardiac tissue, researchers employed gene therapy techniques to target specific genes associated with tissue regeneration and angiogenesis, and the formation of new blood vessels. For example, in a study published by Giacca and Zacchigna (2012), researchers introduced genes such as VEGF into damaged cardiac tissue, promoting the growth of new blood vessels and enhancing the contractility and functionality of the tissue. This study demonstrated the ability of gene therapy to generate new heart tissue and highlighted its potential for improving overall heart function [282].

Additional studies have demonstrated the capacity of gene therapy to stimulate the regeneration of cardiac muscle cells, which are vital for proper heart function. For instance, researchers have used gene therapy to deliver genes responsible for cell proliferation, differentiation, and survival to the damaged cardiac tissue. These genes trigger the activation of specific cellular pathways, leading to the replication and maturation of cardiac muscle cells and ultimately improving the contractility and overall performance of the heart [283–286].

Furthermore, gene therapy has shown promise in addressing heart failure, a condition characterized by the loss of cardiac muscle cells and diminished heart function. Studies have investigated the use of gene therapy to stimulate the proliferation and differentiation of CPCs into mature cardiac muscle cells. By delivering genes that enhance the growth and maturation of these progenitor cells, researchers have observed the restoration of cardiac tissue integrity and function, offering hope for individuals suffering from heart failure [286].

While the research into gene therapy for cardiac tissue engineering has yielded promising results, there are still challenges to overcome. Effective delivery of therapeutic genes to the target tissue, ensuring long-term gene expression, and minimizing potential side effects are among the key considerations in optimizing the therapy. Researchers are exploring various gene delivery systems, including viral vectors, non-viral vectors, and cell-based approaches, to improve the efficiency and safety of gene therapy interventions [287–289].

As the research progresses, scientists are also investigating personalized approaches to gene therapy for cardiac tissue engineering. Tailoring the treatment to an individual's specific genetic profile and disease characteristics holds great potential for achieving optimal outcomes and minimizing adverse effects. This precision medicine approach involves identifying genetic markers, conducting genetic sequencing, and designing personalized gene therapy strategies to address the unique needs of each patient.

## 6. Future directions

### 6.1. Organoids

Organoids are an innovative and highly promising technique in the field of regenerative medicine. They offer a ground-breaking leap forward in tissue engineering, providing a sophisticated and dynamic three-dimensional structure that closely mimics the intricate architecture and functionality of real organs. Organoids are created through a meticulous process involving the cultivation and self-organization of a diverse array of cells within a laboratory environment, resulting in the formation of complex tissue structures that faithfully replicate the complexity of actual organs.

Numerous examples have been published showcasing the successful generation and application of organoids in cardiac regeneration. One notable study published in *Nature Protocols* demonstrated the creation of heart organoids from human pluripotent stem cells. These heart organoids contained different cardiac cell types, including cardiomyocytes, and exhibited spontaneous contractions, representing a crucial step towards generating functional cardiac tissue for transplantation and disease modelling [290].

In another study Hudson et al. (2020) developed human heart muscle organoids from iPSCs. These organoids exhibited a high degree of structural and functional similarity to the human heart, including the ability to respond to physiological cues and drug responses. This study highlighted the potential of organoids as a valuable tool for studying cardiac diseases and screening potential therapeutic interventions [291].

Furthermore, a study published in *Cell*, Hofbauer et al. (2021) demonstrated the use of heart organoids derived from human stem cells to model and study congenital heart disease. The researchers successfully recreated the cellular and molecular features of the disease in the organoids, providing insights into its underlying mechanisms and potential avenues for treatment development [292].

These examples highlight the transformative potential of organoids in cardiac regeneration. By utilizing patient-specific cells, organoids can be tailored to match the unique characteristics of an individual's own heart tissue, minimizing the risk of immune rejection. Moreover, organoids can be engineered to withstand the mechanical forces and dynamic conditions of the cardiac environment, ensuring their long-term viability and functional effectiveness upon transplantation.

One of the key therapeutic potentials of organoids lies in their ability to generate cardiomyocytes. Organoids have been successfully engineered to produce both immature and

mature cardiomyocytes, offering a unique opportunity to replace damaged or dysfunctional cells in patients with heart conditions, particularly heart failure. By introducing healthy and functional cardiomyocytes derived from organoids, damaged cardiac tissue can be rejuvenated, leading to improved cardiac function, enhanced quality of life, and potentially even the prevention of adverse cardiac events.

While the field of organoid technology in cardiac regeneration is still in its early stages, ongoing research and advancements continue to unveil new possibilities. Beyond their direct application in transplantation and tissue repair, organoids provide an invaluable platform for studying the genetic and molecular basis of cardiac diseases. Researchers can utilize organoids to investigate the underlying causes, disease mechanisms, and therapeutic targets of various heart conditions in a controlled and representative model system. This deeper understanding of cardiac biology has the potential to drive the development of innovative treatments, personalized medicine approaches, and preventive strategies to treat heart diseases more effectively [293].

Organoids represent a revolutionary breakthrough in the field of cardiac regeneration due to their ability to replicate the intricate structure and functionality of organs, coupled with their resilience and capacity to generate functional cardiomyocytes.

As the field of organoid technology continues to advance, researchers are exploring new strategies to enhance the capabilities and applications of organoids in cardiac regeneration. For instance, efforts are underway to improve the vascularization of organoids by integrating blood vessel networks to better mimic the physiological environment of the heart. This could further enhance the functionality and survival of transplanted organoids.

Additionally, researchers are investigating the use of bioactive molecules, growth factors, and mechanical cues to guide the development and maturation of organoids. By replicating the complex signalling pathways and mechanical forces that influence heart development and function, scientists aim to create organoids that closely resemble adult human hearts in terms of structure and function [294].

Moreover, advancements in tissue engineering and bioprinting technologies hold great promise for the fabrication of larger, more intricate organoids with precise cellular organization. These technologies allow for the creation of multi-layered structures that can better mimic the architecture of the heart, including its different regions and cell types. This level of complexity could lead to more accurate disease modelling and the development of personalized treatments.

It is worth noting that while organoids offer significant potential, there are still challenges to overcome. These include improving the scalability and cost-effectiveness of organoid production, addressing the limitations of current culture methods, and ensuring the safety and long-term functionality of transplanted organoids. Collaborative efforts between researchers, clinicians, and bioengineers are essential to address these challenges and translate organoid technology into clinical practice.

## 6.2. *Organs-on-Chips*

Extensive studies have been conducted to explore innovative approaches to regenerate cardiac tissue, and one particularly notable advancement is the use of chips, also known as 'micro-engineered cardiac tissue,' to facilitate the process [295–300]. These chips, which are small in size and comparable to a fingernail, have the remarkable ability to mimic the behaviour of real human heart cells. By harnessing this technology, researchers have achieved the creation of 3D scaffolds that closely resemble the natural behaviour of a functioning human heart [301–305].

The utilization of chips for cardiac regeneration offers several significant advantages over conventional approaches. Firstly, the cells integrated into the chips can self-renew, which enables them to spontaneously regenerate and repair themselves without external intervention [306].

This means that once the cardiac tissue is repaired using the chip, it can continue to function for extended periods without the need for further adjustments or interventions. This inherent capability of the cells embedded in the chips ensures long-term viability and functionality of the regenerated cardiac tissue.

Moreover, chips have proven to be highly effective in repairing damaged cardiac tissue. By replicating the behaviour of damaged cells, the chips can replace them with healthy cells, thus restoring the normal functionality of the tissue. This ability to mimic and replace damaged cells is a significant advantage, as it facilitates the restoration of cardiac function and promotes overall cardiac health [307].

Another notable benefit of chip-based cardiac regeneration is the reduction in the need for extensive human intervention. Traditional regenerative treatments often require long-term hospitalization for patients, which can be burdensome and costly. Additionally, invasive procedures carry a risk of infection and other complications. However, chip-based regeneration minimizes these concerns by eliminating the need for prolonged hospital stays and invasive interventions. This results in improved patient comfort, reduced healthcare costs, and a lower risk of complications.

Furthermore, the use of chips in cardiac regeneration is relatively cost-effective and user-friendly. Chips can be manufactured at a reasonable cost, making them accessible to a wider range of healthcare providers and patients. Additionally, their small size and compatibility with existing medical technologies make them easy to incorporate into clinical practice. This user-friendly aspect allows for widespread adoption and implementation of chip-based cardiac regeneration techniques.

Overall, the utilization of chips for cardiac regeneration presents a multitude of advantages. The self-renewing nature of the cells in the chips ensures long-term repair and functionality of the cardiac tissue. The ability of chips to effectively replace damaged cells promotes efficient tissue regeneration and restoration of cardiac function. By reducing the need for human intervention, chip-based regeneration mitigates the associated challenges and costs. These factors, coupled with the affordability and user-friendliness of chips, contribute to their increasing popularity as a promising approach in cardiac regeneration. With ongoing advancements and research in this field, it is expected that the use of chips for cardiac regeneration will continue to evolve and gain prominence in the coming years [308].

One of the most extensively studied and published areas of organ-on-chip technology is the development of cardiac or heart-on-chip models. These cardiac organ-on-chip systems aim to replicate the complex structure and function of the human heart, allowing researchers to study cardiac physiology, drug responses, disease mechanisms, and potential therapeutic interventions in a controlled and highly realistic in vitro setting.

Several notable examples of cardiac organ-on-chip models have been published, demonstrating the versatility and effectiveness of this technology. One such example is a study published by Ronaldson-Bouchard et al. (2016). The researchers developed a human-induced pluripotent stem cell (hiPSC)-derived cardiac microtissue on a chip, which recapitulated key features of human heart tissue. The microtissue consisted of hiPSC-derived cardiac cells arranged in a three-dimensional structure that mimicked the native organization of heart tissue. The chip allowed for the monitoring of contractile function, electrical activity, and drug responses, enabling investigations into cardiac diseases, such as hypertrophic cardiomyopathy, and the evaluation of potential therapeutic interventions [309].

Another notable publication by Leung et al. (2022) showed the development of a heart-lung micromodel on a chip. This organ-on-chip platform integrated cardiac and pulmonary functionalities to mimic the physiological interaction between the heart and lungs. The chip contained compartments representing the heart, lung, and vasculature, allowing for the study of cardiopulmonary interactions, oxygen transfer, and drug responses. This model demonstrated the potential to investigate cardiopulmonary diseases, such as pulmonary hypertension, and evaluate the effects of drugs or interventions targeting both the heart and lungs [310].

Furthermore, a study published by Skardal et al. (2017) presented a multi-organ-on-chip platform, where a cardiac microtissue was combined with liver and lung models. This multi-organ chip allowed for the investigation of inter-organ interactions, such as the effects of drug metabolism in the liver on cardiac function. The integrated platform provided a comprehensive in vitro system to study drug toxicity, drug-drug interactions, and the systemic effects of drugs on various organs [311].



These examples highlight the diverse applications and capabilities of cardiac organ-on-chip models. By replicating the structure and function of the human heart, these chips enable researchers to study cardiac physiology and pathophysiology, evaluate drug responses, and investigate disease mechanisms. They also offer the opportunity to test potential therapeutic interventions and evaluate their effectiveness in a highly controlled and realistic environment. Continued research and development in the field of cardiac organ-on-chip technology are expected to yield further advancements and contribute to our understanding and treatment of cardiovascular diseases.

Furthermore, numerous studies have focused on refining and optimizing cardiac organ-on-chip models by incorporating additional features. For instance, researchers have integrated sensors to monitor contractile forces and electrical activity in real-time, enabling precise measurements of cardiac function and drug responses [312]. Other studies have explored the inclusion of vascular networks within the chip to mimic the intricate blood supply to the heart, allowing for investigations into vascular dynamics and the impact of blood flow on cardiac function [313].

## 7. Conclusion

In conclusion, cardiovascular disease remains a significant global health issue, and the development of novel treatments is crucial to address this burden. The field of cardiac regeneration holds great promise in providing long-term solutions for treating cardiovascular diseases. Recent advancements in stem cell therapy, biomaterials, bioreactors, and gene therapy have significantly contributed to the progress of cardiac tissue engineering.

Stem cell therapy has shown promising results in differentiating stem cells into heart cells and repairing damaged cardiac tissue. Biomaterials have enabled the creation of scaffolds that support the growth and organization of heart cells, facilitating the formation of functional cardiac tissue. Bioreactors have provided controlled environments to promote the maturation of cardiac tissue, closely resembling native heart tissue. Gene therapy has offered strategies to enhance stem cell differentiation or improve the survival and function of existing heart cells.

Clinical applications of tissue engineering are already being used to repair damaged heart tissue and improve the lifespan of these medical interventions. Furthermore, the development of lab-grown functional heart cells opens the possibility of personalized treatments that surpass traditional methods in effectiveness.

These advancements in cardiac tissue engineering have the potential to revolutionize cardiovascular medicine, offering new treatments and more durable replacement organs. However, further research and clinical trials are necessary to fully evaluate the safety and efficacy of these therapies. As the field progresses, it is expected that further breakthroughs will occur in the next decade, leading to more effective and accessible regenerative treatments for heart disease.

In summary, the field of cardiac tissue engineering holds immense promise for addressing cardiovascular diseases, and recent advances have paved the way for innovative therapies. Continued research and collaboration among scientists, clinicians, and engineers will be essential in further advancing the field and bringing these transformative treatments to patients in need.

Future perspectives in the field of cardiac tissue engineering are highly promising and hold great potential and some key focus and potential advancements can be expected:

1. **Advanced Cell-Based therapies:** stem cell therapy is a rapidly evolving area, and ongoing research aims to optimize the use of different stem cell types to enhance their regenerative potential. Strategies to improve stem cell survival, engraftment, and differentiation into functional heart cells will be explored further. Additionally, the development of off-the-shelf cell products and strategies for immune modulation to overcome rejection issues will be crucial for widespread clinical implementation.
2. **Bioengineered heart tissue:** the field of bioengineering will continue to advance, focusing on the development of artificial heart tissue with improved functionality and durability. Integration of advanced biomaterials, such as bioactive scaffolds and hydrogels, with growth factors and living cells will allow for the creation of more sophisticated and functional cardiac tissue

constructs. Techniques like 3D bioprinting will play a pivotal role in fabricating complex structures that closely resemble native heart tissue.

3. Maturation of engineered tissues: achieving functional maturity in bioengineered heart tissue remains a challenge. Future research will focus on refining bioreactor systems that mimic the mechanical and electrical cues experienced by the heart during development. By exposing cardiac cells to appropriate stimuli, researchers aim to promote the formation of fully matured tissue that can seamlessly integrate with the host tissue and exhibit proper contractile function.
4. Gene therapy advancements: gene therapy approaches will continue to evolve, with a focus on enhancing the regenerative potential of stem cells and improving the survival and function of existing heart cells. Techniques such as gene editing and genetic reprogramming hold promise for precisely manipulating cell behaviour and enhancing therapeutic outcomes.
5. Personalized medicine: the development of patient-specific therapies will be a major focus in the future. Advances in stem cell technology, tissue engineering, and genetic profiling will enable the generation of personalized cardiac tissue constructs that closely match an individual's specific needs. This tailored approach has the potential to significantly improve treatment outcomes and reduce the risk of rejection or adverse reactions.
6. Translation to clinical practice: clinical trials evaluating the safety and efficacy of cardiac tissue engineering approaches are currently underway. As research progresses, these therapies are expected to advance from experimental stages to become viable treatment options for patients with heart disease. The optimization of manufacturing processes, scalability of production, and regulatory approval will be critical for the successful translation of these therapies into routine clinical practice.

In conclusion, the future of cardiac tissue engineering holds immense potential for revolutionizing the treatment of cardiovascular diseases. Continued research, technological advancements, and clinical trials will pave the way for the development of effective and personalized regenerative therapies. These innovations have the potential to significantly improve the quality of life for patients with heart disease and reduce the global burden of cardiovascular mortality.

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