Narrative Review Article

Biological and Methodological Barriers in Cardiac Regeneration: A Review of Current Challenges & Emerging Strategies

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ABSTRACT

Heart failure following a myocardial infarction (MI) remains a major global health challenge. This is largely attributable to the adult human heart's limited innate regenerative capacity, which results in ventricular fibrosis, remodeling, and the formation of scar tissue. Early clinical trials evaluating bone marrow-derived mononuclear cells (BMMNCs) and mesenchymal stromal cells (MSCs) demonstrated acceptable safety profiles. Still, they produced only modest improvements in cardiac function and failed to replicate the robust benefits observed in preclinical models. This review examines the biological and methodological barriers responsible for this translational gap. Biologically, therapeutic efficacy is limited by the hostile post-infarction environment, which is characterized by hypoxia, inflammation, and oxidative stress, all of which contribute to extensive donor cell death, poor cell retention, and inadequate electrical integration with host myocardium. Methodologically, progress is hindered by small clinical trial sizes with limited statistical power coupled with high heterogeneity in cell preparations, dosing, and delivery routes. Furthermore, the widespread reliance on surrogate imaging endpoints, particularly on the left ventricular ejection fraction (LVEF), rather than definitive clinical outcomes, such as mortality, heart-failure hospitalization, functional status, and quality of life, limits the interpretation of therapeutic efficacy. In response to these limitations, the field is shifting toward cell-free therapies utilizing extracellular vesicles and exosomes to deliver cardiovascular bioactive molecules, as well as direct cardiac reprogramming to convert resident scar-forming fibroblasts into functional cardiomyocytes. Successfully overcoming these translational barriers will require standardized trial designs, rigorous product characterization criteria, and greater emphasis on clinically meaningful endpoints.

Keywords: Cardiac Regeneration; Translational Barriers; Myocardial Infarction; Heart Failure; Direct Cardiac Reprogramming

INTRODUCTION

Heart failure is characterized by the inability of the heart to pump sufficient blood to meet the body's metabolic demands because of structural or functional cardiac abnormalities. This condition is associated with ventricular fibrosis, adverse cardiac remodeling, and an increased risk of arrhythmias (1). Myocardial infarction (MI), resulting from prolonged interruption of coronary blood flow, causes extensive cardiomyocyte death and remains one of the leading causes of heart failure globally (1).

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The five-year mortality rate following an MI event has declined substantially, from approximately 70% in the 1970s to less than 45% today, largely because of advancements in reperfusion strategies and evidence-based pharmacological therapies (2). Despite these improvements in survival, many patients continue to develop chronic heart failure because the adult human heart possesses minimal intrinsic regenerative capacity and responds to ischemic injury primarily through fibrotic scar formation (3).

Cell-based therapies initially emerged as a promising approach to regenerate damaged myocardium. Early clinical trials utilizing bone marrow–derived mononuclear cells (BMMNCs) and mesenchymal stromal cells (MSCs) demonstrated acceptable safety and feasibility but produced only modest therapeutic benefits and had high variability (4-6). The subsequent development of human-induced pluripotent stem cells (iPSCs) renewed interest in generating patient-specific specific cardiomyocytes, though initial expectations were quickly tempered by biological and manufacturing hurdles (7-9).

As experimental evidence accumulated, researchers observed that transplanted cells rarely survived long-term or achieved durable engraftment despite occasional functional improvements (7). These findings shifted the focus of the cardiac regeneration field from therapeutic effects toward paracrine signaling (10). Consequently, increasing attention has been directed toward cell-free strategies, such as secretome- and extracellular vesicle–based therapies, which seek to harness these restorative signals without the complexities of live-cell delivery.

Despite these innovations and evolving paradigms, a critical translational gap persists, as shown in Table 1. This review analyzes the biological and methodological barriers hindering the clinical translation of cardiac

Table 1. Comparative analysis of cardiac regeneration strategies. *Summary of the primary mechanisms of action, major advantages, principal limitations, clinical status and representative references for stem cell transplantation, cell-free therapies, and direct cardiac reprogramming.*

Therapy Type	Mechanisms of Action	Major Advantages	Principal Limitations	Clinical Status	Key References
Stem Cell Transplantation (BMMNCs, MSCs, iPSCs)	Historical Focus: Direct cell replacement and physical tissue engraftment to rebuild lost cardiomyocytes. Current Understanding: Primarily acts via paracrine signaling rather than long-term engraftment for BM-MNCs/MSCs.	Proven clinical safety profile for adult cell types iPSCs offer an unlimited, patient -matched cell supply capable of generating true cardiomyocytes	Extensive acute cell death (>90% in days) Low cell retention and limited electromechanical integration Arrhythmogenic risk from immature iPSC-derived cardiomyocytes	Numerous completed and ongoing phase 1-3 clinical trials Overall functional improvements in major adult cell-trials (BMMNCs, MSCs) remain modest to neutral IPSC-derived therapies are currently in early-phase clinical testing	(6, 7, 9)
Cell-Free Therapy (Extracellular Vesicles & Exosomes)	Direct delivery of the paracrine secretome (proteins, lipids, microRNAs) to activate endogenous cardioprotective and regenerative pathways	Reduced risk of immune rejection, tumorigenesis, and microvascular embolization. Enables highly standardized, scalable, and consistent manufacturing	Rapid clearance and short half-life of vesicles in circulation. Requires optimized, targeted delivery vehicles. Dosing, kinetics, and potency protocols remain complex and non-standardized	Robust preclinical success across small and large animal models Transitioning toward early-phase clinical design and initial human safety evaluations	(11, 12, 21)

Continued Table 1. Comparative analysis of cardiac regeneration strategies. *Summary of the primary mechanisms of action, major advantages, principal limitations, clinical status and representative references for stem cell transplantation, cell-free therapies, and direct cardiac reprogramming.*

Therapy Type	Mechanisms of Action	Major Advantages	Principal Limitations	Clinical Status	Key References
Direct Cardiac Reprogramming	*in situ transdifferentiation:* using lineage-specific transcription factors or microRNAs to convert resident scar-forming fibroblasts directly into functional cardiomyocytes	Harnesses endogenous cell populations while simultaneously reducing local myocardial fibrosis Bypasses *ex vivo* cell culture and transplantation hurdles	Low conversion efficiency *in vivo* Risk of off-target reprogramming in non-cardiac cell types Requires precise, target vector delivery methods to the injury zone	Many in early preclinical stages Demonstrates functional improvements and scar reduction in rodent coronary ligation models	(20, 21)

regeneration therapies. Specifically, it evaluates how the hostile post-infarction microenvironment, intrinsic donor-cell limitations, and heterogeneity in clinical trial design impede therapeutic success while highlighting emerging strategies designed to overcome these barriers.

BIOLOGICAL BARRIERS

The adult human heart possesses only a limited capacity to replace damaged cardiomyocytes. Current evidence indicates that, at approximately 25 years of age, nearly 1% of cardiomyocytes are renewed annually, with this regenerative capacity declining progressively with age until it approaches negligible levels (11). Following myocardial infarction (MI), prolonged interruption of coronary blood flow results in extensive cardiomyocyte death. The damaged myocardium is subsequently replaced by fibrotic scar tissue that lacks the contractile properties of healthy cardiac muscle. Consequently, scar formation reduces cardiac contractility, promotes adverse ventricular remodeling, and ultimately contributes to the development of heart failure (12).

Many preclinical studies rely on small animal models, particularly mice, and frequently report encouraging improvements in left ventricular ejection fraction (LVEF). However, mice and humans differ substantially in physical domains, including immune response, cardiac environment, and overall biological makeup. Consequently, extrapolating findings from small neonatal animal models to adult human disease may overestimate therapeutic efficacy and translational potential.

Furthermore, neonatal mammals, including mice during the first postnatal week of life, possess a remarkable but transient capacity for complete cardiac regeneration following injury. In contrast, cardiomyocyte renewal in adult humans is restricted to a baseline turnover rate of roughly 0.5–1% annually (13). Consequently, the widespread use of neonatal rodent models represents an important limitation of preclinical cardiac regeneration research because their inherently high regenerative capacity does not accurately reflect the biological environment of the adult human heart.

Following an MI, the affected tissue area develops into a hostile microenvironment. Characterized by severe ischemia, the region is subjected to profound hypoxia, elevated levels of reactive oxygen species, and nutrient deprivation, as highlighted in Figure 1. Post-transplantational studies of BMMNCs and MSCs have demonstrated that more than 90% of transplanted cells die or are cleared within only a few days after delivery (14). Over time, progressive ventricular fibrosis further increases tissue stiffness, limiting donor-cell retention and survival while reducing the likelihood of successful tissue integration.

Beyond the hostile microenvironment, the structural and functional immaturity of transplanted cardiomyocytes presents another major barrier to successful cardiac regeneration. If donor cardiomyocytes, specifically iPSC-derived cardiomyocytes, remain immature or fail to match the host cell lineage, they are unable to form the intercalated discs and gap junctions required for proper electromechanical coupling with host tissue. Poor electrical integration increases the risk of ventricular arrhythmias while limiting meaningful contributions to myocardial contractility, thereby reducing overall therapeutic efficacy (15).

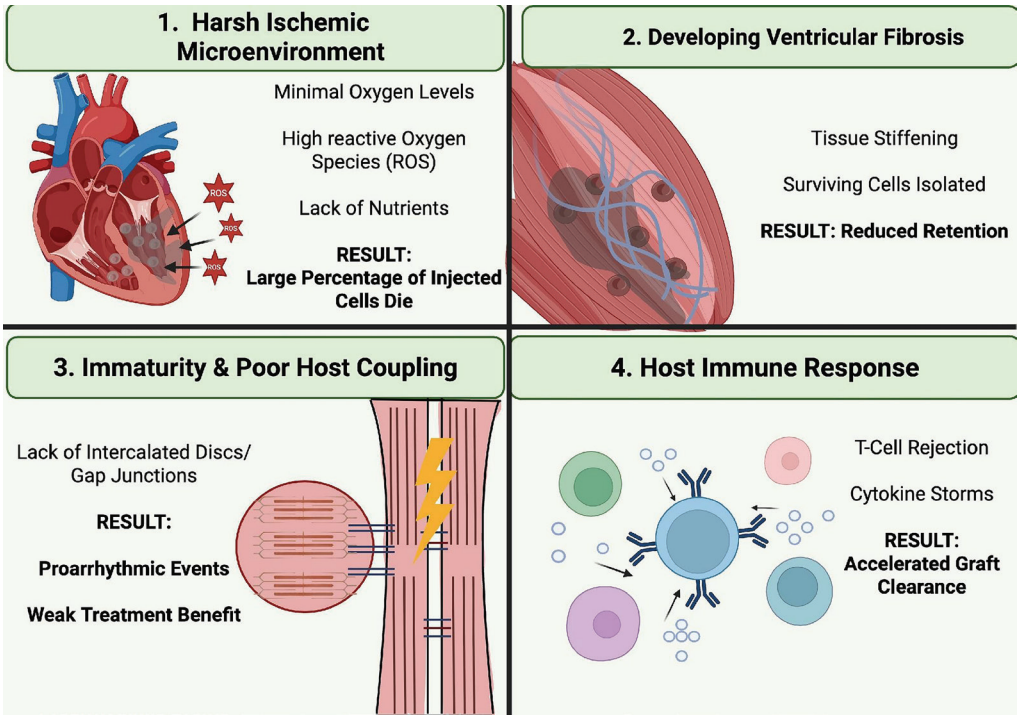

Figure 1. ***Sequential Biological Barriers to Post-MI Cell Transplantation.*** *The sequential and compounding biological barriers limiting the survival, retention, electrical and mechanical coupling, and long-term integration of transplanted stem cell-derived cardiomyocytes in the adult human post-myocardial infarction microenvironment. Created in BioRender. Vad, V. (2026) https://BioRender.com/4ospfig.*

METHODOLOGICAL BARRIERS

Beyond biological limitations, methodological weaknesses in clinical trial design and execution have impeded the accurate evaluation of cardiac regeneration therapies and contributed to inconsistent assessments of their therapeutic efficacy.

Many early- and mid-phase clinical trials enrolled relatively small patient cohorts, resulting in insufficient statistical power to evaluate definitive clinical endpoints such as all-cause mortality and heart-failure hospitalization (16). Furthermore, inadequate blinding and weak allocation concealment compromised investigator and participant masking. These limitations likely influenced semi-objective outcome measures, including functional class or other symptom-based scoring, thereby blurring the true therapeutic effect (17).

Additionally, many studies relied heavily on surrogate imaging measures, particularly LVEF via echocardiography or cardiac magnetic resonance imaging (MRI), rather than prioritizing hard clinical outcomes. Improvements in LVEF of only 2-3% do not consistently correlate with long-term survival or other patient-centered outcomes (18). Consequently, reliance on surrogate imaging markers limits the ability to determine whether modest functional improvements translate into meaningful clinical benefit.

Substantial heterogeneity also existed across clinical trials with respect to cell type, including BMMNCs, MSCs, and iPSC-derived cardiomyocytes, as well as cell dose, which ranged from approximately 10^6 to more than 10^9 cells, and delivery strategy. Delivery strategies varied widely, encompassing intracoronary infusion, transendocardial injection, and epicardial delivery.

In addition, cell preparations were frequently administered without standardized assessments of cellular potency or viability. The timing of cell delivery also varied considerably, ranging from a few days after myocardial infarction to several months later, thereby encompassing both the acute inflammatory phase and the chronic fibrotic stage of myocardial infarction. Similarly, follow-up durations ranged from three months to several years. Collectively, these methodological differences hinder meaningful comparisons across studies and increase the likelihood of misleading conclusions, as illustrated in Figure 2.

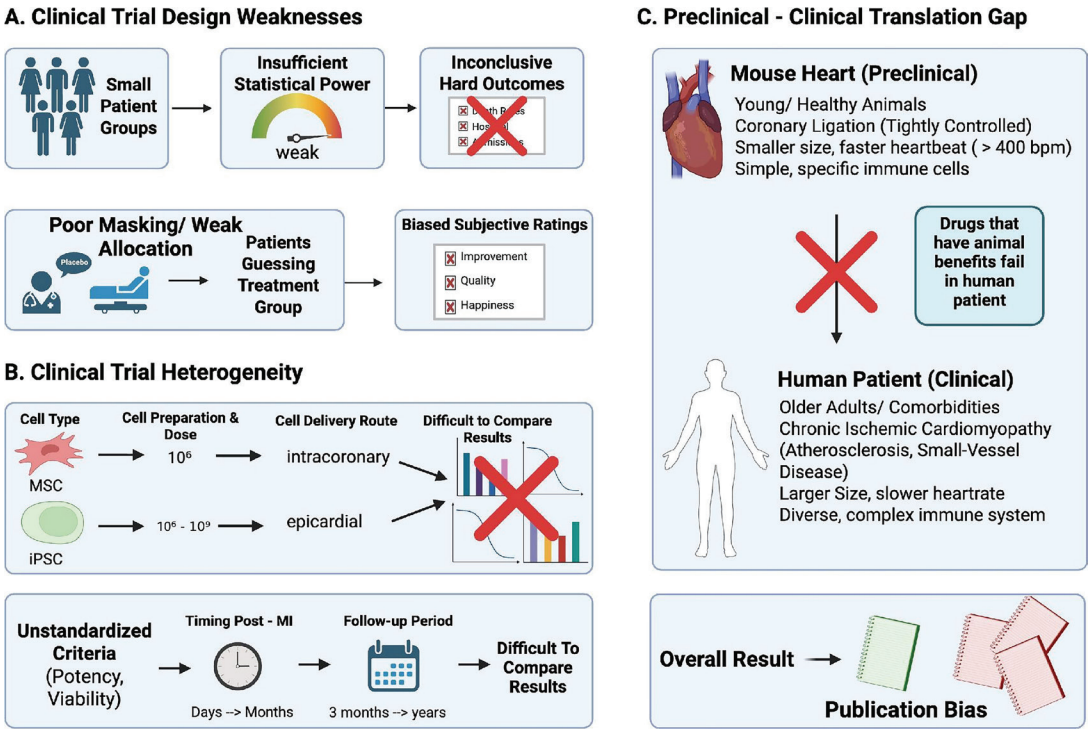

Figure 2. Methodological and Model Limitations in Cardiac Regeneration Trials. *Systemic methodological challenges in preclinical translation gaps in cardiac regeneration research. (A) Limitations in clinical trial design that skew efficacy assessments. (B) Methodological heterogeneity across clinical trials that prevents reliable cross-study comparisons. (C) Disparities between rodent models and multi-morbid, aging human populations may produce overestimated therapeutic efficacy. Created in BioRender. Vad, V. (2026) https://BioRender.com/r16211n.*

Publication bias represents another important methodological challenge. The preferential publication of positive findings, together with the underreporting of neutral or negative results, distorts the overall body of evidence and delays recognition of important methodological limitations.

Although preclinical models can offer critical insights into disease mechanisms, they do not fully recapitulate the biological complexity of human cardiovascular disease. Most animal studies utilize young, healthy animals and coronary litigation models, in which the blockage is created surgically under tightly controlled conditions. These models fail to replicate the widespread atherosclerosis, microvascular disease, advanced age, and multiple comorbidities that characterize patients with chronic ischemic cardiomyopathy.

Murine hearts differ substantially from the human heart in several fundamental respects. They are considerably smaller, exhibit resting heart rates that frequently exceed 400 beats per minute, and possess distinct electrophysiological and action-potential characteristics. In addition, important differences exist in both systemic and cardiac immune-cell populations between rodents and humans. Consequently, therapies demonstrating promising efficacy in rodent models often show limited or no benefit in clinical trials. This discrepancy reflects not only species-specific biological differences but also the greater clinical complexity and heterogeneity of human patient populations.

LESSONS LEARNED

Over the past two decades, cardiac regeneration research has progressively shifted away from whole cell transplantation toward therapies that exploit paracrine signaling and *in situ* direct cardiac reprogramming.

Cell-free therapies, such as extracellular vesicles (EVs) and exosomes, aim to capture the restorative signaling of stem cells while avoiding the risks associated with whole-cell delivery, including graft failure, immune rejection, and tumorigenesis. Preclinical studies in both small- and large-animal models of myocardial infarction

have demonstrated that MSC-derived EVs reduce infarct size, promote angiogenesis, and preserve left ventricular function (19). Because EVs are non-replicative, cell-free entities capable of transporting concentrated loads of cardioprotective microRNAs, proteins, and lipids, their manufacturing and quality control can be regulated better than live-cell products.

Concurrently, researchers have developed direct reprogramming strategies to convert endogenous cardiac fibroblasts, which typically drive scar-formation in injured myocardium, into induced cardiomyocyte-like cells. In a rodent model of chronic myocardial infarction, increased expression of cardiac-specific transcription factors converted approximately 2% of resident fibroblasts into induced cardiomyocytes (20). Treated hearts demonstrated reduced fibrosis and improved systolic function. Moreover, this transdifferentiation process appeared to reduce fibrosis among non-reprogrammed fibroblasts, suggesting an additional antifibrotic effect of cardiac reprogramming (21).

Although current reprogramming efficiency remains too low for immediate clinical application, the current results have demonstrated a realistic path forward. Ultimately, this approach may allow the regeneration of injured myocardium by converting abundant resident cardiac fibroblasts directly into functional cardiomyocytes.

For these emerging methods to succeed in clinical practice, future trials must establish increased standardization. Recent efforts to establish consensus definitions for cell-based and extracellular vesicle therapies highlight the need for standardized criteria to assess product identity, purity, biological potency, and viability. Likewise, consensus protocols are needed for dosing, delivery methods, and the optimal timing of therapy following myocardial infarction.

In parallel, cardiovascular clinical trials should emphasize standardized core outcome sets that combine imaging-based assessments with clinically meaningful endpoints, including mortality, heart-failure hospitalization, functional status, and quality of life. Future investigations should employ adequately powered, prospective, randomized, and appropriately blinded study designs. Given the mixed outcomes and substantial heterogeneity observed in previous cell-therapy trials, studies evaluating *in situ* cardiac reprogramming should also adopt standardized protocols and validated outcome measures to facilitate meaningful comparisons across investigations.

CONCLUSION

The journey of cardiac regeneration research over the last two decades has been characterized by promising advancements and important limitations. Early strategies centered on direct cell transplantation highlighted the complexity of the adult human heart and demonstrated that effective cardiac repair requires more than simply replacing lost cardiomyocytes. These findings have redirected the field toward therapeutic approaches that better align with the biological characteristics of the post-infarction microenvironment.

Emerging strategies, including cell-free signaling via EVs and direct cellular reprogramming, reflect an effort to work in tandem with endogenous repair pathways rather than override them. Although these approaches show considerable promise, important challenges remain, including limited reproducibility, incomplete cardiomyocyte maturation, suboptimal electrical integration, and uncertainties regarding long-term safety. Continued research is therefore needed to optimize delivery strategies, improve cellular maturation, and establish standardized methods for the characterization and quality control of extracellular vesicle-based therapies.

Future research must prioritize a rigorous evaluation of off-target effects in reprogramming strategies, particularly focusing on unintended cellular phenotypes and arrhythmogenic risk. Greater use of clinically representative large-animal models will also be essential to better approximate human cardiovascular physiology and improve the predictive value of preclinical studies. Furthermore, future clinical trials should emphasize clinically meaningful endpoints, including mortality, heart-failure hospitalization, functional capacity, and quality of life, while incorporating adequately powered, randomized, and standardized study designs.

Although substantial challenges remain, continued advances in regenerative biology, translational research, and clinical trial methodology provide a strong foundation for future progress. Addressing the biological and methodological barriers outlined in this review will be critical to realizing the clinical potential of cardiac regeneration therapies for patients with heart failure following myocardial infarction.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

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