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Mitochondrial transplantation: a promising strategy for treating degenerative joint diseases

Hong Luo[1†], Yue Lai[2†], Weili Tang[1], Guoyou Wang[1*], Jianlin Shen[3,4*] and Huan Liu[1*]

Abstract

The prevalence of age-related degenerative joint diseases, particularly intervertebral disc degeneration and osteoarthritis, is increasing, thereby posing significant challenges for the elderly population. Mitochondrial dysfunction is a critical factor in the etiology and progression of these disorders. Therapeutic interventions that incorporate mitochondrial transplantation exhibit considerable promise by increasing mitochondrial numbers and improving their functionality. Existing evidence suggests that exogenous mitochondrial therapy improves clinical outcomes for patients with degenerative joint diseases. This review elucidates the mitochondrial abnormalities associated with degenerative joint diseases and examines the mechanisms of mitochondrial intercellular transfer and artificial mitochondrial transplantation. Furthermore, therapeutic strategies for mitochondrial transplantation in degenerative joint diseases are synthesized, and the concept of engineered mitochondrial transplantation is proposed.

Keywords Degenerative joint diseases, Mitochondrial transplantation, Intervertebral disc degeneration, Osteoarthritis, Engineered mitochondria

Introduction

Degenerative joint diseases encompass a range of age-related disorders affecting the musculoskeletal system, with intervertebral disc degeneration (IVDD) and osteoarthritis (OA) being the most prevalent. These diseases are frequently associated with factors such as trauma, obesity, infections, and autoimmune disorders [1, 2]. IVDD and OA are major causes of pain and dysfunction in patients, significantly diminishing quality of life and resulting in high socioeconomic costs.

For the treatment of degenerative joint disease, there is no modality that directly restores tissues and cells after injury. In the early stages of the disease, measures such as physical therapy and pain medications are often used to reduce pain and improve function. However, they do not address the underlying cause of the patient's pain. In cases of advanced IVDD, surgical interventions such

†Hong Luo and Yue Lai contributed equally to this work.

*Correspondence:
Guoyou Wang
wangguoyou1981@swmu.edu.cn
Jianlin Shen
shenjianlinchina@126.com
Huan Liu
20016040@163.com
[1]Department of Orthopedics, The Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, Luzhou 646000, Sichuan, China
[2]Department of Orthopedics, Affiliated Hospital of Guangdong medical University, zhanjiang 524000, China
[3]Central Laboratory, Affiliated Hospital of Putian University, Putian 351100, Fujian, China
[4]Department of Orthopedics, Affiliated Hospital of Putian University, Putian 351100, Fujian, China

as decompression, spinal fusion, or disc replacement are employed, albeit with variable success rates [3]. Similarly, individuals with severe OA frequently opt for joint replacement surgery, which carries risks of complications such as superficial and deep infections at the implant site, along with decreased joint mobility, all of which can further compromise the patient's quality of life [4, 5].

Although the pathogenesis of IVDD and OA is complex, it is broadly believed to be closely related to the nucleus pulposus cells (NPCs) and chondrocytes [6]. NPCs are the main functional cells of the intervertebral disc, which can be metabolized by synthesis and catabolism to maintain intervertebral disc homeostasis. Damage to NPCs caused by cellular senescence, apoptosis, necrosis, and inflammatory responses is a determining factor in promoting the onset and progression of IVDD. During the development of OA, chondrocytes control articular cartilage homeostasis by controlling Extracellular matrix (ECM) metabolism.

Mitochondria are intracellular energy centers that directly regulate the production of Adenosine triphosphate (ATP) and play an important role in the regulation of reactive oxygen species (ROS) production, intracellular metabolism, apoptosis and autophagy. Current research concludes that age-related mitochondrial dysfunction and associated oxidative stress may be the main cause of NPC and chondrocyte damage as well as articular cartilage destruction [7, 8]. As individuals age, various stressors can cause mitochondrial impairment, disturbing mitochondrial dynamics and quality control mechanisms, thereby elevating ROS levels. This leads to disturbances in calcium homeostasis and the release of pro-apoptotic factors. Excessive ROS accumulation further inflicts oxidative damage on mitochondrial DNA and proteins, compounding mitochondrial dysfunction [9, 10].Therapeutic advances have been made in various means of alleviating mitochondrial dysfunction, scavenging ROS and antioxidants, such as Mitoquone (MitoQ) [11], exosomes [12], and various types of active substances of traditional Chinese medicine [13]. These interventions are mainly applied in in vitro and animal model studies [14, 15], only a few early therapies can be applied in the clinic. With the important role of dysfunctional mitochondria in the development of degenerative joint diseases, researchers have focused on a method that can directly improve damaged mitochondria - mitochondrial transplantation. Healthy mitochondria can be extracted and transplanted into mitochondrial dysfunction cells, which can improve mitochondrial function, reduce oxidative stress, and decrease necrotic apoptosis in cells [16, 17]. This article reviews the mitochondrial dysfunction that occurs in degenerative joint disease, describes the mechanisms of mitochondrial transfer, and summarizes the therapeutic strategies as well as the developmental potential of mitochondrial transplantation for the treatment of degenerative joint disease.

Mitochondrial dysfunction in orthopedic degenerative joint diseases

Mitochondria and oxidative stress

Oxidative stress reflects an imbalance between intracellular ROS production and the action of the antioxidant system, and is an important factor contributing to damage in NPCs and chondrocytes. Mitochondrial dysfunction generates large amounts of ROS such as superoxide anion and hydrogen peroxide (H_2O_2) to affect the cellular antioxidant system. Studies have shown that menaquinone-induced mitochondrial H_2O_2 modulates MAPK signaling, thereby inducing human chondrocyte death [18]. Oxidative stress-induced ROS overload also opens the mitochondrial permeability transition pore (MPTP) and decreases the mitochondrial membrane potential, releasing intermembrane proteins such as cytochrome C and apoptosis-inducing factors to induce cell death [8]. In addition, these oxidized substances damage the mitochondrial respiratory chain protein complex, which further generates ROS, creating a positive cycle. Cellular senescence is also significantly affected by oxidative stress, with large amounts of ROS attacking mitochondrial DNA, lipids, and proteins, all of which alter microenvironmental homeostasis and mechanical properties of the intervertebral discs and bone joints [19, 20]. Superoxide dismutase 2 (SOD2) is an antioxidant enzyme distributed in the inner mitochondrial membrane, and in the early stages of cartilage injury, a decrease in SOD2 may increase ROS, potentially exacerbating inflammation, which can lead to OA progression [21]. Researchers have attempted to activate SOD2 by other means to achieve ROS scavenging, improve mitochondrial metabolism, and ultimately increase chondrocyte viability [22].

Mitochondrial autophagy

Mitochondrial autophagy is a process that selectively removes damaged mitochondria, thereby maintaining mitochondrial mass and reducing cellular damage. This process is mediated by two distinct molecular pathways: the PRKN-dependent pathway and the PRKN-independent pathway. The PRKN-dependent pathway is mediated by PTEN-induced putative kinase (PINK1) and PRKN, an E3-ubiquitin ligase, and has been extensively investigated in a variety of cell types. The PRKN-independent pathway relies on mitochondrial receptor proteins, including BNIP3 (BCL2-interacting protein 3), NIX (Nip3-like protein X), FUNDC1 (FUN14-domain containing protein 1), and FKBP8 (FK506-binding protein), which initiate mitochondrial autophagy by recruiting autophagosomes to damaged mitochondria through binding interactions with LC3. Inhibition of either of

these two major pathways results in impaired mitochondrial autophagy, inducing the accumulation of dysfunctional mitochondria. Ultimately, this can influence the progression of numerous aging and degenerative diseases including IVDD and OA [23, 24]. Mitochondrial autophagy-related proteins (including LC3B, PINK1, and Parkin) were significantly elevated in an artificially established rat OA model. Inhibition of autophagy results in the inability of cells to effectively remove dysfunctional mitochondria, thereby impairing apoptosis and ECM degradation in NPCs and chondrocytes, ultimately leading to degenerative joint lesions [25]. Mitochondrial autophagy is also influenced by oxidative stress. Wang et al. demonstrated that oxidative stress induces mitochondrial autophagy in NPCs, and depletion of PINK1 impairs this autophagy process, exacerbating NPC senescence. It has been suggested that PINK1 facilitates the removal of damaged mitochondria through a mitochondrial autophagy mechanism under oxidative stress, thereby alleviating NPC senescence [26].

However, despite the increase in mitochondrial autophagy with disease progression in IVDD and OA, controversy remains regarding its role in degenerative joint diseases. Some researchers have identified a positive correlation between PINK1 expression and the progression of IVDD during NPC senescence induced by mechanical stress of varying durations. It is speculated that mitochondrial autophagy mediated by the PINK1 pathway serves as a predominant mechanism of cellular senescence under stress. They proposed that the protective effect of mitochondrial autophagy against oxidative stress may become ineffective under conditions of excessive compression. Xu et al. reported that strong oxidative stress activates mitochondrial autophagy, and that NPCs can be protected from apoptosis by inhibiting excessive mitochondrial autophagy [27]. A plausible explanation for these findings is that overactivated mitochondrial autophagy removes a greater number of mitochondria than the normal threshold, ultimately accelerating NPC senescence. Simultaneously, mitochondrial autophagy may exhibit varying effects in different cell types and under diverse stimuli [23, 28].

Imbalance in mitochondrial dynamics

Mitochondrial dynamics has become a current research hotspot, mainly referring to the fact that mitochondria can respond to metabolic changes by controlling the processes of fusion and fission when a stressor stimulates the cell. Mitochondrial fusion is a process that enables partially damaged mitochondria to complement healthy ones, thereby allowing defective mitochondria to acquire the essential components necessary for restoring mitochondrial function. Conversely, mitochondrial fission generates fragmented mitochondria, facilitates the expulsion of damaged molecules and metabolic waste, and enables the separation of compromised mitochondria from their parent mitochondria [29]. Dynamin-related protein 1 (Drp1), mitochondrial fission 1 protein (Fis1) and mitochondrial fission factor (MFF) are known to be involved in mitochondrial fission. Mitochondrial fission is mediated by mitofusin 1 (Mfn1), mitofusin 2 (Mfn2) and optic atrophy 1 (OPA1). In excessive mechanical stress-induced IVDD, increased expression of DRP1, MFF, and Fis1 and decreased expression of Mfn1 and Mfn2 were observed in NPC. Researchers prevented excessive mitochondrial fission and slowed down the progression of IVDD by upregulating Sirtuin-3 (SIRT3) [30]. Similarly, inflammation- and stress-induced inhibition of mitochondrial fusion and increased mitochondrial fission are key factors accelerating mitochondrial damage and contributing to senescence and apoptosis in NPCs and chondrocytes [31, 32]. It is increasingly evident that rebalancing the relationship between mitochondrial fusion and fission represents a potent strategy for mitigating mitochondrial dysfunction.

Mitochondria and inflammation

High levels of ROS and inflammatory responses characterize degenerative bone and joint diseases, both of which drive disease onset and progression [33]. It is well established that oxidative stress, driven by mitochondrial dysfunction, initiates the release of ROS, activates NOD-like receptor family, pyrin domain-containing protein (NLRP) inflammatory vesicles, and stimulates the production of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), by chondrocytes or NPCs [34, 35]. The activation of the TNF-α/NF-κB pathway further promotes the expression of tumor necrosis factor-alpha (TNF-α) and matrix metalloproteinases (MMPs) [36, 37], both of which are implicated in the breakdown of the extracellular matrix, cartilage damage, and inflammatory responses.

The accumulation of damaged mitochondria ultimately leads to an imbalance between pro-inflammatory cytokines and anti-inflammatory factors, resulting in the increased levels of IL-1β, interleukin-6 (IL-6), interleukin-17 (IL-17), and TNF-α, among others. As a classical inflammatory factor, IL-1β promotes the release of chemokines, such as monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1α), which facilitate the recruitment of inflammatory cells to the joint site; it also induces the production of various enzymes that mediate the release of prostaglandin E2 (PGE2) and nitric oxide (NO), thereby exacerbating local inflammatory responses [38]. IL-1β and TNF-α induce damage to mitochondrial DNA (mtDNA), decrease ATP production and mitochondrial transcription, ultimately leading to mitochondrial dysfunction.

In this context, elevated NO production is a critical factor contributing to the accumulation of mtDNA damage [39]. Consequently, targeting mitochondrial dysfunction to suppress inflammation has emerged as a promising therapeutic strategy for the management of degenerative joint diseases.

Mitochondria and apoptosis

Apoptosis, which is also known as programmed cell death, is the orderly process of cell death. Apoptosis is mainly divided into endogenous pathways (mitochondria-centered) and exogenous pathways [40]. In unfavorable microenvironments, mitochondrial dysfunction increases endogenous apoptosis. Stimuli such as mechanical stress [41], inflammatory factors [42], and oxidative stress [43] alter the permeability of the mitochondrial membrane, causing the mitochondrial permeability transition pore to open, releasing leading to a large amount of cytochrome C. Cytochrome C binds to apoptotic protease-activating factor 1 and induces the formation of the apoptotic complex, followed by the sequential activation of caspase-9 and caspase-3/7, leading to the apoptosis of NPCs and advancing the process of IVDD. Similarly mitochondrial damage leading to chondrocyte apoptosis and reduced production of extracellular matrix components, including collagen and proteoglycans, is a key factor contributing to OA cartilage degeneration [44]. Other mitochondria-related modes of cell death are present during arthropathic degeneration, including necroptosis [45, 46], pyroptosis [46, 47], and iron death [48]. Different molecular mechanisms in various cell types mediate different patterns of cell death, and intervening in these patterns through the mitochondrial pathway is emerging as a new therapeutic point (Fig. 1).

Mitochondrial transplantation therapy

Concepts

Mitochondrial transplantation is a strategy to extract functional mitochondria from normal cells and transplant them into target tissues to treat diseases caused by mitochondrial dysfunction. This innovative procedure is derived from the documented occurrences of mitochondrial transport between cells. Under a spectrum of pathological and physiological states, it has been noted that intracellular mitochondria can engage in translocation through structures such as tunneling nanotubes (TNTs), extracellular vesicles (EVs), and gap junction channels (GJCs) [49, 50]. The trigger point for mitochondrial metastasis in pathological conditions is impairment of mitochondrial function or disruption of the cellular environment, such as ROS generation and accumulation, release of damaged mitochondria, and inflammatory responses [51, 52]. Homeostasis of the cellular environment can be maintained by mitochondrial transfer in

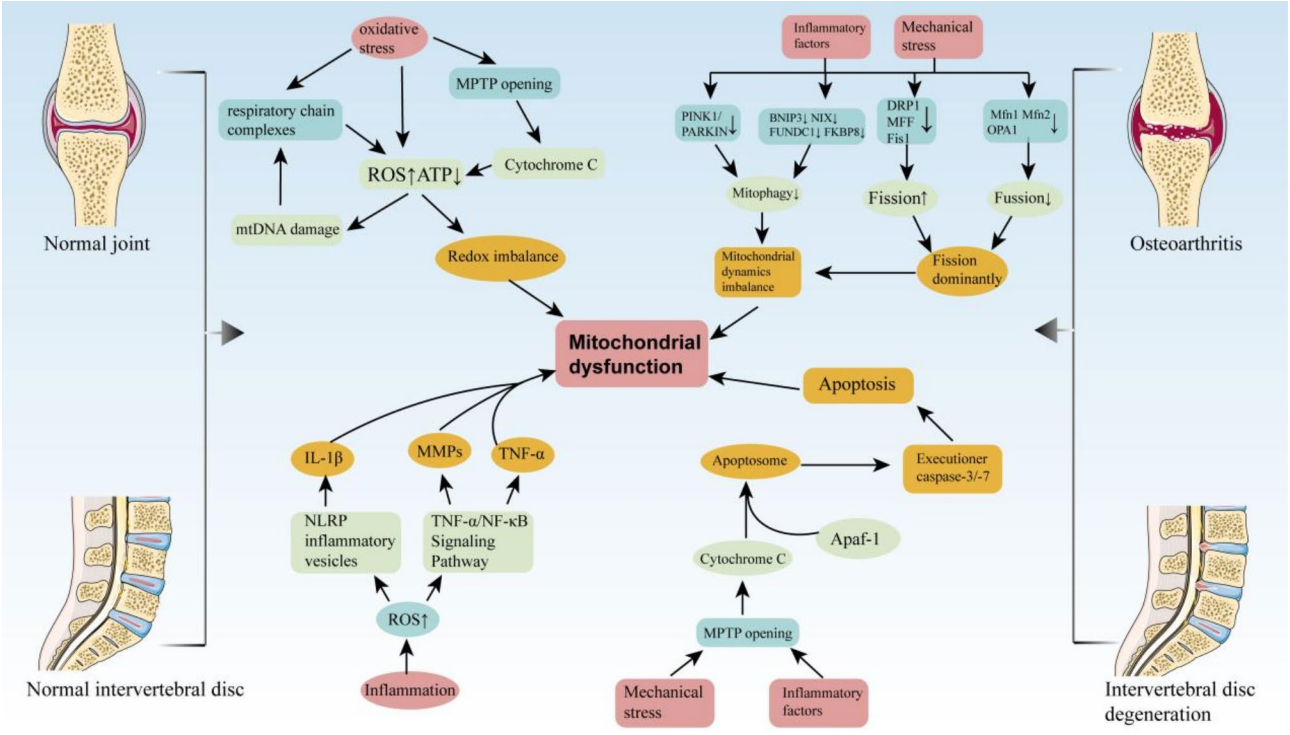

Fig. 1 Schematic representation of mitochondrial dysfunction in orthopaedic degenerative joint diseases. Excessive oxidative stress generates large amounts of ROS, which inhibit the cellular antioxidant system and promote inflammation. Stressors such as mechanical stress and inflammatory factors stimulated by stressors inhibit mitochondrial autophagy and mitochondrial dynamics, accelerate mitochondrial damage, and activate apoptosis

response to metabolism, oxidative stress, or external stress stimuli.

Intercellular mitochondrial transfer

Tunneling nanotubes

Rustom originally identified TNTs as a new type of intercellular communication channel [53]. TNTs are tubular cytoplasmic extensions of plasma membrane origin, which are bridge-like membrane structures based on fibrous actin (F-actin) with diameters ranging from 50 to 700 nm. TNTs connect the plasma and cytoplasm of different cells, enabling long-distance interconnection and communication between cells. Transportation of organelles such as mitochondria, Golgi and lysosomes and substances such as microRNAs (miRNAs), lipids and proteins can be carried out through these nanotubes [54]. Utilizing TNTs as a bridge for mitochondrial transport protects cells with mitochondrial dysfunction under pathological conditions. Hang et al. found that mesenchymal stem cells (MSCs) can establish TNTs with damaged cells and exert anti-apoptotic effects through functional mitochondrial transfer of these nanotubes to the latter [55]. Yao et al. determined that MSC can make mitochondrial transfers to neuronal cells to alleviate oxidative stress induced by iron death, and determined that the transfer channels involved are dominated by TNTs [56].

Extracellular vesicles

Extracellular vesicles are heterogeneous populations of vesicles secreted by different cell types. Through the release of EVs, donor cells can deliver cargoes such as mitochondria, proteins, and mtDNA to other cells. EVs are produced through plasma membrane vesiculation and outgrowth, and Zhou et al. defined the population of EVs in which mitochondrial components are enriched as MitoEVs [57]. Upon arrival at the recipient cell surface, MitoEVs can undergo mitochondrial translocation by direct fusion, endocytosis and other interactions [58, 59]. Mitochondria contained in MitoEVs can be categorized into three types: intact mitochondria, mitochondrial components, and free mtDNA [60], which correlate with the type of secreting cell and isolation method, among others. Mitochondrial components delivered by EVs can complement the mitochondrial network of recipient cells and play a role in regulating metabolism and maintaining cellular homeostasis [61].

Gap junctions channels

Gap junctions are specialized intercellular channels based on six connexin (Cx) adjacent hemichannels (HC), which form a hydrophilic pathway between them across two adjacent plasma membranes and the cytosolic space [62]. They are the most direct cellular channel for exchanging materials between cells, and current research suggests that mitochondria can also be transported through this channel. Current researchers believe that connexin 43 (Cx43) is required for mitochondrial transfer, and that through Cx43-GJCs, ultra-purified bone marrow mesenchymal stem cells (BMSCs) can transfer mitochondria to cells in direct contact [63]. There is also evidence that Cx43-based GJC may be associated with TNTs. Yao et al. found that overexpression or silencing of CX43 regulated the formation of TNTs, significantly affecting mitochondrial transfer between induced pluripotent stem cell (iPSC)-MSCs and epithelial cells [64]. Some researchers have employed biomaterials technology to enhance GJCs by using iron oxide nanoparticles (IONPs) to promote the formation of gap junction channels containing Cx43 and enhance intercellular mitochondrial transfer from MSC to injured cells [65].

Cell fusion

Cell fusion is the process by which two or more cells merge cell membranes and share organelles, cytoplasm and even nuclei [66]. Permanent cell fusion allows for the formation of new cells with unique nuclear structures, while partial cell fusion allows for organelle exchange and intercellular information exchange. Adrien et al. found that Human mesenchymal stem cells can facilitate the return of cardiomyocytes to a progenitor-like state through cell fusion, in which the transfer of stem cell mitochondria is required for reprogramming [67], However, in eukaryotes, cell fusion occurs rarely under normal physiological conditions and is mediated by fusogen proteins and dynamin [68, 69], so this is not a major mechanism for mitochondrial transfer.

Artificial mitochondrial transplantation

Unlike intercellular mitochondrial transfer, artificial mitochondrial transplantation (AMT) is a process that primarily involves mitochondrial isolation, delivery and internalization into target cells or tissues. The origin of the mitochondria in this context is of primary concern, for example, mitochondria in the heart, skeletal muscle and liver can differ in proteome and function [70]. MSCs, as excellent candidates in the field of cell therapy, are self-regenerating pluripotent stem cell progenitors and are a good choice for transferring functional mitochondria to myeloid and chondrocytes [71]. The use of skeletal muscle cells as a source of mitochondrial transplantation has been investigated for short-term improvement in the bioenergetics of the subject cardiomyocytes [72], and it also has the potential to be transferred into myofibroblasts and human fibroblasts with mitochondrial DNA mutations [73], which makes it one of the effective alternative sources of mitochondrial transfer. The current continuous improvement of differential centrifugation

and the construction of buffer systems for in vitro isolation of mitochondria have led to improved purification isolation and preservation of mitochondria [74, 75]. This method first removes intact cells in the tissue by low-speed centrifugation, followed by high-speed centrifugation to concentrate the mitochondria and separate them from other organelles [76]. In addition to centrifugation, commercial kits for mitochondria are also an effective measure. McCully et al. constructed an effective isolation procedure by applying filters that easily isolated mitochondria from liver and skeletal muscle tissues [77]. Centrifugation and filtration can be combined with each other to isolate mitochondria, and Adlimoghaddam et al. improved the time-consuming centrifugation step by utilizing three different direct filters combined with centrifugation to rapidly isolate purified mitochondria [78]. In vivo, mitochondrial transplantation can be performed by direct injection, intra-arterial administration, or systemic administration. In diseases with a fixed location such as degenerative joint disease, local direct injection administration is a better option [17]. After injection of mitochondria into the target site, mitochondrial internalization is largely dependent on cellular macropinocytosis. Kitani et al. demonstrated that isolated naked mitochondria could be internalized into template cells by simple co-incubation [79]. Rossi et al. found that isolated mitochondria were biologically active and could be actively internalized by renal proximal tubular cells in a dose-dependent manner [80]. Sercel et al. constructed the MitoPunch a pressure-driven device for forcible transfer of isolated mitochondria to target cells. This device improves transfer efficiency while stably retaining transplanted mtDNA [81].

In the AMT, due to the overly drastic means of separation and the dangers involved in transport, the use of mitochondrial EV as a carrier transport has been chosen as an alternative to free mitochondria [82, 83]. With the help of EV, mitochondria can be better integrated into the cell. Cell-penetrating peptides (CPPs), short polypeptides that help target molecules penetrate cell membranes, can both aid in the delivery of drugs to reach intracellular mitochondria [84] and facilitate the uptake of translocated mitochondria into the cell [85], thereby improving mitochondrial function. Despite confirming that these methods can enhance mitochondrial uptake in cells, they are currently in the early stages of research and lack assessment of safety and efficacy.

Therapeutic potential of mitochondrial transfer in degenerative joint diseases

Mitochondrial transfer in OA

As early as 2019, the first study provided evidence supporting mitochondrial transfer between MSCs and chondrocytes. Chondrocyte mitochondrial dysfunction was also found to be an important trigger for in vitro mitochondrial donation by MSCs [86]. In 2020, Wang et al. demonstrated mitochondrial translocation from BM-MSC to OA chondrocytes in vitro. By co-culturing the two types of cells, new mitochondria were brought to OA chondrocytes and mitochondrial function was improved, rescuing damaged chondrocytes from mitochondrial dysfunction and cartilage degeneration. However, this study did not address the potential mechanism of mitochondrial transfer [87]. Mechanical stress can also be a contributing factor to OA progression, and Fahey et al. found that when cartilage is mechanically injured, MSCs can migrate to the area of injury and extend cellular processes deep into the microfracture, delivering mitochondria to chondrocytes through gap junctions. Inhibition of Cx43-based signaling prevented this process [88]. Korpershoek et al. experimentally identified three mechanisms responsible for mitochondrial transport, i.e., direct cell-to-cell contact, TNT, and EV, and demonstrated that mitochondria are transferred from MSCs to target cells via these pathways for chondrogenicity in humans [89]. It was also confirmed that mitochondrial transfer occurs at 4 h from the start of co-culture. The study also confirmed that mitochondrial transfer occurs at 4–16 h from the start of the co-culture.

While these above mitochondrial transfers between cells have yielded good results, because spontaneous MT transfer is still relatively rare and not every tissue and organ exhibits strong metastatic capacity, current treatments usually rely on isolated MT transfer rather than enhancing spontaneous transfer. Some investigators have directly extracted simple mitochondria and used this as an intervention. Li et al. performed the first OA-associated exogenous artificial mitochondrial transplantation therapy by adopting an intra-articular injection of myofibroblast-derived mitochondria, which acted to maintain cellular homeostasis, regulate inflammation, inhibit ROS activity, and activate autophagy in a rat model of OA, and ultimately ameliorated OA caused by mitochondrial dysfunction [17]. The researchers also used non-contact MT transfer via MSC-derived mitoEV. They characterized MSC-derived mitoEV and determined that MT in EV can be taken up by chondrocytes without direct cell-to-cell interaction [90]. More dysfunctional MTs were found in mitoEV after the use of mitochondrial inhibitors, suggesting that stressful situations MSC release unhealthy MTs by this means. After treatment with mitoprotective peptides, MSC similarly increased the output of functional MTs in mitoEV. However, mitoEV-mediated transfer was found to be less efficient than direct MT transfer in their study, which may be related to the experimental design including incubation time and MV number [88]. Yu et al. showed that BMSCs-derived microvesicles better enhanced chondrocyte mitochondrial

function compared to exosomes, probably because BMSCs-derived microvesicles can carry functional mitochondria. Microvesicles were stronger than equivalent doses of mitochondria in improving IL-1β-induced mitochondrial dysfunction [12]. Kim et al. chose to address the problem of less efficient MT uptake by encapsulating MT in fusion liposomes and delivering it into chondrocytes via membrane fusion. Through in vitro and in vivo experiments, it was shown that the membrane fusion approach provided a more rapid and safe transfer of MT compared to the endocytosis route [91].

Mitochondrial transfer in IVDD

Hu et al. showed that BMSCs could effectively reduce the apoptosis rate of NPC after inflammatory stimulation through TNTs-mediated mitochondrial translocation and paracrine effects. In this process, they found that the mitochondrial directional transfer may be induced by IL-1β stimulation [92]. However, it was suggested that the paracrine mechanism may play a major role in this protective process. The study of Chen et al. also came to the same conclusion, but they clarified that the mitochondrial pathway plays an important role in preventing compression-induced apoptosis of NPCs [93]. The study by Yang et al. directly clarifies a novel mechanism by which BMSC rescues damaged NPC, namely TNT-mediated mitochondrial transfer. Following mitochondrial dysfunction in NPC, NPC acquired mitochondria from BMSC in a concentration-dependent manner. These transferred functional mitochondria promoted the restoration of the mitochondrial respiratory chain and increased mitochondrial membrane potential in NPC cells, while decreasing ROS levels and apoptosis rates [94]. Chen et al. found that MSC-derived exosomes contain mitochondrial proteins that can provide these complementary substances to NPCs to restore damaged mitochondria and play a role in suppressing oxidative stress and inflammatory responses [95]. However, these studies on IVDD are currently focused on in vitro and intercellular transfer, with little progress on in vivo disease modeling and artificial mitochondrial transplantation (Fig. 2). (Table 1).

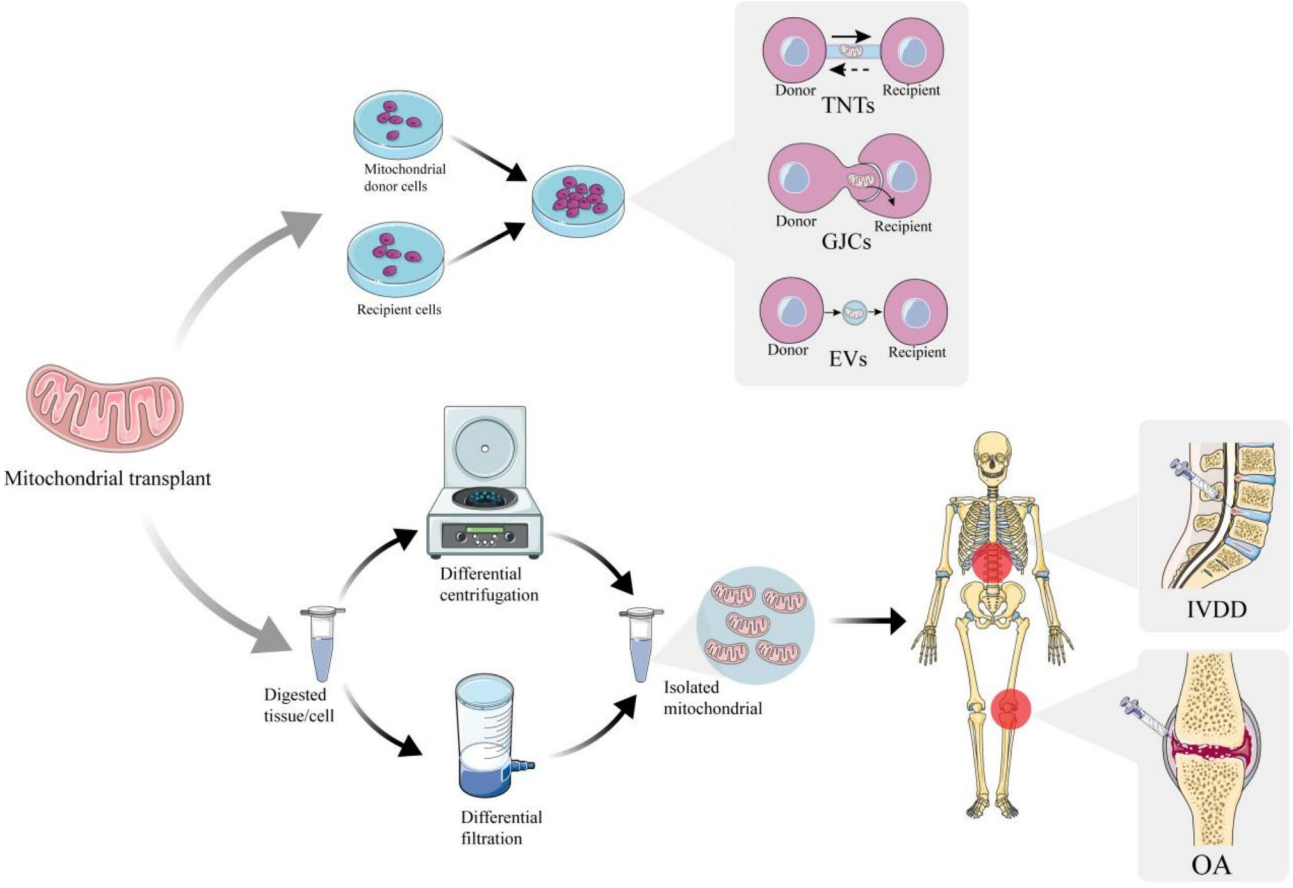

Fig. 2 Natural mitochondrial transfer and artificial mitochondrial transplantation for the treatment of IVDD and OA. natural mitochondria are transferred via intercellular TNTs, GJCs, and EVs, and artificial mitochondrial transplantation undergoes mitochondrial segregation and transport for eventual internalisation into the target cells

Table 1 Research reports of mitochondrial transplantation for degenerative joint diseases

Mitochondrial source	Recipient	Condition	Mechanism	Therapeutic outcome	References
Horse MSCs	Horse Chondrocytes	In vitro	/	Demonstrate the presence of mitochondrial transfer	[86]
Rat BM-MSCs	rats chondrocytes	In vitro	/	Improving mitochondrial function, cell proliferation, and inhibiting apoptosis in chondrocytes	[87]
Murine MSCs	Murine chondrocytes	In vitro	gap-junction	MSC transfers mitochondria to stressed chondrocytes	[88]
hMSCs	Human Chondrocytes	In vitro	TNTs, cell-cell contact and EVs	Treatment of mitochondrial dysfunction and subsequent ROS accumulation	[89]
L6 cells human muscle Cells	Rat and human chondrocytes	In vitro and in vivo	Mitochondrial transplantation	Reduced transcript levels of IL-1β, TNF-α, matrix metallopeptidase 13 and MCP-1 in cartilage	[17]
Murine BMSCs hBMSCs	Murine articular chondrocytes	In vitro	EVs	MSC-EV contains functional mitochondria and can be taken up by chondrocytes	[90]
L6 cells hBMSCs	C28/I2 cell	In vitro and in vivo	Mitochondrial transplantation	Increased cartilage expression and decreased inflammatory cytokine expression	[91]
Rat BMSCs	Rat NPC	In vitro	TNTs	Amelioration of mitochondrial dysfunction and apoptosis	[94]
C57BL/6 BMSCs	C57BL/6 NPC	In vitro	EVs	Inhibition of inflammatory mediators and activation of NLRP3 inflammasome	[95]

The potential for engineered mitochondrial transplantation

Mitochondrial grafts alone are susceptible to damage during extraction and preservation, and relying on endocytosis for uptake may not meet the efficiency expectations raised by the researchers. In view of these shortcomings of naked mitochondrial transplantation, more and more studies have been devoted to the development of engineered mitochondrial interventions, which we categorize as endogenous and exogenous interventions.

Endogenous interventions

Employing an endogenous approach, parental cell-based engineering techniques are utilized to modulate the source cell through diverse methodologies prior to mitochondrial isolation. This aims to enhance the yield and functional integrity of the harvested mitochondria, and it encompasses the production of mitochondria with desirable characteristics via genetic engineering. Sun et al. used Alda-1 to activate mitochondrial aldehyde dehydrogenase 2 (ALDH2) in rat cardiomyoblast cells. The mitochondria obtained through this intervention were more viable than the original mitochondria, and the mitochondrial membrane potential mediated fusion was more efficient. At the same time, the mitochondria were more viable after transplantation, ultimately significantly enhancing the therapeutic effect on myocardial ischemia/reperfusion (I/R) injury [96]. Huang et al. treated mesenchymal stem cells with pioglitazone (Pg) and iron oxide nanoparticles (IONP), and the intervention promoted mitochondrial biogenesis in the cells, ultimately increasing the quality and quantity of mitochondria [97]. There are also means such as herbal monomers [98] that can improve mitochondrial biogenesis to optimize intracellular mitochondria, but whether treated mitochondria can achieve greater gains in mitochondrial transplantation needs to be followed up.

In the context of mitochondrial transplantation, the integration of mtDNA into recipient cells is a critical aspect. One study used MitoPunch to deliver mitochondria containing mtDNA with a chloramphenicol resistance point mutation; the transferred mtDNA replaced the endogenous mutant mtDNA and improved respiration in metabolically impaired cells [99]. There are many tools available to intervene with mtDNA such as Mitochondrial-targeted restriction endonucleases (mitoREs), transcription activator-like effector nuclease(TALEN) and Zinc-finger nucleases(ZFN) [100, 101]. It raises an intriguing prospect whether these gene-editing technologies could be employed to modify donor cell mitochondria before isolating the specifically engineered organelles for transplantation. However, this concept is not without challenges; editing efficiency, potential off-target mitochondrial effects, and the ethical implications of genetic manipulation are all areas requiring thorough investigation.

Exogenous interventions

Bioengineering techniques and chemical modifications

The core problem of mitochondrial transplantation is how to deliver it to a specific tissue or organ, and bioengineering techniques can provide a promising solution. Triphenylphosphine cation (TPP+) and its derivatives have been used to target mitochondria for drug delivery [102, 103], and researchers are now applying them to mitochondrial transplantation. Xu et al. modified mitochondria with TPP-(cysteine- alanine-glutamine-lysine)

(TPP-CAQK), which markedly improved its targeting affinity [104]. Using an intravenous route of transport, the modified mitochondria were specifically targeted to the site of spinal cord injury and were taken up by target macrophages to exert protective effects. A similar mitochondrial design is found in myocardial ischemia. Because of the dangers of local delivery of drugs to the myocardium, the choice has been made to use TPP+in combination with the specific peptide CSTSMLKAC (PEP) to modify mitochondria, taking the form of an intravenous injection, where mitochondrial complexes in the bloodstream can be delivered to the injured myocardium in the presence of the targeted peptide [105]. Mitochondria modified by a polymer of dextran (Dex) and TPP (Dex-TPP) were internalized 3-fold more than naked mitochondria in various cell lines [106, 107]. Liposomes have also been used in mitochondrial modification, with one study utilizing artificial cations and lipids to encapsulate mitochondria, which was able to reduce the exposure of mitochondrial components and reduce the resulting potential for damage. Another study used membrane fusion as a strategy, employing a variety of liposomes bound to mitochondria to form fusion mitochondrial capsules (FMCs), and determined that they had higher stability and membrane fusion efficiency, with greater improvements in safety and delivery rates [91]. There are currently other means of modifying isolated mitochondria such as cationized gelatin nanospheres (cGNS) [108], dextran [109], and Pep-1 [110]. The safety, accuracy and efficiency of mitochondrial transplantation have been significantly improved by these specific bioengineering tools.

Physical interventions

Superior mitochondrial quality and transplantation outcomes can be achieved by applying physical interventions. Photobiomodulation (PBM) has been observed to modulate mitochondrial membrane potential, attenuate ROS, and augment ATP synthesis [111]. One study has used PBM in a rat model of spinal cord injury (SCI). Zhu et al. isolated mitochondria from rat platelets, and then used 810 nm laser irradiation as an intervening factor in both in vivo and in vitro experiments. The combination of laser light and mitochondria was found to promote neuronal uptake of exogenous mitochondria, a process in which connexin 36, a specific connexin involved in message exchange between neurons, plays a major role [112]. Another study used ultrasound to treat platelets, which maximized the release of mitochondria from platelets in contrast to thrombin, repeated freezing and thawing, and photoactivation, and the mitochondria obtained were smoothly taken up by endothelial cells and performed their functions [113]. The introduction of exogenous plasmid DNA into isolated mitochondria by electroporation has been reported as a possible new therapeutic approach for mitochondrial diseases, but the ability of the modified mitochondria to be taken up by the cells has not been studied more thoroughly [114, 115]. In addition, physical means such as magnetic fields and freeze-thaw were determined to act on intracellular mitochondria, but the effects on isolated mitochondria are still being further explored [116, 117].

In summation, mitochondrial engineering is implemented to (1) increase targeted concentration, (2) safeguard mitochondrial integrity, (3) amplify mitochondrial functionality, and (4) optimize cellular uptake of mitochondria (Fig. 3).

Conclusion and perspectives

In the realm of regenerative medicine, mitochondrial transplantation is emerging as a promising therapeutic approach for addressing mitochondrial-related degenerative joint conditions. The pathophysiologic processes associated with mitochondrial dysfunction in IVDD and OA can be alleviated by transplanting new mitochondria. However, direct mitochondrial transplantation encounters challenges, including immune rejection, reduced activity, and suboptimal transplantation efficiency. In response to these shortcomings, the use of engineered mitochondrial interventions to improve their performance is anticipated to become an important area of future research. Advancements in bioengineering and chemical modification techniques have shown promise in augmenting mitochondrial targeting and protecting these organelles in various extracellular environments; the use of tools such as MitoPunch can significantly improve the throughput and efficiency of mitochondrial transplantation. Future research efforts should focus on establishing more standardized engineered mitochondrial transplants that utilize bioengineering and other techniques to modify mitochondria in order to increase the efficiency of mitochondrial transplants while maximizing their efficacy.

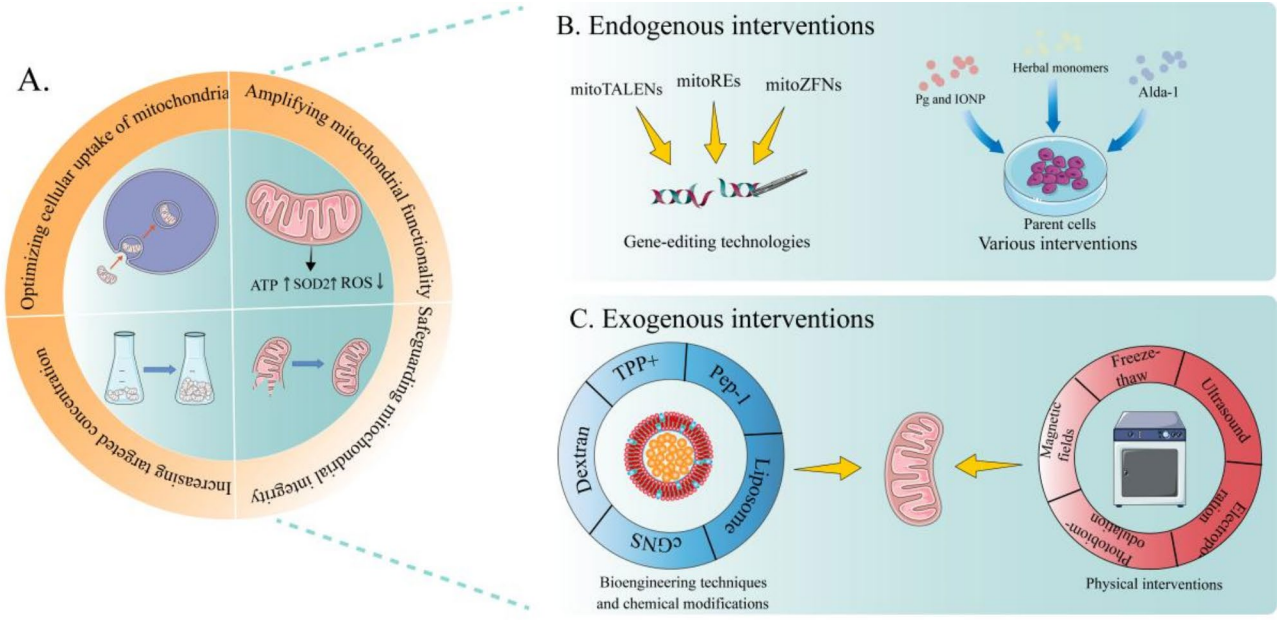

Fig. 3 Schematic diagram of engineered mitochondrial transplantation. **A**. By engineering mitochondria, the target concentration can be increased, (2) mitochondrial integrity can be safeguarded, (3) mitochondrial function can be enhanced, and (4) cellular uptake of mitochondria can be optimised. **B**. Endogenous interventions, an engineering approach based on parental cells using gene editing techniques and other interventions, to obtain mitochondria of higher quality. **C**. Exogenous interventions, the Methods of intervention against isolated mitochondria, including bioengineering techniques with chemical modifications, and physical interventions

Abbreviations

AMT	Artificial mitochondrial transplantation
ATP	Adenosine triphosphate
BNIP3	BCL2-interacting protein 3
Drp1	Dynamin-related protein 1
ECM	Extracellular matrix
EVs	Extracellular vesicles
Fis1	Mitochondrial fission 1 protein
FKBP8	FK506-binding protein
FUNDC1	FUN14-domain containing protein 1
GJCs	Gap junction channels
IL-1β	Interleukin-1 beta
IVDD	Intervertebral disc degeneration
MCP-1	Monocyte chemotactic protein-1
MFF	Mitochondrial fission factor
Mfn1	Mitofusin 1
Mfn2	Mitofusin 2
MIP-1α	Macrophage inflammatory protein-1 alpha
MitoQ	Mitoquone
MMPs	Matrix metalloproteinases
MPTP	Mitochondrial permeability transition pore
MSCs	Mesenchymal stem cells
mtDNA	Mitochondrial DNA
NIX	Nip3-like protein X
NLRP	NOD-like receptor family, pyrin domain-containing protein
NO	Nitric oxide
NPCs	Nucleus pulposus cells
OA	Osteoarthritis
OPA1	Optic atrophy 1
PGE2	Prostaglandin E2
PINK1	PTEN-induced putative kinase
ROS	Reactive oxygen species
SCI	Spinal cord injury
SIRT3	Sirtuin-3
SOD2	Superoxide dismutase 2
TNF-α	Tumor necrosis factor-alpha
TNTs	Tunneling nanotubes

Authors contributions

Hong Luo: Writing – review & editing, Writing – original draft. **Yue Lai**: Writing – review & editing, Supervision, Funding acquisition.**Weili Tang**: Writing – review & editing, Supervision. **Guoyou Wang**: Conceptualization, Supervision, Funding acquisition. **Jianlin Shen**: Writing – review & editing, Writing – original draft, Conceptualization. **Huan Liu**: Writing – review & editing, Writing – original draft, Conceptualization.

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Availability of supporting data

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Data availability

No data was used for the research described in the article.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

All authors concur with publishing the study at present version. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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