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COMPENDIUM ON MIGRATION OF MITOCHONDRIA BEYOND CELL BOUNDARY: BIOLOGY, PHYSIOLOGY, AND THERAPEUTIC POTENTIAL

Mitochondrial Transfer to Endothelial Cells: Mechanisms, Evidence, and Therapeutic Potential

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ABSTRACT: Mitochondria are increasingly recognized as central regulators of vascular health, shaping endothelial cell function through roles that extend far beyond energy production. In addition to coordinating redox balance, calcium dynamics, and biosynthetic support, recent studies have revealed that mitochondria participate in intercellular communication, with evidence of transfer events emerging in vascular contexts. Parallel efforts have advanced the deliberate delivery of exogenous mitochondria from preclinical proof-of-principle studies to first-in-human trials, demonstrating that freshly isolated organelles can be harvested and administered in real-time to critically ill patients with favorable early outcomes. The mechanisms underlying these benefits remain incompletely defined, and strategies for efficient and scalable delivery are still emerging. In this review, we prioritize recent evidence linking mitochondrial function to endothelial cell physiology, highlight the nascent but growing field of mitochondrial transfer in the vasculature, and examine how mitochondrial transplantation is evolving from experimental concept to clinical translation. Together, these advances point to new therapeutic avenues for preserving vascular integrity and treating disease.

Key Words: cell communication ■ endothelial cells ■ mitochondria ■ regenerative medicine ■ therapeutics

The vascular endothelium maintains tissue homeostasis by integrating metabolic, mechanical, and inflammatory signals across organ systems, and endothelial dysfunction contributes to a wide spectrum of cardiovascular and systemic diseases.[1] Mitochondria are increasingly recognized as central regulators of these processes.[2] Endothelial cells (ECs) maintain energy homeostasis through tightly coordinated glycolytic and mitochondrial oxidative phosphorylation that support their diverse physiological roles and adaptability to stress. Pyruvate generated from glycolysis can either be reduced to lactate in the cytoplasm through lactic fermentation or be oxidized to CO_2 in the mitochondria via the tricarboxylic acid cycle and electron transport chain, providing metabolic flexibility according to oxygen availability and cellular demand. Mitochondria, therefore, contribute to functions that extend well beyond bioenergetics, positioning them as key determinants of vascular tone, permeability, and angiogenesis. In addition, recent studies suggest that mitochondria participate in intercellular communication.[3,4] Transfer between neighboring or supporting cells has been documented in multiple contexts, suggesting that similar processes may influence vascular repair and adaptation. Parallel efforts have tested the transplantation of exogenous mitochondria, with preclinical and early clinical studies suggesting that mitochondrial supplementation may restore vascular function under stress. These developments broaden the view of mitochondria from auxiliary metabolic units to central participants in EC physiology and vascular health. This review examines the emerging evidence that positions mitochondria as key regulators of EC function, focusing on their roles in vascular health, intercellular transfer, and the evolving field of mitochondrial transplantation.

MITOCHONDRIAL BIOLOGY IN ECs

ECs line the inner surface of blood vessels and maintain vascular homeostasis by coordinating metabolic,

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Nonstandard Abbreviations and Acronyms

ARRDC1	arrestin domain–containing protein 1
CoA	coenzyme A
CypD	cyclophilin D
Drp1	dynamin-related protein 1/dynamin-1-like protein
EndMT	endothelial-to-mesenchymal transition
eNOS	endothelial NO synthase
ER	endoplasmic reticulum
EV	extracellular vesicle
IsoLG	isolevuglandin
MCU	mitochondrial calcium uniporter
Mfn2	mitofusin-2
MSC	mesenchymal stromal cell
mtDNA	mitochondrial DNA
mtROS	mitochondrial ROS
NF-κB	nuclear factor-κB
oxLDL	oxidized low-density lipoprotein
PDH	pyruvate dehydrogenase
PDHA1	pyruvate dehydrogenase E1 alpha
QC	quality control
ROS	reactive oxygen species
SERCA2a	sarco/ER Ca2+-ATPase
TGF	transforming growth factor
TNF-α	tumor necrosis factor-α
TNT	tunneling nanotube
Vegfr3	vascular endothelial growth factor receptor 3

signaling, and biosynthetic functions that regulate vascular tone, permeability, and angiogenesis.[5,6] These functions rely on a finely tuned interplay between energy metabolism and signal transduction, enabling ECs to rapidly adapt to changing hemodynamic and biochemical environments.[2,7] Although ECs have traditionally been viewed as predominantly glycolytic—generating roughly 75% to 85% of their ATP through this pathway even in the presence of oxygen[8–10]—recent evidence indicates that mitochondrial respiration contributes more meaningfully to endothelial metabolism than previously appreciated. Mitochondrial oxidative phosphorylation functions in coordination with glycolysis, with pyruvate oxidation serving as a point of metabolic coupling. This interplay helps preserve redox balance and supports key endothelial functions, including barrier integrity, nitric oxide (NO)-mediated vasorelaxation, and angiogenic signaling.[11,12] In line with this, endothelial fatty acid oxidation–derived acetyl-CoA (coenzyme A) has been shown to preserve endothelial identity and suppress EndoMT, while mitochondrial ATP production is required for calcium-dependent NO-mediated control of vascular tone.[13,14] Thus, mitochondria are increasingly recognized not only for their metabolic contribution but also as dynamic signaling hubs that integrate redox control, calcium handling, and biosynthetic support for vascular integrity.[10,15–17]

Rather than serving solely as static energy generators, EC mitochondria are spatially and functionally specialized to influence vascular tone, angiogenic capacity, barrier function, and inflammatory responses.[18] Frequently positioned near the nucleus or at cell–cell junctions, they modulate gene expression, NO bioavailability, and intracellular Ca^{2+} dynamics.[19–22] By buffering cytosolic calcium during stimulation, mitochondria shape the amplitude and frequency of Ca^{2+} oscillations that activate eNOS (endothelial NO synthase), thereby fine-tuning NO-mediated vasodilation.[15,16]

Mitochondria also regulate reactive oxygen species (ROS) generation and scavenging to maintain redox homeostasis. Within physiological limits, mitochondrial ROS (mtROS) act as key signaling molecules that promote angiogenesis and preserve endothelial quiescence.[23] In ECs, ROS production occurs through tightly regulated enzymatic reactions within the respiratory chain, even under conditions of mitochondrial stress. Mechanistically, mtROS arise from reverse electron transport at complex I and other controlled redox reactions coupled to changes in ATP and nicotinamide adenine dinucleotide $(NAD)^+$ levels, mitochondrial membrane potential ($\Delta\psi$), and matrix pH.[24] When these regulatory systems are disrupted, impaired Sirt3 (sirtuin 3) activity and diminished antioxidant capacity lead to redox imbalance and oxidative stress. Under these pathological conditions, excessive ROS impair NO signaling, activate inflammatory cascades, and trigger apoptosis.[5,15,16] Through this integration of metabolic, redox, and calcium signals, mitochondria function as central orchestrators of vascular homeostasis, adjusting EC phenotype in response to growth factors, hypoxia, or shear stress.[15,16]

The requirement for intact mitochondrial metabolism becomes evident in angiogenesis. In mouse embryonic lymphatic ECs, genetic inactivation of QPC (ubiquinone-binding protein), a subunit of respiratory chain complex III, disrupts lymphatic vessel formation during midgestation and downregulates key transcriptional regulators, such as Vegfr3 (vascular endothelial growth factor receptor 3) and Prox1 (prospero homeobox 1).[25] In human umbilical vein ECs, pharmacological inhibition of complex III lowers the NAD^+/NADH ratio and suppresses proliferation.[26] Endothelial-specific deletion of complex III in vivo markedly reduces angiogenesis in the postnatal retina, lung, and tumor vasculature, underscoring the essential role of oxidative phosphorylation in EC proliferation and vessel growth.[26] These findings, together with recent metabolic profiling of brain microvessels,[12] further highlight that mitochondrial respiration is not auxiliary but essential for sustaining endothelial bioenergetic and proliferative demands. At the level of pyruvate utilization,

knockdown of *PDHA1* (pyruvate dehydrogenase E1 alpha) increases apoptosis in angiogenic tip cells, while blockade of mitochondrial pyruvate import with UK5099 reduces tip-cell numbers.[9] In the chorioallantoic membrane assay, *PDHA1* suppression decreases vascular branching and sprout length, demonstrating that mitochondrial respiration is indispensable for sprouting angiogenesis ex vivo.[9]

Within this context, controlled mitochondrial metabolism acts as a proangiogenic cue, supporting tip-cell formation and directional sprouting.[25,26] This reflects a broader principle in EC biology: redox signaling is dose-dependent, with regulated mtROS enabling angiogenic signaling, whereas excessive ROS disrupt NO signaling, exacerbate inflammation, and promote apoptosis, culminating in microvascular rarefaction.[15,16]

Mitochondria further shape endothelial calcium homeostasis.[27] By buffering cytosolic Ca^{2+} during stimulation, they fine-tune the magnitude and kinetics of Ca^{2+} transients that govern eNOS activation and NO production.[15,16] Through these interconnected redox-calcium axes, mitochondrial signaling determines whether ECs remain quiescent, adopt an activated phenotype, or enter a proangiogenic state.[15,16]

In sum, ECs rely on a coordinated contribution from both glycolysis and mitochondrial respiration, and mitochondria serve as central integrators of signaling and biosynthetic networks. By coordinating NO, ROS, and Ca^{2+} signaling, they preserve barrier integrity, sustain angiogenic potential, and enable adaptive responses to environmental stress.[5,15,16] This broader physiological footprint underpins the therapeutic rationale—discussed in later sections—for strategies that preserve, enhance, or replace mitochondrial function, including intercellular mitochondrial transfer and engineered mitochondrial transplantation.[9,25,26]

MITOCHONDRIAL DYSFUNCTION AND VASCULAR DISEASE

ECs rely on intact mitochondrial function to preserve vascular integrity and respond adaptively to hemodynamic and metabolic challenges. Mitochondrial dysfunction disrupts this balance, triggering maladaptive responses that reshape endothelial identity, impair barrier function, and promote cell loss (Figure [A]). These disturbances can manifest as apoptosis, proinflammatory activation, or defective vascular repair—responses that underpin a wide spectrum of vascular pathologies.[28] Importantly, these pathways are not isolated: mitochondrial Ca^{2+} handling, mitophagy, and mtROS generation intersect extensively, and dysregulation in one domain often propagates dysfunction in the others. For clarity, we discuss each process in its own context below, while recognizing their mechanistic interdependence.

Mitochondria-Driven Apoptotic Pathways

Mitochondrial dysfunction engages intrinsic apoptotic pathways in ECs, contributing to vascular injury and degeneration.[5,29,30] In models of acute stress, such as heat-induced injury, EC apoptosis is mediated by Ca^{2+}-dependent mitochondrial signaling that bypasses death receptor and ER stress pathways.[31] Elevated cytosolic Ca^{2+} promotes Apaf-1 (apoptotic protease activating factor 1) induction, caspase-9 and -3 activation, PARP (poly [ADP-ribose] polymerase) cleavage, and nDNA fragmentation, accompanied by increased mtROS.[31]

Central to this process are mitochondria-associated membranes, specialized ER-mitochondria contact sites that regulate Ca^{2+} transfer.[32] ≈75% of the Ca^{2+} released from the endoplasmic reticulum (ER) is resequestered by the SERCA2a (sarco/ER Ca^{2+}-ATPase), whereas Ca^{2+} transfer to mitochondria occurs through a coordinated complex in which the outer-membrane VDAC (voltage-dependent anion channel) channels are physically coupled to ER-localized IP_3 (inositol 1,4,5-trisphosphate) receptors, allowing Ca^{2+} to enter the intermembrane space. Uptake of Ca^{2+} into the mitochondrial matrix is then mediated by the MCU (mitochondrial calcium uniporter) complex in the inner membrane, where it regulates tricarboxylic acid cycle flux and oxidative phosphorylation.[33] Knockdown of *TMEM215* (transmembrane protein 215) enhances mitochondria-associated membrane formation, reduces ER-mitochondria distance, and leads to mitochondrial Ca^{2+} overload and apoptosis—a phenotype reversible by IP_3 receptor or MCU inhibition.[30,34]

Pathological stressors in vascular disease activate similar mechanisms. oxLDL (oxidized low-density lipoprotein), a major atherogenic factor, disrupts mitochondrial membrane potential ($\Delta\Psi m$) and triggers cytochrome c release, leading to caspase activation through Apaf-1 binding.[35–37] In ischemia/reperfusion injury, excessive mtROS oxidizes cardiolipin, destabilizing cytochrome c anchorage and further amplifying apoptosis.[38]

Apoptotic signaling is accompanied by dynamic remodeling of the mitochondrial network. Proapoptotic stimuli activate Drp1 (dynamin-related protein 1/dynamin-1-like protein)-mediated fission, shifting mitochondrial morphology from tubular to fragmented.[39–41] Members of the Bcl-2 (B-cell leukemia/lymphoma 2) family, such as Bax (Bcl-2 associated X-protein) and Bak (Bcl-2 homologous antagonist/killer), contribute to this remodeling, although fragmentation alone is not sufficient to induce apoptosis.[33,40]

Mitochondrial Quality Control in Endothelial Homeostasis and Disease

Mitochondrial quality control (QC)—encompassing dynamics, mitophagy, and biogenesis—is essential for maintaining EC function. Disruption of this network contributes to vascular pathology across diverse disease

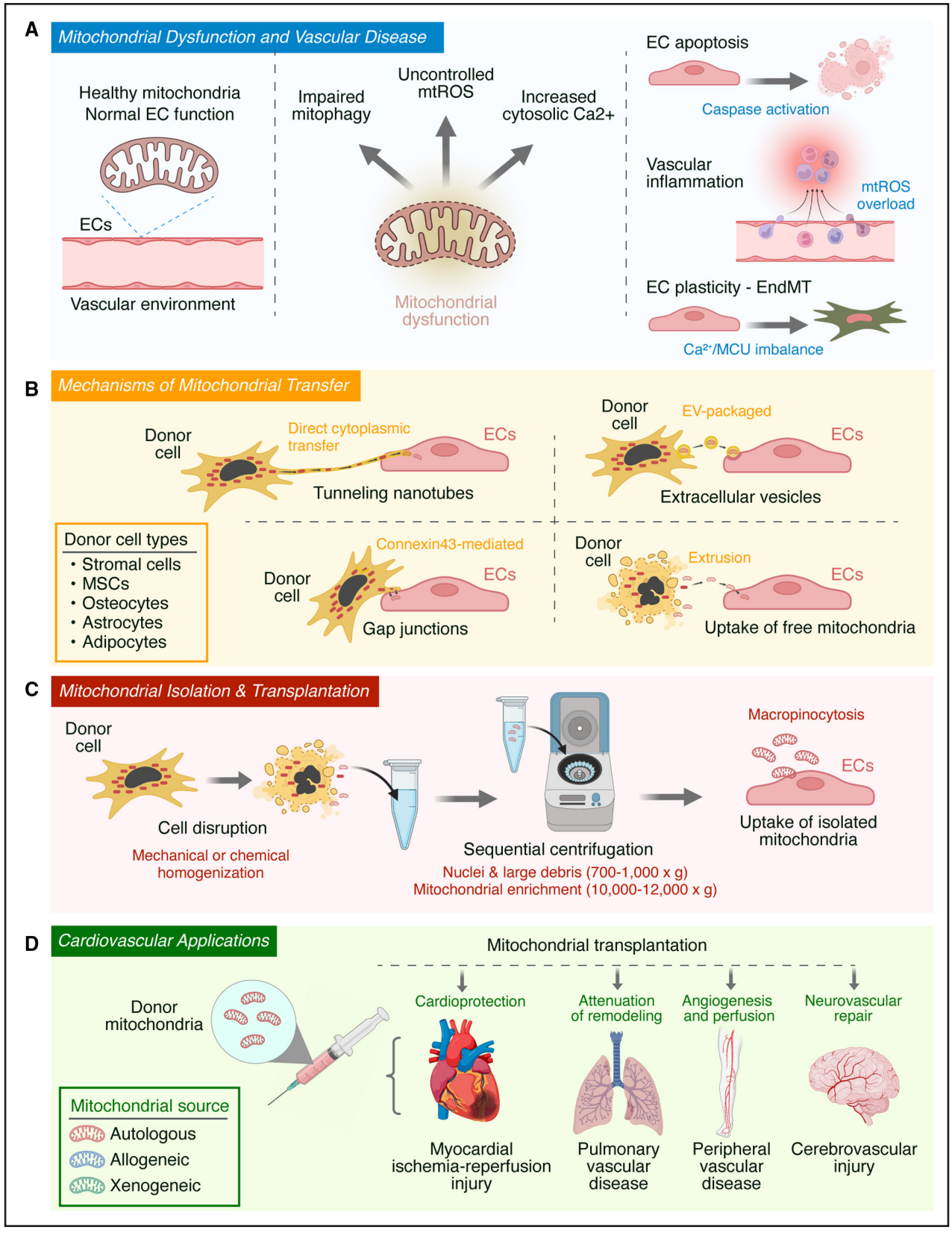

Figure. Conceptual framework of mitochondrial dysfunction, transfer, and transplantation in vascular biology.
A, Mitochondrial dysfunction in endothelial cells (ECs) contributes to vascular pathology by disturbing quality control, redox balance, and calcium handling. These alterations converge on maladaptive outcomes, including apoptosis, vascular inflammation, and loss of endothelial identity. **B**, Intercellular mitochondrial transfer has emerged as a key form of communication with ECs. Donor cells—including stromal cells, mesenchymal stromal cells (MSCs), astrocytes, osteocytes, and adipocytes—donate mitochondria through mechanisms such as tunneling nanotubes, (*Continued*)

contexts. In hypertension, mitochondrial QC is perturbed by an imbalance between GCN5L1 (general control of amino acid synthesis 5 like 1)-mediated acetylation and Sirt3-mediated deacetylation, leading to hyperacetylation of CypD (cyclophilin D). This posttranslational modification sensitizes the mitochondrial permeability transition pore to opening, triggering metabolic collapse and increased mtROS production.[11]

Mitophagy, the selective removal of damaged mitochondria, plays a particularly nuanced role. Loss of major vault protein impairs Parkin-mediated mitophagy by stabilizing NEDD4L (neural precursor cell expressed developmentally downregulated gene 4-like), a negative regulator of Parkin. This leads to mtROS accumulation, vascular smooth muscle cell proliferation, neointima formation, and accelerated atherosclerosis.[42] Conversely, excessive mitophagy may also be maladaptive. In cardiac microvascular ischemia/reperfusion injury, persistently elevated Parkin activity—indicated by increased LC3-II, a lipidated form of LC3 (microtubule-associated protein 1A/1B-light chain 3), and SQSTM1/p62 (sequestosome 1) accumulation—leads to EC loss and impaired angiogenesis. These phenotypes are rescued by genetic or pharmacological suppression of mitophagy.[43]

Together, these findings underscore the importance of a homeostatic mitophagy threshold in vascular health. Both impaired and excessive mitochondrial clearance can destabilize endothelial integrity, indicating that QC pathways must be precisely tuned to preserve vascular function.

mtROS and Vascular Inflammation

Dysregulated enzymatic mtROS production is a central driver of endothelial inflammation. In human aortic ECs, suppression of the mitochondrial uncoupling protein UCP2 (uncoupling protein 2) elevates mtROS levels and induces inflammatory gene expression.[44] Proinflammatory cytokines, such as TNF-α (tumor necrosis factor-α), further exacerbate this response by shifting EC metabolism toward glycolysis and activating PDH (pyruvate dehydrogenase), which enhances mitochondrial acetyl-CoA production.[45] The resulting increase in histone acetylation at inflammatory gene loci sustains a transcriptionally permissive chromatin state.[45]

Emerging evidence suggests that immune–endothelial cross talk may further reinforce these inflammatory circuits.[46] Activated monocytes can transfer mitochondria and extracellular vesicles (EVs) to ECs, triggering both TNF-α and type I interferon signaling cascades.[46] This interplay establishes a feedforward loop in which inflammation augments mtROS production, and mtROS, in turn, amplifies inflammatory signaling.

Sustained oxidative stress has pathological consequences for vascular structure and function. Elevated mtROS levels promote endothelial dysfunction, impair angiogenesis, delay reendothelialization after injury, and contribute to fibrotic remodeling and arterial stiffening.[15] In patients with hypertension, oxidative mitochondrial injury in endothelial progenitor cells compromises their reparative potential,[47] underscoring the broader impact of mitochondrial redox imbalance on vascular homeostasis.

Endothelial Plasticity and Mitochondrial Regulation

Mitochondrial dysfunction influences EC plasticity, including transitions toward mesenchymal-like states.[48] A well-characterized example is endothelial-to-mesenchymal transition (EndMT), in which ECs lose canonical endothelial identity—downregulating junctional and homeostatic genes—while acquiring mesenchymal markers. This phenotypic shift contributes to fibrosis, vascular remodeling, and atherosclerotic lesion formation.[49,50]

Recent evidence links EndMT to altered mitochondrial calcium handling. Single-cell analyses reveal that during EndMT, proliferative, tip-like, and transitioning EC subtypes exhibit increased mitochondrial Ca^{2+} uptake, coinciding with transcriptional upregulation of MCU complex components, including MCU, MICU1 (mitochondrial calcium Uptake 1), MICU2 (mitochondrial calcium Uptake 2), and MCUR1 (mitochondrial calcium uniporter regulator 1).[49] Functional studies support a causal role: MCU overexpression accelerates mesenchymal reprogramming, whereas pharmacological or genetic inhibition of MCU attenuates EndMT-associated transcriptional changes.

Mitochondrial QC also shapes EC plasticity. Activation of mitophagy—particularly via the AMBRA1 (activating molecule in Beclin-1-regulated autophagy 1)/Parkin axis—suppresses EndMT. Src kinase inhibition induces this mitophagy pathway and prevents mesenchymal transition.[51] In contrast, impairing mitophagy genetically or pharmacologically promotes EndMT progression.[52] These findings highlight a mechanistic link between mitochondrial integrity and endothelial fate decisions, suggesting that disruptions in mitochondrial homeostasis not only impair function but may also reprogram EC identity in disease.

Figure Continued. extracellular vesicles (EVs), gap junctions, or release and uptake of free mitochondria. These exchanges restore EC bioenergetics, enhance angiogenic competence, and sustain vascular integrity. **C**, Building on these endogenous pathways, mitochondrial transplantation provides a therapeutic strategy in which intact organelles are isolated from donor cells and delivered to recipient ECs. Preclinical studies have demonstrated uptake and functional integration of exogenous mitochondria, supporting vascular repair and regeneration. **D**, Translational efforts now extend to cardiovascular applications, where mitochondrial transplantation has shown promise in models of myocardial ischemia-reperfusion, pulmonary hypertension, peripheral ischemia, and cerebrovascular injury. Both autologous and allogeneic sources are under investigation, with early phase clinical trials beginning to test feasibility and safety. EndMT indicates endothelial-to-mesenchymal transition; mtROS, mitochondrial reactive oxygen species; and MCU, mitochondrial calcium uniporter.

Disease Contexts: Mitochondrial Dysfunction Across Vascular Pathologies

Endothelial mitochondrial dysfunction is increasingly recognized as a central player in the pathogenesis of diverse vascular diseases. While many of the molecular defects converge on oxidative stress, bioenergetic insufficiency, and impaired QC, the specific manifestations and consequences vary across disease contexts.

In atherosclerosis, dysfunctional mitochondria contribute to disease initiation and progression by promoting endothelial inflammation and apoptosis. oxLDL induces mitochondrial membrane depolarization and cytochrome c release, triggering caspase activation and EC loss. Simultaneously, mtROS activate NF-κB (nuclear factor-κB) signaling, upregulating adhesion molecules and cytokines that drive monocyte recruitment. Impaired mitophagy, particularly due to defective PINK1 (PTEN-induced putative kinase 1)/Parkin signaling, exacerbates these events and accelerates lesion formation.[53] Importantly, EC-specific deletion of mitochondrial proteins such as PGAM5 (phosphoglycerate mutase family member 5) or Parkin sensitizes vessels to inflammatory stimuli, further underscoring the importance of intact QC pathways.[54,55]

In hypertension, chronic hemodynamic stress leads to elevated mtROS in ECs, which in turn impairs NO bioavailability and promotes vasoconstriction. mitochondrial permeability transition pore opening, mediated by hyperacetylated CypD, has been implicated in this process, linking defective QC to redox imbalance and endothelial dysfunction.[11] In the context of endothelial progenitor cells, increased mtROS also reduces regenerative capacity, impeding reendothelialization after injury.[47,56] At the level of mature endothelium, oxidative mitochondrial injury further promotes lipid peroxidation and the generation of IsoLGs (isolevuglandins), which form stable protein adducts, disrupt signaling, and exacerbate vascular inflammation. These IsoLG–protein adducts interfere with eNOS coupling, activate NF-κB, and compromise barrier integrity, thereby amplifying vascular dysfunction.[57,58] Moreover, damaged or oxidized mitochondria released extracellularly can expose bacterial-like lipids such as cardiolipin, eliciting inflammatory responses.

Ischemia/reperfusion injury presents a unique context where mitochondrial dynamics and QC timing are critical. The abrupt reoxygenation phase generates a burst of mtROS, leading to cardiolipin oxidation, cytochrome c release, and endothelial apoptosis.[59] Although basal mitophagy serves a protective role, excessive Parkin-mediated mitophagy during reperfusion may deplete mitochondria to the point of compromising energy supply, impairing repair and angiogenesis.[60]

In diabetes and metabolic syndrome, persistent hyperglycemia drives excessive mtROS production in ECs, leading to cytoskeletal disorganization and defective migration. This compromises angiogenic responses and predisposes the vasculature to impaired wound healing.[61,62] Moreover, chronic oxidative burden sensitizes ECs to pro-EndMT signaling, particularly in the presence of TGF (transforming growth factor)-β or inflammatory cytokines, accelerating vascular rarefaction and fibrosis.[63] Impaired mitochondrial DNA (mtDNA) integrity has also been shown to reduce endothelium-dependent vasodilation in metabolic syndrome, and experimental restoration of mitochondrial function—either by repairing mtDNA lesions or by introducing healthy mitochondria—can improve vasodilatory responses, underscoring the importance of mitochondrial quality for vascular tone.[64]

Cerebral small vessel disease and blood-brain barrier dysfunction are increasingly associated with endothelial mitochondrial defects. In brain microvascular ECs, mtROS disrupts tight junction integrity, alters ABC (ATP-binding cassette) transporter function, and enhances leukocyte adhesion, contributing to blood-brain barrier breakdown.[65,66] This mechanism is implicated in neurovascular complications of aging, stroke, and Alzheimer's disease, where vascular oxidative injury precedes neuronal degeneration.[67,68]

MITOCHONDRIAL TRANSFER TO ECs

Over the past decade, mitochondrial transfer has emerged as a previously underappreciated mode of intercellular communication, with significant implications for tissue repair and regeneration. Initially characterized in bone marrow–derived mesenchymal stromal cells (MSCs), this process involves the delivery of functional mitochondria to neighboring cells through tunneling nanotubes (TNTs), EVs, or cell fusion. In multiple tissues—including lung, heart, and central nervous system—MSC-mediated mitochondrial donation has been shown to rescue recipient cells from oxidative injury, restore bioenergetics, and facilitate repair after injury. This concept has expanded the traditional view of mitochondria from static energy producers to dynamic organelles with paracrine-like roles in homeostasis and healing.

Although this paradigm is well established in several organ systems, evidence for mitochondrial transfer involving ECs is only beginning to surface (Table 1). Studies have shown that ECs are not merely passive recipients but may actively uptake exogenous mitochondria, especially in contexts of metabolic stress or vascular injury. Early work in retinal and pulmonary vascular models demonstrated that mitochondria derived from pericytes or MSCs can improve EC viability and enhance angiogenic function under ischemic or inflammatory conditions. More recently, emerging data from vascular organoid models suggest that mitochondrial transfer may also play a role in developmental vasculogenesis, with mural cells acting as donors to nascent ECs.

One of the earliest demonstrations came from ischemia-reperfusion models in vitro, where ECs subjected to oxygen–glucose deprivation were found to

Table 1. Examples of Mitochondrial Transfer From Donor Cells to ECs

Donor cell/source	Recipient ECs	Mode of transfer	Key outcomes	Reference
Human BM-MSCs	HUVECs subjected to oxygen–glucose deprivation/reoxygenation	TNT-like structures (in vitro)	Restored aerobic respiration, reduced apoptosis, and rescued injured ECs	Liu et al[69]
Human EPCs	Human brain ECs (primary cultures, OGD model)	Free mitochondria (in vitro)	Increased ATP and mtDNA copy number, elevated TOM40 expression, and restored barrier integrity and angiogenic activity	Hayakawa et al[70]
Human BM-MSCs	HUVECs stressed with cytarabine (Ara-C)	TNTs (in vitro)	Restored bioenergetics, proliferation, migration, and angiogenic capacity and reduced apoptosis	Feng et al[71]
Human CMEC/D3 (endothelial cell–derived microvesicles)	Human CMEC/D3 cells and brain ECs in acute mouse slices	EV-mediated delivery (in vitro and ex vivo)	Increased ATP production and enhanced survival under hypoxia	D'Souza et al[72]
Mouse adipocytes	Mouse adipose ECs	EVs and free mitochondria (in vivo)	Increased mitochondrial uptake by ECs, uptake varied with diet, and inhibited by long-chain fatty acids	Borcherding et al[73]
Mouse astrocytes	Mouse bEnd.3 cells (in vitro) and mouse cerebral microvascular ECs (in vivo)	Direct transfer via astrocytic endfeet (in vitro and in vivo)	Maintained BBB integrity, Mfn2 deficiency caused BBB leakage, and process declined with aging	Liu et al[74]
Mouse astrocytes	Mouse brain microvascular ECs (capillaries, in vivo)	Direct transfer and EV-mediated delivery (in vivo and in vitro)	Increased mitochondrial transfer with aging, maintained BBB integrity, and potential compensatory mechanism for EC dysfunction	Velmurugan et al[75]
Mouse osteocytes	ECs of TCVs	Mitochondrial donation	Maintained TCV network; prevented regression after osteocyte ablation; restored EC proliferation, migration, and tube formation; and accelerated angiogenesis and bone repair	Liao et al[3]
Human MSCs	HUVECs, ECFCs, and WAT-ECs (engraftment models)	TNTs (in vitro and in vivo)	Enhanced EC bioenergetics and engraftment, promoted functional vessel formation, and blocking TNTs impaired transfer and engraftment	Lin et al[4]
Mouse BM-MSCs	MPMECs and ARDS model	TNTs (in vitro and in vivo)	Reduced ROS and apoptosis, reactivated TCA cycle, increased fatty acid synthesis and HGF/VEGF release, and promoted EC proliferation and vascular regeneration	Wang et al[76]
Mouse stromal ADRCs	Mouse hindlimb ECs (in vitro and in vivo, ischemia model	Gap junctions (connexin43) and TNTs	Enhanced mitochondrial biogenesis and angiogenesis in ECs and promoted vascular repair in ischemic limbs	Che et al[77]

Examples summarize reported instances of mitochondrial transfer to ECs, highlighting donor cell diversity, transfer modes, and functional outcomes. ADRC indicates adipose-derived regenerative cell; Ara-C, arabinosylcytosine; ARDS, acute respiratory distress syndrome; BBB, blood-brain barrier; BM-MSC, bone marrow–derived mesenchymal stem cell; CMEC, cerebral microvascular endothelial cell; EC, endothelial cell; ECFC, endothelial colony-forming cell; EPC, endothelial progenitor cell; EV, extracellular vesicles; HGF, hepatocyte growth factor; HUVEC, human umbilical vein endothelial cell; Mfn2, mitofusin-2; MPMEC, mouse pulmonary microvascular EC; mtDNA, mitochondrial DNA; OGD, oxygen–glucose deprivation; ROS, reactive oxygen species; TCV, transcortical vessel; TNT, tunneling nanotube; TOM40, translocase of the outer membrane 40; VEGF. vascular endothelial growth factor; and WAT-EC, white adipose tissue endothelial cell.

receive mitochondria from adjacent mesenchymal stem cells. The transfer occurred through TNT-like structures and was largely unidirectional—from stem cells into the injured ECs. This exchange restored aerobic respiration, reduced apoptosis, and highlighted mitochondrial donation as a potential rescue mechanism in vascular injury.[69]

Further evidence came from models of chemotherapy-induced endothelial injury, where cytarabine-stressed human umbilical vein ECs were shown to establish TNT connections with bone marrow–derived mesenchymal stem cells. Through these conduits, MSCs delivered mitochondria into the injured ECs, rescuing their bioenergetic function. The donated organelles reduced apoptosis, restored proliferative and migratory capacity, and reestablished angiogenic competence. These findings reinforced the view that mitochondrial transfer is not a passive byproduct of coculture but an active rescue mechanism that safeguards endothelial integrity under cytotoxic stress.[71]

Evidence has also come from studies of EVs. In cultured human brain ECs (human cerebral microvascular EC/D3), microvesicles—but not exosomes—were shown to transfer polarized mitochondria to stressed ECs, boosting ATP production and improving survival under hypoxic conditions. When applied to acute mouse brain slices, these EVs also delivered mitochondria to neurons, suggesting that vesicle-mediated transfer can extend beyond in vitro culture systems.[72] These observations point to EVs as a conduit for mitochondrial exchange in vascular contexts.

Extending this concept beyond local vascular interactions, mitochondria can also reach ECs via systemic routes. Recent work has shown that adipose tissue serves as a source of EV–encapsulated mitochondria that circulate in the bloodstream. Vesicles released from different fat depots—including epididymal white, inguinal white, and interscapular brown adipose tissue—were found to deliver mitochondria not only to immune cells such as macrophages but also to vascular ECs. Notably, this transfer was sensitive to dietary lipid intake, which altered the efficiency and destination of mitochondrial delivery. Together, these findings suggest that

adipose-derived mitochondria contribute to systemic metabolic adaptation and identify ECs as relevant targets of long-range mitochondrial trafficking[73]

Building on these observations, adipose-derived stromal populations were also recently shown to donate mitochondria directly to ECs in a murine hindlimb ischemia model. Mechanistic studies demonstrated that transfer occurred through connexin 43–based gap junctions and TNTs, leading to enhanced mitochondrial biogenesis and angiogenic activity in ECs and promoting vascular repair in ischemic limbs. Importantly, partial inhibition of mitochondrial transfer diminished the proangiogenic benefits of these stromal populations, establishing intercellular mitochondrial exchange as a central mechanism underlying their therapeutic efficacy.[77]

Additional work has implicated endothelial progenitor cells as a source of extracellular mitochondria. In experimental models, endothelial progenitor cells released mitochondria that were subsequently internalized by brain ECs, leading to increased ATP levels, improved barrier function, and enhanced angiogenic activity. These findings support the idea that progenitor-derived mitochondrial donation can stabilize the cerebral endothelium and promote vascular repair.[70]

Disease-specific contexts have also provided new insights. In acute respiratory distress syndrome, MSCs were shown to transfer mitochondria to pulmonary microvascular ECs both in vitro and in vivo, reducing oxidative stress, reactivating the tricarboxylic acid cycle, and enhancing proangiogenic signaling. This transfer ultimately supported vascular regeneration in injured lungs.[76] Similarly, in models of diabetic kidney disease, MSCs donated mitochondria to glomerular ECs, improving mitochondrial function, reducing apoptosis, and alleviating renal oxidative stress and fibrosis. These results demonstrate that mitochondrial transfer is a key component of endothelial repair in renal pathology.[78]

More definitive in vivo evidence has recently come from the central nervous system. Using a transgenic model, investigators demonstrated that *Dmp1* (dentin matrix acidic phosphoprotein 1)-expressing astrocytes regulate blood-brain barrier integrity through direct mitochondrial transfer to adjacent ECs. This donation occurs via astrocytic endfeet, and disruption of the process—through conditional deletion of Mfn2 (mitofusin-2) in astrocytes—abolished mitochondrial transfer and led to overt blood-brain barrier leakage. Notably, the same pathway appears to decline with age, as reduced Mfn2 expression in astrocytes diminishes transfer efficiency and compromises barrier function. These findings provide compelling mechanistic evidence that intercellular mitochondrial exchange is not only a rescue response but also a constitutive process essential for sustaining endothelial barrier integrity in vivo.[74] Independent studies employing inducible mitochondrial reporters further revealed that astrocytic mitochondrial transfer to ECs and pericytes not only occurs physiologically but actually increases with age, suggesting a role in vascular adaptation during brain aging.[75]

Complementary evidence has emerged from the skeletal system. Osteocytes were recently shown to sustain the ECs of transcortical vessels through mitochondrial donation. Genetic ablation of *Rhot1* in osteocytes, which impairs mitochondrial trafficking, caused regression of the transcortical vessel network. Conversely, delivery of osteocyte-derived mitochondria restored endothelial function and accelerated angiogenesis during cortical bone repair, positioning mitochondrial transfer as a homeostatic regulator of vascular niches in bone.[3]

In the setting of vascular regeneration, MSCs themselves have been shown to facilitate EC engraftment through mitochondrial donation. Under stress, MSCs transfer mitochondria to ECs via TNTs, enabling the latter to survive, integrate, and form functional vessels in ischemic tissues. Blocking mitochondrial transfer abolished EC engraftment, whereas direct mitochondrial transplantation into ECs recapitulated the effect, underscoring the functional centrality of mitochondrial donation in vascular repair.[4]

Taken together, the available studies demonstrate that ECs can acquire mitochondria from diverse stromal partners—including MSCs, astrocytes, and osteocytes—across several organ systems (Table 1). The conduits vary (TNTs, EVs, or cytoplasmic bridges), as do the physiological contexts (ischemia, chemotherapy, acute respiratory distress syndrome, diabetic kidney disease, aging, bone remodeling, or regenerative transplantation). Despite the diversity of mechanisms and contexts, the findings converge on a common theme. Mitochondrial transfer improves endothelial bioenergetics, promotes survival, and contributes to the preservation of vascular integrity. What was once observed only in isolated in vitro systems has now been substantiated by in vivo evidence, suggesting that intercellular mitochondrial exchange may contribute to vascular homeostasis and adaptation (Figure [B]).

MECHANISMS MEDIATING MITOCHONDRIAL TRANSFER INTO ECs

Intercellular mitochondrial transfer is not a unitary process but rather a collection of mechanisms that allow organelles to move between cells. The best-established routes include TNTs, EVs, and mitochondrial extrusion followed by uptake (Figure [B]; Table 1). Although these pathways have been described in multiple cell types, evidence in ECs has begun to clarify how each operates in vascular contexts, yet important uncertainties remain regarding their relative contribution and regulation.

Tunneling Nanotubes

TNTs are thin, actin-based cytoplasmic bridges that physically connect donor and recipient cells, enabling direct transfer of organelles. First described as dynamic extensions allowing cytoplasmic continuity, TNTs have since been recognized as important mediators of mitochondrial trafficking.[79] Their architecture is supported by F-actin and sometimes microtubules, allowing organelles and macromolecules to be directly transported between cells across intercellular distances.[80–84]

In the context of ECs, TNT-mediated transfer is one of the most clearly demonstrated routes of mitochondrial exchange. In vitro studies of ischemia-reperfusion injury showed that MSCs form TNT-like structures with stressed pulmonary microvascular ECs, donating mitochondria that restore membrane potential and improve respiration.[85] Disruption of TNT formation through the knockdown of trafficking proteins, such as TNFAIP2 (TNF alpha induced protein 2) or Miro1 (mitochondrial Rho GTPase 1), substantially reduced transfer efficiency and impaired EC angiogenic function.[4] Similarly, in models of cytotoxic injury, MSC-to-EC TNTs rescued endothelial survival and restored migratory and angiogenic capacity, reinforcing the notion that TNTs function as an active rescue mechanism rather than an epiphenomenon.[69,71]

At the molecular level, TNT transport depends on motor–adaptor complexes. The mitochondrial GTPase Miro1 recruits TRAK (trafficking kinesin protein) adaptors, which couple mitochondria to kinesin and dynein for microtubule-based transport. Structural work has resolved the Miro1-TRAK1 complex, clarifying how this interface allows motor recruitment and directional trafficking.[86] In addition, Miro proteins interact with myosin XIX to facilitate actin-dependent mitochondrial movement.[87] Functional studies have confirmed that Miro1 overexpression enhances TNT-mediated donation from MSCs to ECs, while its depletion abolishes transfer and blunts endothelial rescue.[88] These data establish TNTs not only as structural conduits but as mechanistically regulated pathways critical for mitochondrial delivery to ECs.

Thus, TNTs represent one of the best-documented mechanisms of mitochondrial uptake by ECs, with functional consequences that include improved survival, enhanced respiration, and restoration of angiogenic competence under stress.

Extracellular Vesicles

EVs represent another route by which mitochondria can be transferred from one cell to another. These membrane-bound particles, including microvesicles, exosomes, and migrasomes, are secreted into the extracellular space as carriers of proteins, nucleic acids, and organelles.[89,90] The packaging of mitochondria within EVs enables long-range delivery, including through circulation, and positions them as a systemic mode of organelle exchange.

For ECs, evidence of EV-mediated mitochondrial transfer is growing. In cultured human brain ECs, microvesicles—but not exosomes—delivered polarized mitochondria that enhanced ATP production and improved survival under hypoxia.[72] Application of these vesicles to acute brain slices further showed transfer into neurons, underscoring their capacity to distribute mitochondria across multiple cell types. Beyond local contexts, adipose tissue has emerged as a systemic donor of mitochondria via EVs. Vesicles released from white and brown adipose depots have been tracked delivering mitochondria to immune cells and, importantly, to vascular ECs. Dietary lipid status influenced this transfer, altering efficiency and targeting, thereby linking metabolic state to endothelial mitochondrial acquisition.[73]

Molecularly, EV biogenesis is regulated by ESCRT (endosomal sorting complexes required for transport) machinery and Ca^{2+}-dependent scission processes.[91–93] Uptake by recipient ECs occurs primarily through endocytosis, a pathway sensitive to NAD^+/CD38/cADPR (cyclic ADP-ribose)/Ca^{2+} signaling.[93] Some mitochondria contained within EVs integrate into the cytoplasm, while others undergo lysosomal degradation. Migrasomes, formed during cell migration, have also been identified as carriers of mitochondria, with regulators such as Miro1, KIF5B (kinesin family member 5B), and Drp1 implicated in their biogenesis.[94] ARRDC1 (arrestin domain–containing protein 1) has been highlighted as essential for packaging mitochondria into vesicles.[95]

The physiological significance of EV-mediated mitochondrial transfer to ECs is still being clarified, yet current findings indicate that it may contribute both locally and systemically, particularly under conditions such as ischemia or metabolic stress.

Extrusion and Uptake of Free Mitochondria

A less common but biologically intriguing route is the extrusion of free mitochondria into the extracellular space, followed by uptake by recipient cells.[96] Stressed or dying cells can actively expel mitochondria, a process often linked to oxidative damage or mitochondrial QC.[97] These organelles may then be internalized by neighboring cells, potentially contributing to intercellular adaptation.

Although this process has been documented in other systems, direct evidence in ECs remains limited. However, experimental mitochondrial transplantation demonstrates that ECs are capable of internalizing exogenous mitochondria when provided artificially, leading to enhanced bioenergetics and improved vascular function.[4] This confirms the capacity of ECs to internalize mitochondria, though whether this occurs naturally in vascular physiology remains to be established. Mechanistically, internalization has been linked to macropinocytosis and fusion proteins such as syncytins, which may mediate membrane interactions.[98,99]

The therapeutic implications of this mechanism are substantial, as exogenous mitochondrial transplantation builds directly on this natural capacity of ECs to accept and use free mitochondria. Still, its role in physiological endothelial biology requires further investigation.

Other Mechanisms

Other modes of mitochondrial transfer beyond TNTs, EVs, and free organelle uptake have also been described. Recent work demonstrated that adipose-derived stromal populations donate mitochondria to ECs through connexin 43–based gap junctions, establishing direct junctional communication as a bona fide route of mitochondrial exchange in vascular contexts.[77] In contrast, evidence for transient cell fusion events or cytoplasmic bridges during angiogenesis remains limited, and their contribution to mitochondrial trafficking is still speculative.[96,100,101] As imaging and lineage-tracing approaches continue to advance, it is likely that additional or hybrid mechanisms of transfer into ECs will be identified.

In summary, although the diversity of transfer routes highlights the versatility of intercellular communication, it also raises open questions about their relative importance in endothelial physiology. Clarifying when and how each mechanism predominates, and how these pathways are regulated in vivo, will be critical for understanding their contribution to vascular health and disease.

MITOCHONDRIAL TRANSPLANTATION

The recognition that mitochondria can move between cells in vivo has naturally prompted efforts to harness this process in controlled settings. Although the discovery of TNTs, EVs, and other transfer routes came later, investigators as early as the 1980s and 1990s had already begun to ask whether isolated mitochondria could be deliberately introduced into recipient cells to restore function. This concept, initially termed mitochondrial transformation, was explored in pioneering studies where mammalian cells acquired antibiotic resistance encoded by donor mtDNA. Remarkably, the phenotype persisted even without selective pressure, showing that transplanted mitochondria could replicate and express within the new host environment.[102,103] These early findings established that mitochondria were not only energy-producing organelles but also vehicles of heritable traits, underscoring the deep functional interplay between nuclear and mitochondrial genomes.

A second major advance came with studies using ρ0 cells—mammalian cells depleted of mtDNA and therefore incapable of oxidative phosphorylation. These cells, dependent on pyruvate and uridine supplementation for survival, regained respiratory competence after the introduction of donor-derived mitochondria.[104] This approach provided direct evidence that exogenous mitochondria could restore bioenergetic capacity in cells otherwise incapable of mitochondrial respiration, establishing a fundamental principle of complementation. Together, these early studies defined mitochondria as transferable and functional organelles, not merely as static cellular components, and they laid the foundation for what has become known as mitochondrial transplantation.

As the field evolved, the terminology shifted from transformation to transplantation, reflecting a broader recognition that mitochondria could be isolated, transferred, and functionally integrated in a therapeutic or experimental context. Although mitochondrial transfer typically refers to physiological, cell-to-cell exchange processes, mitochondrial transplantation has come to denote deliberate experimental or therapeutic introduction of isolated mitochondria into recipient cells or tissues.[105] This distinction is important: transfer emphasizes endogenous biology, whereas transplantation implies intentional intervention.

The shift in nomenclature also aligns with advances in methodology. Early mitochondrial transformation studies focused primarily on demonstrating genetic complementation, but modern mitochondrial transplantation emphasizes the delivery of respiration-competent organelles to restore cell function in injury or disease. As noted in recent consensus discussions, maintaining clarity between these terms helps avoid confusion and better situates experimental findings within either natural physiology or therapeutic intervention.[105,106]

Mitochondrial transplantation generally proceeds in 2 steps: isolation of intact, respiration-competent organelles from donor cells or tissues, followed by their introduction into recipient cells, where they can integrate and augment bioenergetic function. The feasibility of this approach rests on the ability of mitochondria to withstand extracellular manipulation, retain respiratory activity, and be internalized through endogenous uptake pathways.[105]

Mitochondrial Isolation

The standard method for isolating mitochondria is differential centrifugation, which balances yield with preservation of function. Donor cells are gently disrupted—commonly by mechanical homogenization, sonication, or nitrogen cavitation—followed by sequential centrifugation steps. Low-speed spins (≈700–1000×*g*) remove nuclei and large debris, while medium-speed spins (≈10 000–12 000×*g*) enrich the mitochondrial fraction; repeated washes improve purity.[105] Further separation can be achieved using density gradients (sucrose or Percoll), which help distinguish mitochondria from lysosomal or peroxisomal contaminants.[105]

Alongside these classical approaches, newer methods have emerged that emphasize speed and reproducibility. Immunomagnetic capture targeting outer-membrane proteins, such as TOMM20 (translocase of outer mitochondrial membrane 20), uses antibody-conjugated beads to rapidly isolate mitochondria with high specificity. By

reducing processing time and contamination, this technique offers clear advantages in translational and clinical contexts, where standardized preparations are essential.[107]

Together, conventional centrifugation and newer immunomagnetic approaches provide complementary strategies, with trade-offs between scalability, purity, and clinical applicability, for generating mitochondria suitable for transplantation.

Delivery Approaches

After isolation, mitochondria must be introduced into recipient cells. In most experimental systems, this occurs spontaneously through endocytic pathways such as macropinocytosis, allowing extracellular organelles to cross the plasma membrane and integrate into the cytoplasm.[104] This natural uptake is the predominant route described in the literature and forms the basis for most transplantation studies, including those in endothelial systems.[4] The capacity of cells to internalize mitochondria without additional manipulation underscores the feasibility of the approach and has enabled widespread investigation of mitochondrial transplantation in vitro and in vivo.

To complement this intrinsic capacity, several strategies have been developed to enhance uptake or adapt transplantation to specialized settings. Microinjection represents one of the earliest approaches: by directly introducing mitochondria into the cytoplasm, investigators bypassed membrane barriers entirely. Although mainly applied in zygotes and embryos to study mtDNA inheritance and mitochondrial disease, the technique remains technically demanding and unsuitable for scalable applications.[108]

Other efforts have focused on membrane-active carriers. Cell-penetrating peptides, such as Pep-1, have been conjugated to mitochondria to promote translocation across cellular membranes. This approach achieved efficient internalization into mammalian cells, restoring bioenergetic function and improving survival under stress.[109] Lipid-based carriers provide another avenue. Early studies showed that cationic liposomes like Lipofectin could drive mitochondrial uptake by fibroblasts.[110] More advanced strategies, including MitoCoat (1,2-dioleoyl-3-trimethylammonium-propane [DOTAP]/ 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine [DOPE]–based emulsions) and fusogenic liposomes, have since improved uptake efficiency and reduced lysosomal degradation, with benefits reported in models of neuronal injury and cartilage repair.[111,112]

Although spontaneous uptake through endocytic pathways remains the dominant mechanism of mitochondrial transplantation, these engineered approaches demonstrate that uptake can be enhanced or tailored for particular applications, broadening the potential scope of therapeutic use.

Despite these technical advances, the functional consequences of mitochondrial transplantation remain an active area of debate. Two central questions continue to shape the field: (1) whether exogenous mitochondria persist as respiration-competent organelles after internalization, and (2) how a relatively small number of donor mitochondria could exert measurable effects on host bioenergetics.[113,114] Recent studies suggest that transplanted mitochondria may not need to serve as long-term ATP-producing units; instead, their principal effect may be to initiate mitophagy and stimulate remodeling of the host mitochondrial network, promoting clearance of damaged organelles and restoration of metabolic homeostasis.[4] This emerging view reframes mitochondrial transplantation from direct bioenergetic supplementation toward a potential strategy for restoring mitochondrial QC. The impact of mitochondrial transfer is therefore likely to be context-dependent, influenced by delivery method, cellular environment, and the metabolic state of recipient cells.

Donor Source Considerations

A central question in mitochondrial transplantation concerns the origin of donor organelles. Autologous transplantation, in which mitochondria are harvested from a patient's own cells or tissues and reintroduced into that same individual, minimizes immunologic risk and has been the preferred strategy in early preclinical studies.[115–117] Compatibility between mitochondrial and nuclear genomes reduces the likelihood of immune recognition and ensures functional integration, making this approach conceptually straightforward though limited by the practicality of harvesting sufficient donor tissue. In disease conditions characterized by systemic oxidative stress, metabolic disruption, or chronic inflammation, the functional status of mitochondria obtained from the patient may vary, and it remains uncertain whether such mitochondria will consistently provide optimal benefit when transplanted. This consideration suggests that future work will be needed to evaluate tissue sources, develop quality assessment criteria, and explore conditioning strategies to ensure that autologous mitochondria are functionally suitable for transplantation in different clinical contexts.

Allogeneic transplantation, in which mitochondria are derived from a donor of the same species, offers greater scalability and the potential for standardized off-the-shelf preparations. However, variability in mtDNA haplotypes and heteroplasmy introduces uncertainty regarding persistence and function, while also raising the possibility of immune activation.[118,119] Supporting this concern, recent in vivo work has shown that even naturally occurring, nonpathogenic differences between mtDNA haplotypes can disrupt mito–nuclear coordination and lead to cardiometabolic dysfunction, pulmonary hypertension, and systemic frailty in mice.[120] These observations suggest that mtDNA compatibility may influence transplantation outcomes even within a species and warrant continued evaluation in the context of allogeneic approaches. To mitigate these risks, several strategies have been proposed, including

donor screening for mitochondrial quality, haplotype compatibility, and pathogen status, as well as technical approaches such as lipid/EV-mimetic coatings or transient immunomodulation to reduce host responses.[117]

Xenogeneic transplantation, involving transfer across species, has largely remained experimental. The coevolution of nuclear and mitochondrial genomes means that mismatched combinations are prone to mitonuclear incompatibility, often accompanied by immune activation.[121,122] This evolutionary constraint is considered a major barrier to cross-species mitochondrial transfer and helps explain why therapeutic efforts in the field are focused almost entirely on autologous or allogeneic human mitochondria. Classic studies using xenomitochondrial cybrids and cross-species mtDNA replacement models further demonstrated that even small evolutionary differences between mitochondrial- and nuclear-encoded components can disrupt respiratory chain assembly, reduce Complex I and IV activities, and increase oxidative stress.[123–125] These findings provide additional mechanistic context for why cross-species transplantation remains confined to experimental settings.

An additional factor influencing mitochondrial transplantation outcomes is the tissue of origin of donor organelles. Mitochondrial content, respiratory capacity, and stress tolerance differ across cell types and organs, and thus, they could perform differently if used as sources for mitochondrial transplantation. Comparative studies have shown that mitochondria from different tissues display distinct calcium retention capacities, permeability transition thresholds, and propensities for ROS generation.[126] For instance, brain mitochondria are generally more resistant to permeability transition and oxidative stress than those from the liver. These organ-specific differences highlight the need to identify the most compatible and functionally resilient mitochondrial sources for use in transplantation into ECs and vascular repair.

MITOCHONDRIAL TRANSPLANTATION IN CARDIOVASCULAR APPLICATIONS

ECs have been shown in several preclinical models to internalize isolated mitochondria, demonstrating that these organelles can integrate functionally within vascular systems. A recent in vivo and ex vivo study with isolated human ECs demonstrated that exogenous mitochondria enhance endothelial bioenergetics, reduce apoptosis, and improve angiogenic activity.[4] Mechanistically, these effects were linked to activation of mitophagy, which stimulates mitochondrial biogenesis and other cytoprotective pathways in the recipient ECs. In the same study, mitochondrial transplantation also improved EC survival in regenerative settings and supported the formation of functional vessels in ischemic tissues.[4] These observations support the concept that ECs are receptive to exogenous mitochondria and suggest that they could be important targets in therapeutic transplantation strategies (Figure [C]).

More broadly, mitochondrial transplantation has progressed from a conceptual approach in isolated cell systems to a strategy with growing experimental support in selected preclinical vascular models.[118,127,128] Preclinical studies suggest that exogenous mitochondria can be taken up by cardiovascular cells in vivo, where they may help restore oxidative metabolism, reduce injury, and promote repair. Cardiac applications in preclinical animal models have received particular attention, with additional work extending to the peripheral circulation, lung, and brain. Across these settings, mitochondrial transplantation has been reported to improve vascular function and tissue recovery, highlighting its potential relevance to vascular biology and disease (Table 2).

Myocardial Ischemia-Reperfusion Injury

One of the best-developed preclinical lines of evidence for mitochondrial transplantation comes from studies of myocardial ischemia-reperfusion injury. Initial work showed that mitochondria isolated from autologous skeletal muscle and injected into ischemic myocardium at the time of reperfusion improved postischemic recovery. Notably, protection was observed not only with immediate administration but also when delivery was delayed by up to 2 hours, suggesting that the therapeutic window extends beyond the acute phase of ischemia.[133]

Large-animal studies have reinforced these findings. In porcine models, mitochondrial transplantation reduced infarct size and improved indices of contractile function, including ejection fraction, maximum rate of pressure change, and end-diastolic pressure.[133] Repeated intracoronary administration before ischemia (prophylactic delivery) further decreased infarct burden and preserved regional and global myocardial performance.[132] Transplanted mitochondria were internalized by host cardiomyocytes and ECs, retained membrane potential, and supported oxidative phosphorylation. Persistence of donor mitochondria for weeks after delivery has been documented, raising the potential persistence rather than short-term supplementation.[131]

Applications have also extended to cardiac transplantation. In porcine allografts, mitochondrial delivery during organ storage and at reperfusion extended the allowable cold ischemia time to nearly 30 hours while maintaining contractile function.[131] These findings suggest that mitochondrial transplantation may be useful as a potential therapy for acute ischemic injury as well as a preservation strategy in transplantation, where survival of both endothelial and parenchymal cells is critical to graft viability.

Peripheral Vascular Disease

The rationale for mitochondrial transplantation in peripheral vascular disease lies in its potential to restore

Table 2. Preclinical Examples of Mitochondrial Transplantation for Vascular Applications

Animal model	Mitochondrial source	Key outcomes	References
Mouse (C57BL/6J)–acute limb ischemia (tourniquet model)	Skeletal muscle (syngeneic)	Reduced infarct size and apoptosis, preserved muscle viability, and restored gait and limb function	Orfany et al[129]
Rat (Sprague-Dawley)–chronic hypoxia, pulmonary hypertension	Femoral artery smooth muscle (syngeneic)	Reduced hypoxia-induced vasoconstriction, attenuated vascular remodeling, prevented and reversed pulmonary hypertension	Zhu et al[130]
Mouse (C57BL/6J) –heart transplantation (prolonged cold ischemia)	Donor heart muscle (syngeneic)	Preserved graft beating and contractility, improved ejection fraction and shortening fraction, and reduced necrosis and inflammation	Moskowitzova et al[131]
Pig (Yorkshire) –myocardial infarction (ischemia-reperfusion, preischemic MT)	Skeletal muscle (autologous)	Reduced infarct size, improved ejection fraction and coronary blood flow, enhanced regional contractility, and conferred prophylactic cardioprotection	Guariento et al[132]
Pig (Yorkshire) –myocardial infarction (ischemia-reperfusion, delayed MT)	Skeletal muscle (autologous)	Reduced infarct size, improved left ventricular function and pressure recovery, and preserved cardiomyocyte survival	Blitzer et al[133]
Mouse–ischemic stroke (tMCAO)	Placenta (cryopreserved)	Reduced infarct size, preserved antioxidant enzyme activity, and improved neurological outcomes	Nakamura et al[134]
Rat (Sprague-Dawley) –MCT+aortocaval shunt–pulmonary hypertension	Soleus muscle (allogeneic)	Increased lung ATP, restored pulmonary artery contractility, reduced BNP levels, and improved right ventricular function	Hsu et al[135]
Mouse–ischemic stroke (photothrombosis)	Freshly isolated (not specified)	Reduced infarct volume and pyroptosis, promoted neurogenesis, improved cognitive and emotional function, evidence of fusion with microglial mitochondria	Sun et al[136]
Mouse (C57/BL6)– hindlimb ischemia (PAD model)	hMSC (xenogeneic)	Improved perfusion and blood flow recovery and promoted EC proliferation, M2 macrophage polarization, and angiogenesis	Zeng et al[137]
Rat (Sprague-Dawley)–LPS-induced acute lung injury	Soleus muscle (allogeneic)	Preserved alveolar-capillary barrier, improved oxygenation, and reduced inflammation and neutrophil infiltration	Pang et al[119]

Examples summarize preclinical studies of mitochondrial transplantation in vascular disease models. BNP indicates brain natriuretic peptide; ECs, endothelial cells; LPS, lipopolysaccharide; MCT, monocrotaline; MT, mitochondrial transplantation; PAD, peripheral artery disease; and tMCAO, transient middle cerebral artery occlusion.

bioenergetics and promote angiogenesis in ischemic tissues. In murine models of hindlimb ischemia, intramuscular delivery of isolated mitochondria reduced apoptosis across muscle groups, increased ATP content, and improved limb function, as assessed by treadmill-based DigiGait analysis.[129] Treated animals demonstrated earlier restoration of motor capacity compared with controls. Mechanistically, transplanted mitochondria influenced the local tissue environment by promoting M2-like macrophage polarization and inducing browning of white adipose tissue, both processes associated with the release of proangiogenic factors that support vascular regeneration.[129,137]

Most experimental work to date has focused on acute ischemia, but interest is extending toward chronic peripheral artery disease. By enhancing endothelial survival and neovascularization, mitochondrial transplantation could improve tissue perfusion in ischemic limbs. Although direct clinical studies in peripheral artery disease are not yet available, these preclinical findings provide proof of concept and highlight potential avenues for translation. The established capacity of ECs to internalize exogenous mitochondria in vitro further supports the feasibility of targeting vascular cells in ischemic muscle.

Pulmonary Vascular Disease

Mitochondrial transplantation has also been explored in pulmonary vascular disorders, where mitochondrial dysfunction contributes to endothelial and smooth muscle cell abnormalities. In endotoxin-induced acute lung injury, intravenous delivery of isolated mitochondria increased pulmonary ATP levels, improved arterial oxygenation, and reduced alveolar–capillary leakage.[119] These effects were accompanied by restoration of eNOS expression in pulmonary ECs and attenuation of inflammatory damage to the vascular barrier.

More recent work has extended these findings to transplantation settings. In porcine and human ex vivo lung perfusion systems, delivery of mitochondria isolated from porcine heart reduced pulmonary vascular resistance, improved gas exchange, and suppressed proinflammatory signaling.[138] Mechanistically, exogenous mitochondria reduced oxidative byproducts, promoted glutathione synthesis, and induced autophagy in recipient lung cells, including ECs—effects that were blunted by pharmacological inhibition of the MEK (mitogen-activated protein kinase)–autophagy pathway.

Pulmonary hypertension has also emerged as a potential target. In rodent models, mitochondrial transplantation attenuated vascular remodeling, normalized right ventricular mass and wall thickness, and improved pulmonary artery reactivity.[130,135] Collectively, these findings support the concept that mitochondrial transplantation can modulate endothelial and vascular homeostasis in the experimental lung models, with therapeutic implications for both acute injury and chronic remodeling.

Cerebrovascular Injury

The brain vasculature also appears amenable to mitochondrial transplantation. In murine models of focal cerebral ischemia-reperfusion, delivery of mitochondria—whether intravenously or directly into the ischemic region—reduced infarct size, improved neurological recovery, and enhanced cognitive outcomes.[134,136] Mechanistic ex vivo and in vitro work demonstrated that exogenous mitochondria were internalized by microglia and fused with endogenous organelles, where they suppressed pyroptosis, increased ATP production, and promoted neurogenesis. The microglial protein S100A9 was identified as a mediator of this process, facilitating mitochondrial uptake and amplifying anti-inflammatory and proregenerative effects.[134]

Placenta has also been investigated as a donor source. Cryopreserved placental mitochondria maintained high viability, preserved membrane potential, and retained antioxidant enzymes, making them comparable to skeletal muscle–derived preparations. When administered immediately after reperfusion in mouse stroke models, placental mitochondria reduced infarct volume, highlighting the feasibility of banked tissue as a source for therapeutic transplantation.[136]

These findings suggest that mitochondrial transplantation can engage multiple components of the neurovascular unit—including ECs, microglia, and pericytes—to preserve barrier integrity and promote functional recovery after cerebral ischemia. The demonstration that cryopreserved mitochondria remain effective further underscores a potential path toward clinical translation.

Collectively, studies in diverse vascular contexts indicate that transplanted mitochondria are not limited to parenchymal cells but are also internalized by ECs. Endothelial uptake has been associated with improved barrier integrity, reduced apoptosis, and enhanced angiogenic capacity under stress. While functional recovery is usually measured at the tissue or organ level, these findings suggest that endothelial repair may represent an important component of the therapeutic effect.

Overall, preclinical work supports mitochondrial transplantation as a potential strategy to protect and restore the vasculature, with effects documented in the selected preclinical models of the heart, skeletal muscle, lung, and brain (Figure [D]). Although these preclinical findings are promising, most evidence comes from animal models, and translation to human vascular disease remains to be established. Continued efforts to clarify the mechanisms and durability of endothelial uptake will be essential for translating these observations into clinical practice.

CLINICAL AND TRANSLATIONAL OUTLOOK

Clinical translation of mitochondrial transplantation has begun to move from preclinical promise into early human testing (Table 3). Building on decades of animal work across myocardial ischemia-reperfusion, hindlimb ischemia, pulmonary injury, and stroke models, early phase studies are now evaluating feasibility, safety, and practical delivery in patients, including questions of dose, timing relative to reperfusion, and route of administration (intramyocardial, intra-arterial, intravenous).[96,118,127]

One of the first clinical applications of mitochondrial transplantation was carried out at Boston Children's Hospital in pediatric patients with severe myocardial dysfunction requiring extracorporeal membrane oxygenation. Autologous mitochondria were isolated from skeletal muscle and injected into ischemic myocardium, identified by echocardiography. The procedure was performed rapidly at the bedside, was well tolerated, and several patients showed measurable improvement in ventricular function, allowing weaning from circulatory support.[139] This experience underpins an ongoing clinical study (URL: https://www.clinicaltrials.gov; Unique identifier: NCT02851758) designed to further evaluate the safety and feasibility of autologous mitochondrial transplantation in this high-risk population. Although still preliminary and small in scale, the findings provide proof-of-concept that mitochondrial harvest and delivery can be accomplished in critically ill patients and may support myocardial recovery.

Parallel efforts are also examining cerebrovascular disease. At the University of Washington, a first-in-human trial (URL: https://www.clinicaltrials.gov; Unique identifier: NCT04998357) is testing the intra-arterial infusion of autologous mitochondria in adults with acute ischemic stroke undergoing thrombectomy. In this

Table 3. Examples of Clinical Trials of Mitochondrial Transplantation for Cardiovascular Applications

Indication	Donor source	Delivery site/procedure	NCT number
Cerebral ischemia (acute stroke, thrombectomy)	Skeletal muscle (autologous)	Intra-arterial infusion into occluded brain artery during reperfusion	https://www.clinicaltrials.gov; Unique identifier: NCT04998357
Severe myocardial dysfunction requiring ECMO	Skeletal muscle (autologous)	Intramyocardial injection during ECMO support	https://www.clinicaltrials.gov; Unique identifier: NCT02851758
Coronary artery bypass grafting with LV dysfunction	Skeletal muscle (autologous)±MSC-derived exosomes	Intracoronary and intramyocardial injection at time of surgery	https://www.clinicaltrials.gov; Unique identifier: NCT05669144
Polymyositis/ dermatomyositis	Umbilical cord MSCs (allogeneic, PN-101)	Intravenous infusion	https://www.clinicaltrials.gov; Unique identifier: NCT04976140

Examples are registered trials listed on ClinicalTrials.gov. PN-101 is an umbilical cord MSC-derived mitochondrial product developed by Paean Biotechnology. ECMO indicates extracorporeal membrane oxygenation; LV, left ventricle; MSCs, mesenchymal stem cells; and NCT, National Clinical Trial.

design, mitochondria are harvested from a small muscle biopsy during the procedure, isolated at the bedside, and infused through the microcatheter into the occluded artery. Early phase 1 results have shown the approach to be feasible and safe, with no major periprocedural complications reported.[116] Beyond its immediate objectives, the trial is notable in that it integrates organelle delivery into a standard interventional workflow, raising the possibility that mitochondrial supplementation could augment existing reperfusion therapies by mitigating vascular and parenchymal injury at the time it is most likely to occur.[140]

Beyond these early cardiac and stroke applications, other groups are exploring broader strategies. A phase I/II trial at Tehran University (URL: https://www.clinicaltrials.gov; Unique identifier: NCT05669144) is testing autologous mitochondrial transplantation, mesenchymal stem cell–derived exosomes, and their combination in patients with severe left ventricular dysfunction undergoing coronary artery bypass grafting. This multiarm design will help assess whether mitochondria alone are sufficient or whether pairing them with exosome-mediated paracrine support can enhance recovery.

Efforts to develop off-the-shelf mitochondrial products are also emerging. Paean Biotechnology has advanced PN-101, an allogeneic preparation derived from umbilical cord mesenchymal stem cells. A first-in-human Phase I/II trial in South Korea tested PN-101 in patients with refractory polymyositis and dermatomyositis, reporting that intravenous administration was safe and showed preliminary signs of clinical benefit.[141] While distinct from cardiovascular applications, this study represents a milestone for scalability and commercialization, as it demonstrates that donor-derived mitochondria can be standardized and delivered across individuals. The results will help clarify whether allogeneic products can complement, or in some settings replace, autologous transplantation—particularly in acute vascular emergencies where harvesting patient-derived mitochondria may be impractical.

Across these first-in-human studies, a consistent theme is the feasibility of isolating and delivering respiration-competent mitochondria to target tissues in real time.[96,118,127] Approaches ranging from intramyocardial injection to intra-arterial infusion have proven technically achievable and, to date, safe in early cohorts. These results justify cautious expansion into larger, controlled trials, even as key questions remain about the persistence, biodistribution, and mechanistic basis of benefit.[113] The translational path mirrors that of other biologic therapies: beginning with autologous strategies to establish proof of principle, then evolving toward scalable allogeneic products. For vascular medicine, the convergence of preclinical efficacy, mechanistic insight, and emerging human data marks the point at which mitochondrial transplantation is shifting from an experimental concept to a potential therapeutic modality.

FUTURE DIRECTIONS AND CONCLUDING REMARKS

The recognition that mitochondria are not only metabolic organelles but also dynamic mediators of endothelial health is reshaping our understanding of vascular biology. Evidence from both experimental and clinical contexts has demonstrated that EC function is intimately linked to mitochondrial integrity and that exogenous mitochondria can restore or augment vascular performance under stress. These insights open a new therapeutic horizon, but they also highlight the many unanswered questions that will define the trajectory of the field in the coming decade.

A central priority is to delineate the role and mechanisms of mitochondrial transfer in the vascular system, both in health and disease. Reports of intercellular mitochondrial exchange are accumulating across multiple organs and cell types, yet evidence in ECs and blood vessels is only beginning to emerge. Progress in this area will require not only improved imaging and labeling strategies but also the development of genetically engineered models with mitochondria-specific reporters in defined vascular lineages. Such tools would enable rigorous tracing of transfer events in vivo and provide the mechanistic insight necessary to understand how mitochondrial exchange contributes to vascular homeostasis, repair, and pathology. In addition, because ECs exhibit organ- and vascular bed–specific metabolic and functional heterogeneity,[1,142] it will be important to determine whether mitochondrial transplantation exerts distinct effects in different tissue contexts.

Building on this physiological perspective, mitochondrial transplantation represents a related but distinct paradigm: rather than tracing endogenous transfer events, it entails the deliberate isolation and delivery of mitochondria into target tissues. In recent years, experiments in ECs and preclinical models of the vascular system have established proof of principle for this approach. Clinical translation has followed rapidly, with early trials demonstrating that mitochondria can be harvested and administered in real-time to critically ill patients, supported by encouraging safety signals. Yet the basis for their therapeutic benefit remains unresolved, and direct evidence of transplanted mitochondria integrating into vascular cells is still sparse. Whether improvements in organ function result from bioenergetic rescue of ECs, modulation of inflammatory pathways, or paracrine signaling triggered by transient uptake remains to be determined. Genetically engineered mouse models featuring mitochondria-specific fluorescent reporters in vascular lineages, several of which are commercially available, will be valuable for visualizing transplanted organelles in vivo and for defining whether and how they persist and integrate within host mitochondrial networks. Clarifying these mechanisms will be essential for optimizing

therapeutic design, defining the requirements for persistence, and establishing meaningful end points for clinical monitoring.

Another frontier for clinical translation is technology development. Current protocols rely on freshly isolated, autologous mitochondria—a practical approach for proof-of-concept but constrained in scalability. Progress in cryopreservation, encapsulation, and biomaterial scaffolding may enable mitochondria to be stabilized, targeted, and released in a controlled manner. Complementary strategies, such as engineering mitochondria to resist oxidative stress or to preferentially integrate into ECs, could further enhance efficacy. Collectively, these innovations will determine whether mitochondrial transplantation can progress from a highly individualized procedure to a broadly applicable therapeutic platform.

Vascular medicine is uniquely positioned to drive this translational trajectory. ECs lie at the interface of every organ system, and their dysfunction is a unifying feature of ischemic, inflammatory, and degenerative diseases. Restoring mitochondrial health in ECs could therefore yield benefits that extend beyond the vasculature, shaping tissue regeneration, immune responses, and metabolic balance. Embedding mitochondrial transplantation within existing interventional workflows—such as reperfusion procedures or surgical revascularization—offers a pragmatic near-term pathway, allowing the technology to enhance rather than replace established therapies at the moment of greatest vulnerability.

Looking ahead, progress will require a dual agenda: elucidating the biology of mitochondrial transfer in vascular systems and refining the translational toolkit to make interventions practical, safe, and scalable. This will depend on close collaboration between vascular biologists, bioengineers, immunologists, and clinicians, as well as the development of robust metrics—functional assays, imaging platforms, and molecular signatures—that can track mitochondrial health and exchange in vivo.

Mitochondrial transplantation is still in its early stages of clinical development, yet it has already challenged conventional assumptions about organelle autonomy and therapeutic feasibility. Its promise in vascular health lies not only in immediate applications but also in what it reveals about the plasticity of EC biology. Defining how mitochondria are exchanged in the vasculature, clarifying the mechanisms by which transplanted organelles confer benefit, and establishing strategies for scalable delivery will be essential steps toward making mitochondrial transplantation a transformative therapy—one that can restore EC function, preserve vascular integrity, and expand the therapeutic landscape for vascular disease.

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