Review

Harnessing Induced Pluripotent Stem Cells for Cardiac-Related Pain: Advances, Models, and Therapeutic Prospects

Boya Liao , Kin-ho Aaron Lee , Fei Meng , Jiangwei Wu , Jinghan Yang , Sau-ching Stanley Wong *

Posted Date: 11 October 2025

doi: 10.20944/preprints202510.0878.v1

Keywords: induced pluripotent stem cells; cardiac-related pain; iPSC-CMs

 doi:10.20944/preprints202510.0878.v1

Review

Harnessing Induced Pluripotent Stem Cells for Cardiac-Related Pain: Advances, Models, and Therapeutic Prospects

Liao Boya, Lee Kin-ho Aaron, Meng Fei, Wu Jiangwei, Yang Jinghan, Wong Sau-ching Stanley *

Department of Anaesthesiology, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

* Correspondence: wongstan@hku.hk

Abstract

Cardiac pain is difficult to diagnose and treat in part because existing models fail to capture human neuro-cardiac signaling. This review examines how iPSC-derived cardiomyocytes and sensory neurons in co-culture and organoid systems model ischemic and neuro-immune mechanisms, and how such platforms enable regenerative and neuromodulatory translation. We identify key barriers (maturation, heterogeneity, scalability, safety) and propose priorities to bridge discovery and clinical application.

Keywords: induced pluripotent stem cells; cardiac-related pain; iPSC-CMs

1. Introduction

Cardiac-related pain encompasses chest discomfort arising from underlying heart pathology [1]. It is most commonly a manifestation of myocardial ischemia, which occurs when coronary blood flow fails to meet myocardial oxygen demand [2]. Clinically, this pain is a hallmark of conditions such as angina pectoris and acute myocardial infarction; patients typically describe it as a pressure, squeezing, or tightness in the chest that may radiate to the neck, jaw, shoulder, or arms [3]. Because ischemic heart disease is a leading cause of death worldwide, timely recognition of cardiac pain is critical [4]. Cardiac pain can present acutely (as in myocardial infarction) or chronically (as in stable angina), and it often varies with activity and rest [5,6].

Major forms of cardiac-related pain include stable angina pectoris, acute myocardial infarction, pericarditis and microvascular angina which share a common pathophysiology: myocardial ischemia leads to the release of metabolites (such as adenosine, bradykinin, and lactate) that activate cardiac nociceptors [7]. However, unlike somatic pain, cardiac pain signals are often poorly localized [5]. The heart has relatively sparse sensory innervation, and its afferent fibers converge centrally with somatic pathways, so ischemic pain is frequently diffuse or referred [8]. As a result, patients may report atypical symptoms (e.g. jaw or arm pain, nausea, or shortness of breath) instead of classic chest pressure [9]. This complex presentation underscores the clinical significance of cardiac pain: it is a warning sign of potentially life-threatening heart disease, yet it can be easily missed or misinterpreted.

Psychological variables are increasingly recognized as critical modulators of cardiac pain [10]. Anxiety and emotional distress can amplify pain perception, leading to an increased reliance on psychological rather than purely ischemic determinants of pain [11]. Studies have demonstrated that individuals with high neuroticism exhibit greater pain sensitivity, whereas those with lower neuroticism are more likely to experience silent ischemia. These findings highlight the role of personality traits in shaping subjective pain perception and regulation.

Despite its clinical importance, cardiac-related pain remains challenging to understand and manage. The high prevalence of atypical presentation leading to 26% of MI patients can present

 doi:10.20944/preprints202510.0878.v1

without typical chest pain [12]. For example, many women, elderly patients, and people with diabetes experience "silent" ischemia, presenting with minimal or no pain [13]. Up to one-third of myocardial infarctions may be clinically silent [14]. Besides, the paucity of cardiac sensory endings and the convergence of visceral afferents in the spinal cord lead to imprecise pain perception [15]. Patients may have difficulty pinpointing heart pain, and clinicians may confuse it with non-cardiac causes of chest pain (such as gastrointestinal or musculoskeletal pain) [16]. Likewise, cardiac pain can manifest as shortness of breath, fatigue or jaw/arm discomfort, which complicates diagnosis and delays treatment [14]. This neuroanatomical complexity obscures the link between symptoms and underlying myocardial events [15].

Traditional anti-ischemic drugs have shown limited efficacy in certain patient populations, while anxiolytics and antidepressants, despite their potential to improve psychological well-being, have yet to demonstrate definitive benefits in the direct modulation of non-ischemic cardiac pain [17]. Agents like nitrates, beta-blockers, and revascularization procedures improve blood flow but do not directly address nociceptive signaling [18]. Opioids and analgesics are used cautiously in acute coronary syndromes due to hemodynamic side effects [19]. For chronic angina, options (e.g. ranolazine, nerve stimulation) are limited and often fail to fully alleviate pain [20,21]. Moreover, existing animal models and cell cultures fail to replicate the complex crosstalk between human cardiomyocytes and sensory neurons, and no robust "disease-in-a-dish" model of ischemic chest pain currently exists. This gap hampers translational efforts to identify novel analgesics and cardioprotective strategies.

In summary, diagnosing and treating cardiac-related pain is hindered by variable clinical manifestations, incomplete understanding of pain mechanisms, and an absence of suitable human models. These challenges highlight an urgent need for innovative platforms—such as induced pluripotent stem cell (iPSC)-based systems—to elucidate cardiac nociception and accelerate the development of targeted treatments.

1. Role of Induced Pluripotent Stem Cells (iPSCs) in Regenerative Medicine and Pain Research

iPSCs offer a transformative platform to overcome many of the above challenges [22]. iPSCs are generated by reprogramming adult somatic cells (e.g. skin fibroblasts or blood cells) back to a pluripotent state, as first demonstrated by Takahashi and Yamanaka [23]. This allows creation of patient-specific stem cells without ethical issues associated with embryonic sources [24].

Because they retain the donor's genetic makeup, iPSCs enable personalized disease modeling; for example, cardiomyocytes derived from individuals with heart disease can replicate patient-specific mutations or risk factors [25]. Their pluripotency allows differentiation into virtually any cell type, including functional cardiomyocytes and peripheral sensory neurons, using established protocols to generate beating heart cells or nociceptive neurons in vitro [26–28]. iPSCs are also scalable and amenable to genetic modification with tools like CRISPR, making them ideal for large-scale mechanistic studies and drug screening [28]. Crucially, iPSC-based models bypass interspecies differences, enabling human-specific "disease-in-a-dish" systems that have already advanced research into inherited cardiac conditions and neuropathic pain syndromes under controlled laboratory settings [29,30].

In cardiovascular research, iPSC-derived cardiomyocytes (iPSC-CMs) have proven invaluable [31–33]. These cells beat spontaneously in culture and recapitulate many features of human heart cells [31]. Patient-specific iPSC-CMs have been used to model genetic cardiomyopathies, channelopathies, and the cardiotoxic effects of drugs [34–36]. In experimental studies, transplantation of iPSC-CMs into infarcted hearts of animals has shown improved cardiac function, illustrating their regenerative potential [37]. Such advances position iPSCs as a promising avenue for repairing damaged myocardium and restoring cardiac function.

Importantly for pain research, iPSCs have also been harnessed to generate human sensory neurons [38–40]. Protocols involving small molecules or transcription factors can produce iPSC-

 doi:10.20944/preprints202510.0878.v1

derived nociceptors that express key pain-sensing ion channels (such as Nav1.7 and Nav1.8) [41,42]. These iPSC-derived nociceptors have been used to model inherited pain disorders and to test analgesic compounds, faithfully reflecting patient phenotypes in vitro. By combining iPSC technologies, it is now conceivable to create co-culture systems or organoid models that integrate patient-derived cardiomyocytes and sensory neurons [43]. Such integrated models could mimic how ischemic myocardium communicates with cardiac afferents to produce pain.

Overall, iPSC technology provides a new paradigm for studying cardiac-related pain. By enabling generation of human cardiomyocytes, neurons, and hybrid tissues in the laboratory, iPSCs bridge the gap between bench and bedside. This approach holds promise not only for unravelling the cellular mechanisms of cardiac nociception, but also for screening cardioprotective and analgesic agents on patient-specific tissue.

Table 1. Clinical Forms of Cardiac Pain.

Clinical form	Pain quality / triggers / duration	Underlying pathophysiology	Distinguishing clinical / diagnostic clues	iPSC model relevance (recommended)
Stable angina	Pressure/squeezing; exertion- or stress-provoked; relieved by rest/nitrates	Demand–supply mismatch due to fixed epicardial atherosclerotic stenosis → transient ischemia	Predictable exertional pattern; ischemic ECG changes on stress testing; obstructive lesions on angiography	Patient iPSC-derived cardiomyocytes (iPSC-CMs) for ischemic stress assays, drug response and arrhythmia testing; patient-specific cardiotoxicity screens. [31–37]
Unstable angina / ACS / MI	Severe, prolonged (>20 min), may occur at rest; autonomic features common	Acute plaque rupture with partial/complete coronary occlusion → sustained ischemia and myocyte injury	Dynamic ECG changes; troponin rise (MI); urgent coronary imaging/intervention required	iPSC-CMs to model ischemia–reperfusion injury and cardioprotective drug screens. [31,34,37]
Microvascular angina / Cardiac syndrome X	Angina-like pain with normal epicardial coronaries; exertional or spontaneous episodes	Coronary microvascular dysfunction (endothelial or small-vessel dysfunction) causing ischemia despite patent large arteries	Normal angiogram; abnormal microvascular testing or ischemia on stress testing; higher prevalence in women	iPSC-derived endothelial cells, microvascular organ-on-chip models and co-cultures to study microvascular physiology and test endothelium-targeted therapies. [28,31–33]
Pericarditis	Sharp, pleuritic retrosternal pain; worse with inspiration or supine, relieved by sitting/leaning forward	Pericardial inflammation (infectious, autoimmune, post-MI, uremic, etc.)	Positional pain, pericardial friction rub, diffuse ST elevation on ECG; responds to anti-inflammatories	iPSC co-cultures of cardiomyocytes with immune cells and/or mesothelial cell models to investigate inflammatory signaling and anti-inflammatory interventions. [22,24]
Aortic dissection	Sudden, severe tearing/ripping chest or back pain; radiates; maximal at onset	Intimal tear with blood dissecting between aortic layers →	Sudden onset; asymmetric limb blood pressures; widened mediastinum on	iPSC-derived vascular smooth muscle cells (VSMCs) and ECM models for studying chronic aortopathy

 doi:10.20944/preprints202510.0878.v1

		catastrophic structural failure	imaging; surgical emergency	mechanisms; less useful for acute diagnosis but informative for genetic predisposition research. [22,31]
Referred / ischemia-related neuropathic pain	Pain referred to arm, jaw, neck or back; may be atypical (epigastric/back), common in women/elderly/diabetics	Activation and sensitization of cardiac afferent fibers during ischemia; spinal/central convergence produces referred somatic pain	Atypical distribution; often requires ECG/biomarkers to rule in/out cardiac cause	Co-culture organoids of iPSC-CMs with iPSC-derived nociceptors (nociceptors express Nav1.7/1.8) to model neuron–cardiomyocyte cross-talk and test analgesics. [38–43]
Postoperative / chronic chest pain	Persistent localized or referred chest pain after surgery or myocardial injury; may have burning or hyperalgesia	Nerve injury, scar, chronic inflammation and central/peripheral sensitization	Temporal relation to surgery; neuropathic descriptors; exclude recurrent ischemia	iPSC nociceptor models and inflamed co-culture systems to study chronic sensitization and screen neuromodulatory/analgesic compounds. [38–43]

2. Pathophysiological Mechanisms of Cardiac-Related Pain

2.1. Ischemic Myocardial Injury and Nociceptive Signaling Networks

Coronary oxygen supply-demand imbalance triggers a metabolic crisis that amplifies nociceptive signaling cascades. When myocardial oxygen imbalance exceeds a critical threshold (typically >15 minutes), ATP depletion-induced ionic homeostasis collapse initiates a biphasic pathological process [44,45]. The early phase (20–60 minutes) is characterized by calcium overload-mediated contraction band necrosis, while the late phase involves irreversible damage due to sustained mitochondrial permeability transition pore opening [46]. Notably, this injury exhibits spatial heterogeneity, preferentially affecting hemodynamically vulnerable regions (e.g., subendocardial myocardium and papillary muscles) and forming a "wave-like" necrosis pattern extending toward the epicardium [44].

Dynamic oxygen gradient experiments using iPSC-derived cardiomyocyte models demonstrate that reperfusion injury arises from a spatial and temporal discord between the restoration of energy metabolism and the induction of oxidative stress [47,48]. The early reperfusion period produces reactive oxygen species (ROS) which modify vital contractile proteins like troponin I through oxidation and simultaneously cause peripheral sensitization by activating nociceptive ion channels such as TRPV1/TRPA1 [49–51]. Silent nociceptors in cardiac dorsal root ganglia become active through ASIC3 channels when lactate/proton accumulation creates a "metabolic fingerprint" which serves as molecular targets for iPSC-based neuro-cardiac co-culture systems in pain transmission research [52–54].

Of particular significance, myocardial stunning and hibernation may constitute the microenvironmental basis for chronic angina [55,56]. Emerging evidence from iPSC-engineered myocardial tissue models has elucidated the critical role of intermittent hypoxia in driving HIF-1α-dependent metabolic reprogramming. This adaptive response stimulates sustained nerve growth factor (NGF) secretion, which has been mechanistically linked to aberrant sprouting and hypersensitivity of cardiac afferent nerves [56,57]. Such findings underscore the potential of iPSC-based platforms to recapitulate neuro-cardiac crosstalk, offering a robust framework for identifying neuromodulatory targets through high-throughput drug screening [53,55,58].

 doi:10.20944/preprints202510.0878.v1

2.2. Post-Myocardial Infarction Neuro-Immune Crosstalk

Mitochondrial DNA and HMGB1 released from the infarct core serve as initial signals for neuroimmune communication [59]. The dual activation mechanism of the TLR4/MyD88 axis warrants attention: while initiating an inflammatory storm via NF-κB, it directly activates TLR4+ sensory neurons in dorsal root ganglia [59]. Co-culture experiments with iPSC-derived microglia reveal that IL-1β not only lowers nociceptive neuron activation thresholds through the COX-2/PGE2 pathway but also induces epigenetic modifications of Nav1.8 sodium channels, forming the molecular basis of pain memory [60,61].

Neutrophil infiltration exhibits a dynamic double-edged effect: early-stage MMP-9-mediated perineural degradation facilitates nociceptive signal transmission, while later stages involve resolving D1 secretion to promote inflammation resolution [62]. Recent single-cell sequencing studies identified specific macrophage subsets (CD9+ Trem2+) in infarcted regions that secrete brain-derived neurotrophic factor (BDNF), triggering central sensitization via TrkB receptors [63,64]. This suggests that iPSC-differentiated regulatory macrophages may serve as therapeutic vectors to disrupt neuroimmune vicious cycles.

2.3. Metabolic-Autonomic Axis Dysregulation

Diabetic cardiac autonomic neuropathy fundamentally arises from Schwann cell energy crisis induced by hyperglycemic microenvironments. Mitochondrial pyruvate carrier (MPC) dysfunction leads to impaired lactate shuttling, while endoneurial hypoxia causes paradoxical VEGF downregulation, creating a "dual energy failure" pathology [65,66]. Chemotherapeutic agents (e.g., paclitaxel) synergistically amplify diabetic damage by stabilizing microtubules and disrupting mitochondrial dynamin-related protein (DRP1) shuttling [67].

Advancing iPSC-based models have delineated the temporal dynamics of post-injury sympathetic remodeling, revealing distinct phases of neural plasticity. Early-phase responses (2–3 weeks post-injury) are marked by tyrosine hydroxylase-positive (TH+) nerve hyperinnervation, whereas chronic stages (>8 weeks) exhibit progressive epigenetic suppression of the NGF/TrkA trophic signaling axis [57,68]. These temporal shifts correlate with β-adrenergic receptor desensitization patterns, creating phase-specific therapeutic opportunities—notably, stem cell-derived exosome miR-133 has emerged as a promising modulator of maladaptive neural circuit reorganization [69].

Notably, integrated iPSC-neuromuscular platforms have uncovered IL-6/JAK2/STAT3-mediated dysregulation of neuro-cardiac electrophysiological coupling. This mechanistically links neuroinflammatory signaling to arrhythmogenic pain pathways, providing a plausible explanation for the chest pain symptomatology observed in rhythm disorders [70].

3. iPSC-Derived Models for Studying Cardiac Pain

The utilization of induced pluripotent stem cells (iPSCs) within cardiovascular research is an innovative approach for the comprehension and management of cardiac pain. iPSCs are derived from adult cells that have been reprogrammed to an embryonic-like state, thereby acquiring pluripotency, meaning that they can differentiate into most kind of cells in the human body, including cardiac cells [70]. Differentiation protocols have been developed to induce differentiation of iPSCs into human cardiomyocytes (CMs) with high yield and purity. These iPSC-derived human cardiomyocytes are very similar to naturally occurring CMs in terms of structure and functions, and therefore offers numerous advantages for studying cardiac pain, as it provides a method to explore the mechanisms underlying cardiac disease in details, facilitating novel drug discovery and testing for drug toxicity, as well as a promising source for regenerative therapy for the diseased human heart [58,70].

 doi:10.20944/preprints202510.0878.v1

3.1. iPSC-Derived Cardiomyocytes for Modeling Cardiac Diseases

One of the primary applications of iPSCs is the creation of patient-specific cardiac disease models. Researchers can generate iPSCs from patients suffering from various cardiac conditions and differentiate these cells into cardiomyocytes. These patient-specific cardiomyocytes can subsequently be utilized to study the pathophysiology, cellular and molecular mechanisms of various cardiac diseases and related pain. Numerous inherited cardiac disorders such as channelopathies and arrhythmogenic syndromes have been successfully modelled by iPSC-derived CMs. These include familial long QT syndrome, arrhythmogenic right ventricular dysplasia and catecholaminergic polymorphic ventricular tachycardia. Cardiomyopathies such as hypertrophic cardiomyopathy and dilated cardiomyopathy involve dysfunctional sarcomeric and cytoskeletal proteins, have also been studied using iPSC-derived CMs [55,70].

3.2. Drug Screening and Testing

iPSC-derived CMs enable researchers test therapeutic drugs and develop new treatment strategies in a personalized manner. Traditional methods of drug discovery often rely on animal models, which may not accurately reflect human physiology. By using iPSC-derived cardiomyocytes, researchers can test the efficacy and safety of new drugs on human cardiomyocytes or even heart models. This approach can help identify compounds that improve heart function and alleviate cardiac pain. Additionally, it allows for the assessment of drug toxicity, thereby reducing the risk of adverse effects in clinical trials [58,70].

3.3. iPSC-Derived Sensory Neurons (iPSC-SNs)

Traditionally, pain mechanisms were studied in animal models which may not reflect the exact mechanisms and physiology in human pain due to species-related differences and also contextual differences which generate the pain. iPSC can be induced to differentiate into sensory neurons. This provides a new avenue for studying pain mechanisms, electrophysiology and pathophysiology in human sensory neurons. Scientists has also used multicellular strategies mimicking in vivo micorenvironments, improving cell contact and communication (co-culture), and developing three-dimesional organoid structure of sensory neurons to closely resemble native human sensory system functions. Like iPSC-CMs, iPSC-SNs can also be deployed for screening potential novel therapeutic drugs and testing for drug toxicity [55,58].

4. Therapeutic Applications of iPSCs in Cardiac Pain

Apart from studying cardiac diseases and pain processes, iPSC-CM is also implicated as a direct therapeutic agent for cardiac disease and pain. Stem cell therapy aims to enhance the repair and regeneration of damaged heart tissues, thereby improving blood flow and reducing angina symptoms.

4.1. Cell-Based Cardiac Regeneration

Several preclinical animal studies have shown that cardiac stem cell therapy was feasible and effective in improving cardiac function. Chong et al. injected 1 billion human PSC derived CMs intra-myocardially in to adult macaque monkey heart 2 weeks after acute myocardial ischemia reperfusion injury [71]. Over the next 3 months, it was found that a significant proportion of of the injected PSC-CM survived and were able to form electrical coupling with host myocardium. No teratoma formation or off-target effects were detected. Zhu et al. examined the effect of human PSC-derived cardiovascular progenitor cells (PSC-CVPC) on myocardial infarction in cynomolgus monkeys. In this study, they injected PSC-CVPC into the monkey heart 30 minutes after induction of MI [72]. However, they could not detect any transplanted cells or remuscularization of infarcted heart at 20 weeks after transplantation. The left ventricular ejection fraction improved from 37.5% to 43.5%. The discrepancy might be due to differences in the disease model (I/R vs. MI), timing of cell delivery (2

 doi:10.20944/preprints202510.0878.v1

weeks vs. 30 minutes after ischemic injury), the type and the dose of transplanted cells (1 billion hESC-CMs vs 10 million hPSC-CVPCs) [72].

4.2. Paracrine Effects of Stem Cell Therapy

Various studies have shown that functional recovery can be achieved with stem cell transplantation in myocardial infarction, despite suboptimal survival of the transplanted cells. This leads to the concept of paracrine effects of the stem cells which promotes recovery of the diseased host myocardium. Increased angiogenesis and reduced apoptosis of host myocardium were observed after iPSC-CM transplantation [73]. Anti-apoptotic factors (such as tumour necrosis factor alpha), pro-angiogenic factors (such as interleukin-8, placental growth factor-1, vascular endothelial growth factor), pro-cell migration factors (such as stromal cell-derived factor-1 a , TNF-a, vascular cell adhesion protein 1 VCAM-1, and plasminogen activator inhibitor-1 PAI-1) among other cytokines were detected after iPSC-CM transplantation [74]. These paracrine factors play a significant role in functional recovery of host myocardium even in the absence of successful engraftment of the transplanted iPSC-CM.

4.3. Bioengineering of iPSC-CM to Enhance Therapeutic Effects

One problem with stem cells therapy is the low survival rate of the transplanted cells, partly due to the harsh environment in host myocardium which is often ischemic and possibly with non-functional scar tissues [75]. Two major forms of engineered PSC-CM has been developed to counter the problem, namely injectable biomaterials and engineered cardiac tissue patch [76]. Improved survival of PSC-CMs in these engineered forms increase the chance of mechanical and electrical coupling with host myocardium and prolong paracrine secretion, thereby further improving heart function [77].

Injectable biomaterials or hydrogels contain extracellular matrix proteins such as matrigel, fibrin and collagen as scaffold material. The scaffold material encapsulates the iPSC-CM and promotes the survival and engraftment of transplanted iPSC-CM in host myocardium [73].

Cell sheets of PSC-CM can be stacked together to generate three-dimensional cardiac tissue patch. Initially the number of layers of cell sheet was limited due to inadequate perfusion causing insufficient oxygen and nutrient delivery to the cells [78]. With the use of omental flap which improves blood supply, cell sheets of seven layers have been transplanted into infarcted pig hearts which significantly improved heart contractility from LVEF of 36.8% to 52.1% [79].

3D printing technology has also been used in cardiac tissue engineering and to generate cardiac tissue patch that closely resembles native human heart tissue. Gao et al. used 3D printing technique to produce a cardiac muscle patch containing hiPSC-CMs, hiPSC-ECs, and iPSC-SMCs in 2:1:1 ratio, within a ECM-based scaffold [80]. When transplanted onto the heart, the cardiac muscle patch increased contraction speed and calcium handling in the infarcted myocardium of mice. Graft survival and improved cardiac function were also confirmed 1 month after graft transplantation [81].

Although these tissue-engineered patches have multiple benefits for cell survival, they have limitations such as the need for open-chest surgery, non-migration of transplanted cells into host myocardium, arrhythmogenicity and biodegradability of scaffold biomaterials.

4.4. MicroRNAs and Cell Survival

MicroRNAs have also been shown to have therapeutic role in cardiac regeneration. MicroRNAs are small (20–25 nucleotides), non-coding RNAs that regulatepost-transcriptional gene expression [82]. Some of them promotes angiogenesis and cell survival, while others promote apoptosis. MicroRNA-126 and microRNA-155 overexpression in stem cells prevent cell apoptosis and promote cell survival, migration and angiogenesis [83,84]. On the other hand, microRNA-15, microRNA-34, microRNA-140 and microRNA-320, negatively influenced CM survival and proliferation [85,86]. Inhibition of these microRNAs promotes cell survival and heart regeneration. In vivo manipulation

 doi:10.20944/preprints202510.0878.v1

of the microRNAs has resulted in CM dedifferentiation and improved heart functionality after injury [87]. This provides evidence that microRNA modification and manipulation can be a potential therapeutic target to promote CM survival, whether as a stand-alone therapy or combined therapy with iPSC-CM.

4.5. Exosomes: A Potential Cell-Free Regenerative Therapy

Stem cell-conditioned medium has been shown to promote heart regeneration, further supporting the paracrine hypothesis [88]. Stem cells produce cytokines, growth factors and membranous vesicles called exosomes, which aid intercellular communication [89]. Exosomes are small vesicles that are natural carriers of mRNA, microRNA, and proteins among different cells. They are formed from fusion of multivesicular bodies with the plasma membrane of stem cells [89,90]. They have cardioprotective functions by means of promoting angiogenesis, reducing fibrosis, and preventing apoptosis.

Mouse ESC-derived exosomes have improved heart function and cell proliferation in myocardial infarction (MI) models. MicroRNA-294 in the exosomes was implicated and hypothesized to play a significant role in cardiac regeneration [91]. Similarly, exosomes derived from cardiosphere-derived cells, rich in microRNA-146a, have shown regenerative potential in damaged heart muscles [91].

Extensive ongoing research explores the use of exosomes as an alternative to cell-based stem cell therapy, as the use of exosome itself eliminates some of the major problems associated with stem-cells, such as chromosomal instability, tumorigenicity and scalability [92,93]. While exosomes offer a promising cell-free therapy alternative to stem cells, challenges like exosome-mediated immune reactions and incomplete understanding of their repair mechanisms remain [94].

Table 2. Maturation Strategies for iPSC-CMs.

Strategy	Mechanistic rationale	Typical implementation / examples	Maturity endpoints improved	Key limitations
Electrical pacing / chronic stimulation	Activity-dependent electrophysiological remodeling	Long-term field stimulation in plates or bioreactors (weeks)	AP waveform, ion-channel expression, synchronous contractions	Requires specialized hardware; long culture periods
Hormonal / biochemical supplementation	Recreates endocrine cues of late development	T3/T4, glucocorticoids, IGF, thyroid hormones	Sarcomeric organization, Ca^{2+} handling, metabolic markers	Requires dose/timing optimization; partial effect alone
Substrate engineering / stiffness tuning	Mechanical cues drive structural/contractile maturation	Tunable hydrogels, micropatterned substrates, stiffness matching	Cell alignment, force generation, improved E–C coupling	Material biocompatibility; scale-up challenges
3D culture / tissue engineering	Enhances cell–cell and cell–matrix interactions	Cardiac organoids, engineered heart tissues, stacked cell sheets	Mature sarcomeres, higher contractility, improved metabolism	Perfusion/oxygenation limits; complex assays
Co-culture with non-myocytes	Paracrine and structural support from stromal cells	Endothelial cells, fibroblasts, macrophages co-culture	Vascular cues, ECM deposition, improved electrophysiology	Variable cell ratios; increased complexity
Long-term culture + metabolic conditioning	Drives metabolic switch toward FA oxidation	Extended culture; fatty-acid supplementation; mitochondrial modulators	Mitochondrial maturation, adult-like metabolism	Time-consuming; genomic stability concerns
Bioreactor / perfusion systems	Scalable mechanical, nutritional control and shear	Stirred, perfused, or microfluidic bioreactors	More homogeneous maturation; higher yields	Costly setup; process optimization required

 doi:10.20944/preprints202510.0878.v1

Table 3. Current Therapeutics vs. iPSC-Based Opportunities.

Strategy	Mechanistic rationale	Typical implementation / examples	Maturity endpoints improved	Key limitations
Electrical pacing / chronic stimulation	Activity-dependent electrophysiological remodeling	Long-term field stimulation in plates or bioreactors (weeks)	AP waveform, ion-channel expression, synchronous contractions	Requires specialized hardware; long culture periods
Hormonal / biochemical supplementation	Recreates endocrine cues of late development	T3/T4, glucocorticoids, IGF, thyroid hormones	Sarcomeric organization, Ca^{2+} handling, metabolic markers	Requires dose/timing optimization; partial effect alone
Substrate engineering / stiffness tuning	Mechanical cues drive structural/contractile maturation	Tunable hydrogels, micropatterned substrates, stiffness matching	Cell alignment, force generation, improved E–C coupling	Material biocompatibility; scale-up challenges
3D culture / tissue engineering	Enhances cell–cell and cell–matrix interactions	Cardiac organoids, engineered heart tissues, stacked cell sheets	Mature sarcomeres, higher contractility, improved metabolism	Perfusion/oxygenation limits; complex assays
Co-culture with non-myocytes	Paracrine and structural support from stromal cells	Endothelial cells, fibroblasts, macrophages co-culture	Vascular cues, ECM deposition, improved electrophysiology	Variable cell ratios; increased complexity
Long-term culture + metabolic conditioning	Drives metabolic switch toward FA oxidation	Extended culture; fatty-acid supplementation; mitochondrial modulators	Mitochondrial maturation, adult-like metabolism	Time-consuming; genomic stability concerns
Bioreactor / perfusion systems	Scalable mechanical, nutritional control and shear	Stirred, perfused, or microfluidic bioreactors	More homogeneous maturation; higher yields	Costly setup; process optimization required

5. Challenges and Future Perspectives

Although the application of induced pluripotent stem cells (iPSCs) in cardiomyocyte modelling and myocardial disease research holds significant promise, several critical limitations remain—particularly with regard to cell heterogeneity and maturation.

First, standard differentiation protocols often yield a mixed population of cardiomyocyte subtypes—including ventricular, atrial, and nodal-like cells—each with distinct electrophysiological and contractile properties [95]. This subtype variability can obscure disease-specific phenotypes and complicate the interpretation of functional assays. Additionally, batch-to-batch inconsistencies and clonal variability hinder experimental reproducibility and may exaggerate or mask disease-relevant signals [96,97]. Such heterogeneity presents a major obstacle in complex co-culture systems that aim to model nociceptor–cardiomyocyte interactions. Variations in subtype composition and maturation state can produce inconsistent results in pain-relevant assays, making it difficult to isolate genuine biological effects from experimental noise.

These challenges underscore the need for more refined differentiation protocols, rigorous quality control benchmarks, and subtype-specific purification strategies to fully realize the potential of iPSC-derived cardiomyocytes in both basic research and translational applications.

Another persistent limitation is the immature, fetal-like phenotype retained by iPSC-derived cardiomyocytes [98]. Compared to adult cardiomyocytes, they exhibit different action potential durations and immature ion-channel expression profiles, which can increase susceptibility to arrhythmias [99]. Their calcium handling apparatus also remains underdeveloped, with reduced sarcoplasmic reticulum density and diminished levels of key regulators such as SERCA2a and ryanodine receptors, resulting in inefficient excitation–contraction coupling [100].

Moreover, iPSC-derived cardiomyocytes often display a metabolic profile that diverges from adult physiology. Notably, there is a shift from fatty acid oxidation to glycolysis, accompanied by mitochondrial dysfunction. This metabolic immaturity compromises the cells' ability to model stress

 doi:10.20944/preprints202510.0878.v1

responses such as those triggered by ischemia–reperfusion injury. Consequently, modeling pain-related triggers—such as reperfusion-induced calcium overload or oxidative damage—may yield misleading conclusions if the metabolic and contractile machinery does not faithfully mimic adult pathophysiology. Strategies such as electrical pacing, hormonal supplementation, and substrate engineering are essential to enhance functional maturation [101].

Scaling up the production of iPSC-derived cardiomyocytes also poses a significant challenge [102]. Most differentiation protocols generate only limited numbers of cardiomyocytes—typically in the low millions—which is insufficient for high-throughput drug screening or therapeutic applications. Furthermore, reliance on costly growth factors and small molecules inflates production costs. While cell surface markers like SIRPA and VCAM1 are used for enrichment through fluorescence-activated or magnetic-activated sorting, these methods may introduce shear stress and affect cell viability or phenotype [103]. To overcome these issues, more efficient differentiation matrices, bioreactor systems, and marker-free purification strategies must be developed to improve both yield and quality.

Despite progress, there is currently no consensus on standardized assays for evaluating "pain-relevant" cardiac endpoints. Critical molecular mediators—such as TRPV1 channels, which link nociceptive signaling to cardiomyocyte function—are rarely quantified during differentiation [104]. Likewise, the establishment of neuro-cardiac synapses, key for modeling sensory–myocyte cross-talk, lacks reliable in vitro benchmarks. Commonly used functional readouts, such as calcium transients or contractility, do not necessarily reflect clinically observed pain manifestations like ischemia-induced chest pain or arrhythmogenic discomfort [105]. Developing reproducible, scalable assays that can bridge in vitro findings with in vivo pain phenotypes will be critical for validating future therapeutic targets.

Furthermore, generating iPSC-derived cardiomyocytes that accurately emulate adult heart tissue typically requires prolonged culture periods—often three to four weeks—to reach a late fetal-like stage [27]. During this time, the absence of a supportive cardiac microenvironment, including fibroblasts, endothelial cells, and an appropriate extracellular matrix, limits structural and functional maturation [106]. Extended culture also increases the risk of genomic instability, including chromosomal aberrations and copy number variations, which may compromise reproducibility and safety [107]. Addressing these issues requires the development of co-culture systems, engineered biomaterials that replicate myocardial stiffness, and robust genomic surveillance protocols.

5.2. Ethical and Safety Concerns in Ipsc-Based Therapies

The reprogramming of somatic cells into iPSCs introduces several safety and ethical challenges, particularly when integrating viral vectors or transposons are used. These methods may induce genomic alterations—including single-nucleotide mutations and chromosomal aberrations—that could predispose derived cardiomyocytes to malignant transformation [108,109]. In addition, from the donor cell type can result in aberrant methylation patterns that persist through differentiation, potentially altering gene expression in ways that affect safety and function. Of particular concern is the presence of undifferentiated iPSCs, which carry a well-established risk of teratoma formation after transplantation [110]. Therefore, comprehensive genomic and epigenomic screening at multiple production stages is essential to mitigate these risks.

Although autologous iPSC-based therapies offer the theoretical advantage of immune compatibility, their clinical application is constrained by the time, cost, and complexity involved in generating patient-specific lines [111,112]. As an alternative, allogeneic iPSC banks with selected HLA haplotypes provide a scalable "off-the-shelf" solution [113]. However, even partially matched iPSCs may trigger alloimmune responses or activate innate immunity. Approaches such as partial HLA matching or CRISPR/Cas9-mediated deletion of immunogenic loci are under exploration, though each introduces its own set of technical, regulatory, and ethical challenges [114]. Comprehensive preclinical evaluation of both humoral and cellular immune responses is necessary to ensure long-term graft survival without chronic immunosuppression [115].

 doi:10.20944/preprints202510.0878.v1

The use of donor cells for iPSC generation also raises important ethical considerations regarding informed consent. Donors must be fully aware of the potential uses of their cells—including commercial applications and future genomic analyses—and how their genetic information will be stored, shared, and protected. Adherence to regulatory frameworks such as the General Data Protection Regulation (GDPR) and evolving FDA guidelines is essential for maintaining public trust and supporting large-scale biobanking initiatives [116].

Regulatory frameworks for iPSC-based therapies are still evolving. Agencies such as the U.S. FDA and the European Medicines Agency (EMA) require adherence to Good Manufacturing Practice (GMP) standards during iPSC derivation, cardiac differentiation, and cryopreservation, along with comprehensive safety assessments covering genomic integrity, residual pluripotent cells, and tumorigenicity [117,118]. Clinical trial protocols must also include long-term monitoring and adverse event reporting. Yet, the criteria for determining "acceptable risk" are not fully standardized. Collaborative efforts among academic institutions, industry partners, and regulators are necessary to harmonize guidelines and expedite the safe clinical translation of iPSC technologies.

Finally, while genome editing tools such as CRISPR/Cas9 offer powerful avenues for enhancing cardiomyocyte function or improving stress resilience, they also raise serious ethical concerns. Off-target effects and the potential for dual-use applications necessitate strict oversight [119]. It is imperative that genome editing be used strictly for therapeutic purposes rather than cosmetic or non-medical enhancements. Equitable access to these advanced therapies must also be a priority to avoid exacerbating existing health disparities and to ensure that the benefits of iPSC technologies are accessible to diverse patient populations.

6. Conclusion

Cardiac-related pain arises predominantly from myocardial ischemia and manifests in diverse clinical forms—from stable angina and microvascular angina to acute myocardial infarction and pericarditis [5]. Its diffuse, poorly localized nature stems from sparse cardiac innervation and convergence of visceral and somatic pathways, often leading to atypical presentations and diagnostic challenges. Traditional pharmacotherapies improve perfusion but do not directly address nociceptive signaling, and existing preclinical models fail to recapitulate human neuro-cardiac interactions. iPSC-based platforms overcome many of these barriers: patient-derived cardiomyocytes faithfully model genetic and metabolic contributors to ischemic injury, while iPSC-derived sensory neurons capture human nociceptor physiology [120]. Co-culture and organoid systems further enable mechanistic dissection of neuro-cardiac crosstalk and high-throughput screening of analgesic candidates [121].

Harnessing iPSC models promises to shift cardiac pain management from symptom control toward mechanism-driven interventions. By integrating human iPSC-CMs and nociceptors, researchers can pinpoint novel targets—such as specific ion channels or neurotrophic pathways—that mediate ischemia-induced sensitization. Improved in vitro assays will facilitate more predictive drug screening, reducing reliance on animal models and accelerating translation. Moreover, patient-specific iPSC lines enable personalized risk stratification and may guide individualized therapy choices, particularly in populations prone to silent ischemia. Ultimately, combining regenerative approaches with targeted neuromodulation could yield hybrid treatments that both repair damaged myocardium and dampen maladaptive nociception.

Induced pluripotent stem cells represent a paradigm shift in cardiac pain research and therapy. Beyond modelling, iPSC-derived cardiomyocytes offer prospects for cell-based regeneration of ischemic myocardium, while iPSC-engineered sensory neurons enable unprecedented study of human pain pathways. Advances in bioengineering, genome editing, and co-culture technologies will refine these systems, paving the way for "disease-in-a-dish" platforms that predict clinical outcomes and identify safe, effective analgesic and cardioprotective agents. As protocols mature and standardize, iPSC approaches may transition from the bench to first-in-human trials—ushering in an

 doi:10.20944/preprints202510.0878.v1

era where cardiac pain is not merely managed, but fundamentally understood and treated at its source.

Author Contributions: Dr. LIAO Boya and Prof. LEE Kin-ho Aaron1, Prof. WONG, Sau-ching Stanley: conceptualization, supervision, writing—review and editingMENG Fei1, WU Jiangwei1, YANG Jinghan1, 1#; Mr. Mengfei: researched the data, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: No funding was received to perform this study.

Data Availability Statement: No novel data was generated in the writing of this review article.

Conflicts of Interest: The authors declare no competing interests.

References

1. Pasceri V, Lanza GA, Buffon A, Montenero AS, Crea F, Maseri A. Role of abnormal pain sensitivity and behavioral factors in determining chest pain in syndrome X. J Am Coll Cardiol. 1998;31(1):62–6.
2. Schindler TH, Dilsizian V. Coronary Microvascular Dysfunction: Clinical Considerations and Noninvasive Diagnosis. JACC Cardiovasc Imaging. 2020;13(1 Pt 1):140–55.
3. Shapiro LM, Crake T, Poole-Wilson PA. Is altered cardiac sensation responsible for chest pain in patients with normal coronary arteries? Clinical observation during cardiac catheterisation. Br Med J (Clin Res Ed). 1988;296(6616):170–1.
4. Pastena P, Frye JT, Ho C, Goldschmidt ME, Kalogeropoulos AP. Ischemic cardiomyopathy: epidemiology, pathophysiology, outcomes, and therapeutic options. Heart Fail Rev. 2024;29(1):287–99.
5. Leach A, Fisher M. Myocardial ischaemia and cardiac pain - a mysterious relationship. Br J Pain. 2013;7(1):23–30.
6. Crea F, Camici PG, Bairey Merz CN. Coronary microvascular dysfunction: an update. Eur Heart J. 2014;35(17):1101–11.
7. Zhang JW, Guo Z. [Changes in substance P in myocardium and dorsal root ganglion following coronary artery occlusion in rats]. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2006;18(4):201–5.
8. Foreman RD, Qin C. Neuromodulation of cardiac pain and cerebral vasculature: neural mechanisms. Cleve Clin J Med. 2009;76 Suppl 2:S75–9.
9. Myers DE. Vagus nerve pain referred to the craniofacial region. A case report and literature review with implications for referred cardiac pain. Br Dent J. 2008;204(4):187–9.
10. Altintas E, Yigit F, Taskintuna N. The impact of psychiatric disorders with cardiac syndrome X on quality of life: 3 months prospective study. Int J Clin Exp Med. 2014;7(10):3520–7.
11. Cannon RO, 3rd, Quyyumi AA, Schenke WH, Fananapazir L, Tucker EE, Gaughan AM, et al. Abnormal cardiac sensitivity in patients with chest pain and normal coronary arteries. J Am Coll Cardiol. 1990;16(6):1359–66.
12. Uretsky BF, Farquhar DS, Berezin AF, Hood WB, Jr. Symptomatic myocardial infarction without chest pain: prevalence and clinical course. Am J Cardiol. 1977;40(4):498–503.
13. Johnson BD, Shaw LJ, Pepine CJ, Reis SE, Kelsey SF, Sopko G, et al. Persistent chest pain predicts cardiovascular events in women without obstructive coronary artery disease: results from the NIH-NHLBI-sponsored Women's Ischaemia Syndrome Evaluation (WISE) study. Eur Heart J. 2006;27(12):1408–15.
14. Parry M, Bjørnnes AK, Victor JC, Ayala AP, Lenton E, Clarke H, et al. Self-Management Interventions for Women With Cardiac Pain: A Systematic Review and Meta-analysis. Can J Cardiol. 2018;34(4):458–67.
15. Wang Y, Zeng XL, Gao RR, Wang XM, Wang XT, Zheng GQ. Neurogenic hypothesis of cardiac ischemic pain. Med Hypotheses. 2009;72(4):402–4.
16. Kachintorn U. How do we define non-cardiac chest pain? J Gastroenterol Hepatol. 2005;20 Suppl:S2–5.
17. Szczepanska-Sadowska E. Neuromodulation of Cardiac Ischemic Pain: Role of the Autonomic Nervous System and Vasopressin. J Integr Neurosci. 2024;23(3):49.
18. Piegza M, Wierzba D, Piegza J. Cardiac syndrome X - the present knowledge. Psychiatr Pol. 2021;55(2):363–75.

 doi:10.20944/preprints202510.0878.v1

19. Tavenier AH, Hermanides RS, Ottervanger JP, Ter Horst PGJ, Kedhi E, van 't Hof AWJ. Risks of Opioids in ST-Elevation Myocardial Infarction: A Review. Drug Saf. 2018;41(12):1303–8.
20. Imran TF, Malapero R, Qavi AH, Hasan Z, de la Torre B, Patel YR, et al. Efficacy of spinal cord stimulation as an adjunct therapy for chronic refractory angina pectoris. Int J Cardiol. 2017;227:535–42.
21. Ayyasamy L, Bagepally BS. Cost-utility of Ranolazine for Chronic Stable Angina Pectoris: Systematic Review and Meta-analysis. Clin Ther. 2023;45(5):458–65.
22. Yan W, Xia Y, Zhao H, Xu X, Ma X, Tao L. Stem cell-based therapy in cardiac repair after myocardial infarction: Promise, challenges, and future directions. J Mol Cell Cardiol. 2024;188:1–14.
23. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–76.
24. Liu G, David BT, Trawczynski M, Fessler RG. Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications. Stem Cell Rev Rep. 2020;16(1):3–32.
25. Pozo MR, Meredith GW, Entcheva E. Human iPSC-Cardiomyocytes as an Experimental Model to Study Epigenetic Modifiers of Electrophysiology. Cells. 2022;11(2).
26. Oliveira NC, Russo FB, Beltrão-Braga PCB. Differentiation of peripheral sensory neurons from iPSCs derived from stem cells from human exfoliated deciduous teeth (SHED). Front Cell Dev Biol. 2023;11:1203503.
27. Feyen DAM, McKeithan WL, Bruyneel AAN, Spiering S, Hörmann L, Ulmer B, et al. Metabolic Maturation Media Improve Physiological Function of Human iPSC-Derived Cardiomyocytes. Cell Rep. 2020;32(3):107925.
28. Hendriks D, Clevers H, Artegiani B. CRISPR-Cas Tools and Their Application in Genetic Engineering of Human Stem Cells and Organoids. Cell Stem Cell. 2020;27(5):705–31.
29. Kamdar F, Klaassen Kamdar A, Koyano-Nakagawa N, Garry MG, Garry DJ. Cardiomyopathy in a dish: using human inducible pluripotent stem cells to model inherited cardiomyopathies. J Card Fail. 2015;21(9):761–70.
30. Kalia AK, Rösseler C, Granja-Vazquez R, Ahmad A, Pancrazio JJ, Neureiter A, et al. How to differentiate induced pluripotent stem cells into sensory neurons for disease modelling: a functional assessment. Stem Cell Res Ther. 2024;15(1):99.
31. Hu Y, Chen L, Zhao S, Feng R, Cao X, Chen G, et al. Transcriptomics, proteomics, metabolomics and network pharmacology reveal molecular mechanisms of multi-targets effects of Shenxianshengmai improving human iPSC-CMs beating. Clin Transl Med. 2023;13(6):e1302.
32. Prondzynski M, Berkson P, Trembley MA, Tharani Y, Shani K, Bortolin RH, et al. Efficient and reproducible generation of human iPSC-derived cardiomyocytes and cardiac organoids in stirred suspension systems. Nat Commun. 2024;15(1):5929.
33. Joshi J, Albers C, Smole N, Guo S, Smith SA. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) for modeling cardiac arrhythmias: strengths, challenges and potential solutions. Front Physiol. 2024;15:1475152.
34. Lee J, Termglinchan V, Diecke S, Itzhaki I, Lam CK, Garg P, et al. Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy. Nature. 2019;572(7769):335–40.
35. Pang L, Cai C, Aggarwal P, Wang D, Vijay V, Bagam P, et al. Predicting oncology drug-induced cardiotoxicity with donor-specific iPSC-CMs-a proof-of-concept study with doxorubicin. Toxicol Sci. 2024;200(1):79–94.
36. Cai D, Wang X, Sun Y, Fan H, Zhou J, Yang Z, et al. Patient-specific iPSC-derived cardiomyocytes reveal aberrant activation of Wnt/β-catenin signaling in SCN5A-related Brugada syndrome. Stem Cell Res Ther. 2023;14(1):241.
37. Lin Y, Sato N, Hong S, Nakamura K, Ferrante EA, Yu ZX, et al. Long-term engraftment and maturation of autologous iPSC-derived cardiomyocytes in two rhesus macaques. Cell Stem Cell. 2024;31(7):974–88.e5.
38. Hirano M, Huang Y, Vela Jarquin D, De la Garza Hernández RL, Jodat YA, Luna Cerón E, et al. 3D bioprinted human iPSC-derived somatosensory constructs with functional and highly purified sensory neuron networks. Biofabrication. 2021;13(3).

 doi:10.20944/preprints202510.0878.v1

39. Ahn K, Park HS, Choi S, Lee H, Choi H, Hong SB, et al. Differentiating visceral sensory ganglion organoids from induced pluripotent stem cells. Nat Methods. 2024;21(11):2135–46.
40. Schwartzentruber J, Foskolou S, Kilpinen H, Rodrigues J, Alasoo K, Knights AJ, et al. Molecular and functional variation in iPSC-derived sensory neurons. Nat Genet. 2018;50(1):54–61.
41. Alsaloum M, Labau JIR, Liu S, Estacion M, Zhao P, Dib-Hajj F, et al. Contributions of Na(V)1.8 and Na(V)1.9 to excitability in human induced pluripotent stem-cell derived somatosensory neurons. Sci Rep. 2021;11(1):24283.
42. Shin SM, Itson-Zoske B, Fan F, Xiao Y, Qiu C, Cummins TR, et al. Peripherally targeted analgesia via AAV-mediated sensory neuron-specific inhibition of multiple pronociceptive sodium channels. J Clin Invest. 2024;134(13).
43. Takayama Y, Kida YS. In Vitro Reconstruction of Neuronal Networks Derived from Human iPS Cells Using Microfabricated Devices. PLoS One. 2016;11(2):e0148559.
44. Heusch G. Myocardial ischemia/reperfusion: Translational pathophysiology of ischemic heart disease. Med. 2024;5(1):10–31.
45. Li WG, Xu TL. ASIC3 channels in multimodal sensory perception. ACS Chem Neurosci. 2011;2(1):26–37.
46. Halestrap AP. What is the mitochondrial permeability transition pore? J Mol Cell Cardiol. 2009;46(6):821–31.
47. Rexius-Hall ML, Khalil NN, Escopete SS, Li X, Hu J, Yuan H, et al. A myocardial infarct border-zone-on-a-chip demonstrates distinct regulation of cardiac tissue function by an oxygen gradient. Science Advances. 2022;8(49):eabn7097.
48. Hidalgo A, Glass N, Ovchinnikov D, Yang SK, Zhang X, Mazzone S, et al. Modelling ischemia-reperfusion injury (IRI) in vitro using metabolically matured induced pluripotent stem cell-derived cardiomyocytes. APL Bioeng. 2018;2(2):026102.
49. Xiang M, Lu Y, Xin L, Gao J, Shang C, Jiang Z, et al. Role of Oxidative Stress in Reperfusion following Myocardial Ischemia and Its Treatments. Oxid Med Cell Longev. 2021;2021:6614009.
50. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation. 1977;56(5):786–94.
51. Rexius-Hall ML, Khalil NN, Escopete SS, Li X, Hu J, Yuan H, et al. A myocardial infarct border-zone-on-a-chip demonstrates distinct regulation of cardiac tissue function by an oxygen gradient. Science Advances.8(49):eabn7097.
52. Immke DC, McCleskey EW. ASIC3: a lactic acid sensor for cardiac pain. ScientificWorldJournal. 2001;1:510–2.
53. Bernardin AA, Colombani S, Rousselot A, Andry V, Goumon Y, Delanoë-Ayari H, et al. Impact of Neurons on Patient-Derived Cardiomyocytes Using Organ-On-A-Chip and iPSC Biotechnologies. Cells. 2022;11(23).
54. Sutherland SP, Benson CJ, Adelman JP, McCleskey EW. Acid-sensing ion channel 3 matches the acid-gated current in cardiac ischemia-sensing neurons. Proceedings of the National Academy of Sciences. 2001;98(2):711–6.
55. Oh Y, Cho G-S, Li Z, Hong I, Zhu R, Kim M-J, et al. Functional Coupling with Cardiac Muscle Promotes Maturation of hPSC-Derived Sympathetic Neurons. Cell Stem Cell. 2016;19(1):95–106.
56. Govoni S, Pascale A, Amadio M, Calvillo L, D'Elia E, Cereda C, et al. NGF and heart: Is there a role in heart disease? Pharmacological Research. 2011;63(4):266–77.
57. Li CY, Li YG. Cardiac Sympathetic Nerve Sprouting and Susceptibility to Ventricular Arrhythmias after Myocardial Infarction. Cardiol Res Pract. 2015;2015:698368.
58. Labau JIR, Andelic M, Faber CG, Waxman SG, Lauria G, Dib-Hajj SD. Recent advances for using human induced-pluripotent stem cells as pain-in-a-dish models of neuropathic pain. Experimental Neurology. 2022;358:114223.
59. Jia L, Lee S, Tierney JA, Elmquist JK, Burton MD, Gautron L. TLR4 Signaling Selectively and Directly Promotes CGRP Release from Vagal Afferents in the Mouse. eneuro. 2021;8(1):ENEURO.0254–20.2020.
60. Samad TA, Moore Ka Fau - Sapirstein A, Sapirstein A Fau - Billet S, Billet S Fau - Allchorne A, Allchorne A Fau - Poole S, Poole S Fau - Bonventre JV, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. (0028-0836 (Print)).

 doi:10.20944/preprints202510.0878.v1

61. Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature. 2001;410(6827):471–5.
62. Ji RR, Xu ZZ, Wang X, Lo EH. Matrix metalloprotease regulation of neuropathic pain. Trends Pharmacol Sci. 2009;30(7):336–40.
63. Xiong T, Chen Y, Liu C, Li Y, Zhang Y, Chang Q. Integrative single-cell and spatial transcriptome analysis reveals the functions of TREM2(high) macrophages and infarct border dynamics post-myocardial infarction. Mol Cell Biochem. 2025.
64. Yu X, Liu H, Hamel KA, Morvan MG, Yu S, Leff J, et al. Dorsal root ganglion macrophages contribute to both the initiation and persistence of neuropathic pain. Nature Communications. 2020;11(1):264.
65. Viader A, Golden JP, Baloh RH, Schmidt RE, Hunter DA, Milbrandt J. Schwann Cell Mitochondrial Metabolism Supports Long-Term Axonal Survival and Peripheral Nerve Function. The Journal of Neuroscience. 2011;31(28):10128.
66. Quansah E, Peelaerts W, Langston JW, Simon DK, Colca J, Brundin P. Targeting energy metabolism via the mitochondrial pyruvate carrier as a novel approach to attenuate neurodegeneration. Molecular Neurodegeneration. 2018;13(1):28.
67. Canta A, Pozzi E, Carozzi VA. Mitochondrial Dysfunction in Chemotherapy-Induced Peripheral Neuropathy (CIPN). Toxics. 2015;3(2):198–223.
68. Descalzi G, Ikegami D, Ushijima T, Nestler EJ, Zachariou V, Narita M. Epigenetic mechanisms of chronic pain. Trends Neurosci. 2015;38(4):237–46.
69. Tang X, Leng M, Tang W, Cai Z, Yang L, Wang L, et al. The Roles of Exosome-Derived microRNAs in Cardiac Fibrosis. Molecules. 2024;29(6).
70. Song M, Choi DB, Im JS, Song YN, Kim JH, Lee H, et al. Modeling acute myocardial infarction and cardiac fibrosis using human induced pluripotent stem cell-derived multi-cellular heart organoids. Cell Death & Disease. 2024;15(5):308.
71. Chong JJH, Yang X, Don CW, Minami E, Liu Y-W, Weyers JJ, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014;510(7504):273–7.
72. Zhu K, Wu Q, Ni C, Zhang P, Zhong Z, Wu Y, et al. Lack of Remuscularization Following Transplantation of Human Embryonic Stem Cell-Derived Cardiovascular Progenitor Cells in Infarcted Nonhuman Primates. Circ Res. 2018;122(7):958–69.
73. Oikonomopoulos A, Kitani T, Wu JC. Pluripotent Stem Cell-Derived Cardiomyocytes as a Platform for Cell Therapy Applications: Progress and Hurdles for Clinical Translation. Mol Ther. 2018;26(7):1624–34.
74. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci. 2020;77(9):1745–70.
75. Madonna R, Van Laake LW, Davidson SM, Engel FB, Hausenloy DJ, Lecour S, et al. Position Paper of the European Society of Cardiology Working Group Cellular Biology of the Heart: cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure. Eur Heart J. 2016;37(23):1789–98.
76. Reis LA, Chiu LL, Feric N, Fu L, Radisic M. Biomaterials in myocardial tissue engineering. J Tissue Eng Regen Med. 2016;10(1):11–28.
77. Tachibana A, Santoso MR, Mahmoudi M, Shukla P, Wang L, Bennett M, et al. Paracrine Effects of the Pluripotent Stem Cell-Derived Cardiac Myocytes Salvage the Injured Myocardium. Circ Res. 2017;121(6):e22–e36.
78. Das S, Nam H, Jang J. 3D bioprinting of stem cell-laden cardiac patch: A promising alternative for myocardial repair. APL Bioeng. 2021;5(3):031508.
79. Kawamura M, Miyagawa S, Fukushima S, Saito A, Miki K, Ito E, et al. Enhanced survival of transplanted human induced pluripotent stem cell-derived cardiomyocytes by the combination of cell sheets with the pedicled omental flap technique in a porcine heart. Circulation. 2013;128(11 Suppl 1):S87–94.
80. Gao L, Kupfer ME, Jung JP, Yang L, Zhang P, Da Sie Y, et al. Myocardial Tissue Engineering With Cells Derived From Human-Induced Pluripotent Stem Cells and a Native-Like, High-Resolution, 3-Dimensionally Printed Scaffold. Circ Res. 2017;120(8):1318–25.

 doi:10.20944/preprints202510.0878.v1

81. Dvir T, Kedem A, Ruvinov E, Levy O, Freeman I, Landa N, et al. Prevascularization of cardiac patch on the omentum improves its therapeutic outcome. Proc Natl Acad Sci U S A. 2009;106(35):14990–5.
82. Hodgkinson CP, Kang MH, Dal-Pra S, Mirotsou M, Dzau VJ. MicroRNAs and Cardiac Regeneration. Circ Res. 2015;116(10):1700–11.
83. Zhang L, Ouyang P, He G, Wang X, Song D, Yang Y, et al. Exosomes from microRNA-126 overexpressing mesenchymal stem cells promote angiogenesis by targeting the PIK3R2-mediated PI3K/Akt signalling pathway. J Cell Mol Med. 2021;25(4):2148–62.
84. Moutabian H, Radi UK, Saleman AY, Adil M, Zabibah RS, Chaitanya MNL, et al. MicroRNA-155 and cancer metastasis: Regulation of invasion, migration, and epithelial-to-mesenchymal transition. Pathology - Research and Practice. 2023;250:154789.
85. Fu J, Imani S, Wu MY, Wu RC. MicroRNA-34 Family in Cancers: Role, Mechanism, and Therapeutic Potential. Cancers (Basel). 2023;15(19).
86. Liang Y, Li S, Tang L. MicroRNA 320, an Anti-Oncogene Target miRNA for Cancer Therapy. Biomedicines. 2021;9(6).
87. Duelen R, Sampaolesi M. Stem Cell Technology in Cardiac Regeneration: A Pluripotent Stem Cell Promise. EBioMedicine. 2017;16:30–40.
88. Li H, Zuo S Fau - He Z, He Z Fau - Yang Y, Yang Y Fau - Pasha Z, Pasha Z Fau - Wang Y, Wang Y Fau - Xu M, et al. Paracrine factors released by GATA-4 overexpressed mesenchymal stem cells increase angiogenesis and cell survival. (1522-1539 (Electronic)).
89. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478).
90. Emanueli C, Shearn AI, Angelini GD, Sahoo S. Exosomes and exosomal miRNAs in cardiovascular protection and repair. Vascul Pharmacol. 2015;71:24–30.
91. Lazar E, Benedek T, Korodi S, Rat N, Lo J, Benedek I. Stem cell-derived exosomes - an emerging tool for myocardial regeneration. World J Stem Cells. 2018;10(8):106–15.
92. Abdulmalek O, Husain KH, AlKhalifa H, Alturani M, Butler AE, Moin ASM. Therapeutic Applications of Stem Cell-Derived Exosomes. Int J Mol Sci. 2024;25(6).
93. Rezabakhsh A, Sokullu E, Rahbarghazi R. Applications, challenges and prospects of mesenchymal stem cell exosomes in regenerative medicine. Stem Cell Res Ther. 2021;12(1):521.
94. Liao Z, Zeng J, Lin A, Zou Y, Zhou Z. Pre-treated mesenchymal stem cell-derived exosomes: A new perspective for accelerating spinal cord injury repair. European Journal of Pharmacology. 2025;992:177349.
95. Ben-Ari M, Schick R, Barad L, Novak A, Ben-Ari E, Lorber A, et al. From beat rate variability in induced pluripotent stem cell-derived pacemaker cells to heart rate variability in human subjects. Heart Rhythm. 2014;11(10):1808–18.
96. Morrison G, Liu C, Wing C, Delaney SM, Zhang W, Dolan ME. Evaluation of inter-batch differences in stem-cell derived neurons. Stem Cell Res. 2016;16(1):140–8.
97. Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, et al. Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res. 2021;56:102512.
98. Vučković S, Dinani R, Nollet EE, Kuster DWD, Buikema JW, Houtkooper RH, et al. Characterization of cardiac metabolism in iPSC-derived cardiomyocytes: lessons from maturation and disease modeling. Stem Cell Res Ther. 2022;13(1):332.
99. Casini S, Verkerk AO, Remme CA. Human iPSC-Derived Cardiomyocytes for Investigation of Disease Mechanisms and Therapeutic Strategies in Inherited Arrhythmia Syndromes: Strengths and Limitations. Cardiovasc Drugs Ther. 2017;31(3):325–44.
100. Hwang HS, Kryshtal DO, Feaster TK, Sánchez-Freire V, Zhang J, Kamp TJ, et al. Comparable calcium handling of human iPSC-derived cardiomyocytes generated by multiple laboratories. J Mol Cell Cardiol. 2015;85:79–88.
101. Lu Y, Liu Y, Yan Y, Fooladi S, Qyang Y. Advancements in techniques for human iPSC-derived cardiomyocytes maturation: mechanical and electrical stimulation approaches. Biophys Rev. 2025;17(1):169–83.

 doi:10.20944/preprints202510.0878.v1

102. Thomas D, Cunningham NJ, Shenoy S, Wu JC. Human-induced pluripotent stem cells in cardiovascular research: current approaches in cardiac differentiation, maturation strategies, and scalable production. Cardiovasc Res. 2022;118(1):20–36.
103. Hsieh A, Feric NT, Bogojevic D, Radisic M. Methods for Cardiomyocyte Isolation from Heterogeneous Cell Populations for Regenerative Medicine and Tissue Engineering Applications. Journal of Biomaterials and Tissue Engineering. 2014;4(11):845–67.
104. Schoepf CL, Zeidler M, Spiecker L, Kern G, Lechner J, Kummer KK, et al. Selected ionotropic receptors and voltage-gated ion channels: more functional competence for human induced pluripotent stem cell (iPSC)-derived nociceptors. Brain sciences. 2020;10(6):344.
105. Smulders MW. Diagnostic evaluation of chest pain: The role of non-invasive cardiac imaging. 2020.
106. Sharma D, Jia W, Chiu A, Jang HJ, Leonov V, Chen Z, et al. Human iPSC-derived cardiac-specific extracellular matrix scaffolds for cardiomyocyte maturation and post-myocardial infarction repair. Bioact Mater. 2026;55:114–30.
107. Yoshihara M, Hayashizaki Y, Murakawa Y. Genomic Instability of iPSCs: Challenges Towards Their Clinical Applications. Stem Cell Reviews and Reports. 2017;13(1):7–16.
108. Poetsch MS, Strano A, Guan K. Human Induced Pluripotent Stem Cells: From Cell Origin, Genomic Stability, and Epigenetic Memory to Translational Medicine. Stem Cells. 2022;40(6):546–55.
109. Liu X, Li C, Zheng K, Zhao X, Xu X, Yang A, et al. Chromosomal aberration arises during somatic reprogramming to pluripotent stem cells. Cell Div. 2020;15(1):12.
110. Liu Z, Tang Y, Lü S, Zhou J, Du Z, Duan C, et al. The tumourigenicity of iPS cells and their differentiated derivates. J Cell Mol Med. 2013;17(6):782–91.
111. Cerneckis J, Cai H, Shi Y. Induced pluripotent stem cells (iPSCs): molecular mechanisms of induction and applications. Signal Transduct Target Ther. 2024;9(1):112.
112. Neofytou E, O'Brien CG, Couture LA, Wu JC. Hurdles to clinical translation of human induced pluripotent stem cells. J Clin Invest. 2015;125(7):2551–7.
113. Kim A, Lee KG, Kwon Y, Lee KI, Yang HM, Habib O, et al. Off-the-Shelf, Immune-Compatible Human Embryonic Stem Cells Generated Via CRISPR-Mediated Genome Editing. Stem Cell Rev Rep. 2021;17(3):1053–67.
114. Ji TT, Niu SS, Fang MH, Xu LX, Wang X, Zou J, et al. Genome editing HLA alleles for a pilot immunocompatible hESC line in a Chinese hESC bank for cell therapies. Cell Prolif. 2023;56(5):e13471.
115. MacDonald KP, Hill GR, Blazar BR. Chronic graft-versus-host disease: biological insights from preclinical and clinical studies. Blood. 2017;129(1):13–21.
116. Alkhatib R, Gaede KI. Data Management in Biobanking: Strategies, Challenges, and Future Directions. BioTech (Basel). 2024;13(3).
117. Martins F, Ribeiro MHL. Quality and Regulatory Requirements for the Manufacture of Master Cell Banks of Clinical Grade iPSCs: The EU and USA Perspectives. Stem Cell Rev Rep. 2025;21(3):645–79.
118. Generali M, Kehl D, Meier D, Zorndt D, Atrott K, Saito H, et al. Generation and purification of iPSC-derived cardiomyocytes for clinical applications. Stem Cell Res Ther. 2025;16(1):189.
119. Bonowicz K, Jerka D, Piekarska K, Olagbaju J, Stapleton L, Shobowale M, et al. CRISPR-Cas9 in Cardiovascular Medicine: Unlocking New Potential for Treatment. Cells. 2025;14(2).
120. de Lange WJ, Farrell ET, Kreitzer CR, Jacobs DR, Lang D, Glukhov AV, et al. Human iPSC-engineered cardiac tissue platform faithfully models important cardiac physiology. Am J Physiol Heart Circ Physiol. 2021;320(4):H1670–h86.
121. Ni B, Ye L, Zhang Y, Hu S, Lei W. Advances in humanoid organoid-based research on inter-organ communications during cardiac organogenesis and cardiovascular diseases. J Transl Med. 2025;23(1):380.