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REVIEW

Infarct Zone: a Novel Platform for Exosome Trade in Cardiac Tissue Regeneration

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Abstract

The global incidence of coronary artery diseases (CADs), especially myocardial infarction (MI), has drastically increased in recent years. Even though the conventional therapies have improved the outcomes, the post-MI complications and the increased rate of recurrence among the survivors are still alarming. Molecular events associated with the pathogenesis and the adaptive responses of the surviving myocardium are largely unknown. Focus on exosome-mediated signaling for cell-cell/matrix communications at the infarct zone reflects an emerging opportunity in cardiac regeneration. Also, cardiac tissue engineering provides promising insights for the next generation of therapeutic approaches in the management of CADs. In this article, we critically reviewed the current understanding on the biology of cardiac exosomes, therapeutic potential of exosomes, and recent developments in cardiac tissue engineering and discussed novel translational approaches based on tissue engineering and exosomes for cardiac regeneration and CADs.

Keywords Cardiosomes · Exosomes · Extracellular vesicles · Infarct zone · Myocardial infarction · Tissue engineering

Abbreviations

AAA+	ATPases associated with diverse cellular activities
ADAM10	Disintegrin and metalloproteinase domain-containing protein 10
ARF6	ADP ribosylation factor 6
CABG	Coronary artery bypass graft
CAD	Coronary artery diseases
CPCs	Cardiac progenitor cells
CTE	Cardiac tissue engineering
CVDs	Cardiovascular diseases
ECM	Extracellular matrix
ESCRT	Endosomal sorting complex required for transport
EVs	Extracellular vesicles
ICAM1	Intercellular adhesion molecule 1
ILV	Intra-luminal vesicles
IZ	Infarct zone
LFA1	Lymphocyte function-associated antigen 1
LV	Left ventricle
MI	Myocardial infarction
SNAP	Soluble *N*-ethylmaleimide-sensitive factor attachment proteins
SYLT4	Synaptotagmin-like 4
TGN	Trans-Golgi network
Tsg101	Tumor susceptibility gene 101

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Introduction

Coronary artery diseases (CADs), especially myocardial infarction (MI), are the leading cause of mortality throughout the globe and in every ~ 40 s, one MI case is reported in the USA. Also, ~ 720,000 peoples have a new cardiac event and ~ 335,000 patients suffer from recurrent attacks every year [1]. The diagnosis of MI is mainly based on the combination of electrocardiographic (ECG) findings and serum biomarkers, including troponin, creatine kinase, and others [2]. The molecular pathogenesis for acute MI has been attributed to coagulative necrosis of the myocardium [2]. The advancements in cellular and molecular biology have improved our understanding of the pathogenesis of MI at cellular, molecular, and genetic levels. The dynamic alterations in cell biology and the structural and functional changes in the key biomolecules

associated with the myocardium collectively contribute to the pathobiology of MI. The role of oncosis (series of cellular events following an injury leading to cell death), apoptosis, autophagy, and necrotic cell death and the central role of mitochondria-mediated ischemic injury response, reperfusion injury, and myocardial conditioning have been unveiled [3]. However, the exact molecular events associated with the pathogenesis and the adaptive responses of the surviving myocardium are largely unknown.

The pathology of cardiac tissue, especially at the infarct zone (IZ) (area in the heart tissue which is at the maximum risk of damage after MI), following MI was reported to be aggravated by the persistent immune response leading to left ventricular remodeling and subsequent heart failure [4, 5]. Remodeling of left ventricle (LV), extra cellular matrix (ECM) disorganization, thinning of LV wall, and scarring are the major clinical conditions associated with MI. The conventional treatment strategies include coronary artery bypass graft (CABG), intravascular stenting and pharmaceuticals such as statins, thrombolytic drugs, angiotensin-converting enzyme (ACE) inhibitors, and beta-blockers. Even though these therapies attenuate the symptoms, the regeneration of the IZ is still challenging [6, 7]. In addition, the structural and functional remodeling associated with cardiac ECM fibrosis has been the major histological hallmark of MI and subsequent heart failure. The molecular mechanisms underlying the formation of pathologic ECM is largely unknown, and the therapeutic strategies specifically targeting the myocardial regeneration following the fibrosis at IZ have not been reported [8]. It has been estimated that approximately 50 g cardiac muscle become dysfunctional following MI resulting in the irreversible loss of ~ 1 billion cardiomyocytes. Also, the inherent cardiac repair mechanisms are insufficient to replenish the lost cardiomyocytes and to regenerate cardiac ECM at the IZ, thereby leading to chronic impairment and subsequent end-stage cardiac failure [9]. In this article, we critically reviewed the current understanding of the cellular and molecular communications associated with MI and shed light to the advancements in translational approach to rejuvenate the IZ.

Infarct Zone: the "*Playground*" for Cardiac Repair

MI is a localized event of left ventricle which leads to the activation of systemic and localized inflammatory responses. The inflammation results in the increased inflow of acute phase proteins, pro-inflammatory mediators, immune cells, and stem cells that communicate with each other to stabilize the IZ which is initiated by the adhesion of myeloid cells and subsequently to scar tissue formation [10]. Furthermore, the tissues that generate inflammatory cells, such as bone marrow and spleen, are activated following the MI via cytokine signaling [10]. The repair responses at the IZ are orchestrated with a cascade of biochemical events which involve an inflammatory phase followed by reparative phase. The balance between these two phases is critical for the proper healing, and disturbances in these phases result in increased cell loss, defective scar tissue formation, contractile dysfunction, infarct expansion, and chamber dilation [11]. The key events associated with cardiac repair, as reported in the literature [12–24], are given in Tables 1, 2, and 3. However, the actual cellular and molecular mechanisms underlying the inflammation and LV remodeling are largely unknown.

Recent findings revealed that the serum of MI patients is capable of eliciting anti-inflammatory effects without altering the electrical integrity of the heart thereby protecting the IZ expansion. However, the ventricular fibrillation associated with MI transforms healthy cardiomyocytes towards pro-inflammatory phenotype [10]. These findings suggest a possible communication among the resident cells such as cardiomyocytes, cardiac fibroblasts, endothelial cells and resident stem cells, and the recruited cells such as immune cells and stem cells. However, the cell-cell and cell-ECM communication at the IZ are yet to be unveiled.

Exosomes: the Key Regulator for Cell-Cell and Cell-ECM Communication

The communication between/among various cell types and ECM is significant in regulating physiological and pathological responses. Among several modes of biological communications, the exosome-mediated signaling constitutes an important pathway for cell-cell/ECM interactions. The involvement of exosomes has been identified in the pathology and healing responses associated with several diseases including cardiovascular diseases (CVDs) [25–28]. The exosomes are capable of delivering and engulfing several signaling molecules to and from adjacent/distant cells and ECM suggesting their active involvement in biological responses [29]. Moreover, the molecular content of exosomes varies in response to biological stimuli such as alterations in O_2 content, nutrient status, and various stresses [30].

Exosomes are extracellular vesicles (EVs) released from the cells to the surrounding biological fluid including plasma, milk, saliva, sweat, tear, semen, and urine [31]. The exosomes are homogeneous vesicles of 50–100 nm diameter formed by the fusion of endosomes with the plasma membrane. Also, the cells release larger heterogeneous vesicles with size up to 2 μm called microvesicles that are formed due to budding or shedding of cellular membrane [32]. Similar to microvesicles, the exosomes carry cargo to deliver either to adjacent or remote locations within the body [31]. The contents of exosomes include genetic materials such as mRNA, miRNA, and traces of DNA and proteins such as growth factors, mediators of gene expression including the transcription factors and cytokines [33]. The exosomes exhibit diverse functions [34–46], as shown in Fig. 1. The exosomes are

Table 1 The biological and immunological events associated with inflammatory phase of the remodeling of IZ following MI

Inflammatory phase		
Biological events	Cellular response	References
Hypoxia	Ischemia	[12]
Ischemia	Insufficient supply of O_2 and nutrients to IZ distal to the occlusion site, necrosis, apoptosis, and autophagic death of cardiomyocytes and parenchymal cells	[12]
Loss of vascular integrity	WBC infiltration	[13]
Reperfusion	Reactive oxygen species (ROS) generation, complement activation	[14]
Oxidative stress	Mitochondrial dysfunction, cell, and ECM damage	[15]
Activation of damage associated molecular patterns (DAMPs)	DAMPs bind to PRRs of immune cells and surviving myocardial parenchymal cells to trigger sterile inflammation	[16]
Secretion and activation of chemokines/cytokines	Upregulation of IL-1, TNF-α, IL-6, and IL-18 and others amplify inflammation via downstream MAPK and NF-κB signaling, extend inflammation to surviving parenchymal cells which express the receptors for these cytokines, and acilitating the recruitment of more immune cells	[11]

decorated with biomarkers such as Alix, CD3, CD9, CD63, CD81, CD146, and HSP70 which may vary depending on the type and status of the cells that have been exploited for their detection and quantification [31].

Since the classical definitions to distinguish exosomes and EVs are unavailable and due to the close similarities between the both, the terminology of "exosomes" used in this article is applicable to "EVs" as well. Because, the published literature employed both terms, exosomes and EVs, for versatile tissue regenerative strategies.

Biogenesis

Exosomes are derived from endosomes as inward budding of endosomal membrane resulting in the formation of intraluminal vesicles (ILV). Depending on the signals, the ILVs are degraded in lysosomes or fused with plasma membrane for extracellular release as exosomes [25, 47]. The biogenesis and loading of exosomes are tightly regulated by the endosomal system mediated by trans-Golgi network (TGN) which maintains the dynamicity of the recycling of multiple receptors. This facilitates the incorporation of the contents in the exosomes to reflect the physiological status of the cell [25]. The exosome biogenesis pathways differ depending on the content, composition, and architecture of the secreted exosomes which can be either endosomal sorting complex required for transport (ESCRT) pathway or ESCRT-independent pathway [48]. ESCRT pathway is based on protein sorting to endosomal membrane and inward budding whereas ESCRT-independent pathway is based on lipid microdomains, lipid rafts, and/or tetraspanins [48–51].

ESCRT exists as four multi-protein complexes designated as ESCRT-0, ESCRT-1, ESCRT-2, and ESCRT-3, and the accessory proteins such as Alix and VPS4 (vacuolar protein

Table 2 The cellular events associated with inflammatory phase of the remodeling of IZ following MI

Cells	Inflammatory response	References
Cardiomyocytes	Necrotic and surviving cardiomyocytes stimulates inflammatory responses, DAMP activation, and ROS generation	[11]
Endothelial cells	Promotes the infiltration of immune cells and stem cells to IZ	[17]
Neutrophils	DAMP signaling, secretion of inflammatory mediators, and ECM degradation	[11]
Monocyte subpopulations	Early-phase pro-inflammatory $Ly6C^{hi}$ monocytes activates via MCP-1 receptor to trigger phagocytosis and ECM degradation and inflammation, where the late-phase anti-inflammatory $Ly\text{-}6C^{lo}$ monocytes act via CX_3CR1 to facilitate myofibroblast accumulation, angiogenesis, and ECM deposition	[18]
Lymphocytes	Subpopulations of $CD4^+/CD8^+$ T-cells, $Foxp3^+$ regulatory cells (Tregs), invariant natural killer (iNK) T-cells, and γδT-cells aid in wound healing	[11]
Fibroblasts	DAMP signaling and cytokine secretion, ECM synthesis, and fibrosis	[19]
Mast cells	Perivascular mast cells release TNF, histamine, and tryptase to trigger inflammation	[11]
Macrophages	Two subsets: pro-inflammatory M1 and anti-inflammatory M2 populations	[20]
Dendritic cells	Mo/Mϕ, $CD11c^+$ dendritic cells activate scarring and angiogenesis	[21]

Table 3 The cellular and biochemical events associated with reparative, proliferative, and maturation phases of the remodeling of IZ following MI

Reparative and proliferative phase		
Biochemical events	Pathological effects	References
Suppression of inflammation	Decrease in pro-inflammatory cytokines, hypoxia, acidosis, and neutrophil density and increase in anti-inflammatory cytokines, M2 macrophages, lipid-derived pro-resolving mediators, and pro-fibrosis mediators	[11]
Fibroblast activation and scarring	Transdifferentiation of cardiac fibroblasts to the synthetic phenotype called myofibroblast	[22]
ECM remodeling	Formation of mechanically weak provisional matrix comprising fibrin and fibronectin is formed at IZ which matures by collagen deposition	[23]
Maturation phase		
Biochemical events	Pathological effects	References
Scar maturation	ECM cross linking, deactivation of reparative cells, withdrawal of fibrogenic growth factors, clearance of matricellular proteins, and reduction in ECM synthesis and myofibroblast density	[24]

sorting- associated protein 4) bind to these complexes. ESCRT-0, ESCRT-1, and ESCRT-2 complexes are mainly involved in sequestering the ubiquitinated proteins at the endosomal membrane whereas ESCRT-3 facilitates the membrane budding and the scission of ILVs [52, 53]. The oligomerization of the exosomal components initiates the formation of membrane domains, which stabilizes and grows beyond a critical size to bud off. Also, the tension between liquid-ordered and disordered domain boundaries has been considered to be the driving force underlying the biogenesis of exosome [54]. Moreover, the ESCRT complexes are mainly associated with cargo processing and loading. The involvement of chaperons such as Hsc70 (heat shock cognate 70) is also associated with the incorporation of cytosolic constituents to the exosomes in most cell types [55].

Tumor susceptibility gene 101 (Tsg101) is associated with ESCRT-1 complexes with ubiquitinated cargo proteins and activates ESCRT-2 which in turn initiates the oligomerization

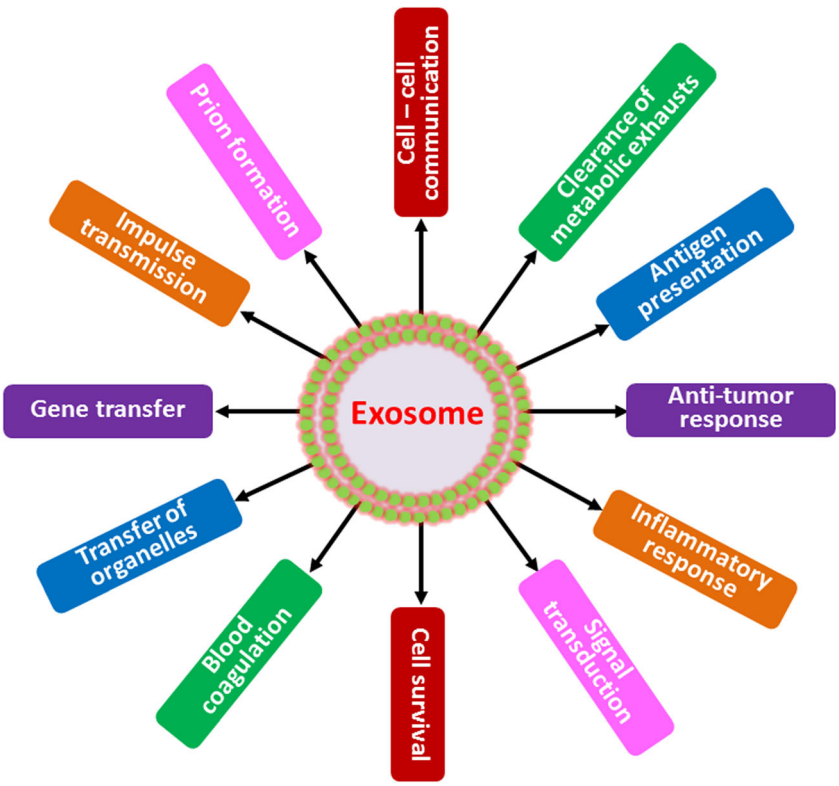

Fig. 1 Schematic diagram showing the general functions of exosomes

and subsequent formation of ESCRT-3 complex. The ubiquitination was originally considered for the sole tag for proteasomal degradation of proteins. However, the functional role of ubiquitination in sorting the membrane proteins in the TGN for lysosomal targeting has been established [56]. Also, the site of ubiquitin linkage determines the fate of tagged protein. For instance, ubiquitination at Lys-48 drives the proteins for proteasomal degradation whereas Lys-63 linked ubiquitin chains are targeted to membrane bound vesicles such as exosomes [57]. The active ESCRT-3 complex recruits deubiquitinating enzymes to remove ubiquitin tag from the cargo. More than 95 deubiquitinating enzymes have been identified in human genome which are either Zn-metalloproteases or cysteine protease [58]. For example, ubiquitin-specific peptidase 8 (USP8) is a cysteine protease, mainly found in cytoplasm and endosomes of several cell types, which degrades Lys-48, Lys-63, and Lys-6 linked ubiquitin chains [59]. The ESCRT-3 complex is then disassembled by AAA+ (ATPases associated with diverse cellular activities) following the sorting of cargo proteins in ILVs [60, 61].

Alix, a vacuolar protein sorting factor, interacts with Tsg101 and activates ESCRT-mediated ILV formation [62]. Also, Alix interacts with CD63 tetraspanin along with the transmembrane proteins syndecan and syntenin to induce the budding of exosomes [48]. For instance, the exosomal secretion of transferrin receptor occurs via its interaction with Alix, however independent of ubiquitination [63]. However, the sorting and loading of proteolipid protein in the exosomes of oligodendritic cells do not require either Alix or Tsg101 [49]. These studies suggest the existence of versatile mechanisms (ESCRT-dependent and ESCRT-independent) for exosome proteins in different cell types. The major molecular events associated with exosome formation and release are depicted in Fig. 2.

Regulation of Exosome Release

The release of exosomes is operated via two mechanisms: inducible or constitutive depending on the cell sources [64]. The constitutive secretion pathway mainly depends on Rab GTPases whereas inducible secretion is regulated by various cellular activities [65]. Cellular stimuli such as increased Ca^{2+} influx, DNA damage, extra cellular ATP, hypoxia, infection, and immune stimulation have been identified to be the trigger

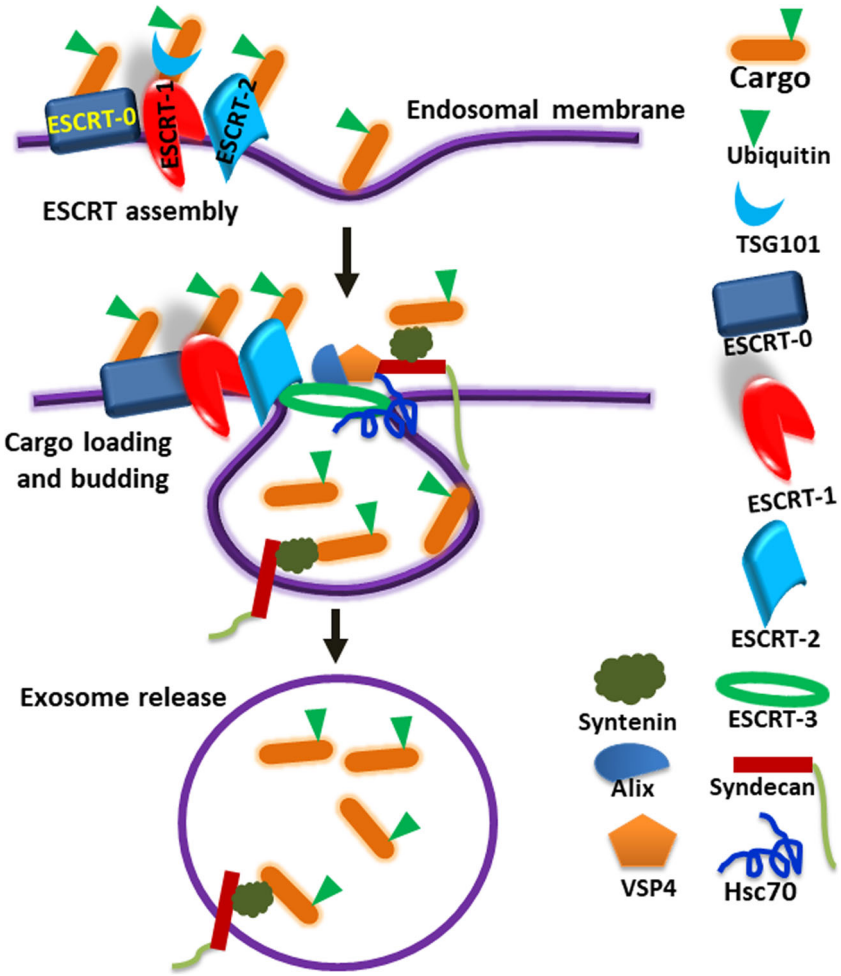

Fig. 2 ESCRT assembly, cargo loading, and exosome formation: The exosome formation initiates by the assembly of ESCRT complex in the endosomal membrane. The ubiquitinated cargo is processed by the sequential interaction between ESCRT-0-2 which in turn recruits and assembles the ESCRT-3 and the adapter proteins such as Alix, VSAP4, and Hsc70. Transmembrane proteins such as syndecan and the protein associated with cargo loading such as syntenin also form the part of ESCRT-3 complex. ESCRT-3 is responsible for the budding of the vesicle from endosome

for exosome release [65]. Gupta and Knowlton reported that mild hypoxia triggered doubling of exosome release by the cardiomyocytes within 2 h [66]. Moreover, the release of exosome has been reported to be influenced by p53-mediated genes and also on lipid mediators such as diacylglycerol [25]. Molecular mechanisms underlying the exosome release are unexplored arena in exosome biology and warrants further investigations.

The transport, docking, and fusion of exosomes with the plasma membrane are driven by the co-ordination of cytoskeletal components, tethering factors, Rab GTPases, SNAP (soluble N-ethylmaleimide-sensitive factor attachment proteins), and SNARE (receptors for SNAP). The SNARE family of proteins such as YKT6, VAMP7, VAMP3, and syntaxin-1a is critical for membrane fusion during the formation of exosomes [67]. Among the 60 identified Rab proteins, Rab11, Rab27, Rab35, and Rab22a have been identified to be associated with the regulation of exosome release [67]. Interestingly, the Rab proteins which are not directly involved in exosome biology also contained in the ILVs which warrants further research [68]. In addition, ARF6 (ADP ribosylation factor 6) activates its effector enzyme phospholipase D2 (PLD2) to interact with syntenin and Alix which in turn facilitates exosome release [69]. Also, the hypoxia triggers the exosome release via hypoxia-inducible factors (HIF): HIF-1α and HIF-2α [67]. In addition, the recent findings revealed that IFN-1 slows down the exosome release by activating the degradation of Tsg101 [70].

Along with the SNARE, the cytoskeletal proteins such as actin and microtubules, molecular motor proteins such as kinesins and myosins, and molecular switches (small GTPases as mentioned above) are also involved in the release of exosomes [36]. The Rab GTPases act via their downstream effector molecules. For instance, the silencing studies revealed the active involvement of two effector molecules, SYLT4 (synaptotagmin-like 4) and exophilin 5, in Rab27-mediated exosome release [71]. Observations from several models revealed the existence of versatile stimuli and diverse mechanisms for exosome synthesis and secretion [36]. For example, the depolarization of plasma membrane triggers the rapid secretion of exosomes by the neural cells, and the cross-linking of CD3 in T-cells stimulates release of exosomes in a similar manner [72, 73]. Alterations in the intracellular Ca^{2+} concentration also act as trigger for the release of exosomes [74]. The major signals responsible for exosome release are depicted in Fig. 3.

Exosome-Cell Communication: a "*Molecular Dialect*"

The exosomes were originally identified as a disposal system for cellular garbage from the cells having poor lysosomal activity. Later, the physiological roles of exosomes have been unveiled which are mainly mediated via cell-cell communication and signaling [65]. The exosomes usually communicate in a juxtacrine manner by receptor-ligand interaction (cell-to-exosomes or vice versa) and subsequent activation of intracellular signaling. The fragments of degraded exosomal membrane proteins act as ligands for cell surface receptor to facilitate the internalization of exosomes to deliver the cargo resulting in the activation of downstream signaling in the

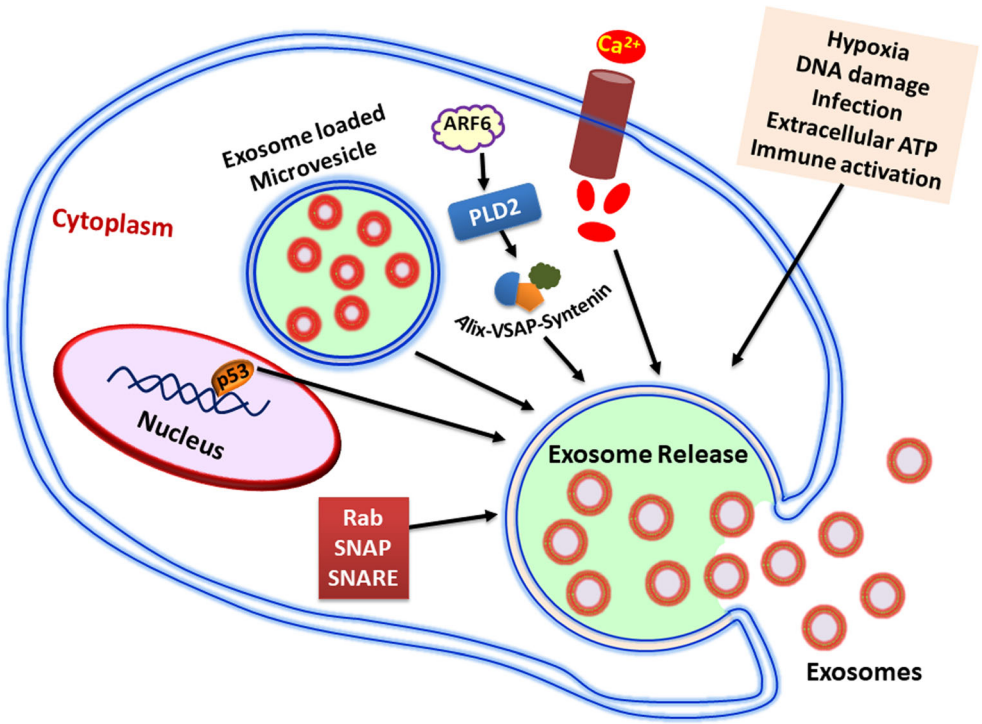

Fig. 3 Factors influencing the release of exosomes: the stimuli such as increased Ca^{2+} influx, DNA damage, extracellular ATP, hypoxia, infection, and immune stimulation activates the release of exosomes. The p53-mediated genes trigger exosome release. Rab GTPases, SNAP, and SNARE are critical for membrane fusion during the formation and release of exosomes. ARF6 activates its effector enzyme PLD2 to interact with syntenin and Alix to facilitate the release of exosome

recipient cells [65, 75]. Intercellular adhesion molecule 1 (ICAM1) from the surface of exosomal membrane of dendritic cells interacts through lymphocyte function-associated antigen 1 (LFA1) of antigen-presenting cells and T-lymphocytes for their uptake [75, 76]. Similarly, the involvement of several receptors and ligands such as CD51, CD61, CD11a, CD54, CD9, CD81, lactadherin, and CD91 (the receptor for several heat shock proteins) and the lipid molecule phosphatidylserine has been identified in multiple cell types [77–79].

The proteolytic processing of transmembrane is also possible within the intracellular sites including exosomes and microvesicles. The degradation fragments of such proteins trigger the exosome release and signaling [65]. For example, the neural adhesion molecule L1 and CD44 undergo proteolytic processing in microvesicles by the ADAM10 (disintegrin and metalloproteinase domain-containing protein 10) which are released by the exocytosis. Similar mechanism has been reported for CD46 and tumor necrosis factor receptor 1 (TNFR1) [65, 80, 81]. Furthermore, proteins such as galactin-5 and -9 were reported to be associated with the signaling of exosomes by macrophages and $CD4^+$ T-cells [36].

The internalization of exosomes is the ideal mechanism for the delivery of exosomal contents, especially the genetic materials, to the target cell. The fusion of exosomes with the plasma membrane of the recipient cell depends on the physiochemical status of the membrane. For example, the fluidity of exosomes and plasma membrane is closely similar at pH 5 and this acidic pH has been considered to be ideal for fusion whereas in neutral pH, the membranes become rigid which hurdles fusion [65, 82]. Phagocytosis, macropinocytosis, and endocytosis are the other mechanisms adopted for exosome-mediated communication [79, 83].

The understanding regarding the biology of exosomes is necessary to explore the interplay of the pathological/regenerative mediators at the IZ. Also, it is reasonable to speculate that the basic mechanism of exosome release and function would be similar irrespective of the difference in cell/tissue types, despite the tissue-specific molecular mechanisms. The specific signaling pathways and molecular mediators associated with the IZ-exosomes are largely unknown; however, the published literature on this aspect is limited which warrants further research. Based on the current understanding of exosome biology, the extrapolation of the general principles of exosome transit with respect to the diverse cellular milieu at the IZ could unveil the molecular mechanisms underlying the cardiac tissue regeneration.

Cardioprotection at the Infarct Zone: "*the Exosomes in Action*"

As discussed above, the membrane-bound vesicles fall into three main categories: exosomes, microvesicles, and apoptotic bodies. Exosomes are endosome-derived vesicles released through the fusion of multivesicular bodies with the plasma membrane. Microvesicles (~ 20 nm to 2 μm) arise by the direct budding of plasma membrane whereas the apoptotic bodies (~ 50 nm to 5 μm) emerge due to the blebbing of apoptotic cells. Majority of studies used the term "exosomes" to define the isolated vesicles as no current technique, other than by size, is available to distinguish between the exosomes and microvesicles [84]. These vesicles play potential roles in the pathogenesis and healing responses of CVDs. For example, the increased contents of platelet-derived vesicles and tissue factor-loaded microvesicles have been associated with familial hypercholesterolemia as these vesicles possess procoagulant and atherogenic activities [85]. In addition, the endothelial vesicles contribute to the dysfunction of endothelium and subsequent CADs [86]. The increased pool of leukocyte-derived vesicles in atherosclerotic patients suggest their potential use in the diagnosis of early-stage atherosclerotic alterations [87]. Moreover, the role of vesicles in microcalcification and plaque rupture in MI pathology has been unveiled [88]. The apoptotic bodies are formed in the later stage of apoptosis which are engulfed by phagocytes and smooth muscle cells [89]. However, the signaling pathways mediated by apoptotic bodies in the pathogenesis of CVDs remain unexplored.

The functional role of exosomes in cardiac repair following MI has gained prior attention to CVD researchers. The exosomes released from red blood cells (RBCs), white blood cell (WBC) sub-types, platelets, and endothelial cells carry biological information of regenerative, diagnostic, and therapeutic potential for coronary heart diseases (CHDs) [90]. Also, the exosomes derived from human atherosclerotic plaques affect inflammation, cell proliferation, thrombosis, calcification, and vascular responses which impact the severity of MI [90]. In addition, the exosomes released at the vicinity of IZ may carry the regenerative mediators; however, the information regarding regenerative exosomes is obscure. The screening of such regenerative components, the identification of the sources of regenerative exosomes, and the determination of appropriate signals which triggers the synthesis and release of regenerative exosomes could open immense translational opportunity in regenerative cardiology. However, extensive research is warranted to translate the regenerative exosomes to therapeutic arena. This section reviews the current developments in exosome biology and their potential therapeutic aspects for taming the IZ.

Cardiosomes and Cardiac Tissue-Derived Exosomes

The cardiomyocytes at the IZ have been reported to secrete exosomes which are collectively referred as "cardiosomes" which possess significant regenerative potential [25]. The major sources of exosomes with cardiac regeneration potential, as reported in the literature, is displayed in Table 4.

Table 4 Major sources of exosomes with cardiac regeneration potential

Exosomes	Source	Function
Cardiosomes	Cardiomyocytes	Cardiac regeneration and immunomodulation
Stem cell-derived exosomes	Various stem cells	Prevention of apoptosis, angiogenesis, antioxidant response, cell proliferation, and cardioprotection
Circulatory exosomes	Circulating leukocytes and platelets	Pro-coagulation, vaso-relaxation, and inhibition of autophagy
Cardiac tissue-derived exosomes	Cardiac telocytes, cardiac fibroblasts	Immunomodulation, cardioprotection, and cell differentiation

The secretion of cardiosomes from neonatal rats was increased during hypoxic insult in which HSP60 was identified to be the major content of those hypoxic exosomes [91]. Extracellular HSP60, a mitochondrial chaperone, has been considered as DAMP (damage-associated molecular patterns) which acts via TLR4 receptors [92]. HSP60 exhibits immunomodulatory and pro-inflammatory functions; however, it is still in debate [93], and its role in cardiac regeneration is largely unknown. Similarly, the exosomes containing tumor necrosis factor-α (TNF-α) were elevated at the IZ following MI which is released in response to hypoxia mediated by HIF-1α signaling, and elevated level of TNF-α results in the death of cardiomyocytes at the IZ [94]. Interestingly, a population of exosomes with TNF receptor (TNFR) has been identified in human plasma which modulates TNF-α-mediated cell loss and inflammation [95].

Recent reports revealed the cardioprotective effects of exosomes released from cardiac telocytes and cardiac progenitor cells by preventing the apoptosis of cardiomyocytes and improving the cardiac function following MI [96]. The electron micrographs of left ventricular cardiomyocytes from human patients displayed increased exosomes suggesting their possible role in cardiac rejuvenation. However, the cardiosomes varied in their size (40–300 nm), electron microscopic pattern (electron dense or electron lucent), and contents [97]. Majority of cardiosomes express flotillin and caveolin-3 on their surface [98]. Interestingly, the exosomes of border zone cardiomyocytes exhibit cup-shaped architecture embedded within the sarcomeres [99]. Moreover, the exosomes originated from the cells of IZ release their contents to general circulation targeting the cells at remote locations and may serve as next-generation biomarkers for MI [100].

Generally, the micro niche of parental cells influences the quality and the contents of released exosomes. For instance, the HL-1 cardiomyocytes treated with TGF-β2 and PDGF-BB displayed considerable alterations in the quality, quantity, and the content of the released exosomes. The fibroblast cells which received TGF-β2/PDGF-BB-stimulated exosomes displayed alterations in their expression profile [101]. Also, the naïve exosomes released from parental cells at the IZ exert strong protective/regenerative potential on the recipient (surviving) cells [102]. However, the identification and manipulation of naive exosomes are challenging owing to the versatility of their contents and biological effects. On the other hand, exosomes can be effectively modified to be used as delivery vehicles for CVD drugs [103]. These findings suggest that the cardiosomes play a significant role in driving the phenotypic switch of cardiac fibroblasts at the IZ. However, the literature on cardiosomes is limited and further research is warranted to understand their translational and diagnostic significance. Based on the information obtained from other cell types, it is logical to speculate that the exosomes released from the surviving/dying/stressed cardiomyocytes modulate the molecular signaling to transform non-cardiomyocyte cell populations to functional cardiomyocytes and to improve the cardiac function.

Stem Cell-Derived Exosomes

The MSC-derived exosomes possess the potential to regenerate the injured cardiac tissue by preventing cell death and promoting the angiogenesis to restore the blood flow. Timmers et al. [104] reported that the intravenous administration of MSC-conditioned medium significantly reduced the IZ more than 50% in swine and rat models of MI. The animals which received the conditioned medium exhibited improved cardiac function and minimal oxidative stress suggesting the therapeutic potential of MSC-derived exosomes [105]. Moreover, the hostile microenvironment at the IZ following MI stimulates the release of exosomes from bone marrow-derived dendritic cells (BMDCs). These exosomes significantly improved the cardiac function in MI mice following left coronary ligation [106]. Such exosomes, released from IZ, are taken up by $CD4^+$ T-cells in the spleen and trigger the downstream signaling to enhance the expression and release of various cytokines and activate the Treg cells for cardioprotection [106, 107].

The molecular transit of exosomal mediators released at the IZ facilitates the cardiac repair by inducing angiogenesis, cell proliferation, and prevention of cell death by apoptosis, necrosis, and pyroptosis [108]. For instance, the MSC-derived

exosomes delivered at the IZ significantly reduced the fibrotic reactions [108]. The experimental evidences demonstrate that the exosomes released from the cardiac progenitor cells (CPCs) in the damaged heart improves the cardiac function. However, the contents of exosomes largely depend on the pathological/physiological status of CPCs which warrants thorough screening [109]. The cardiosphere-derived cells secrete miR-146a enriched exosomes to confer cardioprotection from MI [110]. Minghua et al. [111] demonstrated the cardioprotective effects of exosome-derived miR-24 from the plasma of experimental rats which acts by silencing the expression of the pro-apoptotic protein Bim.

Circulatory Exosomes

A drastic increase in the circulating vesicles has been observed in human plasma following acute MI which was associated with the obstruction of micro vessels [112]. However, those circulating exosomes exhibited pro-coagulation potential and promoted vaso-relaxation by inhibiting endothelial NO pathway [113, 114]. Moreover, the ischemia and activation of platelets at the IZ stimulate the release of exosomes from endothelial cells, platelets, and leukocyte subpopulations [67]. Moreover, the hypoxia-challenged H9c2 cardiomyoblasts released miR30a via exosomes which function to inhibit autophagy by targeting HIF-1α [115]. Also, similar increase in exosomes was observed in pericardial fluid following MI that contained clustrin which, in turn, facilitates epithelial-to-mesenchymal transition in epicardial cells [67]. On encountering such exosomes, the expression profile of epicardial cells was altered with the co-expression of smooth muscle actin (SMA) and c-Kit (stem cell biomarker) [116]. Also, the clustrin-containing exosomes have proven their potential to increase angiogenesis and to improve cardiac function [116, 117].

The level of miR-208a in the circulating exosomes has been correlated with cTnI levels post-CABG surgery suggesting its diagnostic potential [118]. Similarly, miR-192, miR-194, and miR-34a have been considered as prognostic biomarkers, in which miR-34a is highly expressed at IZ following MI and is incorporated to the exosomes of cardiomyocytes and fibroblasts [119, 120]. Similarly, the macrophage-derived exosomes carry miR-155 which prevents the cardiac regeneration following the MI and the exosome-mediated transfer of miR-155 to cardiac fibroblasts inhibits their proliferation, thereby accelerating inflammation [121]. Interestingly, the macrophages receive miR-155-enriched exosomes from endothelial cells and interferes with the polarization switch of macrophages (retains M1 status) [122]. In addition to miR-155, pro-inflammatory miRNAs such as miR-19, miR-21 miR-146, and miR-223 were also increased in macrophage-derived exosomes during CHDs [123].

Cardiac Tissue Engineering: a New Avenue for Exosome Application

The cardiomyocytes are terminally differentiated cells with limited ability for self-regeneration which warrants the need of novel therapeutic strategies for rejuvenating the injured heart following MI [124, 125]. The advancements in implant biology and biomaterials science have paved ways for the design and development of several cardiac implants including artificial valves and left ventricular assist devices which helped to prolong the life of cardiac patients. However, their limited life span, high risk of thrombosis and infections, and adverse immune reactions hurdle the performance of cardiac implants [126, 127]. Hence, the heart transplantation forms a viable option for the management of end-stage cardiac complications. However, the limited availability of donor hearts for transplantation and the post-transplant complications demand for effective alternative approaches with minimal side effects. In addition, the repair strategies such as cell delivery have been examined by several researchers; however, the lack of 3D biomimetic microenvironment, imbalanced oxygen tension at the IZ, reactive oxygen species (ROS), and hyperactivation of immune responses (especially due to the hyper secretion of matrix metalloprotease (MMPs) and pro-inflammatory cytokines) result in the failure of such approaches [128–131].

Cardiac tissue engineering (CTE) emerged as a potential alternative to replace/regenerate the damaged heart tissue [132, 133]. CTE is based on multidisciplinary approaches by adopting the principles of cell biology, biomaterials science, engineering, and medicine; however, it is still in infancy [134]. Biomaterial scaffolds form an inevitable part of CTE in which biocompatible polymeric materials (natural and synthetic) have been extensively employed [7]. Among the versatile polymeric biomaterials, hydrogel sub-sets have been widely employed owing to their superior biomimetic characters. The diverse qualities of CTE hydrogels were detailed in our recent publications [127], [133, 135–138]. However, the application of hydrogel-based biomaterials for exosome-mediated CTE and cardiac regeneration remains unexplored and the published literature regarding the same are unavailable. This section throws light to the concepts and possibilities mainly based on the biomaterials scaffolds which could offer promising opportunities for exosome-mediated CTE to accelerate cardiac regeneration.

Currently, very few clinical trials based on exosomes have been registered, mostly for cancer applications, and the information regarding exosome-based therapy for MI is unavailable in the literature [139, 140]. However, the understanding of exosome biology is advancing, and exosome-based therapeutic strategies are expected to be launched in the near future. Also, the information regarding hydrogel-mediated exosome delivery is limited in the literature. Interestingly, the

hydrophilic polymers stabilize exosomes by interacting with the side chains extending from exosomes by imparting physical and hydrogen bonding with the polymers in aqueous solution. PEG (polyethylene glycol) has revealed promising effects of stabilizing membrane-bound vesicles, and PEG displays the benefit of wide range of molecular weight, FDA approval, and anti-fouling nature [141]. These properties of PEG can be effectively utilized for the fabrication of exosome-loaded hydrogel scaffolds for CTE. Hydrophilic polymers such as PVA (polyvinyl alcohol) and naturally available heteropolysaccharides such as alginate, OPP (*O*-palmitoyl pullulan), chitosan, and hyaluronic acid can also be utilized for designing exosome-loaded CTE hydrogels [142].

Recently, Han et al. demonstrated the successful delivery of exosomes using silk fibroin hydrogels to treat age-induced vascular dysfunction. The study provided a universal strategy for using silk fibroin to deliver therapeutic exosomes for multiple diseases including CHDs [143]. Schneider et al. assessed the secretome of chondrocytes encapsulated in PEG hydrogels and revealed the ability of the hydrogels to preserve the secretome under diverse culture conditions [144]. Li et al. reported a cell-free tissue engineered bone substitute by combining exosomes derived from human MSCs with poly(lactic-co-glycolic acid) scaffolds. The bone construct accelerated the restoration of critical-sized calvarial defects in mouse model [145]. Similarly, Zhang et al. showed that exosomes loading enhanced the osteoinduction potential of β-TCP (tricalcium phosphate) which act by activating PI3K/Akt signaling in human bone marrow MSCs [146].

Limited information is available regarding the exosome-loaded biomaterial scaffolds for cardiac tissue engineering/regenerative applications suggesting that exosome-mediated CTE is still in its infancy. Unfortunately, very minimal information is available for other diseases also. However, based on the available information, it can be confirmed that the exosomes possess immense therapeutic potential for taming the IZ and the combination of such exosomes with CTE strategies would provide better clinical outcomes. Simultaneous incorporation of therapeutic exosomes, signaling mediators, and cells for IZ regeneration would facilitate to establish a proper communication between loaded exosomes with loaded cells, loaded exosomes with host cells, exosomes released from loaded cells with the host cells, and the exosomes released from the host cells with the loaded cells. The transit of these exosomes at the bio-interface between the implant and IZ would accelerate the regeneration of the surviving heart tissue to re-establish a functional myocardium. The hypothetical communication among exosomes from diverse cell sources at the interface is displayed in Fig. 4.

Future Perspectives

Recent research highlights the therapeutic potential of cardiosomes released from the injured/infarcted heart and the exosomes from several cell sources to regenerate the IZ by facilitating the juxtacrine/paracrine communications. However, there are several unaddressed challenges that warrant further research before arriving at a conclusion. The

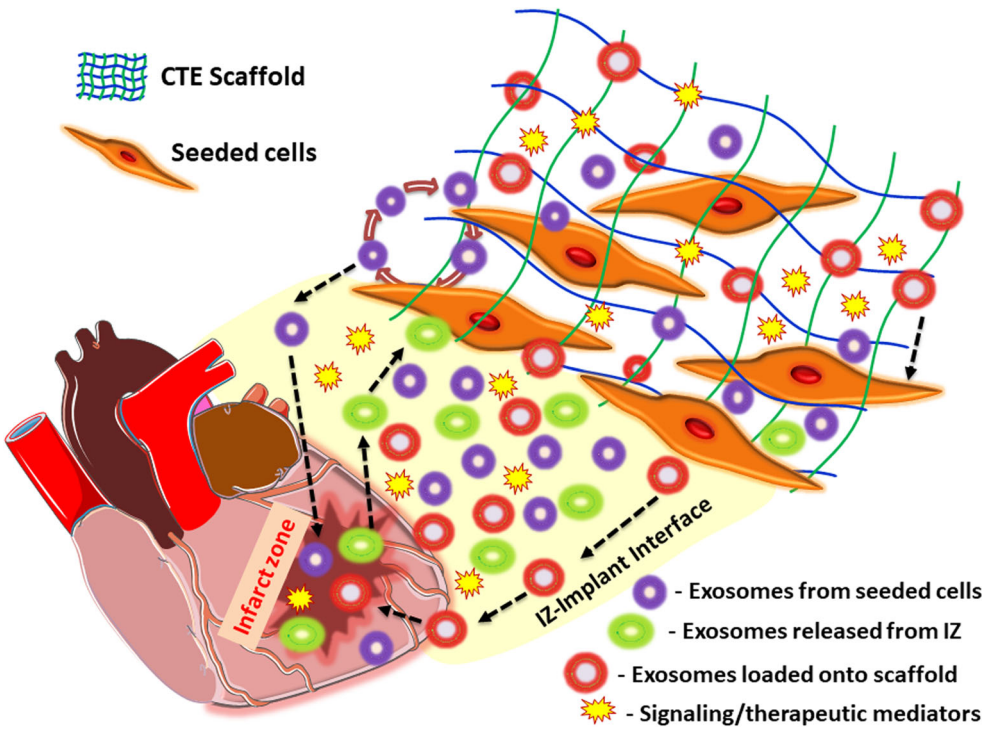

Fig. 4 The transit of exosomes at biomaterial-infarct zone (IZ) interface: The hypothetical CTE strategies to establish a proper communication of exosomes of versatile origin at the biomaterial-IZ interface. The exosomes of therapeutic potential loaded to the biomaterial scaffold interact with the loaded cells and the host cells, exosomes released from loaded cells interact with the host cells and the cells seeded onto CTE scaffold, and the exosomes released from the host cells interact with the loaded cells to accelerate the regeneration of a functional myocardium at the IZ

information regarding the specific cell types initiating the signaling at IZ, the diversity in their sources, quality and quantity, fate, targets and potential downstream signaling, effect on cell reprograming, recruitment and differentiation of stem cells to the IZ, remote signaling, and regulation of exosome signaling is currently unavailable. Moreover, the practical challenges including their smaller size, difficulty to study their effects under physiological conditions, dynamic release profile, dose, off-target effects, tracking, and inefficiency of purification and quantification techniques hurdle the translational applications of exosomes in the management of CVDs.

Even though the exosomes offer an effective cell-free therapeutic strategy, reproducibility in harvesting and purification and maintenance of their sterility would require further standardization for good manufacturing practices (GMPs) for therapeutic interventions. Also, the dosage regimen and route of administration remain debatable. Most of the animal studies on the therapeutic effects of exosome were conducted by the administration of exosomes immediately following the induction of MI, which is not practical in human clinical scenario. In addition, the long-term effects of exosome-based therapeutic strategies and the metabolism of exosomes warrant further research.

In general, the exosome-mediated effects are influenced by multiple factors, most of which are not fully elucidated. On the other hand, there is a multifold increase in cardiosome research aiming to unveil the cell-cell, cell-ECM, and tissue-tissue communications, to identify novel biomarkers for MI, to understand the pathology/regenerative mechanisms, and to discover potential candidates for translational cardiology. Furthermore, the advent of CTE has given new avenues for the therapeutic applications of exosomes; however, this emerging field of medicine is still in infancy. However, the multidisciplinary approaches to combine CTE and therapeutic exosomes for their sustained release at the IZ would facilitate better communications within/among the seeded/host cells which in turn accelerate the healing responses and prevents the infarct expansion. The functional applicability of exosomes in the re-establishment of electrical and mechanical circuits using CTE scaffolds in the surviving myocardium following MI remains as an unattended area of research in cardiac regeneration. Nonetheless, significant progress has been achieved in the area of therapeutic exosomes for cardiac applications during the recent years which would pave multiple ways for the development of exosome-based therapeutic strategies for cardiac tissue engineering and regeneration.

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