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Nano-Theranostics approaches and Mitochondrial Targeted Drug Delivery: Alzheimer 's disease therapeutic interventions

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Abstract

One of the most frequent brain diseases, Alzheimer's is defined by poor cognitive function brought on by the build-up of Beta Amyloid plaques and the gradual death of neurons. Glucose metabolism and the development of amyloid plaques are being studied together. Under physiologically normal circumstances, glucose is the primary substrate for the adult human brain. The prodromal phases of AD are significantly influenced by glucose hypometabolism. Hypometabolism of glucose in the brain is a clear sign of mitochondrial dysfunction and bioenergetic system impairment. By regulating energy synthesis and cell death, mitochondria play a crucial role in the functioning of cells. Increased formation of reactive oxygen species (ROS) and oxidative stress are a result of mitochondrial dysfunction, which also accelerates the development of Alzheimer's disease. For the maintenance of balance, autophagy is crucial because it selectively destroys damaged mitochondria. AD affects this route for mitochondrial breakdown. Targeting specific mitochondrial ligands by interventions along this pathway might be a useful therapeutic approach. Due to a number of biological obstacles, this method has significant limitations. As a result, many nanocarriers have been created to improve drug delivery effectiveness. All potential nanotechnology-based treatments for AD have been examined in this study, with a particular emphasis on medication delivery to the mitochondria.

Keywords: Alzheimer’s disease; Quantum Dot Nanoparticles; PINK1; Theranostic; mitochondria; drug delivery

Abbreviations (arranged in order of appearance in the text)

Alzheimer’s disease: AD ; Reactive oxygen species: ROS ; Presenilin1: PS1 ; Presenilin2: PS2 ; Amyloid precursor protein: APP ; Neurofibrillary tangles: NFT; Oxidative phosphorylation: OXPHOS ; Neurodegenerative illnesses: NDDs; Blood-brain barrier: BBB; Amyloid-β protein: Aβ ; Engineered nanomaterials: ENMs ; Central nervous system: CNS ; International Organization for Standardization: ISO ; Poly D, L-lactic acid: PLA ; Poly lactic-co-glycolic acid: PLGA ; Polyethylene glycol: PEG ; Poly lactic acid: PLA ; Sulphur nanoparticles: SNPs ; Cerebrospinal fluid: CSF ; Phosphorylated tau: P-tau ; Adenosine triphosphate: ATP ; Endoplasmic reticulum: ER ; Nicotinamide adenine dinucleotide: NAD+ ; Mitofusins: MFN ; Dynamin-related protein 1: DRP1 ; Nuclear respiratory factor: NRF ; Mitochondrial transcription factor A: TFAM ; Mitochondrial DNA: mtDNA ; Mitochondrial Reactive oxygen species: mtROS ; Nicotinamide

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adenine dinucleotide phosphate (NADPH) oxidases: NOX ; Cyclooxygenases: COX ; Guanosine triphosphate: GTP ; Mitochondrial fission factor: MFF ; Mitochondrial dynamics proteins: MID ; Mitochondrial fission 1 protein: Fis1 ; Mitochondrial membrane: OMM ; Familial AD: FAD ; Insulin-like growth factor: IGF ; Phosphoinositide-3-kinase: PI3K ; Amnestic mild cognitive impairment: aMCI ; Chaperone-associated autophagy: CMA ; AMP-activated kinase: AMPK ; Mechanistic target of rapamycin complex 1: mTORC1 ; Transcription factor EB: TFEB ; Autophagy-related genes: ATGs ; Atg/Unc52-like kinase 1: ULK1 ; PTEN-induced putative kinase 1: PINK1 ; Optineurin: OPTN ; Nuclear domain 10 protein 52: NDP52 ; Adenovirus E1B 19 kDa-interacting protein 3: BNIP3 ; BNIP3-like protein X: NIX ; Induced pluripotent stem cells: iPSCs ; Magnetic resonance imaging: MRI ; Positron emission tomography: PET ; Nanosilver: nano-Ag ; Hexamethylene tetramine: HMT ; Thioflavin T: ThT ; Surface Plasmon Resonance: SPR ; Gold nanoparticles: AuNPs ; Phosphate buffer saline: PBS ; Scanning tunneling microscope: STM ; Quartz Crystal Microbalance: QCM ; Mono-crystalline iron oxide nanoparticles: MIONPs ; Ultra-super magnetic iron oxide nanoparticles: USIONPs ; Congo red-loaded magnetic nanoparticles: CR-MNPs ; Acetylcholinesterase: AchE ; N-methyl D-aspartate receptor: NMDAR ; Monoclonal antibodies: mAbs ; Fragment crystallizable: Fc ; Cell penetration peptides: CPP ; Quantum Dot Nanoparticles: QD NPs ; TPP-modified molybdenum QD: TPP-MoS_2-QD ; Selenium quantum dots: SeQDs ; Dihydrolipoic acid: DHLA ; Kalopanacis Cortex extract capped gold NPs: KC-GNs ; Poly(amidoamine) Dendrimers: PAMAM ; Poly propylene imine: PPI ; Memantine hydrochloride: MEM ; Extracellular vesicles: EVs ; Methylated -cyclodextrin: Me—CDs ; Polyrotaxane: PRX ; Wheat germ agglutinin: WGA ; Tacrine hydrochloride: THA ; Poly n-butyl cyanoacrylate: PBCA ; Donepezil: DZP ; Solid lipid NP: SLN ; Nanostructure lipid carrier: NLC ; Galantamine Hydrobromide: GAL ; Bone marrow-derived mesenchymal stem cells: BM-MSCs ; Epigallocatechin-3-gallate: EGCG ; Casein phosphopeptide: CPP ; Chitosan: CS; Cetyltrimethylammonium bromide: CTAB ; γ-poly glutamic acid: γ-PGA ; N-Methyl-D-aspartate: NMDA ; Alpha-amino-3- hydroxy-5-methyl-4-isoxazole-propionic acid: AMPA ; Hydroxyquinoline: CQ ; Deferoxamine: DFO ; Trientine: TETA ; Curcumin-encapsulated Pluronic F127 nanoparticles: FCur NPs ; Oxytocin: OT ; Oxytocin (OT)-loaded angiopep-2-modified chitosan nanogels: AOC NGs ; Mitochondrial permeability transition pore: mPTP ; Methylene blue: MTC ; Superoxide dismutase: SOD ; Diethyl (3,4-dihydroxyphenethylamino) (quinoline-4-yl) methyl-phosphonate: DDQ ; Peroxynitrite: ONOO ; Zinc oxide nanoparticles: ZnONPs ; Nickel oxide NP: NiONP

1. Introduction:

Alzheimer's disease (AD), the most prevalent form of dementia in older people, is a chronic, progressive neurological ailment for which there are currently no drug or other form of treatment can halt or slow the progression of AD. The phenotype of this disease includes significant memory loss, as well as sporadic memory impairment in the early phases of the illness. The main pathologic characteristic is primarily brought on by the progressive and specific loss of neurons in the forebrain as well as other parts of the brain. In recent decades, a variety of factors, including age,

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gender, weight, exercise, toxins, brain injury, and usual hereditary factors (such as genetic mutations in the PS1, PS2, and APP proteins in familial AD), have also contributed to the beginning and development of AD pathogenesis. Worldwide, 36 million people over 65 have dementia caused by AD. By 2030, that figure is expected to double to 66 million, and by 2050, it will reach 115 million. The World Alzheimer Report estimates that the expense of dementia worldwide in 2015 was $818 billion [1]. According to extensive study, numerous cellular mechanisms, including amyloid beta generation, mitochondrial structural and functional alterations, hyper phosphorylation of Tau and NFT creation, inflammatory responses, and neuronal loss, have been implicated in the etiology of AD. Major areas of current research include mitochondrial abnormalities, phosphorylated Tau, and amyloid beta-induced synaptic damage. Early stages of the illness process include mitochondrial oxidative damage and synaptic degeneration [2]. In AD brain autopsy, the pathological hallmarks observed are intracellular neurofibrillary tangles (NFTs) and extracellular plaques primarily in the neocortex, hippocampus, and other subcortical regions important for cognitive activity. Although numerous studies have substantially increased our understanding of AD, more research is still needed to elucidate the precise mechanism underlying its intricate etiology. The studies were also carried out to further understand the pathogenesis of AD in greater detail, and as a result of various such studies it was observed that the mitochondrial defects and oxidative stress may exhibit a significant role in the early pathology of AD[3]. Probably this is the reason why the existing therapies have limited effects and do not cure the disease. Mitochondria is an important cellular organelle and plays a key role in producing energy, maintaining Ca^{2+} homeostasis, metabolizing carbon, producing intermediates for cellular development, and inducing apoptosis. In order to maintain Ca^{2+} homeostasis, mitochondria provide the buffering machinery, which aids in the excitation of neuronal cells. Additionally, it shields neuronal cells throughout their lengthy neuronal lifetimes from a number of stresses. Therefore, mitochondria play a key role not only in oxidative phosphorylation (OXPHOS) and the formation of reactive oxygen species (ROS), but also in intracellular communication and stress response[3–5]. Due to their high level of dynamic activity, mitochondria also influence the morphology of neuronal cells and where they are located inside these cells. This wide range of operations that mitochondria are capable of supporting are combined with the carefully controlled dynamics of mitochondria. It includes a variety of processes, including the autophagy of damaged mitochondria and fission, fusion, transportation,

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and synthesis. Mitochondrial reactions to environmental stimuli are crucial for maintaining energy balance in cells with high energy requirements, such as brain cells. In order to sustain the synaptic actions, mitochondria must go through the characteristic structure of nerve cells. Under various internal and external inputs, mitochondrial dynamics and metabolism are easily changed to eventually control cellular homeostasis [5,6]. Due to their high level of dynamic activity, mitochondria also influence the morphology of neuronal cells and where they are located inside these cells. This wide range of operations that mitochondria are capable of supporting are combined with the carefully controlled dynamics of mitochondria. It includes a variety of processes, including the autophagy of damaged mitochondria and fission, fusion, transportation, and synthesis. Mitochondrial reactions to environmental stimuli are crucial for maintaining energy balance in cells with high energy requirements, such as brain cells. In order to sustain the synaptic actions, mitochondria must go through the characteristic structure of nerve cells. Under various internal and external inputs, mitochondrial dynamics and metabolism are easily changed to eventually control cellular homeostasis. As a result, the abnormalities of the neurological system and mitochondrial deficiencies appear to be closely associated, and this suggests that they may be a contributing factor to a variety of neurodegenerative illnesses (NDDs)[7–9].

According to data, AD sufferers' blood-brain barrier (BBB) is defective and permeable [10]. Functionalized nanomaterials, which have been applied widely to inhibit amyloid-β protein (Aβ) aggregation, show enormous potential in the field of prevention and treatment of Alzheimer’s disease (AD). The BBB regulates the movement of biomolecules in both directions between brain cells and blood arteries. The BBB poses a serious challenge for the development of drug delivery systems to the brain. In order to facilitate drug administration, various ligands with the appropriate ligand density and receptor affinity are attached to the surface of NP and their physicochemical properties are altered. Nanoparticles, which are basically bodies of a specific material that are 100 nm or less in size, are a common by product of industry. The use of engineered nanomaterials (ENMs) is growing in popularity as a result of their tiny size and relatively high surface to volume ratios, which increase the chances of nanoparticle exposure [11]. Additionally, nanoparticles have the ability to enter the brain. It is believed that nanoparticles that adhere to the nasal mucosa of the olfactory bulb travel via the olfactory nerve to the olfactory bulb, where they can circulate to the brain and potentially affect the health and activities of the brain [12]. Since they enable for precise site-specific delivery of drugs, which might limit potential adverse effects, nanoparticles have been

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extensively researched as drug delivery systems in recent years [13]. Though they can improve medication targeting and increase drug availability in the CNS, nanoparticles have also been looked into for the treatment of AD [14]. For the development of alternate drug delivery therapies for all phases of Alzheimer's disease, nanotechnology (NT) offers a novel strategy. The nano particles (NPs) used in NT have at least one dimension less than 100 nm [15]. NPs are described as nano-objects with all three exterior dimensions at the nanoscale by the International Organization for Standardization (ISO) [16]. NPs have a number of benefits over conventional drug delivery molecules. They are extremely small and have a high surface-to-volume ratio, which makes it easier for them to interact with biomolecules. To change their passage across biological barriers, they can be created in a variety of shapes and sizes, including spherical, cubic, and rod-like forms. NPs can be employed for both the diagnosis and therapy of diseases. By adsorbing, entrapping, or forming covalent bonds, they can bind with a wide range of desirable ligands to acquire new physiological, therapeutic, or diagnostic capabilities, The potential for these particles to trigger an innate immune response must be taken into account, too. Numerous nanoparticles, including numerous metal and metal-oxide nanoparticles, have been demonstrated to have pro-inflammatory effects in in vitro and in vivo immune response studies [17]. Alzheimer's disease and other CNS illnesses commonly involve inflammation [18]. The shape of a particle and what, if anything, coats its exterior, along with a number of other characteristics, all have a significant role in whether it significantly affects the immune system [13]. Due to this variety, immunological responses to nanoparticles are not only highly diverse, but they can also frequently be tailored to prevent unwanted immune responses and, in certain circumstances, even to elicit favourable ones [19]. NPs are either inorganic chemicals, natural polymers, or synthetic polymers for the therapy or diagnosis of AD. Poly (ethylenimine), poly (alkylcyanoacrylates), poly (amidoamine) dendrimers, poly (-caprolactone), poly (D, L-lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), and polyesters made of poly (lactic acid) (PLA) are a few examples of synthetic polymer-based NPs. Gold, silica, and carbon are a few examples of inorganic materials for medicinal NPs [20]. Recent research suggests that particular sizes, shapes, and types of sulfur nanoparticles (SNPs) can have the moderating effect against AD pathogenesis, despite the potential implications of certain types of nanoparticles in accelerating AD development [21]. The effects of three different types of brain-targeting sulfur nanoparticles (RVG@Met@SNPs) with new morphologies—volute-like, tadpole-like, and sphere-like on Aβ

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aggregation, their capacity to penetrate the BBB, and their general neurotoxicity —have been studied. According to the findings, smaller nanoparticles, like sphere-shaped SNPs, may decrease Aβ peptide aggregation (61.6%) and boost cell viability (92.4%), in contrast to other nanoparticles that may promote Aβ peptide aggregation and worsen AD pathogenesis [22].

Thus, this article tends to deliberate and provide an insight on brief pathophysiology of AD underlying its association with mitochondrial targets and summarizing the current nanotheranostic approaches to alleviate AD.

2. Role of Aβ amyloid in AD:

Toxic changes are occurring in the brain at the very early stages of Alzheimer's, including aberrant protein buildups that result in amyloid plaques and tau tangles. In persons with sporadic Alzheimer's disease, brain Aβ is high[23]. The primary component of brain parenchymal and vascular amyloid, Aβ is neurotoxic and contributes to cerebrovascular lesions. The exact mechanism by which Aβ builds up in the brain and causes cell illness is still a mystery. Evidence from pathologic, genetic, biologic, and biomarkers has suggested that the β-amyloid peptide (Aβ) plays a significant role in the onset of Alzheimer's disease (AD) [3,7,24]. While amyloid plaques are a neuropathological indicator of AD, Aβ is a normal peptide produced throughout life. It is remarkable that APP, one of the most researched proteins in science, has an undefined natural function. A possible normal function of Aβ is even more uncertain. Nevertheless, synaptic activity, the most distinctive and typical aspect of the nervous system, stimulates Aβ synthesis and secretion. The small Aβ peptide, which can be up to 42 or 43 amino acids long, is therefore not necessarily harmful and may even serve a physiological purpose, whereas amyloid plaques, which are made up of many highly aggregated Aβ fibrils, are an abnormal clinical lesion [23,25]. This naturally occurring protein congregates abnormally in the Alzheimer's brain to produce plaques that build up between neurons and impair cell function. Currently, tau and amyloid Aβ proteins are the two most promising targets for treating Alzheimer's disease. Aβ produces oligomers and extracellular plaques in AD pathology, which can spread the disease by migrating from cell to cell [26]. The amyloid cascade hypothesis, which links Aβ aggregation and cognitive symptoms of AD, is currently supported by a vast body of independently gathered genetic, neuropathological, and experimental data. According to the amyloid cascade hypothesis, a critical stage in the development of Alzheimer's disease is the deposition of the amyloid-peptide in the brain parenchyma (AD). According to biological research [27,28], mutations in the proteins APP and

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PS 1 and 2 result in increased levels of the disease-related Aβ42 and/or other more aggregation-prone forms of Aβ. Finally, biomarker studies on cerebrospinal fluid (CSF) reveal that the disease-associated Aβ42 peptides decrease 1-2 decades before AD symptoms appear [29,30]. Aβ has emerged as the top suspect in the pathogenesis of AD as a result of these factors.

3. Role of Tau & phosphorylated tau in AD:

According to Hanseeuw et al. (2019), tau NFTs are more frequently associated with neuronal loss and clinical symptoms. While Aβ may set off a chain of events, tau impairment is more likely the effector molecule of neurodegeneration. In addition to stabilizing microtubules and participating in their dynamics, tau is involved in myelination, axonal transport, neurogenesis, motor function, learning, and memory, as well as neuronal excitability, glucose metabolism, iron homeostasis, and DNA protection, among other physiological processes[31]. It is crucial to note that whereas Aβ buildup is a hallmark of AD, tau pathology can also be found in a class of neurodegenerative disorders called tauopathies that are distinct from AD, Tau is primarily expressed in the central and peripheral nervous systems, where it is found in the highest concentration in the axons of nerve cells[25]. According to Morris et al. (2015), tau is subject to a number of posttranslational modifications in the brain, including phosphorylation, acetylation, methylation, glycation, isomerization, O-GlcNAcetylation, nitration, sumoylation, ubiquitination, and truncation. However, it is still unclear what function each of these modifications serves for tau. Phosphorylation is the most researched tau posttranslational modification. There is mounting evidence that phosphorylated Tau affects the axonal transport of proteins, vesicles, and subcellular organelles, including mitochondria in AD neurons, contributing to the pathogenesis of AD [32]. In AD, deposition of tau aggregates follows a highly stereotyped pattern, beginning in the entorhinal cortex and hippocampus before propagation to other regions. Their stereotypical appearance in nerve cells underlies Braak's stages of AD , according to which inclusions form first in subcortical regions, trans entorhinal cortex, and entorhinal cortex (stages I and II). They then appear in the hippocampal formation and some parts of the neocortex (stages III and IV), followed by most of the neocortex (stages V and VI). Individuals at stages I and II are asymptomatic, some individuals at stages III and IV show signs of memory impairment, and those at stages V and VI suffer AD [33–35].

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Neuropathological diagnostic criteria for AD include amyloid plaques made mostly of aggregated Aβ and neurofibrillary tangles made of tau, a protein associated with microtubules. In familial forms of AD, the amyloid precursor protein (APP) is mutated. Although phosphorylated tau (P-tau) and amyloid β are involved in the progression of the disease [36], growing evidence suggests that P-tau plays a significant role in the pathogenesis of AD by impairing the axonal transport of subcellular organelles, such as mitochondria, lysosomes, vesicles, and proteins, to nerve terminals from the cell soma [22,35]. Due to its connection to synaptic dysfunction and Alzheimer's disease (AD), phosphorylated tau (P-tau) has attracted a lot of interest as a potential therapeutic target.

Research has shown that over expressed normal Tau and/or overexpressed mutant Tau in neurons become hyper phosphorylated, causing oxidative stress, mitochondrial dysfunction, synaptic deprivation, and neuronal damage. This was discovered using brain tissues from transgenic mouse models of Tau, APP/PS1, and 3XAD.Tg (Ribarič, 2021). Several research groups have reported oxidative damage, defective mitochondrial activities, disrupted calcium homeostasis, and defective mitochondrial function in 3xTg-AD mice 43–47 and APP/PS1,48, both of which are mouse models that produce hyper phosphorylated Tau, supporting the hypothesis that phosphorylated Tau is involved in mitochondrial dysfunction and synaptic damage in AD [26]. Studies were conducted to find the relationship between Aβ and phosphorylated Tau using postmortem brain tissues from AD patients at various stages of disease progression, including AD patients at late disease progression who displayed cognitive loss, from control subjects without AD, from AβPP, AβPP xPS1, and 3xTg-AD mice, and from control subjects. The study additionally looked at the location of monomeric and oligomeric Aβ with phosphorylated Tau using immune histological and double-immunofluorescence studies, as well as AD postmortem brains. In AD-affected neurons, it was discovered that phosphorylated Tau interacting with monomeric and oligomeric forms of Aβ. Furthermore, as AD advanced, these contacts grew more frequent. The localization of monomeric and oligomeric A with phosphorylated Tau was discovered using double-labeling studies of monomeric and oligomeric Aβ and phosphorylated Tau, demonstrating that Aβ and Tau interact more as AD advances. Based on these results, we concluded that inappropriately phosphorylated interacts with Aβ and that this interaction harms synapses and neuronal structure and function, causing cognitive impairment in AD patients. Overall, these investigations provided compelling evidence that hyper phosphorylated Tau is

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associated with cellular alterations predominantly linked to mitochondrial malfunction and synaptic impairment in brain tissue from AD patients [35].

4. Synaptic loss in AD:

Synapse loss is an early and constant symptom of Alzheimer's disease (AD), and the degree of dementia is strongly correlated with the extent of synapse loss. According to some research, AD is an extreme and accelerated form of age-related memory decline [37], but once this accelerated process starts, it takes on a pathogenic profile that is not present in healthy aging. A growing number of studies supports Aβ's physiological function in normal synaptic transmission. When synaptic activity increases -secretase activity in organotypic hippocampal slices, the resultant Aβ peptides suppress excitatory transmission through AMPA and NMDA receptors, suggesting a role for Aβ in homeostatic plasticity [38].

Synaptic terminals actively carry impulses between neurons and process information in healthy, undamaged synapses [22,39]. However, in old people and AD patients [40,41].undamaged synaptic terminals showed alterations that cause cognitive loss. In a study of synaptic loss in the cerebellum, which is unaffected by AD, and hippocampus, which is affected by AD, researchers discovered no statistically significant differences in the synapse-to-neuron ratio in cerebellar samples from adult individuals without AD, nonelderly patients with AD, and elderly patients with AD. However, the synapse-to-neuron ratio in samples from the hippocampus decreased more than 50% in adults without AD and in elderly patients with AD [39,41,42].

These findings imply that in AD, the loss of synapses is restricted to the affected brain region [24]. A loss of synapses in AD patients may more strongly correlate with cognitive decline than the number of Aβ plaques and NFTs, according to several morphological and ultrastructural studies that found a 25%–30% decrease in synapses in the cortex of AD patients and a 15%–35% decrease in synapses per cortical neuron [43,44]. Additionally, recent quantification studies of synaptic proteins in Alzheimer's disease (AD) patients and non-demented healthy control adults revealed decreased levels of the presynaptic vesicle proteins synaptophysin, synaptotagmin, and Rab3a; the postsynaptic proteins synaptopodin, neurogranin, and PSD95; and the synaptic membrane proteins GAP43 and synaptobrevin in the AD patients[22,41,45]. These findings imply that membrane-bound, presynaptic, and postsynaptic proteins may play a crucial role in the development of AD and that the loss of synapses and synaptic proteins may be restricted to brain

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regions that are already impacted by AD. This loss of synapses and synaptic proteins has been attributed to soluble Aβ, which is thought to be localized at synaptic terminals. Axonal degeneration and impaired mitochondrial axonal transport appear to coexist with the loss of synapses and synaptic proteins that happens prior to neuronal death in AD patients[6,42,46]. In AD neurons, Aβ accumulated at synapses, according to numerous study teams. These findings strongly imply that mitochondrial dysfunction and defects in AD neurons are the primary causes of synaptic degeneration and synaptic functional failure.

5. Mitochondrial dysfunction and defects in AD:

The mitochondrion is a crucial organelle for calcium homeostasis and metabolism in neurons. The synthesis of ATP, lipid biogenesis, control of reactive oxygen species (ROS), and calcium clearance are a few of the essential biological functions that mitochondria perform [4]. Furthermore, mitochondria are extremely dynamic and can fuse, expand, and move down microtubule tracks to make sure they are dispersed to the periphery of neuronal cells. From impeded neural development to diverse neurodegenerative disorders, mitochondrial malfunction and altered dynamics are seen in a wide range of circumstances. The role of mitochondria on axon branching, synaptic operations, calcium control with the ER, glial cell function, and neurogenesis has been demonstrated to have major impact in AD [47].

a. *Role in the loss of neuronal plasticity and synaptic plasticity*

The enormous energy needs of neurons, which are highly polarised cells, are mostly met by mitochondria. Mitochondria adjust in correspondence to altered neuronal energy states to support energy balance and nervous system function. Modifications in the form, function, and position can be seen as a result of this adaptation, also known as mitochondrial plasticity[48]. The synapse, where mitochondria play a crucial role in both pre- and postsynaptic processes, is the principal site of energy consumption in neurons. Mitochondria may play significant roles in managing key neuroplasticity processes, such as brain differentiation, neurite protruberance, neurotransmitter deliverence, and dendritic remodelling, by producing energy (ATP and NAD+), controlling subcellular $Ca^{2+,}$ and maintaining redox homoeostasis [4]. The presynaptic terminals and the length of the axons both contain mitochondria, which react to electrical activity as well as the activation of

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growth factors and neurotransmitter receptors (Bray, 2019). The ability of neural stem cells to self-renew, a characteristic of all stem cells, may be influenced by the mitochondria [48]

b. Mitochondrial dynamics in axonal transport

It is important to emphasise the immediate relationship between the transport system and the mitochondrial fusion/fission machinery. The RhoT/Trak complex physically interacts with mitofusins (MFN1 and MFN2). Both the anterograde and retrograde transport are significantly decreased when MFNs are inhibited *in vivo* and in cultured neurons. Transport within the mitochondria has also been linked to the fission protein named dynamin-related protein 1 (DRP1). Both *in vitro* and *in vivo*, DRP1 function inhibition impairs mitochondrial transport to dendrites in Purkinje cells [49]. DRP1 is also crucial for the dispersal of mitochondria in the nerve terminals of dopamine neurons as it interconnects with the dynein-dynactin complex to modify dynein-based retrograde transport, according to recent research (Mandal & Drerup, 2019).

c. Mitochondrial biogenesis

Mitochondrial biogenesis is crucial for maintaining mitochondrial homeostasis throughout the life cycle of the organelle so that eukaryotic cells can function normally [4]. Nuclear respiratory factor 1 (NRF 1), nuclear respiratory factor 2 (NRF 2), which regulates the nuclear genes that resembles a mitochondrial protein, and mitochondrial transcription factor A (TFAM), which promotes transcription and replication of mtDNA, are some factors that control mitochondrial biogenesis [49]. Peroxisome proliferation activator receptor gamma-coactivator 1 (PGC-1), the principal regulator of mitochondrial biosynthesis, controls the expression of NRF 1, NRF 2, and TFAM (Son & Han, 2018). PGC-1, NRF 1, NRF 2, and TFAM expression levels get considerably lowered in AD hippocampus tissues, indicating diminished mitochondrial biogenesis [49,50].

d. Mitochondrial functions

It is known that mitochondria produce 90% of the cellular ROS. Oxidative stress (OS), which results in oxidative damage that affects several cellular components including lipids, DNA, and proteins, is caused by an abnormality in balance between the making and breaking of mitochondrial reactive oxygen species (mtROS) [51]. This imbalance is caused by an excess production of ROS and/or a decrease in antioxidant defence activity.

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Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) and, to a lesser extent, other enzymes such cyclooxygenases (COX), lipoxygenases, xanthine oxidases, and cytochrome P450 enzymes are the principal producers of mitochondrial ROS inside the mitochondria [51]. Furthermore, the electron transport chain is inherently dripping; even under physiological environment, 0.2–2% of the negatively charged particles produced by the respiratory chain are not linked to the resemblance of ATP but instead pay major role in the premature interaction of oxygen, which results in the production of superoxide anion (O_2) or hydrogen peroxide (H_2O_2) [52].

The mitochondrial electron transport chain's Complex IV (also known as cytochrome c oxidase, COX) is the ultimate complex of eukaryotic oxidative phosphorylation and is particularly susceptible in AD [53]. Although COX deficiencies in AD are well known, recently the genetic link between COX-related genes and AD has also been established [53] which extensively reported that AD patients exhibit deficiencies in the complexes of the mitochondrial respiratory chain, particularly those that reduce the effectiveness of complex IV.

When focusing on the fusion and fission processes, it is important to note that these are primarily controlled by proteins from the family of big GTPases called dynamin-related proteins [49]. These proteins drive mechanical motion on biological membranes by hydrolyzing GTP. DRP1 physically interacts with several adaptor proteins, including mitochondrial fission factor (MFF), mitochondrial dynamics proteins 49 and 51 (MID49, MID51), and mitochondrial fission 1 protein (Fis1), to move from the cytoplasm to the outer mitochondrial membrane (OMM) [50].

e. Endoplasmic reticulum stress

Endoplasmic reticulum (ER) stress is thought to be a key factor in the build-up of AD. This is primarily due to neuronal malfunction and cell death and is considered to be caused by the piling up of misfolded proteins and the disruption of intracellular calcium homeostasis [5]. Presenilin1 and the amyloid precursor protein (APP) exhibit elevated ER stress

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responses, according to several investigations (PS1). According to several studies, PS1 controls the homeostasis of ER calcium. Sarco/endoplasmic reticulum Ca2-ATPase, a protein that moves calcium from the cytosol to the ER lumen, and ER-associated calcium channels like the inositol trisphosphate receptor and ryanodine receptor are all affected by PS1. PS1 mutations linked to familial AD (FAD) change the calcium transfer activity of the protein. ER cytosolic calcium concentration changes are a strong inducer of ER stress [54].

f. Effects Aβ and Tau on mitochondrial functions

In neurons, he secretases and cleaves the amyloid precursor protein (APP), releasing fragments of amyloid beta 42 (Aβ42) [55]. Aβ42 fragments assemble into insoluble extracellular fibrils of neuritic plaques (NPs), which lead to the formation of neurofibrillary tangles (NFTs). Nevertheless, several molecular, genetic, and contradictory clinical associations have been found. Tau, a key microtubule-associated protein, is important for the functioning of neurons. The proline-rich region of tau interacts with the microtubule surface, which helps to stabilise the microtubules [8]. Microtubule dysfunction is caused by the non-equilibrium of tau binding to the microtubules, which causes tau to aggregate and fibrillate [55]. The movement of axons depends heavily on the microtubule network. Microtubule disruption that results from this is probably what also causes aberrant axonal transport and synaptic dysfunction [55]. The ability of signalling molecules, trophic factors, and vital organelles like mitochondria to move through axons is made possible by tau's substantial impacts on the microtubule network. As a result, tau supports essential cellular regulatory and structural processes [8].

6. Hypo metabolism of Glucose in AD

The only resource that can pass through the blood-brain barrier and support typical neural activities is glucose. Studies have found that AD patients and animal models' peripheral tissues, including their brains, have impaired glucose uptake and metabolism [56]. Before AD pathogenesis, changes in glucose metabolism happen when oxidative damage builds up. Early-stage AD patients have significantly reduced glucose absorption, which suggests that the condition is preceded by altered glucose metabolism and elevated steady-state glucose concentrations [57]. In addition to facilitating glucose metabolism and influencing tau protein and Aβ processing, insulin in the brain

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is essential for learning, memory, neurite growth, and development. Impaired insulin signalling causes PI3K activity to decline, which in turn lowers Akt activity, which is necessary for neuronal survival, plasticity, and metabolism. Additionally, increased GSK3/ activity encourages Tau phosphorylation and Aβ accumulation [56]. When set side by side to age-matched controls, AD patients show drastical and insignificant brain levels of insulin, insulin-like growth factor I (IGF-I), insulin-like growth factor II, and IGF-I receptor [57].

A pre-symptomatic sign of AD called glucose hypometabolism is frequently found alongside early Aβ pathology [58]. Due to its close ties to the majority of the key AD risk factors, glucose hypometabolism is thought to have contributed to the beginning of sporadic AD. It is also seen in AD patients approximately 20 years before the start of clinical outcomes [58]. It happens to be in people with amnestic mild cognitive impairment (aMCI), is largely believed to represent a prodromal stage of AD and is also evident in patients with AD. Additionally, in aMCI patients, glucose hypometabolism may serve as an accurate prediction marker for the emergence of AD [59].

It is not very much evident that abnormality in glucose metabolism can have a cascade of poisonous effects since glucose utilization underpins essential brain functions like energy supply and antioxidant defense [58,59]. As such, disturbances in glucose metabolism likely represent a major underlying cause of disease initiation and progression [57]. However, the precise origins and effects of AD-associated glucose hypometabolism have remained unknown up to this point, impeding the search for a cure.

7. Mitophagy and autophagy dysregulation in AD

Precise mechanisms regulating organelle and protein quality are needed to maintain neuronal structure and functionality. Autophagy and mitophagy are therefore involved in neuronal homeostasis. Functional flaws in removing and recycling intracellular components are important characteristic features of AD disease (AD). Pathological phenotypes of AD may be brought on by compromised activity in several cellular pathways. One of the defining characteristics of AD is mitochondrial dysfunction. A crucial method for regulating the quality of mitochondria is mitophagy, and AD is associated with poor mitophagy [60].

7.1 Autophagy:

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The phrase "autophagy" has been procured from the Greek words "auto" (self) and "phagy" (eating). Autophagy is an evolutionarily-conserved cellular quality control mechanism by which cells selectively or non-selectively clear the damaged proteins, nutrients, or cell organelles. Though under physiological conditions this mechanism is active at the basal level, toxic stimulation, nutrient deprivation, oxidative stress, DNA damage, and protein aggregation are just a few examples of cellular stresses that can also cause autophagy to be activated [34,55]. Autophagy destroys cellular products that, when accumulated, can cause cytotoxicities, such as oxidative stress protein aggregates or mitochondrial dysfunction. After autophagy, degradation products are used for various purposes like protein synthesis and energy production.

7.1.1 Mechanisms of Autophagy

Based on the various methods that the cellular contents are ingested into the lysosome, autophagy may be categorized into three basic classes: micro-autophagy, macro-autophagy, chaperone-associated autophagy (CMA). Both micro-autophagy and CMA are carried out by direct cytoplasmic cargo engulfment into the lysosome. In macro-autophagy, a two- membrane layered transitional vesicle known as an autophagosome engulfs organelles and cytoplasmic components before fusing with lysosomes to produce an autolysosome, which digests the enclosed contents [60].

AMP-activated kinase (AMPK) activation along with the repression of the mechanistic target-point of rapamycin complex 1 (mTORC1) serve as signals for stress situations such as nutrition, growth factor depletion and cellular energy deprivation to promote transcription factor EB (TFEB). Autophagy-related genes (ATGs) and lysosome-related genes are now more easily transcribed because to TFEB. Atg/Unc52-like kinase 1 is also phosphorylated and activated by AMPK (ULK1). The class III PI3K complex I (PI3KC3) component Beclin1, which is further phosphorylated by the active ULK1 complex, causes the phagophore's membrane to form. Additionally, the ULK1 complex phosphorylates ATG9, which induces the recruitment of PI3P-binding proteins and, ultimately, the ATG12-5-16L complex. This combination enhances the elongation of the phagophore membrane, the lipidation of LC3, and the recognition of the target protein. Following autophagosome development and maturation it undergoes vascular fusion with the lysosome to create an autolysosome. Within the acidic lumen of autolysosome, enzymatic hydrolysis facilitates the degradation of macromolecules [60].

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7.1.2 Defective Autophagy in AD

Neurons are post-mitotic, terminally differentiated cells that require high levels of energy and nutrients. To maintain the integrity of neuronal homeostasis autophagy acts as a useful method for the removal and recovery of intracellular components, such as broken-down organelles and clustered proteins. Autophagy dysregulation is regarded as one of the characteristics of AD. Due to the impaired fusion of the autophagosome and lysosome, an abnormal build-up of vacuoles is seen in AD brain and transgenic mice models. However, more research is currently being done to determine the exact molecular pathways that lead to reduced autophagy activity [60].

7.2 Mitophagy

Mitophagy is nothing but the selective autophagy of malfunctioning mitochondria. Due to its probable significance in several disorders, mitophagy has been the subject of investigation in the neoteric years [61].

7.2.1 Mechanism of mitophagy

The mitochondrial depolarization, which also enhances PINK1/Parkin signalling and raises the phospho-ubiquitin conjugation of the outer mitochondrial membrane, stabilises the PTEN-induced putative kinase 1 (PINK1) (OMM). Mitophagy receptors' recognition of the polyubiquitin chain such Optineurin (OPTN) and Nuclear domain 10 protein 52 leads to the development of the mitophagosome (NDP52). Ubiquitin-independent mitophagy is facilitated by BCL2, the adenovirus E1B 19 kDa-interacting protein 3 (BNIP3), and the BNIP3-like protein X (NIX) mitophagy receptors.

7.2.2 Defective Mitophagy in AD

Besides harmful pathogenic protein aggregates, dysfunction of mitochondria also plays a significant part in the occurance of neurodegenerative illnesses like AD. Reactive oxygen species (ROS) levels inside the cell rise as a outcome of reduced mitochondrial activity, accumulating damage to proteins, DNA, and lipids, among other cellular components. Mitophagy is therefore a crucial neural protective mechanism. An earlier investigation revealed impaired mitophagy in the hippocampus of AD patients. Neurons produced from such patients engendered pluripotent stem cells (iPSCs) in the AD animal model [62].

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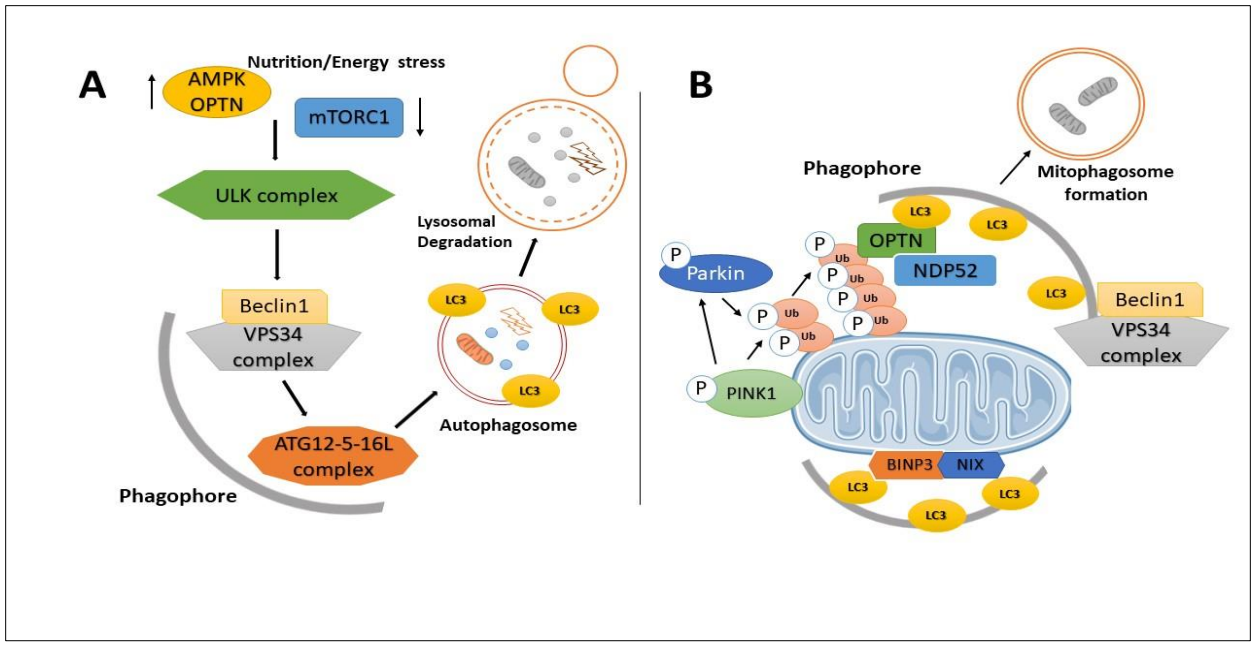

Figure 1: Diagram illustrating the routes for autophagy and mitophagy: A. In response to nutrient or energy stress, AMPK is activated and mTORC1 is suppressed, which increases ULK1 complex activity and stimulates the creation of the VPS34 and ATG5-12-16L complexes which, in turn, stimulates the production of phagophores and autophagosomes. B. Depolarization of the mitochondria stabilizes PINK1 and stimulates PINK/Parkin signaling, which increases OMM's phospho-ubiquitin conjugation. Mitophagy receptors like OPTN and NDP52 identify the polyubiquitin chain, which promotes the formation of mitophagosomes.

8. Nano-based Theranostics in AD:

The difficulty of drugs to enter the brain due to their decreased solubility, limited bioavailability, and the existence of the BBB are the primary obstacles addressed in treating AD Disease. As a result, the BBB breakdown contributes significantly to AD pathogenesis. Due to the current advancements in nanotechnology, it is now possible to treat AD more effectively by finding ways to get drugs over the BBB without experiencing any problems [63].

8.1 Nanotechnology and Diagnosis:

Although there are several conventional diagnostic techniques, they are not very sensitive to the identification of AD. In order to detect AD biomarkers such as A40, A42 insoluble oligomer, Traditional invasive diagnostic procedures such as magnetic resonance imaging

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(MRI), positron emission tomography (PET), sandwich-based ELISA techniques, IP-MS with MALDI-TOF mass spectrometry, immunoassay, NMR spectroscopy, and optical coherence tomography involve the utilisation of bodily fluids such CSF, blood, ocular fluid, and olfactory secretions [64–67].

8.1.1 Nano-diagnostic techniques in vitro for AD: Biosensors for the detection of amyloids responsible for various forms of amyloidosis have been designed using nanotechnological methods which are evident in the prognosis of AD:

i) **Zinc Oxide Nanoflower Platform:**

Zinc oxide (ZnO) is well-known as semiconductor metal oxide nanoparticle which posseses optical attributes. Among all the types of zinc oxide nanostructures, nanoflowers provide larger surface area. By leveraging this characteristic, a reagent less biosensor has been designed and created to detect beta amyloids, a defining feature of AD. Glass slides preparation in this technique includes activation, surface treatment, and electrolase deposition of nano-silver (nano-Ag) thin film [68]. After that, zinc oxide nanoparticles were synthesized, and spin-coated on the glass slides [69]. Subsequently, zinc nanoflowers were grown on the seeded substrate (zinc oxide nanoparticles in 1% acetic acid solution). Then, those treated glass slides were dipped in a growth solution consisting of zinc nitrate hexahydrate and hexamethylene tetramine (HMT) [64]. Only Glass (activated glass slide), Substrate-A (ZnO NFs produced on activated glass slide), Substrate-B (nano-Ag coated glass slide), and Substrate-C (ZnO NFs grown on nano-Ag coated glass slide) were then submerged in thioflavin T (ThT) solution and stored at 4 °C for 12 hours. Substrates that had been coated with ThT were then dried by air in a dust-free environment [70]. Amyloid is very sensitive to conventional ThT assay. ThT increases the intensity of its emission spectrum when it binds to amyloid fibrils, while non-amyloid proteins do not increase their fluorescence intensity when they attach to ThT.

ii) **Surface Plasmon Resonance Nanoparticles:**

Researchers employed Aβ-specific monoclonal antibodies and tau protein-specific monoclonal antibodies. Gold nanoparticles (AuNPs) were coated with streptavidin whereas

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Aβ-primary antibodies were biotin-coated. The SPR surface was put into operation by a self-assembled monolayer of COOH- and OH- thiols. The main antibodies directed against the tau proteins were initially immobilised by enabling them to covalently connect with carboxylic groups. On the sensor surface, a patient's CSF sample was utilised to enable the tau-Aβ complex to bond with any available tau-antibody [71].

The SPR surface was made functional. The main antibodies directed against the tau proteins were initially immobilised by enabling them to covalently connect with carboxylic groups. On the sensor surface, a patient's CSF sample was utilised to enable the tau-Aβ complex to bond with any available tau-antibody [71,72]. The biotin-coated Aβ-primary antibody was then incorporated, and S-AuNP was put to the chip. With the biotin-coated Aβ-antibody, the streptavidin-coated gold nanoparticles will bind precisely since biotin is known to interact with streptavidin. The non-covalently attached particles were ultimately removed, the remaining carboxyl groups were deactivated, and the SPR peak was measured when PBS and aqueous ethanolamine were employed to do so. Based on surface plasmon wavelength spectroscopy, SPR sensors may be employed. The sensor response was represented as a change in the wavelength at which the SPR fall takes place, which is

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commensurate with an alteration in the sensor's refractive index brought on by the molecules adhering to its surface [71]

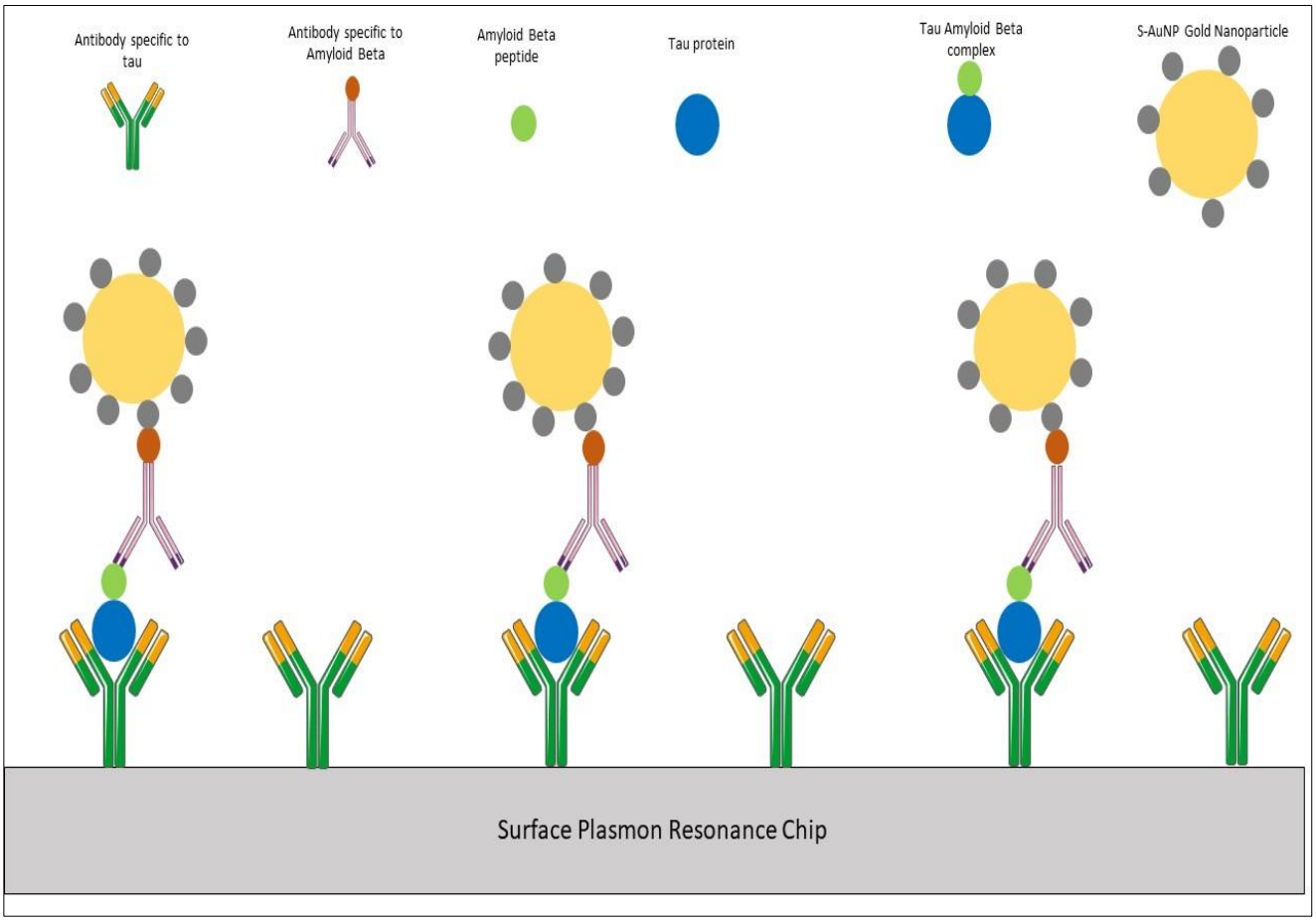

Figure 2: Diagram illustrating how Antibodies specific to tau and amyloid beta work in close proximity with each other, along with streptavidin coated gold nanoparticles and biotin coated Aβ-antibody interaction we can diagnose AD

iii) **Scanning Tunnelling Microscopy:**

Using a scanning tunneling microscope (STM), the AD biomarker was molecularly detected by immobilizing antibodies on a gold (Au) substrate [73]. The Au nanoparticles were utilised in combination with antibodies against Aβ (Aβ 40 or Aβ 42).

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On the surface of the Au substrate, antibodies with a specificity for the Aβ were fixed. After applying the material to the Au-substrate, Aβ-specific antibody coated AuNPs were added to create a sandwich-style binding that was extremely specific. Following washing procedures to get rid of AuNPs' non-specific binding, the surface topography and current profile were noted [65].Therefore, the STM approach enables the ultra-sensitive detection of Aβ by altering the differences between the sample surface and tip of the scanning tunnelling microscope [74,75].

iv) **Quartz Crystal Microbalance (QCM)-Based Detection:**

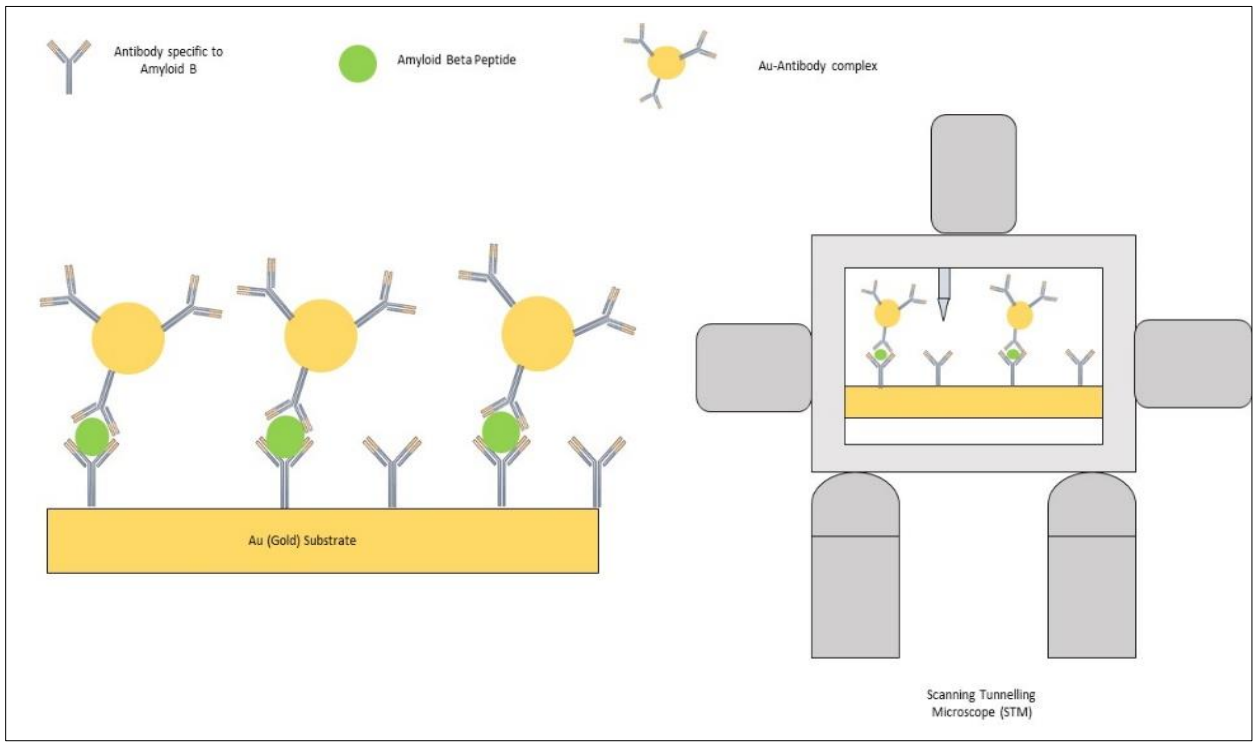

Figure 3: Illustrates how antibodies specific to Aβ binds to Aβ peptide and then goes on to bind with Gold NPs that are being detected by Scanning tunnelling microscope

It is possible to identify neurotoxic A-42 oligomers using two different analytical methods, including Quartz Crystal Microbalance with Dissipation (QCM-D) and Single Molecule Array (Simoa). Both the sensing techniques make use of bapineuzumab, a monoclonal antibody that has a strong affinity for the N-terminal residues 1–5 of the protein Aβ and may be utilized as a capture and identification reagent. We can distinguish between A-42 monomer species and higher aggregates like fibrils using assays created using the two approaches, but we can also precisely identify neurotoxic A-42 oligomers and oligomers. Simoa was discovered to be around 500 times

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better sensitive than the QCM-D approach for the detection of A-42 oligomers, with restrictions of detection upto 0.22 nM and 125 nM, respectively [64,76].

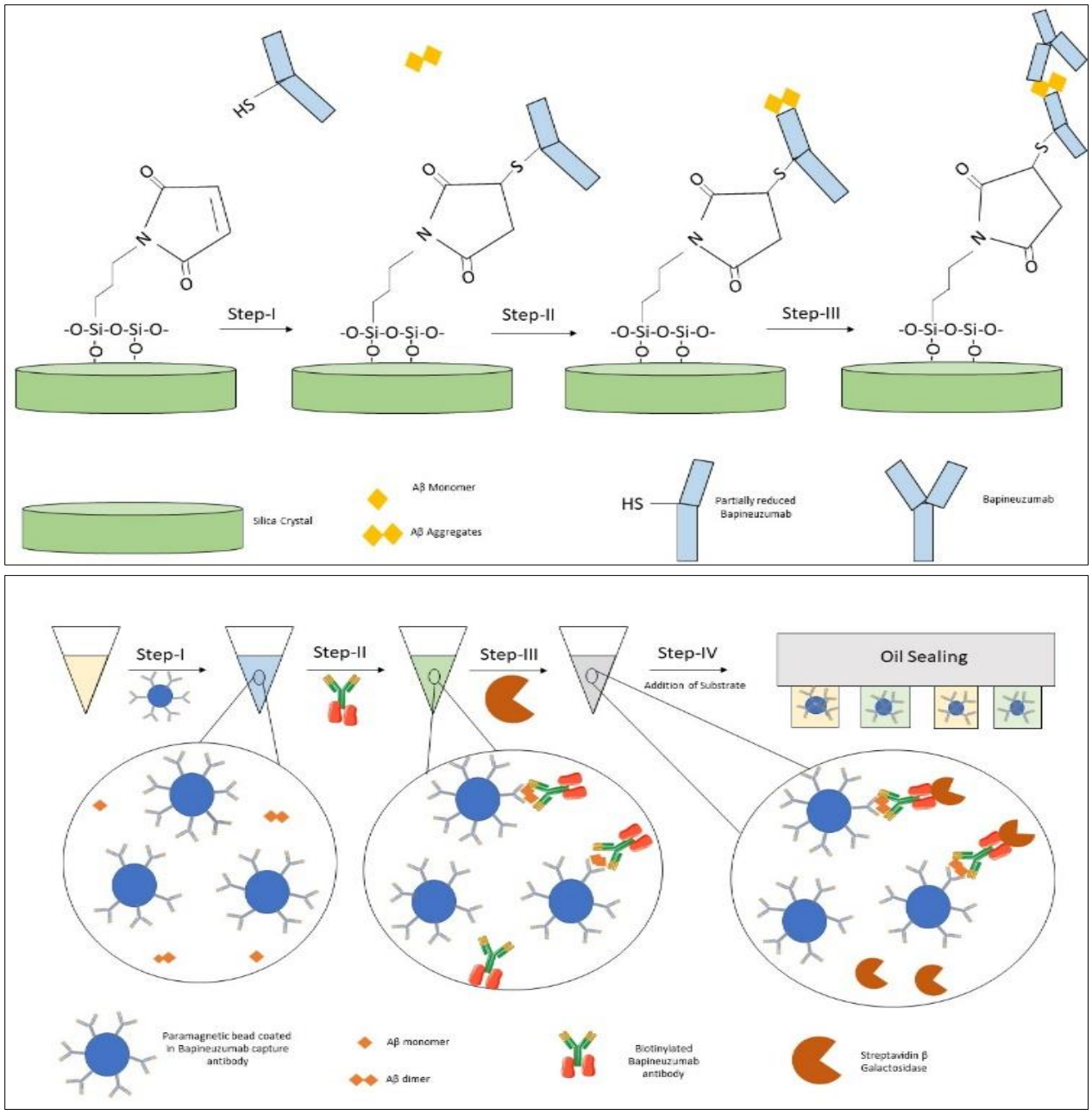

Figure 4: These diagrams illustrate how bapineuzumab, a monoclonal antibody that has a strong affinity for the N-terminal residues 1–5 of the protein Aβ can be utilized as a capture material and assayed by QCM-D & SIMOA techniques where Silica crystals and Paramagnetic bead coated with the MAbs are used respectively. Then they interact with Aβ monomers and dimers(In case of QCM it's later after silica crystal interaction of Partially reduced bapineuzumab). After they interact with partially reduced Bapineuzumab and biotinylated bapineuzumab antibody respectively. Then in presence of Bapineuzumab and Streptavidin β ,galactosidase respectively the diagnosis is carried out.

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8.1.2. In-vivo Nano-diagnostic Methods for AD:

i) Nanoparticles in AD Diagnosis Using MRI: The use of mono-crystalline iron oxide nanoparticles (MIONP's) and ultra-super magnetic iron oxide nanoparticles (USIONP's) as MRI contrast agents can have a considerable impact on the in-vivo detection of AD in transgenic mice models. The simultaneous targeting and imaging of senile plagues is improved by using MIONP and USIONP to target amyloid protein plagues. MRI is the answer to this problem. Amyloid plaques are bonded by a substance called Congo red. Due to their excellent contrast to noise ratio, Congo red-loaded magnetic nanoparticles (CR-MNPs) allow for the precise detection of amyloid protein on MRI [77].

ii) Optical Imaging: Optical imaging techniques known to have precision, affordability and offer a variety of contrast agents. Fluorescent probes and agents like Alexa-fluor-750-conjugated BAM-10 and fluorescein-labeled 10d5 are used to tag antibodies that are specific for Aβ, although the majority of these tags usually are administered locally since they are incapable of penetrating through the BBB. We explicitly target the AD-related biomarker in the optical imaging detection process. A well-known fluorescent probe and amyloid fibril detection agent are thioflavin Ts. Pleated sheets of Aβ aggregates may be recognised by thioflavin T both in-vitro and in-vivo, and Congo Fluorescence imaging may be used to detect red derivative methoxy-X04, Thioflavin S, fluorescent probes like AOI987, and curcumin-derived CRANAD-2 that binds to Aβ [74].

9. Different Types of Therapeutic Approaches:

9.1. Drugs and their associated Challenges in AD:

A whole year of study and discovery has not yet resulted in the creation of a single medication capable of totally curing AD. There are only four authorised medications for treating AD symptoms: donepezil, rivastigmine, galantamine, and memantine [78]. The first three medications are acetylcholinesterase (AchE) inhibitors, which slow down acetylcholine's breakdown in synaptic clefts and so enhance neurotransmission. Memantine, an antagonist of the N-methyl D-aspartate receptor (NMDAR) ion channel, controls Ca2+ entrance into the neurons and prevents glutamate-induced excitotoxicity. Memantine can decrease tau hyperphosphorylation through phosphoprotein phosphatase

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2A activation [79,80]. These drugs can slow the progression of the sickness, but the illness's outcomes cannot be altered. This new generation of medication techniques wants to focus exclusively on the inhibition of Aβ production by BACE1 and BACE2, inhibition of A and tau aggregation, clearance by monoclonal antibodies (mAbs) (passive immunotherapy), suppression of tau phosphorylation, and active immunotherapy approaches, i.e., vaccines, as these have been significant therapeutic targets for AD [81]. In the following *table 1*, we will majorly discuss Aβ directed agents and agents with other targets in the following table.

Table 1*: Drug types, their examples, Mode of action, and Challenges*

Types	***Examples***	***Mode of Action***	***Challenges***	***Reference***
Acetylcholinesterase (AchE) inhibitors	1. Donepezil 2. Rivastigmine 3. Galantamine	Promotion of neurotransmission by reducing acetylcholine hydrolysis in the synaptic cleft.	Can halt the spread of the disease but cannot change how it turns out.	[78]
N-methyl D-aspartate receptor (NMDAR) ion channel antagonist	1. Memantine	Phosphoprotein phosphatase 2A is activated, which controls Ca2+ entrance into the neurons, protects against glutamate-induced excitotoxicity, and can lower tau hyperphosphorylation.	Can halt the spread of the disease but cannot change how it turns out.	[80]

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Combinatorial drugs	Namzaric®	Memantine and donepezil are combined in this medication.	Can halt the spread of the disease but cannot change how it turns out.	(FDA, 2017)
Aβ directed agents (BACE1 & BACE2 Inhibitors	Atabecestat	It is a Prodromal AD drug.	Lacks Efficacy	[83]
Agents with other targets	1. Aducanumab (MAb)	Both brief and prolonged exposure to the amyloid β can result in neurotoxicity and neurodegeneration. Abucanumab specifically binds to the Amyloid complex with high affinity, causing the removal of the Fc area during receptor-mediated phagocytosis; however, it has a	When AD is still in its early stages, it could be beneficial. Due to concerns regarding potential adverse effects including haemorrhage and brain edema, including AD individuals with moderate to severe AD illness are not advised to take Aducanumab.	[84–86]

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		lower affinity for monomers.		

Note. In the above table, we have spoken about several types of medications that are on the market to treat AD, but there are no effective and appropriate treatments to completely eradicate the illness. The majority of the medications listed above are FDA-approved, but several studies indicate they may have serious adverse effects include nausea, diarrhoea, sleep difficulties, exhaustion, vomiting, muscular cramps, anorexia, decreased appetite, headache, skin responses, hallucinations, and urine incontinence.

9.2. Liposome:

Liposomes are a kind of therapeutic carrier nanoparticles (NPs) that are highly flexible and biocompatible [87]. They may readily be functionalized to engage with specific molecular targets and can integrate hydrophilic pharmaceuticals in the aqueous pore or hydrophobic chemicals in a lipid layer [88]. With the advancement in the field of nanotechnology, now we can generate BBB targeting surface-modified liposome ligands to cross the BBB via transcytosis. Recently other than conventional liposomal delivery systems (such as liposomes conjugation with ligands like Tf and Lf to cross over BBB via RMT [89], cell penetration peptides (CPP)-modified liposomes that target different amyloid markers and helps in inhibition of their expression [90] and magnetic liposomal delivery system has been explored to improve the brain drug delivery efficiency [91].

9.3. Quantum Dot Nanoparticles (QD NPs):

QDs are a class of nanoscale semiconductors that are highly stable and have high quantum yield, high absorbency, and high photobleaching resistance [92]. These can be used for the diagnosis and treatment of mitochondrial dysfunction in ADs [62,93,94]. This was verified by Hoshino et al's experiment which prepared mitochondrion-targeted QDs called Mit-8-QD that emitted red fluorescence as compared to non-mitochondrion targeting controls. This red fluorescence was emitted on co-localization with mitochondrion. Also, a TPP-modified molybdenum QD (TPP-MoS_2-QD) was designed to penetrate the BBB and target the mitochondrion. The co-localization levels of TPP-MoS_2-QDs were significantly more

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as compared to control MoS_2-QDs without TPP [95]. It was also found that this type of QDs reduced Aβ mediated ROS and prevented the disappearance of OMM and mitochondrial cristae caused by Aβ [96]. Moreover, TPP-MoS_2-QD reduced neuronal death in AD-infected mice cells as compared to control cells(given no TPP-MoS_2-QDs) thus proving to be a positive theranostic approach in AD [93].

Other strategies to treat AD apart from targeting mitochondria have also been explored. Kajal et al and co-workers engineered graphene carbon dots from ethanolic extracts of flowers from ***Clitoria ternatea*** using a one-step microwave-assisted method for the targeted delivery of drugs. It was observed that these carbon dots showed more percentage of inhibitions than conventional acetylcholinesterase (AChE) inhibitors due to their small size which confers them to the unique ability to cross BBB easily. These NPs prevented the aggregation of Aβ peptides [97]. In another experiment, selenium quantum dots (SeQDs) had been prepared which have high BBB activity and a high cellular uptake rate. Invitro experiments revealed that these QDs interfered with the transformation of Aβ monomers into aggregates thus preventing their accumulation. In vivo experiments revealed that SeQDs possessed antioxidant activity, improved mitochondrial dysfunction, and inhibited abnormally phosphorylated tau protein [98].

9.4. Gold Nanoparticles (Au-NPs):

Due to their extremely small size range (<3mm), good light stability, and high biocompatibility, these types of NPs can easily penetrate through the BBB [72,99]. The treatment of Aβ-induced mitochondrial dysfunction in AD by Au-NPs was confirmed from the experiments of Chiang et al who showed that these types of NPs downregulated the activities of caspase 3/9 [95]. Indicators of mitochondrial function, such as cytochrome C oxidase [COX] activity and mitochondrial morphology, were observed to be normalised by Au-NP in comparison to the control group [100]. These NPs have also been shown to restore the antioxidant levels in vivo and maintained normal function of mitochondria in the brain in AD infected in rat models [101]. As pointed out previously, the overproduction of ROS levels results in mitochondrial dysfunction. Therefore, functionalized Au-NPs like dihydrolipoic acid (DHLA)- functionalized gold nanocluster AuNCs (DHLA-AuNCs) were engineered which reduced ROS levels in BV-2 cell lines having AD. These

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functionalized Au-NPs also eliminated damaged mitochondria by inducing autophagosome formation in this type of BV-2 cell lines [102]. Another functionalized Au-NP is Kalopanacis Cortex extract capped gold NPs (KC-GNs) which was also shown to reduce ROS levels by 60% as compared to controls attenuated neuroinflammation [103] Thus Au-NPs can be a promising theranostic approach to treating damaged mitochondria by relieving oxidative stress and attenuation neuroinflammation.

Zhang et al and his coworkers designed a gold nanoparticle that had maize tetrapeptide anchored to its surface and evaluated the efficiency of the nano-peptide in AD models. The results revealed a decrease in intracellular ROS accumulation, a decrease in the activity of acetylcholinesterase, an upregulation of nuclear erythroid 2-related factor 2, and heme-oxygenase-1 and a downregulation of kelch-like ECH-associated protein 1 relative t untreated AD controls [104]. These NPs can also improve the acquisition and retention of spatial memory and learning in AD mouse models [105]. Other approaches in the treatment of AD include conjugating β-Boswellic Acid to Au-NPs to inhibit tau protein [106] and electrochemical quantification of apoE4 gene by ferrocene-coated Au-NPs. ApoE4 gene not only increases the risk of AD but also lowers the age of its onset and so this approach can help in early diagnosis and designing an effective therapeutic strategy for combating this neurodegenerative disorder [107].

9.5. Dendrimers:

These are synthetic polymers that contain repeating units which are highly branched and arise from a single focal point. They contain a large number of exposed anionic, cationic, or neutral groups which gives them hydrophilic or hydrophobic properties [108]. Their size ranges from 1 nm to 10 nm, and are radially symmetrical, globular, monodispersed, and homogenous [109]. They are extremely used as nanocarriers in drug delivery [110]. There are 2 main types of dendrimers that find use in the treatment of AD. Poly(amidoamine) Dendrimers (PAMAM) have higher degrees of drug loading [111], increased physical or chemical interactions between drug molecules [112] and tertiary amine groups, and increased conjugation degree due to many terminal groups [113]. Phosphorous Dendrimers contain phosphorous as the main inorganic group present in their structures and are extensively used in drug delivery either alone or complexed with other dendrimers [110].

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The major benefits of dendrimers are their adaptability, biocompatibility, ability to load pharmaceuticals into the core and surface, and nano-size. Dendrimers that have been conjugated with ligands can pass through the BBB and improve the uptake of medicines in the brain's target regions.

Recently, it has been seen that oxidative stress and activation of glial cells can induce inflammatory responses in AD. Therefore, a ROS-responsive dendrimer conjugated with peptide had been designed by Liu et al which reduced ROS levels, promoted Aβ phagocytosis, and reduced inflammation in the AD microenvironment by targeting and delivering peptides to the nuclear factor (erythroid-derived 2)-like 2 signaling pathway [111]. G4 poly (propylene imine) (PPI) dendrimers modified with histidine maltose shell NPs had been delivered to AD mouse models which resulted in memory protection [112]. Aβ peptide was also said to attach to sialic acid residues on the cell surface, leading to neurotoxicity [113]. Thus, the removal of sialic acid residues or mimicking the cell surface with sialic acid-conjugated dendrimers can be a potential therapeutic strategy. PAMAM dendrimers containing 32(G3) and 64 (G4) terminal groups and conjugated with sialic acid residues had been shown to reduce neurotoxicity in animal models. Similar results have been found by loading PAMAM dendrimers with an anti-AD drug like Memantine hydrochloride (MEM) [114].

9.6. Liposome-like Vesicles:

These are also known as extracellular vesicles (EVs) which have a size range from 50 nm to 5000 nm in diameter and have an excellent capacity to transfer proteins, lipids, and nucleic acids either to the adjacent or distant cells to bring about various physiological and pathological responses [115]. These vesicles have the capacity for efficient encapsulation of antioxidative and anti-inflammatory compounds like curcumin, easily cross BBB, and have a long shelf life with the stability of up to 6 months [116]. Efficient drug delivery can be carried out efficiently by these exosome-like NPs which possess anti-amyloidogenic activity [117,118].

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9.7. MITO-porters:

These nanoparticles, which resemble liposomes, are used to transport therapeutic proteins and nucleic acids to the mitochondria via fusing of the mitochondrial membrane. When released from the macropinosome, MITO-porters are effectively absorbed into the cell by macro-pinocytosis. They then attach to mitochondria via electrostatic contacts and carry medicines into mitochondria through membrane fusion [119]. The plasma membrane is the initial barrier that is met when substances are delivered intracellularly. High-density octa-arginine (R8) moieties are carried by MITO-porters to facilitate simple uptake via micropinocytosis [119].

Methylated -cyclodextrin (Me—CDs), a synthetic substance created by Yuma Yamada et al. and his associates, was conjugated with acid-labile polyrotaxane (Me-PRX). They have shown that Me—CDs are released from the conjugate complex in the acidic environment of lysosomes and that Me-PRX induced autophagy. So it was proposed that Me-CD interacts with membrane organelles like the ER and mitochondria and clears the membranes of cholesterol. This membrane damage allows Me-PRX to accumulate within these organelles and cause stress which can result in the triggering of autophagy. However, Me-poor PRX's cellular absorption restricts this process. Me-CD modified with folic acid has been shown to be able to trigger autophagy caused by mitochondrial stress. However, given that humans lack folate receptors, folic acid-modified Me-PRX would not be effective for treating AD. As a result, the application of MITO porters enhanced the cellular absorption of Me-PRX. When these nanodevices merged with the mitochondria, the researchers released the Me-PRX, which was then degraded in the mitochondrial intermembrane phase and activated autophagy [120].

9.8. Hormonal NPs

9.8.1. Melatonin-loaded NPs:

It has been reported in the literature that melatonin upregulates antioxidant enzymatic activities while simultaneously downregulating prooxidant enzymatic activity thus projecting nuclear DNA and membrane lipids from oxidative damage [121]. In addition, it has neuroprotective effects against AD. Schaffazick et al.

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created melatonin-loaded NPs utilising a nanoprecipitation technique and Eudragit S100® as the polymer. They induced peroxidation using the free radical ascorbyl and employed liposomes and microsomes as models of the lipid membrane. Melatonin was used to treat these models, and it was shown that it may inhibit peroxidation by up to 51%. However, the type of lipid substrate in which the melatonin was integrated affected how peroxidation worked. Similar tests were carried out in vivo, where melatonin nanoparticles (NPs) were targeted to mice's brains and livers and coated with polysorbate to facilitate simple passage over the BBB. The results revealed a significant decrease in lipid peroxidation in those regions on the administration of a single dose of melatonin NPs as compared to free melatonin. These results suggested polymeric nanocapsules increased the efficiency of the antioxidant activity of melatonin against the oxidation of lipids and could serve as a promising treatment for AD [122].

9.8.2. Vasoactive Intestinal Neuropeptide (VIP) NPs:

According to the research, VIPs can stop cell death linked to cytotoxicity brought on by Aβ [122]. For assessing its neuroprotective effects and biodistribution and brain uptake in mice, Brenneman et al incorporated a ^{125}I-VIP into poly (ethylene glycol)-poly (lactic acid) NPs and modified them with wheat germ agglutinin (WGA). The results revealed an increase in the concentration of the peptide in the brain of mice by 3.5 to 4.7-fold after intranasal administration of ^{125}I-VIP carried by NPs [123].

9.9. Acetylcholinesterase (AChE) NPs:

9.9.1. Nano-delivery of Tacrine:

As an AChE inhibitor, tacrine hydrochloride (THA) was the first drug used to treat AD. However, its use was abandoned due to its brief half-life, hepatotoxic effects, and gastrointestinal adverse effects. Alternative methods, however, have been developed that use the nasal cavity to administer AChE inhibitors through NP [124]. This made it easier for the drug-loaded NP to cross the BBB. To improve nose-to-brain transport of THA, Jogani et al. created a microemulsion delivery

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system conjugated with a mucoadhesive substance. This increased the drug's contact time in the nasal cavity, improved THA absorption into the brain, and decreased systemic distribution and serious adverse effects. Wilson et al. came up with a fresh approach by creating a poly(n-butyl cyanoacrylate) (PBCA) nanoparticle and coating it with polysorbate 80 to administer THA and get beyond its drawbacks. In accordance with the experiment's findings, a 4.07-fold rise in drug concentration was discovered in the mice's brain. Later, the same authors changed the composition of NP to chitosan which has been long-term favored in nanotechnology because of its nontoxicity, controllable polymer degradation rate, and easy modulation of drug release rate. Although the drug loading rate was doubled, the findings showed a comparable amount of THA buildup, indicating that the NP has to be modified in addition to T80. The usage of magnetic compounds resulted in a larger concentration of the desired medication in the target location when subjected to magnetic fields, hence magnetic chitosan NPs loaded with THA were employed in that study [125].

9.9.2. Nanodelivery of Donepezil:

This AChE inhibitor had been developed to overcome the limitations of THA. Donepezil (DZP) has more selectivity to the brain, has a higher inhibitory effect than THA, and possesses a longer half-life and greater bioavailability than other drugs [125]. To enhance the efficiency of drug delivery and destabilize Aβ fibril formation, PLGA NPs had been designed by Baysal et al and these NPs had been with DZP with PEG modification [126]. It has been seen that 60% of the drug was released in 2 hours with a sustained release following up to 70 hours. To administer the AChE inhibitor via the nasal route, Yasir et al developed he loaded the inhibitor onto a solid lipid NP (SLN) [127]. Another recent strategy involves the fabrication of a nanostructure lipid carrier (NLC) loaded with DZP to deliver drugs trans-dermally.

9.9.3. Nanodelivery of Galantamine:

Galantamine Hydrobromide (GAL) is a competitive and reversible AChE inhibitor by acting as an allosteric modulator of acetylcholine receptor [125] Nanoliposomes

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had been designed for the targeted delivery of GAL to the CNS, but results were not satisfactory. So, the search for other strategies continued. One such strategy involved the use of bone marrow-derived mesenchymal stem cells (BM-MSCs) and was co-administered with SLN-GAL NPs which had been shown to restore memory and reduce progressive loss of neurons in animal models [128].

9.10. Polyphenol NPs:

Transformation of polyphenolic antioxidants obtained from dietary plant lignans into physiologically active neuroprotector compounds through the metabolism of gut metabolism. These metabolites exert their neuroprotective effects in AD. For example, the metabolism of plant lignans by gut bacteria produces enterolactone and enterodiol which are AChE inhibitors and can be potentially used in the therapeutics of AD. The two most common polyphenols used in nanotechnology are Catechin and Resveratrol [129].

9.10.1. Catechin loaded NPs:

Catechin is a flavonoid that may be found in foods made from plants. Epigallocatechin-3-gallate (EGCG), an ester of epigallocatechin and gallic acid, is one of these catechins and is most commonly found in tea. EGCG is effective in the treatment of AD and is high in phenolic hydroxyl groups. The ability of catechin-loaded NPs to deliver anti-amyloidogenic peptides and hence promote cell survival against toxic Aβ protein aggregates has recently come to light. It was discovered that casein phosphopeptide (CPP) and chitosan (CS) coated NPs had greater EGCG encapsulation efficiency than CS-tripolyphosphate coated NPs. In the instance of CS-CPP NPs, the burst release was likewise delivered in a manageable way. These NPs containing bioactive peptides were found to be efficient carriers of EGCG [130]. In an alternative approach that involved an oral delivery system, EGCG had been loaded onto SLN. Results showed improved bioavailability, protection of EGCG from degradation due to encapsulation into the SLN, and slow and sustained release of the catechin along with no acute or sub-chronic toxicity [131,132]. To examine possible effects of catechin nano-encapsulation on neuronal survival and morphological abnormalities in primary rat hippocampus neurons, poly (ethylene glycol) and cetyltrimethylammonium

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bromide (CTAB)-modified silica nanoparticles were created. Catechins were then loaded onto these NPs. Release experiments showed that the catechin release profile, which decreased Cu (II)-induced oxidative stress in animal models, was a determinant of these NPs' capacity to load [133]. Other combinations included designing polyethylene glycol-modified PLGA NPs which showed an increase in drug permeance in blood and brain. Recently, Yang et al conducted a study in which the authors designed an NP modified with RD2 peptide and loaded these with EGCG for penetrating the brain tissue and accurately targeting Aβ. Results were quite conclusive and satisfactory which revealed that this therapeutic strategy reduced the expression of proinflammatory expression cytokines like TNF-α and IL-1β, restored neuronal losses and hippocampal damage, and ameliorated spatial memory impairment in AD disease model mice [134].

9.10.2. Resveratrol loaded NPs:

Resveratrol is a polyphenolic phytoalexin which is also known as 3,5,4'-trihydroxystilbene. It possesses antioxidant activity which makes it a useful compound in the treatment of AD. Resveratrol was nano-encapsulated with chitosan (CS) and γ-poly (glutamic acid) (γ-PGA) and delivered to rat models. An increase in the accumulation of this polyphenol was observed in the brain. When in vitro studies had been carried out in H22 mouse models to examine neurotoxicity in AD, the results revealed that these polyphenol-loaded NPs inhibited ferroptosis induced by elastin [135]. Another strategy involved the fabrication of transferosomes using GNPs followed by loading them with resveratrol which showed enhanced behavioral acquisition and spatial memory function in rats [136]. Moreover, the functionalization of SLNs with anti-transferrin receptor monoclonal antibody (OX26 mAb) can function as a carrier to enable targeted delivery of the polyphenol in human brains [129,137].

9.11. Fullerenes

Derivatives of fullerenes, called fullerenols have therapeutic properties for the treatment of AD. They function as scavengers of oxygen and reduce toxicity induced by glutamate, NMDA (N-Methyl-D-aspartate) and AMPA (alpha-amino-3- hydroxy-5-methyl-4-

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isoxazole-propionic acid) as observed in cultured cortical neurons. In order to lessen NMDA receptor-mediated toxicity and apoptotic neuronal death, fullerenols can either inhibit glutamate receptors or infiltrate lipid membranes [138].

9.12. Metal Chelators NPs

It has been observed that Cu^{2+}, Fe, and Zn can induce Aβ aggregation [139]. When endogenous reductants are present, the copper-amyloid complex can accelerate the reduction of dioxygen and create ROS, which can cause neuronal death [98]. Zn plays an important role in neuronal communication by modulating a number of receptors when it is released into synaptic clefts. Additionally, Zn binds to both Aβ and APP; this binding to APP occurs at the Lys 16 position, which may impact the alpha-ability of secretases to cleave the protein, decreasing the amount of soluble APP and increasing the amount of Aβ [139]. Oxidative damage is caused by Fe2+ binding to the N-terminal region of Aβ, changing it, and producing ROS. Meanwhile, Fe3+ activates β-secretase [139]. Organic or inorganic chemicals known as "metal chelating agents" have the ability to bind metal ions and create molecules with a ring structure known as "Chelates". The ligand-binding atoms in chelating agents create covalent and coordinate bonds. Low molecular weight, excellent selectivity, and the ability to penetrate physiological and membrane barriers into regions where a hazardous metal ion is concentrated are all requirements for an efficient metal chelator. It separates the metal from its biological ligand and produces a safe, non-toxic complex with the metal, which promotes its excretion from the deposit site without consuming additional metal ions [139]. The possibility of using metal chelators to reestablish the normal trafficking of metal ions has been seen as a viable strategy to reduce the oxidative stress that kills neurons. A metal chelator such as N, N′-1,10-Bis(naringin) Tri-ethylene-tetraamine is one such method. It prevents Cu2+-induced Aβ aggregation, making it a potentially effective therapeutic strategy for the treatment of AD [98]. Utilizing hydroxyquinoline (CQ) and EGCG moieties to create the lead chemical TGR86, which may sequester Cu2+ from the A complex and hence enhance neuronal cell survival, is another novel therapeutic approach using metal chelators for the treatment of AD. Flavonoids possess natural Fe and Cu chelating abilities. It was shown that Zn chelator (N, N, N′, N′-tetrakis (2-pyridinylmethyl)-1,2-ethylenediamine-TPEN) decreased Zn levels

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and increased longevity and health in transgenic C. elegans. Deferoxamine (DFO), Deferasirox, and Deferiprone as Fe chelators, Trientine (TETA) as Cu chelators, and EDTA as a Zn chelator are further potential metal chelators that might be employed as potential treatment strategies [139].

9.13. Curcumin NPs:

Curcumin is a naturally occurring phytochemical It is a strong anti-inflammatory, antioxidant, and free radical (nitric oxide-based) scavenger that safeguards the brain from lipid peroxidation. It also has a good toxicological profile [140]. Both in vivo and in vitro, it has the capacity to bind with amyloid made of tau protein [140]. Unfortunately, this substance has limited stability since it is quickly hydrolyzed in both alkaline and acidic environments. Additionally, it easily undergoes oxidation or photodegradation, resulting in low bioavailability and little brain absorption [122].

To avoid such problems new efforts are being made to generate new curcumin analogs those having the same biological activity, but under certain physiological conditions, improved pharmacokinetics properties, bioavailability, water solubility index as well as stability [141]. With reference to the work done by Singh et al., they have developed curcumin-encapsulated Pluronic F127 nanoparticles (FCur NPs) those having optimized bioavailability, the ability to go through blood vessels in a faster speed, and improved BBB penetration. They have compared crossing ability of FCur NPs and free curcumin through BBB, used an in vitro BBB model, and tested them by administration into healthy mice. They came to the conclusion that "FCur NPs display 6.5-fold stronger fluorescent intensity in the brain than those from free curcumin. In addition, in vitro comparison with Congo red, a marker for Aβ plaques revealed that encapsulated curcumin maintains its ability to bind to Aβ plaques. FCur NPs exhibited antioxidant and antiapoptotic activity when compared to free curcumin. The combination of in vitro and in vivo results suggests potential utility of the inexpensive FCur NPs as a theranostic agent for AD" [142].

9.14. Nanogels:

Hydrogel solids called nanogels have a three-dimensional network at the nanoscale. They are interconnected, expandable networks with a considerable water-holding capacity. They

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can be made from synthetic polymers, biopolymers, or a mix of the two [143]. By changing their chemical makeup, such as degradability, charge, softness, size, porosity, and amphiphilicity, their physicochemical characteristics may be adjusted [64]. Extracellular senile plaques made mostly of amyloid peptide and intracellular neurofibrillary tangles made of tau protein are present in AD [144]. The hyperphosphorylated Tau protein, which is located in the axons of neurons and is connected to the microtubules, makes up the neurofibrillary tangles. Tau does not connect to the microtubules when it is hyperphosphorylated, which causes cell death [33]. In an aqueous solution, nanogel forms brief hydrophobic pockets that protect the drug contained inside. The AKT pathway was activated as a result of the formulation's ability to bind to the insulin receptor, which in turn began the insulin signaling cascade. Furthermore, when compared to free insulin, in vitro tests revealed that the formulation crossed the blood-brain barrier (BBB), demonstrating that the drug entered the body via the paracellular pathway. According to the study, nanogels may be used to deliver insulin intranasally, allowing it to travel fast from the

olfactory mucosa to the olfactory bulb through the olfactory nerve system. The formulation

showed in vitro that it could protect against the toxicity caused by the amyloid peptide [145]. Numerous studies have suggested that nanogels are potential delivery systems that could be used for the management of AD disease because insulin administration to AD patients (mild or moderate) improves their cognitive abilities, brain function, and preserves the rate of glucose utilisation of the brain [146].

According to recent research, angiopep-2-modified chitosan nanogels (AOC NGs) loaded with oxytocin (OT) can reduce innate inflammatory responses. The research offers a viable early AD preventive method that involves injecting exogenous OT into the brain to have an anti-inflammatory impact [147].

9.15. Antioxidant molecules targeted against mitochondria for AD

All the natural antioxidant molecules available for mitochondrial-targeted therapeutics for AD are listed below with their pharmacological effect and mode of action in the following table 2.

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Table 2 - Therapeutic approaches of Natural anti-oxidants towards AD: [116]

Sl. No.	Bioactive molecule	Pharmacological effect	Mode of action	References
1.	Ubiquinone (also known as CoQ10)	Antioxidant	Controls the formation of ROS, oxidative stress, and inflammation inside mitochondria by acting as a crucial cofactor of the ETC.	[148]
2.	Creatine	Antioxidant/ neuroprotective	Has the potential to produce neuroprotective effects in a variety of neurodegenerative disorders, including AD, by blocking the mitochondrial permeability transition pore (mPTP).	[149]
3.	Ginsenoside Rg3	Antioxidant	Block the signalling pathways for nuclear factor NF-B and mitogen-activated protein kinases to prevent the production of pro-inflammatory mediators.	[150]
4.	Methylene blue (MTC)	Antioxidant	MTC can act as an alternate electron carrier that skips complex I/III and can also trigger autophagy in addition to having an impact on tau aggregation.	[151]

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5.	Mito-TEMPO	Antioxidant	It is a SOD (superoxide dismutase) mimic that can convert ferrous iron into ferric iron and transform superoxide molecules into water. It has also been proven to enhance mitochondrial activity and minimize mitochondria-mediated oxidative stress.	[152]
6.	Mito-VitE	Antioxidant	Prevents lipid peroxidation, which in turn prevents mitochondrial oxidative stress.	[34]

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9.15. Mitochondrial Targeted small molecules towards AD:

All the small molecules available for mitochondrial-targeted therapeutics for AD are listed below with their molecular weight, chemical formula and structure and their properties in the following table 3.

Table 3: Summary of small molecules for the mitochondrial-directed treatment of AD disease

Name	***Molecular Weight***	***Molecular Formula***	***Properties***	***Reference***
DDQ Diethyl (3,4-dihydroxyphenethylamino) (quinoline-4-yl) methyl-phosphonate	430.43 g/mol	$C_{22}H_{27}N_{27}O_5P$	Dopamine derivative that adheres to Aβ; hinders Aβ and Drp1 from binding at the ser8 and Leu34 active sites of the Aβ polypeptide and the ASN16 and Glu16 site of Drp1; interferes with Aβ's ability to interact with mitochondria and synapses.	[153]

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Glutathione (GSH)γ-Glutamyl-Cysteinyl-Glycine γ-Glutamyl-Cysteinyl-Glycine(2S)-2-amino-4-{[(1R)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl}butanoic acid	307.32 g/mol	$C_{10}H_{17}N_3O_6S$	GSH is an inborn antioxidant that reduces free radicals in mitochondria by establishing a bond with the choline ester, which drives GSH to the mitochondrion. It also maintains the equilibrium of protein sulfhydryl molecules and decreases the protein's sulfenic ion through covalent adduction.	[80,153]

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MDivi13-(2,4-Dichloro-5-methoxyphenyl)-2-sulfanyl-4(3H)-quinazolinone	353.217 g/mol	$C_{15}H_{10}Cl_2N_2O_2S$	Promotes mitochondrial fusion genes, inhibits Drp1 and Fis1 overexpression, and prevents mitochondrial degeneration. It also increases cytochrome c oxidase activity, ATP production, and cell survival. decreases lipid peroxidation and H2O2 generation.	[34]

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MitoPBN[4-[4 (1,1-dimethyl-ylethyl) oxidoimino]-methyl]phenoxy]butyl] and triphenyl-phosphonium bromide	590.542 g/mol	$C_{33}H_{37}BrNO_2P$	Conjugated aromatic group (PBN) reduces free radicals and decreases O-induced activation of uncoupled proteins. Coenzyme Q, an antioxidant, and phenyl tert-butyl nitrone make up its composition.	[34]
MitoQ[10-(6-ubiquinonyl) decyltriphenylphosphonium bromide]	665.65 g/mol	$C_{37}H_{46}O_4PBr$	Reduces free radicals, minimises mitochondrial fragmentation, and promotes mitochondrial fusion. A mitochondrial quinolone derivative with a	[34]

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			mitochondria-targeted TPP that has the capacity to accumulate in high quantities in mitochondria (up to 5000–6000). Additionally, it encourages the production of large neurites and scavenges free radicals.	
MitoTEMPO(2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-acylamino)-2-oxoethyl) triphenylphosphonium chloride	510.03 g/mol	$C_{29}H_{35}N_2O_2P\cdots Cl$	Contains a free radical electron that can remove mitochondrial superoxide, has a mitochondria-targeted TPP that scavenges for ROS and mitigates ROS damage, lowers overall	[34]

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			oxidative stress, reduces lipid peroxidation brought on by A, and prevents the formation of O2 radical dots, and increases the quantity and fidelity of mtDNA copies.	
SS-02		Dmt-d-Arg-Phe-Lys-NH2	Mitochondria targeting; aromatic ring; positively charged cation; antioxidant properties	[34]
SS-19		Dmt-d-Arg-Phe-atnDAP-NH2	Mitochondria targeting; aromatic ring; positively charged cation; antioxidant properties	[34]

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SS-20		Phe-d-Arg-Phe-Lys-NH2	Protects mitochondrial cristae, stops the loss of cytochrome c, encourages the restoration of mitochondrial respiration following ischemia reperfusion, and maintains heart function. Mitochondria targeting; aromatic ring; positively charged cation; antioxidant characteristics.	[34]
SS-31 Elamipretide-Bendavia	639.802 g/mol	d-Arg-Dmt-Lys-Phe-NH2 $C_{32}H_{49}N_9O_5$	Preserves heart function, safeguards mitochondrial cristae, stops the loss of cytochrome c,	[34]

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			and promotes the return of mitochondrial respiration following ischemia reperfusion. Mitochondria targeting; aromatic ring; positively charged cation; antioxidant properties. Binds to cardiolipin and localises to IMM.	

10. Toxicities and Challenges associated with Nanoparticles in AD

Nanoparticles (NPs) are known to have widespread biological applications as drug delivery mechanisms due to its exceptional physicochemical and behavioral properties, yet, there are significant uncertainties about the safety of manufactured nanoparticles in people as their use in biological applications expands. By virtue of its minute size and distinctive characteristics, nanoparticles are frequently used in nanomedicine and as drug carriers [154]. However, the toxicity towards healthy human cells, tissues, and organs may also be owing to their crystallinity, solubility, aggregation, surface characteristics, morphology, surface area, and dose-dependent characteristics [155–157]. The ability for the nanoparticles to trigger an innate immune response must be ascertained. Numerous nanoparticles, including metal and metal-oxide nanoparticles, have been reported to induce pro-inflammatory effects in both in vitro and in vivo studies [2].

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Nanoparticles predominantly enter the human body through the respiratory system, wherein they frequently cause inflammatory reactions due to redox stress. Additionally, nanoparticles have the ability to enter the brain. The olfactory nerve is hypothesized to carry nanoparticles that impact on the olfactory mucosa, from where they can travel to the brain and affect brain health and functioning [2]. Furthermore, oxidative stress is one of the frequently studied effects of nanoparticles. Increased production of ROS, which is preferred above antioxidants, leads to an oxidative stress condition. By-products of biological reactions, such as peroxynitrite (ONOO), nitric oxide (NO), hydroxyl radical (OH), hydrogen peroxide (H2O2), and superoxide radical (O2), are the most prevalent ways of ROS generation [158]. The ROS damage proteins, lipids, and the most important biomolecules, which can activate a system similar to NADPH, disrupt the electron transport chain, depolarize the mitochondrial membrane, and affect the mitochondrial structure [159]. In a study conducted by Hou et al. it was observed that Zinc oxide nanoparticles (ZnONPs) cause DNA replication disorders in the G1, M, and G2 phases of the cell cycle pathway in addition to the failure of mini-chromosome maintenance [160]. Nanoparticles also induce cytotoxicity by altering the numerous physicochemical, metabolic, and molecular pathways. Smaller nanoparticles often have bigger surface areas, allowing for interactions with cell constituents such carbohydrates, fatty acids, proteins, and nucleic acids suggesting that particle size may influence cytotoxic efficacy. A major contributor to the cytotoxicity, which is associated with energy and metabolic abnormalities as well as cellular dysregulation is the disturbance of Ca2+ (intracellular calcium). Despite the fact that Ca2+ is one of the key signalling molecules associated with the signal transduction in the metabolic regulations, its elevation has acute toxicity on cellular mitochondria, which results in the induction of apoptosis by preferentially releasing cytochrome c or by enhanced ROS production and opening the inner mitochondrial pore, eventually leading to death of the individual [161]. The cytotoxic action of nanoparticles has recently been demonstrated to not only cause cell death but also to suppress cell growth if cells are arrested in at least one cell cycle phase (G2/M phase, S phase, or G0/G1 phase). Cells that are arrested in the cell cycle either build up significant damage that causes apoptosis or repair the damage. Cell cycle arrest can be specific to certain cell types at particular stages. With reference to the study conducted by Gao et al., nickel oxide NP (NiONP) treatment resulted in a much lower G0/G1 phase in the A549 cell line and a significantly higher G0/G1 phase in the BEAS-2B cell line. The cell cycle is also impacted by the type of nanoparticle. In T-cells, ZnONOs and CuONPS exposure resulted in G2/M phase arrest,

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while TiO2 resulted in S-phase arrest [162]. The primary cause of the process underlying nanoparticle-associated genotoxicity is the increased production of reactive nitrogen (RNS) species and ROS, which causes higher oxidative stress and oxidative damage to the genetic makeup. The interaction of the nanoparticles with the DNA involves the primary toxicity whereas, secondary genotoxicity is contributed by ROS/RNS that the nanoparticles produce. In the indirect primary clastogenic pathway, unsaturated aldehydes produced as a result of primary lipid oxidation by ROS are used for the production of exocyclic DNA adducts [159]. Numerous studies have documented the development of nanoparticles (NPs) like copper oxide (CuO) on biomedical platforms; nevertheless, it's possible that they have the potential to accelerate the process of protein oligomerization. A study by Jaragh-Alhadad and Falahati sought to comprehend how CuONPs affected the oligomerization of β1–42 (Aβ1–42) and related neurotoxicity. Crucial facts concerning the detrimental effects of CuONPs against central nervous system proteins that encourage the development of cytotoxic oligomers were revealed by the study [163]. The analysis of nanoparticle toxicity will pave the way to the development of better and more efficient nanoparticles. Extensive research is thus being carried out to increase the overall knowledge of the effects that nanoparticles have on the environment and public health as well as to advance the development of safer materials.

11. Future scopes and prospects

It has been stated by the World Health Organization (WHO) based on various estimations that the frequency of individuals with dementia will massively quadruple in the coming decade, almost 131 million people worldwide by 2050 [164]. Unsatisfactory outcomes from clinical trials of AD treatments have highlighted the need for further standardization of target populations and monitoring techniques. A further impassable hindrance to the treatment of AD is the Blood-Brain Barrier, which prohibits the conventional medicines from entering the Central Nervous System. Thus, approaches for both early diagnosis and its therapeutic interventions may be the most demanding issues in contemporary medicine. The symptoms of AD can be minimized and temporarily slowed down by the drugs currently available, but the progression of brain damage cannot be prevented [165]. Significant progress has been made in the field of nanotechnology over the past years, particularly in the fields of medicine and material science. The production of different forms of drug-loaded nanocarriers, typically ranging in size from 1-1000 nm, has been

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greatly influenced by the medical use of nanotechnologies [166]. As an alternative to traditional medication delivery techniques, nanotechnology thus offers new possibilities for the treatment of AD. According to recent research, NPs can successfully penetrate the BBB and exhibit suppressive action that can increase specificity and efficiency at optimum pH and temperatures. Also, utilizing therapeutic AD interventions that are mitochondria-targeted, could be further transferred from in vitro and in vivo experiments to human clinical trials. The characteristics, morphology, efficacy, and targeted delivery effectiveness of the nanocarrier can be enhanced by applying surfactants or hydrophilic substances like polyethylene glycol (PEG) to the surface of NPs, thus enhancing the therapeutic efficacy of AD [167,168]. Another popular area of research involves the use of stem cell therapeutics and nanotechnology to treat AD. This new technique controls the proliferation and differentiation of stem cells, or it stimulates tissue healing and repair, using nanotechnology [169]. Liposome based drug delivery is a promising treatment for AD. In terms of therapeutics, liposomes can improve a medicine's efficacy, durability, regulate release, delivery through a variety of routes, direct tissue action, and help minimize unwanted drug toxicity. Liposomes have a number of characteristics that make them superior to other vesicles, which includes the capacity to transport hydrophilic, hydrophobic, and amphipathic drugs, low toxicity, compatibility with the environment outside, not triggering the immune system, and the ability to target the site of action for efficient drug delivery. Liposomes can be modified further to overcome the shortcomings by altering the size and lipid components. Depending on the medicine it comprises and the desired treatment environment, changes are made to a liposome's structure and properties. Target ligand-mediated liposomes and antibodies attached with PEG are two more changes made to liposomes to assure liposome persistence and effective therapy. Ligand targets provide a personalized liposome that can speed up the rate at which drugs accumulate in the intended tissue. The liposome enables better monitoring over how long the therapeutic substance remains in the bloodstream, decreasing toxicity and extending the therapeutic activity [170]. Despite numerous researches, there is still much work to be done in the practical application of nanotechnology in AD therapy. For effective medication delivery to treat patients with CNS illnesses like AD, more powerful and non-toxic nanomedicine formulations are essential [171].

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12. Conclusion

Public health initiatives are focused on preventing AD globally. The intricacy of the disease's symptoms and etiology, our inadequate understanding of its mechanism, and the possibility of a dormant, asymptomatic preclinical period, all contribute to how challenging it is to treat. Despite the fact that a large number of medications are constantly being tested in clinical research for the treatment of Alzheimer's disease, the unusual absence of patient response and occasionally significant side effects makes the use of customized medications obvious. One of the most significant advancements in the treatment of Alzheimers and other associated disorders is Nanotheranostics or the use of nanotechnology, which makes use of the engineered equipment for various biological interactions at the molecular level and also has the potential to revolutionize the treatment of neurodegenerative diseases by inducing biological responses at target sites while lessening adverse effects. The blood-brain barrier shields the brain from toxic drugs, making drug delivery across the BBB a difficult task for neurodegenerative diseases detection, localization, and therapy. Traditional medications frequently fail to cross the BBB, making them ineffective for treating disease. Despite the fact that nanotechnology is being experimented vastly, concerns of translational relevance and safety are to be analyzed. This necessitates a thorough knowledge of how body systems interact with nanomaterials. It has been widely documented that quantum dots, metal NPs, and nanocomposites can cure various neurodegenerative diseases. It is necessary to get past the restrictions put on these NPs. Although the researches that have been conducted in the field have not produced any noteworthy outcomes that could be applied to humans, their effects on the lowering of molecular events leading to neurodegenerative disorders have been notable, so the strategic approach to popularize the use of nano-therapies instead of conventional drugs has a good chance of producing prominent outcomes in the near future.

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