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Cell-cell interactions in the heart: advanced cardiac models and omics technologies

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Abstract

A healthy heart comprises various cell types, including cardiomyocytes, endothelial cells, fibroblasts, immune cells, and among others, which work together to maintain optimal cardiac function. These cells engage in complex communication networks, known as cell-cell interactions (CCIs), which are essential for homeostasis, cardiac structure, and efficient function. However, in the context of cardiac diseases, the heart undergoes damage, leading to alterations in the cellular composition. Such pathological conditions trigger significant changes in CCIs, causing cell rearrangement and the transition between cell types. Studying these interactions can provide valuable insights into cardiac biology and disease mechanisms, enabling the development of new therapeutic strategies. While the development of cardiac organoids and advanced 3D co-culture technologies has revolutionized in vitro studies of CCIs, recent advancements in single-cell and spatial multi-omics technologies provide researchers with powerful and convenient tools to investigate CCIs at unprecedented resolution. This article provides a concise overview of CCIs observed in both normal and injured heart, with an emphasis on the cutting-edge methods used to study these interactions. It highlights recent advancements such as 3D co-culture systems, single-cell and spatial omics technologies, that have enhanced the understanding of CCIs. Additionally, it summarizes the practical applications of CCI research in advancing cardiovascular therapies, offering potential solutions for treating heart disease by targeting intercellular communication.

Keywords Cell-cell interactions, Cardiovascular diseases, 3D models, Cardiac organoid, Multi-omics

Introduction

The adult mammalian heart exhibits an intricate three-dimensional architecture, comprising four distinct chambers that house various cell types, including cardiomyocytes (CMs, such as atrial CMs and ventricular CMs), cardiac fibroblasts (CFs), endothelial cells (ECs), smooth muscle cells (SMCs), pericytes, and immune cells [1]. Cell-cell interactions (CCIs) are vital for both early cardiac development and the preservation of normal cardiac function. For instance, the communication between ECs and CMs is essential not only for maintaining proximity to ensure efficient oxygen and nutrient delivery, but also for regulating contractile state, promoting cardiomyocyte survival, and guiding the structural organization of the heart in its healthy state.

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Acute ischemic injury and myocardial hypertrophy are prevalent cardiac ailments associated with acute and chronic heart diseases, respectively [2, 3]. Following cardiac injury, CMs undergo necrosis, while non-myocyte cells such as CFs, ECs and immune cells proliferate and engage in cross-communication with other cell types, giving rise to a complex network of cardiac interactions [4]. This shift in population dynamics, along with spatiotemporal alterations of CCIs, represents the body's effort to repair the damaged tissue. However, it can also lead to pathological remodeling, such as excessive fibrosis and scarring, ultimately impairing heart function. Cellular communications in this process occur primarily through paracrine/autocrine signaling, direct intercellular interactions, and cell-extracellular matrix (ECM) interactions. Autocrine and paracrine factors released by CFs, ECs, CMs and immune cells in response to injury, along with ECM interactions, orchestrate a series of events critical to cardiac remodeling. Signaling pathways activated during cardiac injury and repair include Wnt/β-catenin, NO, transforming growth factor β (TGF-β), Notch signaling pathways and among others, offering insights into the mechanisms underlying cardiovascular diseases (CVDs) and potential therapeutic strategies (Table 1). Moreover, injury-induced matricellular proteins modulate both cell-cell and cell-matrix interactions [5]. Therefore, investigating intercellular interactions during cardiac injury repair, particularly CF-CM, EC-CM and CM-immune cell interactions, as well as the conversion of CF subtypes, is of pivotal importance.

Despite extensive research on CCIs over the years, comprehensive studies on these interactions and signaling pathways remain limited. After briefly discussing the significance of CCIs in maintaining normal cardiac function, the article further delineates the specific CCIs between major cardiac cell types in diseased hearts, and emphasizes the methods used to study CCIs, with a particular focus on recent advancements in technique development. Finally, it summarizes the current understanding of CCIs' role in the treatment of CVDs, contextualized with examples from recent studies.

Table 1 Signalling pathway-mediated CCIs in the injured heart and their biological effects

Signaling Pathway	Cardiac CCIs	Cell Bioeffects
WNT/β-catenin	a. CM-CF b. EC-CM/CF/ Macrophage c. Epicardial cell-CF d. ICDs	a. Regulate the repair response of CFs and CMs [4] b. Modulate angiogenesis in the infarcted heart [4] c. Promote EMT and CF proliferation [4] d. Maintain normal electrical conduction [6]
TGFβ/SMAD	a. CM-CF b. EC-CM c. CF-Macrophage	a. Promote CF proliferation, differentiation, and synthesis of ECM, induce CM hypertrophy [7] b. Promote EndoMT and provoke apoptosis [8, 9] c. Promote myofibroblast conversion [10]
eNOS/NO	a. EC-CM b. EC-SMC	a. Promote diastolic relaxation, decrease O_2 consumption of CMs, and inhibit CM hypertrophy [11, 12] b. Inhibition of SMC contractile tension and proliferation [12]
NF-κB	a. CM-Macrophage b. EC-CM/CF c. EC- Macrophage	a. Activate macrophages, recruit immune cells promote inflammation [10] b. Trigger inflammation and promote heart remodeling and angiogenesis [10, 13] c. Inhibit migration or mitosis of ECs [14]
PI3K/Akt	a. CM-EC b. Macrophage- CM c. Macrophage- CF	a. Promote angiogenesis and regulate CMs metabolism [11] b. Increase the pyroptosis of CMs [15] c. Reduce collagen production, increase cardiac rupture [16]
JAK/STAT	a. CM-CF b. CF/ CM-Macrophage c. Macrophage-EC	a. Induce CMs and CFs hypertrophy, regulate the behavior of CFs [7, 17] b. Activate immune cells and promote inflammation [18] c. Regulate angiogenesis after MI [10]
MAPK	a. CF/ Macrophage -EC b. ICDs c. CM-CF	a. Promote angiogenesis [19] b. GJ assembly/disassembly, cellular adhesion [6] c. CM hypertrophy and fibrosis [17]

Cell-cell interactions in a healthy heart: sustaining normal cardiac function

In a healthy heart, strong cell-cell connections and cohesive interactions are pivotal for maintaining optimal cardiac function. This section elucidates the interactions between key cell types within the heart under normal conditions (Fig. 1), providing a foundation for understanding how these connections change during disease states.

Cardiomyocytes are pivotal for the heart's systolic and diastolic functions, ensuring efficient blood circulation. They are interconnected by intercalated discs (ICDs),

Table 1 (continued)

Signaling Pathway	Cardiac CCIs	Cell Bioeffects
ERK	a. EC-CM b. Macrophage-EC c. Macrophage-CM d. ICDs	a. Cause cardiomyocyte hypertrophy [20] b. Promote the proliferation of macrophages [21] c. Promote the proliferation and regeneration of CMs [10] d. Cell proliferation [22]
Notch	a. MSC-CM b. MSC-EC c. MSC-CPC	a. Prevent CM apoptosis [23] b. Promote angiogenesis [23] c. CPC proliferation and differentiation [23]

EMT, epithelial-mesenchymal transition; EndoMT, endothelial-mesenchymal transition; MSC, bone marrow derived mesenchymal stem cells; CPC, cardiac progenitor cells; ICDs, intercalated discs; GJ, gap junction

which comprise three major complexes: desmosomes, adhesion junctions (AJs), and gap junctions (GJs) [24]. Each structure has a distinct function: GJs facilitate metabolic and electrical coupling between CMs; AJs connect the actin cytoskeletons of neighboring cells; and desmosomes anchor intermediate filaments, ensuring structural stability [25]. The coordination of electrical activity between CMs vis GJs is critical for maintaining normal heart rhythm, enabling synchronized contraction across the myocardium [26]. Signaling pathways involving adrenergic receptor, protein kinase C (PKC), and sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) play critical roles in controlling desmosomal adhesion and GJs in cardiac cells [27]. Activation of PKC or adrenergic signaling enhances cell cohesion by promoting the location of desmosomal cadherins and connexin 43 (the main GJ protein) at cell-cell junctions, while SERCA inhibition has the opposite effect. Disruption in cell cohesion, similar to GJ inhibition, impairs conduction of excitation and can lead to arrhythmias. These findings suggest potential therapeutic targets for arrhythmogenic cardiomyopathy.

Cardiac fibroblasts are also essential for both normal heart function and in CVDs. CFs are distributed throughout the heart, surrounding CMs and occupying the interstitial spaces between myocardial layers, where they closely interact with CMs [28]. CFs communicate with CMs through paracrine signalling, ECM remodelling, and direct cell-cell contacts [7]. They respond dynamically to changes in mechanical, chemical, and electrical signals by activating cell proliferation and modifying ECM, which regulate myocardial growth, homeostasis, and cardiac repair [29]. CFs are the primary producers of ECM proteins, synthesizing collagens (especially type I and III), fibronectin, elastin, and proteoglycans, which form the structural framework that supports cardiac cells and preserves tissue integrity [30, 31]. In addition to ECM production, CFs regulate its degradation by producing matrix metalloproteinases (MMPs) and their inhibitors (Tissue inhibitors of metalloproteinases, TIMPs). While ECM remodeling helps the heart adapt to stress, injury, or growth, disruption in the balance of ECM synthesis and degradation, driven by paracrine or autocrine signaling, can lead to pathological remodeling. Meanwhile, direct intercellular connections between CMs and CFs, mediated by connexins, enable the exchange of molecular and ionic signals, which is crucial for electrical coupling and fibroblast responses to mechanical stimuli [7].

Endothelial cells can interact directly with neighboring ECs, CMs, SMCs, and leukocytes platelets through GJs and connexins, although further evidence is required to confirm the significance of direct EC-CM interactions in vivo [32]. Key regulators of paracrine interactions between ECs and CMs include angiotensin-II (Ang-II), endothelin-1 (ET-1), nitric oxide (NO), and fibroblast growth factor 2 (FGF2) [11]. Under physiological conditions, ECs sense blood flow, and release autocrine and paracrine signaling molecules, notably NO and ET-1, to regulate vascular smooth muscle contraction [33]. Both ECs and CMs express endothelial NO synthases (eNOS), although ECs produce significantly higher levels. This supports both paracrine and autocrine roles of NO in controlling the relaxation of SMCs in blood vessels and CMs in the heart [34]. Mechanistic studies have also identified a feedback loop among ECs, CMs and SMCs that is regulated by the ET-1 system [35]. When ET-1 binds to the ET_B receptor on ECs, it triggers the release of NO and prostaglandins [36]. Conversely, when ET-1 binds to the ET_A receptor on CMs, it promotes calcium release, leading to CM contraction [37]. Additionally, ET-1 induces potent and long-lasting vasoconstriction by binding to ET_A receptors on vascular SMCs through a paracrine mechanism [37].

In addition to their roles in the immune response, macrophages interact with cardiomyocytes (CMs) and other cardiac cells through both direct and indirect crosstalk to regulate cardiac homeostasis and function [38]. Macrophages can enhance electrical conduction of neighboring CMs via connexin-43-containing GJs, and mediate adaptive myocardial remodeling by sensing mechanical stretch of CMs through physical interaction [39, 40]. Additionally, cardiac macrophages can regulate CM proliferation, hypertrophy, apoptosis, and electrical conduction by secreting cytokines and extracellular vesicles carrying various microRNAs [38]. Conversely, CMs can secrete cytokines to recruit macrophages and modulate their phagocytic activity. These evidences demonstrate that macrophages are crucial participates in cardiac crosstalk under physiological conditions.

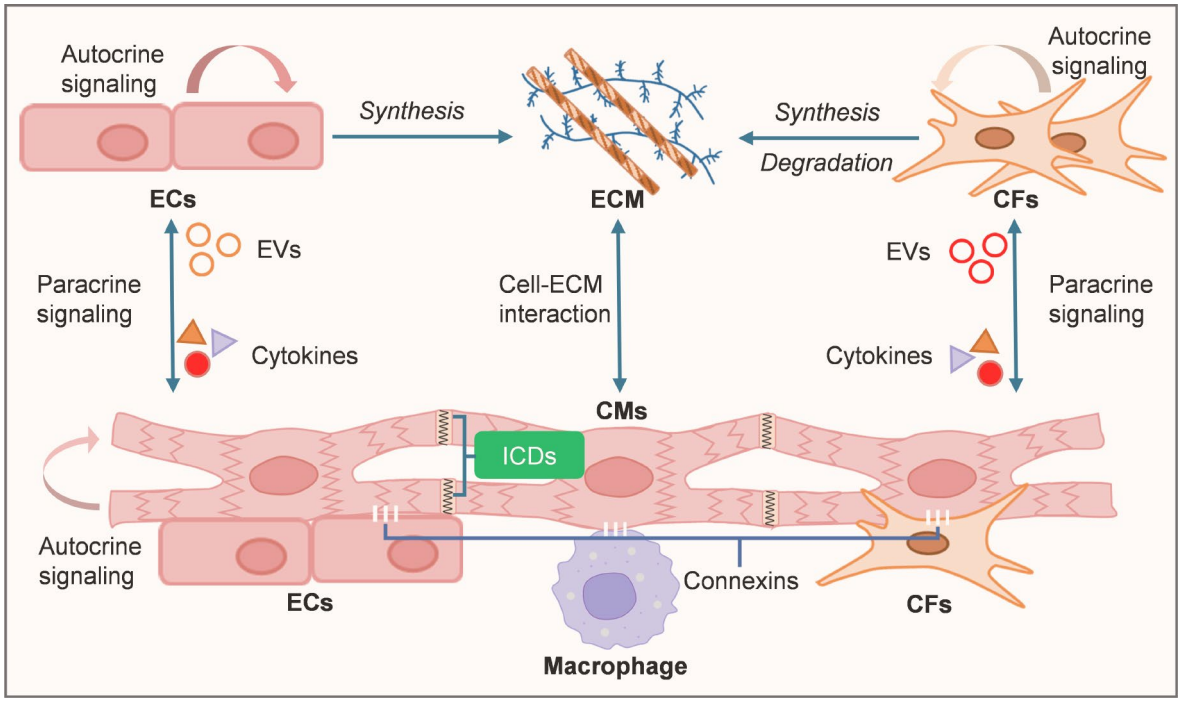

Fig. 1 Cell-cell interactions in normal heart. The cardiomyocytes (CMs), cardiac fibroblasts (CFs), endothelial cells (ECs) and macrophages interact with each other via paracrine, direct interaction, and ECM in normal heart

Cell-cell interactions in the diseased heart: dynamics of communicators and dialogue content

Cardiovascular diseases often manifest as cardiac hypertrophy, fibrosis, ventricular remodelling, and heart failure. Interactions between myocardial and non-myocardial cells are pivotal in the development and remodelling that occurs post-cardiac injury [41]. This section delves into the alterations in key cardiac cell interactions at disease onset and post-injury, highlighting the importance of understanding these interactions for treating heart diseases (Fig. 2).

Disturbed cardiomyocyte intercalated discs in various heart diseases

Intercalated discs (ICDs) serve as conduits for communication and signalling between CMs. Mutations or changes in protein expression associated with the ICD complex can contribute to various heart diseases, including arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy and cardiomyopathic atrophy, ultimately culminating in heart failure [6, 42]. Major signalling pathways associated with ICDs, such as the Wnt/β-catenin pathway, p38 MAPK cascade, and Rho-dependent serum response factor signalling, undergo differential regulation under pathological conditions [6]. While cell-cell coupling was once perceived as a static anatomical structure, recent research underscores the impact of cardiac remodelling processes on GJs in conditions like atrial fibrillation and myocardial infarction. These remodelling processes regulate connexin expression by factors such as angiotensin and endothelin, and local mechanical forces that influence the localization of GJs, contributing to the development of arrhythmogenic cardiomyopathy [43].

Interactions between cardiomyocytes and fibroblasts post-cardiac injury

Following myocardial infarction (MI), heightened cytokine release and inflammatory responses mediate CCIs in the heart, precipitating cardiac remodelling. CMs, immune cells, and CFs under stress produce renin and angiotensin-converting enzyme (ACE), promoting the generation of angiotensin II (Ang II), which exerts a potent fibrogenic effect on CFs via the AT1 receptor. This promotes fibroblast proliferation & survival, myofibroblast transformation, and ECM synthesis [44]. Prominent paracrine signalling factors between CMs and CFs include TGF-β, FGF2, members of the IL-6 family, and interleukin-33 [7]. Under MI conditions, CFs secrete elevated levels of cytokines (including TNF-a, IL-1, IL-6, IL-10, and IL-4) and fibrotic growth factors (such as TGF-β and PDGFs), exerting paracrine effects on CMs [44, 45]. As a pleiotropic cytokine which can be produce by both CMs and CFs, IL-6 plays a central role in hypoxia-induced mitogenic factor-induced CM hypertrophy and myocardial fibrosis. This occurs through the activation of the MAPK and CaMKII-STAT3 pathways, exerting its effects via both autocrine and paracrine

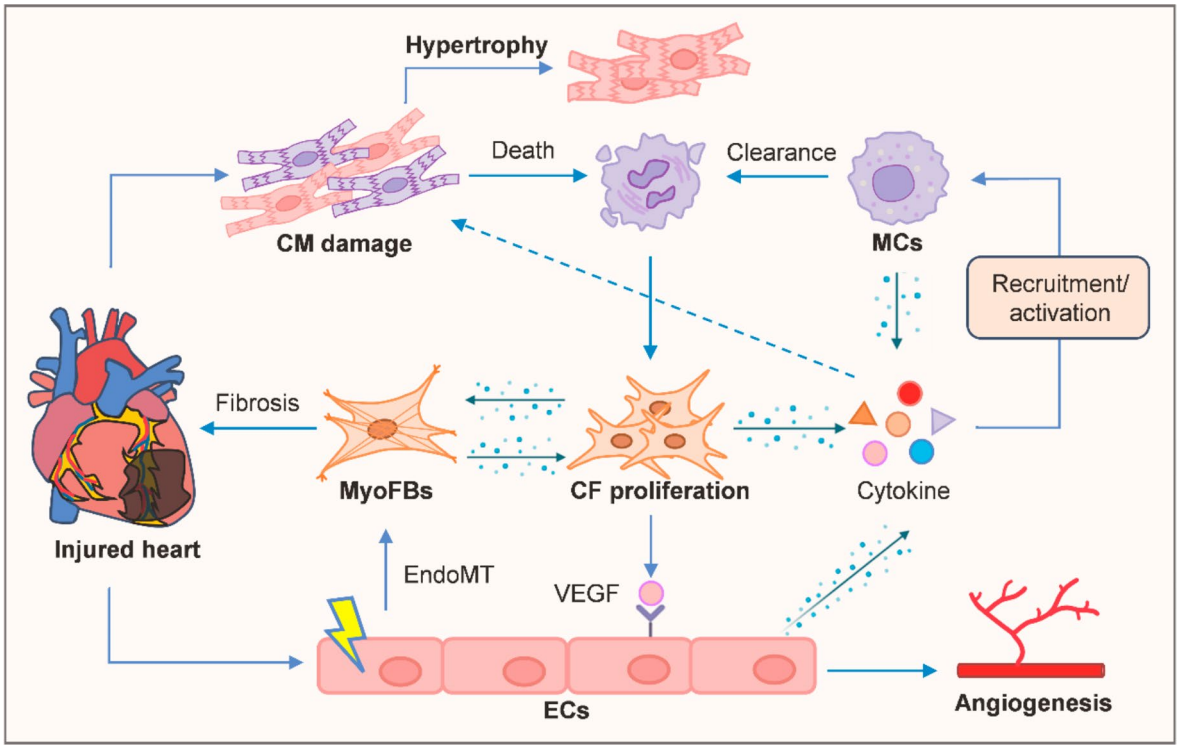

Fig. 2 Cell-cell interactions in the diseased heart. The network of cell-cell interactions following cardiac injury primarily involves cardiomyocyte (CM) damage and death, activation of cardiac fibroblasts (CFs) and endothelial cells (ECs) with paracrine roles, recruitment and activation of monocytes (MCs) to clean apoptotic cells, and transformation of CFs and partial ECs into myofibroblasts (MyoFBs). These MyoFBs can further propagate stimuli, ultimately leading to cardiac fibrosis. Additionally, mechanical and cytokine signals from other cells may induce cardiac hypertrophy in the myocardium under pathological conditions

effects [17]. Given its critical role in mediating the crosstalk between CMs and CFs, IL-6 blockade emerges as a potential therapeutic strategy for addressing cardiac hypertrophy and cardiac fibrosis.

Besides their paracrine function, CFs proliferate and differentiate into myofibroblasts (myoFBs) in response to CMs loss, replacing damaged CMs in injured tissue to maintain cardiac integrity. However, prolonged myoFB activity contributes to fibrotic scarring and cardiac dysfunction [46, 47]. Altered mechanical stress in the injured heart prompts CFs to differentiate into proto-myoFBs, which fully differentiate into myoFBs upon exposure to TGF-β, released by fibroblasts [47]. MyoFBs interact with other cells by secreting factors such as type I collagen, TGF-β, α-SMA, and Ang II, further promoting fibrosis and collagen deposition [48]. Figure 3 briefly illustrates the interaction between CMs and CFs under pathological conditions.

Enhanced EC-CM and EC-CF communications post-cardiac injury

In cardiac pathology, various cardiovascular risk factors can activate cardiac endothelial cells, which, in turn, perform paracrine functions and augment cardiomyocyte signalling [49, 50]. ECs may adopt a myofibroblast phenotype through endothelial-mesenchymal transition, regulated by multiple signalling pathways, such as Wnt and TGF-β [8, 51, 52]. NO is a crucial mediator of EC-CM crosstalk. In response to sustained pressure overload, EC activation may dampen NO signalling while elevating levels of eNOS-derived reactive oxygen species, fostering perivascular inflammation and subsequent myocardial fibrosis [53]. Meanwhile, cardiac ECs contribute to the release of TGF-β factors under hypoxic conditions, thereby promoting endothelial-mesenchymal transition and TGF-β-induced CM apoptosis [54]. This process is triggered via TGF-β receptor activation, and involved in SMAD transcription factor and the eNOS/NO/sGC pathway [9, 54].

Angiogenesis and remodelling are pivotal repair mechanisms in response to cardiac ischemia, addressing not only apoptotic damage to CMs but also vascular injury. The interplay between CFs and ECs significantly influences angiogenesis, facilitated by CFs-secreted various vascular growth factors [30]. CF-derived growth factors, notably fibroblast growth factor and vascular endothelial growth factor (VEGF), stimulate angiogenesis and coronary collateral formation, thereby restoring blood supply to the injured myocardium [55].

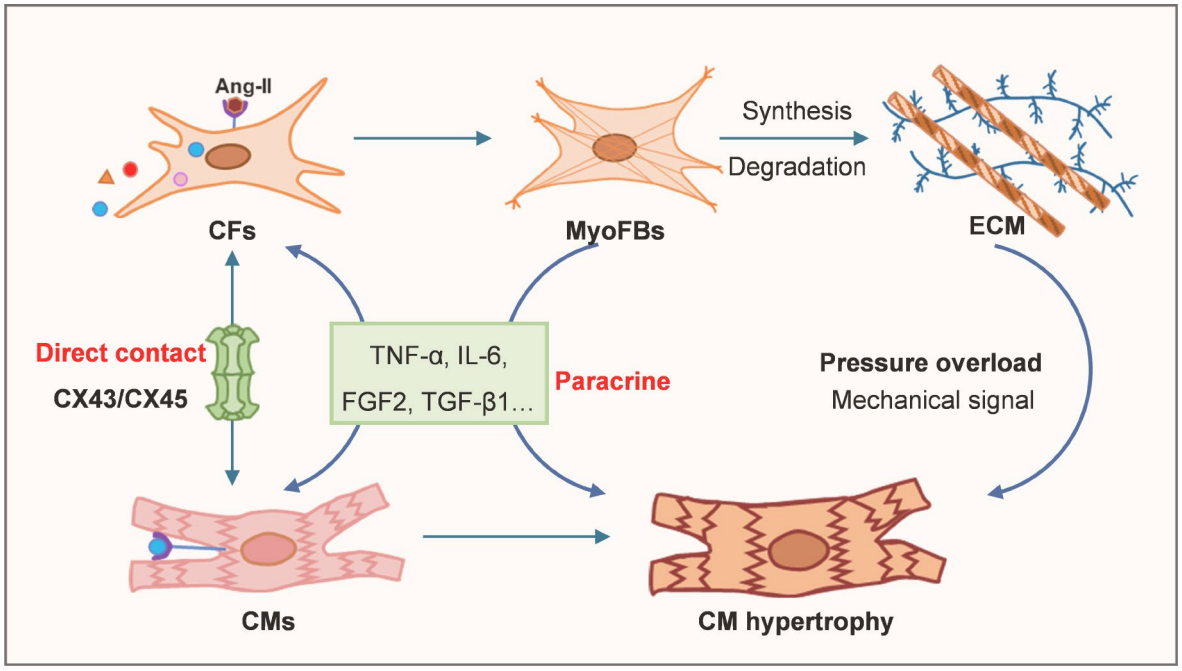

Fig. 3 Interactions between cardiomyocytes and cardiac fibroblasts in diseased heart

Interactions between macrophages and cardiomyocytes: novel approaches for myocardial repair

Extensive research on cardiac resident macrophages (CRMs) has shown their involvement in beneficial responses during tissue repair post-cardiac injury (Fig. 4). CRMs prevent fibrosis, stimulate angiogenesis, improve diastolic dysfunction, and mitigate sudden death during cardiac stress [56, 57]. Macrophages exhibit heterogeneity and coordinating multicellular communication with CMs, stromal, and immune populations, influencing cardiac physiology [58]. During MI, damaged CMs and CRMs recruit monocytes, neutrophils, and lymphocytes by releasing inflammatory factors such as CCL2, IL-1, IL-6, and TNF [13]. CRMs and monocyte-derived macrophages interact with CMs to recruit more immune cell recruitment, initiate inflammatory response, and facilitate effective clearance and degradation of apoptotic CMs [10, 59]. Cardiac macrophages can also produce amphoteric regulatory proteins (AREG), that are pivotal in controlling connexin 43 (CX43) phosphorylation and translocation in CMs, influencing cardiac conduction [57]. Studies on microphage behavior post-MI highlight their potential role in cardiac repair and ventricular remodelling [13, 60].

On the other hand, CM-macrophage interactions can have detrimental effects, providing potential targets for disease treatment. For instance, CM-induced shedding of MERTK in macrophages inhibits phagocytosis, exacerbating inflammation [61]. In the context of diabetes, the activation of toll-like receptor 2 (TLR2) and NLRP3 inflammasome in cardiac macrophages mediates the production of IL-1β, which negatively impacts the electrophysiology in CMs [62]. CCIs between CMs and cardiac macrophages can also occur via macrophage-secreted exosomes. Inhibition of miR-155 transported by exosomes reduces endoplasmic reticulum stress-induced CM apoptosis after MI by suppressing macrophage-induced inflammation [63]. Further investigation of macrophage interactions with other cardiac cells may hold promise for disease treatment.

The investigation of CCIs during heart disease represents a pivotal avenue for advancing CVD treatment. Cardiac fibrosis, a hallmark of adverse cardiac remodelling and eventual heart failure, serves both as a consequence of injury and a critical repair mechanism to prevent cardiac rupture. Ongoing research on CCIs, particularly during cardiac lesions like fibrosis, may yield new clinical applications and therapeutic approaches.

Models utilized for dissecting CCIs in the diseased heart

Investigating CCIs in the heart necessitates systematic approaches that combine various research models and experimental techniques. This section delineates both in vivo and in vitro models and the research strategies applied to study CCIs, highlighting their potential for treating CVDs.

In vivo models: harnessing valuable insights from animal studies

In vivo models can provide realistic and reliable data by closely simulating the original state of CCIs within

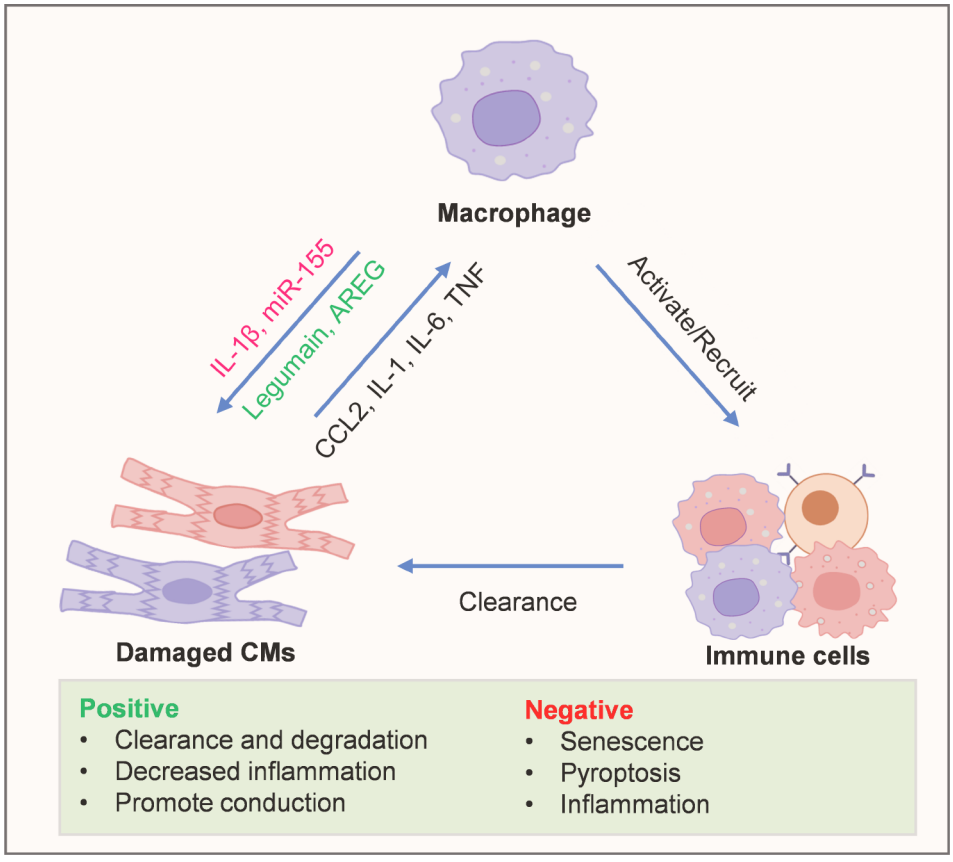

Fig. 4 Cell-cell interactions between cardiomyocytes and cardiac macrophages in diseased heart

disease contexts [64]. Rodents, particularly mice, are widely used in cardiovascular research due to the challenge of obtaining human samples. Besides surgery and drug treatment, knockout or transgenic techniques are employed to construct powerful models for in vivo study of cardiac CCIs [65, 66].

The Wnt/β-catenin signalling pathway serves as a crucial regulatory mechanism for cardiac repair, facilitating interactions among multiple cardiac cell populations post-cardiac injury [4]. The deletion of secreted fucoidan-related protein-2 (SFRP2), a key regulator of the Wnt/β-catenin signalling pathway, reduces fibrosis in mice after myocardial infarction compared to wild-type MI mice [67]. Similarly, another study in a porcine model of reperfusion MI showed that blocking Frizzled signalling is a promising target for improving infarct healing and limiting post-infarct remodelling [68]. However, SFRP2 could also promote angiogenesis and arteriogenesis through crosstalk between CMs and ECs, mediated by endoplasmatic reticulum (ER) stress signalling pathway, ATF6 and cTGF, thereby protecting the heart from myocardial ischemia [69]. These data indicate the multiple-face of SFRP2 and the Wnt/β-catenin signalling pathway during cardiac injury and repair, which is involved in cardiac CCIs.

Blocking direct cell connections or altering the expression of associated genes are also viable research methods. Desmoglein-2 (DSG2) is an important desmosomal adhesion molecule in CMs, and DSG2-W2A mutation disrupts DSG2-mediated CCIs, leading to cardiac fibrosis [70]. Camilla Schinner et al. suggest that dysregulation of integrin-αVβ6 and subsequent TGF-β signalling is a driver of cardiac fibrosis, while blockade of integrin-αVβ6 reduces fibrosis in the DSG2-W2A knock-in mouse model [71].

In conclusion, despite challenges such as species differences and difficulties in isolating and assessing individual cell contributions within complex interactions, animal models remain crucial for studying CCIs in heart disease.

In vitro models: transforming from 2D to 3D

In vitro investigations of cardiac cell interactions necessitate access to cardiac lineage cells. The technique of isolating and purifying various cell types from the hearts of experimental animals has seen significant advancements in recent years [72, 73]. Particularly, human pluripotent stem cell-derived cells such as CMs, ECs, and CFs offer diverse sources for multicellular co-culture in vitro. With the advancement of research on intercellular interactions, in vitro models have evolved from 2D to 3D

models, a progression detailed in this section along with the research significance and application examples of these models.

Two-dimensional co-culture models

Co-culturing specific types of cardiac cells on a flat cell culture dish appears to be the simplest and most direct method for studying their interactions, facilitating the measurement of various parameters such as the secretory/paracrine effects of proteins in the cardiac cell environment, expression of different proteins at various cell-cell interaction sites, and electrophysiological properties of cultured cells [31]. Additionally, CCIs at the 2D level can be examined in a non-contact manner, typically culturing 'B' cells with a medium containing secretion from 'A' cells as a specific additive. Alternatively, co-culture via transwell allows for the evaluation of CCIs through paracrine signalling pathways [74, 75].

Co-culture models have been widely used to study paracrine signalling or direct contact-mediated interactions between cardiac cells. In a CF-CM co-culture model, Mary O. Gray et al. demonstrated that Ang II-induced secretion of TGF-β1 and ET-1 from CFs mediates CF-CM crosstalk and promotes CM hypertrophy in neonatal rat cell culture [76]. Meanwhile, James E. Cartledge et al. revealed that all CF types decrease CM viability and increased CM volume, whereas CM-released soluble mediators affect fibroblast proliferation [77]. In an EC-CM co-culture model, ECs exhibit a protective role in CMs against hypoxic and reoxygenation injury, which is dependent on hypoxia-inducible factor 1α (HIF1α) and NO [50]. Additionally, non-cardiac intercellular co-culture models, like the contact co-culture of SMCs and ECs, have been used to reveal that SMC-EC contact is crucial for BMPR2 to activate Notch1. This activation coordinates metabolism with chromatin remodelling of genes, facilitating EC regeneration, and maintaining monolayer integrity and preserving vascular homeostasis in response to injury [78].

Given the challenges in endogenous repair of damaged CMs, transplantation of ventricular-like pluripotent stem cell-derived CMs (PSC-CMs) has emerged as a promising therapeutic option for MI. Understanding the interaction mechanisms between these exogenous CMs and endogenous cardiac cells is essential to optimize therapeutic outcomes. Researchers utilized a CF-CM co-culture model to study the effects of adult CFs on the electrophysiology of PSC-CMs, and indicated potentially pro-arrhythmic changes of PSC-CM electrophysiology [79]. Encouragingly, a recent study employing three 2D co-culture methods to mimic CCI patterns identified IL-6 and CX43 as critical paracrine and contact mediators, respectively, in the myoFB-to-PSC-CM interaction, suggesting that modulating these pathways may reduce the risk of arrhythmias in cardiac cell therapy [75].

While the various 2D co-culture models described above provide significant insights into CCIs, they are still limited in providing a comprehensive understanding of the interactions within the three-dimensional structure of the heart. Fortunately, several 3D co-culture models have been developed and continue to evolve, offering more physiologically relevant systems for studying CCIs.

Three-dimensional models: cardiac tissue engineering and organoids

The heart's intricate 3D structure is critical for its function, and 3D co-culture models of cardiac cells are designed to better mimic this complexity, allowing researchers to study cell arrangement and interactions more accurately. Two primary approaches in this field include scaffold-based and scaffold-free 3D co-culture systems, which facilitate investigations of cell interactions in both artificial or natural ECM environments [31]. Among various 3D platforms, cardiac tissue engineering (CTE) and human cardiac organoids (hCOs) have gained prominence as in vitro 3D cardiac tissue models in recent years. While CTE shows the potential in the repair of cardiac defects by providing tissue scaffolds that simulate the heart's environment, enabling cell growth, adhesion, and differentiation, hCOs replicate cardiac structure and microenvironment of human heart. Both CTE and hCOs offer in vitro models for studying heart development and disease mechanisms mediated by CCIs. Additionally, there are atypical 3D co-culture models, such as the 3D vascularized cardiac tissue mimic developed by Julian Uwe Gabriel Wagner et al., allowing the exploration of signal crosstalk between cardiac cells in vitro [80]. These advancements are crucial for not only improving the understanding of cardiac CCIs, but also for exploring potential therapeutic strategies for CVDs.

Research on CTE-based CCIs CTE holds significant promise for heart defect repair, offering a range of applications including heart patches, tissue-engineered myocardium, and other bionic tissues [81]. Tissue-engineered scaffolds provide a supportive framework and a conducive microenvironment for cellular activities including adhesion, proliferation, and differentiation in CTE. This field has seen remarkable advancements with the rise of bio-3D printing technologies, which have enabled the fabrication of scaffolds with complex 3D structures and facilitating the integration of various cells into these frameworks [82]. These CTE models are increasingly being used not only for potential therapeutic applications, but also as advanced serve as 3D co-culture systems for investigating CCIs between various cardiac lineage cells, shedding

light on the intricate cellular dynamics underlying cardiac development, physiology and pathology.

Recent studies have explored how CFs, both fetal and adult, affect CMs in CTE models, and uncovered significant effects at both structural and functional levels. A study revealed that the in vitro expanded adult CFs notably impair the electrical and mechanical function of co-cultured CMs [83]. In contrast, another study showed that tissue patches with fetal CFs displayed higher action potential propagation rates and contractile force amplitudes compared to those containing adult CFs [84]. These findings underscore the age-related differences in CF-CM interactions within engineered tissues.

Additionally, EC-CM interactions serve as a cardioprotective mechanism under pathological conditions such as oxidative stress in MI [35, 85, 86]. A study by Aylin Acun and colleagues highlighted that the interaction between ECs and CMs in a CTE model is mediated primarily through secreted factors. The study showed that hypoxia-inducible factor (HIF-1α), secreted from ECs, protects the heart from oxidative damage, highlighting the importance of bidirectional crosstalk between ECs and CMs for EC-mediated cardio protection [86].

Human PSC-CMs, commonly used in CTE models, often exhibit an immature fetal-like phenotype compared to adult CMs. The 3D co-culture of CMs with other cardiac cells in engineered tissues or matrices has been shown to promote cardiomyocyte maturation. These processes are facilitated through mechanisms like the CX43-mediated formation of GJs between neighboring cells and activation of the cAMP pathway [87, 88]. Moreover, multicellular crosstalk was shown to promotes the maturation of CMs in the 3D cardiac microtissues (CMTs) [89]. Transcriptomic analyses have revealed that key Ca^{2+} handling proteins (e.g., RYR2, ATP2A2) and ion channel proteins (e.g., KCNA4, KCND3, and KCNH2) are up-regulated in CMTs compared to the lower-order 2D and 3D cultures of the same composition cells [90]. Notably, three-cell crosstalk in 3D CMTs has been shown to promote postnatal SCN5A switch, from foetal SCN5A isoform containing exon 6 A to the adult SCN5A isoform containing exon 6B, in hPSC-CMs [91]. These findings make CMTs a valuable tool for studying arrhythmia-associated ion channels including those involving in CCIs. Researchers have also employed selective pharmacological ion channel blockers on 3D CMTs to study action potential (AP) alterations, providing a robust platform for in vitro cardiotoxicity assessment [92].

Research on hCO-based CCIs Human cardiac organoids (hCOs) have become invaluable tools for studying multicellular interactions in a 3D environment. These organoids mimic the structure and function of the heart, enabling the study of early heart development, chamber formation, disease modelling, and drug toxicity testing [93–95]. Due to their multicellular nature, hCOs provide an excellent platform to investigate how different cardiac cell types communicate, and insights from these studies can inform the construction of more complex organoids.

A study by Voges et al. demonstrated that adding vascular cells to hCOs improved the overall function and boosted the expression of maturation markers via paracrine mechanisms. Specifically, their research revealed that EC-derived LAMA5 regulated the expression of mature sarcomeric proteins and improved contractility, while paracrine platelet-derived growth factor receptor β (PDGFR-β) signalling from vascular cells enhanced stromal deposition, further boosting the contractility of hCOs [96].

Recently, a novel method for generating vascularized and chamber-like hCOs, called vaschamcardioids (vcCOs) was developed [97]. This method involved encapsulating hPSC-derived blood vessel organoids with ventricular hPSC-CMs, followed by inducing vascular invasion into the myocardium. Researchers used single-cell RNA sequencing (scRNA-seq) to analyze the cellular components and potential CCIs within these vcCOs. They found close interactions of CMs and fibroblasts with most other cell components in the vcCOs, while ECs showed particularly strong connection with CMs, mediated through ligand-receptor pairs involved in angiogenesis and ECM regulation [97]. This highlights the essential role of ECs in facilitating structural and functional coordination within the cardiac organoid environment.

Most current 3D co-culture methods for studying cardiac CCIs involve a defined ratio of multiple cell types combined with either natural or artificial matrix components. In particular hCOs derived from stem cells, using methods that mimic cardiac developmental signals, tend to possess a more complex cellular composition, closely resembling the structure of the natural heart. As transcriptomic technologies advance, hCOS are increasingly recognized as potent models for investigating the cellular and molecular dynamics underlying CCIs.

Application of the high-throughput omics technologies in CCI studies

CCIs in the heart involve variety of complex protein interactions, including ligand-receptor, receptor-receptor, and ECM-receptor interactions. These interactions are dynamic and can shift dramatically under pathological conditions. To unravel the complexity of CCIs and the alterations that occur in disease states, researchers increasingly rely on high-throughput, high-resolution omics technologies. These technologies allow for a comprehensive, integrative analysis of the genome, epigenome, transcriptome, proteome, and metabolome, even

at the single-cell level, providing a multi-dimensional view of cell biology in health and disease. This section delves into the application of transcriptomics, proteomics, and metabolomics for studying cardiac CCIs, highlighting how these approaches deepen our understanding of the molecular mechanisms driving cardiac function and dysfunction.

Transcriptomics-based study of cardiac CCIs

Current transcriptomic technologies, including bulk RNA sequencing, scRNA-seq, single-nucleus RNA sequencing (snRNA-seq), and spatial transcriptomics (ST), provide powerful tools for exploring the spatial and temporal dynamics of gene expression during cardiac development and disease [98]. Among these, scRNA-seq technology has rapidly advanced, allowing for high-resolution analysis of gene expression at the single-cell level, making it instrumental in unravelling the heterogeneity and complexity of RNA transcripts within individual cells. This technology has proven pivotal in identifying different cell types and functional compositions in the highly organized tissues and organisms [99, 100].

Erick Armingol et al. discusses how transcriptomic data could be employed to infer intercellular communication by analysing CCIs through gene expression profiles [101]. Mapping CCI networks using scRNA-seq and ST technologies has been successfully applied to analyse tumor-immune cell interactions [102], suggesting potential applications in cardiovascular research. For instance, both scRNA-seq and snRNA-seq technologies can identify and classify various cardiac cell types, including CMs, ECs, CFs, macrophages, and map their interaction networks. Human and mouse cardiac cell populations and their extensive CCIs have been well studied using these advanced transcriptomics technologies [97, 103–105]. Studies have uncovered the activation of specific cell populations, the switching of cellular subtypes, and signalling crosstalk between different cell types, identifying these processes as pivotal events in the development of hypertrophy or fibrosis [106, 107].

Interactions between cells are often mediated by ligand-receptor binding, which can be predicted from scRNA-seq data. Statistical tools such as CellPhoneDB, CellChat, and CellTalkDB have been developed to predict CCIs between two cell types from single-cell transcriptomics data, identifying how two cell types interact based on their ligand-receptor pairs [108–110]. For example, Li Wang et al. analysed interactions between cells in the left atrium and left ventricle, uncovering distinct cellular interaction networks and cell centers that maintain homeostasis, while non-myocytes were found to play an active role in regulating CM behaviour [111]. Similarly, a widespread presence of CCIs between CMs and noncardiomyocytes was observed in hCOs, further emphasizing the importance of these interactions in maintaining cardiac function and tissue organization [97].

Spatial transcriptomics complements single-cell sequencing by providing spatial information on cell positions, which indicates the likelihood of different cell types interacting based on their proximity describe the relative positions of different cells [112]. For example, a study on mononuclear diploid CMs identified a new subpopulation of non-proliferative cells that has minimal intercellular communication, highlighting the role of microenvironment in CM fate during maturation [113]. Additionally, combining ST with multi-omics technologies enhances the understanding of disease-specific status of major cardiac cell types in CVDs, allowing for more precise spatial environment analysis and assessment of CCIs [114].

Proteomics-based study of cardiac CCIs

Proteomics techniques provide a detailed view of the changes in protein profiles due to CCIs mediated by ligands/receptors, hormones, soluble factors, and extracellular vesicles (EV), offering detailed insights into the mechanisms of CCIs [115]. A variety of high-throughput proteomics methods, such as mass spectrometry (MS), protein pathway arrays, next-generation tissue microarrays, single-cell proteomics, and single-molecule proteomics, are available to researchers, while platforms like Luminex, Simoa, and Olink proteomics, provide tools for proteomics data analysis. These techniques, combined with appropriate algorithms, help to extract valuable proteomics data from clinical and CVD models and uncover the complex interactions and networks involved in cardiac diseases, offering a better understanding of the underlying pathogenesis [116].

Proteomic analysis of plasma samples has been instrumental in identifying circulating protein biomarkers associated with CVDs, which often serve as signaling molecules for CCIs. These biomarkers can be utilized for the early diagnosis of heart failure and the assessment of MI development [74, 117–119]. Additionally, proteomics enables the identification of molecular mechanisms and protein changes in CVDs through preclinical models and translational studies, uncovering potential therapeutic targets [117, 118, 120]. In a recent study, Mark C. Blaser et al. conducted the proteomic comparison of human carotid plaques and calcified aortic valves, revealing that EV-mediated cell-cell communication may be involved in advanced cardiovascular calcification [121]. Furthermore, proteomic analysis of disease models facilitates the identification of key CCIs and cell-ECM interactions, that transcriptomic studies alone may not fully capture. In a study comparing proteomic data from aortic samples of patients with thoracic aortic coarctation to those of normal subjects, Kefeng Zhang et al. identified cell-matrix

interactions related to TGF-β signaling and ECM remodeling as potential contributors to the pathogenesis of thoracic aortic disease [122].

While the single-cell proteomes remain relatively intact, spatial proteomics emerges as a potent technique. By combining high-content imaging, artificial intelligence, single-cell laser microdissection, and ultra-sensitive proteomics, spatial proteomics facilitates the study of spatial organization in tissues, offering deeper insights into fundamental biology such as CCIs between normal and diseased tissue [123]. For instance, a study by Lucia M Moreira et al., investigated the molecular mechanisms of atrial tissue fibrosis in atrial fibrillation, and discovered that atrial CMs produce large amounts of calcitonin, which acts as a paracrine signal regulating the proliferation of neighboring collagen-producing CFs and the secretion of ECM proteins. While transcriptome analysis of human atrial fibroblasts exposed to calcitonin revealed minimal changes, proteomic analysis showed extensive alterations in ECM proteins and pathways related to fibrosis, immune response, and transcriptional regulation [124].

Metabolomics-based study of cardiac CCIs

The pathogenesis of CVD often involves disruptions in cardiac energy metabolism, making metabolomics technology a powerful tool for studying metabolic alterations in conditions such as hypertrophy, ischemia, and MI. These analyses help in identifying novel CVD biomarkers [125, 126], and complement transcriptomics and proteomics to capture the dynamics of certain metabolites, such as lipids, polysaccharides, and vitamins [127, 128]. The primary platforms for metabolomics analysis are nuclear magnetic resonance (NMR) and mass spectrometry (MS). MS, when coupled with gas chromatography (GC), high-performance liquid chromatography (HPLC), and ultra-high-performance liquid chromatography (UHPLC), offers advantages over NMR-based metabolomics in terms of cost-effectiveness, sensitivity, and real-time spatial visualization [125].

Spatial metabolomics techniques, using imaging mass spectrometry (IMS) to localizing metabolites within tissue section, provide valuable insights into the spatial and temporal distribution of metabolites within biological samples, complementing traditional metabolomics and chemical analyses [129]. IMS-based single-cell metabolomics technology was recently applied to investigate the pathogenesis of heart failure. Researchers conducted single-cell lipidomics analyses on CMs isolated from heart failure mice, uncovering close associations with lipoprotein metabolism, transmembrane transport, and signal transduction [130]. A recently developed method integrates IMS-based metabolomics and imaging mass cytometry-based immunophenotyping, enabling the visualization of metabolic heterogeneity and its correlation with specific cell populations at the single-cell level [131]. This approach allows for comprehensive investigations of metabolic processes and interactions between cellular components.

Extracellular vesicles (EVs), which carry nucleic acids, proteins, lipids, and metabolites, play a critical role in intercellular communication by transferring intracellular cargo [132, 133]. In the context of CVD, EVs are involved in a variety of pathophysiological processes and are potential diagnostic and prognostic biomarkers [133]. The application of metabolomic techniques to monitor metabolic alterations in biological fluids, such as urine and blood, offers valuable diagnostic insights into disease states. For example, metabolomic analyses of urinary EVs have provided evidence of exosome metabolism in CVDs, revealing the potential of NMR and MS in assessing the metabolic composition in urinary exosomes [134]. The EVs' diverse cargo makes multi-omics approaches, integrating transcriptomics, proteomics, and metabolomics, an invaluable tool for studying EV involvement in CCIs in CVDs.

Despite advancements in high-throughput and high-resolution detection techniques, most tools for analyzing CCIs focus primarily on protein-related ligand-receptor interactions, overlooking non-protein metabolites. This gap limits the effective use of metabolomic data in CCI analysis. However, the development of MACC, the first knowledgebase focusing on human metabolite-associated intercellular communications, is a promising advancement, offering a new dimension to CCI studies [135]. Additionally, Yuncong Zhang et al. compiled a database, MRCLinkdb, of over 790 human and 670 mouse metabotropic ligand-ligand-receptor (ML-R) interactions. This database and associated algorithms provide new perspectives and prediction tools for studying intercellular communication based on ML-R interactions [136].

As high-throughput omics technologies continue to integrate with various experimental models, the intricate details of cellular interactions in both normal and diseased hearts will be better understood. This will enhancing our understanding of cardiac biology and the mechanisms underlying CVDs.

Conclusion

Cell-cell interactions and cell-matrix interactions are dynamic processes that evolve throughout cardiac development, maintenance of structure and function, disease progression, and repair. A comprehensive understanding of these interactions across various stages offers valuable insights into cardiac pathogenesis and the identification of potential therapeutic targets. Cardiac cells, including CMs, CFs, ECs, CRMs, and others, engage in intricate communication through mechanical, chemical,

and electrical signals to maintain cardiac homeostasis. Disruption of this delicate network can contribute to the onset and exacerbation of heart disease, underscoring the importance of elucidating the mechanisms underlying these interactions for advancing CVD prevention and treatment strategies.

Decades of research across various tissues and disease models have provided essential methodologies for studying such CCIs. Cutting-edge biomedical technologies, such as CRISPR-based gene editing, 3D bioprinting, and multi-omics approaches, have revolutionized the construction of in vivo and in vitro cardiac disease models, and facilitated high-throughput analysis of clinical or experimental samples. The integration of single-cell and spatial transcriptomics, proteomics and metabolomics, especially in human cardiac organoid models, enhances the robustness of research findings by offering a more detail view of cardiac biology at the molecular and cellular levels.

Research into CCIs has garnered significant attention in recent years. Applications include mapping CCIs during the process of cardiac diseases, assessing cardiac degeneration levels by analysing secreted factors of intercellular communication, elucidating the protective effects of specific cell types on the heart under pathological conditions, improving the maturation of hPSC-CMs, CTEs and hCOs, and assessing the compatibility of hPSC-derived cardiac cells or myocardial patches post-transplantation. These multifaceted applications underscore the versatility and potential of CCIs research in advancing cardiovascular therapeutic strategies.

Despite the notable progress, significant challenges remain in CVD research. These include the difficulty of regenerating adult myocardium after injury, the immaturity of patient-specific hPSC-derived CMs, and unresolved issues surrounding the clinical application of cellular therapies and myocardial patch transplantation. Continued exploration of CCIs in the heart will be crucial for overcoming these challenges, fostering the development of innovative therapeutic strategies, and ultimately improving patient outcomes in the fight against CVDs.

Abbreviations

AJs	Adhesion junctions
Ang II	Angiotensin II
CCIs	Cell-cell interactions
CFs	Cardiac fibroblasts
CMs	Cardiomyocytes
CMT	Cardiac microtissue
CRMs	Cardiac resident macrophages
CTE	Cardiac tissue engineering
CVD	Cardiovascular disease
ECM	Extracellular matrix
ECs	Endothelial cells
eNOS	Endothelial NO synthase
ET-1	Endothelin-1
EVs	Extracellular vesicles
GJs	Gap junctions
hCOs	Human cardiac organoids
ICDs	Intercalated discs
MI	Myocardial infarction
MS	Mass spectrum
myoFBs	Myofibroblasts
NO	Nitric oxide
PSCs	Pluripotent stem cells
scRNA-seq	Single-cell RNA sequencing
SMCs	Smooth muscle cells
snRNA-seq	Single-nucleus RNA sequencing
ST	Spatial transcriptomics
TGF-β	Transforming growth factor
vcCOs	Vaschamcardioids
VEGF	Vessel endothelial growth factor

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Data availability
Data sharing is not applicable to this article as no new data were created or analysed in this study.

Declarations

Ethics approval and consent to participate
Not applicable.

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