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Trends in Cell Biology

Focus issue: Mitochondrial Transfer

Review

# Mitochondrial transfer in endothelial cells and vascular health

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**Mitochondria play a vital role in cellular energy metabolism and vascular health, with their function directly influencing endothelial cell (EC) bioenergetics and integrity. Mitochondrial transfer has emerged as a key mechanism of intercellular communication, impacting angiogenesis, tissue repair, and cellular homeostasis. This review highlights recent findings on mitochondrial transfer, including natural mechanisms – such as tunneling nanotubes (TNTs) and extracellular vesicles (EVs) – and artificial approaches like mitochondrial transplantation. These processes enhance EC function and support vascularization under pathological conditions, including ischemia. While early clinical trials demonstrate therapeutic potential, challenges such as mitochondrial instability and scaling host-derived mitochondria persist. Continued research is essential to optimize mitochondrial transfer and advance its application as a therapeutic strategy for restoring vascular health.**

## Highlights

Mitochondrial transfer is a newly recognized form of intercellular communication that supports endothelial function and vascular health.

Mitochondrial dysfunction drives endothelial pathology, contributing to impaired vascular function, inflammation, and metabolic dysregulation.

Endothelial cells acquire mitochondria via tunneling nanotubes (TNTs) and extracellular vesicles (EVs), enhancing resilience under stress and promoting vascular repair.

Beyond energy production, mitochondria regulate reactive oxygen species (ROS) signaling, immune responses, and mechanotransduction, which are critical for endothelial integrity.

Mitochondrial transplantation is emerging as a therapeutic strategy to counteract vascular aging, ischemia, and inflammation.

Challenges remain in optimizing mitochondrial delivery, ensuring functional integration, and translating mitochondrial therapies into clinical practice.

## Mitochondrial transfer in vascular biology

Mitochondrial transfer has recently emerged as a critical mechanism of intercellular communication, influencing both physiological and regenerative processes across various organ systems [1]. In particular, mitochondria can be transferred between cells via natural mechanisms such as TNTs and EVs [2]. In addition, artificial approaches such as mitochondrial transplantation have been explored, with promising therapeutic applications, such as improving cardiac function in clinical settings [3]. Both natural and artificial mitochondrial transfer have been implicated in key cellular functions, including energy metabolism, tissue repair, and cellular homeostasis [4–9]. However, the precise mechanisms and broader physiological roles of mitochondrial transfer remain under investigation, particularly *in vivo*.

While mitochondrial physiology is distinctive across tissues, mitochondria play a central role in EC function and vascular health [10]. ECs are highly reliant on mitochondrial signaling pathways for maintaining homeostasis and responding to mechanical and metabolic cues [11,12]. Mitochondrial dysfunction in ECs has been directly linked to a range of vascular diseases, with deficiencies in mitochondrial genes impairing endothelial function and accelerating disease progression (Figure 1) [13–27]. For instance, 16p11.2 deletion autism syndrome has been associated with reduced PGC-1α expression and impaired mitochondrial biogenesis in 16p11.2 haplodeficient ECs, leading to diminished brain angiogenesis and altered glucose metabolism [13]. Additionally, UCP2 deficiency has been shown to promote the expression of proinflammatory and profibrotic genes, contributing to endothelial inflammation and atherosclerosis [16]. Beyond energy production, mitochondria regulate vascular homeostasis by controlling reactive oxygen species (ROS) signaling, mitochondrial dynamics, angiogenesis, and immune responses, further underscoring their essential role in endothelial biology (Figure 1) [28].

Recent discoveries have shown that mitochondrial transfer between ECs and surrounding cells supports EC function and promotes tissue repair, particularly under conditions of stress or injury.

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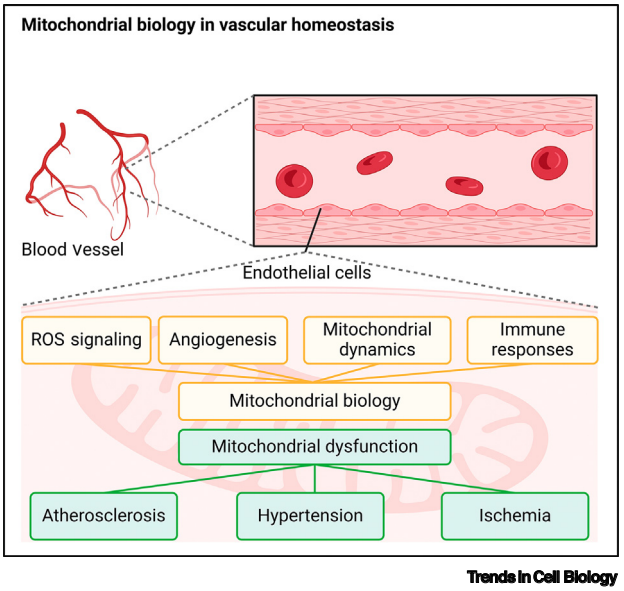



Figure 1. The role of mitochondrial biology in vascular homeostasis. Mitochondria regulate key endothelial functions that maintain vascular homeostasis. Mitochondrial biology influences processes such as reactive oxygen species (ROS) signaling, angiogenesis, mitochondrial dynamics, and immune responses (yellow boxes), which are critical for endothelial cell function. Disruptions in mitochondrial homeostasis can lead to mitochondrial dysfunction, contributing to vascular pathologies such as atherosclerosis, hypertension, and ischemia (green boxes).

This intercellular exchange of mitochondria enhances the regenerative capacity of ECs and contributes to vascular resilience [8,9]. As a result, mitochondrial transfer has emerged as a potential therapeutic target for treating vascular diseases [29].

This review synthesizes the latest findings on mitochondrial transfer, focusing specifically on its role in the vascular system. We examine both natural and artificial mechanisms of transfer, discuss their impact on EC function and vascular health, and explore emerging therapeutic applications. Focusing on recent studies, we aim to provide insights into how mitochondrial transfer may inform novel approaches in vascular biology and regenerative medicine.

## Mechanisms of mitochondrial transfer

Mitochondrial exchange between cells is a fundamental process that supports vascular homeostasis and endothelial function. This transfer occurs through TNTs, EVs, and direct mitochondrial extrusion/internalization, each contributing to different aspects of cellular adaptation and repair. TNTs enable direct organelle transport between cells, enhancing EC respiration, survival, and repair under stress conditions. EVs participate in both localized and systemic mitochondrial transfer, influencing metabolic adaptation and inflammatory signaling in recipient cells. Though less common, mitochondrial extrusion occurs primarily in stressed or dying cells, and recipient ECs may internalize these mitochondria through endocytic pathways. Together, these mechanisms regulate angiogenesis, endothelial resilience, and vascular repair, making mitochondrial transfer a promising target for therapeutic interventions in vascular dysfunction and ischemic conditions.

### Direct cell–cell contact and TNTs

Mitochondrial transfer can occur between directly attached cells or across distances through contact mediated by TNTs. These structures, composed of cytoskeletal elements, were first described as long, thin, actin-based protrusions facilitating intercellular communication [30]. TNTs allow the exchange of various cellular components, including organelles such as mitochondria, and have been implicated in maintaining cellular homeostasis under stress conditions [31].

Current progress in fluorescence and super-resolution microscopy have provided insights into TNT formation [32,33]. For example, the transition from double filopodial bridges to TNTs is triggered by disruptions in adhesion mechanisms, such as cadherin–cadherin interactions at anchoring points [34]. Beyond their role in transporting mitochondria, TNTs serve as pathways for the transfer of other macromolecules, such as proteins and RNAs [35], and even facilitate the spread of pathogens such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [36].

In ECs, TNT-mediated mitochondrial transfer plays a vital role in maintaining vascular homeostasis and promoting repair mechanisms. For instance, mitochondria transferred from mesenchymal stem cells (MSCs) to damaged pulmonary microvascular ECs have been shown to enhance mitochondrial respiration and restore membrane potential, thereby improving EC function and barrier integrity [37]. Also, studies have shown that inhibiting TNT formation via shTNFAIP2 or shMIRO1 in MSCs significantly reduces mitochondrial transfer to ECs, leading to impaired capacity to form blood vessels. TNT-mediated transfer of mitochondria has been shown to enhance vascularization and support the formation of functional blood vessels even under ischemic conditions [9]. Additionally, in ischemia–reperfusion models, MSC-derived mitochondria have been shown to salvage injured ECs via TNT-mediated transfer, highlighting the importance of mitochondrial acquisition in enhancing EC survival and function [38]. Co-culture of injured ECs with MSCs significantly increased basal and maximal oxygen consumption, further demonstrating the metabolic benefits of mitochondrial transfer. These findings underscore the pivotal role of TNT-mediated mitochondrial transfer in endothelial biology.

### EV-mediated transfer

Cells secrete small, membrane-bound particles known as EVs to facilitate intercellular communication [39,40]. Evidence suggests that mitochondria encapsulated within EVs contribute to these processes, though their precise physiological impact remains under investigation. Beyond their role in signaling, mitochondrial EVs have emerged as potential biomarkers of cellular dysfunction, with applications in disease diagnosis and monitoring. Interestingly, dysfunctional mitochondria carried within EVs do not always have a detrimental impact on recipient cells. For example, adipocytes under conditions of obesity release oxidatively damaged mitochondria packaged within EVs, which are subsequently taken up by cardiomyocytes through the circulation [41]. Rather than inducing pathology, this transfer promotes an adaptive response in the heart, bolstering its ability to counteract disease.

The transfer of EV-encased mitochondria through the circulation is not confined to cardiomyocytes. For instance, Borcherding *et al.* documented how dietary lipids can interfere with mitochondrial transfer to macrophages, diverting these organelles into the bloodstream. This study also showed that mitochondria released from adipose tissue supported metabolic adaptation in distant organs, with ECs serving as major recipients of the exogenous mitochondria [4]. Once in circulation, mitochondria originating from various adipose tissue depots – including epididymal white adipose tissue, inguinal white adipose tissue, and interscapular brown adipose tissue – have been shown to transfer not only to macrophages but also to ECs [4]. This underscores the ability of ECs to acquire mitochondria via circulating EVs, suggesting a possible avenue for therapeutic intervention. Notably, EV-mediated mitochondrial delivery through the bloodstream is being explored as a strategy for addressing mitochondrial dysfunction [42].

EV-mediated mitochondrial transfer has been implicated in enhancing endothelial metabolism and function in both *in vitro* and *in vivo* models. For instance, microvesicles containing mitochondria derived from ECs have demonstrated remarkable therapeutic efficacy in ischemic stroke models [5]. In brain ECs, treatment with EVs significantly increased ATP levels and oxygen

consumption rates compared with untreated controls. Furthermore, in middle cerebral artery occlusion models, EV treatment improved post-stroke behavioral outcomes, reducing neurological deficit scores compared with control groups. Homotypic EV donor and recipient cell pairs were found to be more effective than heterotypic pairs in enhancing mitochondrial function in recipient ECs. These findings suggest that EVs derived from ECs themselves may represent an optimized platform for mitochondrial transfer in regenerative medicine.

In summary, EV-mediated mitochondrial transfer is a recognized mechanism in EC function and vascular biology (Figure 2). Ongoing research continues to refine our understanding of its role in mitochondrial health, offering new directions for therapeutic approaches to vascular dysfunction.

## Extrusion and internalization of free mitochondria

Mitochondrial transfer typically occurs through membranous carriers such as TNTs and EVs, but some studies suggest that mitochondria or mitochondrial components can be extruded and internalized as free organelles without the need for these carriers [1]. Free mitochondrial extrusion is often associated with cellular stress responses, including mitochondrial quality control mechanisms and danger signal transduction. For instance, stressed cells undergoing oxidative damage or necroptosis have been shown to release mitochondria into the extracellular environment, where they may influence immune responses and inflammation [43]. This process has been documented in various cell types, but evidence for its occurrence in ECs remains limited.

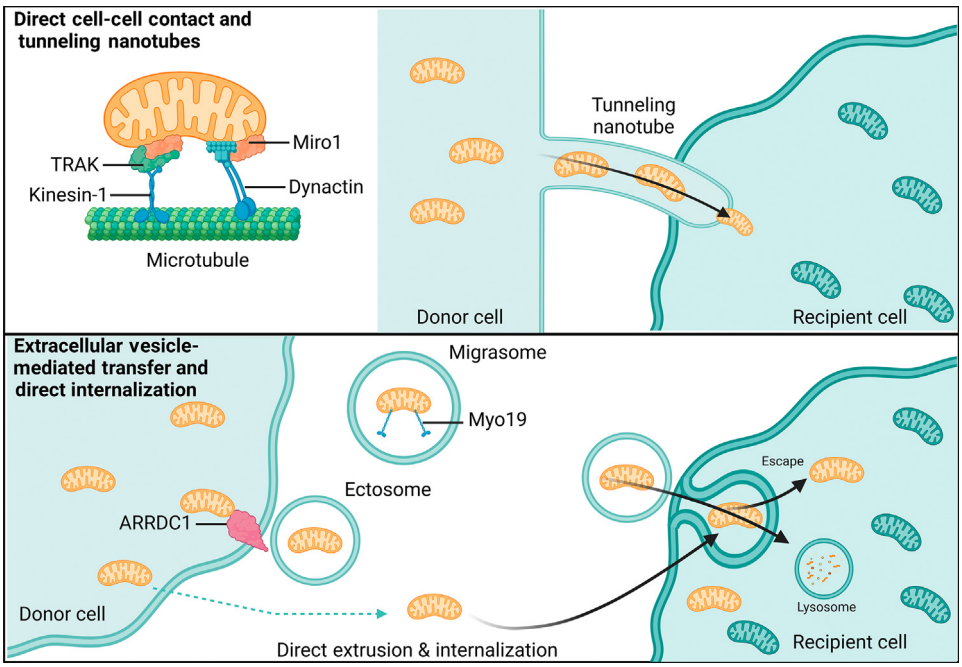



Figure 2. Mechanisms of mitochondrial transfer between cells. Schematic representation of leading mitochondrial transfer mechanisms. Top: tunneling nanotubes (TNTs) facilitate direct cell–cell transfer of mitochondria, driven by microtubule-based transport involving Miro1, TRAK, kinesin-1, and dynein–dynactin complexes. Bottom: extracellular vesicle (EV)-mediated transfer includes ectosomes and migrasomes, which package mitochondria for delivery to recipient cells. Mitochondria within EVs may escape endosomal compartments or be degraded in lysosomes following endocytosis. Key molecular regulators, such as ARRD1C and Myo19, are involved in vesicle formation and cargo transport. In addition to these pathways, direct mitochondrial extrusion and internalization represent an alternative mode of mitochondrial transfer, though their functional significance in endothelial cells remains to be fully characterized.

By contrast, mitochondrial internalization refers to the uptake of free mitochondria by recipient cells, a phenomenon first described as 'mitochondrial transformation' in 1982 [44]. One key demonstration of this process occurred when isolated murine mitochondria were successfully taken up by human ρ0 cells, leading to improved respiratory function [45]. Subsequent studies have identified critical factors regulating mitochondrial uptake, including macropinocytosis and fusion proteins such as syncytin-1 and syncytin-2, which may facilitate interactions between mitochondria and recipient cells [46,47].

The ability of cells to internalize mitochondria has significant therapeutic implications and serves as the foundation for mitochondrial transplantation strategies. Studies have shown that ECs can take up exogenous mitochondria when provided artificially, leading to improved bioenergetics and functional attributes [9]. This suggests that ECs possess the capacity for mitochondrial internalization under certain conditions. However, whether this process occurs naturally in ECs and its physiological relevance in vascular biology remain uncertain. Further research is needed to determine whether ECs naturally engage in direct mitochondrial uptake as part of an intrinsic mechanism for maintaining mitochondrial homeostasis or responding to stress.

### Molecular mechanisms underlying mitochondrial transfer

The transfer of mitochondria through TNTs and EVs involves distinct molecular mechanisms, tightly regulated by key proteins and signaling pathways.

Mitochondrial transfer through TNTs is facilitated by microtubule-based transport mechanisms. This process relies on the recruitment of mitochondrial transport proteins by Miro1 and TRAK adaptors, which link mitochondria to motor proteins kinesin-1 and dynein–dynactin [48,49]. Kinesin-1 moves mitochondria toward the plus end of microtubules through ATP hydrolysis, while dynein drives movement toward the minus end [50]. Recent studies reconstituted the bidirectional transport of Miro1/TRAK complexes along microtubules *in vitro*, revealing that TRAK binding prevents competition between kinesin-1 and dynein [51]. Furthermore, the recruitment of syntaphilin by $Ca^{2+}$ halts mitochondrial movement by immobilizing both motor proteins [51].

However, the formation and release of EVs involve membrane budding and scission, driven by the ESCRT (endosomal sorting complexes required for transport) machinery, including ESCRT-I, -II, and -III [52]. This process is regulated by cytoskeletal reorganization and local increases in membrane fluidity, which are triggered by sharp rises in intracellular $Ca^{2+}$ concentration during the blebbing process [53]. Calcium also regulates EV endocytosis and scission through the calcium-binding protein ALG-2, which assembles the ESCRT-III–Vps4 complex to mediate scission [54]. Once released, mitochondria-containing EVs are taken up by recipient cells via endocytosis [55]. This process involves the $NAD^+$–CD38–cADPR–$Ca^{2+}$ signaling pathway, and some internalized mitochondria are either degraded in lysosomes or escape endosomal compartments to integrate into the recipient cell cytoplasm [7]. Migrasomes, a specialized form of vesicles formed during cell migration, have also been shown to carry mitochondria. Key regulators of mitochondria incorporation into migrasomes include Miro1, KIF5B, and Drp1 [56]. Additionally, arrestin domain-containing protein 1 has been identified as essential for the formation of mitochondria-containing EVs [57].

These molecular mechanisms highlight the diverse pathways by which mitochondria can be transferred between cells, particularly through TNTs and EVs, with implications for maintaining mitochondrial health in ECs and supporting vascular function.

## Functional outcomes of mitochondrial transfer in ECs

Mitochondrial transfer is increasingly recognized as a factor influencing the metabolic and functional state of ECs, potentially enabling them to adapt to diverse physiological and pathological conditions [58,59]. Emerging evidence suggests that ECs, distributed across various organs, may exhibit mitochondrial heterogeneity shaped by the unique properties of mitochondria acquired through intercellular transfer. These variations could contribute to differences in respiratory capacity and functional profiles, depending on the organ of origin [60].

Building on this concept of organ-specific mitochondrial heterogeneity, experimental models have demonstrated that mitochondrial transfer can directly enhance endothelial function and support vascular regeneration. Mitochondrial transfer from MSCs to ECs via TNTs enhances ATP production and mitochondrial respiration, thereby boosting the vascularization potential of ECs [9]. Notably, the transferred mitochondria, although temporarily present within ECs, were effectively cleared through mitophagy, a process that concurrently stimulated mitochondrial biogenesis. This dual mechanism was mediated by the activation of the PINK1–Parkin signaling pathway, which is pivotal for initiating mitophagy. The coordinated removal of dysfunctional or exogenous mitochondria and the subsequent enhancement of mitochondrial biogenesis significantly improved EC functionality (Figure 3). These adaptations translated into superior angiogenesis, as demonstrated in ischemic mouse models, where ECs exhibited sustained vascularization capabilities even in the absence of MSCs [9].

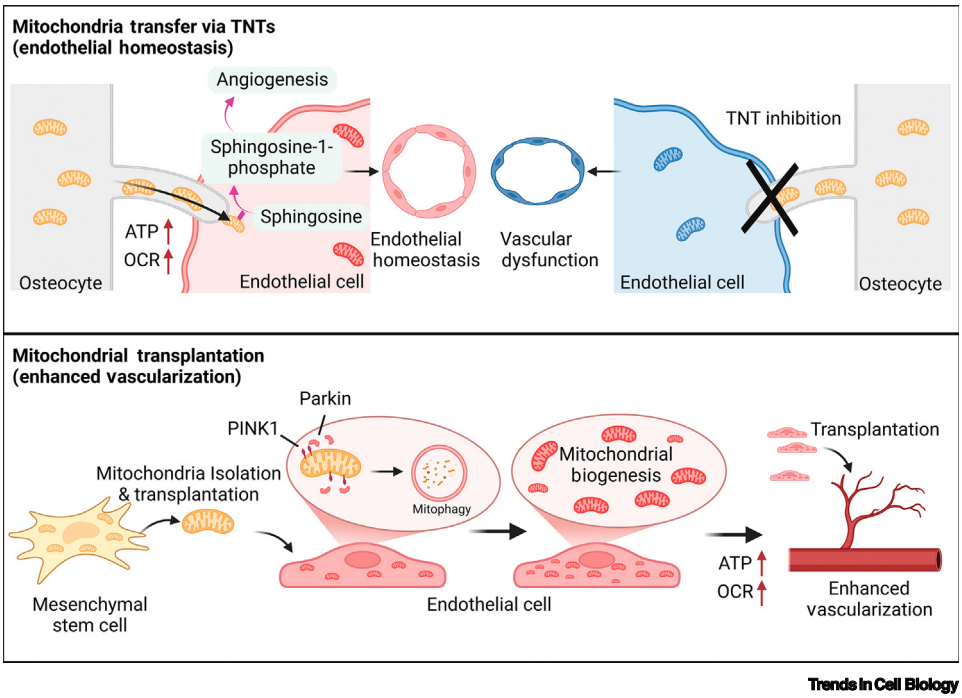



Figure 3. Functional impacts of mitochondrial transfer on endothelial cells (ECs). Top: osteocyte-derived mitochondrial transfer maintains endothelial homeostasis by preventing endothelial dysfunction. Disruption of this transfer, such as through the loss of Rhot1, impairs EC function and leads to vascular dysfunction. Bottom: mitochondrial transfer from mesenchymal stem cells to ECs enhances vascularization by activating the PINK1–Parkin signaling pathway. This process induces mitophagy, stimulates mitochondrial biogenesis, and improves EC function, culminating in enhanced angiogenesis and vascular repair. Abbreviations: OCR, oxygen consumption rate; TNT, tunneling nanotube.

Beyond ischemic models, mitochondrial transfer also plays a critical role in specialized vascular environments. Recent studies have highlighted the importance of mitochondrial transfer from osteocytes in maintaining transcortical vessels (TCVs), which are essential for bone-marrow structure and bone healing. Disruption of this process, such as through Rhot1 deletion in osteocytes, has been shown to result in TCV regression and impaired EC function [8]. Remarkably, osteocyte-derived mitochondria appear to restore EC function while promoting angiogenesis by activating D-sphingosine synthesis and SPHK1-mediated signaling. These findings underscore the potential of mitochondrial transfer to support vascular integrity in bone tissues and accelerate repair processes, offering new insights into its role in specialized vascular niches (Figure 3).

Beyond specialized vascular environments, mitochondrial transfer has also been shown to regulate EC metabolism. Studies using brain ECs demonstrated that mitochondria enclosed within microvesicles improve maximal glycolytic capacity, oxygen consumption rate (OCR), and ATP production [61]. While glycolysis remains the dominant energy pathway in ECs, accounting for >85% of ATP production [62], mitochondrial respiration provides a complementary and critical role in supporting vascularization. Mitochondrial ATP generation is not only more proteome-efficient than glycolysis but is also essential for maintaining $NAD^+$/NADH ratios that drive EC proliferation and angiogenesis [63,64].

Although mitochondrial transfer has been shown to enhance EC metabolism, functional differences may arise depending on the origin of the donor mitochondria. Mitochondria from MSCs, osteocytes, and other cell types may exhibit distinct bioenergetic profiles, metabolic regulatory functions, and signaling capacities. Understanding these variations is crucial for optimizing therapeutic strategies based on mitochondrial transfer. While studies have demonstrated the importance of mitochondrial transfer, the fate and long-term functionality of transferred mitochondria remain poorly understood, particularly *in vivo*.

In pathological conditions, mitochondrial respiration in ECs is frequently compromised, leading to impaired vascular repair and diminished angiogenic capacity [65]. By restoring mitochondrial respiration, mitochondrial transfer provides a mechanistic basis for therapeutic strategies aimed at preserving vascular integrity and function in disease contexts.

## Mitochondrial transplantation: toward clinical applications in vascular disease

Strategies to artificially transplant mitochondria, first explored in the 1980s as a proof of concept, have evolved into a promising therapeutic approach, with emerging evidence supporting their potential applications [44,66–69]. Preclinical studies have demonstrated that mitochondrial transplantation enhances myocardial function and preserves tissue viability in ischemic injury models. In a rabbit model of myocardial ischemia–reperfusion, mitochondrial injection significantly improved left ventricular peak developed pressure and reduced end-diastolic pressure [70]. Infarct size was significantly lower in mitochondrial-transplanted hearts than in vehicle-only treated hearts, and ATP levels in the area at risk were significantly higher in the mitochondrial-transplanted group. Mitochondria were detected within the myocardium post-reperfusion, though they did not integrate into myocytes.

Similarly, in neonatal and pediatric porcine hearts donated after circulatory death (DCD), mitochondrial transplantation was shown to enhance myocardial preservation and mitigate ischemic injury [71]. Following induced circulatory death, these hearts underwent warm ischemia, cold cardioplegia, and *ex vivo* heart perfusion. Mitochondria-treated DCD hearts exhibited significantly preserved left ventricular function, including developed pressure and fractional shortening, reduced infarct size, and extended warm ischemia tolerance. Preclinical studies have demonstrated the

potential of mitochondrial transplantation to improve outcomes in both small and large animal models [72–75].

Building on these preclinical findings, clinical trials have begun evaluating mitochondrial transplantation in human patients. One of the earliest applications targeted ischemia–reperfusion injury in pediatric patients undergoing extracorporeal membrane oxygenation. In this trial (ClinicalTrials.gov Identifier: NCT02851758), autologous mitochondria were injected into ischemic heart tissue in five pediatric patients with myocardial injury, demonstrating reduced myocardial damage and improved cardiac function.

Expanding on this approach, a Phase 1 clinical trial is enrolling 20 adult patients (ages 18–85 years) with cerebral ischemia to assess the safety and feasibility of autologous mitochondrial transplantation during reperfusion treatment (ClinicalTrials.gov Identifier: NCT04998357). Mitochondria will be extracted from a muscle biopsy and infused into the affected cerebral artery. The primary outcome is the incidence of severe adverse events, with secondary measures evaluating functional recovery.

Although these trials mark important early steps in clinical translation, questions remain before mitochondrial transplantation can be widely implemented in practice. Key uncertainties include the fate and functionality of transplanted mitochondria, particularly their survival, retention, and integration within recipient cells [76,77]. Moreover, questions persist about how the delivery of relatively few mitochondria can exert significant effects, such as boosting ATP production or restoring cellular function in recipient cells [2,78]. These concerns underscore the need for further mechanistic studies to refine and optimize this approach by addressing current uncertainties and improving the survival, retention, and activity of transplanted mitochondria.

To overcome these challenges, efforts are under way to address the current limitations of mitochondrial transplantation by developing innovative approaches to improve delivery and efficacy. For example, recent studies have explored the use of nitric oxide-releasing nanomotors and cardiomyocyte membrane coatings to enhance mitochondrial uptake and retention. These modifications allow mitochondria to cross physiological barriers, such as the intestinal epithelium, and target ischemic tissues via systemic delivery. Such advances have demonstrated therapeutic efficacy in preclinical models of ischemic heart disease [79]. While these efforts have predominantly focused on cardiomyocytes, they provide a foundation for future applications targeting ECs to restore vascular integrity and function, particularly in conditions such as chronic ischemic disease.

Looking ahead, the field of mitochondrial transplantation offers an exciting frontier for therapeutic innovation. While significant progress has been made, much-needed research is required to fully elucidate the mechanisms underlying mitochondrial delivery and integration, optimize delivery systems, and ensure safety and efficacy in clinical settings. By addressing these challenges, mitochondrial transplantation could unlock transformative opportunities, not only for vascular health but also for a wide range of conditions driven by mitochondrial dysfunction. The promise of this approach lies in its potential to bridge fundamental science and clinical application, paving the way for groundbreaking treatments in regenerative medicine.

## Concluding remarks

Mitochondria, beyond their role as cellular energy hubs, are integral to maintaining tissue health and function. The importance of mitochondrial health in ECs and the emerging role of mitochondrial transfer in supporting vascular repair and regeneration point to promising opportunities for therapeutic development. Preclinical studies have demonstrated the potential of mitochondrial

### Outstanding questions

What are the mechanistic underpinnings of mitochondrial transfer in ECs, and how do they influence vascular regeneration and the fate of transferred mitochondria?

How can mitochondrial transfer be optimized as a therapeutic strategy for vascular diseases while ensuring stability and functional integration?

What are the key challenges in enhancing the efficiency, survival, and long-term retention of transferred mitochondria in ECs?

To what extent does bidirectional mitochondrial exchange occur in ECs, and what are its functional consequences in vascular biology?

How can biomaterial-based strategies, including encapsulation or scaffold-assisted delivery, improve mitochondrial transplantation outcomes?

What novel methodologies are being developed to overcome mechanistic uncertainties and translational hurdles in mitochondrial transfer research?

transfer to enhance EC function and vascularization, with early clinical trials now exploring its application in disease contexts.

While these advancements are encouraging, several challenges remain (see Outstanding questions). Limitations such as the low stability of mitochondria outside their native environment, inefficiencies in transfer mechanisms, and the difficulty in obtaining sufficient quantities of host-derived mitochondria highlight areas for improvement. A key limitation is the dependence on autologous mitochondria, which may not be feasible for patients with mitochondrial dysfunction. Studies have shown that allogeneic mitochondria can improve organ function in experimental models, though the long-term effects and immunological consequences require further investigation [80,81]. Additionally, mitochondrial storage and preservation present major obstacles to clinical application. Current transplantation methodologies rely on freshly isolated mitochondria, as freeze-thawed mitochondria exhibit increased membrane permeability and outer membrane instability [82]. Developing effective storage strategies will be essential for the widespread clinical adoption of mitochondrial therapies.

Another challenge lies in optimizing mitochondrial delivery strategies. While direct injection has been the primary approach in clinical studies, alternative methods – such as encapsulation within EVs or integration with biomaterial scaffolds – could improve delivery efficiency and mitochondrial retention in target tissues. Recent studies have explored functionalizing mitochondria with biomaterial coatings, enhancing their stability, retention, and targeting abilities *in vivo* [83]. For instance, mitochondria encapsulated with specialized coatings, such as chitosan-based layers or lipid-modified surfaces, have demonstrated improved bioavailability, which may enhance mitochondrial uptake by recipient cells [84]. Additionally, biomaterial scaffolds have long been utilized in tissue engineering to improve cell transplantation outcomes [85], suggesting a potential role in supporting mitochondrial delivery. Incorporating mitochondria into biocompatible scaffolds could enable localized, sustained release in ischemic tissues, improving mitochondrial survival and function post-transplantation. These strategies present new directions for overcoming current limitations in mitochondrial transplantation and warrant further investigation.

Although mitochondrial transplantation has been shown to improve mitochondrial respiration and reduce immune responses in recipient cells, the fate of transferred mitochondria within ECs and the vascular system and the conditions necessary to achieve optimal therapeutic efficacy remain unresolved questions. Addressing these gaps will require advancing our understanding of mitochondrial biology and refining techniques to enhance mitochondrial transfer stability, efficiency, and scalability.

Mitochondrial transfer is increasingly recognized as a bidirectional process occurring between various cell types, including MSCs, SMCs, neuronal cells, and cardiomyocytes [86–88]. However, the extent to which ECs participate in bidirectional mitochondrial exchange remains underexplored. Investigating the functional significance of this process in vascular biology may uncover novel therapeutic opportunities.

Mitochondrial transfer offers a promising approach for developing vascular therapies that utilize safe, autologous materials to restore endothelial and vascular function. With continued research and innovation, this strategy has the potential to address diseases driven by mitochondrial dysfunction while expanding our understanding of vascular biology, mitochondrial therapeutics, and regenerative medicine.

## Declaration of interests

J.M.M-M. is an inventor on a patent application related to this work, filed by the Children's Medical Center Corporation. The authors declare no other competing interests.

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