The Role of Mitochondria in Cartilage Degenerative Disorders

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ABSTRACT

Mitochondria are major organelles that produce energy for cellular metabolism and are vital to proper cell functioning. Metabolic dysregulation is a critical contributing factor to the initiation and development of degenerative and autoimmune diseases, thus is present in cartilage disorders such as osteoarthritis and rheumatoid arthritis. Mitochondrial dysfunction in cartilage diseases is especially interesting because it may present a promising therapeutic and anti-ageing target. By understanding the mechanism of mitochondrial dysfunction, we can aim to upregulate the role of mitochondria in treating cartilage diseases. In this review, we look at the role of mitochondrial dysfunction in degenerative cartilage diseases and mitochondria as tools in the treatment of osteoarthritis and rheumatoid arthritis.

Keywords
Regenerative medicine, Cartilage diseases, Joint degeneration, Mitochondria, Mitochondrial dysfunction.

Introduction to Mitochondria Biology
The mitochondrion is an intracellular organelle in most eukaryotic cells that carries out many essential cellular functions. Mitochondria are the main location for adenosine triphosphate (ATP) production in cells and are thus known as the “power plant” of the cell. The
inner mitochondrial membrane is presented by cristae, which provide a high surface area for housing the protein complexes of the electron transport chain and ATPase, thereby controlling cellular metabolism. Several substances (e.g., pyruvate, glutamine, and fatty acids) enter the tricarboxylic acid cycle in the mitochondrial matrix to generate the electron carriers and transfer their electrons to the electron transport chain embedded in the inner mitochondrial membrane, in a process known as oxidative phosphorylation. This oxidative phosphorylation pathway generates about 90% of cellular ATP in mitochondria [1].

In addition to their primary function, mitochondria play several additional crucial roles. Mitochondria operate as a central hub of both catabolic and anabolic reactions. Acetyl coenzyme A (acetyl-CoA) is condensed with oxaloacetate by citrate synthase in the mitochondria, generating citrate and free CoA. Mitochondria are also involved in heme biosynthesis, indispensable for cellular respiration, energy metabolism, and cell survival. Mitochondria alter their bioenergetic and biosynthetic functions to meet the cell’s metabolic demands and continuously communicate their functionality to the rest of the cell [2]. ATP production requires a continuous flow of electrons through oxidative phosphorylation. As such, mitochondria are the primary source of reactive oxygen species (ROS), including superoxide and H$_2$O$_2$. ROS reflect the degree of cellular oxidative stress, causing severe damage to macromolecules when overproduced [3].

Each mitochondrion has its genome. Many of the mitochondrial DNA (mtDNA) genes encode proteins, while the others are involved in the proteins translation. Such proteins encoded by mtDNA are vital for healthy cellular function. However, the mitochondrial genome is susceptible to oxidative damage. As a result, such injury to the mitochondrial genome has been linked to cell apoptosis, tissue degeneration and other age-related disorders [4].

Conversely, ageing and degenerative diseases are associated with an elevated oxidant state that may cause mitochondrial damage. Several pathologies in this category share the common features of mitochondrial Ca$^{2+}$, ATP, or ROS metabolism disturbances. Some of these disorders include chronic autoimmune diseases (e.g., rheumatoid arthritis) and articular cartilage degenerative diseases (e.g., osteoarthritis). Thus, therapeutic interventions with mitochondria could be effective tool for cartilage diseases [5,6].

In essence, mitochondria are important targets for the treatment strategies. They represent an attractive target for mitochondrial therapy and for treating a range of age-related degenerative diseases.

**Mitochondria as a Therapeutic Target**

The modern approach to restore the mitochondria’s structure and functions include small molecule drugs, biologically active mitochondrial proteins and peptides, and stem cell therapy. The mitochondrial function relies on the electron transport chain to build polarization across the inner membrane, which in turn drives energy production [7]. Mitochondrial dysfunction triggers the cell senescence and death-signalling cascade and results in ageing, organ failure and disease. The functioning of the mitochondrial permeability transition pore regulates the maintenance of the potential of the mitochondrial membrane [8]. Its abnormal opening induces the collapse of the mitochondrial membrane potential and the release of cytochrome c, an apoptotic factor, into the cytosol. A widely used strategy for targeting mitochondria demonstrates the advantage of this remarkable biophysical membrane property: the ability of cationic molecules to attract to and accumulate preferentially within the negatively charged mitochondrial matrix.

Another strategy is based on the affinity of an agent to mitochondrial membrane components. Small molecule drugs or biologically active substances can act on mitochondria through various pathways [9]. These mechanisms include electron transport chain inhibition, uncoupling of oxidative phosphorylation, mitochondrial Ca$^{2+}$ modulation, and oxidative stress control via decrease or increase of mitochondrial ROS accumulation. Other strategies for drug-induced perturbation of mitochondrial biochemistry include the inhibition of peroxidation of the mitochondria-specific phospholipid cardiolipin and targeting other specific mitochondrial proteins [10].

Mitochondrial biochemistry can be damaged by mtDNA binding/oxidation, inhibition of mtDNA synthesis, or modulation of mitochondrial fission/fusion. Recently, compounds that modulate mitochondrial fission/fusion have been proposed as valuable alternatives in treating degenerative diseases [11].

An emerging approach to the selective delivery of bioactive molecules into mitochondria involves using a carrier of short peptide sequences with specific chemical properties. For instance, Horton et al. reported mitochondria-penetrating peptides with alternating cationic and hydrophobic residues [12]. Other types are based on an oligomeric carbohydrate scaffold [13]. The vesicle-based transporter system is another class of mitochondrial delivery vectors that is suitable for importing large or impermeable molecules [13]. The active substance is encapsulated in a liposome and internalized by the cell, and subsequently fuses with the outer mitochondrial membrane.

Another technology to regulate the mitochondrial structure is using of the peptide- and amino acid-based mitochondria-targeted agents. Glutathione plays an important role in protecting cells against oxidants. Two-glutathione molecules react together to form glutathione disulfide after donating an electron to unstable molecules such as ROS. Increasing mitochondrial glutathione can be an effective strategy to prevent mitochondrial oxidative stress. Sheu et al. used a similar approach and prepared choline esters of glutathione and its analogue N-acetyl-L-cysteine for targeting mitochondria [14].

Mitochondrial function and properties depend on the cell type. Thus, a better understanding of mitochondrial properties is of interest to researchers and clinicians, particularly, stem cells for use in anti-
In 2013, Chang and colleagues conjugated isolated mitochondria with chemical compounds and physical methods have been performed on the recipient cells. Assays to optimize transfers involving TNTs is not fully understood. Therefore, it is important to define the directionality of the transport of mitochondria through the activating cell metabolic reprogramming. Mitochondrial transfer between cells, repairing cell damage, and producing TNTs and receiving mitochondria and other intracellular to-cell interaction. Since then, many groups have studied the cells mitochondria after exposure to stress conditions or when their mitochondria after exposure to stress conditions or when keeping them becomes harmful since mitochondria can produce large quantities of ROS.

Mitochondria as the Tool of the Therapy
Despite mtDNA encoding about 60 proteins, mitochondria need around 2000 proteins for their normal function. Most such proteins are synthesized in the cytoplasm through ribosomes encoded in the nucleus. This independence makes them relatively independent from the mitochondrial genome [16]. The nuclear/mitochondrial gene interactions are vital for the translation of proteins and mitochondria respiration. Cells tend to dispose of their mitochondria after exposure to stress conditions or when keeping them becomes harmful since mitochondria can produce large quantities of ROS [17].

Mitochondrial Transfer
Extracellular vesicles (EVs) transport intracellular cargo to other cells, including transporting different substances to mitochondria. EVs are structures surrounded by a lipid bilayer membrane [18] capable of transporting proteins, lipids, carbohydrates, metabolites, small RNAs, and mtDNA. Exosomes are EVs with the greatest size (30-100 nm diameter) [19]. Exosomes could transport mRNA and microRNA to the recipient cells and, therefore, control activities in such cells [20]. Guescini et al. also observed the delivery of mtDNA by exosomes [21].

The mitochondria’s small size and capacity to change shape and length allow them to be transported by subcellular transport mechanisms such as tunnelling nanotubes (TNTs) and EVs. Rustom et al. [22] described TNTs as structures that enable cell-to-cell interaction. Since then, many groups have studied the cells producing TNTs and receiving mitochondria and other intracellular cargo [22]. These studies prove that TNTs are involved in mitochondrial transport between cells, repairing cell damage, and activating cell metabolic reprogramming. The directionality of the transport of mitochondria through the TNTs is not fully understood. Therefore, it is important to define what factors promote the donation of material and their effects on the recipient cells. Assays to optimize transfers involving chemical compounds and physical methods have been performed. In 2013, Chang and colleagues conjugated isolated mitochondria with penetrating peptides to foster their internalization [23]. They used Pep-1, a cell-penetrating peptide, which was developed to make pores in the membrane to deliver the molecules (e.g., oligonucleotides) into the cell. The scientists adapted Pep-1 to conjugate it with isolated mitochondria of human osteosarcoma 143B cells. The combination of Pep-1 and the isolated mitochondria promoted their internalization by fibroblasts involved in a mitochondrial disease myoclonic epilepsy model with ragged red fiber syndrome. As a result, the Pep-1-mediated transfer was more successful in facilitating the internalization of mitochondria than mitochondria alone [23].

The process of mitochondrial transfer allows the re-use of the subcellular organelles of the damaged cells in other cells; this phenomenon is called transmitophagy [24]. The reason for transmitophagy is currently unknown. Understanding the natural transfer of mitochondria by microvesicles and transmitophagy will allow us to find new therapeutic options, which could mediate the recovery of cellular homeostasis and function in degenerative diseases.

Mitochondrial organ and tissue specificity should be considered in the donor mitochondria selection. Mitochondria have different shapes, sizes, energy production potential, and metabolic processes among cell types and stages of differentiation. During cell proliferation, the mitochondria segregate and fuse with other mitochondria [25]. Mitochondria isolated in a specific state, such as proliferation, have a more potent influence on recipient cellular mitochondria networks and are prone to fuse with endogenous mitochondria. Most transfer procedure studies were performed using mitochondria from differentiated cells such as fibroblasts, liver cells and mesenchymal stem cells (MSCs) [26]. Testing these cells at their different states will be essential to understanding how exogenous mitochondria interact with the endogenous organelles, how the cell's phenotype changes after the transfer, and whether metabolic reprogramming is possible.

Most work in mitochondrial delivery between cells involves rescuing damaged cells from healthy ones, such as human MSCs [25]. Some studies have linked such transfer processes to the enhanced immune response of MSCs to macrophages, which is just one example of this mechanism's diverse effects on cells involved in the transfer. MSCs are the optimal cells to transfer mitochondria in bone and cartilage diseases [27], autoimmunity, cancer, and excessive proinflammatory events in sepsis or viral infections [28].

Artificial Mitochondrial Transfer
Considering the symbiotic nature of mitochondria and the cell’s capacity to transfer mitochondria to damaged neighbours, many researchers have developed procedures for artificial mitochondria transfer from one cell to another. The techniques range from simple coincubation of isolated mitochondria and recipient cells to physical approaches to induce integration. For example, through coincubation, Clark and Shay completed the first formal mitochondrial transfer from one xenogeneic cell to another in 1982.
growth etc. Once inside the cell, exogenous mitochondria begin specific signal pathways to induce a response of cell proliferation, cytokines, and other cell components require the activation of or coupled proteins to induce their effects. In contrast, mtRNAs, MSCs use mitochondria because they are independent of receptors to increase [34].

DNA, the generation of ATP synthase, and the rate of respiration of their pluripotency, mitochondria proliferate and the quantity of anaerobic metabolism. However, through differentiation and loss are dormant and immature since they are small and favor differentiation. It has been suggested that stem cell mitochondria mitochondrial transport has shown promising results in healing damaged or stressed cells in vitro and in vivo. Clarification of their mechanisms of action within the cell will allow us to explore and mix artificial mitochondrial transport with other techniques to repair dysfunctional chondrocyte mitochondria.

Mitochondrial Fusion
In 2007, Yoon et al. observed that mitochondria from different species have the ability to fuse with polyethylene glycol [32]. They labelled human and mice mitochondria differently and observed a mix of the two types of mitochondria 45 min after adding the polyethylene glycol and the fusion of mitochondria into hybrids at 4 hours. The fusion of human and animal mitochondria appeared to occur due to the nucleotide similarity between the proteins responsible for this process.

In 2012, Elliott et al. co-incubated mitochondria isolated from immortalized breast epithelial cells with their malignant counterparts. They observed decreased proliferative potential and a higher sensitivity to chemotherapeutic drugs. Remarkably, they also observed that only isolated mitochondria from the immortalized breast epithelial cells, but not the original epithelial cells, could enter the breast cancer cells [33].

Stem Cell Mitochondria
Cells and mitochondria transform during the process of differentiation. It has been suggested that stem cell mitochondria are dormant and immature since they are small and favor anaerobic metabolism. However, through differentiation and loss of their pluripotency, mitochondria proliferate and the quantity of DNA, the generation of ATP synthase, and the rate of respiration increase [34].

MSCs use mitochondria because they are independent of receptors or coupled proteins to induce their effects. In contrast, mtRNAs, cytokines, and other cell components require the activation of specific signal pathways to induce a response of cell proliferation, growth etc. Once inside the cell, exogenous mitochondria begin to fuse with other mitochondria. These mechanisms make the transport of mitochondria through TNTs or vesicles important to their protection and ensure their integrity and stability [35]. Mitochondrium to mitochondrion DNA transfer is one of the fundamental biological processes that could restore its important functions. The pioneering studies of the xenogeneic cell transfer of mitochondria open new horizons for stem cell medicine.

Mitochondrial Dysfunction in Cartilage Diseases
Cartilage diseases represent one of the most common clinical conditions that cause age-related disability and affect patients' quality of life. Most diseases involving cartilage lead to dramatic degenerative changes in the chondrocyte mitochondria, which can govern disease progression.

Osteoarthritis is a chronic degenerative age-related disease that causes progressive articular cartilage degeneration. Rheumatoid arthritis is a chronic inflammatory and autoimmune disorder caused by a complex interplay of different immune and non-immune cell types. Both osteoarthritis and rheumatoid arthritis result in severe cartilage cell damage that affects multiple joints in the body [36].

Metabolic dysregulation is a critical contributing factor to the initiation and development of autoimmunity disease. Many recent studies have focused on the metabolic processes in inflammatory diseases [37]. Mitochondrial dysfunction is well documented in cartilage diseases, including osteoarthritis and rheumatoid arthritis [38] and has been observed in other diseases [39,40]. Since mitochondria contribute to intra- and inter-cellular signalling through the actions of proteins, DNA, lipids, metabolites, and ROS, mitochondrial components are capable of directly activating the immune system.

Mitochondrial Dysfunction in Osteoarthritis
Mitochondrial dysfunction plays a central role in initiating post-traumatic osteoarthritis [38]. Evidence from ex vivo chondral injury models suggests that in cartilage, as in other tissues, mitochondria may act as intracellular mechanotransducers via strain-activated release of ROS [41]. Chondrocyte compression distorts the mitochondrial network, and chondrocyte cytoskeleton dissolution prevents elevated ROS and cell death in injured cartilage. Increased intracellular calcium leads to mitochondrial depolarization, activation of the caspase cascade and chondrocyte apoptosis. Mitochondrial respiratory dysfunction is identified in the acute phase (within 2 hours) after cartilage impact injury [42].

Several in vivo models have assessed mitochondrial function in the sub-acute to early chronic stages after cartilage insult. In animals, chondrocyte respiratory function stays impaired for four weeks after surgical destabilization of the medial meniscus in rabbits [43]. Similarly, in a mouse destabilization of the medial meniscus model, mitochondrial superoxide overproduction occurred in knee cartilage for two weeks postoperatively [44]. Chondrocytes isolated from superoxide dismutase-2 deficient cartilage displayed mitochondrial depolarization, decreased mitochondrial respiratory function, and swollen mitochondria with disrupted cristae structure.
Osteoarthritis is also associated with the age-related decreased numbers of mitochondria and deficiencies in the metabolic biosensors adenosine monophosphate-activated protein kinase and sirtuin-1, which regulate mitochondrial biogenesis [45]. In addition, mitochondria-associated disease pathways are linked to chronic stages of the disease, including decreased synthesis of collagen and proteoglycans and pathologic calcification of cartilage. Furthermore, mutations of mtDNA affecting mitochondrial function are associated with an increased incidence of knee osteoarthritis [46].

Mitochondrial Dysfunction in Rheumatoid Arthritis
Mitochondrial dysfunction also plays a role in rheumatoid arthritis, as dysregulation of immune signalling pathways causes local inflammation in the synovial joint and systemic complications such as an increased risk of cardiovascular diseases. Mitochondrial products have systemic and local functions and additionally are involved in the pathogenesis of rheumatoid arthritis by promoting synovitis via mtDNA in joints [47]. Rheumatoid factor, anti-citrullinated protein antibodies and other autoantibodies are found in many patients with rheumatoid arthritis and are associated with more severe disease [48].

Due to their multiple effects on the cell, mitochondria contribute to disease pathogenesis via metabolic actions and directly affect signalling pathways. The functions of mitochondria in different cell types in rheumatoid arthritis are well known [37]. Mitochondria facilitate immune cell activation and the production of inflammatory mediators, which have mostly been studied in T cells, macrophages, and fibroblasts. Additionally, there is evidence for mitochondrial regulation of endothelial cells, osteoclasts, neutrophils and chondrocytes in arthritis [49].

Mitochondria also have a well-established role in the process of apoptosis. Apoptosis is important for the control of synovial hyperplasia in rheumatoid arthritis, as both extrinsic and intrinsic processes can trigger it. It occurs intrinsically in mitochondria due to oxidative stress, whereas extrinsic routes are inactive in the fibroblast-like synoviocytes of patients with rheumatoid arthritis.

Mitochondrial therapy, especially tissue-specific mitochondrial-based therapy, is becoming an encouraging approach to regenerative medicine. A recently published review demonstrated the growing interest in stem cell-based bioregenerative therapy conducting the regeneration of new chondrocytes [50]. Nalapko et al. (2022) state that multiple preclinical and clinical trials proved that MSCs and chondrocyte stem cell-based agents are potentially useful regenerative technologies for patients with osteoarthritis [50].

Conclusion
The link between the age-related mitochondrial dysfunction and inflammation/tissue damage in skeletal joint pathologies is emerging in cartilage diseases such as osteoarthritis and rheumatoid arthritis. Increasing evidence suggests that this dysfunction occurs early and causally in disease pathogenesis as the accumulation of mitochondrial damage resulting from compromised quality control pathways leads to the development of cartilage damage. By understanding the mechanism of mitochondrial dysfunction, we can aim to upregulate the role of mitochondria in treating cartilage diseases, and new therapeutic anti-ageing paradigms can be developed to restore cartilage integrity.

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References