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Mitochondrial transplantation for cardioprotection and induction of angiogenesis in ischemic heart disease

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Abstract

To date, the regenerative potential of mitochondrial transplantation (MT) has been extensively investigated under several pathologies. Among various cardiovascular diseases, ischemic heart disease (IHD), the most prevalent pathological condition in human medicine, is induced by coronary artery narrowing, or occlusion, leading to bulk necrotic changes and fibrosis within the myocardium. Data associated with the pro-angiogenic activity of mitochondria have not been completely elucidated in terms of cardiac tissue regeneration. Here, we aimed to highlight the recent studies and advantages related to the application of mitochondrial mass in the ischemic myocardium. How and by which mechanisms, mitochondria can reduce aberrant myocardial tissue remodeling via different pathways such as angiogenesis and *de novo* blood formation was discussed in detail. We hope that data from the current review article help us understand the molecular and cellular mechanisms by which transplanted mitochondria exert their regenerative properties in the ischemic myocardium.

Keywords Ischemic heart disease, Mitochondrial transplantation, Cardiac tissue regeneration, Angiogenesis

Introduction

Based on the released data, approximately one-third of all deaths worldwide are caused by cardiovascular diseases (CVDs) [1]. Among several CVD types, IHD is the common cause of heart failure (HF) and the most prevalent pathological condition with a high mortality rate in clinical settings [2, 3]. IHD usually happens due to several etiologies that influence the normal function of the coronary artery [coronary artery disease (CADs)], leading to massive myocardial infarction (MI), aberrant remodeling, loss of contractile efficacy, and eventually congestive heart failure (CHF) [4]. In most circumstances, the stenosis or obstruction of the left anterior descending (LAD) artery or left main stem of the coronary artery results in IHD due to abrupt interruption of blood supply and lack of sufficient O_2 to cardiomyocytes [5–7].

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Under hypoxic conditions, the sudden metabolic switch from aerobic oxidative phosphorylation (OXPHOS) pathway to anaerobic glycolysis reduces the intracardiomyocyte ATP content due to the loss of mitochondrial membrane integrity (ΔΨ↓), making the stressed cardiomyocytes vulnerable to different cell death types such apoptosis, autophagy, necroptosis (RIPK1↑, RIPK3↑, MLKL↑) ferroptosis (Fenton reaction↑, ferrous ions↑, ferrivalent↑, ROS↑) and pyroptosis (GSDMD↑, and Caspase-1↑) [8, 9]. Besides, the continuity of ischemia can sensitize the intra-cardiomyocyte organelles to subsequent irreversible injuries and aberrant extracellular matrix remodeling (ECM) [10].

Because mitochondria are cellular powerhouse subcellular units, restoration of mitochondrial function and number per cell can suitably afford the increased energetic demands of stressed cardiomyocytes after ischemia [11]. In recent years, the number of studies associated with MT has increased in various animal models of ischemia with few clinical trials in humans [12]. It has been thought that the increase of myocardial vascularization is an effective strategy to support appropriate cardiac tissue regeneration and reduce scar tissue volume after ischemia [13]. Whether and how transplanted mitochondria can regulate the function of endothelial cells (ECs) and angiogenesis phenomena needs to be elucidated. In the following sections, details were collected related to the putative regenerative roles of MT, and vascularization in terms of MI.

Energy status in IHD

It has been thought that sudden intracellular ATP depletion and energy stress following ischemic changes increase the number of cardiomyocytes entering apoptosis, and necrosis, leading to prominent pathological outcomes [14]. The concomitant ATP drop and the surplus of mineral phosphate (Pi), free Ca^{2+} cation, lactate, and low pH values reduce the contractibility of cardiomyocytes [15]. Under such circumstances, danger signals [damage-associated molecular patterns (DAMPs)] such as Ca^{2+} ions and ATP are leaked from injured cardiomyocytes into the extracellular matrix (ECM), and taken by local leukocytes, leading to the activation of these cells. The activation of several signaling pathways such as Toll-like receptor (TLR), accumulation of reactive oxygen species (ROS), and production of diverse cytokines and interleukins (ILs), i.e. tumor necrosis factor-alpha (TNF-α) can recruit neutrophils, and other immune cells to the injured sites [16].

As above-mentioned, the energy metabolic switches from mitochondrial OXPHOS to anaerobic glycolysis upon short-, and long-term ischemia [17, 18]. In response to hypoxia, the expression of hypoxia-inducible factor-1 alpha (HIF-1α) leads to concomitant activation of pyruvate dehydrogenase kinase (PDK) and inhibition of pyruvate dehydrogenase complex (PDC), thus reduces the entry of pyruvate to Krebs cycle [19]. The increase of intracellular Ca^{2+}, ROS, and decrease of nitric oxide (NO), Na^+/H^+ exchanger release H^+ into the ECM, and the influx of Na^+ and Ca^{2+} dominates. However, the reduction of pH and Ca^{2+} overload leads to loss of mitochondrial membrane potential (ΔΨ↓) and abnormal opening of mitochondrial permeability transition pores (mPTP). Further ATP depletion and extensive cardiomyocyte swelling (hydropic degeneration) promote subsequent necrotic changes [17, 18]. As above-mentioned, restoration of blood supply can potentiate the hypoxic cardiac tissue to the I/R injury [17, 20, 21]. Commensurate with these conditions, finding novel and sophisticated therapeutic modalities in MI patients is at the center of debate [20]. It is postulated that supplying the minimum ATP levels required for the maintenance of cellular homeostasis can increase the resistance mechanisms in injured and sensitive cardiomyocytes to restore their function and metabolic activity [22]. Among several organelle types, mitochondria are specific ATP-generating entities and exist in various tissues with varied shapes and numbers [22]. Under physiological and pathological conditions, mitochondrial mass can be altered to support and match the cardiomyocyte energy demand by the regulation of fusion, fission, biogenesis, and death [23, 24]. In normoxic conditions, aerobic OXPHOS is the dominant ATP production pathway from fatty acids and low glycogen stores [25]. In response to transient hypoxia and mild oxidative stress, the process of fatty acid breakdown is reduced in cardiomyocytes while the simultaneous entry of glucose is enhanced to glycolysis for ATP production and ion homeostasis [26, 27]. Of note, in severe hypoxic conditions and ischemia, the lack of glucose uptake by cardiomyocytes along with excessive intracellular H^+ leads to severe acidosis and glycolysis inhibition [28, 29].

Regarding the fact that cardiomyocytes possess higher mitochondria mass (~30% of total cell volume), the maintenance and restoration of mitochondrial function is crucial during MI and other CVDs [30, 31]. The release and accumulation of Ca^{2+} ions in cytoplasm lead to loss of mitochondrial membrane integrity and opening of mPTP under hypoxic conditions, leading to mitochondrial swelling and further cardiomyocyte injuries [32]. To date, several pharmacological agents and factors have been used to regulate mitochondrial function at the molecular levels during pathological conditions within the heart [33]. The neutralization of ROS and production of ATP by mitochondria along with the reduction of excessive Ca^{2+} have been touted as a therapeutic strategy in cardiomyocytes under stressful conditions. In this regard, cyclosporine A, a calcineurin inhibitor, blocks the

mPTPs by direct interaction with cyclophilin [34]. Other strategies are based on the regulation of respiratory chain ROS-producing enzymes such as complex I, xanthine oxidase, and NADPH oxidase or direct neutralization of free radicals [17]. For instance, α-tocopherol, coenzyme Q10 (CoQ10), and α-lipoic acid exhibit cardioprotective effects by the reduction of oxidative and nitrosative stress (NO↓) [35]. To overcome Ca^{2+} overload, chelating agents and antagonists such as EDTA analogs are beneficial to stop the apoptotic and necrotic changes. Other compounds such as metformin, dichloroacetate (DCA), and polyphenols directly influence mitochondria and exert cardioprotective effects [17, 36, 37]. It has been shown that the levels of CoQ10 are reduced under pathological conditions [38]. In the swine model of acute MI (AMI), 4-week administration of CoQ10 (400 mg/day) can improve the antioxidant proteins within the mitochondrial matrix [39]. In CAD patients, 150 mg CoQ10 can restore the function of antioxidant enzymes and blunt oxidative stress [40]. The acceleration of electron transfer from respiratory chain complexes I and II to complex III increases ATP production in the inner mitochondria membrane [38]. It was shown that the antioxidant properties of α-lipoic acid are associated with the activation of the PI3K/Akt/Nrf2 pathway and inhibition of excessive autophagic response. In support of this notion, simultaneous ingestion of vitamin E, and α-lipoic acid protects cardiac tissue via the reduction of lipid peroxidation in the I/R injury rat model [41]. Likewise, α-tocopherol with putative anti-oxidant and anti-inflammatory properties have been used for the reduction of excessive remodeling after MI [42, 43]. Inside the cells, AMP-activated protein kinase (AMPK) acts like an ATP biosensor and is activated in response to energy stress. Under such conditions, the activation of AMPK reduces the anabolic metabolism via the phosphorylation of key signaling pathway effectors involved in mitochondrial homeostasis [44]. On the other hand, the reduction of mitochondrial ATP provokes AMPK activity via the release of Ca^{2+} [45]. Thus, drugs and compounds targeting AMPK such as metformin can be used for the alleviation of ischemic injuries. Based on previously published data, metformin (200 mg/kg) can blunt ROS production, mitochondrial swelling, and cardiac cell depolarization, leading to a reduction of infarct size, and improvement of myocardial function in MI patients [33]. Notably, DCA, a PDK inhibitor, restores ATP production via a switch from glycolysis to OXPHOS in the perfused heart [46]. In response to DCA administration and ATP production, lactate levels, and acidosis were reduced [47].

Mitochondria as alternative therapeutics in CVDs

In recent years, stem cell-based approaches have been used for the alleviation of pathological conditions such as CVDs [48]. In this scenario, stem cell transplantation, and/or secretome components [i.e. exosomes (Exos)] have been used in different in vitro, and in vivo models to restore the function of injured cardiomyocytes [49]. MT is an innovative and burgeoning therapeutic modality in several pathological conditions like CVDs (Table 1) [50]. Of note, mitochondria for transplantation purposes can be isolated from varied stem cell types of different tissues with suitable functions [51]. It is postulated that the injected mitochondria can foster the process of healing via restoration of ATP production inside the cardiac cells after being exposed to pathological conditions [52]. Besides, the regulation of Ca^{2+} ions, ROS homeostasis, and activation of survival mechanisms are the main therapeutic roles of mitochondria in the target cells [53, 54]. In contrast to whole-stem-cell therapies, mitochondria exhibit size ranges between 250 and 1000 nm with less possibility of vascular occlusion and promotion of all-reactive immune cells [55]. Of course, it should not be forgotten that the bidirectional and unidirectional transfer of mitochondria occurs under physiological and pathological conditions. Besides, the improvement of metabolic status in injured cardiomyocytes, mitochondrial donation can stimulate the proliferation and differentiation of local cardiac tissue stem cells toward mature and functional lineages [50]. Among various available cell sources, autologous skeletal myocyte mitochondria have been extensively used in CVD conditions [56]. Autologous mitochondria transplantation is available under circumstances without genetic mitochondrial diseases [57]. In pediatric patients with the I/R injury, the injection of autologous myocyte mitochondria was done on the pericardium during the extracorporeal membrane oxygenation with the improvement of ventricular function [58]. Likewise, the injection of autologous mitochondria in children with refractory cardiogenic shock resulted in similar therapeutic outcomes [59]. Besides the examples of MT in humans, several animal models of CVDs with mitochondria transplantation have been conducted yet. For instance, the infusion of mitochondria (6×10^9 particles) in six boluses via an intracoronary artery in female MI Yorkshire pigs enhanced local blood perfusion and diminished local fibrosis, leading to suitable myocardial function [60]. In healthy pigs, the injection of ^{18}F-rhodamine-6G–labeled mitochondria via intracoronary infusion led to the localization of transplanted mitochondria in the left ventricle where the possibility of ischemic changes is high. Besides, the injection of xenogeneic iron oxide-labeled human cardiac fibroblast mitochondria in healthy pigs was detected inside cardiomyocytes and ECs using Prussian blue staining [60]. These data indicate

Table 1 Some in vivo experiments related to mitochondria transplantation in ischemic heart disease

Cell source	Administration route and dose	Outcomes	Ref
Allogeneic mitochondria isolated from LV	Intramyocardial injection just before ischemic/reperfusion injury in rabbits with LAD coronary ligation	LVPDP↓, LVEDP↓, and SS↓, Infarct size↓, CK-MB↓ and cTnI↓, ATP content↑	[120]
Autologous mitochondria isolated from Pectoralis muscle tissue	Intramyocardial injection in rabbits with LAD coronary artery ligation	Lack of arrhythmia, Myocardial function↑, Infarction area↓, CK-MB↓, cTnI↓, Local ATP content↑, Apoptotic cardiomyocytes ($TUNEL^+$ cells↓ and Caspase-3^+ activity↓), Inflammation (IL-6↓, and TNF-α↓)	[121]
Xenogeneic mitochondria isolated from human cardiac fibroblasts	Intracoronary injection and intramyocardial injection in rabbits with LAD coronary artery ligation	Intracoronary injection leads to the rapid distribution of mitochondria, Intramyocardial injection yielded clusters of mitochondria in limited areas, The most of injected mitochondria were in cardiac ECM and juxtaposed to cardiomyocytes, Infarct size↓, Cardiac function↑	[78]
Mitochondria isolated from pectoralis major muscle	Intramyocardial injection in pigs with temporarily occluded circumflex artery	Injected mitochondria persist in cardiac tissue for 4 weeks, No effects in inflammation response were achieved, CK-MB↓ and cTnI↓, No effects in collagen fiber contents, No effects in cardiac function	[122]
Autologous mitochondria isolated from pectoralis major muscle	Intracoronary injection of mitochondrial particles in a swine model of LAD coronary ligation	Distribution of injected mitochondria in LV↑, LV function↑, Coronary blood flow↑, Infarct size↓	[123]
Mitochondria were isolated from murine gastrocnemius muscle	Intracoronary injection	Necrosis↓, Beating score↑, Neutrophil recruitment↓, Cold ischemia time↓, Ejection fraction↑ and Fast shortening↑, $TUNEL^+$ cells↓	[124]
Mitochondria were isolated from the pectoralis major muscle	Intracoronary injection of mitochondrial particles in a swine model with LAD coronary ligation	Ejection fraction↑ and Fast shortening↑, Infarct size↓, Cardiac function↑	[111]
Mitochondria were isolated from the pectoralis major muscle	Intracoronary injection of mitochondrial particles	Prophylactic myocardial protection the ischemic changes↑, Infarct size↓, Necrotic changes↓, Edema↓, LV function↑	[112]
Mitochondria-rich extracellular vesicles were isolated in supernatants of human-induced pluripotent stem cells-derived cardiomyocytes	Intramyocardial injection of mitochondria in a mouse model of LAD coronary artery ligation	LV remodeling↓, Ejection fraction↑, Energetic stress↓, EVs transfer mitochondrial particles into the hypoxic cardiomyocytes, ATP production occurs in a dose-dependent manner, Contractile profile↑, Cell survival↑	[77]
Mitochondria were isolated from gastrocnemius muscle	Intramyocardial injection of mitochondrial particles in piglets exposed to pulmonary artery banding and right ventricle injury	Contractile profile↑, Right ventricle wall thickness↑, Apoptotic changes↓ ($TUNEL^+$ cells↓), Fibrotic changes↓, ATP content↑, Tricuspid annular plane systolic excursion↑, Fractional area change↑	[56]
Mitochondria were isolated from neonatal mouse ventricular myocytes	Intramyocardial injection of mitochondria one day before induction of doxorubicin-induced heart failure in mice	Contractility↑, mtDNA replication↑, Apoptosis↓, mitochondrial ΔΨ↑	[114]
Adult primary cardiomyocytes from mouse heart	Intravenous injection of mitochondrial particles conjugated with ischemia targeting peptide in mice with LAD coronary artery ligation	LV ejection fraction↑, LV fast shortening↑, Conjugated mitochondria targeting effects↑, Apoptosis↓ (Caspase-3↓, Bax↓, Bcl-2↑). In vitro, incubation of human AC16 cardiomyocytes with conjugated mitochondria led to enhanced internalization rate, respiration capacity (NDUFB8↑, UQCRC2↑, MTCO1↑, and ATP5A↑), contractility capacity, and mitochondrial fusion	[113]
Mitochondria were isolated from hepatic tissue	Intravenous injection of mitochondrial particles in rats with doxorubicin-induced cardiotoxicity	Cardiotoxicity↓, MDA↓, SOD↑, GPx↑, Catalase↑, GSH↑, Ejection fraction↑, Fast shortening↑, Inflammation↓ (IL-1β↓, TNF-α↓, IL-6↓), ATP↑, Necrotic and apoptotic changes↓	[125]
Mitochondria were isolated from human mesenchymal stem cells	Intramyocardial injection of mitochondria-loaded alginate/gelatin hydrogel in a rat model of LAD coronary artery ligation	Angiogenesis↑ (vWF^+ and α-SMA^+ vessels), Fibrosis↓, LV anterior wall thickness↑, and mitochondria were internalized by resident cardiomyocytes	[106]

allogeneic, and xenogeneic mitochondria can be used like autologous mitochondria for the restoration of injured myocardium in different pathological conditions. In an experiment conducted by Yip and co-workers, it was shown that the uptake of liver mitochondria by rat cardiomyoblast H9C2 cells increases the expression of energy biomarkers such as NRF1, and 2, TFAM (mitochondrial replication factor), PGC-1α, estrogen-related receptor alpha (ERRα), and Mfn2 [61]. The injection of liver mitochondrial particles to rat myocardium with doxorubicin-induced dilated cardiomyopathy reduced scar area (Masson's trichrome$^+$ area↓). These features were along with the reduction of γ-H2AX factor, a DNA damage indicator, after mitochondrial injection [61]. The regulation of factors related to oxidative stress (NOX-1↓, NOX-2↓, and p22phox↓), apoptosis (Caspase 3↓, Bax↓, and cleaved PARP↓), and mitochondrial injury (cytochrome C↓, DRP1↓, and cyclophilin D1↓) was detected in the cardiac tissue samples [61]. Data also indicated that exogenous mitochondria had the potential to blunt an excessive autophagic response by the modulation of autophagy-related proteins such as Beclin-1, ATG-5, and LC3-II/-I ratio compared to non-treated rats [61]. Similar to these findings, direct intramyocardial administration of autologous gastrocnemius myocyte-derive mitochondria improved the hypertrophic changes, apoptosis (TUNEL$^+$ cells↓), and fibrosis in a piglet model of LV failure model after ligation of pulmonary artery [56]. It was indicated that the internalized mitochondria increase the ATP in hypertrophied cardiomyocytes and return it to the baseline levels [56]. It is thought that damaged cardiomyocytes can accept mitochondrial particles in a homotypic or heterotypic manner from healthy cardiomyocytes or other cell lineages such as immune cells. For example, CFSE-green/MitoTracker-red labeled CCR2$^+$/CD206$^+$ M2 macrophages can easily transfer their mitochondria to the myocardium niche in mice with doxorubicin-induced heart failure. The same story occurred in the Transwell insert® system in which MitoTracker red labeled M2 type macrophage mitochondria were transferred to rat H9C2 cardiomyoblasts on the bottom surface [62]. Among different cell sources for mitochondrial isolation, mesenchymal stem cells (MSCs) are valid mitochondria cell donors for regenerative purposes [63]. Similar to mitochondria from skeletal myocytes, MSC mitochondria are valid subcellular organelles to reduce myocardial remodeling, and fibrosis in animal models of anthracycline-induced cardiomyopathy. These regenerative properties are achieved via the reduction of ROS, and improvement of OXPHOS [64, 65]. Likewise, the transfer of mitochondria from bone marrow MSCs to H9C2 cardiomyoblasts reduced the apoptotic changes [66]. Of note, the mitochondrial donation capacity of MSCs from various tissue types differ and thus attention should be pointed to the application of suitable MSC sources in CVD subjects [67]. Of note, MSCs are commonly isolated from bone marrow, adipose tissue, dental pulp, Wharton's jelly, umbilical cord blood, and placenta. The isolation of MSCs from other tissue is less common [68]. Whether MSCs from various tissue sources have different mitochondrial activities is at the center of attention. Emerging data have confirmed significant differences in donation properties, and respiration rate of mitochondria isolated from several MSC types, resulting in obtaining variable regenerative outcomes [69]. For instance, bone marrow MSCs possess less mitochondrial donation capacity compared to induced pluripotent stem cells. One reason would be that the levels of Miro1 and TNFαIP2 is higher in induced pluripotent stem cells (iPSCs). Compared to the dental pulp and Wharton's jelly MSCs, bone marrow, and adipose tissue MSCs exhibited better mitochondrial transfer capacity [67]. On the other hand, mitochondrial OXPHOS in dental pulp and umbilical cord MSCs are more apparent related to bone marrow and adipose tissue MSCs [70]. Of course, it should be noted that the process of mitochondrial donation in MSCs is associated with the activation of resistance mechanisms after being transplanted into the hypoxic niche and injured sites. In a better word, MSC types with potential to retain the basal metabolism and respiration can donate the mitochondria to the injured cells in the proximity and/or remote sites. The oxygen consumption rate (OCR) can be also variable between various MSC types isolated from same tissue in which cord blood perivascular MSCs have higher OCR and metabolic activity compared to umbilical cord MSCs. Besides, the umbilical cord lining MSCs exhibited less sensitivity to oxygen-glucose deprivation/re-oxygenation, highlighting their importance within the ischemic conditions [71]. Therefore, the attention should be taken in the selection of appropriate cell source for mitochondrial isolation and transplantation in the targeted injured sites. Whether the internalization properties of mitochondria in the acceptor cells are different needs further investigations. Compared to MSCs, it seems that mature cells possess mitochondria with less bioenergetic activities. For instance, Liang et al. found the higher regenerative potential of human bone marrow MSCs related to HFF-1 fibroblasts in terms of angiogenesis and regeneration in mouse model of MI. Data confirmed the superior anti-apoptotic, anti-oxidative, and pro-angiogenic of MSC mitochondria on human ECs, and mouse ischemic myocardium [72]. As a common belief, high mitochondrial numbers should be applied for the restoration of injured cardiomyocyte function via supplying energy requirements [73]. Although the heart encompasses a higher mitochondrial content, the application of autologous heart tissue mitochondria seems actually impossible in

terms of MI which does necessitate the preparation of mitochondria from allogenic and xenogeneic sources. Thus, attempts should be directed toward the isolation of healthy mitochondria from tissues with relatively high mitochondrial contents. Muscle myocytes also contain large amounts of intracellular mitochondria. Despite these advantage, the expansion and proliferation of myocytes is laborious and time-consuming in the laboratory setting [74]. Commensurate with these descriptions, it is postulated that stem cells, especially MSCs, are valid cell source for obtaining mitochondrial mass for regenerative purposes.

Mitochondrial donation and internalization mechanisms

To date, previous studies have indicated the existence of primary and secondary action mechanisms to orchestrate the horizontal mitochondrial transfer between the eukaryotic cells [75]. For example, tunneling nanotubes (TNT), extracellular vesicles (EVs), and gap junctions are mainly involved in the transfer of mitochondrial particles between the cells within the biological system. Along with these mechanisms, direct cell-to-cell fusion also participates in the interchange of cytosolic constituents, especially mitochondria between the juxtaposed cells [76]. As expected, similar mechanisms are also involved in the transfer of exogenous mitochondria into the injured cardiomyocytes (Fig. 1) [77].

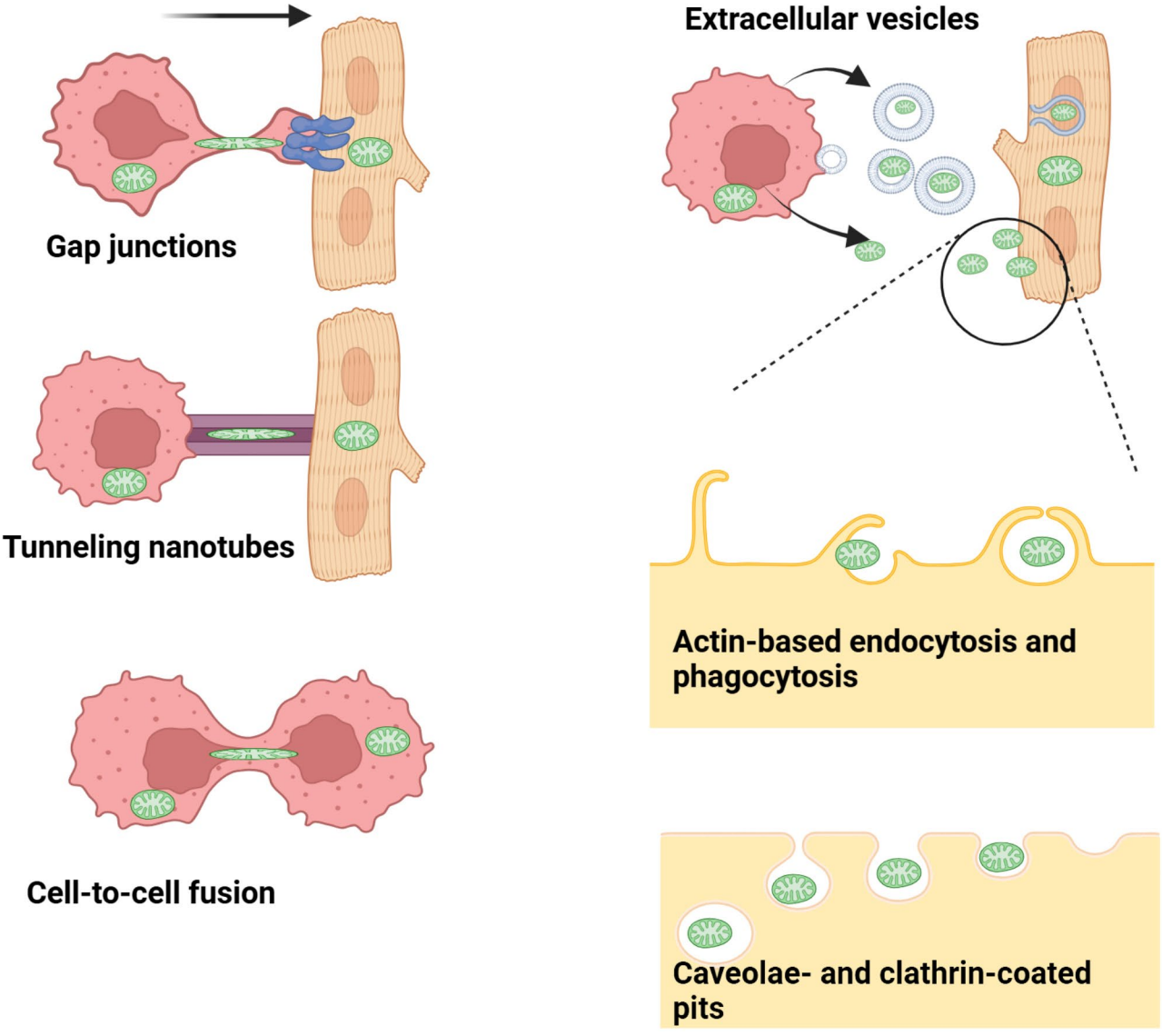

Fig. 1 Different mechanisms are involved in the transfer of mitochondria from other cells into injured cardiomyocytes. Created by BioRender Online

The internalization of exogenous mitochondria to injured cardiomyocytes is crucial for their functionality [78]. This property can restore injured cardiomyocyte function by increasing ATP production, increasing oxygen consumption [79]. One of the underlying mechanisms that modulates cardiac internalization of transplanted mitochondria is actin-dependent endocytosis [79, 80]. Actin is a protein consisting of two subunits, F-actin and G-actin, that make up the main part of the cytoskeleton. The transition between these two subunits initiates endocytosis, which involves the formation of protrusions in the cell membrane, engulfing of exogenous mitochondria, vesicle formation, and vesicle movement [81]. Next, these vesicles fuse with early endosomes and release their contents (i.e., mitochondria) into the host cells. To determine the significance of actin-dependent endocytosis in the uptake of foreign mitochondria by cardiomyocytes, the researchers utilized cytochalasin D. This substance hinders the association of F-actin with cofilin, causing a decrease in actin dynamics activity and internalization of mitochondria [82].

In this regard, Pacak et al. indicated that the inhibition of actin-based endocytosis and phagocytosis using 10 μM cytochalasin D significantly reduced the transfer of hepatocyte mitochondria into the neonate rat cardiomyocytes. They also claimed that the inhibition of other actin-based mechanisms such as caveola-, or -clathrin-dependent axes, TNT formation, and macropinocytosis using methyl-β–cyclodextrin, nocodazole, and 5-(N-Ethyl-N-isopropyl) amiloride, respectively did not alter the rate of mitochondrial entry into the cardiomyocytes [80].

As above-mentioned, macropinocytosis is a type of dynamic endocytosis that relies on actin. In this process, the cell surface ruffles close back against in plasma membrane and creates an intracellular vesicle with a diameter of 0.2 to 10 μm [83]. These vesicles with non-selective mechanisms internalize extracellular macromolecules. Some studies have shown that macropinocytosis plays a significant role in the initial endocytosis of exogenous mitochondria [84, 85]. In this regard, Keitanir and colleagues used inhibitors such as amiloride or 5-(N-ethyl-N-isopropyl) (EIPA) to prove the internalization of mitochondria by micropinocytosis. The incubation of rat H9c2 cardiomyoblasts with amiloride blocked the internalization of mitochondria [86]. The apparent discrepancy between the studies would be presumably related to several factors such as method and time of incubation, and mitochondrial size [80]. For example, caveolae- and clathrin-coated pits can enclose the particles with an average size between 60 and 80 nm, and 85 and 110 nm [74]. Thus, it is logical to hypothesize that the healthy mitochondria with an average size between 0.5 and 1 μm are not internalized by accessory actin-based mechanisms, caveola-, or -clathrin-dependent axes, TNT formation, and macropinocytosis. Besides, the lack of prominent cellular extensions, and lysosome-, caveolae- or autophagosome-related markers along with internalized mitochondria in cultured cardiomyocytes or within the cardiac tissue implies that non-actin-based mechanisms are less likely involved in the uptake of exogenous mitochondria [80]. Commensurate with these statements, one can hypothesize that the mitochondrial fragments have a higher chance of entering the cardiomyocytes via the endocytic pathways and micropinocytosis when compared to large-sized healthy mitochondria. The possibility of homogenous and heterogenous cell-to-cell fusion can also increase cardiomyocyte resistance against different insulting conditions [87]. For instance, it was suggested that the coculture of mouse cardiomyocytes with human adipose tissue stem cells for 7 days led to the heterogenous cell-to-cell fusion, mitochondrial transfer, increase of early-stage cardiac cell factors such as GATA-4, Nkx2.5, and MEF-2 C, and simultaneous reduction of desmin, and cardiac troponin I and α-sarcomeric actinin indicated by in situ hybridization assay [87]. Despite the possibly of synkaryon formation, and cardiomyocyte-to-other cell fusion, this approach is a rare non-classical pathway under in vitro conditions [87].

TNTs are intercellular bridges of horizontally transferring mitochondria or their related byproducts between the cells [88]. TNTs are transient membranes with a width of 50 to 1500 nm that are involved in the transport of proteins, RNA, or intracellular organelles such as mitochondria [89]. In studies targeting the structure of TNTs using molecular and imaging methods, it has been seen that two TNT structures are involved in the transport of mitochondria [90]. F-actin plays an important role in the growth of TNTs to the outward and the appropriate length of protrusion [91]. Based on previous studies, F-actin can transport mitochondria unidirectionally or bidirectionally along the structure of TNTs in a tightly controlled manner. Under such conditions, a continuous network of matrix with the collaboration of cell membranes is created to transfer mitochondria between two cells [92]. TNFaip2/M-Sec is a protein expressed in a wide range of mammalian cells. Recruitment of active RalA by membrane-associated M-Sec leads to membrane deformation and helps in the formation of TNT in cooperation with the exocyst complex and Lst1 [93]. Some research indicates that TNF-α treated MSCs exhibited increased M-Sec expression. This activity triggers the formation of TNT and connects MSCs to cardiomyocytes through the modulation of the NF-κB signaling pathway [36]. Adapter protein complexes consisting of Rho-GTPases (Miro1 and Miro2) are located in the mitochondrial outer membrane (MOM). These factors facilitate the pairing of mitochondria with microtubule motor

proteins and result in mitochondrial exchange between the cells. To regulate and facilitate the movement of mitochondria along microtubules, Miros binds to KIF5, (the kinesin motor protein), with the help of several auxiliary proteins such as TRAK1, TRAK2, MYO10, and MYO19 [94]. Zhang et al. reduced doxorubicin-induced injury in a mouse model by increasing the formation of TNTs and facilitating healthy mitochondria transfer from MSCs (iPSC-MSCs) to injured cells. This study highlights the importance of transferring mitochondria to cardiomyocytes through TNT during ischemic conditions characterized by oxygen and glucose deprivation [95].

Another route in which mitochondria can be transferred from one cell to another is through EV formation. Among different EV types, Exos are nanosized vesicles with the origin of an endosomal system [96]. Studies show that the selection of mitochondrial content by EVs depends on two proteins, optical atrophy 1 (OPA1) and sorting nexin 9 (Snx9) proteins. In the cardiomyocytes and fibroblasts, Mfn1, Mfn2, and OPA1 proteins facilitate the fusion of exogenous mitochondria with endogenous mitochondrial networks [97]. The process of Exo formation involves the generation of late endosomes and multivesicular bodies containing numerous intraluminal vesicles through membrane invagination. In the following steps, their fusion with the plasma membrane results in the release of Exos into the extracellular space and the transfer of cargo to the recipient cells [97]. Integrins on EV surfaces regulate the anchoring of EVs to recipient cells. There are multiple pathways for EVs to enter the recipient cells, such as direct integration with the recipient cell membrane, endocytosis by actin, clathrin, and kaolin, as well as through phagocytosis, and micropinocytosis [98]. One of the important characteristics of EVs in the cardiovascular system is the transfer of intact mitochondria or its components to cardiomyocytes [63]. In ischemic conditions, damaged cardiomyocytes can increase their rescue ability by the uptake of EVs containing respiring mitochondria [77]. Also, studies conducted by Wang et al. showed that MSC Exos can be directed toward the ischemic cardiomyocytes, and alleviate the pathological conditions during AMI [99].

MT for induction of angiogenesis

Reestablishment of blood vessels into the hypoxic and ischemic areas within the cardiac tissue is an effective strategy to reduce the pathological outcomes after IHD [100]. The term angiogenesis or neo-angiogenesis refers to the formation of new vascular units from a preexisting network [101]. Along with angiogenesis, vasculogenesis is also involved in the generation of new blood vessels. This phenomenon is associated with the direct participation of vascular progenitor cells such as endothelial progenitor cells (EPCs) to foster blood perfusion into the hypoxic sites [101]. In the context of MT, the main question is how the internalization of mitochondria by cardiac tissue cells helps to the establishment or increase blood supply into the hypoxic site. The activation of mitochondrial function in parent cells before transplantation seems an actual strategy to increase the angiogenic outcomes. For instance, treatment of EPCs with 200 μM Diazoxide, a selective opener of mitochondrial ATP sensitive K^+ channel, increased the angiogenic behavior with the induction of VEGF, IGF-1, SDF-1α, PCNA, and Bcl-2 compared to the non-treated EPCs, indicating the angiogenic properties and reduction of apoptotic change [102]. It is thought that the influx of K into the mitochondrial matrix can lead to several cardioprotective effects mainly via the inhibition of Ca^{2+} release into the mitochondrial matrix and stimulation of ROS contents [103]. The injection of Diazoxide-treated EPCs into MI rats resulted in a reduction of fibrosis with a concomitant increase of α-SMA, and vWF^+ vascular units [103]. These data indicate that the activation of mitochondrial mass in the transplanted cells can increase their resistance against microenvironmental insults and improve the regenerative outcomes in in vivo conditions. It seems that the activation of mitochondrial function helps the parent cells to secrete several angiogenesis factors as well. In support of this claim, Niagara and co-workers indicated that Diazoxide-treated skeletal myoblasts exhibited more angiocrine activity (HGF↑, FGF↑, IGF-1↑) with reduced apoptotic features (phospho-Akt↑, ΔΨ↑, $TUNEL^+$ cells↓, Annexin-V^+ cells↓, released LDH↓) in in vitro conditions and after being administrated into infarcted myocardium in rats [104]. In another study, the activation of mitochondrial mass in cardiac progenitor cells using resveratrol-loaded nanocarriers led to a significant reduction of fibrosis, and ROS content in murine cardiac tissue following ischemic-reperfusion injury [105]. In recent work, Hassanpour et al. incubated MSCs with 50 μM metformin and 40 μM dichloroacetic acid to improve mitochondrial activity before the isolation and injection into MI rats [106]. Data showed that the functional $MitoTracker^+$ mitochondria were successfully internalized into the cytosol of juxtaposed $actinin^+$ cardiomyocytes. Along with these changes, the injection of ionic cross-linked mitochondria-loaded alginate (3% w/v)/gelatin (1% w/v) hydrogel containing 1 μM pyrrole into the per-infarct area increased prominently the left ventricle thickness and intensity of vWF^+ capillaries and $\alpha\text{-}SMA^+$ arterioles after 14 days in the border zone (Fig. 2) [106]. Even the angiogenic effects were more evident compared to infarcted rats that received mitochondria alone, or mitochondria-free alginate/gelatin hydrogel. One reason would be that direct transplantation contributes to rapid mitochondrial disappearance just a few days after transplantation [107]. Therefore, it seems that the incorporation of

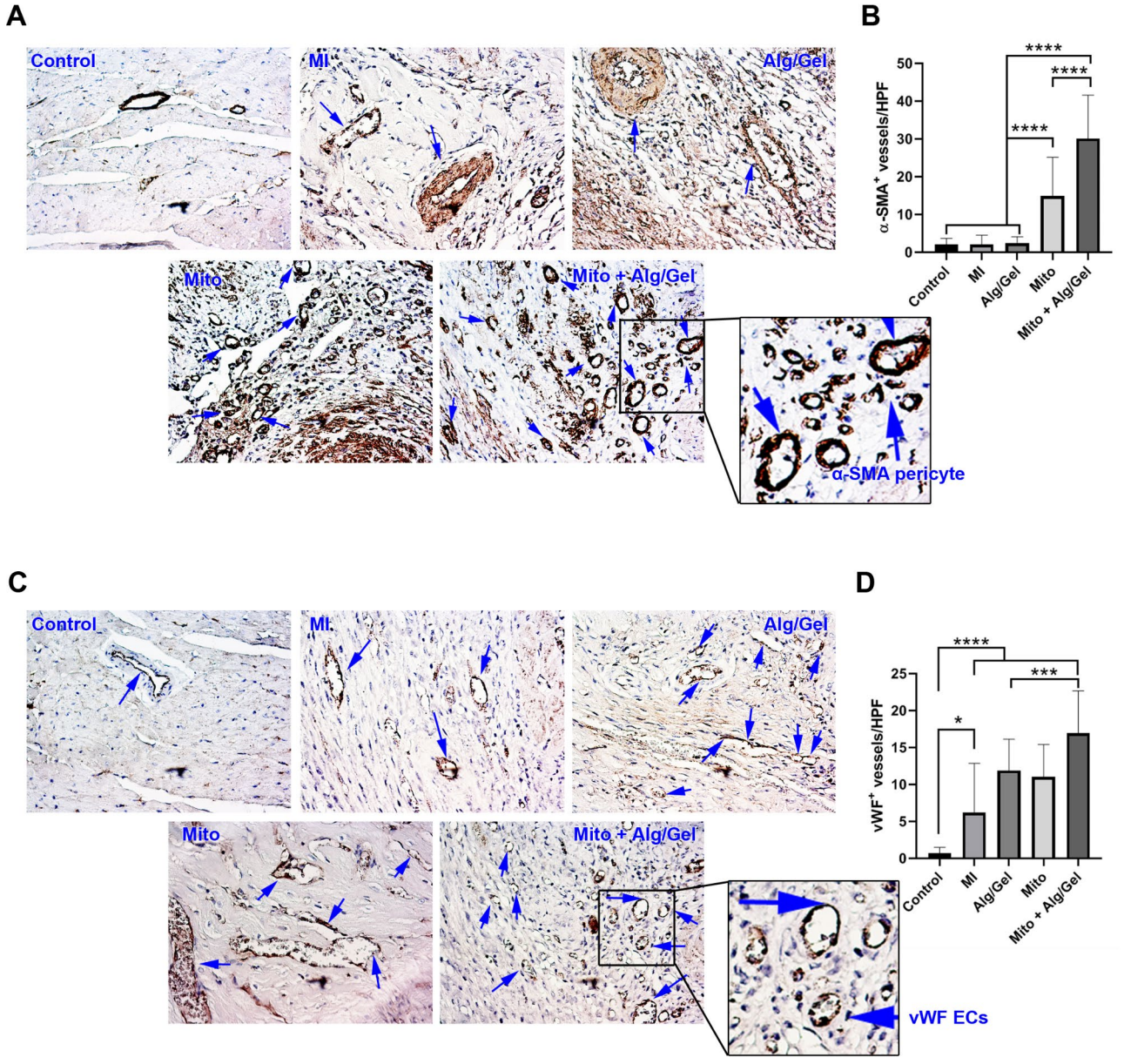

Fig. 2 Monitoring the angiogenic properties of mitochondria-loaded alginate/gelatin hydrogel in a rat MI model using immunohistochemistry staining after 14 days (**A-D**). Mitochondria were isolated from MSCs pre-treated with 50 µM metformin and 40 µM dichloroacetic acid. Mitochondria (Mito), alginate/gelatin (Alg/Gel), or their combinations (Mito + Alg/Gel) were injected into the peri-infarct zone (2×10^7 particles per 1 ml of Alg/Gel hydrogel). Data indicate the promotion of α-SMA$^+$ arterioles (A, and B; blue arrows), and vWF$^+$ capillaries (**C**, and **D**; blue arrows) compared to Control, MI, and Alg/Gel groups ($p < 0.05$). The co-administration of mitochondrial particles with supporting hydrogel increased the angiogenic potential inside the ischemic cardiac tissue. Copyright 2024 [106]. International Journal of Biological Macromolecules

mitochondria with supporting substrates can heighten the regenerative properties of mitochondria by preventing them against mechanical stress during injection into the consolidated cardiac tissue meanwhile simultaneously increasing their retention time at the site of injection [48]. In an experiment conducted by Liang et al., they found that injection of MSC mitochondria into the per-infract zone in MI mice led to a reduction of fibrotic changes, and local induction of vWF$^+$ and α-SMA$^+$ vessels [108].

The increase of local ATP content and neutralization of excessive ROS contents prevent the senile changes of ECs (β-galactosidase↓, and ERK↑) [108]. Of course, it should not be forgotten that the process of cardiac tissue healing will not be initiated immediately after MT. For instance, Mori et al. indicated donor mitochondrial DNA fragments in recipient ventricular cardiomyocytes

3 days using polymerase chain reaction technique after co-transplantation of 1×10^6 human adipose tissue MSCs and fibrinogen-thrombin solution into MI rats (Fig. 3) [107]. Compared to in vivo conditions, data revealed the transfer of mitochondria to cardiomyocytes within the first 24 h in the hypoxic co-cultured system. Likewise, the transfer of MSC mitochondria to juxtaposed cardiomyocytes was indicated in contrasted sections using electron microscopy [107]. These effects along with cardiac function indices were blunted in the presence of a gap junction inhibitor namely a-glycyrrhetinic acid, indicating the importance of physical contact in the phenomenon of mitochondrial donation. Of note, these data also indicate the possibility of xenogeneic MT within the rat cardiac tissue and the presence of donated mitochondrial DNA content about 56 days post-transplantation [107]. However, the mitochondrial transfer time can be lessened when isolated mitochondria are directly injected compared to the parent cells harboring those mitochondria. Of course, attention should be taken that most fractions of injected mitochondria are not internalized into the local cardiomyocytes. Along with this claim, Cowan et al. found that the xenogeneic human green-colored $MTCO2^+$ cardiac fibroblast mitochondria transplantation in a rabbit MI model led to the accumulation of injected mitochondria in the interstitial spaces just a few minutes while the most of injected mitochondria are juxtaposed to cardiomyocytes (Fig. 4) [78]. They found that the injected mitochondria tend to enter cardiomyocytes over time and colocalize with red-colored WGA^+ rabbit mitochondria [78]. One can hypothesize that prolonged retention time within the interstitial space can contribute to shorter retention time because of rapid mitochondrial washout from the injection site. Whether some mechanisms can be exploited for improving the entry of allergenic/xenogeneic even autologous mitochondria into cardiomyocytes needs further investigation. Data showed that intracoronary artery injection led to rapid and evenly distribution of 18 F-rhodamine 6G and iron oxide nanoparticles labeled mitochondria in the Langendorff-perfused rabbit hearts indicated by PET and μCT images compared to direct injection into the ischemic sites [78]. Irrespective of the delivery route, the process of mitochondrial internalization and reactivation inside the target cell cytosol is closely related to the accelerated healing process and regenerative outcomes.

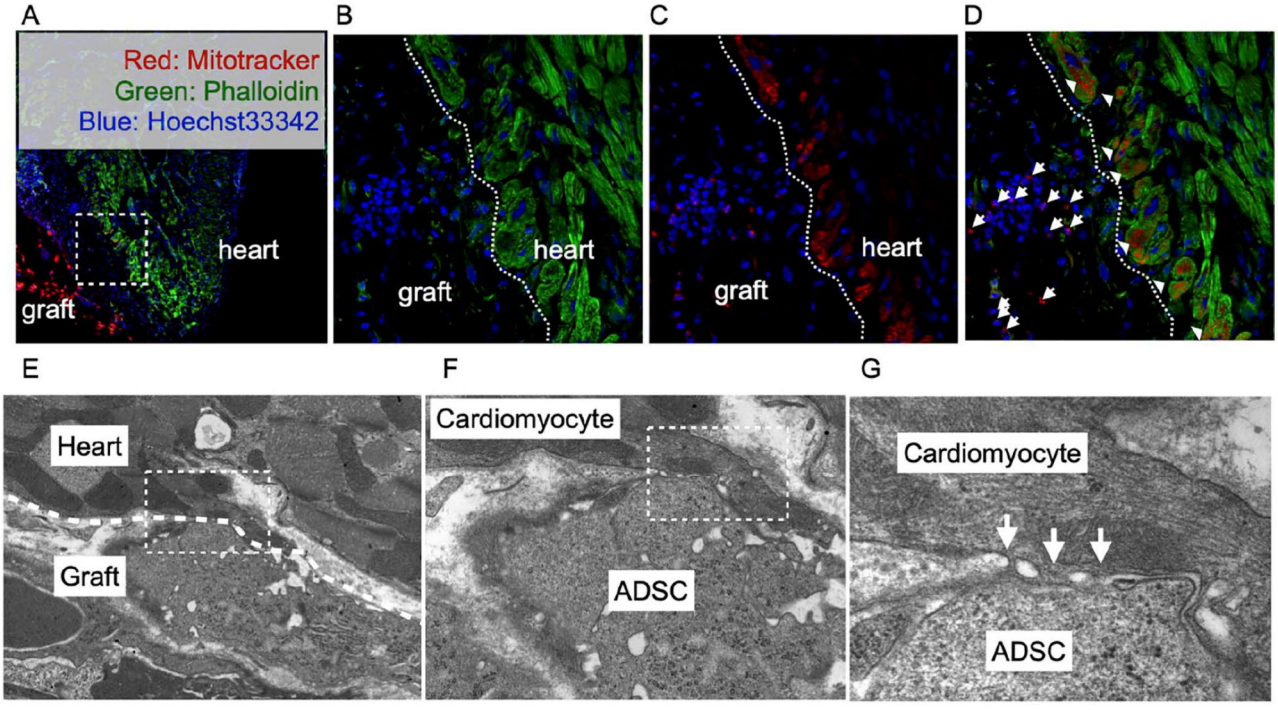

Fig. 3 Monitoring the transfer of mitochondria from pre-labeled MitoTracker Red^+ ADSCs into the cardiomyocytes in rat MI model. About 1×10^6 ADSCs were incorporated inside the thrombin and fibrin solution and injected into the infarct zone two weeks after LAD coronary artery ligation. Immunofluorescence images indicate the existence of red-colored ADSC mitochondria in rat Green $Phalloidin^+$ cardiomyocytes at the epicardial region (**A**; Control), and MI rats (**B** and **C**). Magnification of dashed box line of panel **A** (**D**; Arrows: mitochondria in ADSCs; arrowheads: mitochondria in rat cardiomyocytes). Blue Hoechst 33,342 stain was used for counterstaining. Transmission electron microscopy images of ADSCs and site of transplantation (**E**). Panel **F** is the magnified dashed rectangle indicated in panel **E**. Panel **G** is the magnified dashed rectangle indicated in panel **F**. White arrows indicate the fusion between the transplanted ADSCs and local cardiomyocytes. Copyright 2023 [107]. Cell Transplantation

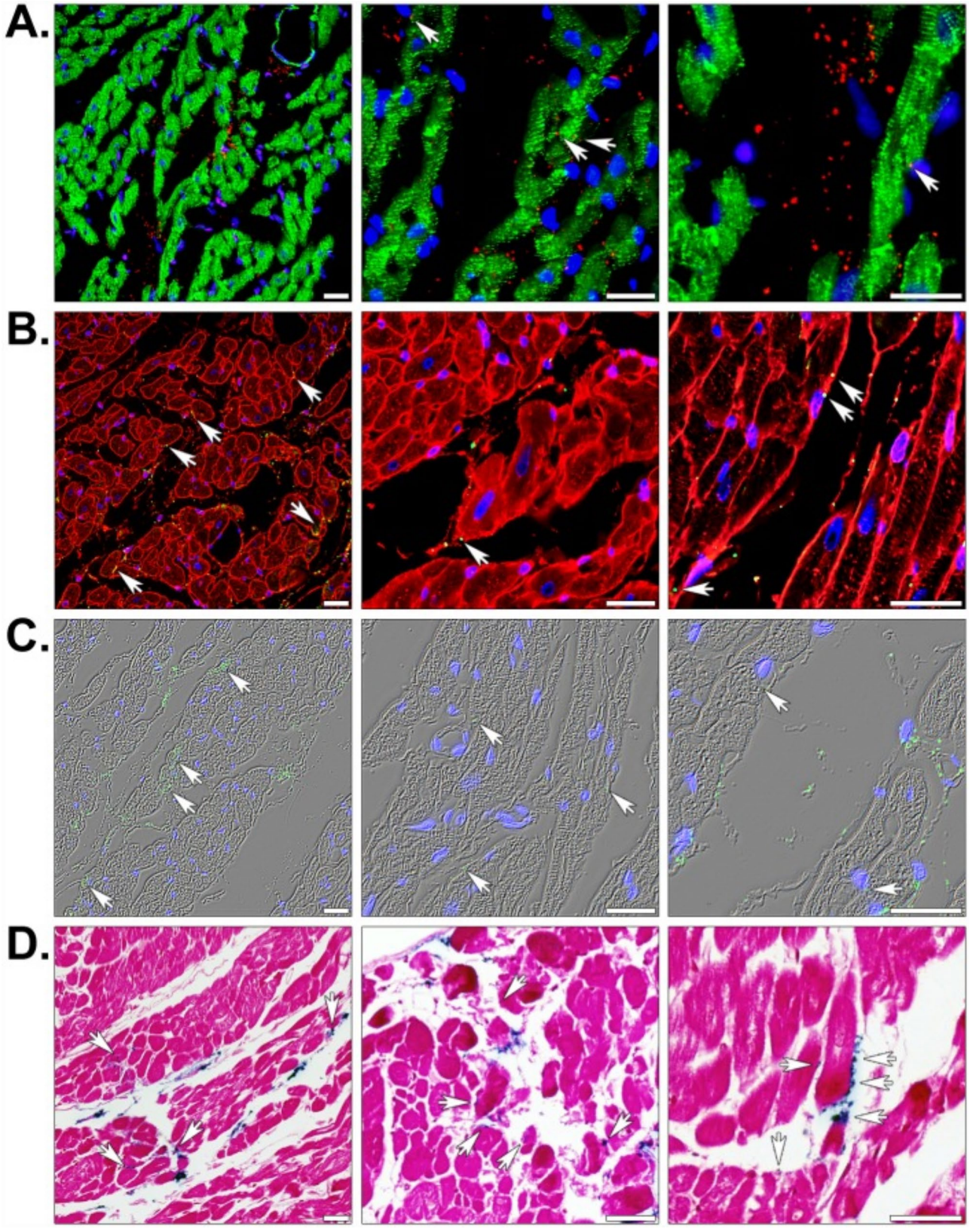

Fig. 4 (See legend on next page.)

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Fig. 4 Histological examination of rabbit ischemic cardiac tissue after injection of human cardiac fibroblast mitochondria (**A**-**D**). Immunofluorescence images indicate that the majority of injected mitochondrial particles stained with red-colored human-specific mitochondrial marker MTCO2 were dispersed inside the cardiac ECM and periphery of cardiomyocytes (**A**). The internalized green-colored 113–1 human mitochondria are co-localized with the rabbit red-colored WGA sarcolemma (**B**). Phase contrast illumination of images revealed appropriately the localization of MTCO2-stained mitochondria after being injected into the ischemic sites (**C**). Detection of magnetic iron oxide nanoparticle-loaded mitochondria using Prussian blue (blue) and pararosaniline (pink) staining (**D**). Scale bar: 25 μm. All mitochondrial particles were indicated by white arrows in all panels. Nuclei were stained with blue-colored DAPI. Copyright 2016 [78]. PLOS ONE

Limitation of MT and novel approaches

As above-mentioned, cardiac function is improved following MT via engaging different intracellular mechanisms [77, 109]. Of note, the intensity, and type of these mechanisms are affected by factors such as the mitochondrial administration route, dose, and donor cell source [110]. Of note, it has been declared that MT before induction of ischemia and following the ischemic conditions are beneficial in CVD candidates [111, 112]. As above-mentioned, transplantation of mitochondria is done directly into the injured myocardium, intracoronary artery, and/or systemic circulation [74, 110]. Of note, direct injection approaches into the ischemic myocardium increase the loss of mitochondrial integrity due to the firm consistency of cardiac tissue as compared to soft microenvironments. Therefore, attempts should be directed toward the development of feasible and standard administration routes. It is mighty that the direct injection of mitochondrial particles does not yield higher retention time and exogenous mitochondria are eliminated a few weeks after injection [112]. On the other hand, this approach agglomerates the injection cargo at specific sites while systemic transplantation and intracoronary administration lead to the even distribution of mitochondria through the myocardium [78]. The systemic injection increases off-target effects in which administrated mitochondria are seen in non-cardiac tissues, leading to significantly reduced therapeutic properties [113].

To circumvent these pitfalls, several studies have been conducted to increase the on-target homing properties of mitochondria within the myocardium. In an experiment, Sun et al. designed a sophisticated delivery system to increase the delivery efficiency of mitochondria to cardiomyocytes after crossing the endothelial layer in animal models [113]. For this purpose, they coated the cardiomyocyte-derived mitochondrial surface with CSTSMLKAC (PEP) and lipophilic connector namely triphenylphosphonium cations (PEP-TPP-mitochondria) (Fig. 5) [113]. To show appropriate mitochondrial localization, the Cyanine5 (Cy5) dye was conjugated with the lysine (K) residue of the PEP sequence to produce fluorescent PEP(Cy5). The procedure was followed by the incubation of MitoTracker Green-labeled mitochondria with PEP(Cy5). The incubation of adult cardiomyocytes and AC16 cells with PEP(Cy5)–TPP–mitochondrial particles indicated the existence of exogenous particles inside these cells along with internal MitoTracker Red-colored mitochondria after a few hours. Due to the close interaction of PEP with ligands expressed in the ischemic area, the systemic injection Cy5-labeled PEP-TPP-mitochondria increased on-target localization within the injured myocardium and improved OXPHOS (*NDUFB8*↑, *UQCRC2*↑, *MTCO1*↑, and *ATP5AC*↑) inside the cardiomyocytes following the systemic administration in a mouse model of cardiac tissue I/R [113]. Along with these effects, apoptotic changes (Casapase-3↓, and Bax/Bcl2 ratio↓), immune cell infiltration ($CD11b^+F4/80^+$ macrophages), and inflammatory response (NLRP3↓, IL6↓, and IL1β↓) were blunted compared to the groups that received non-conjugated mitochondria. The metabolic adaptation and close interaction between the injected mitochondria and cardiomyocytes should be precisely pointed out before any manipulation (Fig. 5) [114].

Compared to the aged cell mitochondrial contents, the function, number, and integrity of mitochondria are suitable in neonate cells. However, the availability of neonate cells is challenging for clinical purposes [114, 115]. The enrichment protocols and injection route can affect the mitochondria membrane integrity. Upon entry to host cardiomyocytes, the membrane potential of exogenous mitochondria is restored [114]. There are conflicting debates related to the precise action of mitochondria in acceptor cells within the cardiac tissue. For instance, it has been shown that exogenously injected mitochondria improved the function of ventricular cardiomyocytes but not atrial cardiomyocytes [114]. This may relate to the source of mitochondrial parent cells. However, transplantation of autologous mitochondria isolated from cardiac tissue, soleus, and gastrocnemius muscles restored the cardiomyocyte contractility and reduced apoptosis rate without significant differences concerning mitochondrial source [56].

Clinical trials

Emerging data have revealed the eligibility of mitochondrial donation in human patients with IHD (Table 2). The first pilot clinical application of autologous mitochondria injection was done in five pediatrics with I/R myocardial damage (NCT02851758) and continued to 24 patients [58]. Searching for registered clinical trials at "https://clinicaltrials.gov" with keywords "Myocardial Infarction, Ischemic Heart Disease, Ischemic, and

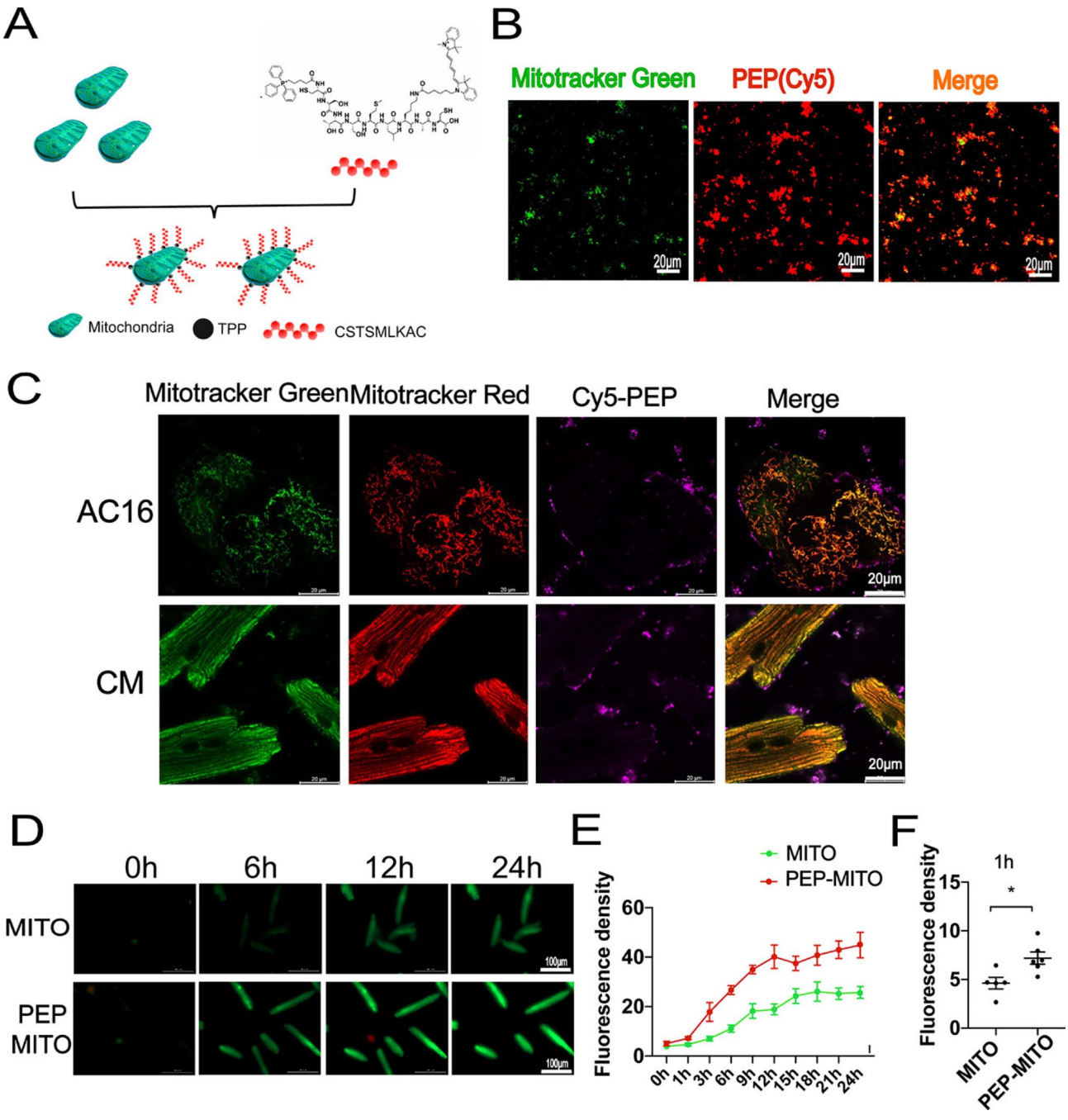

Fig. 5 Conjugation of isolated mouse cardiomyocyte mitochondria with ischemia targeting peptide (PEP) using triphenylphosphonium (TPP) cations. Schematic representation of PEP-TPP-mitochondria (**A**). Immunofluorescence images of mitochondria pre- and post-staining with PEP (Cy5). Scale bar: 20 μm (**B**); Monitoring the internalization of MitoTracker Green^{+} PEP-TPP-mitochondria by MitoTracker Red^{+} human AC16 cardiomyocytes and primary cultured mouse cardiomyocytes (CM) after 3 h (**C**). During the mitochondria internalization, the targeting peptide was eliminated and bared mitochondrial particles were guided into the human AC16 and mouse cardiomyocyte cytosol. Scale bar: 20 μm; Cellular uptake of naïve and conjugated mitochondria. MitoTracker Green + PEP-TPP-mitochondria were internalized much more compared to the natural mitochondria without targeting PEP (**D-E**; Scale bar: 100 μm). *$p < 0.05$; One-Way ANOVA with Tukey post hoc analysis. Copyright 2023 [113]. ACS Nano

mitochondria" resulted in about sixteen registered clinical trials which just two trials were associated with (MT) in MI subjects. Other primary registration websites were searched for more registered clinical trials in this topic by mentioned keywords, however just one registered trial was found in IRCT. Regarding the trend of in vivo studies, it is expected to increase the clinical trials using MT for cardiac protection. Based on the results, the direct injection of $1 \times 10^{7} \pm 1 \times 10^{4}$ mitochondria in pediatric patients with ischemic reperfusion myocardial injury did

Table 2 Clinical trials in mitochondria transplantation for ischemic cardiomyocytes rehabilitation

Title	Case NO.	Identification number	Cell source for mitochondria	Dose and administration route	Results	Ref
Autologous mitochondrial transplantation for cardiac ischemic injury	5 patients	Not registered	Healthy rectus abdominis muscle	Intramyocardial injection of $1\times10^{8}\pm1\times10^{5}$ mitochondrial particles	Improved ventricular function	[58]
Autologous mitochondrial transplantation in pediatric patients with cardiac tissue ischemia-reperfusion Injury	Pilot study on 24 patients	NCT02851758	Autologous chest skeletal muscles	ND	ND	[116]
Co-transplantation (intracoronary and intra-myocardial injection) of MSC exosomes and autologous mitochondria in patients subjected to CABG surgery	Phase I, II	NCT05669144	Co-transplantation of autologous pectoralis muscle mitochondria and MSC exosomes	100 µg/ml exosome containing 1×10^{6} mitochondria	ND	
Platelet mitochondria in heart ischemia	Phase I, II	IRCT20210920052524N1	Platelet mitochondria	Intracoronary	ND	

not cause bleeding and arrhythmias at the site of injection. Of 5 subjects, this trial led to the improvement of ventricular function in 4 patients without the necessity for extracorporeal membrane oxygenation support [58]. The injection of 5×10^{7} mitochondria in subjects ($n=10$) who underwent revascularization strategies did not lead to intramyocardial hematoma [116]. Data supported the increase of ventricular strain in patients with MT + revascularization compared to the control revascularization group coincided with the shortening of mean functional recovery time [116]. These features show the regenerative properties of MT in patients with ischemic myocardium along with conventional therapeutic protocols.

Conclusions

MT can restore the function of injured cells, especially cardiomyocytes following ischemic diseases. The donated mitochondria can fuse with the resident mitochondria inside the cardiomyocytes or directly afford the energy demands in stressed cardiomyocytes to inhibit irreversible cellular changes. Future studies are suggested to monitor which mechanisms are directly involved in the escape of internalized allogenic, and xenogeneic mitochondria from lysosomal degradation. Using different molecular and cell-based mechanisms, transplanted mitochondria can regulate the angiogenic behavior of ECs and blood supply into the ischemic sites, leading to the reduction of aberrant cardiac tissue remodeling and scar formation within the myocardium. Irrespective of administration route, and intervals, dose, other parameters can affect the regenerative properties of isolated mitochondrial particles in the targeted tissues. For instance, parent cell metabolic activity, environmental conditions, and genetic traits can influence the dynamic and bioenergetic functions of isolated mitochondria. It is thought that transformed cells are suitable cells for achieving higher mitochondrial content because of their short doubling time and rapid expansion rate [117]. Because mitochondrial metabolites can directly exert epigenetic effects on the host cells, thus it is logical to carefully assess the possibility of permanent phenotype acquisition and specific function after the internalization of transplanted mitochondria in the normal cells [118]. Obtaining healthy mitochondrial particles is a vital step for successful regenerative outcomes under ischemic conditions transplantation. The lack of standard isolation and purification protocols for large-scale mitochondrial isolation can yield different regenerative rates using similar protocols, and make difficult the interpretation of results. Non-standard isolation protocols not only do not supply healthy functional mitochondria but also can exacerbate the stressed cardiomyocyte damage following transplantation under ischemic conditions. It is believed that the non-functional and injured mitochondria can

escape the mitophagy response inside the recipient cells, and prolong the oxidative stress [119]. Because of inherent differences in the metabolic activity, and crista-density of mitochondria in various tissues especially myocardium, future studies should also focus on the finding of efficiency of transplanted non-cardiomyocyte mitochondria in the supply of energy demands in the damaged cardiomyocytes.

Abbreviations

AMPK	AMP-activated protein kinase
CVDs	Cardiovascular diseases
CoQ10	Coenzyme Q10
CABG	Coronary artery bypass grafting
CADs	Coronary artery disease
DAMPs	Damage-associated molecular patterns
DCA	Dichloroacetate
ERS	Endoplasmic reticulum stress
EC	Endothelial cell
EPCs	Endothelial progenitor cells
ERRα	Estrogen-related receptor alpha
Exos	Exosomes
ECM	Extracellular matrix
ECM	Extracellular matrix
EVs	Extracellular vesicles
HIF-1α	Hypoxia-inducible factor-1 alpha
ILs	Interleukins
I/R	Ischemia-reperfusion
IHD	Ischemic heart disease
LAD	Left anterior descending
LV	Left ventricle
MSCs	Mesenchymal stem Cells
mtDNA	Mitochondrial DNA
MOM	Mitochondrial outer membrane
mPTP	Mitochondrial permeability transition pores
MT	Mitochondrial transplantation
NO	Nitric oxide
OPA1	Optical atrophy 1
OXPHOS	Oxidative phosphorylation
PDK	Pyruvate dehydrogenase kinase
ROS	Reactive oxygen species
TLR	Toll-like receptor
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-alpha
TNTs	Tunneling nanotubes

Supplementary Information

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Supplementary Material 1

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Author contributions
P.H., F.S., S.S., S.B., P.K., and S.H.A.T. collected data and prepared the manuscript. R.R. and M.R. supervised the study.

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Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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