Current progress of mitochondrial transplantation that promotes neuronal regeneration

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Abstract

Background: Mitochondria are the major source of intracellular adenosine triphosphate (ATP) and play an essential role in a plethora of physiological functions, including the regulation of metabolism and the maintenance of cellular homeostasis. Mutations of mitochondrial DNA, proteins and impaired mitochondrial function have been implicated in the neurodegenerative diseases, stroke and injury of the central nervous system (CNS). The dynamic feature of mitochondrial fusion, fission, trafficking and turnover have also been documented in these diseases.

Perspectives: A major bottleneck of traditional approach to correct mitochondria-related disorders is the difficulty of drugs or gene targeting agents to arrive at specific sub-compartments of mitochondria. Moreover, the diverse nature of mitochondrial mutations among patients makes it impossible to develop one drug for one disease. To this end, mitochondrial transplantation presents a new paradigm of therapeutic intervention that benefits neuronal survival and regeneration for neurodegenerative diseases, stroke, and CNS injury. Supplement of healthy mitochondria to damaged neurons has been reported to promote neuronal viability, activity and neurite re-growth. In this review, we provide an overview of the recent advance and development on mitochondrial therapy.

Conclusion: Key parameters for the success of mitochondrial transplantation depend on the source and quality of isolated mitochondria, delivery protocol, and cellular uptake of supplemented mitochondria. To expedite clinical application of the mitochondrial transplantation, current isolation protocol needs optimization to obtain high percentage of functional mitochondria, isolated mitochondria may be packaged by biomaterials for successful delivery to brain allowing for efficient neuronal uptake.

Keywords: Mitochondrial dynamics, Mitochondrial therapy, Neurodegenerative diseases, Stroke, Neuronal regeneration

Background

Mitochondria are double-membraned cytoplasmic organelles that generate the majority of adenosine triphosphate (ATP) via oxidative phosphorylation. In addition to energy production, mitochondria also function in the biosynthesis of fatty acids, cellular calcium buffering, and act as a platform to integrate cell signalling circuitry that modulates cell survival, immune response, and autophagy [1, 2]. It has been hypothesized that mitochondria evolved from engulfed prokaryotic bacteria so that they possess their own circular DNA (mitochondrial DNA, mtDNA) encoding 37 genes and 13 mitochondrial proteins. Together with nuclear encoded mitochondrial proteins, they maintain mitochondrial integrity [2–4].

Research in the past decade has unveiled that mitochondria are dynamic bioenergetic organelles undergoing controlled fusion, fission, transport, and targeted turnover. Mitochondrial population and quality are controlled in part by dynamic morphogenesis. Initiation of mitochondrial fission starts with recruiting cytosolic dynamin-related protein 1 (Drp1) to mitochondrial outer membrane and forming Drp1 oligomers at candidate fission site, which is marked by ER-mitochondria contact region. Drp1 oligomers then constrict mitochondrial membrane upon GTP hydrolysis to divide mitochondria.
of body's energy. Thus, mitochondrial localization within dendrites and axons supply energy as well as to maintain calcium homeostasis [14]. It is thus not surprising to find that mitochondrial distribution and transport are essential for synaptogenesis and dendritic spine formation during development as well as for regulating neuronal activity and behaviour [11, 14]. The dependency of neuronal function and structure on mitochondrial integrity and dynamics is echoed by increasing studies that demonstrate mitochondrial dynamic abnormalities in the well documented neurodegenerative diseases, such as Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), ischemic stroke and traumatic brain injury (TBI) [15–17] . To this end, better understanding the mechanism underlying defective mitochondrial dynamics and function in these diseases would provide insights into the improvement of clinical treatment. In this review, we summarize and discuss recent reports that lead to the emerging mitochondrial therapy.

**Mitochondrial dynamics and diseases**

**Neurodegenerative diseases**

Due to the complexity and therapeutic setbacks of current treatment for neurodegenerative diseases, increasing attention points to the mitochondria-related pathogenesis [15, 18]. Reduced utilization of glucose in the brain measured by flurodeoxyglucose positron emission tomography (FDG PET) suggests metabolic defect in AD brain and prompts the exploration of the role of mitochondria in AD pathogenesis [19]. In AD, increased S-nitrosylation at Cys644 and phosphorylation at Ser616 of Drp1 protein enhance the GTPase activity and lead to mitochondrial fragmentation [20, 21]. Inhibition of Drp1 in AD models restores amyloid beta (Aβ)-mediated mitochondrial dysfunction, synapse damage, and cognitive impairment. Increase of mitochondrial fragmentation in AD subjects could also result from up-regulated fusion proteins (Drp1, Fis1) and down-regulated fusion proteins (Mfn1, Mfn2, OPA1) that partially contribute to gradual neuronal loss and synaptic impairment [22–26]. In addition, the absence of an autophagy/mitophagy regulator PTEN-induced putative kinase protein 1 (PINK1) on OMM within neurofibrillary tangles of AD brain fails to recruit Parkin protein upon membrane depolarization and thus underlies the accumulation of damaged mitochondria in AD patients [27]. Intra-hippocampal injection of PINK1-expressing construct to transgenic mice that overexpress human form of mutant amyloid precursor protein effectively alleviates Aβ-mediated mitochondrial dysfunction and rescues the mitophagy defect via recruiting autophagy receptors (nuclear dot protein 52 kDa, optineurin) to damaged mitochondria to activate mitophagy signalling [26, 28]. PD-associated leucine-rich repeat kinase 2 (LRRK2) mutant and HD-associated mutant huntingtin protein (mHtt) were found interacting with Drp1 to enhance mitochondrial
fission, accompanied by defective anterograde mitochondrial transport and synapse degeneration [29, 30]. The toxicity of mutant PD-associated proteins, including PINK1, Parkin, LRRK2, protein deglycase DJ-1, vacuolar protein sorting-associated protein 35, and α-synuclein, accounts for mitochondrial fission, impaired mitophagy, and neuronal death in the PD genetic models [15, 31]. Loss of synapses concurred with deficiency of mitochondrial complexes I and IV in PD neurons within substantia nigra (SN) were also observed [32]. For HD patients, mHtt protein directly or indirectly alters mitochondrial morphology, functions, bioenergetics status, and dynamics, mainly in the striatum and cortical cerebrum [33, 34]. In addition to mHtt-Drp1-interaction-mediated mitochondrial fission [35], mHtt interacts with OMM and leads to defect of calcium homeostasis. High sensitivity of mitochondria to calcium-induced permeability transition pore in mHtt-expressing clonal striatal cells (conditionally immortalized cells of striatal origin) and striatal neurons results in increased calcium release in the presence of ROS stress [36–38]. However, the clearance of defective mitochondria via mitophagy is inhibited due to the binding of mHtt aggregates to the adaptor proteins, such as p62 and huntingtin-associated protein-1, during formation and transport of autophagosomes [39–41]. Notably, it was demonstrated in HD mice that decreased activity of mitochondrial complex IV and reduced ATP production in striatal cells precede neuronal death [42]. Inhibiting mitochondrial citric acid cycle by administrating 3-nitropropionic acid in the animal models resembles the pathology and symptomatology in HD [43, 44].

**Stroke**

Mitochondrial fission was regarded as an early pathological event in ischemic stroke mice and accompanied by morphological change of mitochondria, high level of free radicals, and ATP depletion [45]. In the middle cerebral artery occlusion (MCAO) mice model, mitochondrial fission occurred in penumbra region 3 h after reperfusion [45]. Another study showed that oxygen-glucose deprivation (OGD)-induced mitochondrial fission resulted in neuronal cell death and inhibition of Drp1 by siRNA or pharmacological inhibitors prevented mitochondrial fission, reduced death of cortical neurons and reduced the infarct volume in ischemic stroke mice [46]. PINK1 was reported to prevent subcellular translocation of Drp1 and reversed mitochondrial fission induced by OGD. Knockdown of PINK1 caused an increase in fragmented mitochondria and worsened the collapse of mitochondrial membrane potential [47]. The MCAO mice and hypoxic/ischemic condition in hippocampal neurons suppressed the expression of Mfn2. Overexpression of Mfn2 increased the ratio of Bcl-2/Bax and reduced the cleaved caspase 3 and cytochrome c release after hypoxia [48]. These studies indicate that the excess of mitochondrial fission induced by stroke leads to mitochondrial damage and cell death. Thus, restoration of the imbalanced mitochondrial dynamics may potentially be a way to attenuate stroke-induced neuronal death.

**Traumatic brain injury**

Studies dated back in 1960s revealed increased number of mitochondria following neuro-anatomy of motor neurons [49, 50]. Mitochondrial swelling were observed in isolated sensory ganglions from limb-amputated newt [51] and in dorsal root ganglions after sciatic nerve crush in rat [52]. Dimova et al. performed axonal section on rat hypoglossal neurons and noted the increased clustering of hypertrophic mitochondria around axon hillock along with strong respiration activity (Fig. 1a and b) [53]. Our previous study reported that fragmented mitochondria were increased 24–48 h after injury in primary hippocampal neurons [13]. Another study showed reduced length of mitochondria in hippocampal neurons after TBI in a controlled cortical impact (CCI) mouse model. The aberrant mitochondrial fission was caused by the increase in Drp1 translocation but not total Drp1 level. Excessive Drp1-mediated mitochondrial fission in TBI animals impairs mitochondrial respiration, leads to reactive oxygen species (ROS) overproduction, and neuronal loss [16]. Mitochondrial division inhibitor 1 (Mdivi-1) treatment attenuated the reduction of mitochondrial length and protected new-born neurons in the hippocampus post injury [16]. A recent study reported that Mdivi-1 blocked the induction of mitochondrial fission and mitophagy in a CCI model of moderate TBI [54]. It appears that TBI induces mitochondrial fission and inhibiting fission can reduce the damage caused by TBI. However, another study on TBI model of rats suggests that the change of mitochondrial fission/fusion dynamics depends on injury severity. The expression level of the genes involved in fission and fusion were down-regulated and up-regulated, respectively, following a mild TBI. In contrast, mitochondrial fission was increased following a severe TBI [55]. Due to the complexity of TBI, it remains debatable whether mitochondrial fission enables higher mobility of mitochondria to the injury site for regeneration or is a result of tissue damage. Nevertheless, these two conclusions do not necessarily conflict with each other.

**A new paradigm of therapeutic strategy: mitochondrial therapy**

**Mitochondrial dynamics and neuronal regeneration**

As accumulating data demonstrate the interplay between defective mitochondrial biogenesis and diseases, several lines of evidence reveal dynamic morphogenesis during neuronal regeneration. Our laboratory previously reported
that increased mitochondrial fusion promoted survival of hippocampal neurons in response to low-dose ionizing radiation (Fig. 1b) [56]. Interestingly, in response to TBI, mitochondrial fission was increased in hippocampal neurons allowing faster mobilization of smaller/fragmented mitochondria to the injury site, likely to facilitate regeneration process [13]. Along this line, live cell imaging of regenerating neurons after laser axotomy of γ-aminobutyric acid motor neurons of C. elegans and Mauthner axons of zebra fish suggests that increased number of mitochondria translocated in injured axons and that mitochondrial mobility is positively correlated with axonal regeneration [57, 58]. Furthermore, genetic knockout of Snph, a gene encoding mitochondria-anchoring protein syntaphilin, improved mitochondrial motility in axons after in vivo sciatic nerve injury and enhanced axonal regeneration [59]. Similarly, overexpression of mammalian-specific mitochondrial protein Armcx1 in adult retinal ganglion cells mobilized mitochondria in axons and promoted neuronal survival as well as axonal re-growth [60].

These studies raise a possibility that higher mitochondria number and motility in injured neurons may provide better regenerative capacity both in the peripheral nervous system and the central nervous system (CNS) (Fig. 1c) [61–63].

**Mitochondrial therapy**

The concept of “mitochondrial medicine”, which refers to medical intervention targeting mitochondria, boots a new line of biomedical endeavor. Mitochondrial therapy aims to restore mitochondrial functions, such as mildly inducing mitochondrial uncoupling, boosting energy production, and antagonizing the release of ROS. New drugs in forms of mitochondrial membrane uncoupling agents (eg. 2,4-dinitrophenel, uncoupling protein-2, uncoupling protein-3), electron transfer chain-boosting substrates (eg. dichloroacetate, thiamine), metabolism modulators (eg. Metforin) and antioxidants (eg. coenzyme Q10, MitoQ, RP103) have been developed or preclinically tested [2, 64, 65]. By the end of July 2018, there were more than 400 completed or ongoing clinical trials for mitochondria-targeted medical intervention registered at ClinicalTrials.gov. However, there is currently no medicine to cure mitochondria-related diseases caused by inefficient energy production, and the loss of normal physiological ROS function. Therefore, a new paradigm of mitochondrial therapy based on organelle delivery strategy was established. Supplement of healthy mitochondria into cells containing damaged mitochondria was beneficial to improve energy generation, reverse excessive ROS production, and restore mitochondrial function. Findings in recent years
| Disease or injury state | Source of mitochondria | Recipient | Method of delivery | Mechanism of mitochondria uptake | Outcome | Reference |
|------------------------|------------------------|-----------|-------------------|---------------------------------|---------|-----------|
| In vitro               |                        |           |                   |                                 |         |           |
| Ischemia               | MMSCs                  | Cortical neurons & astrocytes | Co-culture | Cytosol transfer | Improved cell viability | Babenko et al., 2015 |
| UV light damage        | PC12                   | PC12      | Co-culture        | TNTs                           | mtDNA transfer | Wang and Gerdes, 2015 |
| Ischemia/reperfusion   | BM-MSCs                | H9c2      | Co-culture        | TNTs                           | Reduced apoptosis process | Han et al., 2016 |
| TBI                    | Cortical neurons       | Hippocampal neurons | Add in medium     | Not discussed                  | Enhanced neuroregeneration | Chien et al., 2018 |
| In vivo                |                        |           |                   |                                 |         |           |
| Acute lung injury      | mBMSCs; hBMSCs         | Aveolar epithelia | Intranasal instillation | Cx43-dependent nanotubes and micro-vesicles formation | Increased alveolar [ATP] and abrogated ALI pathologies | Islam et al., 2012 |
| In situ blood-perfused regional ischemia | Autologous rabbit muscle cells | Myocardial cells | Injection of mitochondria-containing respiration buffer | Actin-dependent organelle-to-cell transfer | Decreased myocyte necrosis and enhanced post-ischemic function | Masuzawa et al., 2013 |
| Transient focal cerebral ischemia | Mouse cortical astrocytes | Peri-infarct cortex | Direct injection or autologous secretions | Integrin-mediated astrocyte-to-neuron mitochondrial transfer | Promoted adjacent neuronal survival and plasticity after injury transfer | Hayakawa et al., 2016 |
| Parkinson’s disease    | PC12; human osteosarcoma cybrids | PD rats/ brain neurons | Local injection at medial forebrain bundle | Pep-1-mediated cell-penetrating mitochondrial delivery | Improved locomotive activity and attenuated deterioration of dopaminergic neurons | Chang et al., 2016 |
| Acute myocardial infarction | Autologous porcine muscle cells | Myocardial cells | Injection of mitochondria-containing respiration buffer | Not discussed | Enhanced myocardial cell viability following ischemia and reperfusion | Kaza et al., 2017 |
| Parkinson’s disease    | HepG2                  | Multiple tissues | Intravenous injection | Not discussed | Increased ETC activity, decreased ROS formation, apoptosis and necrosis | Shi et al., 2017 |
| Spinal cord injury     | PC12; syngeneic muscle cells | Brain macrophages, endothelium, pericytes, glia | Microinjection at mediatolateral grey matter | Zipper-like actin-mediated phagocytosis | Maintenance of acute mitochondrial bioenergetics, enhanced behavioral recovery | Gollihue et al., 2017 |
| Non-alcoholic fatty liver disease | HepG2                  | Multiple tissues | Intravenous injection | Not discussed | Decreased lipid content and restored cellular redox balance | Fu et al., 2017 |
| Acetaminophen-induced liver injury | HepG2                  | Multiple tissues | Intravenous injection | Not discussed | Increased hepatocytes energy supply, reduced oxidation stress | Shi et al., 2018 |

MMSCs mesenchymal multipotent stroma cells, PC12 pheochromocytoma cell line, TNTs tunneling nanotubes, BM-MSCS bone marrow-derived mesenchymal stem cells, H9c2 heart myoblast cell line, TBI traumatic brain injury
m/hBMSCs mouse/human bone-marrow-derived stromal cells, Cx43 connexin 43, HepG2 hepatocellular carcinoma cell line, ETC electron transfer chain, ROS reactive oxygen species
have demonstrated the promising outcome upon receiving mitochondrial delivery using in vitro and in vivo models (Table 1) and in several completed or on-going clinical trials (Table 2) [2, 66]. In the following section, we will review recent application of mitochondrial delivery techniques in experimental animals modelling human diseases and highlight the therapeutic potential of delivering isolated mitochondria for the management of neurodegenerative diseases, cerebral stroke, and TBI.

**Mechanism of mitochondrial uptake by cells**

Mechanisms underlying mitochondrial internalization have been reported (Table 1 and Fig. 2) [67]. Organelle transfer through cell-to-cell fusion or via mitochondria-containing vesicles was observed in bone-marrow-derived stroma cell-to-lung epithelium mitochondrial transfer to mitigate acute lung injury [68]. Tunneling nanotubes (TNTs)-dependent mitochondrial transfer has been well-characterized [68–70]. This actin-based structure was found to mediate mitochondrial exchange between healthy and UV stress-damaged PC12 cells to prevent damaged cells from apoptosis. Nanotube-mediated mitochondrial transfer from co-cultured mesenchymal stem cells to epithelium was reported to rescue cigarette smoke-induced lung damage [71]. Notably, recent study discovered an intriguing mechanism by which stroke-induced activated astrocytes released mitochondria-containing particles and these particles entered damaged neurons through actin-dependent endocytosis to prevent neuronal death [72, 73].

**Mitochondrial delivery for neurodegenerative diseases, cerebral stroke and TBI**

As in vivo mitochondrial supplementation in cardiac ischemia models set a milestone for organelle delivery-based therapy, this approach was also applied to neurodegenerative diseases, cerebral stroke, and TBI. Hereinafter, we review the approach of mitochondrial delivery in degenerating, hypoxic, or injured nervous system.

**Neurodegenerative diseases**

Due to limited understanding of molecular basis underlying AD pathogenesis, available drugs approved by the Food and Drug Administration of the United States for AD, such as acetylcholinesterase inhibitors galantamine, donepezil, and rivastigmine, can simply relieve the symptoms [74, 75]. Since the 1980s, many studies have revealed mitochondrial abnormalities in the AD subjects, including structural change, deficiency of Kreb cycles enzymes, reduced cytochrome oxidase activity, and the disturbance of calcium homeostasis [76–79]. Mitochondrial delivery in AD model was originally conducted in the in vitro cybrid cell system. Cybrids were generated by fusing mtDNA-depleted human neuroblastoma cell line, SH-SY5Y, or teratocarcinoma cells Ntera2/D1 (NT2), with mitochondria from platelets of AD patients [80, 81]. Reduced activity of mitochondrial complex IV, elevated ROS production, higher cytosolic calcium concentration, and defective cytochrome c oxidase, were found in the AD cybrids compared to non-AD control cybrids. Based on these discoveries, mitochondrial cascade hypothesis in the pathogenesis of sporadic AD was then proposed by Khan et al, suggesting that baseline mitochondrial function and durability determine aging-related mitochondrial changes and would progress to AD [82, 83]. Although pre-clinical studies on many anti-oxidants, such as α-tocopherol, for treating AD were found effective in experimental AD animal models, few clinical trials have succeeded. Given the complexity of AD pathophysiology as well as limited efficiency of drug delivery, improved therapeutic strategy of mitochondrial therapy is needed.

Mitochondrial dysfunction aggravates the progression of PD, manifested by increased oxidative stress, dysregulated bioenergetic homeostasis, and reduced viability of affected SN dopaminergic neurons. While mitochondria-targeting antioxidant was considered of great potential for treating PD, existing agents have limited effect on preventing PD from deterioration even if there was promising outcome in animal models and pre-clinical tests [84, 85]. For example, antioxidant drugs, coenzyme Q10 and creatine monohydrate, failed to significantly alleviate the progression in patients with PD in the clinical

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**Table 2** Registered interventional studies for mitochondrial transplantation on ClinicalTrials.gov

| Conditions/Diseases | Status | Phase | Intervention | Mitochondria donor | NCT number |
|---------------------|--------|-------|--------------|--------------------|------------|
| Age-related deterioration of oocyte quality | Withdrawn | I&2 | Injection of autologous mitochondria to the oocytes during ICSI | Autologous granulosa cells | NCT01631578 |
| Infertility | Completed | NA | Autologous micro-injection of mitochondria into the oocytes | Autologous ovarian stem cells | NCT02586298 |
| Mitochondrial diseases: Pearson Syndrome | Not yet recruiting | Early 1 | Mitochondria augmentation therapy: transplantation of autologous stem cell enriched with MNV-BLD | Autologous peripheral hematopoietic stem cells | NCT03384420 |
| Extracorporeal membrane oxygenation complication | Recruiting | NA | Autologous mitochondria injected or infused into the ischemic myocardium | Autologous skeletal muscle cells | NCT02851758 |

NA not applicable, ICSI intracytoplasmic sperm injection, * MNV-BLD refers to blood-derived mitochondria
Therefore, instead of targeting a single specific aspect of mitochondrial function, supplementing healthy mitochondria to damaged regions in PD brain may potentially be an innovative strategy for improving clinical outcome. To this end, several studies set out to examine the efficacy and feasibility of mitochondrial delivery in inhibiting PD progression. Chang et al. demonstrated that cell-penetrating peptide-based mitochondrial delivery in 6-hydroxydopamine (OHDA)-treated PC12 cells rescued mitochondrial respiratory function, improved cell viability, and promoted neurite growth when treated the PC12 cells with nerve growth factor [88]. Xenogeneic/allogeneic injection of mitochondria into medical forebrain bundle (MFB) of 6-OHDA-unilaterally infused PD rats enhanced the survival of dopaminergic neurons as well as effectively sustained mitochondrial functions by restoring the normal level of mitochondrial complex I-IV and relieving mitochondrial oxidative stress in vivo. Upon receiving supplemented mitochondria, protein levels involved in mitochondrial fusion (Mfn2, OPA1), fission (Drp1), and deterioration (Parkin) in dopaminergic neurons within SN were restored. In addition, mitochondrial transplantation in MFB improved locomotive activity of 6-OHDA-induced PD rat. In the other study conducted by Shi et al., MPP (1-methyl-4-phenyl-pyridinium)-treated SH-SY5Y cells incubated with intact isolated mitochondria improved cell viability in a dose-dependent manner [89]. ATP production, mitochondrial complex I activity and cell survival were rescued after mitochondrial supplementation while the level of ROS significantly lowered, compared to MPP+ control cells. The initial report by Shi et al. showed that systemic intravenous mitochondrial administration to respiratory chain inhibitor MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced PD mouse model prevented PD progression [89]. In vivo distribution of intravenously-injected mitochondria was found in multiple organs, including brain, 2 h after intravenous injection. As a result, striatal mitochondria in MPTP-induced PD mice showed increased ATP content, restored mitochondrial complex I activity, and decreased ROS production with improved locomotor activity.

**Stroke**

Current intervention for stroke is limited owing to narrow therapeutic time window after the occurrence of ischemic stroke. Ischemia-induced OGD in affected regions leads to low ATP production, excessive ROS release from mitochondria, ionic disequilibrium across mitochondrial membranes, and eventually programmed cell death [17, 90]. As accumulating evidence links mitochondrial deficit to brain
impairment following ischemic stroke, therapeutic regimen was developed aiming to restore mitochondrial physiology. In light of new concept of intercellular organelle-transfer, Hayakawa et al. demonstrated that CD38 signalling mediated release of functional mitochondria from activated astrocyte. These mitochondria then entered damaged cortical neurons, restored ATP level and neuronal viability after OGD injury. Treatment with extracellular mitochondria-containing particles, released from cultured astrocytes in a mouse model of focal cerebral ischaemia, provided neuroprotection. In vitro astrocyte-to-neuron mitochondrial delivery and in vivo astrocyte-derived mitochondrial transfer promoted neuronal survival, plasticity, as well as improved behavior outcome [72]. Besides, it has been reported that mitochondria are transferred from mesenchymal multipotent stromal cells to co-cultured neurons. Intravenous administration of mesenchymal multipotent stromal cells to MCAO rats reduced infarction area and improved post-stroke neurological indexes. Treatment of “primed” stem cells, which had been previously co-cultured with neuron cells, caused a more pronounced beneficial outcome in rats after stroke [73]. Transfer of exogenous mitochondria via local intracerebral or systemic intrarterial injection reduced brain lesion, cell death, and restored motor function in MCAO rats [91]. In addition, autologous mitochondrial transplantation has been studied in rabbit ischemic heart model. After regional ischemia, autologous skeletal muscle-derived mitochondria were injected into ischemic zone of heart prior to reperfusion. Mitochondrial transplantation significantly reduced myocyte necrosis, infarction volume and improved post-ischemic recovery of cardiac function without eliciting any immune or inflammatory response. Moreover, biochemical markers of myocardial infarction, creatine kinase-muscle/brain isoenzyme and cardiac troponin I, were reduced after mitochondrial transplantation [92]. Follow-up study using porcine cardiac ischemia/reperfusion model showed similar results in that autologous mitochondrial transplantation enhanced post-ischemic myocardial cell viability, reduced infarction size and deceased myocardial injury biomarkers [93]. These successful cases highlight the effective mitochondrial therapy in post-stroke neuroprotection, preserving cell viability and promoting functional recovery.

**Clinical application of mitochondrial transplantation**

*Techniques for mitochondrial delivery*

The effectiveness of mitochondrial therapy is expected to be variable among patients due to the heterogeneity of pathogenesis and efficiency of mitochondrial internalization into the affected tissues. Successful uptake of mitochondria by target tissues depends on the amount, quality of mitochondria and proper routes of organelle delivery. Therefore, better understanding of the mechanisms underlying mitochondrial delivery and cellular uptake will facilitate the translation of mitochondrial transplantation in clinic.

A number of in vivo studies documented feasible approaches of mitochondrial transplantation, including microinjection directly to affected sites in SCI, stroke, and PD models [88, 92–94], and intravenous administration in PD and fatty liver models [89, 100]. In PD, to improve functional incorporation of supplemented mitochondria, a novel strategy of peptide-mediated allogenic mitochondrial delivery (PMD) was applied to neurotoxin-induced PD rats. Direct microinjection of Pep-1-modified allogenic mitochondria into MFB promoted cellular uptake of
mitochondria compared to the injection of naïve mitochondria or xenogenic PMD. It was clear that PMD successfully rescued impaired mitochondrial respiration, attenuated oxidative damage, sustained neuron survival, and restored locomotor activity of PD rats [88]. Nevertheless, the conjugation ratio of Pep-1 and mitochondria should be optimized to avoid undesired mitochondrial aggregation. Moreover, the conjugation time and human manipulation should be minimized before clinical translation.

Another study systemically administered isolated mitochondria via tail vein improved locomotor activity in PD mouse model, albeit differential distribution of injected mitochondria in brain, heart, liver, kidney, and muscle [89]. The feasibility of intravenous mitochondrial delivery was achieved by smaller size of the organelle (~1 μm in diameter) compared to that of red blood cells (6–8 μm in diameter) and that supplemented mitochondria are not to be incorporated into red blood cells to interfere oxygen transport.

**Clinical trials**

The burgeoning of mitochondrial therapy opened a new era for reversing mitochondrial function in human diseases. Thus far, few registered clinical trials for treating neurodegenerative diseases, stroke, or TBI based on mitochondrial delivery technique have been launched. To date, there is only one completed trial which aimed to treat infertility by autologous mitochondrial injection into oocytes (Table 2, NCT#02586298). Autologous ovarian mitochondria were isolated prior to in vitro intracytoplasmic sperm injection (ICSI). The outcome was determined by ongoing rate of pregnancy within 12 weeks after mitochondrial therapy, as the improvement in preimplantation genetic screening and embryo quality were also evaluated. An ongoing trial tries to demonstrate the feasibility of mitochondrial transplantation, using autologous mitochondrial injection (Table 2, NCT#02851758), for rehabilitating myocardial ischemia/reperfusion injury and is currently recruiting participants. Mitochondria will be isolated from autologous skeletal muscle from patients undergoing surgical re-operation or catheterization and directly injected into affected myocardium or proximal aorta, or via intracoronary infusion. The outcome will be measured by the safety and the improvement of ventricular function after therapeutic intervention.

**Conclusions**

Previous proposals for treating mitochondrial dysfunction have been targeting specific mitochondrial residents and fusion/fission regulators [64, 65]. The outcome of these approaches has not been satisfactory. The emerging line of approach is to supplement freshly isolated mitochondria (mitochondrial transplantation) to injury sites. Alternatively, in the case of stroke, to activate astrocyte for releasing mitochondria-containing particles for intercellular transfer of mitochondria (to neurons). Our previous work showed that supplement of freshly isolated mitochondria promoted neurite re-growth and restored the membrane potential of injured hippocampal neurons [13]. Nonetheless, it is conceivable that clinical translation of mitochondrial delivery on TBI would face great challenge. For instance, checkpoint at the blood brain barrier should be considered to improve the effectiveness and the volume used would also be a limiting factor. The therapeutic outcome of mitochondrial transplantation largely depends upon the isolation protocol, quality of isolated mitochondria, and tissue-specific differential uptake. Biocompatible materials for packaging mitochondria may facilitate the delivery and the subsequent uptake by cells. For clinical application, it is more feasible to isolate mitochondria from peripheral tissues to obtain sufficient amount of allogeneic mitochondria for the treatment of CNS diseases. Based on our experience, the percentage of functional mitochondria after isolation and the quality maintenance over time are crucial measurement for the success of promoting neuronal regeneration. While published data showed that peptide-based allogeneic mitochondrial delivery successfully entered target cells and recovered damaged tissues without triggering significant immune response in PD model, the efficacy of PMD in cerebral stroke and TBI patients has yet to be determined [88]. More importantly, regenerative outcome characterized by neurite re-growth, de novo synaptogenesis, and the restoration of neuronal activity should be inclusively evaluated in addition to the maintenance of cell survival. Thus, future efforts on the feasibility and efficacy of allogeneic mitochondrial delivery on treating a wide range of mitochondria-related diseases will expedite the clinical translation.

**Abbreviations**

6-OHDA: 6-hydroxydopamine; AD: Alzheimer’s disease; ATP: adenosine triphosphate; Aβ: amyloid β peptide; Bax: Bcl-2-associated X protein; CCI: controlled cortical impact; CNS: central nervous system; Drp1: dynamin-related protein 1; FiS1: mitochondrial fusion 1 protein; HD: Huntington’s disease; IMM: inner mitochondrial membrane; LRRK2: leucine-rich repeat kinase 2; MCAO: middle cerebral artery occlusion; Mdivi-1: mitochondrial division inhibitor 1; MFB: medical forebrain bundle; MnF1: mitofusin-1; MnF2: mitofusin-2; mHtt: mutant huntingtin protein; MPP: 1-methyl-4-phenylpyridinium; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mtDNA: mitochondrial DNA; OGD: oxygen–glucose deprivation; OMM: outer mitochondrial membrane; OPA1: optic atrophy protein 1; PD: Parkinson’s disease; PINK1: PTEN-induced putative kinase protein 1; PMD: peptide-mediated mitochondrial delivery; ROS: reactive oxygen species; SCI: spinal cord injury; SN: substantia nigra; TBI: traumatic brain injury; TNTs: Tunneling nanotubes

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**Authors’ contributions**

LC and CYC conceived and designed the content of the paper. CYC and MZL contributed to literature search and Table preparation. MZL contributed...
to figures preparation. LC, CYC, and MZL wrote the manuscript. All authors read and approved the final manuscript.

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