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REVIEWS

Review

Advances in Alzheimer's Diagnosis and Therapy: The Implications of Nanotechnology

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Alzheimer's disease (AD) is a type of dementia that causes major issues for patients' memory, thinking, and behavior. Despite efforts to advance AD diagnostic and therapeutic tools, AD remains incurable due to its complex and multifactorial nature and lack of effective diagnostics/therapeutics. Nanoparticles (NPs) have demonstrated the potential to overcome the challenges and limitations associated with traditional diagnostics/therapeutics. Nanotechnology is now offering new tools and insights to advance our understanding of AD and eventually may offer new hope to AD patients. Here, we review the key roles of nanotechnologies in the recent literature, in both diagnostic and therapeutic aspects of AD, and discuss how these achievements may improve patient prognosis and quality of life.

Why AD Therapeutic Approaches Fail: Are We Using the Right Diagnostic and Therapeutic Strategies?

AD is the most prevalent neurodegenerative disorder leading to memory loss and progressive and permanent deterioration of cognitive function. Approximately 5%, 20%, and 33% of people aged over 65, 80, and 90 years, respectively, live with AD [1]. The prevalence of AD increases yearly; by 2050 the number of affected individuals is expected to be more than 115 million people worldwide [2,3]. Despite considerable research efforts, current diagnostic and therapeutic approaches are ineffective for the prevention or treatment of AD. The causes and etiology of AD remain elusive and AD therapy/prevention is known as one of the most high-risk approaches in the pharmaceutical industry. A detailed understanding of the etiology and molecular mechanism of AD pathogenesis is fundamental to the identification of new therapeutic targets to reduce cognitive decline and/or improve memory function.

To date, many biological pathways and molecules have been claimed to be the main cause of AD. Among them, amyloid beta (Aβ) has emerged as the primary focus of studies on neurodegeneration (i.e., the amyloid cascade hypothesis). Many scientists support the popular amyloid cascade hypothesis, which suggests that self-assembly of Aβ monomers into soluble oligomers (AβOs) and/or insoluble fibrils results in neural death, synaptic dysfunction, and cognitive impairment. Therefore, many current therapeutic approaches are based on targeting the production, fibrillation, and clearance of Aβ. Although Aβ-related treatment strategies are more advanced than other strategies, they have had no clinical benefits. Current approved approaches to neurotransmitter modulation only ameliorate symptoms.

Trends

Nanotechnology offers a multitude of diagnostic, mechanistic, and therapeutic tools for Alzheimer's disease (AD).

Nanobased approaches are already providing new insights to address the pathogenesis of AD. Nanotechnology addresses the multifaceted nature of age-related degeneration, while simplistic linear models of AD, such as amyloid cascade, have failed to address it.

Nanoparticles have the utility to address each compartment and phase of the disease in a highly sophisticated manner.

Nanotechnology offers new hope for AD where conventional approaches have stalled.

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The disappointing results of AD treatment are due to the reliance on a simplistic, linear view of AD and in part are related to the inability to detect the disease in its early stages before behavioral symptoms and memory loss. As AD pathogenesis is multifactorial, it is hard to discover the biomarkers that predict disease progression from a mild state to profound memory loss. Intense studies are being focused on the detection of Aβ and tau proteins (i.e., the microtubule-associated proteins that contribute mostly to modulating the stability of microtubules in neurons) as AD biomarkers. In addition, large-scale genomics, proteomics, and metabolomics studies have identified several biomarkers that distinguish patients and predict disease development from mild cognitive impairment (MCI) to AD [4–7]. Besides biomarkers, monitoring the patterns of multivalent cations (e.g., zinc, copper, iron) in the blood plasma showed promising outcomes in the detection of AD [8]. As the net charge of Aβ proteins is negative, the multivalent cations can bind to Aβ proteins and accelerate the oligomerization and fibrillation process.

Despite impressive progress in preclinical studies, almost all Aβ-related diagnosis/treatment approaches thus far have failed in clinical translation [9–14], which might be related to the secondary role of Aβ in the progression of AD. Thus, there is an urgent need to re-examine the validity of the Aβ-targeting approaches and to develop new effective strategies [15]. Besides the issues with Aβ-targeting approaches, the inability of diagnostic/therapeutic agents to cross the blood–brain barrier (BBB) and targeting multiple molecules involved in AD pathogenesis are other major issues in the field [16]. The therapeutics also exhibit poor solubility, bioavailability, and targeting potency in reaching their pathogenic targets. This has encouraged the development of new diagnostic/therapeutic agents to overcome these limitations.

Nanotechnology-Based AD Diagnosis and Therapy

Recent developments in nanotechnology have provided an unprecedented opportunity to improve the prevention, early detection, and treatment of AD. Because of inherently valuable properties such as high surface-to-volume ratio, ease of surface functionalization with desired ligands, and potential ability to cross biological barriers, NPs are increasingly recognized as promising candidates for AD diagnosis and therapy [16]. NPs are objects that have at least one dimension below 100 nm and are extensively used for drug delivery across the BBB, imaging and early detection of plaques and AβOs, inhibition of Aβ fibrillation, clearance of preformed fibrils, oxidative stress and/or neuroinflammation suppression, metal-chelation therapy, and photothermal therapy (Figure 1) [17–24]. This review outlines the potential application of NPs in AD diagnosis and therapy.

Nanodiagnostic Approaches for AD Diagnosis

AD is clinically characterized as the accumulation of Aβ plaques and neurofibrillary tangles of hypophosphorylated tau proteins in the brain, with symptoms of loss of mental faculties, memory retention, and independence [25,26]. The Aβ peptide is the main constituent of senile plaque (i.e., extracellular deposits of Aβ in the brain's grey matter) and a key pathological hallmark of AD. Due to its likely vital role in the pathogenesis, Aβ imaging in the brain is a promising approach for AD diagnosis [27].

Nanodiagnostic approaches have shown promising potential in the early detection and diagnosis of AD. For example, it was demonstrated that Fe biometal specifically accumulates in amyloid plaque core (APC) as crystalline magnetic NPs and has superparamagnetic properties, which can be used for AD diagnosis [28]. Nanosized imaging agents or nanocarriers loaded with imaging agents have been developed to detect Aβ plaques with high specificity. To date, several nanodiagnostic approaches have been developed to image and/or monitor Aβ in the brain (Table 1) [28–35]. For example, Cheng and colleagues [30] synthesized curcumin-conjugated superparamagnetic iron oxide NPs (SPIONs) coated with polyethylene glycol

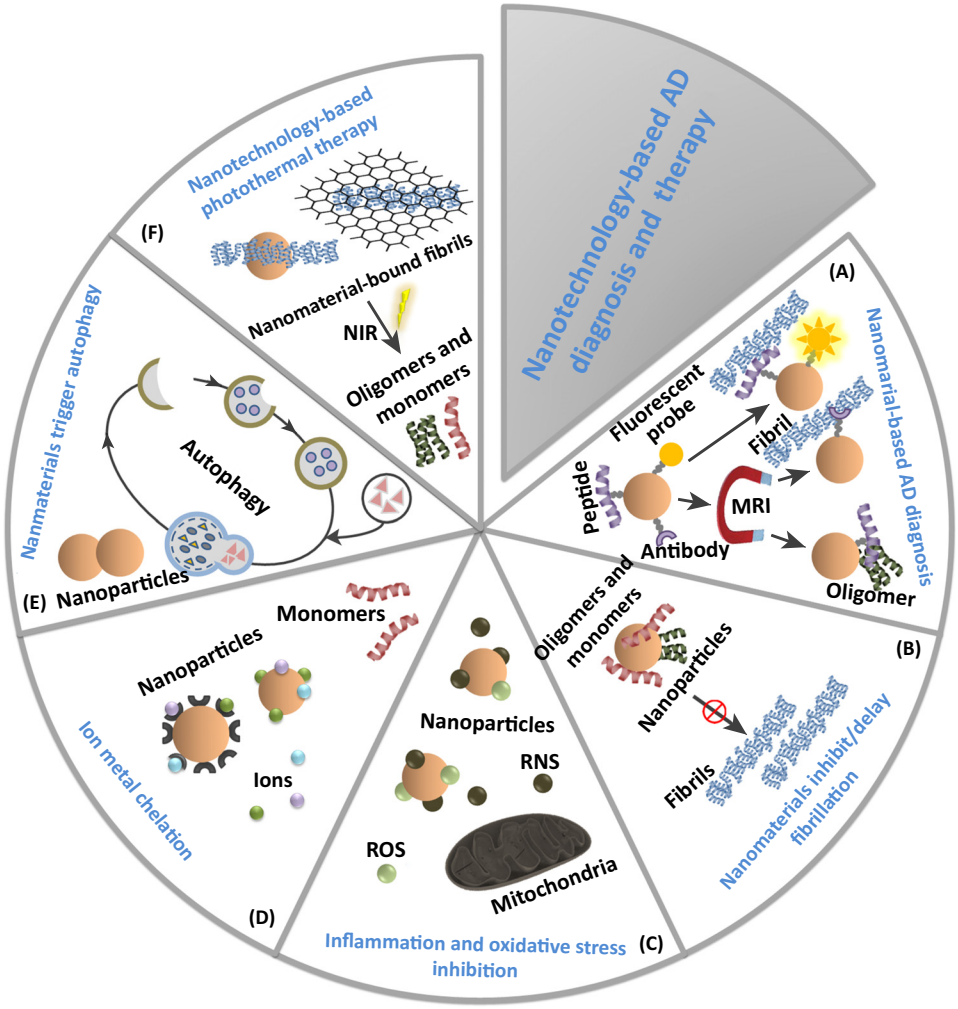


Figure 1. Schematic Representation of Nanotherapeutic and Nanodiagnostic Approaches Developed for Alzheimer's Disease (AD) Diagnosis and Therapy. Antibody/peptide/probe-functionalized nanoparticles (NPs) are used to detect amyloid beta (Aβ) via targeted MRI and fluorescence quenching (A). NPs can adsorb Aβ monomers/oligomers and delay/inhibit the fibrillation process (B). NPs have the capacity to prevent oxidative stress and inflammation via capture of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (C). NPs can inhibit amyloid fibrillation via ion metal chelation (D). Single-wall carbon nanotubes amend abnormal degradative pathways (defective autophagy and lysosome dysfunction) (E). Graphene-bound fibrils are destabilized to monomers and oligomers through exposure to near-IR irradiation (NIR) (F).

(PEG)–polylactic acid (PLA) that penetrated into brain and located Aβ plaques. Importantly, for many NPs, the use of PEG could increase NP circulation half-life, which is essential to improve the targeting capacity of NPs.

In conjunction, immune-based nanodiagnostic approaches have been developed to increase the specificity of contrast agents for Aβ/tau detection. Extensive and promising work has been conducted in this field. For example, Sillerud and coworkers [31] demonstrated that anti-Aβ-functionalized SPIONs cross the BBB and specifically bind to amyloid plaques. In a similar study, magnetic nitrogen-doped graphene functionalized with Aβ antibody showed high selectivity for Aβ. This immunomagnetic nanosensor was used for electrochemical detection of Aβ [32]. In another study Demeritte and colleagues [33] modified magnetic core–plasmonic

Table 1. Nanotechnology-Based Strategies for Diagnosis and Early Detection of AD

NP	Surface modification	Application	Mechanism of action	NP size	Model of use	Remark	Refs
Liposome	Liposome NPs were modified with amyloid-targeting ligand (ET6-21) and gadolinium	Targeted MRI of amyloid plaques	ET6-21 increased the targeting efficacy of NPs Gadolinium increased the contrasting and imaging efficacy	148.9 ± 19.5 nm	*In vivo* (intravenous injection)	NPs pass through BBB	[29]
SPIO	Curcumin-functionalized SPIONs were coated with PEG–PLA	Targeted MRI of amyloid plaques	Curcumin increased the targeting efficacy of NPs PEG–PLA coat increased NP circulation half-life and enhanced NP transport across BBB	69.7 ± 1.7 nm	*In vivo* (intravenous injection)	NPs pass through BBB	[30]
SPIO	SPIONs were functionalized with antibody (anti-AβPP)	Targeted MRI of amyloid plaques	Antibody increased the targeting efficacy of NPs	9.5 ± 1.0 nm	*In vivo* (intravenous injection)	NPs pass through BBB	[31]
Magnetic nitrogen-doped graphene	Magnetic nitrogen-doped graphene was functionalized with Aβ antibody	Targeted detection of amyloid plaques	Antibody increased the targeting efficacy of NPs	Magnetic NP: 10–20 nm Graphene: 1 μm	*In vitro*	Aβ was detected by electrochemical assay	[32]
Magnetic core–plasmonic coat nanomaterials linked to hybrid graphene oxide	Magnetic core–plasmonic coat nanomaterials linked to hybrid graphene oxide were modified with both tau and Aβ antibodies	Targeted detection/ separation of amyloid plaques and tau	Both tau and Aβ antibodies increased targeting efficacy of NPs	Magnetic core–gold plasmonic shell NP: 50 nm	*In vitro*	Easy and fast method for detection of both Aβ and tau	[43]
Gold	Gold NPs were modified with tau antibody	Targeted detection of tau protein in CSF	Tau antibody increased targeting efficacy of NPs	40–50 nm	*In vitro*	Tau protein was detected using two-photon Rayleigh scattering assay	[34]
SPIO	SPIONs were coated with ganglioside carbohydrate (sialic acid)	Targeted MRI of amyloid plaques	Ganglioside carbohydrate (sialic acid) increased targeting efficacy of NPs	5–10 nm	*In vitro* and *ex vivo*		[35]
Iron oxide (magnetic NPs)	AβO antibody was conjugated with carboxylate-functionalized magnetic NP	Early detection of AD in early stages. Targeted MRI imaging of AβOs	AβO antibody increased the targeting efficacy of NPs for detection of AβOs in CSF	12–16 nm	*In vivo* intranasal inoculation	NPs pass through BBB	[18]
Mixture of magnetic particles and gold NPs	AβO antibody was conjugated to magnetic particles. AβO antibody conjugated gold NPs were functionalized with double-strand DNA	Early detection of AD in early stages Measurement of low concentration of ABOs in Antibody-CSF	Antibody-functionalized NPs attached to AβOs and formed a sandwich complex, which was then separated and heated; after heating, the released DNA and AβO concentrations were measured	30 nm	*In vitro*	A biobarcode amplification strategy was used to measure AβO concentration	[41]

coat nanomaterials linked to hybrid graphene oxide with both tau and Aβ antibodies, which allowed selective screening for tau and Aβ in patients' blood with impressive selectivity. Another study conjugated gold NPs with tau antibodies to detect tau protein in the cerebrospinal fluid (CSF) [34].

Additional conjugation with contrast agents may mediate specific binding to amyloid plaque and increase the specificity of plaque detection. Kouyoumdijan and coworkers [35] functionalized magnetic NPs, as a contrast agent, with ganglioside carbohydrate (sialic acid). These NPs, with strong affinity for Aβ, exhibited high targeting efficacy for the detection and contrasting of Aβ against the surrounding environment.

Early Detection of AD

The poor prognosis of AD is in large part due to the typically late diagnosis of the disease. AD comprises two phases, asymptomatic and symptomatic, that progress over a long period of time [5]. This disease develops in five stages: the preclinical stage and MCI, followed by mild, moderate, and finally severe dementia. The cognitive decline progresses gradually and meets criteria for dementia after a long period of time. It has been argued that in the onset of clinical dementia, extreme brain impairment occurs before the dementia phase of AD [36].

Aβ fibrillation, which triggers biological events and leads to cognitive decline, starts decades before the onset of symptoms (e.g., memory loss). Recent studies suggest that therapeutic strategies may be more effective in the early stages of AD. The asymptomatic nature of this pathological process makes it difficult to diagnose. It is therefore imperative to identify and screen for biomarkers of Aβ fibrillation and such has been the effort of many studies. Giannakopoulos and coworkers [37] concluded that there is no direct correlation between the insoluble fibrillar Aβ species and disease stage. Furthermore, they were unable to find it in the initial stages of AD. By contrast, Aβ oligomers (AβOs) are known to be neurotoxic biological products and emerge in the earliest stages of AD as specific byproducts of AD initiation and development [38–40]. Thus, AβOs have garnered much attention as potential biomarkers of early-stage AD.

Several nanodiagnostic approaches have been developed to detect AβOs during the early stages of AD (see Table 1 for details). For example, Viola and colleagues [18] conjugated AβO-selective antibodies to magnetic nanostructures (SPIONs) for MRI. After intranasal administration in mice (5xFAD), this nanoprobe crossed the BBB and reached the brain, where the AβO antibody-functionalized SPIONs located and bound to their targets with high selectivity and sensitivity. Through this study the authors obtained valuable information about the structure and site of AβOs. Consequently, this MRI nanoprobe can also be used to evaluate the therapeutic/toxic impacts of new drugs developed for treatment.

The Mirkin group [41] developed a highly sensitive nanodiagnostic approach to measure the AβO level in CSF. For the first time, they used NPs as DNA carriers to estimate the AβO concentration in the CSF of patients via a biobarcode assay. They designed and executed an elegant experiment in which CSF was simultaneously exposed to both AβO antibody-conjugated magnetic particles and AβO antibody-conjugated gold NPs functionalized with double-stranded DNA. The specific binding of antibodies to AβOs led to the formation of a sandwich complex comprising magnetic particles and gold NPs. The complex was easily separated through exposure to an external magnetic field and washing. The NP-bound DNA strands were then heated and the released DNA barcodes measured by a scanometric assay that quantified silver deposition on the gold NP via an image analyzer. They were then able to detect and measure the low concentration of AβOs in the CSF of patients with MCI (early stages of AD).

Due to their dynamic structure, the oligomeric species have differing conformations. It is difficult to screen a therapeutic/diagnostic agent specifically targeting oligomers. The Kayed group [42] showed that gold NPs stabilize amyloidogenic protein in an oligomeric conformation. The stabilized oligomers had a common epitope segment that could potentially be targeted by therapeutics/diagnostics.

Brain Drug Delivery in AD: NP Transport across the BBB

The BBB is a protective and dynamic interface that separates the brain from extracerebral blood circulation and controls the transport of biomolecules into the brain [43]. This natural barrier hinders drug transport into the central nervous system (CNS) and therefore limits developments in the diagnosis and treatment of neurodegenerative disorders [16]. The therapeutic efficacy of drugs developed for CNS disorders thus strongly depends on their ability to override the BBB and arrive at the target site. NPs optimized in terms of their physicochemical properties, with long blood circulation times and able to carry drugs/biomolecules with sustained-release abilities, can be designed to pass the BBB (through several mechanisms including adsorptive and receptor-mediated transcytosis, carrier proteins, and the transcellular lipophilic pathway) and thus can be considered a strong alternative to traditional drug delivery systems (see Figure 2 and Table 2 for details). Such NPs may have effective roles in the diagnosis and treatment of AD. For example, rivastigmine-loaded poly(*n*-butylcyanoacrylate) NPs modified with polysorbate 80 exhibited a promising ability to pass through the BBB via receptor-mediated endocytosis and deliver therapeutic concentrations of rivastigmine, a cholinesterase inhibitor, to the brain [44]. Zhang and colleagues [45] used *Solanum tuberosum* lectin (STL)-functionalized polyethylene glycol-polylactide-polyglycolide (PEG–PLGA) NPs for effective delivery of basic fibroblast growth factor to the brain. The STL ligand mediated the entrance of NPs into brain through binding to its corresponding receptor on the nasal epithelial membrane. Another group used curcumin–lipid derivative-loaded nanoliposomes functionalized with antitransferrin antibodies, which were effectively taken up by brain capillary endothelial cells through transferrin receptor-mediated transcytosis [46]. Wang and coworkers [47] engineered trimethylated chitosan-conjugated PLGA NPs that delivered coenzyme Q into the brain

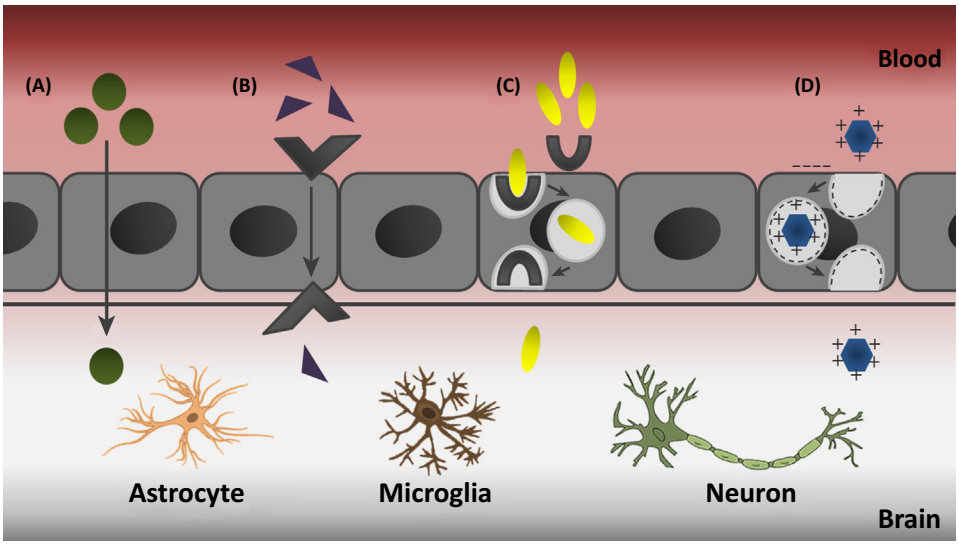


Figure 2. Schematic Representation of Nanoparticle (NP)-Transporting Pathways across the Blood–Brain Barrier (BBB). NPs can pass through the BBB via various mechanisms, such as the transcellular lipophilic pathway (A), carrier proteins (B), receptor-mediated transcytosis (C), and adsorptive transcytosis (D).

Table 2. Nanotechnology-Based Delivery for AD Diagnosis, AD Therapy, and AβO/Fibril Clearance

NP	Surface modification	Application	Mechanism of entrance/action	NP size	Animal model	NP administration	Remark	Refs
Poly(*n*-butylcyanoacrylate)	Poly(*n*-butylcyanoacrylate) nanoparticles were modified with polysorbate 80	Rivastigmine delivery to brain	Polysorbate 80 adsorbs apolipoprotein E and B and passes through the BBB via receptor-mediated endocytosis	40.5 ± 6.9 nm	Wistar rat	Intravenous injection	Rivastigmine improves AD treatment via inhibition of cholinesterase	[44]
PEG–PLGA	PEG–PLGA was functionalized with STL	Basic fibroblast growth factor delivery to brain	STL specifically attaches to its corresponding receptor on the nasal epithelial membrane and mediates NP transport into brain	104.8–118.7 nm	Sprague–Dawley (SD) rats (Aβ25–35 and ibotenic acid were injected into hippocampus of SD rats)	Intranasal administration	Fibroblast growth factor induces neurite growth of neurons and prevents neuron impairment	[45]
Chitosan		Rivastigmine delivery to brain		183–341 nm	Wistar rat	Intranasal administration		[48]
Liposome	Liposome was coated with antitransferrin antibody	Curcumin–lipid delivery	NPs were taken up by brain capillary endothelial cells through transferrin receptor-mediated transcytosis	116–159 nm				[46]
PLGA	PLGA NPs were functionalized with trimethylated chitosan	Coenzyme Q delivery to brain	Trimethylated chitosan mediates NP passage across BBB via absorption-mediated transcytosis	150 nm	APP/PS1 transgenic mice	Intravenous injection		[47]
PEG–PLA	PEG–PLA NPs were functionalized with B6 peptide	Neuroprotective peptide (NAPVSIPQ) delivery to brain	This nanostructure transported drug in brain via lipid raft- and clathrin-mediated endocytosis	100–120 nm	Balb/C nude and ICR mice	Intravenous injection		[49]
PEG-Au	PEG-Au was functionalized with penetratin and ruthenium		Lipid raft-mediated endocytosis	105 nm	Nude mice	Intravenous injection		[50]
PEGylated PLA polymer	Polymer was functionalized with two targeting peptides: TGN and QSH		TGN and QSH targeting peptides facilitate BBB transport and targeting of Aβ, respectively	96–107 nm	Nude mice and ICR mice	Intravenous injection		[52]
ApoE3-rHDL	ApoE3-rHDL	Degradation/clearance of preformed Aβ fibrils	The nanostructure induced uptake and subsequent destabilization of Aβ in microglia and astrocytes by triggering intracellular lysosome traffic	21.7 ± 7.9 nm	Senescence-accelerated prone mouse (SAMP8)	Intravenous injection	Nanostructures passed through BBB; those that remained in blood induced Aβ clearance by liver cells	[20]

Table 2. (continued)

NP	Surface modification	Application	Mechanism of entrance/action	NP size	Animal model	NP administration	Remark	Refs
rHDL	Peptide (NAP)-loaded monosialotetrahexosylganglioside-modified rHDL (GM1-rHDL)	Aβ efflux through BBB degradation/clearance of preformed Aβ fibrils Delivery of neuroprotective peptide (NAP) into brain	GM1-rHDL specifically binds to Aβ and induces the degradation/clearance of preformed Aβ fibrils by microglia	23.67 ± 6.68 nm	ICR mice (Aβ25–35 and ibotenic acid were injected into hippocampus of ICR mice)	Intranasal administration	Nanostructures pass through BBB, bind to Aβ, and induce microglia-mediated fibril clearance	[98]
Gold nanorod		Degradation/clearance of preformed Aβ fibrils	Targeted disaggregation of preformed fibrils with femtosecond laser irradiation	Length: 40 nm Diameter: 10 nm		*In vitro*	This strategy provides a unique opportunity for rapid/targeted disaggregation of preformed fibrils	[66]
Graphene oxide nanosheets	Graphene oxide nanosheets were modified with ThS	Degradation/clearance of preformed Aβ fibrils Monitoring Aβ fibril destabilization in real time	ThS-functionalized graphene oxide nanosheets specifically bind to beta-sheet structure in Aβ fibrils; they destroy fibrils after laser irradiation and local heating.	Thickness of nanosheets: 1.2 nm		*In vitro*	The fluorescence intensity of ThS increases on binding to a beta-sheet structure; ThS-functionalized graphene oxide provides a unique opportunity to monitor hyperthermia therapy/fibril destabilization in real time	[21]

via adsorption-mediated transcytosis, ultimately inhibiting cognitive decline in APP/PS1 transgenic mice (an animal model of AD). Another approach to passing through the BBB is to use the nose-to-brain transport pathway. For example, this pathway was used to deliver rivastigmine-entrapped chitosan NPs into the brains of Wistar rats [48].

One effective strategy to bypass the BBB is the use of targeting peptides at the surface of NPs. For example, PEG–PLA NPs functionalized with B6 peptide can effectively transport a loaded neuroprotective drug into the brain through lipid rafts. The delivered drug significantly improved the disease symptoms and quality of life in a mouse model of AD (Balb/C nude mice) [49]. The penetrating peptide facilitated the passage of PEG-Au NPs labeled with ruthenium, as an imaging fluorescent dye, across the BBB. This nanostructure delayed Aβ fibrillation and disaggregated preformed fibrils. The ruthenium's fluorescent properties also enabled real-time tracking of the drug delivery process [50].

Although many nanocarriers have been developed for brain drug delivery, few nanocarriers could specifically transport therapeutic or diagnostic agents to the damaged site. Random spreading of these agents in the brain tissue might have several adverse effects. For example, untargeted distribution of nerve growth factor in the brain may lead to sympathetic neuron hyperplasia [51]. Therefore, improving the targeting capability of nanocarriers is crucial for the treatment of CNS disorders. To this end, a PEGylated PLA polymer was functionalized with two targeting peptides (TGN and QSH) that facilitate BBB transport and targeting of Aβ, respectively [52]. These peptides increased the targeting capability of nanocarriers for brain drug delivery and AD therapy.

A major limitation of targeted NPs is the formation of a protein shell at the surface of the NPs (the so-called 'protein corona'), which may shield the targeting species at the surface of NPs and cause mistargeting [53]. To overcome this issue, additional modifications (e.g., using zwitterionic coatings) should be performed at the surface of NPs, which makes clinical translation of the NPs more complicated. We have recently reviewed these major issues for the targeting and clinical translation of NPs and readers are referred to these publications for more in-depth details [54,55].

NPs Inhibit and Delay Aβ Fibrillation

Nanotechnology-based AD therapy predominantly focuses on the inhibition and delay of Aβ fibrillation. Aβ fibril formation is a nucleation-dependent self-assembly process that follows sigmoidal kinetics, where the process initiates with a lag phase (oligomer/critical nucleus formation) followed by elongation (oligomer polymerization) and fibril maturation (Figure 3). With their large surface-to-volume ratio, NPs efficiently adsorb monomers, oligomers, critical nuclei, and protofibrils and therefore influence the nucleation process and lag/elongation time (Figure 3 and Figure 4A) [56]. In some cases the NPs themselves act as preformed nuclei that rapidly adsorb Aβ peptides, increase local Aβ concentration, and enhance fibrillation [57].

Depending on their physicochemical properties (e.g., size, charge, surface modification, composition, shape) and concentration, NPs can have dual effects (i.e., inhibition or acceleration) on fibril formation [58–61]. The ratio of NP surface area to Aβ peptide largely determines the NP behavior. For example, a high concentration of polystyrene NPs provided a large surface area that adsorbed free Aβ and inhibited fibrillation, while a low concentration acted as preformed seeds that enhanced the fibrillation rate [58]. NPs of different sizes, shapes, and/or surface charges have different affinities to Aβ monomers/oligomers and therefore have diverse effects on the fibrillation process. Previous studies showed that small, negatively charged iron oxide NPs exert more inhibitory effects than their large, positively charged counterparts [59]. Other experiments demonstrated that negatively charged gold, inorganic

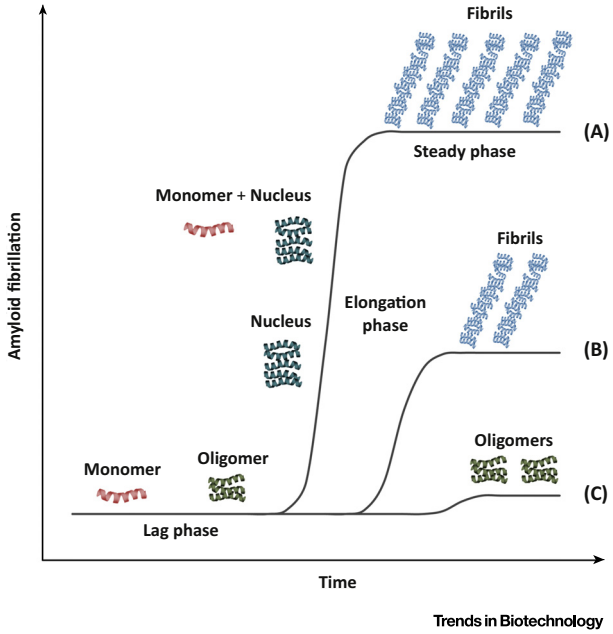


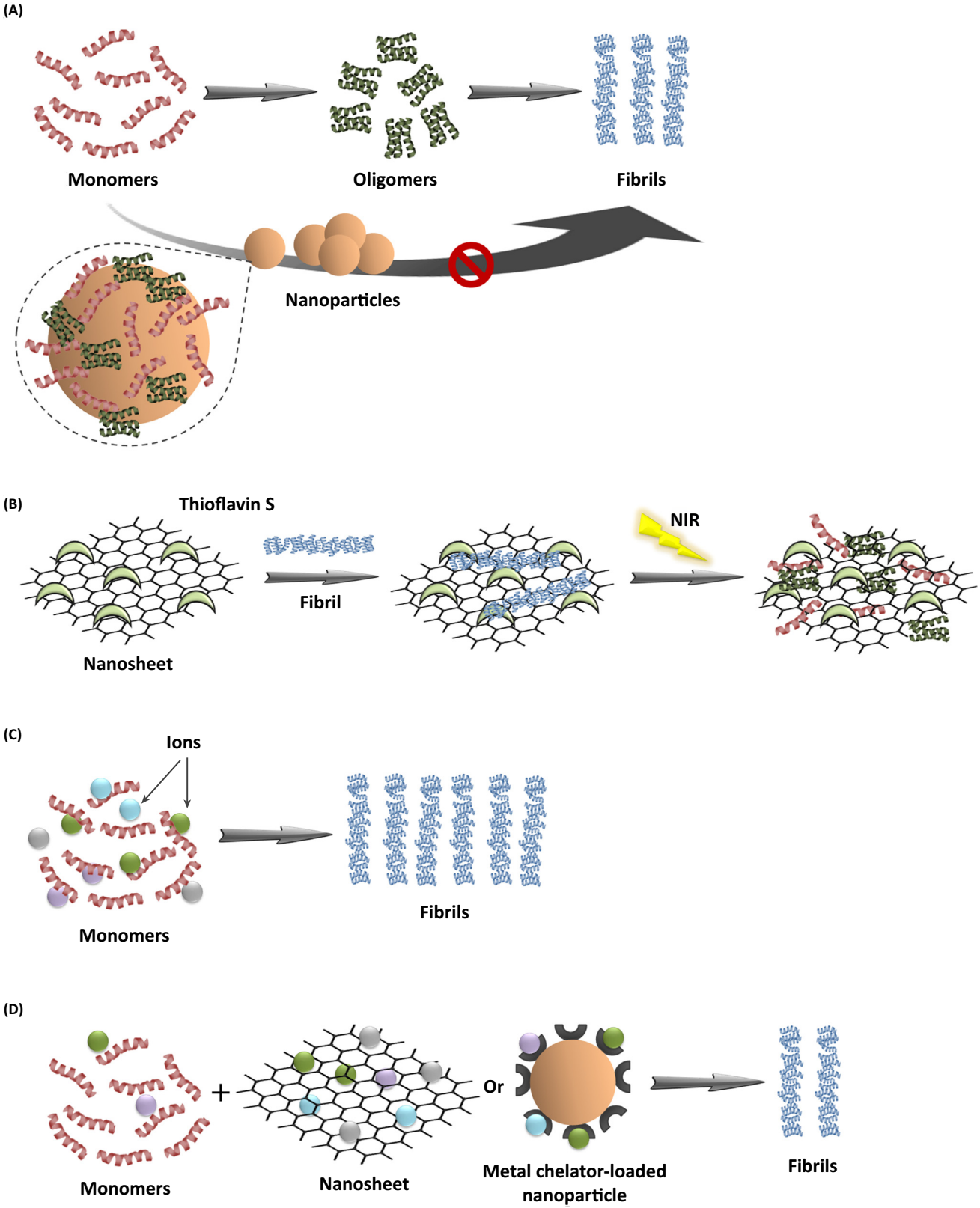
Figure 3. Schematic Representation of the Amyloid Beta (Aβ) Fibrillation Process. Aβ fibrillation is a nucleus-dependent process that follows sigmoidal kinetics, wherein the process initiates with a lag phase followed by elongation and fibril maturation (A). NPs can delay the fibrillation process via extending the lag phase (B). NPs can prevent fibril formation via capture/blocking of Aβ monomers/oligomers (C).

CdTe, and graphene oxide NMs capture Aβ monomers or oligomers to delay fibrillation [19,61,62]. Another study demonstrated that small, spherical gold NPs have stronger anti-fibrillation effects than large, rod-shaped NPs [60].

NPs Enhance Amyloid Oligomer/Plaque Clearance

As oligomers and mature fibrils are the main pathological hallmarks of middle- to late-onset AD [63,64], destabilization of preformed oligomers and plaques can be recognized as an effective strategy for the treatment of AD. Nanotherapeutic approaches are mainly focused on the prevention, inhibition, or interruption of the fibrillation process, especially in the early stages (i.e., the lag/elongation phase). With regard to the late stage of AD, (nano)therapeutics are being developed to destabilize preformed oligomers and fibrils. However, a major challenge in the field is that destabilization of mature fibrils will produce small amyloid fibrils and oligomer-like structures, which have much stronger neurotoxic effects than their larger counterparts [65]. However, fibril destabilization can reduce amyloid plaque deposition on the neuron membrane. Therefore, both scientists and clinicians should find a balance between the predetermined dual effects (both adverse and therapeutic) of fibril destabilization strategies.

Major developments in nanotechnologies that specifically target amyloid oligomers/fibrils are summarized in Table 2. These strategies have exhibited potential therapeutic roles in the clearance of AβOs/fibrils in animal models. For instance, Song and colleagues [20] demonstrated that apolipoprotein E3-reconstituted high-density lipoprotein (ApoE3-rHDL) nanostructures could successfully pass through the BBB and specifically target AβOs. The nanostructure entered microglia and astrocytes, triggered intracellular lysosome trafficking pathways, and destabilized the AβO. The remaining NPs that could not pass through the BBB captured Aβ in the blood circulation and accumulated and were digested in liver cells. In another study Liao and colleagues [19] showed that carboxyl-conjugated gold NPs could destabilize amyloid fibrils to form amorphous aggregated species. Although fibril disaggregation may increase the



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oligomer-like structure, they found that the achieved amorphous species were less toxic than the amyloid plaques. This could be a result of the dual effects of fibril destabilization, as discussed previously. Notably, most of the *in vivo* tests have been performed in animal models and there are very few reports in humans. It is noteworthy that the majority of studies were performed in animal models of AD except for few studies that considered human cases (e.g. [18,46]).

Hyperthermia is another employed strategy for the destabilization of preformed amyloid oligomers/fibrils. In this case hyperthermic NPs (e.g., metallic and magnetic) demonstrated promising therapeutic potential. Due to their high optical absorption in the near-IR (NIR) region, gold- or carbon-based nanostructures are widely used for hyperthermia therapy. Gold NPs can generate local heat for targeted destabilization of Aβ fibrils with femtosecond laser irradiation [66]. Qu and coworkers [21] developed thioflavin S (ThS)-functionalized graphene oxide nanosheets that traversed the BBB and selectively destabilized amyloid fibrils following NIR laser irradiation. ThS fluorescence was increased on binding to ordered β-sheet structures in fibrils, which was used to monitor Aβ decay (Figure 4B). A potential adverse effect of hyperthermia approaches is the capacity of the generated heat to affect the normal function of nearby healthy cells/neurons. Therefore, future research in the field should be more focused on monitoring the safety and therapeutic efficacy of hyperthermic NPs in animal models of AD.

Nanotherapeutic Approaches Targeting Concurrent Disorders Associated with AD Occurrence/Progression

A big challenge in AD therapeutic approaches is the suitability of targeting Aβ fibrils/oligomers and tau protein. Aβ monomers play several critical roles in the physiological functions (e.g., synaptic activity) of brain tissue. Therefore, excessive removal of Aβ monomers through therapeutic approaches may affect normal synaptic activity [67,68]. The other key role of Aβ monomers in brain tissue is their antioxidant and antimicrobial activities, which can protect the tissue against excessive oxidative stress and microbial infection [69–71].

Many theories have been postulated on the mechanism of Aβ's involvement in AD development. To the best of our knowledge, no single theory, however, has been able to completely explain the molecular details of how AD develops. AD pathogenesis is a multifactorial process affected by Aβ fibril/oligomer, tau phosphorylation, inflammation, oxidative stress, mitochondrial dysfunction, autophagy, metal/multivalent ion imbalance, and complex crosstalk between genetic and environmental factors [15,72–77]. The convoluted nature of this disease suggests that combination therapy may be more effective than monotherapy. Targeting concurrent disorders associated with AD may be considered a more promising strategy to control AD progression than focusing on the Aβ production/fibrillation. Table 3 summarizes major efforts in the use of combination therapy to inhibit/delay AD progression.

Oxidative stress is a physiological phenomenon with wide-ranging implications in neurodegenerative diseases [78]. Mitochondria are highly susceptible to oxidative and nitrosative stress leading to excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively. Mitochondrial dysfunction induces the production of amyloidogenic types of Aβ and triggers the mitogen-activated protein kinase (MAPK) pathway [79]. Excessive levels of Aβ peptides then lead to mitochondrial dysfunction [80], ratcheting the pathogenesis of AD. Therefore, nanotechnology-mediated antioxidative therapy and ROS/RNS scavenging are potential strategies to prevent AD and brain injury [81]. Dowding and colleagues [82]

Figure 4. Nanoparticle Impacts on Aβ Fibrillation and Fibril Clearance. NPs inhibit/delay the fibril formation via capturing free monomers and/or oligomers (A). The amyloid fibrils attached to Ths-conjugate graphene were destabilized after NIR irradiation and local heating (Photothermal degradation of amyloid fibrils) (B). Metal ions bind to Aβ peptides and accelerate the fibril formation (C). Nanochelators and/or metal-chelator nanocarriers capture metal ions and inhibit/delay the fibril formation (D).

Table 3. Strategies for Targeting Concurrent Disorders Associated with AD

NPs	Concurrent disorders targeted by NPs	Mechanism of action	NP size	Model of use	Remark	Refs
Cerium oxide	Oxidative stress Nitrosative stress Neuroinflammation	Cerium oxide NPs localized in neuron mitochondria and scavenged ROS and RNS compounds	3–8 nm	*In vitro*	Alternative to current antioxidants	[82]
TPP-conjugated cerium oxide	Oxidative stress Nitrosative stress Neuroinflammation	Cerium oxide NPs specifically localized in neuron mitochondria and scavenged ROS and RNS compounds	3 and 10 nm	*In vitro* and *In vivo* (injection into subicula of 5XFAD mice)	TPP increases targeted delivery of cerium oxide NPs in neuron mitochondria	[22]
Quantum Oxidative stress dots	Oxidative stress Inflammation	Quantum dots bypassed the biological barriers and penetrated into brain Bioluminescence resonance energy transfer to quantum dots emitted light that effectively extinguished inflammation and oxidative stress	N/A	*In vitro*	This strategy overcomes the challenges associated with low-level laser therapy	[85]
Single-wall carbon nanotubes	Defective autophagy Lysosome dysfunction	Single-wall carbon nanotubes suppress mTOR signaling pathway and thereby amend the autophagy impairment and lysosomal dysfunction	1–2 nm	*In vitro*		[87]
Metal Ultrathin g-C_3N_4	Metal ion imbalance in brain	This nanostructure captured Cu^{2+} ions and thereby prevented Aβ fibrillation It also destroyed preformed Aβ fibrils	~60 nm	*In vitro*	Nanochelators may be promising alternatives to current metal chelation therapy	[89]
Mesoporous Metal ion silica	Metal ion imbalance in brain	Mesoporous silica NPs specifically delivered the metal chelators to cells producing H_2O_2; these NPs released the loaded metal chelators when exposed to increased H_2O_2 concentration	~100 nm	*In vitro*	Smart metal chelator nanocarriers overcome the challenges (e g., BBB passage, side effects, active targeting) associated with traditional metal chelatio [23] therapy	[23]

demonstrated that cerium oxide NPs can specifically localize in neuron mitochondria, scavenge/capture ROS/RNS, and reduce mitochondrial oxidative stress, neuroinflammation, and AD progression. These NPs are a promising alternative to current antioxidant agents. To improve the delivery of nanoceria into neuron mitochondria, Kwon and colleagues [22] conjugated the NPs with the lipophilic cation triphenylphosphonium (TPP). FITC-TPP–nanoceria showed greater localization in mitochondria than FITC-nanoceria alone.

In recent years, low-level laser therapy has emerged as a new therapeutic strategy for diseases affected by oxidative stress and inflammation [83]. Because of its limited penetrative capacity, however, it has had limited use in AD diagnosis and therapy [84]. NPs have the potential to overcome this limitation and provide an unprecedented opportunity to apply this therapeutic approach in other oxidative stress/inflammation-related diseases. For example, it was demonstrated that light-emitting quantum dots could access the brain and reduce inflammation and oxidative stress induced by excessive Aβ peptides using laser therapy [85].

Autophagy is a lysosomal degradative pathway used to remove damaged organelles and macromolecules and maintain neural hemostasis. It also plays a key role in the clearance of aggregated proteins [86]. Deficiency in the degradation of damaged proteins and organelles results in loss of homeostasis, neural death, and AD development [75]. Xue and colleagues [87]

created single-wall carbon nanotubes that amended defective autophagy and lysosome dysfunction in glial cells from a CRND8 transgenic mouse model of AD, which are responsible for the clearing/degradation of excessive Aβ within lysosomes, via inhibition of the mTOR signaling pathway. These NPs may serve as neuroprotective agents that reverse the abnormal degradative pathways in damaged glia cells.

Metal ion homeostasis and distribution is another factor that determines neuronal function and AD progression. Metal ions play a major role in the creation and clearance of Aβ by regulating enzymes responsible for Aβ generation or degradation [88]. The binding of various metal ions (e.g., copper, iron, manganese, zinc, aluminum) to Aβ results in Aβ precipitation and fibril formation, oxidative stress, ROS generation, and synaptic dysfunction (Figure 4C). Metal ion chelation therapy and prevention of metal-induced Aβ fibrillation have been proposed as possible methods to control AD. Li and colleagues [89] developed ultrathin graphitic-phase carbon nitride (g-C_3N_4) as a nanochelator that specifically captured Cu^{2+} ions and thereby prevented Aβ fibrillation (Figure 4D). This nanostructure destabilized the Aβ fibrils formed in conjugation with Cu^{2+} ions. Smart mesoporous silica nanocarriers have also been used for targeted delivery of metal chelators to cells that produce H_2O_2. These H_2O_2-sensitive nanocarriers release the loaded chelator when exposed to high concentrations of H_2O_2 (Figure 4D) [23]. The application of nanochelators and/or chelator-loaded nanocarriers may overcome the challenges (e.g., BBB passage, side effects, active targeting) associated with traditional metal ion chelation therapy.

Challenges of Nano-Based Strategies for Diagnostic and Therapeutic Applications

It is well recognized that the NP surface will be immediately covered by biomolecules (protein corona) on introduction to physiological fluid [53], and Aβ peptides 'see' the protein corona-coated NPs *in vivo*. To study the impacts of NPs, the effects of corona-coated NPs on the fibrillation process should be evaluated; however, this important matter has not been considered in the majority of the current literature. The formation of a protein corona layer is demonstrated to empower the inhibitory potency of silica, polystyrene, and graphene oxide nanostructures by extending the lag time (the time needed for oligomer/critical nucleus formation) [62,90]. Another important issue in the field is the role of disease-specific protein corona, where disease type can change the *in vivo* fate of NPs [91]. It means that identical NPs may have different therapeutic/toxic impacts in different patients [92]. The incubation temperature is another variable parameter that affects the protein corona of NPs and thus can change the interactions of Aβ peptides with the NPs. For example, it was shown that polystyrene NPs have an accelerating effect on the fibrillation process at 37°C but are inhibitory at 42°C [93]. These studies demonstrate that multiple hidden factors at the nano–bio interface should be considered in future studies of nanotechnology-based AD diagnosis/therapy to provide robust and accurate outcomes. In addition, it is possible that different types of amyloidal proteins (which might have toxic effects on some biological systems/organs; e.g., pancreas) contribute to the composition of the corona and thus one may expect that NPs could act as 'infectious amyloids' or 'cross-seeding amyloids' transmitting amyloid from one biological system (e.g., cells) to another.

Another major challenge for the field is to fabricate NPs with long blood circulation times. Targeting NPs need a long blood-residency time to pass through the BBB and hit their target. However, most of the developed NPs do not have this capacity and a large portion of them accumulate in the liver and spleen.

The potential toxicity of NPs is another important challenge that should be considered in detail. The majority of the studies monitored the short-term toxicity of NPs and there are very few

reports on their long-term toxicity. Most of the current studies that evaluate the effects of NPs on the kinetics of Aβ fibrillation *in vitro* are poorly correlated with *in vivo* conditions. To mimic the *in vivo* environment, future studies need to develop novel protocols to monitor long-term Aβ fibrillation, which may take several years to be completed. The time present and dose of NPs in a patient's brain should be optimized to inhibit the fibrillation process. In this case, the main concern would be the adverse effects of NPs in such long-term treatment.

Concluding Remarks and Future Perspectives

We have reviewed recent advances in nanomedicine for the diagnosis and treatment of AD. Although nanotechnology-based therapeutic/diagnostic approaches have been promising in overcoming the unmet challenges, there has been no success in clinical translation of these approaches. One of the main reasons for this wide gap between bench discoveries and clinical translation is that most efforts are focused on the targeting of AβO and fibrils, which ignores other factors involved in the progression of AD. In addition, poor understanding of the nano–bio interface has made many current studies less useful than they were supposed to be. For example, a large part of the *in vitro* studies in the field has ignored the crucial role of the protein corona at the surface of NPs before the interaction of NPs with Aβ products. To accelerate the clinical translation of NPs in both the diagnosis and the treatment of AD, future studies should be focused on five important items (see Outstanding Questions): (i) revising current protocols to consider ignored factors at the nano–bio interface and reduce the misinterpretation of outcomes [94–96]; (ii) probing the adverse effects of NPs in fibril fragmentation, as they may lead to the production of more toxic short fibrils and oligomer-like structures exacerbating neuron loss; (iii) recognizing new therapeutic targets (i.e., misfolded tau protein, which is another shared pathological hallmark of neurodegenerative diseases); (iv) developing multifunctional NPs with mutitherapeutic capacities (e.g., delivering a wide range of therapeutic molecules to control tau phosphorylation, inflammation, oxidative stress, and mitochondrial dysfunction); and (v) considering challenges in the manufacture of controllable and reproducible NPs [97].

Outstanding Questions

How have diagnostics for AD been able to make steady progress over the past two decades?

Are we using appropriate diagnostic strategies for AD?

Is Aβ an initiator or an AD cofactor?

Are today's therapeutic agents heading in the right direction or should we explore alternatives?

How can the use of nanotechnologies help the discovery of new diagnostic or therapeutic agents?

How can nanotechnologies change our simplistic views of AD pathogenesis?

How can nanocommunities accelerate the translation of NPs to the clinic for patients with AD?

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