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REVIEW

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Current status and new horizons in stem cell therapy in cardiovascular regenerative medicine (CaVaReM): an update

Fateme Moradi Moraddahande[1†], Seyed Mahdi Emami Meybodi[2†^], Maryam Matin[3], Nafiseh Soleimani[2], Navid Ghasemzadeh[4] and Ali Dehghani Firoozabadi[2*]

Abstract

Cardiovascular diseases (CVDs) and, consequently, heart failure are life-threatening diseases, and they should be considered a worldwide health primacy. Conventional medical treatments have been unable to tackle the burden of disease thoroughly, and the limited availability of organ donors continues to pose a significant hurdle. This pressing need has prompted researchers to explore innovative regenerative techniques and expedite their progression into clinical trials, instilling fresh hope in patients who do not respond adequately to traditional therapies. Cardiac regeneration is an extensive approach that strives to repair irreversibly damaged heart tissue through groundbreaking scientific advancements, encompassing stem cells, tissue engineering, and cell-free therapies, collectively known as cardiovascular regenerative medicine (CaVaReM). The primary vision for CaVaReM is to develop regenerative-based therapies that can effectively cure cardiovascular disorders. This innovative field focuses on repairing and restoring damaged heart tissue, improving cardiac function, and ultimately enhancing the quality of life (QOL) in patients suffering from heart diseases. This review categorized cardiac regenerative therapies' present status, future opportunities, and challenges.

Keywords Regenerative medicine, Stem cells, Cardiovascular disease, Tissue engineering

Introduction

As per the World Health Organization (WHO), CVDs accounted for 17.9 million deaths in 2019, constituting a staggering 32% of all deaths worldwide. It is anticipated that CVDs will persist as the primary cause of mortality worldwide, with projections indicating an annual death toll of 23.3 million by 2030. Healthcare costs associated with CVDs continue to escalate, amounting to an astounding $300 billion annually [1]. CVDs encompass various diseases affecting the heart and blood vessels, such as coronary heart disease and heart failure (HF). Notably, CVDs remain the leading cause of global mortality, with a higher annual death toll than any other cause [2, 3].

However, prescription medications may also have adverse effects. Consequently, optimizing treatment

†Fateme Moradi Moraddahande and Seyed Mahdi Emami Meybodi contributed equally to this work.

Seyed Mahdi Emami Meybodi†.: Deceased.

*Correspondence:
Ali Dehghani Firoozabadi
ali.dehghani.molmed@gmail.com; a.dehghanifiroozabadi@ssu.ac.ir
[1] Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Yazd Cardiovascular Research Center, Non-Communicable Diseases Research Institute, Shahid Sadoughi University of Medical Sciences, Yazd 8917945556, Iran
[3] Alborz University of Medical Sciences, Karaj, Iran
[4] Department of Clinical Biochemistry, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran

for individual patients should be carefully assessed on a risk versus benefit-basis. Personalized medicine and risk stratification are essential in determining the most appropriate therapeutic strategies for patients following MI, including targeted pharmacological therapies, lifestyle interventions, and novel regenerative medicine approaches. Furthermore, ongoing research is essential to enhance the timely identification of patients at risk of HF and to provide better healthcare solutions to prevent or reverse the deleterious effects of MI [4, 5].

At the forefront of modern medical innovation, regenerative medicine (RM) represents an evolving interdisciplinary field that synergizes medicine and cutting-edge science. This integration aims to facilitate the reconstruction of cells, tissues, and organs, with the overarching objective of reinstating normal physiological function. As RM holds the promise of transforming the future of medicine, it addresses the constraints of existing therapeutic approaches while offering enduring solutions to enhance patients' quality of life significantly. The rapid convergence of multidisciplinary technological approaches, from traditional transplantation and replacement therapies to cell-engineered therapies, fosters innovative regenerative medicine strategies. This convergence also involves cutting-edge technologies related to small molecule therapeutics and pioneering advances in therapeutic compounds and development (Fig. 1) [6].

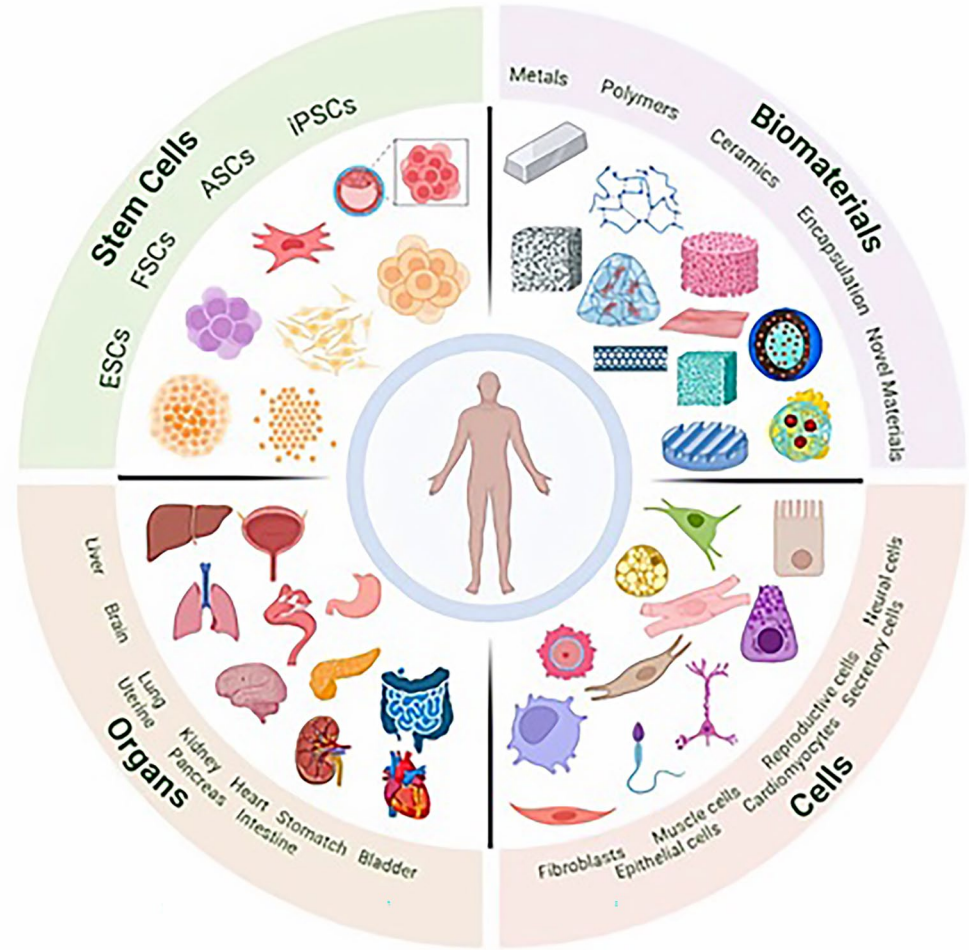

Fig. 1 Significant strides in SC-based regenerative medicine point toward a promising future. Owing to their remarkable capacity to differentiate into different cell lineages, SCs present immense potential for pioneering discoveries and post-regenerative medicine. The diverse range of SC types includes ESCs, tissue-specific or adult SCs, and iPSCs, all of which possess the unique ability to differentiate into numerous cell types, such as osteoblasts, chondrocytes, adipocytes, and other specialized cells both in vitro and in vivo. For regenerative medicine strategies to yield successful outcomes, the employment of essential biomaterials has become imperative in addressing degenerative diseases. Ideally, these materials should not only be capable of replacing damaged tissue but also function as effectively as the original tissue or stimulate the regeneration of the original tissue in organs, such as the liver, brain, lungs, kidneys, and heart

Ischemia–reperfusion injury and subsequent systemic inflammation lead to oxidative stress-induced progressive loss of cardiomyocytes through apoptosis and necrosis. Standard invasive and non-invasive cardiac therapies have been unable to repair damaged cardiac tissue post-AMI successfully. Thus, developing successful cardioregenerative therapies has become a potential strategy to address this unmet clinical need.

Researchers and clinicians focus on the following regenerative medicine approaches to improve cardiac function and outcomes for patients suffering from AMI and its long-term consequences. Utilizing various stem cell (SC) types, such as mesenchymal SCs (MSCs), cardiac SCs (CSCs), and induced pluripotent SCs (iPSCs) can replace damaged cardiac cells, promote angiogenesis, and stimulate the heart's endogenous repair mechanisms. Introducing therapeutic genes, RNA molecules, or genome editing tools modify cellular functions, promote tissue regeneration, and prevent adverse remodeling following AMI. Developing biomaterials, scaffolds, and 3D-printed cardiac patches can also support the growth and integration of new cardiac tissue. Harnessing the therapeutic potential of paracrine factors, extracellular vesicles, and other bioactive molecules derived from SCs or other cell types stimulates cardiac repair and regeneration.

Preliminary findings from preclinical investigations and early stage clinical studies have showcased the potential of these regenerative medicine strategies, instilling fresh optimism for patients grappling with AMI and other CVDs. An expanding body of evidence corroborates the therapeutic utility of SC therapies in augmenting cardiac function across AMI and ischemic cardiomyopathy animal models. Moreover, the feasibility and safety of diverse cellular routes have been substantiated in many preclinical and clinical studies [7]. However, evidence suggests that cell-based therapies can only moderately improve cardiac function in diseased hearts, and the results of clinical studies remain controversial and inconsistent [8]. Several parameters must be considered to optimize cell-based therapies' therapeutic efficiency and success. CVDs remains the leading global cause of morbidity and mortality, with HF being a particularly devastating consequence of irreversible cardiomyocyte loss following MI. In contrast to lower vertebrates such as zebrafish, the adult mammalian heart exhibits a very limited regenerative capacity, which has led to decades of research into cell-based, molecular, and bioengineering approaches aimed at restoring cardiac tissue. Despite numerous preclinical advances, the field of cardiac regeneration remains highly controversial and fragmented, characterized by mixed outcomes, translational challenges, and ongoing debates about the biological feasibility of true myocardial regeneration in humans.

This review provides a comprehensive and up-to-date analysis of the critical aspects affecting the optimization of SC therapies for cardiac regeneration. By examining the unique properties and regenerative potential of various SC types, such as MSCs, iPSCs, and CSCs, we aim to identify the most suitable cell type for specific cardiac conditions. Furthermore, we assess the benefits and risks associated with SC pre-differentiation and the utilization of growth factors and cell culture optimization to enhance therapeutic outcomes. Our review's unique contribution lies in its thorough evaluation of the complex interplay between donor cell characteristics, recipient immune response, and patient-specific factors, highlighting the importance of tailored cell-based treatments for individual patients. By integrating knowledge from preclinical and clinical studies, we offer valuable insights into the challenges and potential solutions for improving treatment efficacy, consistency, and safety in regenerative cardiac therapy.

Cell-based therapy in CVDs: regenerative surgery

In the quest for effective therapeutic interventions for advanced ischemic heart disease and post-infarction HF, regenerative therapy employing progenitor cell transplantation has surfaced as a burgeoning strategy. The underlying principle of this methodology entails the introduction of immature progenitor cells into the impaired cardiac tissue, anticipating that these cells will facilitate the development of novel blood vessels and cardiac muscle. A critical point to acknowledge in this context is that adult cardiomyocytes constitute terminally differentiated cells, inherently exhibiting restricted potential for proliferation and regeneration. It challenges cardiac repair, since the heart relies on cardiomyocyte maturation and differentiation for proper function [9]. It is now understood that around 1% of cardiomyocytes are renewed annually, and approximately 40% of cardiomyocytes in the human heart are generated postnatally. However, the reason why effective repair does not occur following MI remains a subject of active investigation by researchers seeking to restore damaged cardiac tissue. One common approach to address this issue is replacing the damaged tissue by transplanting cells capable of generating new cardiomyocytes, a process known as de novo cardiomyogenesis. Accumulating evidence from various clinical trials has underscored the therapeutic promise of SC therapy in addressing CVDs. These encouraging findings have incentivized researchers to concentrate on pinpointing the most efficacious cell sources and ideal cell types for transplantation procedures [10].

Gaining insights into the various cell types and learning from clinical studies in myocardial regenerative medicine is crucial, as multiple SC types have been employed in this area, including pluripotent SCs (PSCs), CPCs, and ESCs and their derivatives. The primary objective of cardiac cell therapy in cardiovascular medicine is to stimulate angiogenesis and vasculogenesis, ensuring the delivery of essential oxygen and nutrients to both chronically ischemic myocardial tissue and newly implanted progenitor cells. It is necessary to administer or release factors that stimulate and maintain signaling pathways supporting the survival, proliferation, and differentiation of transplanted SCs. Bone-marrow mesenchymal SCs (BM-SCs), including MSCs and hematopoietic stem cells (HSCs), have demonstrated significant promise in preclinical and clinical studies by promoting angiogenesis and enhancing cardiac function. These BM-SCs exert their therapeutic effects by secretion of paracrine factors, which promote angiogenesis, reduce inflammation, and improve cardiomyocyte survival.

In addition, their potential to differentiate into various cardiovascular cell types, such as endothelial and smooth muscle cells, further contributes to their regenerative capacity in damaged heart tissue. CPCs, which inherently reside within the heart, can differentiate into endothelial and smooth muscle cells, making them a promising option for regenerative therapies. This attribute enables CPCs to participate actively in tissue repair and regeneration processes. While ESCs and their derivatives showcase the capability to differentiate into multiple cardiac cell types, ethical considerations and the looming risk of teratoma formation have impeded their widespread clinical adoption. iPSCs, reprogrammed adult cells with pluripotency, have also shown potential in cardiac regeneration but require further research to address safety concerns. Understanding the different cell types and their applications in cardiac cell therapy is crucial for developing effective therapeutic strategies. By promoting angiogenesis, vasculogenesis, and the survival of implanted cells, these approaches aim to enhance cardiac function and reduce the adverse effects of ischemic heart disease [11].

Which cell type is suitable for cardiac cell therapy?

Endowed with distinctive characteristics that render them invaluable for regenerative medicine, SCs epitomize a unique category of specialized cells that have yet to evolve into a particular cell type. This attribute empowers them to differentiate into a myriad of specialized cells. SCs can undergo extensive division while preserving their undifferentiated state, thus facilitating the long-term maintenance and expansion of the SC pool. Notably, these cells possess the extraordinary capacity to differentiate into more specialized cells, making them an ideal choice for addressing various conditions, replenishing damaged tissue, and exploring diverse SC types for their potential therapeutic applications [12].

The classification of SCs can be premised upon various salient characteristics, encompassing anatomical origin, morphological attributes, functional properties, differentiation potential (potency), surface markers, transcription factors (TFs), and gene expression profiles. Ontogenetically, SCs can be sorted into ESCs, which originate from pre-implantation stage embryos, adult SCs (ASCs) that populate diverse tissues within the body, and iPSCs derived through genetic reprogramming of adult cells. From a morphological standpoint, SCs exhibit distinct size, shape, and surface features. Functionally, these cells assume specific roles within their native environments, predominantly participating in regeneration processes. Concerning differentiation potential, SCs span a spectrum from pluripotency, characterized by the capacity to differentiate into cells of all three germ layers, to unipotency, wherein cells are restricted to a single cell lineage. This range of potency provides a basis for further categorizing SCs based on their capacity to differentiate into specific lineages. Unique cell surface markers, such as proteins, facilitate the identification of SCs from other cell types. TFs are integral in preserving SC properties and inducing differentiation, while distinct gene expression profiles differentiate SCs from other cellular entities (Fig. 2).

Another method of classifying SCs is rooted in their origin, which can be attributed to ESCs, ASCs, and iPSCs. ESCs are obtained from the inner cell mass of blastocyst-stage embryos and are regarded as pluripotent, harboring the capability to differentiate into any cell type within the body. While ESCs exhibit promising potential in regenerative medicine, ethical concerns regarding their derivation from embryos persist. ASCs, also called somatic SCs, are dispersed throughout various cell types and tissues within the mature body, with their presence noted in bone marrow, peripheral blood, and adipose tissue. ASCs are generally considered multipotent, capable of differentiating into multiple cell types, albeit with more restricted differentiation potential than ESCs. These cells can be isolated with less ethical contention, mainly acquired from medical waste materials, such as discarded tissue following surgical procedures. Illustrative examples encompass HSCs and MSCs. iPSCs, conversely, are adult cells that have undergone genetic reprogramming to attain an embryonic SC-like state, conferring pluripotency and the capacity to differentiate into diverse cell types. The derivation of iPSCs circumvents the ethical dilemmas associated with ESCs and holds significant potential for tailored regenerative medicine applications.

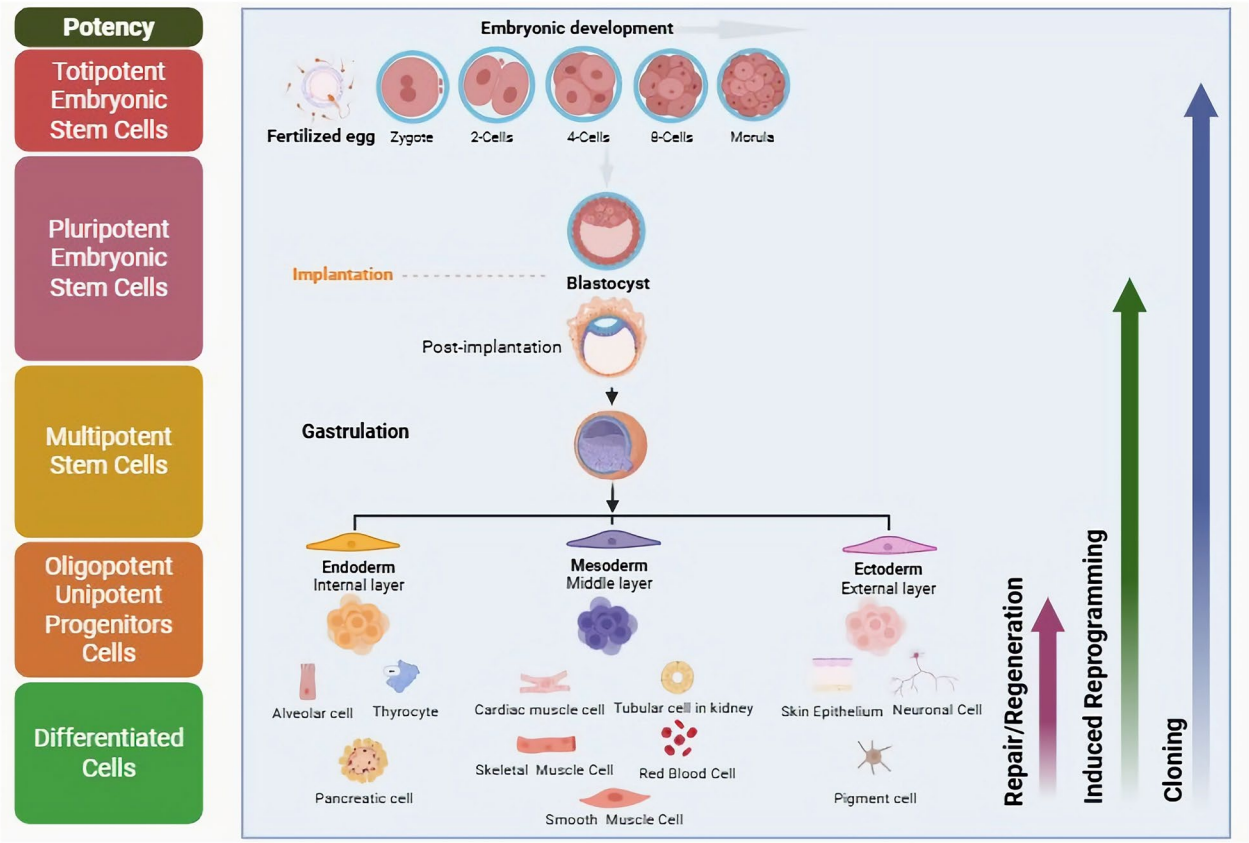

Fig. 2 Diagram illustrates the sequential process of embryonic differentiation, showcasing the gradual transition from a single fertilized cell to specialized tissue-specific lineages. This progression involves a steady decrease in potency as cells become increasingly specialized. At the pinnacle of the diagram, a fertilized egg or zygote symbolizes the totipotent stage, where the cell can differentiate into any cell type. Through cell division, the zygote develops into a blastocyst, comprising an inner cell mass and a surrounding layer of cells called the trophoblast. The inner cell mass consists of pluripotent cells capable of differentiating into any cell type within the three germ layers. As the blastocyst undergoes further differentiation, it segregates into three distinct germ layers: ectoderm, mesoderm, and endoderm. These layers serve as the foundational building blocks for the diverse tissues and organs of the human body. As the outermost layer, the ectoderm gives rise to the skin, intricate nervous system, and sensory organs. The mesoderm, situated between the ectoderm and endoderm, forms the muscular system, skeletal structures, blood vessels, and connective tissues. Finally, the innermost layer's endoderm develops into the vital internal organs and the complex digestive system [14]

As researchers continually strive to harness the immense potential of SCs for therapeutic applications, it is essential to address several challenges in this field. Notably, the potential risk of tumorigenicity associated with using SCs requires ongoing evaluation and the development of more efficient and reliable reprogramming methods. In the quest to maximize the benefits of SC-derived therapies, scientists are exploring various sources of SCs, each presenting its unique advantages and obstacles. The collective efforts of ongoing research aim to identify, refine, and optimize these distinct SC types, ultimately paving the way for effective and transformative treatments across a broad spectrum of diseases [12, 13].

Cardiomyocytes and resident cardiomyocytes

Fetal and neonatal cardiomyocytes have demonstrated the ability to proliferate and contribute to cardiac tissue repair. However, this regenerative capacity is limited in adult mammalian hearts. Fetal and neonatal cardiomyocytes, during early development, have a higher proliferative capacity, which allows for the growth and maturation of the heart. Some studies suggest that this regenerative potential may persist shortly after birth but declines rapidly with age. In contrast to fetal and neonatal cardiomyocytes, adult cardiomyocytes exhibit a notably restricted ability to divide and replenish themselves. As a result, adult hearts struggle to regenerate functional myocardium after injury, such as a heart attack [15]. Researchers are actively investigating the mechanisms that govern cardiac regeneration to develop therapeutic strategies that promote cardiomyocyte renewal and improve heart function after injury.

Some potential approaches include stimulating endogenous cardiac SCs, inducing cardiomyocyte proliferation,

and introducing exogenous SCs or their derivatives into the injured heart. Several approaches are being explored to modulate cardiomyocyte plasticity and enhance their regeneration capacity, including genetic manipulation of cardiomyocytes or CPCs to overexpress specific TFs or growth factors that promote proliferation and regeneration. For example, studies have shown that introducing genes such as cyclin A2, cyclin B1, or microRNA-590-3p can stimulate cardiomyocyte cell cycle re-entry and improve cardiac function after injury [16]. Small molecules or drugs activate signaling pathways that promote cardiomyocyte proliferation. Examples include periostin, neuregulin, and fibroblast growth factor1 (FGF1), which have shown some promise in preclinical studies by enhancing cardiomyocyte proliferation and reducing scar formation after cardiac injury [17]. Targeting epigenetic factors that regulate gene expression and cellular reprogramming can help boost cardiomyocyte plasticity and regeneration. Histone deacetylases (HDACs) and DNA methyltransferases are enzymes that small molecules or inhibitors can target to modulate gene expression and enhance cardiac repair [18]. Although these strategies have exhibited potential in preclinical investigations, additional research is required to refine their efficacy, safety, and applicability in clinical contexts. A more profound comprehension of the molecular mechanisms governing cardiomyocyte regeneration will likely pave the way for devising more potent therapeutic interventions to address cardiovascular disorders. Elucidating the complex mechanisms that govern cardiomyocyte regeneration is crucial for identifying new therapeutic targets and devising groundbreaking strategies to bolster the heart's innate healing capacity. A deeper understanding of these processes will significantly advance cardiovascular medicine and pave the way for more effective treatment approaches, ultimately improving clinical outcomes and quality of life for patients afflicted by CVDs.

Non-cardiomyocytes

Advances in scientific inquiry have uncovered the remarkable plasticity and regenerative capacity of non-cardiomyocyte cells within the heart, including endothelial cells, CPCs, epicardial cells, and cardiac fibroblasts, following cardiac injury. These cell types have demonstrated significant potential for contributing to cardiac repair processes. Endothelial cells, which line the blood vessels in the heart, have been shown to facilitate cardiac repair by stimulating angiogenesis, the growth and formation of new blood vessels, and the secretion of paracrine factors that enhance cardiomyocyte survival and function. CPCs, as a resident SC population in the heart, hold the potential to differentiate into numerous cardiac cell types, such as cardiomyocytes and endothelial cells, further emphasizing their essential role in cardiac regeneration [19]. They are essential to heart development and can be triggered upon injury to promote tissue repair. The epicardium, a thin layer of cells enveloping the heart's outer surface, has been found to exhibit a remarkable capacity for regeneration following injury. Epicardial cells can undergo a process called epithelial-to-mesenchymal transition (EMT), which enables them to differentiate into various cell types, such as fibroblasts and smooth muscle cells. This cellular plasticity significantly contributes to tissue repair and fibrosis in response to injury.

The fibroblast and other predominant cell types within the heart are critical in maintaining the myocardium's structural stability and overall health. In response to injury, fibroblasts can proliferate and differentiate into myofibroblasts, which are instrumental in forming scar tissue and remodeling damaged heart tissue. These processes are essential for maintaining cardiac function following injury and underscore the importance of investigating cellular plasticity within the heart to develop novel therapeutic strategies for cardiovascular disorders. These studies highlight the dynamic and complex nature of the heart's response to injury, involving a coordinated interplay between various cell types. Satellite cells, also known as muscle SCs, are crucial for skeletal muscle regeneration. Satellite cells between the basal lamina and the sarcolemma of mature muscle fibers typically remain quiescent until external signals prompt them into action. In skeletal muscle injury or damage, satellite cells become activated by signaling molecules, including growth factors and cytokines, released from damaged muscle fibers and immune cells. Once activated, satellite cells divide, creating new muscle fibers. This remarkable regenerative capacity makes satellite cells an essential focus of investigation in muscle regeneration. It holds promising implications for developing therapeutic interventions to enhance muscle repair following injury or disease [20]. Myoblasts then differentiate into myocytes, which fuse with the existing muscle fibers to form new multinucleated muscle cells, promoting muscle regeneration. Some satellite cells retain their SC properties, self-renewing to maintain the reserve of dormant satellite cells for future muscle regeneration needs.

Macrophages and other immune cells play crucial roles in modulating the regenerative capacity of various tissues, including skeletal muscle and heart. Macrophages are immune cells that play a dual role in tissue regeneration. During the early phase of tissue repair, they exhibit a pro-inflammatory phenotype (M1), which helps clear cellular debris and stimulate inflammation. Later, macrophages switch to an anti-inflammatory phenotype (M2), promoting tissue repair, angiogenesis, and

regeneration by releasing growth factors and cytokines [21–23]. T cells, especially regulatory T cells (Tregs), contribute to tissue regeneration by modulating inflammation, promoting angiogenesis, and enhancing the function of tissue-specific SCs. Neutrophils are early responders to tissue injury and help clear cellular debris through phagocytosis. They also release cytokines and chemokines, attracting other immune cells to the injury site and initiating tissue repair processes [24, 25]. Dendritic cells serve as a critical link connecting the innate and adaptive immune systems, and their role in modulating immunological processes is vital for regulating tissue regeneration. Their exceptional ability to impact innate and adaptive immunity establishes dendritic cells as a central orchestrator in coordinating successful regenerative responses. Mast cells release various mediators, including histamine and cytokines, which can promote angiogenesis, stimulate the recruitment of immune cells, and modulate tissue remodeling [26, 27]. The coordinated interplay between immune and resident tissue cells is essential for successful tissue regeneration. Further understanding of these mechanisms may help develop novel therapeutic approaches to enhance the regenerative capacity of various tissues and promote tissue repair in various pathologies.

Endogenously cardiac progenitor cells (CPCs)

CPCs indigenous to the heart have exhibited considerable promise in promoting cardiac tissue renewal and regeneration. These resident SCs are primarily localized in specific heart regions, such as the atrial apex and the outflow tract. CPCs can be obtained from different sources, including the epicardium, myocardium, and perivascular regions. Characterized by their SC properties, CPCs demonstrate both self-renewal and multipotency. This unique combination enables them to differentiate into various cardiac cell types, such as cardiomyocytes and endothelial cells, making CPC a highly versatile cell population for potential therapeutic applications in cardiac regeneration [28]. CPCs contribute to cardiac repair and regeneration through multiple mechanisms. They can directly replace lost cardiomyocytes by differentiating into new functional heart muscle cells and secreting various growth factors, cytokines, and exosomes, promoting angiogenesis, reducing inflammation, and stimulating endogenous cardiac repair mechanisms. CPCs can fuse with existing heart muscle cells, enhancing their regenerative capacity and interacting with immune cells, modulating inflammation and promoting a regenerative microenvironment. Due to their regenerative properties, CPCs have emerged as a potential therapeutic option for treating various cardiac conditions, such as MI, HF, and congenital heart defects [19]. However, CPCs are currently subject of debate regarding their potential for regenerating cardiomyocytes in vivo. Early studies suggested that CPCs, particularly those expressing the cKit+ marker, could transdifferentiate into new cardiac myocytes upon infarction injury. However, more recent research has challenged this notion, providing evidence that contradicts the regenerative capabilities of CPCs. A seminal study by van Berlo et al. [29] and numerous other investigations have demonstrated that cKit+ cells in the heart are primarily endothelial and have a negligible capacity to differentiate into cardiomyocytes in vivo. These findings, verified by multiple studies, indicate that CPCs should no longer be considered regenerative cells with the ability to generate new cardiomyocytes after cardiac injury. Consequently, the focus of cardiac regenerative research has shifted toward investigating alternative cell types and therapeutic strategies that may hold greater promise for restoring cardiac function following injury or disease.

Adult SCs

The bone marrow, a pliant and spongy tissue located within bones, such as the arms and legs, harbors a diverse array of SCs and progenitor cells. Among these cell types are HSCs, EPCs, and MSCs. In response to tissue damage, these cells are mobilized from the bone marrow and subsequently recruited to the injury site, which is crucial in promoting tissue repair. Emerging evidence suggests that BM-SCs, particularly MSCs, exhibit therapeutic potential in treating CVDs, such as MI and HF. MSCs can differentiate into various cell types, including cardiomyocytes, and exhibit potent paracrine effects by releasing growth factors and exosomes. These factors can promote angiogenesis, reduce inflammation, and stimulate endogenous repair mechanisms, improving cardiac function and structure.

HSCs constitute a scarce cell population within the bone marrow, accounting for merely 0.01% of the total nucleated cells. Despite their rarity, HSCs possess a distinct capacity for self-renewal and differentiation into all blood cell lineages, including erythrocytes, leukocytes, and platelets. This remarkable attribute enables HSCs to play a vital role in preserving the blood and immune systems throughout an individual's lifetime. Several studies have investigated the potential of HSCs in treating CVDs. Transplantation of HSCs has been shown to improve cardiac function and increase ejection fraction in some preclinical and clinical trials. HSCs may differentiate into cardiomyocytes or fuse with existing heart muscle cells, thereby contributing to cardiac muscle regeneration. They can secrete various paracrine factors, promoting angiogenesis, reducing inflammation, and stimulating endogenous repair mechanisms. Some studies suggest

that HSCs may transdifferentiate into endothelial cells, contributing to forming new blood vessels and vascular repair [30].

Apart from their direct regenerative capacity, specific BM-SCs, such as EPCs, have been found to secrete a diverse range of growth factors and cytokines. These secreted factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF), have been revealed to promote angiogenesis, improve cell survival, and stimulate tissue repair mechanisms.

Overall, BM-SCs, including HSCs and EPCs, hold promise for treating CVDs, but more research is needed to optimize their therapeutic potential and understand their precise mechanisms of action. A systematic review of preclinical and clinical studies has suggested that HSC transplantation can improve cardiac functional outcomes, measured by various parameters, such as left ventricular end-systolic volume (LVESV) [31]. While these findings are promising, it is essential to consider that the results may vary among studies, and further large-scale clinical trials are required to confirm the safety and efficacy of HSC transplantation for treating CVDs. In addition, optimizing the dose, timing, and delivery methods for HSC therapy is crucial to ensure the best outcomes for patients.

EPCs, a unique type of BM-SCs, hold significant promise for cardiovascular repair due to their distinctive characteristics. EPCs can differentiate into endothelial cells, which form the lining of blood vessels. These cells express specific surface markers, such as CD34, CD133, and vascular endothelial growth factor receptor 2 (VEGFR2). EPCs represent a heterogeneous population of cells, which can be further categorized into two primary subtypes based on their surface marker expression and angiogenic potential: early and late EPCs. Early EPCs, also known as angiogenic progenitor cells or colony-forming unit endothelial cells (CFU-ECs), are considered by the expression of CD34 and VEGFR-2/KDR surface markers. They have a robust capacity to promote angiogenesis, mainly through paracrine signaling and the secretion of pro-angiogenic factors. Late EPCs, also called circulating angiogenic cells (CACs) or endothelial colony-forming cells (ECFCs), express markers, such as CD34, CD133, VEGFR-2/KDR, and CD31. They are more mature and possess a higher proliferative and differentiation potential than early EPCs. Late EPCs can directly contribute to blood vessel formation by differentiating into endothelial cells and integrating into the existing vasculature [32]. EPCs preserve endothelial integrity and foster angiogenesis, forming new blood vessels. These cells are mobilized from the bone marrow in response to various stimuli, such as tissue ischemia or injury, and can migrate toward sites of endothelial damage. Upon arrival at these damaged sites, EPCs facilitate vascular repair and regeneration, ultimately promoting the restoration of blood vessel function and tissue perfusion. Due to their angiogenic potential, EPCs have been investigated as a therapeutic agent for treating CVDs, such as MI, peripheral artery disease, and ischemic stroke. Preclinical and clinical studies have demonstrated that EPC therapy can improve blood flow, enhance tissue perfusion, and better functional outcomes [33]. However, it is essential to note that the therapeutic efficacy of EPCs can be affected by factors, such as patient age, disease severity, and comorbidities. Further research is needed to optimize EPC therapy and understand the precise mechanisms involved in their angiogenic and regenerative actions.

Some studies have demonstrated that early EPCs can express specific receptors and proteins that contribute to their angiogenic and regenerative functions. Phosphodiesterase type 5 (PDE5) is an enzyme responsible for cyclic guanosine monophosphate (cGMP) degradation. cGMP is a crucial signaling molecule that participates in numerous physiological processes, including vasodilation and the widening of blood vessels to increase blood flow. Inhibition of PDE5, either pharmacologically or through overexpression, has increased cGMP levels and enhanced EPC function, promoting angiogenesis and vascular repair. CXCR4 is a chemokine receptor expressed on EPCs that interacts with its ligand, stromal cell-derived factor-1 (SDF-1). The SDF-1/CXCR4 axis plays an essential role in the homing and migration of EPCs to injury sites, where they can contribute to blood vessel repair and regeneration. An increased expression of CXCR4 on EPCs can enhance their responsiveness to SDF-1 gradients and improve their therapeutic potential [34].

Bone-marrow-derived mononuclear cells (BMNCs) represent a multifaceted population of cells that can be obtained from bone marrow or recruited into the bloodstream upon the administration of granulocyte colony-stimulating factor (G-CSF). BMNCs consist of diverse cell types, including HSCs, MSCs, progenitor cells, and an assortment of immune cells. While BMNCs have demonstrated potential in fostering cardiac regeneration, their capacity to directly differentiate into cardiomyocytes is limited [35, 36]. Instead, BMNCs are believed to support cardiac repair by secreting paracrine factors that promote angiogenesis, decrease inflammation, and improve the heart's overall function following injury. A meta-analysis of clinical trials investigating the use of BMNCs in patients with CVDs revealed that transplantation of BMNCs may provide some beneficial effects, including reduced hospitalization rates for congestive heart failure (CHF), fewer reinfarctions, and decreased cardiac-related mortality [37]. However, these findings

should be interpreted with caution due to the heterogeneity of the studies and potential publication bias. Further research is needed to understand better the optimal cell types, delivery methods, and patient populations that would benefit most from BMNC therapy in treating CVDs. In addition, exploring strategies to enhance the regenerative potential of BMNCs, such as by genetic modification or preconditioning, may help improve their therapeutic efficacy.

MSCs, which can be sourced from multiple adult tissues, show low levels of MHC-I and lack MHC-II molecules. This unique characteristic reduces the likelihood of eliciting an immune response upon transplantation. In addition to their immunomodulatory properties, MSCs possess multipotent differentiation capabilities and the ability to stimulate angiogenesis. These attributes make MSCs an attractive candidate for regenerative therapies. Several studies have demonstrated that MSC transplantation can enhance cardiac function, as indicated by improved left ventricular function (LVF), reduced cardiomyocyte apoptosis, and diminished fibrosis [38]. In addition, MSCs may promote cardiomyocyte differentiation, further enhancing their therapeutic potential. Furthermore, optimizing the source of MSCs, delivery methods, and combination with other treatments, such as chemotherapy, may help maximize the benefits of MSC therapy for treating CVDs.

Adipose tissue has recently gained attention as a promising source of SCs for regenerative therapies, particularly in the context of adipose-derived SCs (ADSCs). These cells, which can be isolated from adipose tissue, exhibit a rapid proliferation rate and differentiation into multiple cell types, such as cardiomyocytes. Several studies have successfully demonstrated the differentiation of ADSCs into cardiomyocytes using various methods, highlighting the potential of these cells in cardiac regeneration. ADSCs also exhibit immunomodulatory properties and paracrine effects that can contribute to tissue repair and regeneration [39]. Researchers have employed various strategies to enhance the differentiation process, including pro-cardiogenic factors, such as bone morphogenetic protein 2 (BMP2), VEGF, TGF-β1, or a combination of these factors. These factors help promote the differentiation of ADSCs into cardiomyocytes, which may contribute to improved cardiac function in the context of CVDs [40]. Some advantages of using ADSCs for regenerative therapies include their abundance and accessibility, as they can be easily obtained from adipose tissue through minimally invasive procedures such as liposuction [41, 42].

Despite the promising attributes of ADSCs, further investigations are required to refine the isolation, expansion, and differentiation protocols to maximize their therapeutic potential. Moreover, assessing the safety and efficacy of ADSCs in clinical settings remains a critical area of focus in advancing their translational application. Addressing these challenges will be instrumental in realizing the significant potential of ADSCs as a therapeutic option for CVDs and other conditions necessitating tissue regeneration [43]. Recent studies have employed 5-azacytidine treatment to promote the differentiation of ADSCs into cardiomyocytes, highlighting the ongoing efforts to develop effective strategies for enhancing the cardiogenic potential of these cells [44, 45]. This study treated ADSCs with 5-Aza to inhibit DNA methylation and induce their differentiation into cardiomyocytes. The researchers observed an upregulation of specific cardiomyocyte markers in the cultures treated with this protocol, such as sarcomeric alpha-actinin (Serca2), GATA4, and connexin-43 (Cx43). Serca2 is a critical structural protein involved in the contraction of cardiac muscle cells, GATA4 is a transcription factor essential for heart development, and Cx43 is a gap junction protein that plays a crucial role in the electrical coupling of cardiomyocytes [46]. The upregulation of these markers suggests that 5-Aza treatment effectively promoted the differentiation of ADSCs into cardiomyocytes. This study provides valuable insights into potential strategies for guiding the differentiation of SCs into specific cell types, which could be beneficial for regenerative therapies in the context of CVDs. However, further research is necessary to establish the safety and efficacy of such approaches before they can be translated into clinical applications.

MSCs derived from bone marrow (BM-MSCs) represent another source of SCs capable of differentiating into functional cardiomyocytes. Several growth factors and signaling pathways, including FGF, PDGF, and TGF-β, have been implicated in promoting the differentiation of BM-MSCs into cardiomyocytes. FGF, a protein involved in diverse cellular processes, such as differentiation, proliferation, and migration, has been demonstrated to enhance the differentiation of BM-MSCs into cardiomyocytes. PDGF is another growth factor that can stimulate the differentiation of BM-MSCs into cardiomyocytes by promoting the expression of cardiac-specific markers and proteins. TGF-β is a multifunctional cytokine that plays a crucial role in cell growth, differentiation, and extracellular matrix production. It has been shown to induce the differentiation of BM-MSCs into cardiomyocytes, likely by activating specific signaling pathways. These growth factors can trigger various signaling pathways, such as the PI3K/Akt and Wnt/β-catenin pathways, which regulate cell survival, proliferation, and differentiation. By modulating these pathways, the growth factors can stimulate the differentiation of BM-MSCs into cardiomyocytes,

potentially offering a therapeutic approach to treating CVDs [47].

Umbilical cord-derived mesenchymal SCs (UC-MSCs) present an alternative source of MSCs, offering several advantages over BM-MSCs. UC-MSCs are easily accessible, conveniently stored and transported, and exhibit multipotency. These cells can be non-invasively collected from the Wharton's jelly of the umbilical cord following childbirth, providing a readily available and ethically uncomplicated source of SCs. The ease of procurement and the potential to establish UC-MSC banks make these cells appealing for regenerative therapies. UC-MSCs can be cryopreserved and transported relatively easily, facilitating their use in regenerative medicine applications. Like BM-MSCs, UC-MSCs can differentiate into various cell types, such as cardiomyocytes, making them a potential therapeutic option for treating CVDs. Due to these advantages, UC-MSCs have gained considerable interest in regenerative medicine and are being investigated for their potential therapeutic applications. A study investigated the effects of 5-azacytidine (5-Aza) treatment on Wharton's jelly derived mesenchymal SCs (WJ-MSCs) to promote their differentiation into cardiomyocytes. The results showed that treating WJ-MSCs with 5-Aza induced epigenetic changes in the cells, which primed them for differentiation into cardiomyocytes. After 5-Aza treatment, the WJ-MSCs showed upregulation of several cardiomyocyte markers, including α-actinin, GATA4, and SERCA, indicating that the cells were differentiating into cardiomyocytes [48].

This study suggests that 5-Aza treatment can induce epigenetic changes in WJ-MSCs, promoting their differentiation into cardiomyocytes. These findings have important implications for developing regenerative therapies for CVDs, as WJ-MSCs may serve as a valuable source of cardiomyocytes for transplantation or tissue engineering applications.

In a research study, scientists explored the potential of a specific inhibitor, CHIR99021, to enhance the differentiation of Wharton's jelly derived MSCs (WJ-MSCs) into cardiomyocytes. They treated WJ-MSCs with CHIR99021, an inhibitor of glycogen synthase kinase-3β (GSK3β), which is known to play an essential role in different cellular processes, including cell differentiation. They observed that CHIR99021 treatment augmented the differentiation potential of WJ-MSCs into cardiomyocytes, as evidenced by the increased expression of cardiomyocyte-specific markers [49]. Transcriptome analysis revealed an upregulation of critical genes associated with cardiomyocyte differentiation and function, such as GATA4, SERCA, and other factors, in the CHIR99021-treated WJ-MSCs. This study demonstrates that CHIR99021 can enhance the differentiation of WJ-MSCs into cardiomyocytes, potentially offering a novel strategy for regenerative therapies in CVDs. Further research is needed to optimize the conditions for cardiomyocyte differentiation and explore the potential of CHIR99021-treated WJ-MSCs in preclinical and clinical settings.

Other sources of MSCs have been explored for their potential in CaVaReM, such as dental pulp SCs (DPSCs) and skin-derived SCs (SDSCs). DPSCs can be isolated from the dental pulp tissue of extracted teeth. They exhibit multipotency, immunomodulatory properties, and the ability to differentiate into various cell types, including cardiomyocytes. Recent studies demonstrated that DPSCs could improve cardiac function and promote angiogenesis in a rat model of MI [50].

Fetal SCs

Fetal and prenatal SCs are a valuable source of various types of SCs, which possess unique properties and differentiation potential that can be harnessed for CaVaReM. Amniotic Fluid SCs (AFSCs) are isolated from amniotic fluid rich in nutrients and growth factors. They possess a high proliferation rate and low immunogenicity, making them suitable for allogeneic transplantation. In addition to their potential to differentiate into cardiomyocytes, AFSCs can also secrete paracrine factors that promote angiogenesis, reduce inflammation, and enhance cardiac function.

Placenta-derived MSCs (PMSCs) and MSCs derived from chorionic plates (CP-MSCs) can be obtained from the placenta, which is generally discarded after childbirth. These cells possess immunomodulatory properties, low immunogenicity, and high proliferative capacity. Both PMSCs and CP-MSCs have shown promising results in preclinical models of MI, promoting cardiac repair and regeneration.

Fetal cardiac progenitor cells (FCPCs) are another type of fetal SC showing potential in CaVaReM. They are derived from the fetal heart and can be identified by specific cell surface markers, such as c-kit, Sca-1, and PDGFRα. These cells exhibit high proliferative potential and can differentiate into multiple cardiac cell types, including cardiomyocytes and endothelial cells. Several trials have validated the cardiogenic potential of FCPCs both in vitro and in vivo. In vitro, FCPCs have been successfully differentiated into beating cardiomyocytes under specific culture conditions. In vivo, transplantation of FCPCs into animal models of MI has resulted in improved cardiac function, increased angiogenesis, and reduced fibrosis. While FCPCs show great promise, their clinical translation is limited by ethical concerns and technical challenges associated with their isolation and expansion [51].

Embryonic SCs (pluripotent SCs)

ESCs are pluripotent cells harvested from the inner cell mass of blastocysts, exhibiting the remarkable ability to differentiate into diverse cell types representing all three primary germ layers: endoderm, mesoderm, and ectoderm. This pluripotent nature has positioned ESCs as a prominent focus in regenerative medicine research, particularly within cardiovascular therapies [52]. Several protocols have been developed to differentiate ESCs into cardiomyocytes in vitro efficiently. These ESC-derived cardiomyocytes (ESC-CMs) exhibit many features of native cardiomyocytes, such as electrical coupling, spontaneous contractions, and expression of cardiac-specific markers. ESCs that are differentiated into cardiomyocytes express various cardiac-specific markers, which can be used to confirm their identity and functionality. ESC-CMs express sarcomeric proteins, such as myosin heavy chain (MHC), cardiac troponin T (cTnT), and cardiac troponin I (cTnI), which are involved in muscle contraction. ESC-CMs also express various ion channels and receptors that are essential for the electrical coupling and contractility of cardiomyocytes, such as connexin-43 (Cx43), potassium channels (e.g., KCNQ1), and L-type calcium channels (e.g., CACNA1C). ESC-CMs express several cardiac TFs, including GATA4, NKX2.5, and MEF2C, which play crucial roles in cardiac development and function [53].

In preclinical studies, transplantation of ESC-CMs into injured hearts has shown promising results. The transplanted cells can integrate into the host myocardium, form electromechanical connections with the surrounding cardiomyocytes, and promote cardiac remodeling, neovascularization, and reduced scar formation. These effects collectively improve cardiac function and overall cardiac repair [54].

Induced pluripotent stem cells (iPCs)

iPSCs represent an up-and-coming SC source generated by reprogramming somatic cells through the ectopic expression of specific TFs, commonly krüppel-like factor 4 (Klf4), octamer-binding transcription factor 4 (Oct4), SRY-box transcription factor 2 (Sox2), and c-Myc, collectively known as the Yamanaka factors. This reprogramming process effectively reverts the cells to a pluripotent state, enabling them to differentiate into various cell types, including cardiomyocytes. Several protocols have been established to differentiate human iPSCs into cardiomyocytes, which can be generally classified into two main strategies. Embryoid body (EB) formation involves the aggregation of iPSCs into three-dimensional EBs, which mimic early embryonic development. The EBs are then cultured in specific conditions that promote the differentiation of iPSCs into cardiomyocytes. Another approach is monolayer differentiation; in this approach, iPSCs are cultured as a monolayer and exposed to a series of growth factors, small molecules, or other signaling cues that guide their differentiation into cardiomyocytes. Both approaches have successfully generated hiPSC-CMs that exhibit typical cardiomyocyte features, such as spontaneous beating, expression of cardiac markers, and response to pharmacological agents. However, further optimization and standardization of differentiation protocols are needed to ensure the consistency, purity, and functionality of hiPSC-CMs for potential clinical applications [55, 56].

The differentiation of iPSCs into cardiomyocytes typically involves a multi-step process. Initial protocols often employ growth factors and small molecules that modulate signaling pathways, such as the Wnt, Activin/Nodal, and BMP pathways, to induce mesoderm differentiation. Subsequent exposure to specific factors such as Wnt inhibitors and GSK3B inhibitors promotes the differentiation of mesoderm cells into cardiac progenitors [57]. Finally, the addition of factors that promote cardiac maturation, such as thyroid hormone and ascorbic acid, helps drive the formation of functional cardiomyocytes. However, the translation of iPSC-based therapies to clinical settings is not without challenges. Key concerns include tumorigenicity, immunogenicity, genetic instability, and the limited scalability of iPSC production [58]. In addition, current differentiation protocols often yield cardiomyocytes that resemble fetal cells rather than mature adult cells, which may limit their therapeutic potential [59]. To overcome these challenges, researchers are actively investigating novel strategies, such as improving differentiation protocols, enhancing cell engraftment and survival, and addressing safety concerns. Close collaboration between scientists, clinicians, and engineers is essential to fully harness the potential of iPSCs for cardiovascular medicine. As the field continues to evolve, iPSCs hold immense promise for transforming our understanding and treatment of cardiovascular diseases.

There are inherent differences in cardiac physiology between species, which may limit the translatability of findings from animal studies to humans. However, hiPSC-CMs offer a valuable human-based model to study cardiac biology and disease. The use of human embryos for deriving pluripotent SCs raises ethical concerns. In addition, the invasive nature of collecting somatic cells for generating iPSCs can pose practical challenges. Traditional methods for generating iPSCs and differentiating them into cardiomyocytes can be inefficient, time-consuming, and expensive, hindering their widespread use. However, ongoing advancements in reprogramming and differentiation technologies aim to address these

limitations. In addition, genetic and epigenetic differences between donors can influence the characteristics and functionality of hiPSC-CMs, leading to variations in their therapeutic potential and underscoring the need for personalized approaches in SC-based therapies. The viability and long-term survival of transplanted hiPSC-CMs can be challenging due to factors, such as immune rejection, cell death, and poor engraftment. Overcoming these hurdles is crucial for realizing the full potential of hiPSC-CMs in regenerative medicine [60, 61].

Despite these challenges, hiPSC-CMs are a valuable tool for studying cardiac development, disease modeling, drug screening, and exploring cell-based therapies for CVDs. Ongoing research addresses these limitations and refining protocols for the efficient generation and functional maturation of hiPSC-CMs [62, 63].

The current situation in SC-based therapy for cardiovascular regeneration

Numerous research endeavors and early stage clinical trials are actively exploring the potential applications of multiple SC types, such as MSCs, in cardiovascular medicine. These studies aim to evaluate safety, efficacy, and optimal delivery methods. The results from both basic and clinical studies in cell-based therapy for cardiovascular regeneration suggest that the potential therapeutic benefits are mainly derived from indirect paracrine or indirect cell–cell interaction mechanisms, along with some direct approaches.

SCs can secrete pro-angiogenic factors that enhance the growth of new blood vessels, thereby improving blood flow and oxygen delivery to the damaged heart tissue. SCs can also produce anti-fibrotic factors that help reduce scar formation and prevent detrimental ventricular remodeling following an MI. Some SCs have been shown to improve the survival of cardiomyocytes and enhance the recruitment of endogenous SCs to the injury site. Direct differentiation and incorporation of injected SCs into the myocardium can compensate for the extensive loss of cardiomyocytes following an MI.

Preclinical research has provided substantial evidence supporting the ability of SCs to enhance cardiac function, stimulate angiogenesis, and decrease scarring in animal models of CVDs. However, translating these findings to human clinical trials has encountered challenges due to species variations and other factors. While some clinical trials have demonstrated modest improvements in cardiac function following SC therapy, others have shown mixed results or negligible benefits. These discrepancies can be attributed to differences in study design, patient selection, cell type, delivery method, and the timing of administration. The field faces several challenges, including ethical concerns, donor variability, poor cell survival and engraftment, and more efficient and cost-effective protocols for generating and differentiating SCs. Addressing these issues is crucial for advancing SC-based therapies in CaVaReM.

Combining these direct and indirect mechanisms is believed to contribute to the beneficial effects observed in SC-based therapies for cardiovascular regeneration, including improved cardiac function and ventricular contraction. However, further research is needed to fully understand and optimize these mechanisms to maximize the therapeutic potential of SCs in treating CVDs [64].

Trans-differentiation of SCs

The possibility of ASCs, specifically those derived from bone marrow, to transdifferentiate into cardiomyocytes within the heart has been a topic of controversy. Some research has shown that BM-SCs can differentiate into endothelial cells and cardiomyocytes. However, various other studies have challenged the idea that bone-marrow SCs possess the capacity to differentiate directly into cardiac tissue. Instead, these studies propose that the observed improvements in heart function following SC therapy may result from alternative mechanisms, including paracrine signaling, immunomodulation, and cell fusion [65, 66].

Preclinical studies involving SC-based approaches have shown that only a few cells directly differentiate into cardiomyocytes in MI animal models. This limited cardiomyogenic differentiation has prompted researchers to investigate alternative mechanisms by which SCs contribute to cardiac regeneration, aiming to optimize cell-based therapies for treating ischemic heart disease [67–69]. Recent clinical studies have indicated that CPCs minimally contribute to de novo cardiomyocytes and new myocardium formation after MI [70]. However, it has been observed that cardiac-resident c-Kit-positive cells can differentiate into cardiac endothelial cells during development and adulthood. These findings suggest that ASCs may possess an intrinsic capacity to adopt a cardiovascular fate within the cardiac microenvironment [71, 72]. Despite this potential, the transdifferentiation capacity of ASCs, such as CPCs, could be more efficient, as these events are infrequent. Current research efforts aim to understand the mechanisms underlying SC differentiation better and develop strategies to enhance their therapeutic potential for cardiac repair and regeneration [73, 74].

Paracrine effect

Another approach in CaVaReM involves harnessing the paracrine signaling effects of SCs. This strategy assumes that SCs, upon local implantation or systemic infusion, secrete various bioactive molecules that act in a paracrine

fashion in response to the local environment. These paracrine factors can synergistically promote tissue repair, angiogenesis, and regeneration, ultimately improving cardiac function [75]. Paracrine signaling triggered by SCs influences extracellular contacts and the surrounding cardiovascular microenvironment, leading to activation or inhibition of local signaling pathways independent of cell–cell interaction with the host myocardial cells. It is proposed that the functional benefits detected after SC injection in animal models of CVDs might be correlated with the secretion of paracrine biomolecules, which regenerate the cardiac tissue and diminish pathological cardiac remodeling, induce neovascularization, and induce cardiac regeneration (Fig. 3) [76–78].

Upon their injection into infarcted hearts, paracrine signaling pathways initiated by biomolecules secreted by transplanted SCs are crucial to the reparative processes. The release of angiogenic factors such as VEGF, FGF, and HGF by SCs promotes the formation of new blood vessels in the infarcted area. This enhanced angiogenesis improves blood supply and oxygenation to the damaged tissue, fostering tissue repair and regeneration. Paracrine factors such as interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1Ra), and tumor necrosis factor-alpha-stimulated gene/protein 6 (TSG-6) exhibit anti-inflammatory and anti-fibrotic properties, limiting scar tissue formation and improving cardiac function. SDF-1, IGF-1, and PDGF promote cell survival, differentiation, and proliferation, contributing to tissue regeneration. SC-derived paracrine factors can modulate the immune response by inducing a shift from a pro-inflammatory to an anti-inflammatory environment, facilitating tissue repair and regeneration [79] (Table 1). By exploiting the paracrine effects of SCs, researchers aim to develop cell-free therapeutic strategies that can harness the benefits of SC therapy without the potential risks associated with cell transplantation, such as immune rejection, tumorigenesis, and long-term cell engraftment issues [76, 80].

Although the improvement of SC transplantation has shown considerable results in cardiac repair and regeneration, many studies have focused on MSCs secretome and their released EVs as a novel approach to managing various CVDs and cardiovascular-related diseases. New observations further suggest that cell-based therapy depends partly on the paracrine activity of the injected cells rather than their integration/transdifferentiation into the myocardium. Paracrine communication describes the action of secreted messengers, including regulatory factors, growth factors, cytokines,

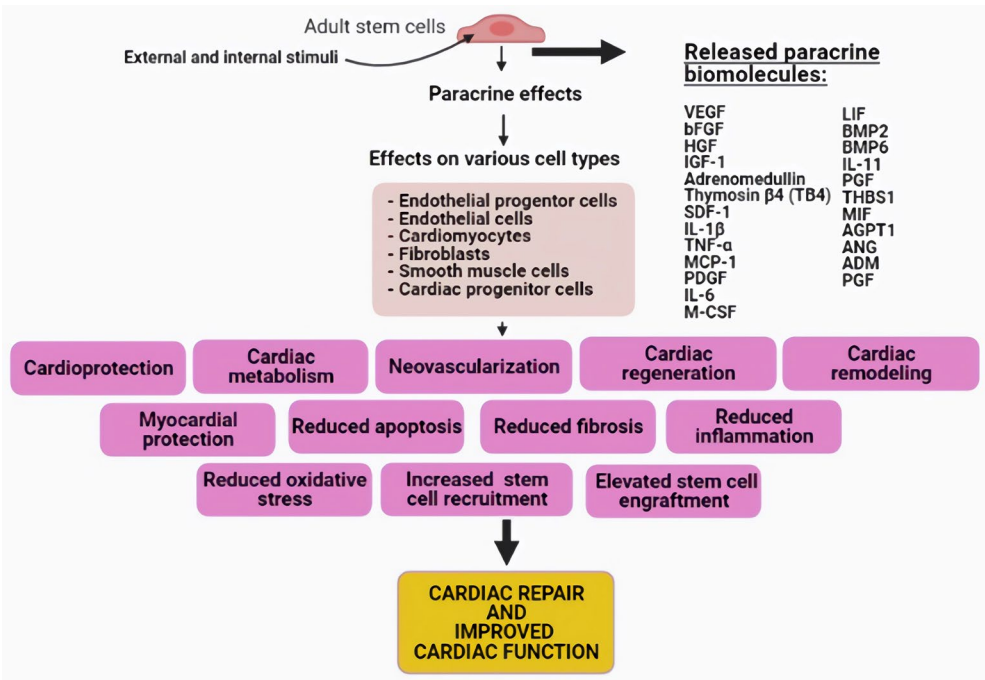

Fig. 3 Paracrine signals mechanisms in SC-mediated cardiac repair. The figure depicts the paracrine signaling mechanism involved in SC-mediated cardiac repair, the secretion of paracrine factors by SCs in response to external and internal signals, and local environmental cues such as ischemic conditions. These biologically active substances influence various cell types, such as cardiomyocytes, endothelial cells, and fibroblasts, promoting angiogenesis, reducing inflammation, inhibiting apoptosis, and stimulating tissue regeneration. Consequently, this results in improved cardiac function and overall cardiac repair

Table 1 Several important adult SC-related paracrine factors are involved in cardiac regeneration

Paracrine factors	Function
Angio-associated migratory protein (AAMP)	Angiogenesis and migration
Angiogenin (Ang)	The formation of new blood vessels through the process of angiogenesis
Vascular endothelial growth factor (VEGF)	Angiogenesis, migration, matrix metalloproteinase activity, αvβ3 activity, Vasodilation (indirectly by NO release), vasculogenesis, and cytoprotection
SC-derived factor-1 (SDF-1 = CXCL12)	Homing, migration, and angiogenesis
Platelet-derived growth factor (PDGF)	Proliferation, cell migration, angiogenesis, and differentiation
Matrix metalloproteinase (MMP)	Proliferation, adhesion, angiogenesis, differentiation apoptosis, and tubule formation
Thrombospondin-1 (THBS1)	Migration, platelet aggregation, cell-to-cell and cell-to-matrix interactions, and angiogenesis
Tumor necrosis factor-α (TNF-α)	Proliferation, matrix degradation
Insulin-like growth factor-1 (IGF-1)	Migration, contractility, and cytoprotection
Endothelin-1 (EDN1)	Proliferation, cytoprotection
Fibroblast growth factor (FGF)	Angiogenesis, proliferation, migration, and cell stabilization
Hepatocyte growth factor (HGF)	Myogenesis, mitogenesis, proliferation, and angiogenesis
Kit ligand/SC factor (KITLG (SCF))	Hematopoiesis, proliferation, and migration
Transforming growth factor-β (TGF-β)	Vessel maturation, cell proliferation and apoptosis

chemokines, and nucleic acids that can be released freely or packaged into EVs. Exosomes, measuring 50 to 150 nm, originate from endosomes and are secreted by all cell types. These vesicles play a critical role in cell-to-cell communication, acting as potent regulators by transporting a diverse range of biomolecules, including regulatory miRNA, peptides, cytokines, signaling lipids, and other bioactive compounds, to recipient cells as cargo.

MSC-derived exosomes have been implicated in regulating various physiological and pathological processes across different models. These exosomes, released by MSCs, have been demonstrated to play critical roles in promoting cardiac regeneration, enhancing angiogenesis, inhibiting cardiac fibroblast proliferation, and modulating immune cell activity within the infarcted region [81–84]. The multifaceted effects of MSC-derived exosomes on cardiac tissue repair and immune regulation have positioned them as a promising therapeutic tool for treating CVDs. Several preclinical studies have substantiated the efficacy of these exosomes in significantly reducing infarct size and improving cardiac function following injury [85–89].

Interestingly, several studies showed that cell-free exosome-based therapies promote angiogenesis, thereby up-regulating several pro-angiogenic and angiogenesis-associated factors [90–92]. Recent findings have revealed that exosomes derived from ADMSCs can influence the expression of angiogenesis-related genes in endothelial cells [93, 94]. Specifically, ADMSC-derived exosomes were shown to up-regulate the pro-angiogenic genes Angiopoietin-1 and its receptor Tie-2 while also suppressing the anti-angiogenic factors Vasohibin-1 and Thrombospondin-1 (TSP-1) [95, 96]. These expression changes were mediated by the activation of the Ark12 and Akt signaling pathways in endothelial cells upon exposure to ADMSC-derived exosomes [97].

The application of exosomes derived from SCs, particularly MSCs, presents several benefits compared to the direct transplantation of SCs. These advantages include reduced immunogenicity, minimizing the risk of immune rejection, and negligible risk of tumor formation, addressing concerns associated with SC therapies [98–100]. Moreover, repeated administration of exosomes derived from BMMSCs has demonstrated excellent tissue penetration capacity without causing significant adverse reactions, such as fever or allergic hemolytic reactions [98]. Despite the numerous benefits of MSC-derived exosomes as a therapeutic tool, their clinical application still faces several challenges. These challenges include insufficient targeting ability, limited efficacy, and stability of exosomes upon transplantation [101–103]. In response to these hurdles, novel strategies are continuously being developed to enhance the biological function of MSC-derived exosomes. Some of these approaches include preconditioning MSCs before exosome isolation, engineering exosomes to improve their stability and targeting ability, and utilizing exosomes as targeted drug delivery systems [104–108] (Fig. 4).

Neovascularization

CVDs, such as MI, are primarily initiated by endothelial dysfunction. Therapeutic angiogenesis, which promotes cardiac tissue restitution through endothelial cell growth and the production of growth factors, has emerged as a potential treatment strategy. SCs can secrete paracrine factors, including pro-angiogenic

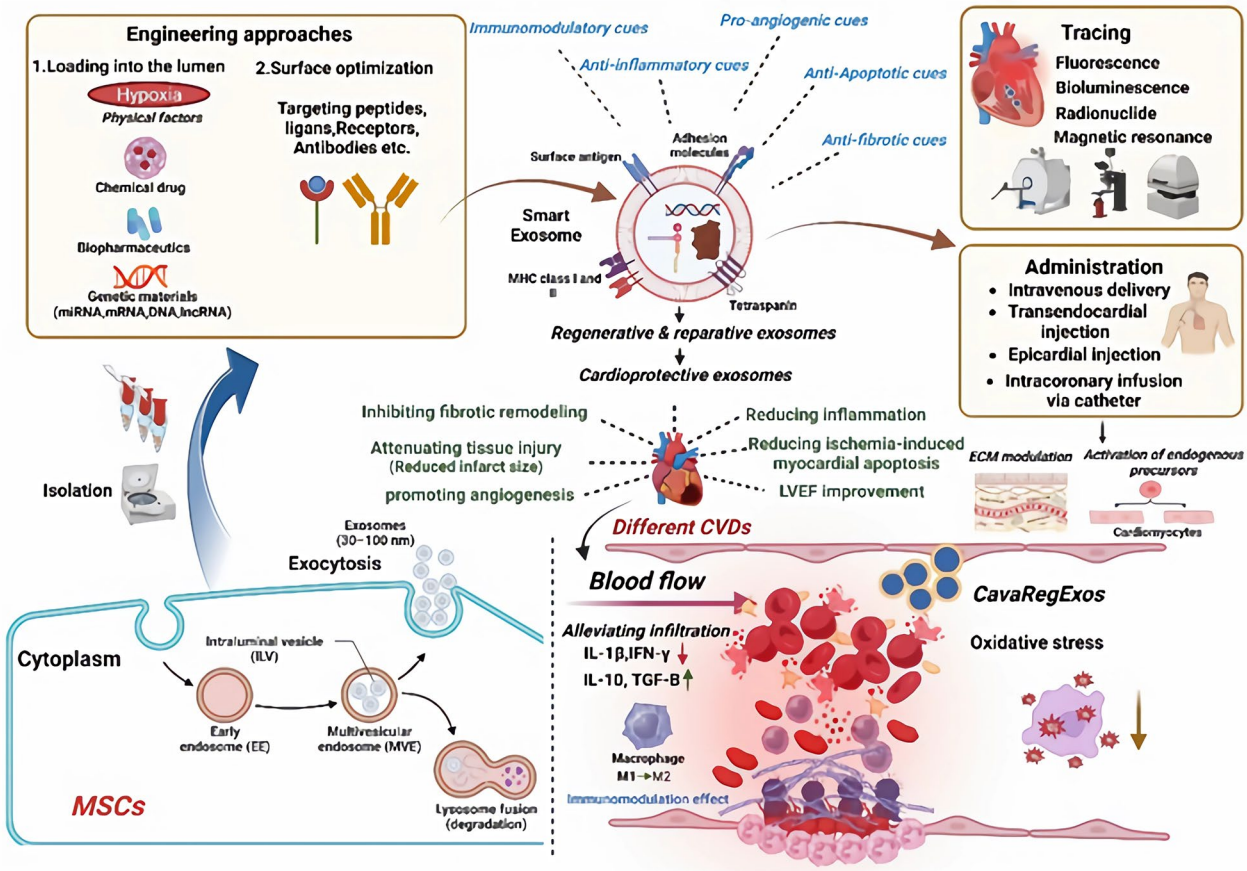

Fig. 4 Schematic presentation of engineered exosomes as a desirable therapeutic tool for CVDs. Exosomes, derived from various cell types, are engineered using strategies such as genetic modification, loading with therapeutic cargo, and surface functionalization to enhance their targeting and therapeutic potential. These engineered exosomes can be administered through various routes (e.g., systemic, local, or targeted delivery) to reach the heart directly via cell–cell contact or circulation. Upon reaching the targeted site, engineered exosomes benefit inflammation, angiogenesis, apoptosis, and fibrosis, thereby addressing the critical pathological processes involved in CVDs. Exosomes can be labeled with tracking agents to ensure specific delivery and monitor treatment effectiveness. They can also be functionalized with targeting ligands, allowing for precise visualization and evaluation of their therapeutic impact. In summary, the schematic presentation highlights the versatility of engineered exosomes as a promising therapeutic tool for CVDs, offering targeted delivery of therapeutic agents and the potential to modulate multiple pathological processes associated with CVDs

mediators, which can induce the formation of new blood vessels (Fig. 5). These SC-secreted paracrine molecules, such as VEGF, bFGF, and IGF-1, have been shown to increase capillary density in the infarct border zone in cardiac tissue, albeit with varying efficiency [109, 110]. In vitro studies have demonstrated the angiogenic potential of SC-derived paracrine factors. For example, conditioned medium from cultured SCs has been shown to promote endothelial cell proliferation and tube formation, indicating a pro-angiogenic effect. In vivo studies have also demonstrated the efficacy of SC-based paracrine therapies in promoting angiogenesis and improving cardiac function in MI animal models [111, 112].

Due to the limited differentiation potential of MSCs into endothelial cells, the neovascularization process, particularly angiogenesis, is mainly stimulated by the secretion of various growth factors, such as VEGF. VEGF is well-known for promoting the proliferation and migration of endothelial cells, ultimately triggering neovascularization. Enhancing the potency of MSCs to induce efficient angiogenesis holds promise for potential clinical applications of MSC-based therapies. Highlighting therapeutic angiogenesis as one of the future approaches in cell therapy can lead to developing novel strategies for treating CVDs, such as MI. Overexpressing pro-angiogenic factors such as VEGF or other growth factors in MSCs can enhance their

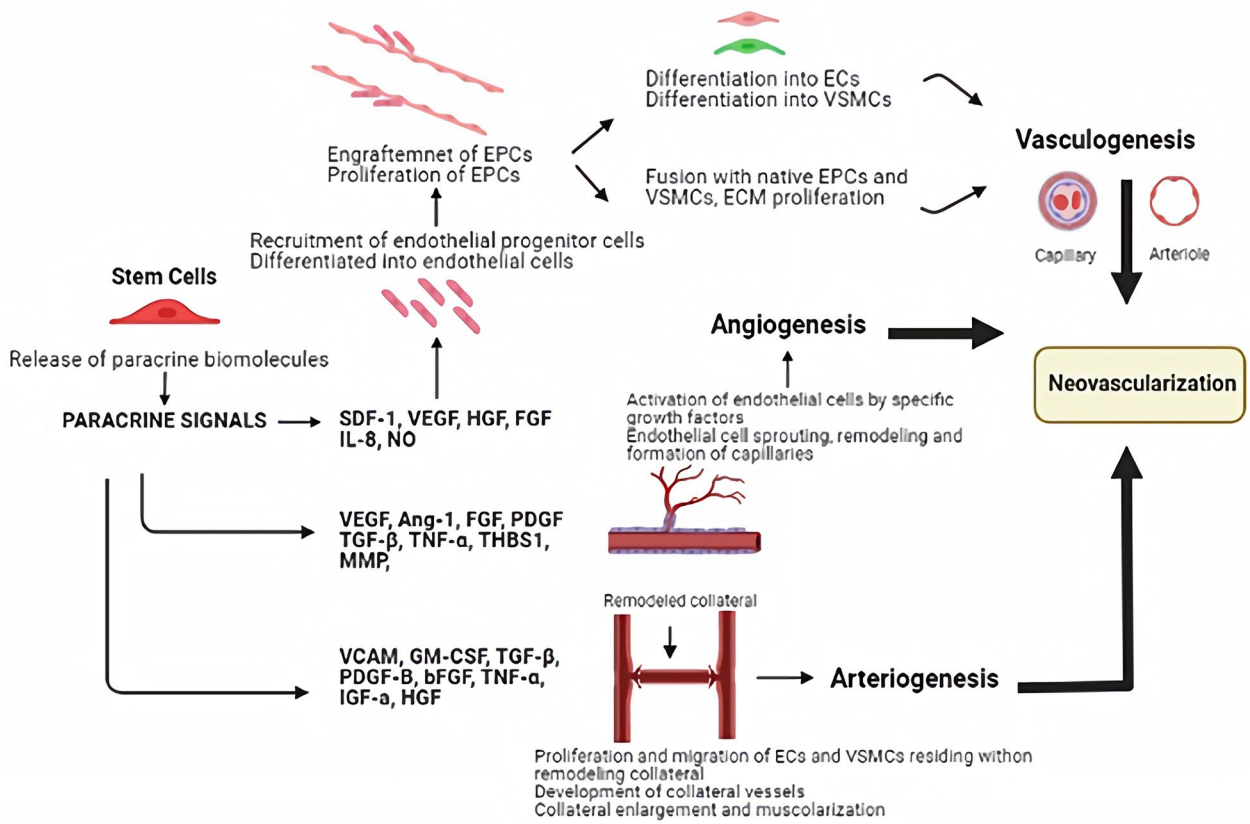

Fig. 5 Neo-vascularization mechanisms are crucial for improving blood flow and oxygen supply in ischemic tissues, such as those affected by diabetes or MI. In these conditions, increased oxidative stress and vascular damage stimulate the expansion of the vascular network. Adult SCs play a vital role in this process by secreting paracrine molecules that promote neo-vascularization through angiogenesis and arteriogenesis. Angiogenesis refers to forming new blood vessels from pre-existing ones, involving endothelial cell sprouting and splitting. Angiogenic factors, such as VEGF, mainly mediate this process. Arteriogenesis, conversely, involves the growth and development of pre-existing collateral arteries into functional collateral vessels. This process helps compensate for blood flow loss in occluded arteries and improves tissue perfusion. Bone-marrow-derived SCs, including EPCs, play a critical role in these processes. EPCs differentiate into endothelial cells and contribute to forming primitive vascular networks. The secretion of pro-angiogenic and pro-arteriogenic factors by SCs further supports the development and maturation of these new blood vessels

ability to promote neovascularization. Priming MSCs with growth factors or small molecules can increase their angiogenic potential and improve their therapeutic efficacy in vivo. Co-administering MSCs with other pro-angiogenic agents or cells can lead to synergistic effects, promoting more robust neovascularization and improving cardiac function. Isolating and utilizing extracellular vesicles derived from MSCs, such as exosomes, can provide a cell-free approach to harnessing the therapeutic benefits of MSC-secreted paracrine factors. By optimizing the angiogenic potential of MSCs, researchers aim to develop more effective cell-based therapies for treating CVDs and promoting tissue regeneration. Further research is needed to establish these approaches' safety, efficacy, and long-term effects in preclinical models and clinical trials [109, 113–115].

Novel SC-based therapeutic strategies for CVDs

Numerous preclinical investigations have shown that administering SC therapy in animal models of MI can result in an approximate 11% improvement in LVEF. This observed enhancement in LVEF indicates that SCs possess the potential to promote cardiac function following MI, highlighting their therapeutic promise for treating CVDs [116]. However, the translation of these results to clinical settings has been less pronounced. Some clinical trials have shown a small but significant improvement in cardiac output of 2% to 4% following SC therapy [117]. The variability between different clinical trials has led to discrepancies in the reported improvement in LVEF. Studies with lower variability tend to report more minor improvements in LVEF, suggesting that factors such as patient heterogeneity, cell dosage, delivery methods, and timing of administration may influence the therapeutic

outcomes [118]. In light of the inconsistent clinical outcomes associated with SC therapy for CaVaReM, developing novel strategies to optimize SC-based cardiac regeneration is crucial. The unique physiological and pathological profile of each patient and the specific context of their heart disease can considerably influence the efficacy of cardiac SC therapies.

Several strategies have been proposed to overcome these obstacles, which can be broadly classified into genetic engineering and non-genetic modification-based approaches.

Pharmacological preconditioning of the SCs (preconditioning strategy in SC transplantation therapy)

Ischemic preconditioning, initially described by Murray et al., is a process in which the heart is subjected to brief episodes of ischemia followed by reperfusion. Their research discovered that four cycles of 5-min left circumflex coronary artery occlusion significantly reduced MI size by approximately 75% when a subsequent prolonged ischemic event took place [119]. Preconditioning activates various signaling pathways that generate a protective memory, reducing ischemic damage. These signaling cascades involve the release of several mediators, including opioids, reactive oxygen species (ROS), adenosine, and nitric oxide. Activation of these signaling molecules leads to the involvement of other factors, such as protein kinases (e.g., Akt and PKC), inhibitory G-proteins, and mitochondrial ATP-sensitive potassium (KATP) channels. The activation of these signaling pathways ultimately converges on the mitochondria, which play an essential role in mediating the cardioprotective effects of ischemic preconditioning. By modulating mitochondrial function and promoting cellular adaptation to stress, ischemic preconditioning helps preserve cardiac function and reduces the extent of tissue damage during subsequent ischemic events. This phenomenon has significant implications for developing innovative therapeutic approaches in managing ischemic heart diseases [118].

On the other hand, pharmacological preconditioning involves administering drugs that protect the heart against acute MI just before its onset. This approach may be more suitable and cost-effective for clinical applications and efficient SC-based therapies [120]. As previously mentioned, SCs have the potential to exert their effects by stimulating paracrine pathways. It has been confirmed that drug-mediated preconditioning and modulation of specific signaling pathways can alter SC biology, thereby enhancing their regenerative potential for heart repair. For instance, pretreatment with specific agents, such as oxytocin, can promote the secretion of various cytokines and growth factors such as FGF-6 and EGF in MSCs. It also can enhance the cardioprotective effects of MSCs and improve their therapeutic potential for treating ischemic heart diseases [121]. Co-culture of primed-MSCs has been revealed to increase the capacity of SCs and improve cardiovascular function by 50% in preclinical MI animal models. The potential efficacy of pharmacological preconditioning has also been evaluated, with promising results. For instance, injecting trimetazidine-primed-MSCs into MI-affected hearts led to significant improvements in cardiac function and a 10% reduction in fibrosis compared to control groups. It demonstrates the potential benefits of preconditioning MSCs with cardioprotective agents before cell transplantation [122]. Another example involves priming MSCs with melatonin, which increases cell viability and improves graft retention after intramyocardial injection. This melatonin-primed MSC therapy enhanced left ventricular function and reduced left ventricular wall thickness 8 week post-injection [123]. Preconditioning strategies have been reported to boost the therapeutic potential of SCs in treating MI. For instance, preconditioning AD-MSCs with 17β-estradiol or the phosphodiesterase-5 inhibitor sildenafil (Viagra) or through knockdown with a silencing vector has been shown to increase the reparative effects of these cells in a MI mouse model. This preconditioning approach led to significantly improved cardiac function, a 4% reduction in fibrosis area, increased angiogenesis, and elevated VEGF and bFGF secretion. These findings indicate that modulating specific signaling pathways in SCs can enhance their regenerative capacity and paracrine factor secretion [124, 125].

In addition, selective activation of specific intracellular pathways, such as Rho-dependent signaling, can promote the adhesion capacity of MSCs. This further underscore the importance of optimizing SC function through pharmacological agents or genetic manipulation to achieve better therapeutic outcomes. In another example, pharmacological agents have been used to reprogram iPSCs into pacemaker-like cells. This was achieved by applying small molecules, such as the FGF signaling inhibitor PD 173074 and the ALK5 inhibitor SB431542, which target specific signaling pathways crucial for cardiomyocyte differentiation [126].

In addition to the compounds, as mentioned earlier, several other small molecules have been utilized to modulate SC function and enhance their cardiac differentiation potential. CHIR99021, a GSK-3 inhibitor, is known to activate the Wnt signaling pathway and has been shown to promote cardiac differentiation in SCs. Y-27632, a Rho-kinase inhibitor, has been found to improve SC survival and cardiac differentiation by modulating cytoskeletal organization and cell adhesion. IWR-1, IWR-2, and IWR-4-3 are Wnt signaling inhibitors that

can manipulate SC fate and enhance their differentiation toward a cardiac lineage.

By targeting specific signaling pathways involved in SC maintenance, proliferation, and differentiation, these pharmacological agents can optimize SC-based therapies for CVDs. Ongoing research continues to explore the most effective combinations and concentrations of these compounds to achieve the desired therapeutic outcomes and minimize potential side effects [127–130].

Cytokine and growth factor-based preconditioning

Prior research has established that priming MSCs with inflammatory cytokines such as IFN-γ and TNF-α can augment their therapeutic potential. This approach aims to precondition the MSCs, making them more responsive and effective when administered for therapeutic purposes. Preconditioning MSCs with IFN-γ has been shown to induce the secretion and release of various immunomodulatory molecules, including CCL2, CCL7, CXCL9, CXCL10, CXCL11, and CXCL16 [131]. Furthermore, exposing MSCs to a supernatant rich in IFN-γ and TNF-α can increase the expression of various secretory factors in MSCs, including the chemokine CCL5. These observations indicate that acute inflammatory stimulation can potentially augment the immunomodulatory and regenerative properties of MSCs [132]. The use of cytokines and growth factors to modulate SC potency before transplantation into injured hearts has shown promise in enhancing the therapeutic outcomes of SC-based therapies (Table 2). Various growth factors can be utilized to prime SCs, encouraging their differentiation into cardiogenic lineages. FGFs, particularly FGF2, are potent stimuli that drive SC differentiation into cardiomyocytes, ultimately boosting their therapeutic efficacy. Another factor, BMP4, promotes cardiogenic differentiation in SCs, enhancing their capacity to repair damaged heart tissue. By utilizing these growth factors to prime SCs before transplantation, researchers aim to increase their therapeutic potential for regenerative medicine, particularly in cardiovascular disorders [133]. Exposing MSCs to cytokines and growth factors such as TGF-β, BMP-4, and FGFs can induce cardiogenic conversion and improve their therapeutic efficacy. Preconditioning SCs with these factors prior to transplantation has been demonstrated to improve LVEF in patients with ischemic HF [134].

Several studies have reported that priming of EPCs before transplantation can enhance their pro-angiogenic potential. It promotes angiogenesis and network formation, ultimately improving cardiac function in the context of ischemic heart diseases. On the other hand, transplantation of SDF-1-primed-MSCs in a rat model of MI significantly improved cardiac function. Left ventricular EF was improved by 5–7.5%, and there was a 50% reduction in infarct size, fibrotic tissue, and remodeling, leading to better regeneration [135]. Interestingly, the impact of preconditioning on SC efficacy can vary depending on the specific factors involved. For instance, priming bone marrow and Wharton's jelly derived MSCs with CXCR4 agonists had the opposite effect on myocardial regeneration. In some trials, preconditioning with CXCR4 agonists did not improve cardiac function [136].

Inflammatory priming of MSCs has been shown to improve their therapeutic potential in MI. For instance, priming bone-marrow-derived MSCs with TNF-α has been found to enhance their immunomodulatory capacities and increase the production of paracrine factors, such as TGF-β1, interleukin 6 (IL-6), and cyclooxygenase 2 (COX2). These changes contribute to improved post-ischemic myocardial functional recovery compared to control groups [137]. It has been proposed that the therapeutic activity of TGF-α primed MSCs is related to the upregulation of G-CSF, which stimulates MSCs and improves their cardioprotective effects. It highlights the importance of paracrine factor secretion by MSCs in mediating their therapeutic effects in CVDs [138]. Hormonal priming of MSCs has also been shown to enhance their survival and induce paracrine properties post-transplantation in vivo MI models. For example, treating MSCs with erythropoietin (EPO) or other hormones can improve their survival in the ischemic environment, increasing the secretion of factors that promote angiogenesis, reduce inflammation, and reduce scar formation [139].

Lineage-specific differentiation can be induced in SCs by exposing them to specific cytokines, growth factors, or other signaling molecules. Previous studies have demonstrated the potential of such approaches in directing cell fate determination [140, 141]. For instance, exposing MSCs to a cardiogenic cocktail containing factors such as FGF2, BMP4, cardiotrophin-1, and alpha-thrombin has been revealed to hasten their differentiation into cardiomyocytes. These factors play crucial roles in inducing mesenchymal-to-cardiogenic lineage conversion, thereby increasing the potency of cardiomyogenesis [142]. The TGF-β superfamily, which includes TGF-β, BMP4, and activin, has been implicated in the induction of NKX2.5 expressions in embryonic SCs. NKX2.5 is a critical transcription factor involved in cardiomyocyte differentiation. By promoting the expression of NKX2.5 and other cardiogenic markers, such as GATA4, MEF2C, and ISL1, at both the transcript and protein levels, these growth factors can help guide SCs toward a cardiomyocyte fate [141].

Preconditioning SCs with specific factors before transplantation has shown promise in enhancing their

Table 2 Priming of MSCs by growth factors, cytokines, and other stimuli molecules

Type of priming	Type of cells	Routs of infusion	Outcome in vitro/in vivo	References
FGF-2, IGF-1, BMP-2	Rat BM-MSCs	Local transplantation	• Enhanced gap junction formation • Smaller infarcted size in MI models • Better cardiac function	[149]
VEGF	Mouse-BM-MSCs	IV infusion	• Reduced levels of cellular stress • Up-regulation of prosurvival factors via phosphorylation of Akt and Bcl-xL • Improved cardiac function • Enhanced cell survival and engraftment • Reduced cellular stress	[150]
EGF	MSCs	IM-injection	• Cell growth • Vascular tissue repair • Increased cell adhesion, migration, and survival	[151]
DNP	Bone marrow	In vitro/in vivo (rats)	• DNP preconditioning increased the expression of certain proteins (Bcl-2, Bcl-xL, and HIF-1α) in MSCs, which are known to promote cell survival and protect against cell death • Rats receiving DNP-preconditioned MSCs showed improved heart function, reduced scar tissue formation, and increased blood vessel growth compared to those receiving untreated MSCs • DNP-preconditioned MSCs were found to secrete higher levels of growth factors and cytokines that promote tissue repair and angiogenesis	[152]
DMOG	Bone marrow	In vitro/in vivo (rats)	• DMOG preconditioning led to increased HIF-1α levels in BM-MSCs, promoting the expression of genes associated with angiogenesis and cell survival • In vitro experiments showed that DMOG-preconditioned BM-MSCs had improved survival and migration abilities, as well as enhanced pro-angiogenic properties • Transplantation of DMOG-preconditioned BM-MSCs into the ischemic hearts of rats resulted in improved cardiac function, reduced scar tissue formation, and increased blood vessel growth compared to untreated BM-MSCs	[153]
Curcumin	Adipose tissue (rat)	In vitro/in vivo (rats)	• Pretreatment with curcumin enhanced the survival of ADSCs under oxidative stress conditions, which mimic the environment found in ischemic hearts • Curcumin-pretreated ADSCs demonstrated increased secretion of vascular endothelial growth factor (VEGF), a protein that promotes angiogenesis and tissue repair • Transplantation of curcumin-pretreated ADSCs into ischemic rat hearts resulted in better heart function, reduced scar tissue formation, and increased blood vessel growth compared to untreated ADSCs • Curcumin pretreatment also led to decreased apoptosis and increased cell retention within the heart tissue, which can improve the overall therapeutic potential of SC therapy	[154]
Ang1	Bone marrow (rat)	In vitro/in vivo (rats)	• Increased cell survival due to Akt phosphorylation and increase expression of Bcl-2	[155]
Oxytocin	Bone marrow (diabetic rat)	In vitro/in vivo (rats)	• Oxytocin treatment was found to restore the angiogenic capacity of MSCs collected from diabetic individuals by promoting the secretion of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietin-1 • Oxytocin-treated diabetic MSCs demonstrated improved survival and enhanced migration abilities under conditions that mimic the diabetic microenvironment • In a diabetic rat model of hind limb ischemia, transplantation of oxytocin-treated diabetic MSCs led to increased blood flow recovery and better overall therapeutic outcomes compared to untreated diabetic MSCs	[156, 157]

therapeutic potential for treating CVDs. Notably, injecting these primed cells has been demonstrated to be both practical and safe for patients. Preconditioning SCs with IGF-1 and VEGF has been demonstrated to exert cardioprotective effects by promoting cell survival by activating the PI3K/Akt signaling pathway. It leads to increased expression of the anti-apoptotic protein Bcl-2, which contributes to improved cell survival in cell-based treatments [143]. In addition, VEGF preconditioning has been found to protect SCs by promoting proliferation, decreasing apoptosis, and increasing cell survival while minimizing senescence under hyperglycemic conditions. These beneficial effects are particularly relevant for patients with diabetes, who often suffer from cardiovascular complications and impaired wound healing [144].

An alternative approach in SC-based therapy involves using an SC-conditioned medium (SC-CM), which contains various soluble factors secreted by SCs. SC-CM has been demonstrated to effectively promote the proliferation and regeneration of other cells under different environmental conditions [145]. For example, preconditioning diabetic AD-MSCs with myogenic medium obtained from stress-induced injury cardiomyopathy in a hyperglycemic context enhanced proliferation, angiogenesis, and overall cardiac outcomes in a diabetic rat model. It suggests that SC-CM can improve the therapeutic potential of SCs, particularly under pathological conditions [146]. It is proposed that inducing oxidative stress in neonatal rat cardiomyocytes under hypoxic conditions can lead to the release of VEGF in the conditioned medium, promoting angiogenesis. In vitro studies have demonstrated that ischemic preconditioning can stimulate the production of angiogenic factors, such as VEGF, FGF, and others, leading to cardioprotection, improved blood vessel growth, and increased cell survival [147].

SC-conditioned medium containing the soluble factors shows great potential in boosting the therapeutic effectiveness of SC-based treatments. Further investigations are required to gain a deeper understanding of the mechanisms responsible for the beneficial effects of SC-conditioned medium and to refine its application in clinical settings for treating CVDs and other conditions [148].

Environmental preconditioning of SCs

Accumulating evidence suggests that preconditioning SCs by exposing them to various environmental cues can enhance their capacity to modulate the harsh ischemic and inflamed microenvironment in injured tissues [158]. These environmental cues may include factors, such as immune rejection, tissue damage, oxidative stress, tissue fibrosis, inflammation, extracellular matrix breakdown, and other components of the post-acute ischemic environment [159, 160]. However, chronic exposure to an unstable microenvironment may lead to apoptosis, reduced survival, and decreased engraftment and retention of the implanted SCs, which could diminish their therapeutic potential [161]. Several studies have demonstrated that hypoxic preconditioning can improve the viability, differentiation, and proliferation of SCs under severe hypoxic conditions (2.5% oxygen). Hypoxia-induced factors (HIFs) are crucial in mediating these adaptive responses, leading to the upregulation of various growth factors that promote cell survival and angiogenesis [160–163].

Hypoxic preconditioning of CPCs loaded onto implantable sheets significantly enhanced cell survival, blood flow recovery, and capillary density (25%) in a mouse model of chronically infarcted hearts. This preconditioning approach also improved cardiac function through reduced collagen deposition and better preservation of the infarct border zone [164]. Overall, preconditioning various SC types under low oxygen levels has been shown to improve their migration and protect them from ischemic damage. It enhances SC survival and angiogenic capabilities, improving engraftment, integration, and functional recovery after transplantation in injured tissues, particularly in CVDs [164–166]. Several studies have confirmed that hypoxic preconditioning of MSCs leads to elevated release of pro-angiogenic factors, such as VEGF and HGF, contributing to vascular protection. These findings emphasize the potential of hypoxia-preconditioned MSCs to effectively stimulate tissue regeneration and angiogenesis in the surrounding tissue through the upregulation of various growth factors and cytokines. The upregulated factors include VEGF, angiopoietins, HIF-1, erythropoietin, PDGF, FGF, and their respective receptors, such as CXCR4. These molecules promote angiogenesis, cell survival, and tissue repair under ischemic conditions [163, 165–167]. In addition, studies have demonstrated that environmental cues, such as nutrient levels, can regulate the expression of angiogenic factors like PDGF, which are critical for angiogenesis in ischemic tissues. By fine-tuning these factors through preconditioning strategies, researchers aim to optimize SC-based therapies and enhance their therapeutic potential in treating ischemic injuries and other related conditions [168].

In-situ preconditioning

In situ preconditioning is an approach in regenerative medicine that aims to improve the therapeutic potential of SCs or other therapeutic agents by exposing them to specific environmental conditions or stimuli before transplantation. This approach is designed to improve cell survival, engraftment, and overall effectiveness of the therapy. In the context of CaVaReM, in situ

preconditioning may involve exposing SCs or other therapeutic agents to various factors such as growth factors, cytokines, hypoxic conditions, or mechanical stimuli before injecting them into the damaged heart tissue. The goal is to precondition the cells better to withstand the harsh environment of the infarcted myocardium, enhance their paracrine effects, and promote their integration with the host tissue. Some potential benefits of in situ preconditioning include improved cell survival and retention in the target tissue, enhanced differentiation into desired cell types (e.g., cardiomyocytes), increased secretion of paracrine factors that promote angiogenesis, anti-apoptosis, and anti-fibrosis, better integration with the host myocardium.

In the study conducted by Yao Liang Tang et al., the researchers examined the impact of in situ hypoxic preconditioning on the therapeutic potential of CPCs in a rat model of MI. The results showed that hypoxic preconditioning improved the survival and retention of transplanted CPCs within the infarcted heart tissue. This improvement was linked to increased expression of anti-apoptotic proteins, such as Bcl-2, and reduced expression of pro-apoptotic proteins, such as Bax. Hypoxic preconditioning promoted the secretion of pro-angiogenic factors, such as VEGF and SDF-1α by CPCs, which contributed to increased blood vessel formation in the infarcted area. Hypoxic preconditioning of CPCs reduced cardiomyocyte apoptosis in the peri-infarct region, possibly through paracrine mechanisms involving the secretion of growth factors. The treatment with hypoxic-preconditioned CPCs resulted in better preservation of cardiac function, as indicated by increased LVEF and fractional shortening, as well as reduced infarct size and myocardial fibrosis. These findings demonstrate that in situ hypoxic preconditioning can significantly enhance the survival, angiogenic potential, and therapeutic efficacy of CPCs for treating MI in a preclinical setting. Further studies are warranted to explore the potential of this strategy in clinical applications [169, 170].

In another research, investigators focused on creating a nanocomposite hydrogel system designed for in situ preconditioning of SCs to improve their therapeutic effectiveness in promoting angiogenesis. To achieve this, the researchers developed a nanocomposite hydrogel system comprising gold nanoparticles, upconversion nanoparticles (UCNPs), and poly(L-lactide-*co*-glycolide) (PLGA) nanoparticles integrated into a gelatin-based hydrogel carrier, termed GelMA. This innovative system facilitates the controlled release of SDF-1α and basic FGF, which play critical roles in angiogenesis. The in situ priming of SCs using the nanocomposite hydrogel system enhanced the expression of angiogenic factors, such as VEGF, angiopoietin-1 (Ang-1), and PDGF-B. When applied in a mouse model of hindlimb ischemia, the nanocomposite hydrogel system promoted blood vessel formation, increased blood perfusion, and improved limb salvage, indicating its potential therapeutic efficacy for treating ischemic diseases. The PLGA nanoparticles within the hydrogel allowed for the controlled release of SDF-1α and bFGF, ensuring sustained and localized delivery of these angiogenic factors at the target site. In conclusion, this study demonstrated the potential of a nanocomposite hydrogel system for in situ preconditioning of SCs to enhance their angiogenic potential, which could be a promising strategy for treating ischemic diseases and promoting tissue regeneration [171].

In a study, researchers employed a combination of BM-MSCs, genetically engineered hepatocyte growth factor-expressing MSCs (HGF-MSCs), and decellularized-ECM hydrogel to induce in situ preconditioning. Their findings revealed that this combinatorial approach substantially improved the survival and retention of BM-MSCs within the infarcted hearts of rats with MI. The study demonstrates that in situ preconditioning with a mixture of cells and supportive biomaterials can significantly enhance the therapeutic potential of SC-based interventions. Optimizing the microenvironment within the infarcted myocardium enables better cell survival and retention, ultimately promoting more effective cardiac repair and regeneration [172].

Although in situ preconditioning has demonstrated potential in preclinical studies, more research is required to fine-tune the preconditioning protocols and evaluate their efficacy in clinical settings. Furthermore, a deeper understanding of the mechanisms responsible for the positive effects of in situ preconditioning is crucial to harness its therapeutic potential fully.

Genetic-based SC modifications

SC transplantation has demonstrated a moderate or subtle improvement of cardiac function following myocardial injury. Although SCs display long-term regeneration, they have several limitations, including low viability and poor engraftment in the cardiac environment due to hostile ischemic microenvironment and immune system reaction. The rapid disappearance of transplanted cells post-transplantation remains a challenge in cell-based therapies. Genetically modified MSC therapy has emerged as a promising field of research aimed at addressing this issue [173–176]. By Engineered SCs can be genetically engineered ex vivo before transplantation to enhance transplanted cell viability and engraftment [120, 176, 177]. Numerous viral and non-viral vector-based gene delivery methods have been applied for MSC transfection [178]. The main approaches to manipulating SCs have been the overexpression of specific proteins

by DNA delivery, gene editing (CRISPR/Cas9, TALENs), gene silencing techniques, and miRNA-based technologies [136, 179].

Paracrine signaling, involving the release of various messengers, is crucial for the regenerative capacity of SCs. The expression of trophic factors normally secreted by SCs to support cardiac regeneration following heart damage can be artificially induced. In the early 2000s, a pioneering study by Mangi et al. demonstrated that MSCs genetically modified to overexpress Akt exhibited enhanced cell survival and prevented myocardial remodeling in infarcted hearts. It led to improved cardiac performance compared to control MSCs expressing a reporter gene, highlighting the potential of genetic modification in boosting the therapeutic efficacy of MSC-based treatments for CVDs [180].

Studies have suggested several strategies to counter the rigorous, harsh microenvironment (inflammation storm, oxidative stress) following cardiac damage [181–183]. It has been revealed that equipping MSCs with lipocalin 2 (Lcn2) plays a protective role under oxidative stresses and maintains their viability [184]. Moreover, other studies confirmed that manipulating the integrin signaling pathway is a promising target to improve SCs tethering and attachment to the ECM of the damaged cardiac tissue and improve cardiac performance in mouse and pig models of MI [185–187].

In another studies, MSCs, which were genetically modified to produce/overexpress SDF-1 and CXCR4 proteins, enhanced the mobilization and homing of progenitor cells toward the myocardium [188–191]. Kanwal Haneef et al. reported that transfected MSCs with IL-7, one of the main lymphopoietic growth factors, increased the fusogenic properties of MSCs. They enabled MSCs to release paracrine and autocrine signals contributing to cardiac recovery in a rat model of MI [192]. Taken together, selective targeting of transfected MSCs has been a promising approach to over-express therapeutic factors and pro-survival genes.

In previous studies, genetic-based SC modifications have been extensively investigated. It was found that MSCs show higher paracrine activity by producing higher levels of different growth factors such as IGF, bFGF, EGF, and VEGF compared to native MSCs. Genetically modified SCs may improve the efficiency of next-generation SC-based therapies and stimulate myocardial regeneration in adult mammalian hearts [193, 194].

Viral transduction is a widely used method for genetic delivery because of its high transduction efficiency in target cells. However, using viruses in clinical trials poses unique challenges, such as mutagenesis, oncogenesis, and immune reactions against transduced cells. These issues can be addressed by employing non-integrating viral vectors. Moreover, the random insertion of DNA into the chromosomes of target cells can result in dysregulation of proto-oncogenes or disruption of tumor suppressor genes, potentially leading to malignant transformation.

One of the most powerful technologies for targeted insertion of therapeutic genes into the SCs without undesired activation of oncogenic genes is CRISPR/Cas9 gene editing technology [195, 196]. The combination of CRISPR/Cas9 gene-editing technology and SCs has ushered in a new era in regenerative medicine [197]. This powerful alliance has empowered researchers to precisely modify SCs and direct their differentiation into specific cell types for functional analysis and clinical transplantation purposes [198, 199]. CRISPR/Cas9 technology has revolutionized the creation of cellular models for cardiac diseases by facilitating the introduction of precise missense or frameshift mutations in specific genes. This powerful tool has enabled researchers to [200, 201]. By employing CRISPR/Cas9-mediated gene editing, scientists can generate various cardiac cell models, each carrying specific genetic alterations that mimic various aspects of CVDs [200, 202–204].

Epigenetic modifications, such as miRNA applications, offer a unique approach to influence cellular behavior with transient effects, unlike DNA-based methods that result in permanent genetic changes. Previous studies have demonstrated the potential of miRNA-mediated ex vivo modification of SCs to modulate their functional properties, leading to increased growth factor secretion and angiogenic effects, ultimately improving cardiac function in injured hearts. Epigenetic modifications are reversible and do not alter the DNA sequence, providing a potential advantage over DNA-based approaches. However, miRNAs can have multiple targets and may be prone to off-target effects, warranting careful consideration during their application. Moreover, the expression of targeted genes can be regulated by several miRNAs, necessitating an understanding of potential compensatory mechanisms to ensure precise and effective modulation of gene expression.

Roles of biomaterials in CaVaReM

Significant progress has been made in developing innovative biomaterials with properties akin to native cardiac tissue, such as elasticity, electrical conductivity, and mechanical strength. These engineered materials act as scaffolds that support cell growth, adhesion, and differentiation, thereby fostering the generation of functional heart tissue. In parallel, advancements in cell-based therapies have yielded promising outcomes in preclinical investigations. Multiple cell types, including iPSCs and MSCs, are being actively researched for their potential in regenerative medicine, particularly for treating CVDs.

These cells have the potential to differentiate into cardiomyocytes, vascular cells, and other cell types necessary for cardiovascular tissue regeneration. The application of 3D bioprinting technology has enabled the fabrication of complex, patient-specific cardiovascular constructs with high precision and resolution. The potential applications of these technological advancements are vast, including the generation of functional cardiovascular tissues and organs for transplantation and drug screening and development. Integrating bioactive molecules and growth factors in tissue engineering has positively affected cell proliferation, differentiation, and angiogenesis, ultimately improving the overall functionality of engineered cardiovascular tissues [205].

In the quest to recreate functional cardiovascular tissues, scientists have engineered a range of biomaterials that effectively mimic the native biological and mechanical features of heart tissues. These scaffolds provide a supportive framework for cell growth, adhesion, and differentiation, ultimately fostering tissue regeneration and restoring tissue function. Introducing bioactive molecules into these scaffolds has demonstrated significant promise in bolstering cell survival, promoting angiogenesis, and improving overall tissue integration. By incorporating these elements, researchers aim to create a more favorable environment for tissue regeneration and functional recovery [206, 207]. In recent years, cutting-edge fabrication methods such as electrospinning and 3D bioprinting have produced intricate scaffolds with complex geometries and hierarchical structures, allowing for a more accurate recreation of native tissue architecture. Among the various types of scaffolds developed, hydrogel-based scaffolds stand out due to their high-water content, facilitating efficient nutrient and oxygen diffusion.

In addition, these scaffolds can be conveniently combined with cells and growth factors, creating a supportive microenvironment conducive to tissue regeneration. Decellularized ECM-based scaffolds, derived from native tissues, retain the crucial components of the ECM. This feature enhances cell adhesion, growth, and differentiation, fostering an environment that promotes tissue regeneration and functional recovery [208]. In addition, synthetic polymer-based scaffolds offer tunable mechanical and degradation properties, allowing for tailored scaffold design based on specific requirements. The emergence of 3D bioprinting technology has revolutionized scaffold fabrication, creating patient-specific, anatomically accurate scaffolds with high precision and resolution. The development of advanced bioinks, printable materials containing cells and biomaterials, has further enhanced the potential of 3D bioprinting for cardiovascular tissue engineering applications. Researchers have successfully demonstrated the feasibility of 3D-printed scaffolds for creating functional cardiac patches and vasculature in preclinical studies.

Microfluidics-based approaches enable precise control and manipulation of small amounts of fluids, typically at the microliter or nanoliter scale. These approaches have been increasingly applied in various fields, including biomedical research, drug development, and diagnostics. Lab-on-a-chip (LOC) devices integrate multiple laboratory functions onto a single microfluidic chip, allowing for high-throughput, automated analysis of biological samples. LOC devices have diverse applications, including cell culture, drug screening, and diagnostics. Another promising application of microfluidic technology lies in organ-on-a-chip (OOC) systems. These microfluidic devices are engineered to replicate the structure and function of human organs, such as the heart and lung, on a miniature scale. OOC systems allow studying organ-level physiology and modeling drug testing in a controlled, human-relevant environment [209, 210]. Microfluidic devices can create dynamic cell culture environments that mimic in vivo conditions better, promoting more accurate and physiologically relevant results [211]. This approach can be applied to studying cell–cell interactions, migration, and tissue development.

Droplet microfluidics is a cutting-edge technique involving microdroplet generation and precise manipulation within microfluidic devices [212]. This method benefits high-throughput single-cell analysis, sequencing, and drug encapsulation and delivery applications. By integrating microfluidic channels, droplet microfluidic devices enable the encapsulation of individual cells or molecules within discrete droplets [213]. This compartmentalization allows for the isolation, processing, and analysis of single cells or biomolecules at an unprecedented scale and efficiency. In addition, droplet microfluidics facilitates the encapsulation and controlled release of drugs or therapeutic agents. This feature enables targeted drug delivery and holds great potential for improving the efficacy and safety of various treatments. Microfluidics-based approaches offer several advantages, including reduced sample and reagent consumption, improved experimental control, and enhanced sensitivity and specificity. As a result, they have the potential to revolutionize various aspects of biomedical research and diagnostics, enabling more efficient, accurate, and cost-effective solutions. Microfluidics-based approaches have been employed in 3D cardiac tissue engineering to create more complex and functional tissue models. Microfluidic devices enable the development of microphysiological systems, such as heart-on-a-chip or cardiac tissue-on-a-chip, which can recapitulate critical aspects of the heart's structure and function. These systems can be used for drug screening, disease modeling, and studying

cardiac cell behavior in a controlled environment [214, 215]. Microfluidic devices can facilitate the formation of perfusable vasculature within the engineered tissue, improving nutrient transport and overall tissue viability. Microfluidics allows for the precise control of factors, such as fluid flow, oxygen levels, and growth factor gradients, enabling the recreation of physiological conditions found in native cardiac tissue. It can promote cell differentiation, maturation, and alignment, improving tissue functionality. By integrating microfluidic devices with biosensors and other analytical tools, researchers can perform high-throughput screening of drugs, biomaterials, or other factors affecting cardiac tissue development and function. Microfluidic devices allow for the development of co-culture systems, where multiple cell types (e.g., cardiomyocytes, endothelial cells, and fibroblasts) can be cultured together to mimic better the cellular complexity and interactions found in native cardiac tissue. Overall, the use of microfluidics-based approaches in 3D cardiac tissue engineering can advance our understanding of cardiovascular biology, improve drug development, and contribute to developing more effective therapeutic strategies for treating CVDs.

Bioreactors have been designed to apply mechanical stimuli, such as cyclic strain and pressure, to promote the maturation and alignment of cardiac cells within engineered tissues [216, 217]. It has improved functional properties, such as enhanced contractility and electrophysiological function. Hybrid systems incorporating electrical stimulation have demonstrated improved synchronization of cardiomyocyte beating and enhanced cellular organization within engineered cardiac tissues. Bioreactors, in combination with advanced scaffold materials, have facilitated the development of more complex and biomimetic cardiac tissue models. These systems provide a supportive environment for cell growth and differentiation, as well as the application of mechanical and electrical stimuli to promote tissue maturation.

On the other hand, scaffold-free bioprinting techniques have been employed to create 3D cardiac tissue models by assembling cardiac cell-laden spheroids into larger tissue constructs. These spheroids promote cell–cell interactions and facilitate the formation of functional cardiac tissue. Advances in bioprinting technology have enabled the direct printing of cells without scaffold materials. This approach has been applied to create cardiac patches and other tissue models with improved cellular organization and function. Scaffold-free bioprinting has also created vascular networks within engineered cardiac tissues, improving nutrient transport and overall tissue viability [218, 219].

Biomaterial-based approaches are currently being developed to enhance cardiac regeneration, addressing the challenge of poor SC retention and engraftment post-transplantation. Approximately 95% of transplanted SCs are cleared within the first few hours after transplantation, necessitating alternative strategies to improve cell retention and therapeutic efficacy [220, 221]. Tissue engineering-based strategies provide an effective solution by promoting the incorporation of bio-implantable cell-based materials into the host cardiac tissue. This approach offers several advantages, such as increased cell engraftment and retention by mitigating cell clearance due to high perfusion during the systolic phase of the cardiac cycle. Moreover, tissue engineering strategies provide mechanical support to damaged myocardial tissue during the post-infarction healing process. Integrating biomaterials with therapeutic cells can help maintain their viability and function, enhancing their regenerative potential. Combining biomaterial-based therapies with bioreactive molecule-based approaches may further improve the efficacy of cell-based treatments by reducing the need for repeated cell infusions. It is vital, since the half-life of cells in vivo can be limited, resulting in a decline in their therapeutic effects over time [222].

Biomaterials employed in cardiac tissue engineering can help neutralize the adverse tissue remodeling that often leads to heart failure. These materials promote angiogenesis, decrease cell death, and enhance cardiac tissue formation by stimulating cell proliferation. However, it is essential to consider several properties when selecting biomaterials for cardiac tissue engineering applications [223]. They should not elicit adverse host tissue responses, such as inflammation or immunogenic reactions. Materials should possess mechanical properties similar to native cardiac tissue to ensure seamless integration and functionality. Biomaterials should degrade at a controlled rate, allowing sufficient time to form new functional cardiac tissue. They should not induce cell death or negatively impact cellular functions.

In addition, biomaterials should be able to reduce inflammation in the local microenvironment to facilitate cell engraftment and integration with host cardiac tissue [224, 225]. In summary, selecting appropriate biomaterials for cardiac tissue engineering applications is critical for optimal therapeutic outcomes. These materials must exhibit a combination of biocompatibility, cardiac compatibility, biodegradability, minimal cell toxicity, and anti-inflammatory properties to support cardiac regeneration and repair effectively.

Cardio-immunology of cardiac tissue

Cardioimmunology, a rapidly evolving field, studies the intricate interplay between the immune system and the cardiovascular system, particularly during MI and subsequent heart remodeling. SC therapy, another promising

area of research for treating CVDs, can potentially harness and modulate the immune response to improve treatment outcomes. In the context of MI, various immune cells, such as macrophages, dendritic cells (DCs), and mast cells (MCs), play crucial roles in acute inflammatory responses and tissue remodeling. Resident cardiac immune cells contribute to steady-state heart protection, while external immune cells participate in inflammation and healing processes post-MI. SC therapy holds the potential to modulate the immune response in the infarcted myocardium. Transplanted SCs can regulate inflammation by secreting anti-inflammatory cytokines, promoting the resolution of inflammation, and enhancing tissue regeneration [226]. Moreover, SCs can modulate the adaptive immune response by interacting with T-helper cells, T-regulatory cells, and other immune cell types. The integration of cardioimmunology and SC therapy can lead to innovative strategies for treating CVDs. By considering the complex immune landscape in the heart, tailored SC therapies can be developed to fine-tune the immune response, promoting tissue repair and regeneration while reducing adverse remodeling and inflammation. Future research in this area should focus on understanding the cellular and molecular mechanisms underlying the interplay between SCs and the immune system, paving the way for more effective and targeted treatments for patients suffering from MI and other cardiovascular conditions.

Major clinical trials in CaVaReM

The field of CaVaReM has seen considerable progress through several groundbreaking clinical trials (Table 3). These trials have explored the efficacy and safety of various regenerative strategies, particularly those involving MSCs, BM-MSCs, and novel peptides or biologics. This chapter summarizes major completed and ongoing clinical trials that have advanced our understanding of regenerative therapies for CVDs.

The CONCERT-HF (Combination of Mesenchymal and c-kit+ CSCs as Regenerative Therapy for HF) trial was a randomized, placebo-controlled study that investigated the combined effect of MSCs and c-kit+ CSCs in ischemic HF. The results demonstrated that combination therapy was safe and showed improved patient-reported outcomes and reduced heart failure-related events, although primary endpoints were not met statistically [227].

The SENECA trial is another significant study that examined the safety and feasibility of administering allogeneic MSCs to cancer survivors with anthracycline-induced cardiomyopathy. This study provided early evidence that MSC therapy in this population is safe and well-tolerated, supporting future efficacy trials [228].

The DREAM-HF trial (double-blind randomized assessment of clinical events with allogeneic mesenchymal precursor cells) assessed the impact of mesenchymal precursor cells (MPCs) in chronic HF patients. Results indicated that MPC therapy reduced major adverse cardiovascular events and improved patient quality of life over standard care [229].

CardiAMP Heart Failure Trial currently in Phase III, investigates autologous BMMNCs delivered using the CardiAMP system for patients with post-myocardial infarction HF. Preliminary findings suggest improved cardiac function, with final results expected in 2025 [230].

The CATO trial focuses on the intravenous administration of umbilical cord-derived MSCs in patients with ischemic cardiomyopathy. This Phase II trial, ongoing in the United States, aims to evaluate both the safety and regenerative efficacy of allogeneic MSC delivery.

The Metcela JRM-001 Trial, conducted in Japan, is a clinical study examining the effects of autologous CSCs therapy in pediatric patients with congenital heart disease. After resolving previous manufacturing concerns, the trial has resumed its Phase III enrollment. The primary objective of this trial is to gather long-term safety and efficacy data for this innovative therapeutic approach in treating children with heart conditions [231].

These trials represent pivotal efforts in translating regenerative biology into therapeutic strategies for CVDs. While some have already shown clinical promise, others remain in progress and may define the future of personalized, regenerative therapies for HF and related conditions.

Limitations, consensus, and future directions

In light of the current evidence and reflective of the comprehensive critique presented by Liu et al., it is evident that cardiac regeneration science stands at a critical juncture. While significant progress has been made in uncovering cellular and molecular pathways that may enable tissue repair, the field continues to wrestle with several foundational challenges: the low replicative potential of adult cardiomyocytes, the lack of consistent functional integration of transplanted cells, the ambiguous regenerative role of stem-cell-derived exosomes, and immune compatibility barriers. The consensus emerging from both developmental and translational studies suggests that complete, large-scale cardiomyocyte replacement in the adult human heart is unlikely under current therapeutic paradigms. Instead, strategies may increasingly shift toward modulating the microenvironment, enhancing paracrine support, and promoting adaptive remodeling rather than full regeneration [248].

Our review seeks to offer novel insight by bridging the gap between mechanistic discovery and clinical

Table 3 Clinical trials of stem cell administration for cardiovascular diseases

Study Type	Disease	Cell Type	Delivery System	Mechanism of Action	Outcome	Refs.
Phase II, open-label, single-center clinical trial.	Ischemic heart failure	Autologous adipose tissue-derived mesenchymal stromal cells	Intramyocardial injections	Paracrine effects of the mesenchymal stromal cells, which are thought to promote angiogenesis, reduce inflammation, inhibit apoptosis, and decrease fibrosis in the damaged heart muscle.	Treatment was safe and feasible. While there was no significant improvement in the primary endpoint of left ventricular ejection fraction (LVEF) at 6 months, there were positive signals, including improvements in the 6-min walk test, quality of life scores, and a reduction in NT-proBNP levels in a subgroup of patients.	[232]
Randomized, single-blind, placebo-controlled, phase I/II clinical trial	Acute Myocardial Infarction	Bone-marrow-derived mesenchymal stromal cells	Intracoronary injection	Secretion of paracrine factors that can reduce inflammation, inhibit cardiomyocyte apoptosis, promote angiogenesis and modulate the immune response to reduce scarring and encourage repair of the damaged heart muscle	Both single and double doses of allogeneic MSCs were safe and feasible for patients after AMI. While there were no significant improvements in the primary endpoint of LVEF at 6 months compared to the placebo group, some secondary endpoints suggested potential benefits. Specifically, the study observed a significant reduction in infarct size in the group that received a single dose of MSCs	[233]
Multicenter, double-blind, randomized, placebo-controlled, phase II clinical trial	Chronic ischemic heart failure	Allogeneic adipose tissue-derived mesenchymal stromal cell	Intramyocardial injection	Secretion of various bioactive molecules that can modulate the local inflammatory response, reduce fibrosis, and promote angiogenesis, thereby improving the function of the existing heart tissue	Trial did not meet its primary endpoint, which was a significant improvement in the LVEF at 6 months compared to the placebo group. However, a post-hoc analysis suggested a potential benefit in a subgroup of patients with higher levels of systemic inflammation. The treatment was found to be safe and well-tolerated.	[234]

Table 3 (continued)

Study Type	Disease	Cell Type	Delivery System	Mechanism of Action	Outcome	Refs.
Phase III, randomized, sham-controlled, double-blind clinical trial	Chronic heart failure	Immunoselected mesenchymal precursor cells	Transendocardial intramyocardial injection	Paracrine activity of the MPCs, which secrete a range of bioactive molecules. These molecules are thought to reduce inflammation, inhibit cardiac myocyte apoptosis, decrease fibrosis, and promote angiogenesis, thereby improving the overall function and environment of the heart muscle without the cells themselves transforming into new cardiac tissue.	It demonstrated a significant and durable improvement in health-related quality of life, as measured by the Kansas City Cardiomyopathy Questionnaire (KCCQ), over a 12-month period compared to the sham-control group. The cell therapy was found to be well-tolerated	[235]
Phase I clinical trial	Hypoplastic left heart syndrome (HLHS)	Human umbilical cord blood-derived mononuclear cells (UCB-MNC)	Intramyocardial injection	Differentiation into cardiac cell subtypes	Increased cardiac performance at 3- and 6-month monitoring in neonates having HLHS	[236]
Multicenter, randomized clinical trial	Chronic ischemic cardiomyopathy	CD133+ bone-marrow isolated cells	Infusion into infarcted area after coronary artery bypass grafting (CABG)	Angiogenesis and tissue repair	Increased left ventricular sizes on MRI following 6 months of treatment	[237]
Randomized Phase III trial	Post-infarction patients with chronic ischemia	CD133+ cells	Intramyocardil injection	Cardiac restoration through circulating cells (CD133+ EPCs, thrombocytes)	Enhanced infarction area healing in chronic ischemia individuals	[238]
Phase II randomized comparative trial	Dilated cardiomyopathy	Bone-marrow mononuclear cells (BMMC) and mesenchymal stem cells (BMSC)	Intracoronary infusion	Heart healing is facilitated by paracrine signaling, which could potentially lead to differentiation into cardiac lineages	Both BMSC and BMMC enhanced left ventricular ejection fraction (LVEF) and New York Heart Association (NYHA) class at 3 months; however, only BMSC preserved improvements in LVEF and NYHA at 12 months compared to the control group; no significant variations in major adverse cardiac events (MACE) were observed between the groups	[239]
Phase II, randomized, blinded trial	Ischemic cardiomyopathy	Human bone-marrow-derived mesenchymal stem cells	Transendocardial stem cell injection (TESI)	Paracrine communication and heterocellular interaction enhance heart healing, immunomodulation, and scar formation	100 million doses demonstrated a considerable enhancement of the left ventricular ejection fraction. decreased blood TNF-α concentrations demonstrating lowered inflammation; fewer heart failure rehospitalizations in the greater dose group	[240]

Table 3 (continued)

Study Type	Disease	Cell Type	Delivery System	Mechanism of Action	Outcome	Refs.
Phase II-A, single-blind, placebo-controlled, crossover randomized trial	Nonischemic Cardiomyopathy	Bone-marrow-derived mesenchymal stem cells	Intravenous infusion	Paracrine signals and anti-inflammatory mechanisms modulate the systemic immune system, thereby mitigating chronic inflammation in heart failure	Safe with no increase in major adverse events; improved 6-min walk distance (+36.47 m); no significant change (LVEF) or ventricular volumes; magnitude of natural killer cell reduction correlated inversely with LVEF improvement	[241]
Multinational randomized, double-blind, sham-controlled trial	Ischemic heart failure	Mesenchymal stem cells conditioned with cardiogenic factors	Endomyocardal injection via retention-enhanced catheter	Cardiac rejuvenation involves increased development and paracrine actions supporting retrograde remodeling	The primary composite endpoints were neutral in general at 39 weeks; the subgroup with baseline left ventricular end-diastolic volume (LVEDV) of 200–370 mL (60% of patients) demonstrated significant advantages, such as enhanced 6-min walk distance, minimized left ventricular end-systolic volume (LVESV), and axially beneficial fatalities and heart failure incidents; no distinction in dedicated complications; and a lower rate of unexpected cardiac death in the treatment group	[242]
Phase I safety and feasibility trial	Severe ischemic left ventricular dysfunction	Human embryonic stem cell (hESC)	Epicardial delivery embedded in fibrin patch during CABG surgery	Cardiac renewal through differentiation into cardiovascular progenitors and paracrine actions; repair of tissues and expansion	No tumor growth or arrhythmias were observed following a median monitoring of 18 months; three patients experienced silent alloimmunization; there was improvement in symptoms accompanied by enhanced systolic movement in the medicated segments	[243]
Phase I randomized, open-label	Non-ischemic dilated cardiomyopathy	Allogeneic and autologous bone-marrow-derived human mesenchymal stem cells (hMSCs)	TESI via NOGA catheter into 10 LV sites	Immunomodulation diminishes chronic inflammation; paracrine communication facilitates heart healing and neovascularization	Allogeneic hMSCs were reliable and demonstrated superior clinical effectiveness than autologous hMSCs, comprising an 8% rise in LVEF, increased 6-min walk distance, higher quality of life ratings (MLHFQ), enhanced endothelial operation, and greater TNF-α reduction	[244]

Table 3 (continued)

Study Type	Disease	Cell Type	Delivery System	Mechanism of Action	Outcome	Refs.
Phase 2, randomized, clinical trial	Advanced heart failure	Mesenchymal precursor cells (MPCs) derived from bone marrow	Intramyocardial injection during (left ventricular assist device)LVAD implantation surgery	Immunomodulation by the polarization of macrophages to an anti-inflammatory phenotype; attenuation of inflammation, facilitation of myocardial restoration, and reversal of remodeling	No significant difference in effective temporary weaning off LVAD assistance at 6 months in comparison with sham; medication was safe with no rise in side outcomes; earlier phase revealed possible benefit, but this trial did not establish efficacy for LVAD emptying	[245]
Double-blind, placebo-controlled, dose-ranging safety trial	Acute myocardial infarction	Bone-marrow-derived human mesenchymal stem cells (hMSCs),	Intravenous infusion	Targeting damaged myocardium; immunomodulation; paracrine communication facilitating heart regeneration and reversal remodeling	Demonstrated safety without an elevation in side effects relative to placebo; decreased occurrences of ventricular tachycardia; enhanced pulmonary functioning; increased worldwide symptom scores; augmented left ventricular ejection fraction; and reversal remodeling in the anterior myocardial infarction subgroup as assessed by cardiac MRI	[246]
Phase I/II, randomized, double-blind, controlled trial	Acute myocardial infarction	Allogeneic bone-marrow-derived mesenchymal stromal cells	Intravenous infusion	Immunomodulation and anti-inflammatory properties; paracrine signaling facilitating heart regeneration	no major side events connected to Stempeucel; greater ejection fraction at 6 months (43.06% to 47.80%), however, not statistically significant when compared with placebo; There are no significant variations in perfusion ratings or infarct volume at 6 months	[247]

application, especially through an integrated view of exosomal signaling, next-generation gene-editing tools, and multimodal biomaterial platforms. Unlike traditional reviews that focus solely on regenerative mechanisms, we emphasize the importance of functional validation, biomechanical integration, and long-term safety, drawing from both animal models and human trial data.

Ultimately, the future of cardiac regenerative medicine may depend less on a single revolutionary breakthrough and more on the careful integration of multiple, incremental strategies, grounded in developmental biology and shaped by a clear understanding of the limitations and ethical boundaries. Only through such a nuanced approach can we move toward durable and meaningful therapies for the millions suffering from heart failure worldwide.

Conclusion

Cardiovascular medicine has witnessed significant advancements in both SC and biomaterial research. Notably, the utilization of pluripotent SCs, such as ESCs and iPSCs, has shown great promise in differentiating into various cardiovascular cell types, including cardiomyocytes, endothelial cells, and vascular smooth muscle cells. MSCs have also demonstrated potential in promoting cardiac regeneration through their anti-inflammatory properties and angiogenic effects. Recent research has identified and isolated resident CPCs, which can differentiate into multiple cardiovascular cell types, thus contributing to heart regeneration. Novel biomaterials have been developed with improved biocompatibility, mechanical properties, and bioactivity, providing better support for cell growth and differentiation in engineered cardiac tissues. The advent of bioactive molecules incorporated into scaffolds or delivery systems has significantly improved cell survival, differentiation, and tissue integration in cardiac tissue engineering. These innovations have opened new avenues for developing advanced therapeutic strategies to stimulate cardiac regeneration. Recent advancements in bioprinting and microfluidic-based approaches have further revolutionized the field, creating more intricate and functional cardiac tissue models. These cutting-edge technologies enable high-throughput screening, vascularization, and precise control over cellular organization, thereby enhancing the potential for successful tissue regeneration. The convergence of advancements in SC technologies and biomaterial research has the potential to reshape the landscape of CaVaReM. By combining the regenerative capacity of SCs with the supportive and instructive properties of advanced biomaterials, we can move closer to developing effective therapies for treating CVDs.

Despite substantial advancements in CaVaReM, various concerns and challenges must be addressed to ensure successful clinical translation and widespread adoption of these innovative therapies. Obtaining adequate, high-quality, safe, and clinically relevant cells remains challenging. The use of autologous cells can be limited by factors, such as patient age, health status, and tissue availability. In contrast, the use of allogeneic cells raises concerns about immune rejection and disease transmission. Achieving long-term survival, retention, and functional integration of transplanted cells within the host myocardium remains a significant challenge. Ischemia, inflammation, and immune response can negatively impact cell survival and engraftment. Engineered cardiac tissues often lack sufficient vascular networks, limiting tissue viability and functionality. Developing strategies to promote angiogenesis and ensure adequate perfusion within the constructs is essential. Developing biomaterials with optimal mechanical, structural, and biochemical properties that can effectively integrate with host tissues and promote tissue regeneration remains an ongoing challenge.

While some studies have demonstrated the potential benefits of SC therapy, such as improvements in LVEF and a reduction in infarct size, these findings have not been consistently replicated across various trials. For instance, the CHART-1 trial revealed neutral primary outcomes and no significant difference in serious adverse events between treatment and control groups [249]. Similarly, the CAREMI trial suggested considerable variability in patient responses, as evidenced by a wide confidence interval for the estimated treatment effect [250]. Furthermore, safety concerns and modest improvements in LVEF have raised questions about the efficacy and risk–benefit balance of SC therapy. As a result, many trials have not progressed to later phases, contributing to the perceived stagnation in the field of regenerative cardiac therapy. To overcome these challenges and advance the field, researchers should prioritize standardization across all stages of clinical trial development and execution. This includes establishing consistent methodologies for patient selection, cell preparation, and outcome assessment. In addition, proactive surveillance of trial status by clinical trial registries could facilitate more effective monitoring and communication of research findings. While SC therapy holds promise for the treatment of cardiovascular conditions, it is essential to carefully consider the limitations and conflicting evidence from recent clinical trials. A balanced discussion of these issues will help guide the development of more effective and safe regenerative therapies for patients in need.

Translating laboratory-scale research into clinically relevant, large-scale production of cells, biomaterials, and

tissue-engineered constructs is a complex process that requires addressing regulatory, economic, and technical hurdles. The relevance and translatability of preclinical animal models to human CVDs remain a concern. Efforts should focus on developing more predictive and human-relevant models to improve therapeutic strategies' translation into clinical applications. Addressing these challenges and concerns will be critical for advancing the field of CaVaReM and bringing innovative therapies to patients in need. Continued multidisciplinary research efforts spanning SC biology, biomaterials science, and engineering will be essential for overcoming these hurdles and realizing the full potential of this promising field.

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