Chapter 1

Introduction

1.1 EFFECTIVE DESIGN OF NANOMATERIALS FOR MITOCHONDRIA-TARGETED NANOTHERAPEUTICS

Medicine refers to the science, engineering, and practice of treating, diagnosing, monitoring, predicting, curing and preventing diseases.[1] The majority of individuals link nanomedicine to pharmacological formulations, wherein soft or hard nanoparticles of nanometre scale are administered to humans for diagnostic and therapeutic purposes.[2] This domain encompasses a broader spectrum of research and development. Nanomedicine is distinct from other medical fields since it entails the creation and utilisation of materials and technologies at the nanometre scale to perform their functions.[3–5] Nanomedicine encompasses the utilisation of nanoparticles for precise drug administration, imaging, and regenerative medicine. These innovations possess the capacity to transform healthcare by delivering more accurate and efficacious therapies for diverse medical diseases. Nanomedicine is an interdisciplinary domain that integrates nanoscience, nanoengineering, and nanotechnology with the life sciences. Nanomedicine is anticipated to facilitate the creation of superior devices, pharmaceuticals, and various applications for the early diagnosis and treatment of numerous diseases, characterised by high specificity, efficacy, and personalisation, aimed at improving patient's quality of life.

Nanomedicine has experienced a notable shift towards the targeted delivery of therapeutic drugs to subcellular organelles, especially mitochondria, to enhance treatment efficacy and reduce systemic side effects. Utilising subcellular targeting to improve the therapeutic index is a compelling approach, as it facilitates the direct delivery of therapeutic drugs to their intracellular areas of action, hence enhancing efficacy and minimising off-target consequences. This precision medicine strategy offers significant potential to transform the treatment of many diseases by optimising therapeutic efficacy and reducing possible harm. By precisely targeting mitochondria, researchers may improve the effectiveness of treatments for conditions such as cancer, neurological disorders, and metabolic diseases (**Figure 1.1**). This focused strategy may result in a reduction of adverse reactions in patients, thus enhancing their quality of life.[6]

Nanotechnology is an innovative domain set to substantially improve medical treatments by utilising the characteristics of materials at the atomic and molecular levels. These materials can assume various configurations, including spheres, dots, platelets, tubes, dendrites, and rods, each designed to address unique therapeutic and diagnostic requirements. Moreover, nanoparticles might possess neutral, positive, or negative charges, offering diverse possibilities for numerous biomedical applications. In the realm of neurodegenerative illnesses, nanotechnology provides novel approaches for diagnostics and therapies. The diminutive size, remarkable stability, and prolonged serum half-life of nanomaterials render them particularly effective in tackling the obstacles posed by these illnesses. A major obstacle in the treatment of neurodegenerative illnesses is the blood-brain barrier (BBB), a highly selective barrier

that restricts the transport of therapeutic medicines to the brain. Nanomaterials offer an advanced solution to surmount this obstacle, facilitating the effective delivery of antioxidants and other neuroprotective substances into the brain. Furthermore, nanoparticles serve as protective carriers, safeguarding sensitive medicinal compounds from enzymatic breakdown or premature elimination, thus improving their efficacy and bioavailability. Specific nanomaterials, like platinum nanoparticles (PtNPs) and cerium oxide nanoparticles (nanoceria), exhibit inherent antioxidant characteristics. These materials can scavenge reactive oxygen species (ROS) and alleviate oxidative stress, a characteristic of numerous neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and stroke. [7,8]

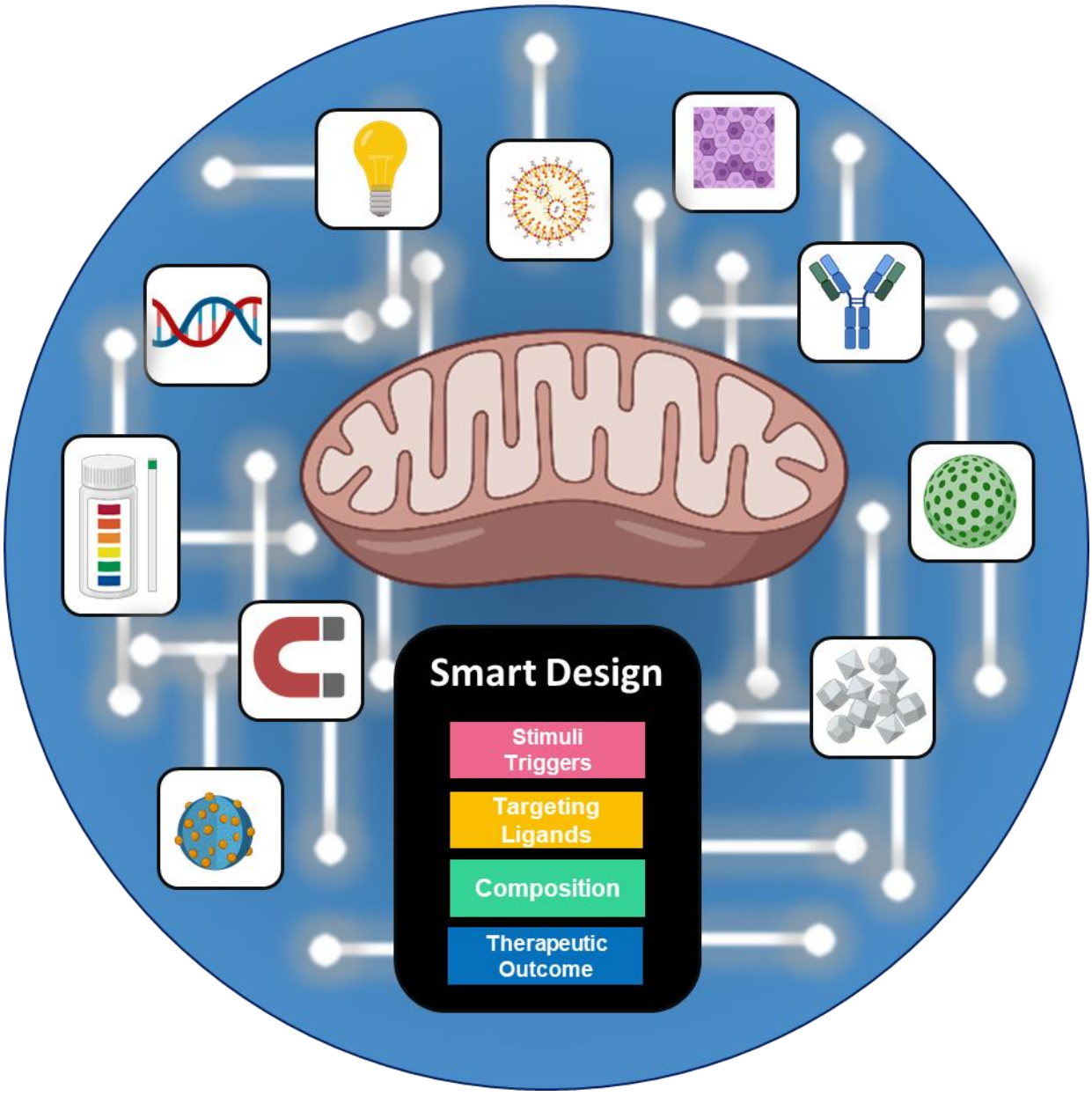


Figure 1.1 Intelligent design of nanotherapeutics: Mitochondria play a vital role in regulating cellular survival and apoptosis. They have become prominent targets in organelle-specific therapy. This illustration highlights

a collection of current developments in the design methodologies of multifunctional mitochondria-targeted nanotherapeutics.

The antioxidant properties of these nanoparticles mitigate oxidative damage, diminish neuroinflammation, and facilitate cellular recovery in brain tissue. Furthermore, nanomaterials provide supplementary benefits in the treatment of neurodegenerative disorders. Their adjustable surface characteristics facilitate functionalisation with targeting ligands, permitting accurate delivery to certain cellular or molecular targets in the nervous system. This feature amplifies the therapeutic efficacy of antioxidants and other pharmaceuticals by assuring their effective delivery to targeted areas while reducing off-target effects. Recent research has shown the capacity of nanomaterials to transform antioxidant therapy for neuroprotection. Their capacity to reduce oxidative damage, promote neural network repair, and improve neuronal survival highlights their potential as a revolutionary method for addressing neurodegenerative illnesses. Ongoing breakthroughs in nanotechnology have the potential to transform treatment strategies for many complex illnesses, providing hope for enhanced patient outcomes and recovery.

1.2 STRUCTURE, FUNCTION AND ASSOCIATED DISORDER RELATED TO MITOCHONDRIA

Mitochondria, as intricate organelles with unique structural characteristics, are considered the most suitable targets for targeted therapeutic interventions. Their distinctive architecture consists of two membranes and four separate compartments: the outer mitochondrial membrane (OMM), the intermembrane space (IMS), the extremely lipophilic inner mitochondrial membrane (IMM), and the mitochondrial matrix. These components collaborate in a unique manner to facilitate the diverse tasks of the mitochondria.[9] The OMM contains voltage-dependent anion channels (VDAC), essential for modulating the passive transit of small molecules into IMS. This characteristic renders the OMM comparatively permeable to tiny molecules, facilitating the transport of ions and metabolites essential for cellular activity. Conversely, the IMM demonstrates a highly selective permeability, remaining predominantly impermeable to tiny ions, including hydrogen ions (H^+). The structural intricacy of the IMM is augmented by its widespread invaginations, termed cristae, which considerably expand its surface area. The cristae serve as a framework for the construction of several protein complexes essential for mitochondrial respiration, especially the electron transport chain (ETC).[10] The electron transport chain is a complicated system consisting of proton-pumping complexes that facilitate the movement of electrons from donors to acceptors via redox processes. This process produces energy that establishes a proton gradient across the inner mitochondrial membrane, leading to a significant negative membrane potential. The electrochemical gradient, or proton motive force, serves as a vital energy store for adenosine triphosphate (ATP) synthase in the creation of ATP. The IMM's function in ATP synthesis is enhanced by its connection to oxidative phosphorylation and the Krebs cycle, both essential for energy metabolism. This complex structure positions mitochondria as a key target for therapeutic investigation, especially in disorders characterised by mitochondrial malfunction (**Figure 1.2**).

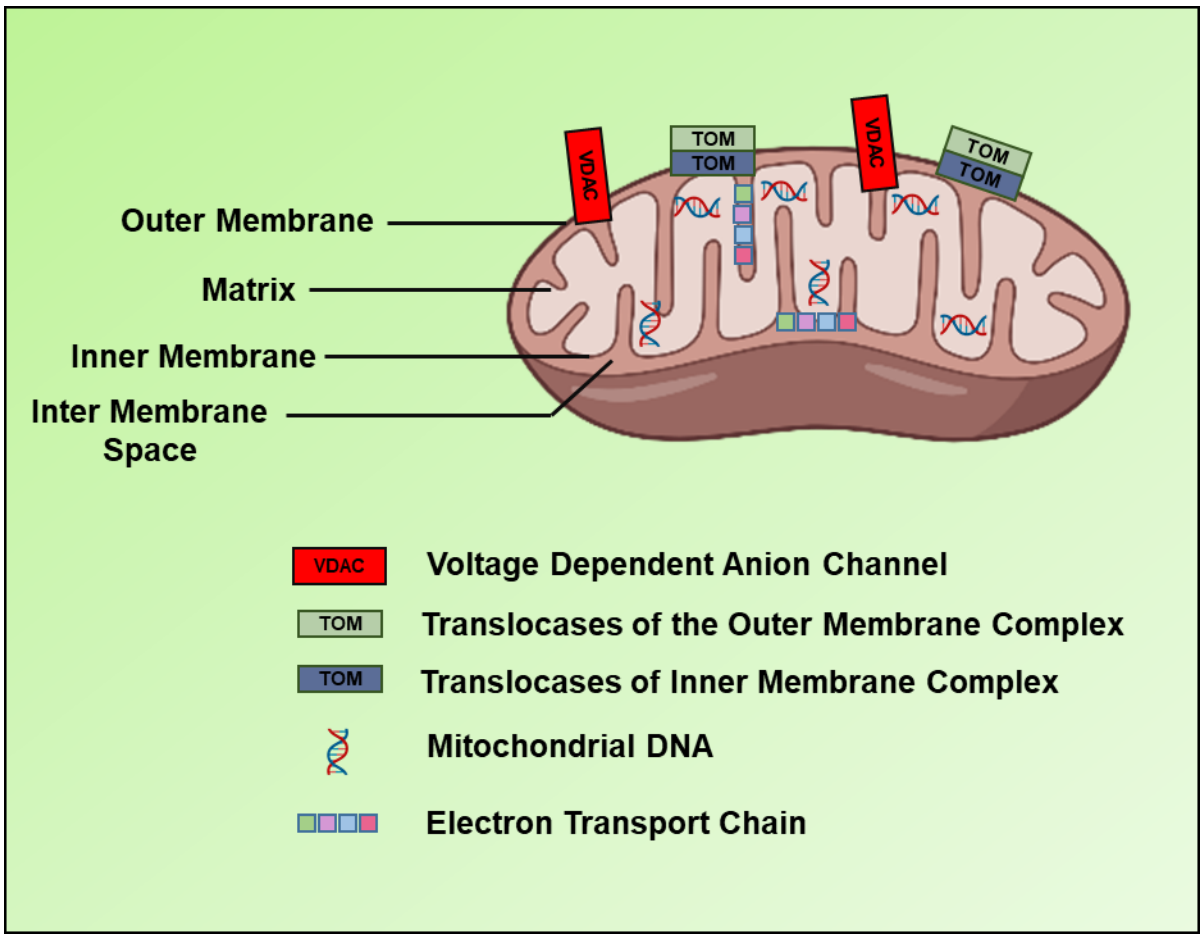


Figure 1.2 The illustration portrays the intricate architecture of the mitochondrion, highlighting essential components such as membrane potential, cardiolipin, translocase complexes (TOM/TIM), and receptor affinities crucial for targeted mediated therapy. It highlights the potential for advanced therapy strategies through targeted mitochondrial modulation.

Mitochondria provide a dual and paradoxical function in cellular processes, essential for both energy productions vital for life and the onset of controlled apoptosis pathways. Regulated cellular apoptosis is essential for preserving tissue equilibrium and overall well-being. Nonetheless, disruptions in these systems lead to numerous clinical diseases. Excessive or abnormal cell death is a characteristic of neurodegenerative illnesses, including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. Conversely, inadequate or suppressed cell death can facilitate disorders such as cancer, which is marked by resistance to apoptosis, uncontrolled cellular proliferation, metastatic capability, dysregulated metabolic pathways, genomic instability, and inflammation that advances tumour progression. There is considerable interest in techniques to modulate cell death pathways in the setting of mitochondrial disorders. A fundamental difficulty in cancer therapy is the emergence of acquired resistance to mitochondria-mediated apoptosis, which greatly restricts the effectiveness of most anticancer treatments. The apoptotic pathway mediated by mitochondria is generally regulated by the Bcl-2 protein family, which is situated on the OMM. These proteins are classified according to their function as either pro-apoptotic or anti-apoptotic regulators. They regulate apoptosis by modulating the permeability of the outer mitochondrial membrane, a crucial step that initiates the release of cytochrome c into

the cytoplasm. This release triggers the caspase cascade, resulting in programmed cell death. Therapeutic resistance frequently results from the overexpression of anti-apoptotic Bcl-2 family members, which obstruct the activation of apoptosis and provide survival benefits to cancer cells. This condition is especially pronounced in specific cancer types, where drug resistance presents a considerable obstacle to treatment efficacy. The complex relationship between mitochondrial function and cell death underscores the necessity of focussing on mitochondrial pathways in the creation of effective treatments for various diseases, including neurodegeneration and cancer.[11,12]

1.3 DISCOVERY OF MITOCHONDRIOTROPHIC

In recent years, the covalent modification of bioactive molecules with mitochondria-targeted "vehicles" has attracted considerable interest because of its straightforward production methods and remarkable targeting efficacy.[13] This method utilises the distinctive bioenergetic characteristics of mitochondria, specifically the negative membrane potential across the inner mitochondrial membrane. Positively charged compounds, including lipophilic cations, are inherently attracted to the mitochondrial matrix, accumulating contrary to their concentration gradient. Various kinds of lipophilic cations have been utilised for this purpose, including alkyl triphenylphosphonium (TPP+) cations, rhodamine derivatives, cyanine cations, and cationic peptides. When conjugated with medicinal drugs or probes, these compounds dramatically improve mitochondrial absorption by utilising the electrochemical gradient. Initially, these chemicals were crucial for investigating fundamental elements of mitochondrial biology, including the coupling mechanism between the electron transport chain and ATP production.[14] Furthermore, they functioned as essential instruments for assessing mitochondrial membrane potential, elucidating the dynamic mechanisms of mitochondrial activity. Recently, these targeted cationic compounds have been utilised in the formulation of therapeutics for diseases linked to mitochondrial malfunction, such as neurodegenerative disorders, metabolic syndromes, and specific types of cancer. Enhancing mitochondrial localisation through lipophilic cation changes increases the bioavailability and functional efficiency of medicinal medicines in mitochondria. This targeted approach facilitates enhanced control of mitochondrial activity, presenting intriguing opportunities for addressing illnesses associated with mitochondrial malfunction while reducing off-target consequences. Mitochondria-targeted chemicals have emerged as essential instruments for research and therapeutic applications, enhancing our comprehension and management of cellular dysfunction.[15]

1.3.1 Mitochondria Targeting Small Molecules

In 1969, Skulachev and his team produced a breakthrough discovery by identifying the TPP+ cation as the inaugural mitochondria-targeting chemical.[16] This discovery facilitated a more comprehensive knowledge and utilisation of delocalised lipophilic cations (DLCs), noted for their quick and efficient uptake by mitochondria independent of specialised transport systems. Subsequently, numerous DLCs have been created and employed, such as triphenylphosphonium (TPP), dequalinium (DQA), rhodamine 123, and (E)-4-(1H-indol-3-ylvinyl)-N-methylpyridinium iodide

(F16). The capacity of DLCs, especially TPP+, to specifically target mitochondria has been thoroughly investigated and developed by experts like Mike Murphy. His research revealed that the conjugation of TPP+ with antioxidant compounds, including Mito E and Mito Q, facilitates the targeted transport of these agents to mitochondria. This focused strategy provides substantial cellular defence against oxidative stress by directly reducing mitochondrial damage, a key factor in numerous illnesses, such as neurodegenerative disorders and metabolic diseases.[17] DLCs are typically tiny molecules, rendering them economically viable and chemically stable, especially in organic solvents. The straightforwardness of their chemical conjugation methods increases their adaptability, allowing for facile attachment to nanocarriers or other molecules for mitochondrial distribution. However, at higher quantities, their excessive accumulation within mitochondria might impair the mitochondrial membrane potential, resulting in cellular toxicity. This dose-dependent toxicity highlights the necessity for meticulous optimisation of DLC use in therapeutic applications. DLCs are proficient in transporting lipophilic or tiny polar molecules, but their efficacy in delivering bigger or more complicated payloads is affected by parameters like size, charge, and molecular characteristics of the conjugates.[18] The design and optimisation of DLC-conjugated systems necessitate a sophisticated comprehension of mitochondrial biology and chemical chemistry to achieve optimal performance and minimal side effects. Notwithstanding these obstacles, DLCs continue to be essential instruments for mitochondrial targeting, with extensive uses in both basic research and therapeutic advancement.

1.3.2 Mitochondria Targeting Peptides

Mitochondria-targeted peptides have surfaced as viable substitutes for DLCs in the transport of bioactive chemicals to mitochondria. These peptides can be obtained from natural amino acid sequences that inherently target mitochondria or synthesised de novo to incorporate special characteristics designed for mitochondrial localisation.[19] Synthetic peptides frequently include hydrophobic amino acids like phenylalanine, tyrosine, and isoleucine, in addition to positively charged residues like arginine and lysine, which enhance mitochondrial targeting. The adaptability of these peptides facilitates the conjugation of several compounds via peptide bond formation, rendering them very suitable for numerous mitochondrial applications. An exemplary instance of mitochondria-targeted peptides is the Szeto–Schiller (SS) peptides. These peptides are designed to transport dimethyltyrosine (Dmt), an antioxidant motif, straight to mitochondria. Dmt, a phenolic molecule, exhibits minimal scavenging ability against superoxide radical anions and hydroperoxides. [20,21]

Mitochondria-targeted peptides provide a multifaceted foundation for therapeutic advancement. Their capacity for precise customisation for specific cargo and their multifunctional characteristics offer a means for novel therapies in mitochondrial dysfunction, with potential applications in antioxidant therapy, cancer treatment, and more.

1.3.3 Mitochondria Targeting Polymeric Nanocarriers

Modifying the surface of polymeric nanocarriers with specific ligands, including DLCs and mitochondria-penetrating peptides (MPPs), has demonstrated efficacy in

imparting mitochondria-targeting properties to these nanocarriers.[22] This novel method has facilitated the creation of sophisticated medication delivery systems specifically aimed at targeting mitochondria. Diverse techniques have been utilised to functionalise a broad spectrum of nanocarriers, such as liposomes, dendrimers, polymeric blends, and hybrid polymers, with DLCs and MPPs. These functional alterations allow the nanocarriers to selectively localise within the mitochondria, hence improving the precision and efficiency of drug delivery to this essential organelle. This skill has significant potential to enhance treatment results, especially in situations where mitochondrial dysfunction is pivotal.[23]

Polymeric nanocarriers constructed from biodegradable and biocompatible substances, including poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), poly(glycolic acid) (PGA), and polysaccharides such as chitosan and hyaluronic acid, have arisen as adaptable platforms for therapeutic delivery.[24] These materials are highly esteemed for their superior biodegradability and compatibility with biological systems, rendering them appropriate for various biomedical applications. A significant enhancement in their functionality is the surface modification with DLCs or MPPs, facilitating the precise delivery of therapeutic medicines to the mitochondria via these nanocarriers. This targeted delivery is essential for tackling diseases associated with mitochondrial malfunction, including neurological disorders and cancer. The integration of polyethylene glycol (PEG) as an external polymer layer has markedly enhanced the functional efficacy of nanocarriers. The hydrophilic PEG coating fulfils several functions: it reduces unfavourable interactions with blood components to prevent aggregation and provides a "stealth effect," diminishing detection and clearance by the immune system. Overall, the integration of various nanocarriers as an exterior delivery agent has markedly enhanced the efficacy of mitochondria-targeting therapeutics. This innovation in nanotechnology offers significant potential for more efficient and precise treatment choices in medicine.

1.3.4 Mitochondria Targeting Vesicles

The disclosed options for targeted drug delivery to mitochondria have primarily depended on a one-to-one ratio between the therapeutic cargo and its carrier, often adopting a covalent connection to link the bioactive molecule to the delivery vehicle.[25] A promising technique entails utilising vesicular carriers like liposomes, in which therapeutic chemicals are either encapsulated or coupled to tiny nanoparticles designed for mitochondrial delivery. These systems generally attain mitochondrial targeting by integrating the lipophilic TPP+ cation, which promotes localisation owing to its delocalised positive charge. Mitochondria-targeted liposomes, particularly those utilising dequalinium, have proven effective in transporting various compounds, ranging from tiny chemotherapeutic drugs like paclitaxel to bigger macromolecules like DNA.[26] This versatility stems from their structural adaptation to encapsulate molecules with differing hydrophobicity and molecular weight. Recent improvements feature TPP+-functionalized liposomes engineered for targeted mitochondrial distribution, with their targeting efficacy confirmed through colocalization tests utilising fluorescent labels and MitoTracker, a recognised mitochondrial marker. Biodegradable polymer nanoparticles have also emerged as powerful carriers for mitochondrial delivery. Nanoparticles derived from a TPP+-

modified copolymer, PLGA-b-PEG-TPP+, have exhibited effective mitochondrial targeting in live cells, as validated by fluorescence imaging and colocalization with MitoTracker. Additional advancements encompass mitochondria-targeted coumarin-iron oxide nanoparticles (Mito-CIO), which integrate the photophysical characteristics of coumarin with the hyperthermia-inducing properties of iron oxide under near-infrared (NIR) irradiation. The mitochondrial selectivity of these nanoparticles has been validated through imaging investigations, revealing colocalization with MitoTracker. Furthermore, derivatives of superparamagnetic iron oxide nanoparticles (SPIONs) have been investigated for their distinctive capacity to interfere with the mitochondrial electron transport chain, especially in cancer cells, presenting a novel therapeutic strategy for addressing mitochondrial malfunction. The creation of these hybrid nanoparticles may provide a viable strategy for improving the effectiveness of various mitochondria-targeting therapeutics.[27]

1.4 MITOCHONDRIA-RELATED DYSFUNCTION DISORDERS

Damage to mitochondrial DNA (mtDNA) by reactive radicals is greatly exacerbated due to the mitochondria's inefficient DNA repair mechanisms. All of an individual's mitochondrial DNA copies are genetically identical when they are born; this is called homoplasmy. Heteroplasmy, in which mitochondria carry both normal and mutant mtDNA, has been linked to the onset of several illnesses. The threshold effect describes the cellular defects in the mitochondrial respiratory chain that become apparent when the proportion of mutant mtDNA crosses a crucial threshold. A self-sustaining cycle of electron loss is set in motion by this malfunction, which starts with the respiratory chain leaking electrons. The mitochondrial membrane becomes structurally compromised, leading to reactive radicals and worsening functional deficits. Delays in mitochondrial energy production due to these disturbances hastens cellular ageing and impacts tissue-specific processes that depend on mitochondrial activity. Thus, mitochondrial abnormalities are highly predictive of the development and worsening of diseases associated with mitochondrial dysfunction.[28] The following sections explore several diseases that can be caused by these mitochondrial defects.

1.5 NEUROLOGICAL SPECIFIC DISORDERS

Since mitochondria are pivotal in cellular energy production and homeostasis, their malfunction is associated with a wide range of serious neurological diseases. **Figure 1.3** shows the cell types most impacted by mitochondrial dysfunction and various disorders to which it contributes significantly. Because they source so much of their energy from oxidative phosphorylation (OXPHOS), neurons are especially susceptible to mitochondrial malfunction. Serious problems with how neurons work can result from interference with mitochondrial communication and function.[29] The development and worsening of numerous neurological disorders are aided by mitochondrial localised oxidative stress, which intensifies disturbances in calcium homeostasis and activates apoptotic pathways. Some of these conditions include neurological diseases such as Alzheimer's and Parkinson's, as well as psychological

illnesses such as Huntington's and amyotrophic lateral sclerosis (ALS). In addition, mitochondrial gene mutations are associated with various syndromes, including chronic progressive external ophthalmoplegia, sensory-neural deafness, cardiac conduction abnormalities, retinitis pigmentosa, chronic progressive external ophthalmoplegia, Leber's hereditary optic neuropathy, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibres (MERRF), and neuropathy with ataxia and retinitis pigmentosa (NARP).[30] Neuroprotective techniques in the setting of neurological disorders and traumas have recently been brought to light by research focussing on mitochondrial biogenesis, mitophagy, and dynamics. The incredible mobility and plasticity of mitochondria are essential for the preservation of brain health under stressful environments. This area is still in its infancy, but nanotechnology shows great promise for the design of therapeutic materials that target mitochondria to treat neurodegenerative illnesses.

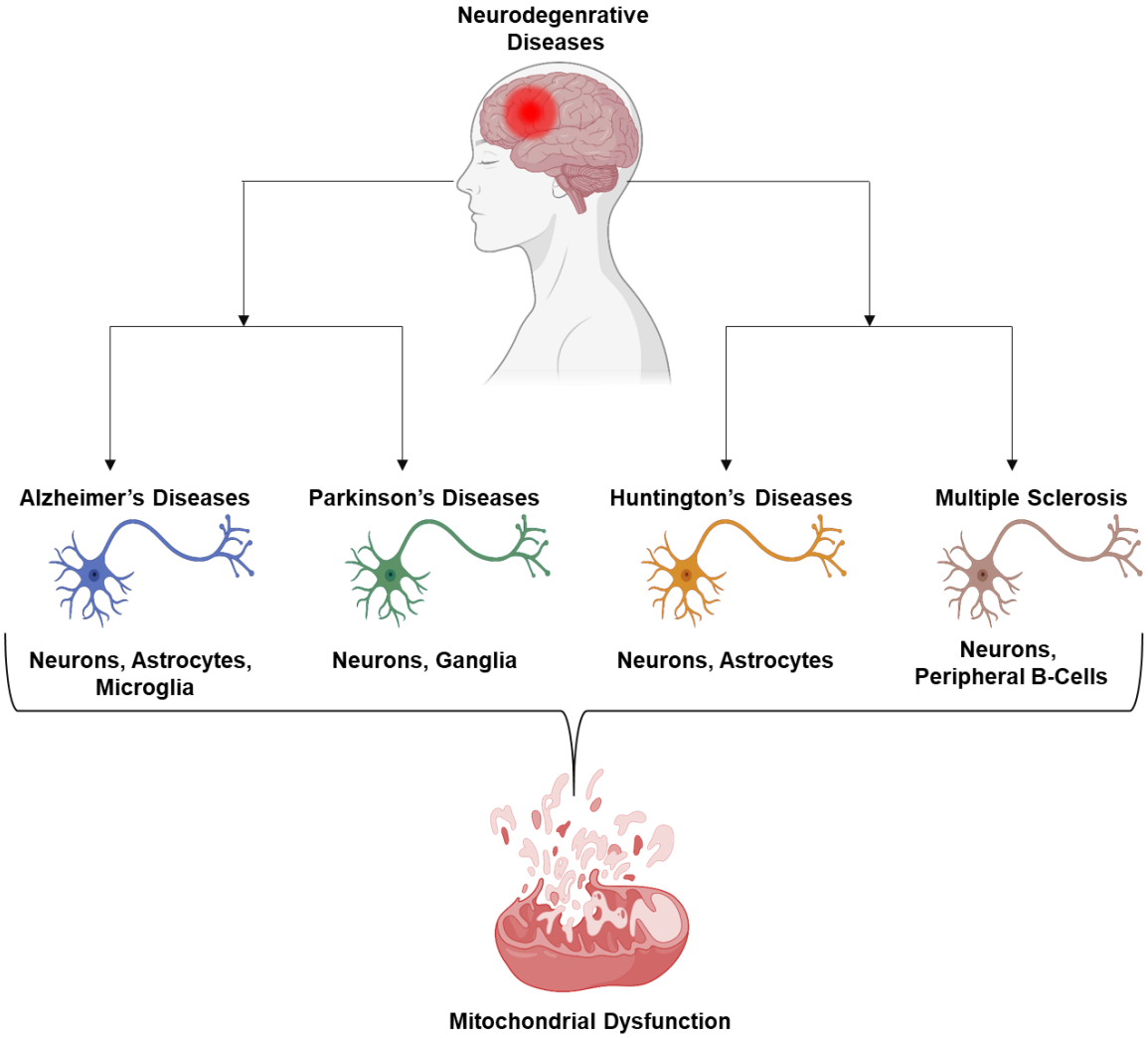


Figure 1.3 Mitochondrial dysfunctions in several neurodegenerative disorders and the particular cell types impacted by these dysfunctions.

1.6 THE FUTURE OF MITOCHONDRIAL NANOMEDICINE

Because of their importance in cellular metabolism, mitochondrial dysfunction is a key component in the development of many illnesses. Precision medicine has the potential to enhance disease management greatly, and nanotechnology provides a revolutionary method for focusing on the mitochondria within sick cells. New methods based on NPs show great promise for delivering drugs to certain cells, organs, or tissues. When these technologies are used on subcellular structures, such as mitochondria, the level of accuracy increases, which could lead to new therapeutic advantages. The mitochondria are a very complicated and dynamic organelle, making it very difficult to target them, even with current advancements. This thesis highlights encouraging studies that show how nanotechnology might be used to target mitochondria. However, it also shows how important it is to carefully evaluate nanomaterials so that their specific qualities can be used for subcellular targeting. Fluorescence imaging is now used in numerous research studies to evaluate nanomaterials' mitochondrial targeting capabilities. Although these imaging technologies offer useful insights, relying only on them to understand mitochondrial association features can be misleading. Accurately characterising the interactions between nano-delivery technologies and mitochondria requires a thorough study utilising many complementary methodologies. In the future, nanotechnology-based solutions can provide creative and effective options for treating disorders related to mitochondrial dysfunction. This can be achieved by careful design, rigorous testing, and strategic deployment. By solving problems at the level of individual organelles, these developments may open up hitherto unexplored possibilities in targeted medicine and completely alter the landscape of therapeutic approaches.

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