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From dysfunction to healing: advances in mitochondrial therapy for Osteoarthritis

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Abstract

Osteoarthritis (OA) is a chronic degenerative joint condition characterised by cartilage deterioration and changes in bone morphology, resulting in pain and impaired joint mobility. Investigation into the pathophysiological mechanisms underlying OA has highlighted the significance of mitochondrial dysfunction in its progression. Mitochondria, which are cellular organelles, play a crucial role in regulating energy metabolism, generating reactive oxygen species, and facilitating essential biological processes including apoptosis. In recent years, the utilisation of exogenous drugs and MT to improve mitochondrial function in chondrocytes has shown great promise in OA treatment. Numerous studies have investigated the potential of stem cells and extracellular vesicles in mitochondrial transfer. This review aims to explore the underlying mechanisms of mitochondrial dysfunction in OA and assess the progress in utilising mitochondrial transfer as a therapeutic approach for this disease.

Keywords Osteoarthritis, Stem cell, Mitochondrial dysfunction, Mitochondrial transfer, Extracellular vesicles

Introduction

Osteoarthritis (OA), a common long-term joint condition, is identified by the breakdown of cartilage, changes in the bone beneath the cartilage, the development of bony outgrowths, joint synovial inflammation, and decreased joint mobility. The condition is one of the most common causes of pain and disability, especially among the elderly [1]. It is estimated that around 10% of males and 18% of females aged ≥ 60 years are affected by OA, resulting in significant burdens on healthcare systems [3]. Unfortunately, there is currently a lack of disease-modifying therapies for OA, and the main treatment strategies still focus on pain relief and improving joint function. Consequently, the treatment of OA remains a noteworthy, unaddressed medical issue and an active field of investigation.

Mitochondria (MT) are cellular organelles responsible for the metabolism and generation of energy in eukaryotic cells. They are essential for maintaining the energy balance within cells [4]. MT take up one-fifth of the area in a eukaryotic cell [5]. Adenosine triphosphate (ATP) generated through the process of oxidative phosphorylation (OXPHOS) is essential for cellular maintenance and regeneration. In addition, OXPHOS also serves as the primary source of reactive oxygen species (ROS) in most tissues [6]. Perturbations in mitochondrial function and metabolism have been linked to many degenerative diseases, including cancer, neurodegenerative disorders, and ischaemic cardiomyopathy [7]. Thus, it is crucial to fully understand the mechanisms behind mitochondrial

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dysfunction to develop effective treatments for these diseases [9].

Mitochondrial dysfunction has the potential to influence multiple pathways implicated in joint degradation, encompassing hypoxia-induced signaling mechanisms within synovial epithelial cells, impaired biosynthetic processes in chondrocytes, and altered growth responses [5]. Chondrocytes are essential for maintaining the balance between the production and breakdown of the ECM in articular cartilage. Damage to chondrocytes manifests primarily through elevated levels of matrix metalloproteinase-3 (MMP-3) and MMP-13, nitric oxide (NO), and inflammatory cytokines, resulting in an imbalance between ECM catabolism and anabolism [12]. This imbalance leads to reduced levels of aggrecan and collagen II, ultimately culminating in the development of OA. Recent studies have elucidated the significant role of mitochondrial dysfunction and perturbed energy metabolism in the aetiology of OA [14]. Chondrocytes isolated from individuals with OA exhibit reduced mitochondrial membrane potential, decreased ATP synthesis, and increased ROS production, increasing oxidative stress and apoptosis, and promoting cartilage degradation [15]. More recently, a mitochondrial DNA (mtDNA) variation (m.16519 C) has also been suggested to be strongly associated with rapid progression of knee OA [18]. As a result of these findings, mitochondrial dysregulation is increasingly recognised as one of the major contributing factors to OA. Therefore, more effective therapeutic strategies targeting mitochondrial metabolism are needed.

With the advancements in stem cell therapy and biomaterials, optimising mitochondrial function provides a new therapeutic approach for treating OA [19]. Additionally, the field of gene therapy is experiencing significant growth, suggesting that biological interventions aimed at modifying OA will be a major treatment approach in the future [21]. Given that chondrocytes are the most important cells in articular cartilage, this article provides a comprehensive review of the underlying mechanism of mitochondrial dysfunction in OA chondrocytes and offers a summary of drugs that restore mitochondrial function in chondrocytes for the treatment of OA. Furthermore, we have evaluated the advancements made in mitochondrial transfer for treating OA, potentially guiding the future of mitochondrial studies aimed at addressing this condition.

Mitochondrial Dysfunction in OA

There is evidence that mitochondrial dysfunction precedes cartilage degradation and contributes to the death of chondrocytes [22]. Multiple factors have been identified as causes of mitochondrial impairment in OA, such as inflammation, ageing, infection, lack of nutrients, and genetic mutations [5]. Oxidative stress is a critical determinant in the induction of mtDNA damage, impairment of mitochondrial respiratory function, and activation of MT-mediated apoptotic pathways. Inflammatory cytokines, such as interleukin-1β (IL-1β)and tumour necrosis factor-alpha (TNF-α) have been documented to diminish mitochondrial activity and ATP production, impair mitochondrial respiration, and contribute to mitochondrial dysfunction in chondrocytes. In addition, gene mutations have also been implicated in mitochondrial dysfunction associated with OA. Aberrant expression of Parkin and P62, which are key mediators of mitophagy, has been observed in OA [25]. Other mechanisms, such as altered mitochondrial biogenesis, have been implicated in the pathophysiology of mitochondrial dysfunction in OA. Dysregulation of the PGC-1α/NRF-1 signalling axis, which serves as a critical regulator of mitochondrial biogenesis, has been observed in OA chondrocytes, resulting in a reduction of mitochondrial mass and function [26]. In summary, various factors contribute to mitochondrial dysfunction in OA.

Structural and locational alterations of mitochondrial components, induced by various factors, can precipitate mitochondrial dysfunction, inflicting significant damage on cells. The resultant mitochondrial dysfunction can trigger extensive cell death, propagating damage across tissues and organs in a cascading manner, akin to a domino effect, and culminating in life-threatening disorders. In OA, mitochondrial dysfunction primarily manifests through decreased ATP production, increased oxidative stress, disrupted mitochondrial dynamics and metabolism, alterations in morphology and function, and impaired calcium homeostasis. These mitochondrial impairments ultimately result in cartilage degeneration (Fig. 1) [5].

Decreased ATP Production

Cellular energy primarily comes from two processes: OXPHOS when oxygen is available and glycolysis when oxygen is not present. Chondrocytes, situated in an environment with relatively low oxygen levels, fulfil a substantial portion of their energy requirements through glycolysis, while OXPHOS accounts for only 25% of the overall ATP production in chondrocytes [29]. Although MT are not the principal energy source for chondrocytes, they perform a vital function in supporting and maintaining chondrocyte glycolysis. In their research, Rajpurohit et al. [30] utilised 2,4-dinitrophenol (2,4-DNP) to isolate electron transport from ATP synthesis, leading to a decrease in ATP generation in chondrocytes without a rise in lactate levels. In another study, researchers reduced ATP production in bovine cartilage after administering the mitochondrial oxidative respiratory chain inhibitor rotenone or the mitochondrial free radical scavenger MitoQ [31]. This phenomenon indicates

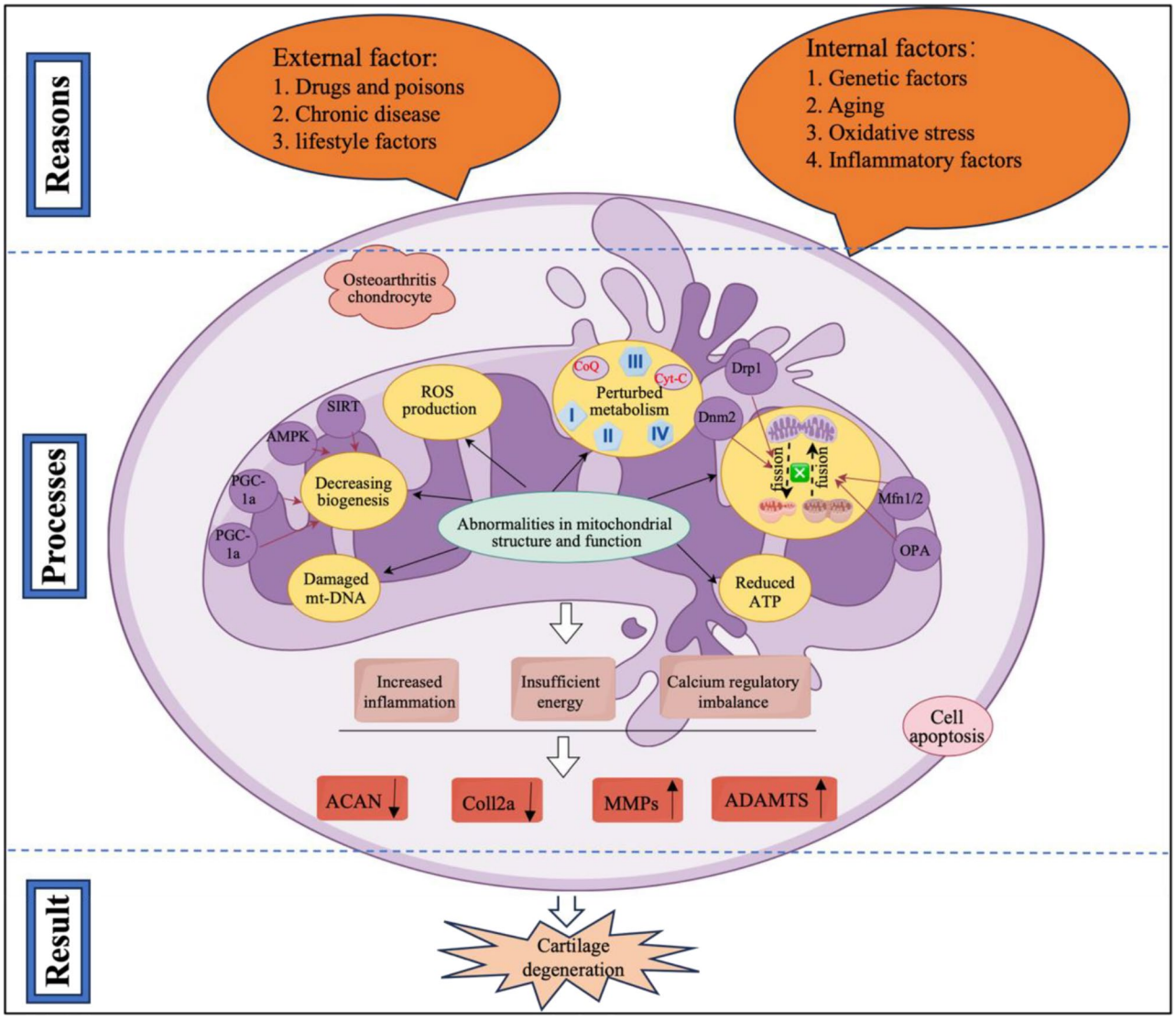

Fig. 1 Mechanism of mitochondrial dysfunction in osteoarthritis (OA)

that chondrocytes still rely on mitochondrial OXPHOS for energy to some extent. In fact, in a hypoxic environment, mitochondrial aerobic respiration is weak, but the oxidants produced promote anaerobic glycolysis and increase ATP production [32]. In OA chondrocytes, the mitochondrial membrane potential is lost and the activity of complexes I, II, and III in the electron transport chain is reduced, resulting in abnormal mitochondrial OXPHOS, ultimately leading to reduced ATP production [33].

Increased Oxidative Stress

A major cause of mtDNA damage, impairment of mitochondrial respiratory function, and activation of MT-mediated cell death pathways is oxidative stress [34]. MT are the primary organelles within cells responsible for producing ROS [32]. Malfunction of MT can trigger the release of mtDNA and mitochondrial ROS (mtROS), leading to activation of the inflammasome and promoting the generation of pro-inflammatory cytokines such as IL-1β and IL-18 in chondrocytes and synovial cells. The buildup of mtROS and mtDNA damage can trigger the nuclear factor-κB (NF-κB) pathway, which serves as the primary controller of inflammation [35]. Activation of NF-κB also enhances the production of MMPs, leading to degradation of the ECM and damage to cartilage. Furthermore, oxidative stress damages protein complexes of the chondrocyte mitochondrial respiratory chain [37]. Elevated ROS levels lead to mitochondrial membrane depolarization, which will further promote the continued generation of ROS. Excessive ROS production and ATP depletion inhibit cell division and disrupt the redox equilibrium [37].

Mitochondrial Dynamics Imbalance

By continually undergoing fission, fusion, and mitophagy, MT maintain a dynamic balance in the mitochondrial network [38]. Mitofusin 1 (Mfn1) and Mfn2 are responsible for merging the outer mitochondrial membrane (OMM), whereas optic atrophy 1 (OPA1) is responsible for merging the inner mitochondrial membrane (IMM). Mitochondrial fusion effectively preserves mtDNA levels and boosts mitochondrial respiration and ATP synthesis [39]. Dynamin-related protein 1 (Drp1) and dynamin 2 (Dnm2) are the main players in mitochondrial fission. Excessive mitochondrial fission in chondrocytes leads to reduced bioenergetic production, impaired calcium regulation, and disruption of redox balance. Mitophagy is a special type of autophagy that is regulated by a variety of autophagy-related proteins and can selectively degrade damaged or redundant MT to maintain mitochondrial health [41]. The balance of mitochondrial dynamics is controlled by these specific genes and proteins and changes according to the needs of the cell or external stimulation [42]. There is evidence that mitochondrial fission is increased and mitophagy and fusion are attenuated in OA chondrocytes. Restoring this balance helps restore cell function [43].

Perturbed Metabolism

Protein complexes in the IMM help move protons through the mitochondrial respiratory chain to produce ATP. Prominent examples of such complexes are NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c (Cyt-C) reductase (complex III), and Cyt-C oxidase (complex IV) [44]. Perturbations in mitochondrial metabolism may lead to disturbances in cellular redox equilibrium and the accumulation of ROS in oxidative stress-related disorders. Recent research suggests that alterations in mitochondrial metabolism could be involved in the development of mild inflammation in OA [5]. During the development of OA, chondrocytes and synoviocytes tend to change their mitochondrial metabolism by switching from OXPHOS to glycolysis, which is mainly controlled by the AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) pathways. At the same time, alterations in lipid and amino acid metabolism have been observed in these cells [45]. Modulating mitochondrial metabolism helps reduce synovial inflammation and slow down the progression of early OA [47]. In addition, the imbalance of mitochondrial metabolic homeostasis in OA chondrocytes is also manifested as abnormal mitochondrial biogenesis, a process that is regulated by various transfer factors such as PGC-1a and AMPK [48]. Correcting abnormalities in mitochondrial biogenesis is also important for the treatment of OA [49].

Aberrant Mitochondrial Morphology and Function

MT are crucial for preserving cellular function. Normal MT are oval in shape with evenly distributed intimal ridges; however, the MT in OA chondrocytes are swollen and spherical in shape, and the intimal ridges are irregularly arranged [51]. These morphological changes are accompanied by a decrease in mitochondrial membrane potential and reduced ATP production, which is an intuitive manifestation of mitochondrial dysfunction [29]. Shen et al. [52] proposed that the AMPK–sirtuin 1(SIRT3) loop has a crucial impact on controlling the advancement and growth of OA, in part by adjusting the quality of chondrocyte MT. Sun-Li Hu et al. [53] also pointed out that preventing mitochondrial fragmentation and reshaping mitochondrial morphology can effectively reduce chondrocyte apoptosis and improve cartilage degradation. In addition, controlling the creation of new MT is essential to preserve their function. Studies indicate a decline in mtDNA levels and a loss of important regulators of mitochondrial biogenesis in OA, such as PGC-1α and mitochondrial transcriptional factor A (TFAM), and reversal of this event can inhibit OA progression [33].

Calcium Dysregulation

Calcium is crucial for cell function and acts as a second messenger in various signalling pathways, controlling processes such as cell growth, contraction, and gene expression. Cells possess the ability to sense alterations in intracellular calcium (Ca2+) levels, including amplitude, duration, frequency, and localisation, and respond appropriately to uphold calcium homeostasis and to mitigate cellular harm [54]. The Ca2+influx and efflux rates between MT must be balanced. An overabundance of Ca2+can lead to the production of ROS, mitochondrial depolarisation, impairment of mitochondrial membrane potential, and apoptosis [55]. Maintaining intracellular Ca2+levels involves the transport of calcium into MT using the mitochondrial calcium uniporter (MCU) and the release of Ca2+through different pathways such as the inositol-1,4,5-trisphosphate receptor (IP3R), the sodium/calcium exchanger, and the mitochondrial permeability transition pore (mPTP) [56]. Abnormal Ca2+accumulation within the mitochondrial matrix can activate the mPTP, a substantial channel located in the IMM that responds to elevated Ca2+levels and ROS [57]. The opening of the mPTP results in the depolarisation of the mitochondrial membrane, causing MT to swell and release calcium and Cyt-C, thereby initiating the apoptotic pathway [57]. Early studies found that calcium balance in OA cartilage tissue is dysregulated [58]. Subsequently, Huser et al. [60] directly confirmed that Ca2+signalling is the key to the mechanical impact of OA. In addition, Zhai et al. [61] reported that the mitochondrial Ca2+level of bone marrow–derived MSCs

(BM-MSCs) derived from the subchondral bone in patients with OA was also significantly higher than in the normal group. When this Ca2+overload was corrected, OA was improved.

MT-Targeting Drugs for OA Treatment

MT are essential to produce energy and to maintain balance within cells, and they may also help regulate cell death to prevent damage to cartilage in joints. Addressing mitochondrial dysfunction represents a hopeful approach to managing OA [62]. Consequently, the development of novel drugs and methodologies centred on repairing and/or restoring mitochondrial function is imperative for the management of OA. Table 1 lists some drugs that have shown promising effects in restoring mitochondrial dysfunction in OA. They include antioxidants, enhancers of mitochondrial biogenesis, regulators of mitochondrial dynamics, and calcium balance stabilisers.

Antioxidants

Appropriate antioxidant strategies aimed at reducing the ROS generated by MT are crucial to protect chondrocytes from oxidative stress damage. Research has shown that compounds such as melatonin and quercetin exhibit efficacy in reducing mtROS accumulation, thereby preventing the deterioration of mitochondrial membrane potential and the release of mitochondrial Cyt-C [63]. Additionally, nanomaterials specifically targeting mtROS have also demonstrated the potential to improve mitochondrial dysfunction [72].

Mitochondrial Biogenesis Enhancers

Mitochondrial biogenesis, a process involving the self-renewal and replication of MT regulated by numerous genes, exhibits abnormalities in OA [48]. Activation of the AMPK/SIRT1/PGC-1a pathway has been shown to boost the generation of MT and to reduce oxidative stress, thereby regulating mitochondrial function and ameliorating OA [33]. Previous studies have indicated that certain compounds, such as puerarin and apple procyanidins, can stimulate the production of new MT in chondrocytes, leading to enhanced mitochondrial functionality, and may serve as promising therapeutic agents for the management of OA [65].

Regulators of Mitochondrial Dynamics

The intricate interplay between mitochondrial fission, fusion, and mitophagy is essential for the preservation of mitochondrial functionality. Perturbation of mitochondrial dynamics is observed in chondrocytes during the pathogenesis of OA [73]. The administration of the mitochondrial inhibitor Mdivi-1 has been demonstrated to attenuate mitochondrial fission-induced damage, leading to a reduction in chondrocyte apoptosis [67]. However, the complexes that promote mitochondrial fusion are less studied and deserve further attention [74]. Besides, removing damaged MT to maintain mitochondrial health has received widespread attention. β-Hydroxybutyrate and protocatechuic aldehyde have been shown to enhance chondrocyte mitophagy, promote the clearance of damaged MT, effectively improve mitochondrial function, and inhibit cartilage degradation in OA [68].

Calcium Balance Stabilisers

In the physiological state, MT regulate calcium homeostasis to ensure the health of cartilage [75]. An imbalance in calcium homeostasis, leading to mitochondrial damage and a series of changes, is considered crucial in chondrocyte apoptosis and cartilage degradation [60]. Research suggests that cyclosporin A and B-type natriuretic peptide can inhibit mPTP opening, protecting MT from Ca2+overload–induced damage [70]. Recently, Zhai et al. [61] synthesised TMA-MSN-TPP-EGTA-PEG (METP) nanoparticles using a composite shell of silica nanoparticles, tetraethylene glycol, and triphenylphosphine. It captured the Ca2+around the MT of mesenchymal stem cells (MSCs) and effectively treated OA [61]. Similarly, Lin [76] found that regulating calcium homeostasis helps slow down the degeneration of intervertebral discs.

Mitochondrial Transfer in OA Treatment

Pharmacological modulation of mitochondrial dysfunction is pivotal for the treatment of MT-related diseases,

Table 1 Treatment strategies for mitochondrial dysfunction

Category	drugs	Mechanism	References
Antioxidants	Melatonin Quercetin	Clearing reactive oxygen species, inhibiting the loss of MMPs, and restoring mitochondrial function	[63]
Mitochondrial biogenesis enhancers	Puerarin Apple procyanidins	Activating the AMPK/SIRT-1/PGC-1α pathway, enhancing mitochondrial biogenesis, and improving mitochondrial function.	[65]
Regulators of mitochondrial dynamics	Mdivi-1 β-Hydroxybutyrate Protocatechuic aldehyde e	Regulating mitochondrial fission and autophagy to maintain mitochondrial homeostasis	[69]
Calcium balance stabilizers	Cyclosporin A B-type natriuretic peptide	Regulating mitochondrial calcium overload and restoring mitochondrial function	[70]

driving significant advancements in mitochondrial medicine and providing valuable insights. However, the development of mitochondrial-targeted therapies is confronted with substantial challenges due to the subcellular localization and complex structure of MT. Therapeutic agents must navigate numerous physiological barriers to reach the target cells and subsequently the MT. Nevertheless, even upon approaching the vicinity of the mitochondria, the highly folded and compartmentalized nature of the inner mitochondrial membrane (IMM) presents a significant obstacle for drug molecules seeking entry [77]. In addition, these drugs often face challenges in achieving effective concentration at lesion sites and within MT due to in vivo barriers and poor selectivity. In particular, the non-selective biodistribution of drugs is a primary factor contributing to suboptimal drug concentrations in targeted organs or tissues [78]. Therefore, there is an urgent need for research and development of novel therapies specifically targeting MT.

Recently, intercellular mitochondrial transfer between mammalian cells has been observed in vitro and in vivo, offering a potential universal solution for treating mitochondrial deficiency of different aetiologies [79]. This mitochondrial transfer facilitates the recovery of damaged cells, enhances OXPHOS, elevates ATP synthesis, and restores mitochondrial functionality [81]. It works in various ways, including reducing oxidative stress [82], promoting mitochondrial fusion [83], and regulating mitophagy [84], among others. As a result of these findings, researchers have begun to focus on the role that mitochondrial transfer plays in disease and explored a variety of new methods and technologies for mitochondrial transfer. Three different approaches – stem cell-based mitochondrial transfer [85], direct transfer of isolated MT [87], and transfer of extracellular vesicle (EV)-encapsulated MT [88] are discussed here as potential ways to ameliorate mitochondrial damage for the therapeutic management of OA (Fig. 2).

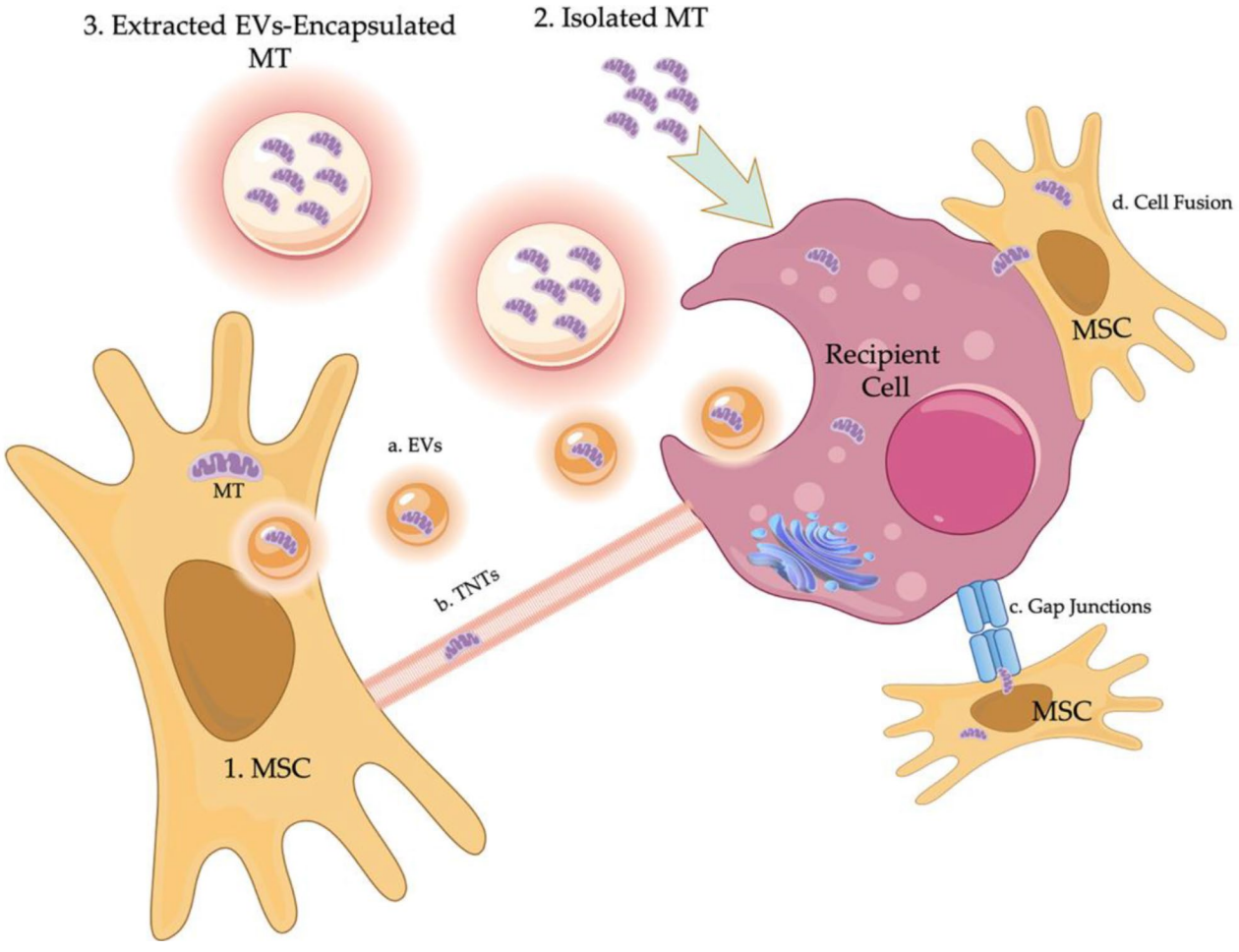

Fig. 2 Mechanism of mitochondrial transfer. (1) Mitochondria (are transferred from mesenchymal stem cells to recipient cells in four ways: (**a**) extracellular vesicles, (**b**) tunnelling nanotubes, (**c**) gap junctions, and (**d**) cell fusion. (2) Transferring isolated mitochondria directly. (3) Transferring EV-encapsulated mitochondria directly

MSC-Mediated Mitochondrial Transfer

Stem cells, as the most undifferentiated cells at the apex of the cellular lineage, exhibit a remarkable ability for differentiation and self-renewal. Furthermore, they possess the capacity to differentiate into a multitude of tissues, organs, or specialised cells within the human body, thus presenting significant potential for applications in engineering and regenerative medicine. Various sources of MSC treatments have demonstrated the ability to inhibit, halt, or potentially reverse cartilage degradation in animal models [89]. In a recent double-blind, randomised phase IIb clinical trial, the authors demonstrated that patients receiving a single injection of adipose-derived MSCs (AD-MSCs) exhibited notable enhancements in their Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores at the 6-month mark, in contrast to the control cohort. Furthermore, there was a deceleration in joint wear progression [92]. The conclusions reached by Sadri et al. [93] were consistent with these results. However, a thorough comprehension of the mechanisms governing MSC therapy for OA is lacking.

Recent research has validated the ability of stem cells to transfer MT to neighbouring cells, resulting in the restoration of cellular respiration, the initiation of cell reprogramming, and ultimately the repair and enhancement of cellular function (Table 2). Undifferentiated MSCs exhibit decreased energy requirements in the glycolytic state, making them promising candidates for mitochondrial transfer [94]. In addition, MSCs possess an exceptional ability to home to diseased tissues, facilitating targeted mitochondrial transfer. These characteristics – combined with the immune privilege, low levels of oxidative damage, and tightly regulated redox balance of MSCs – position them as optimal donor cells for the selective delivery of healthy MT to diseased cells.

Notably, this transfer process is more common in harmful environments than in healthy cells. For example, when exposed to damaged cartilage tissue, MSCs position themselves in the area of matrix injury and extend their MT into chondrocytes located deep within microcracks. In contrast, few cells accumulate in uninjured cartilage [95]. During this process, cytokines and dysfunctional MT are released from injured cells. During this process, cytokines and mtDNA are released from injured cells, serving as indicators of potential damage. These signals stimulate MSCs to transfer their functional MT to aid in the recovery of the impaired cells [96]. On the other hand, a damaged environment will promote the production of more mitochondrial transfer channels, such as tunneling nanotubes (TNTs) [99].

The significance of mitochondrial transfer in the therapeutic efficacy of stem cells for injured tissues is increasingly acknowledged. Although MT are not the primary energy source for chondrocytes, the phenomenon of mitochondrial dysfunction and mitochondrial transfer in OA chondrocytes has attracted research attention [95]. In a co-culture system involving BM-MSCs and osteoarthritic chondrocytes, Wang et al. [51] observed that MSCs promoted the recovery of the chondrocyte mitochondrial membrane potential and increased ATP by transferring their own healthy MT, ultimately reducing the apoptotic rate of chondrocytes and enhancing the function of OA chondrocytes. This finding highlights the role of mitochondrial transfer in OA treatment.

MSCs transfer MT through various mechanisms, such as TNTs, gap junction channels (GJCs), and EVs [97], as well as cell fusion (Fig. 3) [110]. TNTs are membrane-bound cellular conduits capable of directly transferring various cellular components such as endocytic vesicles, lysosomes, MT, and membrane-bound proteins from cell to cell [111]. Hsu et al. [112] utilised anti-mitochondrial

Table 2 Applications of mitochondrial transfer in various systemic diseases

Donor cells	Receptor cells	Result	References
Human bone marrow-derived mesenchymal stem cells	Cells with mtDNA mutations that prevent aerobic respiration (A549 ρ° cells)	Mitochondria are transferred to injured cells and their aerobic respiration is restored	[102]
Human adipose-derived mesenchymal stem cells	Cardiomyocytes	Reprogramming of dividing cardiomyocytes into a viable progenitor-like state via stem cell mitochondrial transfer	[103]
Rat bone marrow-derived mesenchymal stem cells	Rat cardiomyocytes (H9c2 cells) simulating ischemia/reperfusion injury	Mitochondria are transferred to damaged cells through tunnelling nanotubes, enhancing their anti-apoptotic ability	[104]
Human bone marrow-derived mesenchymal stem cells	Injured human umbilical cord vein endothelial cells	Mitochondria are transferred to damaged cells through tunnelling nanotubes, reducing apoptosis and restoring transmembrane migration ability	[105]
Rat bone marrow-derived mesenchymal stem cells	Host cells of cerebral microvasculature in rat stroke model	Significantly improves mitochondrial activity in injured microvasculature through mitochondrial transfer, enhances angiogenesis, reduces the infarct volume, and improves functional recovery	[106]
Mouse bone marrow-derived mesenchymal stem cells	Odontoblast cell line	Mitochondrial transfer relieves pulp damage	[107]

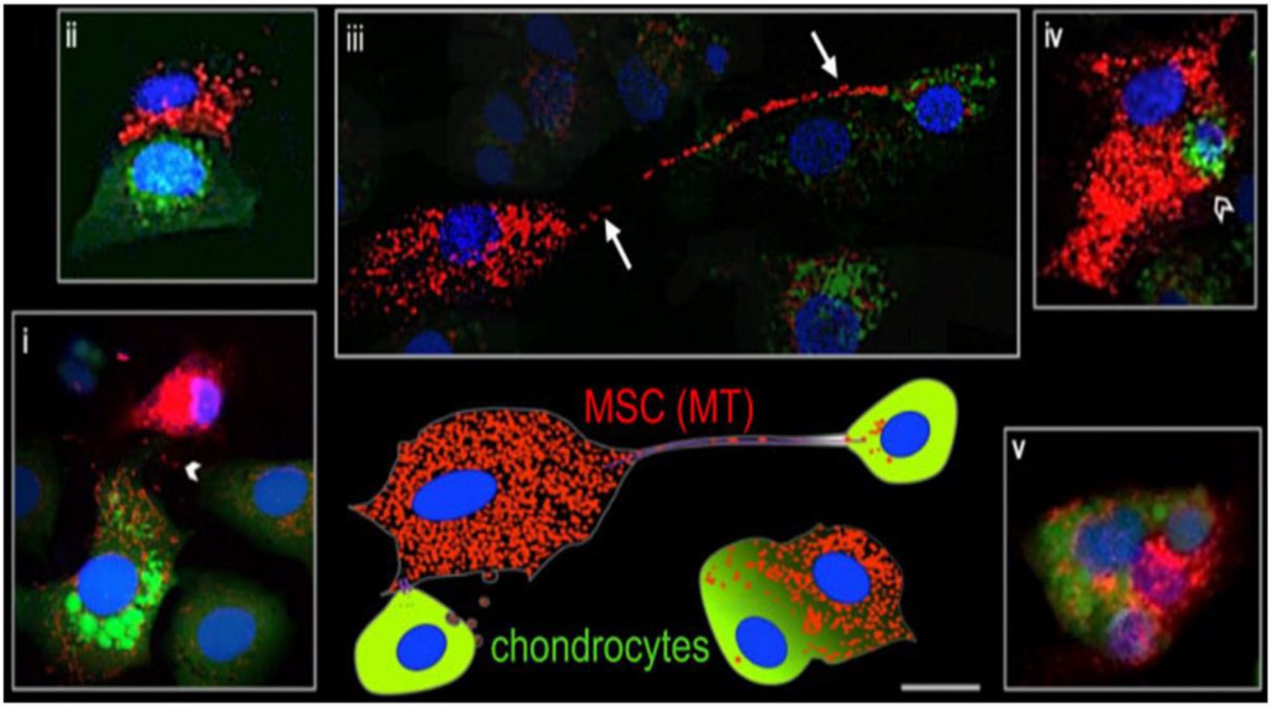

Fig. 3 Laser confocal imaging of mitochondrial transfer. Mesenchymal stems cell transfer mitochondria (red) to chondrocytes (green cytoplasm, blue nuclei) using different methods: (**i**) extracellular vesicles, (**ii**) gap junctions, (**iii**) tunnelling nanotubes, and (**iv** and **v**) cell fusion. Reproduced from a previous publication [95], with permission from the authors

antibodies and MitoTracker along with laser scanning confocal microscopy to study the transmission of MT from human BM-MSCs to mouse liver cells via TNTs. GJCs, which are formed by connexins (CXs), serve as a significant mode of intercellular communication. These pathways enable the transfer of ions and small compounds, such as Ca2+, inositol trisphosphate, cyclic nucleotides, and oligonucleotides, which help to synchronise cellular activities throughout various multicellular tissues [113]. The application of the GJC enhancer retinoic acid resulted in significant augmentation in the quantity of MT transferred from BM-MSCs to neurons. Conversely, the GJC inhibitor 18β-glycyrrhizic acid (18β-GA) reduced mitochondrial transfer. The results indicate that GJCs play a vital role in enabling the movement of MT from stem cells to neurons [81]. Specifically, connexin43 (CX43) is a critical CX protein involved in the establishment of GJCs [97]. Increasing CX43 expression with iron oxide nanoparticles has been shown to improve GJC function and to boost the mitochondrial transfer rate. Conversely, the suppression of CX43 expression eliminates this effect [114]. Moreover, recent reports have demonstrated that the upregulation of CX43 is associated with augmented formation of TNTs, while the employment of short hairpin RNA (shRNA) to suppress CX43 yields contrasting outcomes, suggesting that CX43 also assumes a crucial function in facilitating TNT formation. However, the precise regulatory mechanism remains elusive [115]. Additionally, Miro1 and Miro2, which are two types of Rho-GTPases, interact with other accessory proteins to move MT along the TNTs that connect the two cells [117]. Miro1 upregulation has been shown to enhance mitochondrial transfer by MSCs in cases of myocardial disease [118]. On the other hand, reducing Miro1 expression hinders the development of TNTs, thus blocking the transport of MT from MSCs to endothelial cells [119]. Miro2s participation in the mitochondrial transfer process is also significant [120]. Previous studies have indicated that mitochondrial migration can also occur via EVs, such as exosomes [51]. Phinney et al. [121] discovered that MSCs possess the ability to transfer their own MT into EVs, thereby facilitating the transportation of intact MT or mtDNA to macrophages. This process enhances the bioenergy of macrophages and provides additional evidence supporting the notion that EVs serve as carriers for MT. Additionally, the direct acquisition of MT through cell fusion represents the most straightforward approach [122].

Stem cell injection has been used in the clinical treatment of orthopaedic diseases [124]. However, it is imperative to acknowledge the potential risks associated with stem cell transplantation, including tumourigenicity and immunogenicity. Multiple stem cell types possess the property of tumour tropism [125]. Furthermore, stem cells exhibit a diverse array of surface antigens, such as HLA class 1 antigens, that are absent on the

mitochondrial membrane. Consequently, stem cells possess a higher degree of immunogenicity compared with isolated MT [116]. Another issue that needs to be considered is that the mitochondrial transfer efficiency of stem cell therapy is low. Hence, finding a way to improve the transfer rate is the key to enhancing its efficacy.

Isolated Exogenous Mitochondrial Transfer

Limitations of cell-to-cell MT transfer encompass variability in cell phenotypes, low engraftment and retention rates, and inconsistent clinical outcomes. Therefore, non-contact MT transfer methods are currently being investigated. The phenomenon of intercellular mitochondrial transfer has led researchers to hypothesize that MT may possess the capability to invade cells, and various methods to transfer MT to the recipient cell artificially have been developed. In a seminal study, Tachibana et al. [127] successfully introduced healthy mitochondria into oocytes containing mutated mtDNA, demonstrating potential for the treatment of human genetic mitochondrial disorders and laying the groundwork for further research on mitochondrial transfer. Similarly, Li et al. [81] documented the internalisation of isolated MT in motor neurons following co-culturing under hypoxic conditions for 30 min. This process was concomitant with an elevation in ATP levels and the mitochondrial membrane potential and enhanced neuronal viability. Additionally, the extent of internalisation correlated directly with the concentration of co-cultured MT. Masuzawa et al. [128] validated this effect in in vivo experiments. They isolated MT from the chest muscles of New Zealand white rabbits and immediately injected them into ischaemic hearts. The findings indicated that cardiac cells internalised these MT within 2–8 h of transplantation, leading to increased oxygen consumption, synthesis of high-energy phosphates, and activation of cytokine mediators and protein pathways, ultimately shielding the heart from damage caused by ischaemia–reperfusion. Recently, direct transplantation of MT has been applied to cartilage repair in OA. Kim et al. [129] synthesised fusogenic liposomes encapsulating MT, assisting in their delivery to chondrocytes. Experiments conducted in a lab setting and within living organisms have shown that the use of fusogenic liposomes accelerates and improves mitochondrial transfer, offering a promising approach for enhancing cartilage repair. Inspired by this, researchers have designed different methods in the hope of increasing isolated mitochondrial transfer efficiency (Fig. 4).

Although mitochondrial transfer has shown numerous benefits for cells, the technology is still in its early stages and encounters various obstacles. It is believed that damaged and dysfunctional MT may not provide benefits to host cells and could potentially cause harm [128]. Therefore, it is necessary to obtain fresh, intact, and respiratory-active MT to effectively exert therapeutic effects. Additionally, facilitating the successful entry of MT into recipient cells in adequate amounts and ensuring their complete utilisation presents a significant challenge. In addition to the methods shown in Fig. 4, technologies such as MitoCeption [134] and magnetic nanoparticles [135] have been designed to increase the efficiency of isolated mitochondrial transfer. Nevertheless, the application of these technologies in vivo necessitates additional investigation. Lastly, exogenous MT may selectively degrade after mitochondrial transfer and disappear within a week [136]. Therefore, more complex, minimally invasive methods are needed to isolate fully functional MT from cell extracts. Additionally, less invasive delivery methods need to be developed to fully exploit the beneficial effects of mitochondrial transfer.

EV-Encapsulated Mitochondrial Transfer

Recent research has identified MT-specific cargoes, including DNA, RNA, and proteins in EVs derived from various cell types, such as fibroblasts, neurons, and MSCs [137]. EVs originate from the cell membrane and can be classified based on their size as exosomes, microvesicles, and apoptotic bodies. These vesicles have the ability to transfer their cargo into the cytoplasm of recipient cells, thereby facilitating intercellular communication and modulating the physiological state of the receiving cells [138]. Functioning as pivotal agents in intercellular communication, EVs possess the capacity to selectively bind to particular cells or tissues through receptor-mediated mechanisms, subsequently releasing their contents into the corresponding target structures [139]. This attribute enables EVs to potentially serve as rescuers for recipient cells, while simultaneously preserving the homeostasis of the originating cell. As EV extraction technology has matured, the application of EVs has become increasingly widespread (Fig. 5).

The efficacy of stem cell–derived EVs in the treatment of OA has been proven, and its mode of action is diverse. In a study using mice, the communication between EVs and methyltransferase-like 3(METTL3) results in decreased methylation of Nod-like receptor pyrin domain 3 (NLRP3) mRNA in macrophages, ultimately easing the symptoms of OA in the knee joint [141]. In addition, EVs have the ability to transport microRNAs (miRNA), which effectively inhibits chondrocyte apoptosis [142]. Furthermore, experiments conducted in living organisms and in a controlled environment demonstrate that the use of exosomes can effectively reduce the levels of MMP-13 and a disintegrin and metalloproteinase with thrombospondin motifs 5(ADAMTS-5), thus preventing the degradation of cartilage [143]. However, further investigation is required to fully understand the precise mechanisms underlying EV therapy. Recently, researchers have found

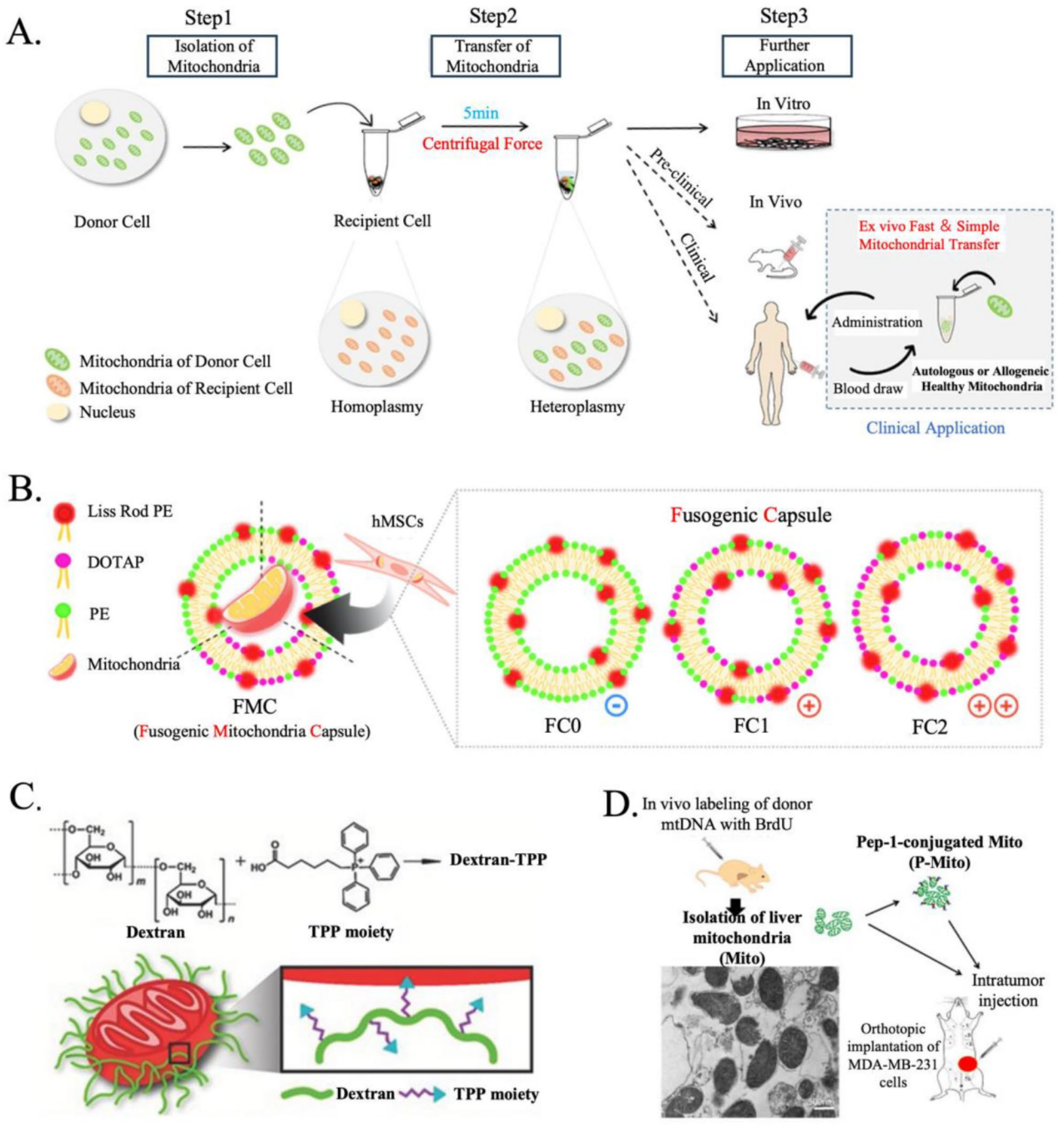

Fig. 4 An illustration of methods to increase the transfer rate of isolated mitochondria. (**A**) Promoting internalisation of isolated mitochondria by centrifugal force [130]. (**B**) Mitochondria are encapsulated in synthetic liposomes to enhance the delivery efficiency [129]. (**C**) Dextran was conjugated with TPP as carriers to increase mitochondrial delivery efficiency [131]. (**D**) Pep-1 peptide binds to mitochondria to enhance delivery efficiency [132]. Each panel has been reproduced from the respective publication, with permission from the authors

that EVs transfer their own MT and mtDNA to recipient cells during the treatment of diseases [144]. In the context of ischaemic stroke, D'Souza et al. [145] revealed that microvesicles, which act as carriers of MT, have the ability to transmit functional MT to chemically impaired brain endothelial cells, thereby enhancing their chances of survival. Similarly to TNT-mediated mitochondrial transfer, this transfer process is less frequent in endothelial cells that are in a healthy state. The utilisation of exosomes derived from AD-MSCs for the treatment of acute lung injury has also been shown to effectively transfer MT, thereby alleviating airway metabolic disturbances

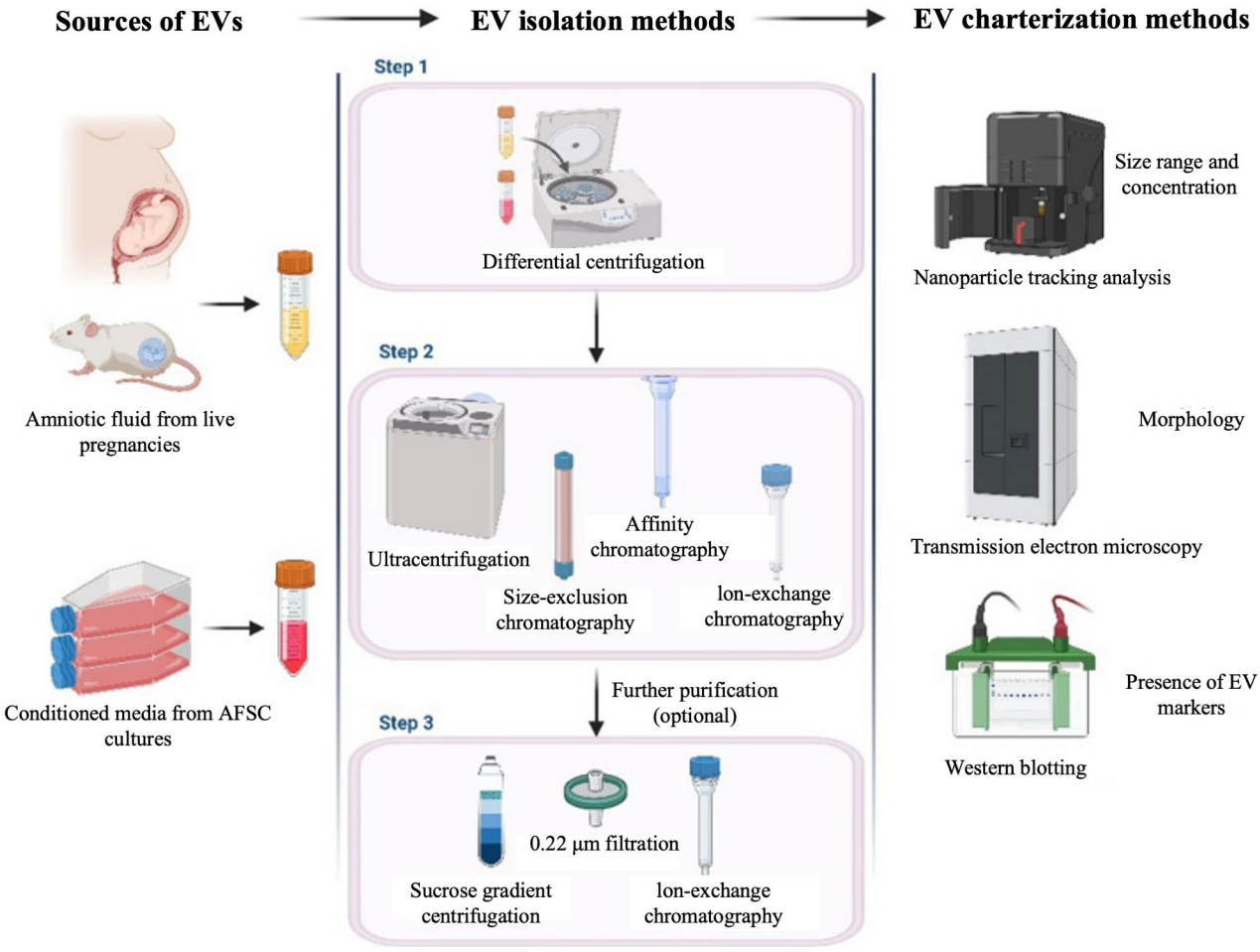

Fig. 5 Isolation methods to obtain extracellular vesicles. Reproduced from a previous publication [140], with permission from the authors

[146]. This finding aligns with the conclusions drawn by Zhang et al. [147] in their study on Alzheimer's disease. Thomas et al. [137] validated that MSCs can package functional MT into EVs and deliver them to chondrocytes, which holds great promise for the treatment of OA.

The utilisation of EVs as vehicles for mitochondrial transfer presents a potential solution to address certain inherent constraints associated with MSC therapy [148]. Moreover, research has indicated that EVs, upon transporting MT, exhibit minimal co-localisation with lysosomes and peroxisomes within recipient cells, thereby diminishing the degradation of exogenous MT [149]. Although this phenomenon has only been illustrated preliminarily, it offers valuable insights for future investigations in the field of mitochondrial therapy. The present obstacle pertains to the insufficiency of MT within EVs, despite their presence. It is imperative to facilitate the incorporation of a greater quantity of MT into EVs by stem cells. Preliminary investigations have substantiated that pre-treating donor cells can augment their survival rate and therapeutic efficacy [150]. In a study focused on obesity, Crewe et al. [151] discovered that elevated energy stress within adipocytes leads to an increased presence of MT enclosed within small EVs (sEVs). These sEVs subsequently migrate to the heart, augmenting its adaptability to cardiac conditions. Recently, novel methodologies have been established for the subfractionation of EV subtypes, allowing for the selective isolation of vesicles containing intact mitochondrial components such as MT, mitochondrial proteins, and mtDNA. These specialised vesicles, known as mitovehicles, appear to provide a more effective means of facilitating mitochondrial transfer [152]. Nevertheless, there have been few studies in this area, and the technology for extracting MT-rich EVs is limited. Hence, there is still a long way to go before this technique can be translated to the clinic.

Conclusion and perspectives

In the pathogenesis of OA, chondrocytes frequently experience mitochondrial dysfunction, resulting in compromised energy metabolism and subsequent cascades of oxidative stress and calcium imbalance following mitochondrial injury. These events significantly impact cell

viability. The advancement of mitochondrial repair therapy has exhibited encouraging outcomes in the reversal of mitochondrial dysfunction, offering novel insights for OA treatment.

In addition to conventional pharmacological repair, mitochondrial transfer has emerged as a promising therapeutic strategy, showing significant advantages in restoring mitochondrial function. There are three established techniques for mitochondrial transfer: stem cell-mediated transfer, isolated exogenous transfer, and EV-encapsulated transfer. Each option has its own advantages and disadvantages. Researchers have conducted extensive research to better exploit the advantages of these solutions. Several studies have proposed that pre-treating stem cells could enhance therapeutic efficacy. For example, Guo et al. [20] isolated MTs from donor BM-MSCs and then transplanted them into BM-MSCs of the same batch and generation. BM-MSCs that underwent autologous mitochondrial transplantation exhibited enhanced bone defect repair capabilities. Specific pharmaceutical agents, including metformin [154], pioglitazone [155], and adiponectin [156], have the ability to activate PGC-1α and AMPK, thereby stimulating mitochondrial biogenesis and increasing the mitochondrial reserves of stem cells. Furthermore, some nanoparticles such as platinum [157], silica [158], and iron oxide [159] have been shown to upregulate CX43 expression and to increase the release of EVs, thereby promoting more effective mitochondrial transfer. On the other hand, strategies to develop advanced technologies to extract higher-quality isolated MTs or MT-rich EVs and enhance efficient mitochondrial delivery are the focus of research. Moreover, in future clinical applications, these technologies face a common problem. Cartilage ECM is characterised by low cellularity and high density, and its small pore size and high charge properties have been shown to impede the diffusion of large particles, including antibodies and other experimental biological factors [161]. This leads to the limitation of mitochondrial transfer therapy, that is, the therapeutic effect on deep-seated chondrocytes will be weaker than that of superficial cells.

Mitochondrial repair technology presents significant potential for the management of OA. The conception of mitochondrial transfer has garnered significant attention in recent years. While the mechanism of mitochondrial transfer remains poorly understood, it holds significant therapeutic promise. In future research, it is necessary to elucidate the molecular and cellular mechanisms involved in mitochondrial transfer and to develop efficient methods for the extraction and delivery of MT, in order to advance their clinical utilisation.

Abbreviations

ATP	adenosine triphosphate
AD-MSCs	adipose-derived MSCs
ADAMTS 5	a disintegrin and metalloproteinase with thrombospondin motifs 5
AMPK	AMP activated protein kinase
BM-MSCs	bone marrow-derived MSCs
18β-GA	18β-glycyrrhizic acid
CX43	connexin43
Cyt-C	cytochrome c
Drp1	dynamin-related protein 1
Dnm2	dynamin 2
2,4-DNP	2,4-dinitrophenol
EVs	extracellular vesicles
ECM	extracellular matrix
GJCs	gap junction channels
IP3R	inositol-1,4,5-trisphosphate receptor
IL-1β	interleukin-1 beta
IMM	inner mitochondrial membrane
METTL3	methyltransferase-like 3
OA	Osteoarthritis
MMPs	matrix metalloproteinases
Mfn1/2	mitofusins 1/2
MSC	mesenchymal stem cell
METP	TMA-MSN-TPP-EGTA-PEG
mtROS	mitochondrial ROS
mtDNA	mitochondrial DNA
mTOR	mechanistic target of rapamycin
miRNA	microRNA
NO	nitric oxide
NF-κB	factor-κB
NLRP3	Nod-like receptor pyrin domain 3
MT	mitochondria
OXPHOS	oxidative phosphorylation
OMM	outer mitochondrial membrane
ROS	reactive oxygen species
SIRT3	sirtuin 1
shRNA	short hairpin RNA
TFAM	mitochondrial transcriptional factor A
TNF-α	tumour necrosis factor-alpha
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

Author contributions

Conceptualization and Design, M.H.-Z. and T.C.; Literature Search and Selection, M.H.-Z., J.F-W. and K.H.-C.; Manuscript Writing, M.H.-Z.; Language Editing, Y.L.,B.T.-L., and J.J.-Z.; Review and Revision, C.X.-G., T.C., and J.Z.-X. All authors have read and agreed to the published version of the manuscript.

Data availability

Due to its nature as a review article, all references are published articles. The data underlying this article are available in the Pubmed.

Declarations

Competing Interest

The authors declare no conflicts of interest.

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