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Applications of Nanotechnology in the Treatment of Alzheimer's Disease

Xiaoqiao Yang

The University of Sydney, Faculty of Medicine and Health, School of Medical Sciences, Camperdown, NSW, 2006, Australia

Corresponding author: xyan7230@uni.sydney.edu.au

Abstract. Alzheimer's Disease (AD) is a neurodegenerative disease associated with progressive memory and cognitive impairment. Due to the devastating social and financial impacts of AD, extensive research is put into gaining a clearer understanding of its pathogenesis and risk factors, as well as the development of treatments that can slow or reverse the disease progression. Despite this, early and accurate detection of AD and the development of curative treatments are yet to be achieved. Further, major challenges remain in the symptomatic treatments available today, as the delivery of Alzheimer's medications is limited by low efficiency due to difficulties in blood-brain barrier permeation and poor absorption. The incorporation of nanotechnology in current and potential treatments present unique opportunities for the delivery of therapeutic agents with increased specificity, lower toxicity and controlled release. In this review, we outline the proposed pathogenesis, current diagnosis and treatment methods of AD, and discuss recent advancements in nanomaterial-based systems that target major hallmarks of AD through different mechanisms, including targeted drug delivery, inhibition of Aβ aggregation, delivery of neuroprotective agents and Aβ removal from the blood.

Keywords: Alzheimer's Disease, Nanotechnology, Drug Delivery

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterised by progressive memory and cognitive decline. As of 2022, The World Health Organisation estimates the global prevalence of dementia to be 55 million cases, with AD accounting for 60-70% of all cases [1]. Furthermore, the number of dementia cases is predicted to rise to 152.8 million in 2050 [2], exacerbating the overwhelming burden of disease associated with dementia. Therefore, the management and treatment of AD have become an increasingly pressing concern. Despite extensive research efforts aimed at reversing the progression of AD, current medications only provide symptomatic treatments that provide short-term relief. It is believed that both genetic and environmental factors can influence the development of AD. Most notably, the apolipoprotein E4 (ApoE4) is believed to be the most prevalent genetic risk factor for AD, where inheriting the ε4 allele can increase the risks of developing AD by 3 times in heterozygotes and by 15 times in homozygotes [3]. However, a comprehensive understanding of the causes, pathology and mechanism of AD is yet to be established. There are various hypotheses postulating the pathogenesis of AD, including the cholinergic, amyloid cascade, tau hyperphosphorylation and neuroinflammation hypotheses.

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Acetylcholine (ACh) is a neurotransmitter produced through the synthesis of acetyl coenzyme and choline, with enzyme choline acetyltransferase (CAT) acting as a catalyst. The normal production and functioning of ACh are critical in maintaining working memory, synaptic plasticity, learning and attention. The cholinergic hypothesis postulates that damages and dysfunction in the cholinergic neurons found in the basal forebrain, along with decreased cholinergic neurotransmission in the cerebral cortex are the contributing factors to the memory impairment and other cognitive symptoms seen in AD.

Amyloid-β (Aβ) accumulation is a pathological hallmark of AD. Aβ peptides can aggregate into various structures, including soluble Aβ oligomers, insoluble Aβ fibrils and large Aβ plaques. While early theories postulate that Aβ plaque formation leads to neurotoxicity, synapse loss, neurodegeneration, and consequently dementia, later studies have found Aβ oligomers to have much greater toxicity compared to Aβ plaques [4]. Aβ deposition and oligomerisation can develop due to elevated Aβ production combined with insufficient Aβ clearance from the brain, resulting in pathology associated with AD [5]. Aβ deposition could trigger astrocyte and microglial activation, leading to reactive oxygen species (ROS) generation through the pro-inflammatory ERK (extracellular signal-regulated kinase) signalling pathway activation, resulting in neuronal damage [6].

Tau is a protein involved in the stabilisation of axonal microtubules. In AD, tau can undergo hyperphosphorylation, leading to reduced affinities and detachment from the microtubules along with conformational change that promotes aggregation [7]. Hyperphosphorylated tau can aggregate into insoluble neurofibrillary tangles (NFTs). Tau is considered as a key biomarker in AD, as tau hyperphosphorylation and NFT accumulation can lead to structural damages to the microtubules in the axons, which can result in compromised axonal transport and synaptic function.

The current diagnosis of AD mostly relies on the exclusion of other possible conditions that can present with similar symptoms. Neuropsychological evaluations can be conducted to assess the cognitive functions, including memory, language ability, executive functions, visual spatial abilities and everyday function [8]. The diagnosis of AD can be aided by imaging techniques such as computerized tomography (CT) and magnetic resonance imaging (MRI). Structural changes of the brain can be visualised using MRI, where hippocampal atrophy has been linked to neuronal degradation [9]. Additionally, visualisation of the cortical thickness may aid in the diagnosis of AD, as studies have found that cortical thinning can indicate neuronal loss, in particular, the cortical thickness of the media temporal lobes is most severely reduced in AD brains [10]. However, some of the aforementioned characteristics are not AD-specific. For instance, hippocampal and cortical volume loss can also be observed in other neurodegenerative diseases, such as Parkinson's Disease. [11]. Only post-mortem histopathological examinations of the brain can provide a definitive diagnosis, where the presence of NFTs and Aβ can be confirmed. In 2020, the U.S. Food and Drug Administration (FDA) approved of flortaucipir, a radioactive probe that can bind to tau fibrils to be used in the identification of NFTs. Studies have revealed the ability to visualise the density and distribution of tau pathology with increased specificity and sensitivity when flortaucipir is used in positron emission tomography (PET) imaging [12].

Cholinesterase inhibitors donepezil, rivastigmine, galantamine and the glutamate antagonist memantine can be prescribed to attenuate the symptoms of AD. In 2021, the Aβ-directed monoclonal antibody aducanumab received FDA approval for treating mild cases of AD. However, its effectiveness remains controversial due to unclear clinical benefits, drug efficacy and safety concerns [13]. In addition, most of the currently approved drugs are administered via the oral route in the form of tablets, which may have drawbacks such as poor absorption and degradation in the digestive tract, difficulties in blood-brain barrier (BBB) permeation and poor control over the dosage that reaches the brain.

The tight junctions of the microvascular endothelial cell lining in the cerebral capillary walls form the BBB [14]. The presence of various cell types (astrocytes, pericytes, microglia and endothelial cells) and the high selectivity of the BBB maintain a highly controlled environment in the brain. The entry of pathogens, large or hydrophilic molecules and the diffusion of solutes in the blood is restricted,

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whereas some small molecules (such as O2 and CO2) can move across the BBB through diffusion. Despite this, only approximately 2% of all small molecules can cross the BBB [14]. Therefore, due to difficulties in crossing the BBB and the removal of drugs by efflux pumps, drug delivery into the brain via the oral and intravenous routes is particularly challenging. Nanoparticles present unique opportunities in crossing the BBB owing to the flexibility in the modifications of their sizes, shapes, surfaces and physiochemical properties based on different synthesis and functionalisation processes. Nanoparticles are small particles (typically 1 to 100 nm in diameter) that can be synthesised from a range of organic, inorganic and carbon-based materials. Nanoparticles show size-dependent permeation across the BBB. A 2020 study investigated the optimal brain delivery size using gold nanoparticles enhanced by focused-ultrasound-induced BBB opening. It was reported that nanoparticles ranging from 3 to 200 nm are able to permeate through the BBB, and while smaller nanoparticles show greater permeation across the BBB, larger nanoparticles (200 nm) show greater accumulation in the brain due to slower clearance from the blood [15]. Furthermore, different functionalisation and conjugation strategies, for instance, the incorporation of specific ligands, have been shown to improve the targeting effects of drug delivery nanosystems. Here, we review various nanosystems that have been proposed as potential therapeutic agents against AD and summarise their key properties (Table 1).

Table 1. Summary of the key properties of the nanoparticles discussed in this review.

Type of nanoparticles	Main mechanism	Size (nm)	Encapsulation efficiency (%)	Reference
GH-loaded flexible Liposomes	Drug delivery	112 ± 8	83.6 ± 1.8.	[17]
Cu/exo-liposomes	Drug delivery	200	93.60 ±4.26	[19]
Ang2-ICA/TSIIA liposomes	Drug delivery	111.26 ± 11.65	92.45 ± 3.20	[21]
CHG NPs	Inhibition of Aβ fibrillisation	110.4 ± 15.6	–	[23]
Chr-Chi NPs	Drug delivery	100-120	73.52 ± 0.31	[24]
Cs@LT-PLGA NPs	Drug delivery	142.3 ± 2.57	83.97 ± 1.03	[28]
AuNPs	Anti-inflammatory and ROS-scavenging activities of AuNPs	20	–	[29]
Au@TPMs	Delivery of therapeutic bioactive peptides	3.5 ± 0.8 and 13.5 ± 1.3	–	[33]
Bucladesine/SPIONs	Drug delivery	20	–	[36]
MCNAs	Magnetic extracorporeal Aβ removal	330	–	[37]

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2. The use of nanoparticles as potential treatments of alzheimer's disease

2.1. Liposomes

Liposomes are small, spherical vesicles that contain one or multiple phospholipid bilayers, which can be synthesised from cholesterol or non-toxic phospholipids. Due to the amphipathic properties of phospholipids, liposomes can undergo self-assembly in an aqueous medium. This mechanism relies on the hydrophobic interactions between water and the non-polar hydrophobic tails, where the polar hydrophilic heads face the water and the tails are shielded from water, forming the exterior and the interior of the lipid bilayer respectively. The surface and structure of liposomes can be modified relatively easily to incorporate important physicochemical properties, such as increased specificity and the ability to carry both hydrophilic and lipophilic drugs. For instance, it has been shown that PEGylation on liposomal surfaces can improve the pharmacokinetics and pharmacodynamics of the liposomal drug delivery systems due to greater stability and retention time, as well as decreased levels of enzymatic degradation and immunogenicity [16]. Furthermore, targeting molecules such as antibodies and cell-penetrating peptides can be conjugated onto the surface of liposomes to increase specificity and lower cytotoxicity, hence potentially reducing side effects for AD patients.

In an earlier study conducted in 2012, Li *et al.* determined the pharmacokinetic efficiency of acetylcholinesterase (AChE) inhibition following treatment using galantamine hydrobromide (GH) loaded flexible liposomes [17]. In this study, GH was encapsulated and transported into the brain using flexible liposomes as drug carriers for AD. Due to the increased fluidity in the liposomal membrane, flexible liposomes have been shown to enhance the entrapment efficiency, transcutaneous drug retention and depth of penetration in *vivo* [18]. To prepare the GH-loaded flexible liposomes, a thin-film homogenisation method was used, and GH was dissolved in aqueous propylene glycol (PG), which acts as a solvent and an edge activator that increases the liposomal membrane fluidity. The entrapment efficiency was calculated to be 83.6 ± 1.8%. Further, the effectiveness of AChE inhibition was tested *in vivo* using rats, where rats underwent intranasal treatment of GH-loaded (3mg/kg) flexible liposomes for 10 days. Control groups were introduced to compare the AChE activity after administration of aqueous GH of varying degrees of encapsulation via different routes, including a group with no treatment, a group that underwent oral administration of aqueous GH solution (3 mg/kg), a group that underwent intranasal administration of GH of the same concentration, and a group was intranasally administered a mixture solution of GH and liposomes. The inhibition of AChE activity was measured quantitatively using a colorimetric method, where spectrophotometry is used to determine the rate at which AChE hydrolyses the acetylcholine iodide substrate into thiocholine [18]. AChE activity assays of rat brain homogenates reveal the greatest inhibition in intranasal administration of GH-loaded liposomes, followed by the intranasal administration of the mixture solution, the intranasal administration of aqueous GH, the oral administration of aqueous GH and the group that received no treatment (Figure 1a). Moreover, it was found that a significantly larger amount of GH reached the brain in a shorter amount of time when the GH-loaded liposomes are delivered intranasally, with the maximum concentration (14 μg/mL) occurring 0.75 hours after the treatment. Subsequently, the cytotoxicity of the treatment was determined using lactate dehydrogenase (LDH) assay, where increased LDH levels indicate greater cell damage. GH-loaded liposomes displayed LDH levels similar to normal cells, however, the cytotoxicity can potentially be reduced further by improving the entrapment efficiency. This study shows great potential in enhancing AChE inhibition and reducing cytotoxicity. However, to enhance the amount of GH that reaches the brain and hence achieve maximum AChE inhibition, a higher entrapment efficiency is required.

In 2021, Fernades *et al.* reported a novel lipid formulation that can be used to synthesise exosome-like liposomes (exo-liposomes) [19]. It was found that the exo-liposomes could be loaded with curcumin (Cur), forming a delivery system that has shown neuroprotective effects against oxidative stress and amyloid-β accumulation. Curcumin is a hydrophobic polyphenol that exhibits anti-inflammatory and antioxidant properties [19]. In the study, PEGylated, curcumin-loaded exosome-like liposomes (Cur/exo-liposomes) were synthesised using various lipids, including cholesterol,

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sphingomyelin, dipalmitoylphosphatidylcholine (DPPC) and PEG-ceramide. The entrapment efficiency was determined using fluorescence microscopy. A higher entrapment efficiency was obtained compared to that reported by Li *et al.*, achieving around 88% without the addition of 1,2-Dioleoyl-3-trimethylammonium propane (DODAP) and 94% with DODAP, as it has been reported that DODAP improves the stability of encapsulation [20]. A range of cells were exposed to different concentrations of curcumin with and without DODAP to test for cytotoxicity *in vitro*, including the neuroblastoma cell line derived SH-SY5Y cells, mouse fibroblasts L929 and mouse microglia BV2. The cell viability was measured using resazurin reduction assays, and damages to the membrane integrity were monitored using trypan blue assays. No cytotoxicity or membrane damage were detected in the SH-SY5Y cells after exposure to the Cur/exo-liposomes of the selected concentrations ([curcumin] = 20 μM, 8 μM; [liposomes]= 200 μM, 40 μM) for 24 hours. Similarly, no cytotoxicity or interference to cell metabolic activity were detected for L929 cells. Haemolysis assays were also carried out to study the potential interactions between Cur/exo-liposomes and erythrocytes. None of the loaded or empty exo-liposomes with curcumin concentration under 500 μg/mL (with or without DODAP) induced haemolysis, which is consistent with the data obtained from the cytotoxicity assays. Further, neuroprotective effects of Cur/exo-liposomes against oxidative stress were assessed. BV2 cells incubated with encapsulated curcumin showed greater viability than BV2 cells incubated with free curcumin when subjected to the oxidative-stress-inducing-agent tert-butyl hydroperoxide (t-BHP) (Figure 1b). While further testing and animal studies need to be conducted to study the immunogenicity, distribution and uptake of curcumin in the brain, and effective dosage of Cur/exo-liposomes before clinical translation, the results show promising potential of Cur/exo-liposomes owing to their biocompatibility, low cytotoxicity and neuroprotection against oxidative stress.

In a latest study, Wang *et al.* developed and assessed an icariin (ICA) and tanshinone IIA (TSIIA) delivery liposome nanosystem that has potential therapeutic applications against AD [21]. The nanosystem consists of liposomes modified with the ligand Angiopep-2 (amino acid sequence: TFFYGGSRGKRNNFKTEEY), which is an oligopeptide that can specifically bind to the low-density lipoprotein receptor-related protein 1 (LRP1). LRP1 are highly expressed in the brain and on the BBB, which enables the entry of Angiopep-2 modified liposomes across the BBB via LRP1-mediated transcytosis. An encapsulation efficiency of 92.45 ± 3.20% was obtained for the Angiopep-2-modified liposomes loaded with ICA and TSIIA (Ang2-ICA/TSIIA liposomes), which is similar to that reported by Fernades *et al.* [19]. The intracellular distribution of liposomes in mouse brain endothelial cell line were determine by loading 6-coumarin, the green fluorescent dye into the liposomes and measuring the intracellular fluorescent intensity after incubation. Angiopep-2-modified liposomes consistently showed significantly greater cell uptake than the unmodified ones, demonstrating how the binding between Angiopep-2 and LRP1 can potentially facilitate and improve intracellular drug accumulation. Further, the neuroprotective effects were assessed through a series of imaging, cognitive and behavioural tests on APP/PS1 AD-expressing double transgenic mice. Not only did the APP/PS1 mice that received the Ang2-ICA/TSIIA liposome treatment show the greatest reduction of cortical and hippocampal Aβ plaque (Figure 1c), significant inhibition of neuroinflammation, oxidative stress and apoptosis was observed in mice that received intravenous injections of Ang2-ICA/TSIIA liposomes. This has likely contributed to their improved cognitive performance, which was confirmed with Morris water maze experiments, in which mice treated with Ang2-ICA/TSIIA liposomes exhibited enhanced learning and memory ability.

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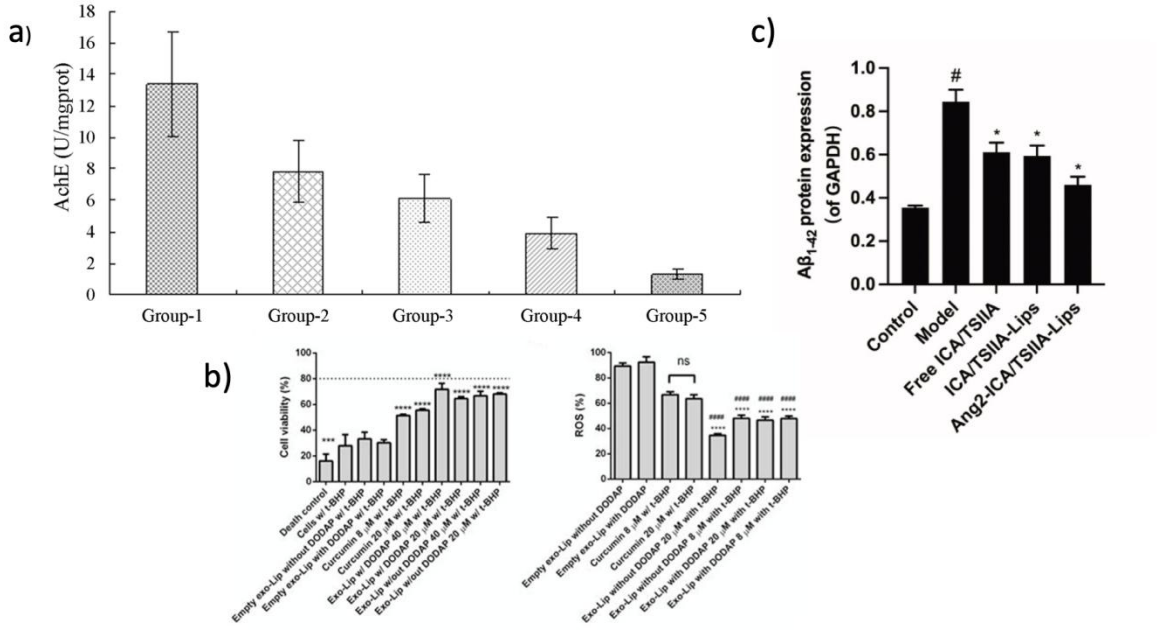

Figure 1. a) GH-loaded flexible liposomes (group 5) show the greatest AchE inhibition, achieving a reduction of approximately 84.6% compared to the group that received no treatment (group 1). Intranasal administration (groups 3-5) is more effective compared to oral administration (group 2) in AChE inhibition. Greater degrees of GH encapsulation (group 5 > group 4 > group 3) correlate with greater AChE inhibition. b) Significant increase in cell viability can be observed in all curcumin- exo-liposomes treated groups regardless of the presence of DODAP, however, 40 μM exo-liposomes with DODAP show the greatest cell viability after exposure to t-BHP. Groups where DODAP is present show greater cell viability. ROS generation is reduced by exo-liposomes with/without DODAP; however, the lowest ROS levels are seen in Cur/exo-liposomes 20 μM without DODAP. c) ICA/TSIIA treatment reduced the Aβ1–42 expression. Greater specificity, i.e., the conjugation of the specific angiopep-2 ligand correlates with greater reduction in the expression of Aβ1–42.

2.2. Chitosan

Chitosan is a polysaccharide chitin derivative that can be produced through deacetylation. Chitosan-based nanoparticles exhibit mucoadhesive properties that arise from the electrostatic interactions between the cationic chitosan and the anionic mucus components and the mucosal epithelium. Additionally, due to its permeation enhancing effects, chitosan can penetrate through the tight junctions of the epithelial cells via paracellular or transcellular transport [22], potentially improving and prolonging the absorption of anti-AD drugs. Therefore, owing to their biocompatibility, biodegradability, nontoxicity and ease of preparation, there has been increased research on the applications of chitosan nanoparticles as potential treatments of AD.

In a 2021, Wang *et al.* reported a theragnostic system where co-assembled chitosan-hyaluronic acid was cross-linked with glutaraldehyde (forming CHG NPs), in turn enabling the targeting and inhibition of Aβ fibrillisation [23]. Interestingly, fluorescent imaging assays found that CHG NPs not only showed binding affinities towards Aβ fibrils, but also Aβ oligomers both *in vitro* and *in vivo*, highlighting the possibility of early AD detection using CHG NPs, prior to Aβ fibrillisation and plaque formation. The *in vitro* inhibition of $A\beta_{40}$ aggregation was monitored using thioflavin T fluorescence assay, which revealed that CHG NPs at low concentrations had little inhibitory effects on $A\beta_{40}$ aggregation (only up to 25% inhibition). However, at high concentrations, significant reduction in $A\beta_{40}$ aggregation was observed (Figure 2a), indicating the binding between the free Aβ monomers and CHG NPs, which could prevent the self-assembly of Aβ monomers into Aβ fibrils. Atomic force

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microscopy (AFM) observed no Aβ fibril formation. The *in vivo* assay of Aβ fibrillogenesis inhibition using $A\beta_{3-42}$-expressing *Caenorhabditis elegans* produced results consistent with the *in vitro* data. Furthermore, MTT assays revealed a 90% cell viability for higher concentrations ([CHG NPs] =720 μg/mL), while no significant effect was observed on cell viability for lower concentrations ([CHG NPs] <180 μg/mL). While the effects of CHG NPs on oxidative stress, tau neurofibrillary tangles and neuroprotection need to be further investigated, this study provides new insights into the developments in AD diagnosis and treatment.

Saleem *et al*. synthesised chitosan nanoparticles loaded with chrysin (Chr-Chi NPs) that exhibit neuroprotective properties and inhibition of Aβ aggregation in zebrafish [24]. Chrysin (5,7-dihydroxyflavone) is a phytochemical that has been reported to display anti-inflammatory properties that result in the downregulation of cyclooxygenase-2 (COX-2) activity via the interleukin-6 (IL-6) signalling pathway [25]. Moreover, Mantawy *et al.* reported the ability of chrysin to downregulate elevated caspase-3 activity associated with doxorubicin-induced cardiotoxicity via the suppression of the p53 pathway [26]. Interestingly, the inhibition of caspase-3 expression has been shown to reduce synaptic dysfunction in mice that exhibit AD-like phenotypes [27], which illustrates the potential for chrysin to inhibit neuronal apoptosis. In this study, $A\beta_{1-42}$ oligomers were intraventricularly injected into the telencephalon of the zebrafish to induce Aβ toxicity. Compromised memory retention, learning and comprehension abilities were observed and confirmed with a series of behavioural assays. An entrapment efficiency of 73.52 ± 0.31% was obtained, which is relatively low compared to the entrapment efficiencies of the liposome NPs discussed in this review [17, 19, 21]. Toxicity analysis revealed no morphological or behavioural abnormalities in zebrafish treated with 1 mg/L Chr-Chi NPs (2 μM, 0.2% Chi NPs) administered via the oral route. A significant reduction of ROS generation (measured by fluorescence in brain homogenates) was seen in zebrafish treated with Chr-Chi NPs compared to the control (ROS generated by and $A\beta_{1-42}$, Figure 2b). Further, increased synaptic integrity and plasticity (measured by synaptophysin levels) were observed in zebrafish treated with Chr-Chi NPs, illustrating the protective effects of this formulation on synapses and $A\beta_{1-42}$-induced neurodegeneration.

In 2020, Dhas *et al.* investigated the use of chitosan as a coating material on lutein-loaded PLGA nanoparticles (Cs@LT-PLGA NPs) for the suppression of oxidative stress in AD [28]. Lutein (β, ε-carotene-3,3′-diol) is a carotenoid that can exhibit antioxidant and anti-inflammatory properties. However, because of its lipophilic nature, lutein has low solubility in water, which contributes to low absorption and bioavailability. Additionally, studies have shown that lutein is susceptible to thermal, oxidative and photo-degradations. Therefore, encapsulation strategies are needed to address the aforementioned issues. Chitosan was used to enhance mucoadehsion and permeation of the nanoparticle formulation via intranasal delivery. An encapsulation efficiency of 83.97 ± 1.03% was obtained for the Cs@LT-PLGA NPs and a uniform coating of chitosan was observed using TEM. Results from the release study showed an initial burst release of lutein for uncoated LT-PLGA NPs, where 25.71 ± 1.35% of the lutein content was released after 6 hours. On the other hand, Cs@LT-PLGA NPs showed a slower, but more sustained release, where no burst release was observed, with 70.12 ± 1.25% of lutein content being released after 96 hours. Further, *in vivo* toxicity study showed no mortality or toxicity-related pathologies in mice that underwent intranasal administration of Cs@LT-PLGA NPs, which is consistent with the *in vitro* cell viability assay. Antioxidant assays demonstrated significant increase in ROS scavenging activities at lower concentrations of Cs@LT-PLGA NPs (5μg/mL), however, at higher concentrations (>5μg/mL), decreased cell viability due to ROS generation was observed (Figure 2c).

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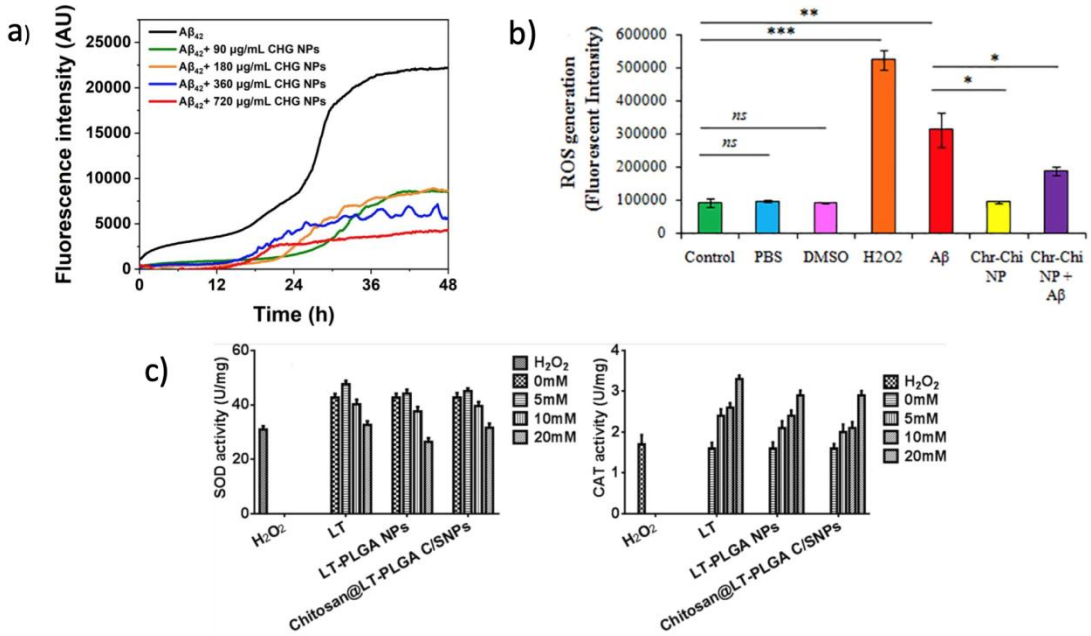

Figure 2. a) The inhibitory effects of CHG NPs of different concentrations on Aβ42 aggregation at high concentrations. Aβ42 aggregation is reduced at all concentrations of CHG NPs, with the greatest inhibitory effects occurring at 720 μg/mL of CHG NPs. b) Chr-Chi NPs significantly reduce Aβ-induced ROS generation compared to the Aβ group, which did not receive treatment. Chr-Chi NPs generate little ROS compared to the control group. c) The greatest ROS-scavenging activities can be seen in 5 mM Cs@LT-PLGA NPs, indicated by increased superoxide dismutase (SOD) and decreased catalase (CAT) levels. Similar trends can be seen in 10 mM Cs@LT-PLGA NPs. However, at 20 mM, the SOD and CAT activities approach the same level as the hydrogen peroxide control due to ROS generation.

2.3. Gold nanoparticles

Gold nanoparticles (AuNPs) play a major role in nanomedicine owing to their unique physiochemical and optical properties, which are able to be modified by altering the sizes and shapes of AuNPs. Due to the ease of synthesis and functionalisation, as well as high biocompatibility, AuNPs have gained great interest in their applications in gene and drug delivery, photothermal therapy for cancer and in medical imaging.

In 2019, dos Santos Tramontin *et al.* studied the ability of AuNPs to reverse okadaic-acid (OA)-induced cerebral tissue damage in rats [29]. In this study, intracerebroventricular injections of OA were administered to rats, which induced AD-like pathologies, including neuroinflammation, decreased brain-derived neurotrophic factors (BDNF) and nerve growth factors β (NGF- β), oxidative stress, and Tau hyperphosphorylation in the cerebral cortex and hippocampus. Following the OA injections, the rats received intraperitoneal injections of 20 nm AuNPs every 48 hours for 21 days. Neuroinflammation was evaluated through pro-inflammatory cytokines interleukin (IL)-1β and TNF-α levels, along with anti-inflammatory cytokines IL-4 levels. Results showed elevated IL-1β and TNF-α levels in rats injected with OA, and rats injected with OA followed by AuNP treatment (OA-AuNP group). The correlation between AuNP treatment and increased IL-1β was investigated in a 2022 study [30]. The study found that gold nanorods induced NLRP3 inflammasome pathway activation, which involves the production of the active protease caspase-1 and cleavage of pro-IL-1β to produce bioactive IL-1β. Despite substantial research on the role of IL-1β in acute neuroinflammation, the effects of chronic IL-1β overexpression in neurodegeneration are lesser-known. Several studies have found that IL-1β-mediated neuroinflammation potentially attenuates AD pathology through microglial

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phagocytosis of Aβ plaque and insoluble $A\beta_{1-40}$ peptides following chronic overexposure to IL-1β [31]. Therefore, since results obtained by dos Santos Tramontin *et al.* only consider the effects of AuNPs on IL-1β and TNF-α levels over a 21-day period, the long-term effects and role of AuNPs in neuroinflammation need further investigation. Moreover, increased cortical and hippocampal IL-4 levels were observed in the AuNP treatment group compared to the OA group. This suggests increased anti-inflammatory activities against elevated pro-inflammatory signalling, since IL-4 is known to inhibit IL-4 and TNF-α production [32]. The cortical and hippocampal levels of BDNF and NGF- β in the treatment and control groups were determined using enzyme-linked immunosorbent assays (ELISA). Decreased BDNF levels can be seen in the OA-treated hippocampus and cortex homogenates compared to the control groups (sham and AuNP-treated). Interestingly, similar BDNF levels are the OA group and the OA-AuNP group. However, rats treated with AuNPs showed improved cognitive performance in the Barnes maze task compared to the OA-treated group, which measured the escape latency and the time spent inside the target quadrant. NGF- β levels followed a similar pattern, with similar cortical and hippocampal levels between the OA group and the OA-AuNP group. OA-induced oxidative stress was measured by the levels of antioxidant compounds, including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Results showed increased cortical and hippocampal SOD (Figure 3a), CAT and GSH activities in OA+AuNP group compared to the OA group, where more pronounced increase occurred in the hippocampus. This demonstrates the ROS-scavenging ability of AuNPs against free radicals including superoxide and hydroxyl anions, as well as the capacity for AuNPs to improve the antioxidant activities of SOD, CAT and GSH.

In a 2021 study, Zhang *et al.* investigated the neuroprotective effects of maize tetrapeptide-anchored gold nanoparticles [33]. Various studies have reported the anti-inflammatory and antioxidant properties of bioactive peptides. For instance, He et al. (2022) found that peptides derived from millet bran significantly reduced the TNF-α, IL-1β and prostaglandin E2 activities *in vivo* and *in vitro* [34]. Moreover, a 2018 study reported the isolation of bioactive peptides from black soybean, which showed the ability to scavenge free radicals [35]. This demonstrates the exciting potential of bioactive peptides in AD management and treatment. In the 2021 study, thiolated maize tetrapeptides (TPMs, amino acid sequence: Leu-Asp-Tyr-Glu) were anchored to the surfaces of AuNPs (3.5 and 13.5 nm). No cytotoxicity was observed after PC12 cells were incubated with Au@TPMs, since MTT assays showed no difference in cell viability between the 3.5/13.5 nm Au@TPM groups and the control group. Interestingly, the cells treated with 3.5 nm Au@TPM showed similar levels of inhibition in L-glutamic-acid-induced apoptotic activities as donepezil hydrochloride (DH), while 13.5 nm Au@TPM were observed to have slightly lower inhibitory effects. Similarly, ROS study showed greater inhibition of L-glutamic-acid-induced ROS accumulation in both 3.5 nm and 13.5 nm Au@TPM treated cells compared to the control, however, the greatest inhibitory effects were seen in DH-treated cells. *In vivo* studies were also conducted, where AD-like pathology was induced in mice through daily subcutaneous injections of D-galactose and intragastric injections of aluminium chloride for 60 days. 3.5 nm and 13.5 Au@TPM treatments were injected intragastrically in the treatment group for 16 days. Significant increase was seen in the acetylcholine (ACh) and choline acetyltransferase (ChAT) levels, whereas AChE levels were significantly decreased. Notably, at high concentrations (1 mg/kg), Au@TMP treatments achieved similar cholinergic and antioxidant effects as DH. Owing to these therapeutic effects, the treatment group exhibited enhanced performance in the Morris water maze experiment, showing the greatest reduction in escape latency when 1 mg/kg of 3.5 nm Au@TMP was administered (lower than that achieved by DH-treated mice). Moreover, increased levels of antioxidant enzymes SOD and GSH-Px were detected following the treatment (Figure 3b).

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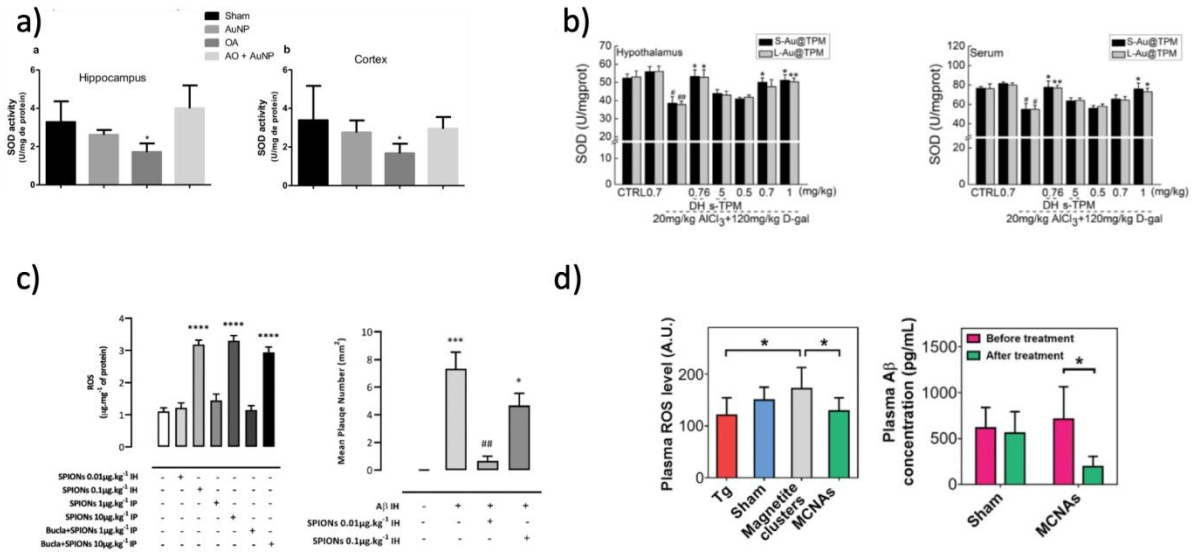

Figure 3. a) AuNPs increase the hippocampal and cortical SOD activities compared to the OA group, a greater increase can be seen in the hippocampus. Additionally, in the hippocampus, SOD activity levels of the OA+AuNP group exceeded that in the sham group. b) Au@TPM treatment significantly increased the SOD activities in the hypothalamus and the serum. No significant differences are observed in the SOD activities in groups treated with 3.5 nm Au@TPM (S) and 13.5 nm Au@TPM (L). c) Groups treated with intrahippocampal administration of SPIONs at 0.01μg/kg show the lowest ROS levels and greatest plaque reduction. Dose-dependent effects can be seen, where lower concentrations appear to be effective in reducing ROS and plaque than higher concentrations. d) MCNAs significantly reduced ROS levels in the plasma to around 130 A.U., which is lower than the ROS levels seen in the sham group. The concentration of plasma Aβ is also significantly reduced after the MCNA treatment.

2.4. SPIONs

Supermagnetic iron oxide nanoparticles (SPIONs) are most commonly made of small maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4) crystals that typically have a size under 100 nm. In recent years, various diagnostic and therapeutic uses of SPIONS have been explored and applied, including their role as an MRI contrast agent, and as drug carriers for targeted cancer and neurodegenerative disease treatment.

In 2021, Sanati *et al.* studied the role of SPIONs in inhibiting Aβ fibrillisation, along with learning and memory functions in rats [36]. 20 nm PEGylated SPIONs and non-PEGylated SPIONs conjugated with the cAMP agonist bucladesine were administered through either intrahippocampal or intraperitoneal routes in $A\beta_{1\text{-}42}$-treated rats. Significant dose-dependent reductions in the average escape latency were seen in rats that received less concentrated SPIONs intrahippocampal or bucladesine+SPIONs intraperitoneal treatments, illustrating the ability for the treatment to alleviate spatial memory deficits. Similar trends were seen in the inhibitory effects of SPIONS on oxidative stress, where 0.01 μg/kg intrahippocampal SPION treatment and 1μg/kg intraperitoneal bucladesine+SPION treatment significantly reduced the levels of key markers of oxidative stress, including ROS (Figure 3c) and malondialdehyde (MDA). Further, 0.01 μg/kg SPIONs greatly reduced the BDNF levels in the Aβ-treated hippocampus, even slightly exceeding the BDNF levels in the group that received no Aβ or SPIONs treatments. BDNF regulates neurogenesis, neuronal differentiation, maintenance and synaptic plasticity, which are crucial to learning and memory. Therefore, increased BDNF levels in AD-like brains could potentially ameliorate the decline in

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cognitive functions. SPIONs treatment also increased cAMP response element-binding protein (CREB) levels in the hippocampus, which is associated with long-term memory formation, neuroplasticity and spatial memory. Moreover, histological analysis measured the mean area of Aβ plaque in thioflavin-S-stained hippocampi, which revealed Aβ plaque reduction in SPION-treated hippocampus, where the most significant reduction was observed at lower SPION concentrations (0.01 μg/kg).

In a 2019 study, Kim *et al.* reported an innovative extracorporeal blood Aβ removal system using core/shell structured supermagnetic magnetite (SPION) and ceria (CeO2) nanoparticle assemblies (MCNAs) [37]. 10 nm SPIONs were synthesised and allowed to form large clusters (approximately 220 nm), which form the supermagnetic core of the MCNA. This enables the magnetic separation of Aβ-containing-MCNA from the blood when placed in an external magnetic field. The SPIONs were then coated with ceria nanoparticles, conjugated with Aβ-specific antibodies and underwent PEGylation, reaching an overall size of approximately 330 nm. Ceria nanoparticles exhibit ROS scavenging and antioxidant properties due to the Ce^{3+}/Ce^{4+} redox reactions that occur on the surface of the ceria lattice in the presence of ROS [38]. MTT assays revealed low cytotoxicity and high cell viability after incubation with MCNAs, which is likely due to PEGylation and the large size of MCNAs that inhibited cellular uptake. The ROS scavenging activities of MCNAs *in vivo* were determined using 5XFAD transgenic mice that express AD-like amyloid pathology. SOD and CAT activities were significantly reduced following the magnetic clearance of Aβ peptides from the blood (Figure 3d). An average of 71% Aβ peptide capture efficiency was achieved and confirmed by reductions in the plasma Aβ concentration and area of Aβ plaque in the cerebral cortex. However, extracorporeal procedures are often invasive and pose risks of infections, especially considering the age demographics of AD patients, who can be more susceptible to severe infections. Therefore, although MCNA treatments showed promising results through the bypassing of the BBB and the extracorporeal removal of Aβ peptides, the risks of infections associated with the procedures require further investigation.

3. Conclusion

Being the 7th leading cause of death [1], growing concerns arise surrounding the social and financial impacts of Alzheimer's Disease. Accurate diagnosis and effective treatments of AD are yet to be developed since the pathogenesis and disease mechanism of AD are not yet completely understood. Therefore, further progress needs to be made in the identification of AD biomarkers through imaging and laboratory tests to enable the early detection of AD. The highly selectivity of the blood-brain barrier microvasculature and inadequate absorption via the oral route pose significant challenges in the effective drug delivery into the brain in current symptomatic treatments of AD. The incorporation of nanotechnology in treatment of AD could be particularly promising owing to the increased specificity and unique physiochemical properties of nanoparticles. In recent decades, many nanoparticle-based therapeutic agents have been proposed to target different aspects of AD, including targeted drug delivery, delivery of antioxidant and anti-inflammatory agents, Aβ fibrillisation and aggregation inhibitors, and Aβ removal from the blood. However, extensive animal and human trials need to be conducted before the implementation of clinical treatment, and the long-term toxicity needs to be studied, since current research mainly focus on examining the short-term cytotoxicity. In addition, combination therapy that targets multiple hallmarks of AD can be explored further. Ultimately, future directions lie in improving the delivery efficiency of current AD drugs, developing potential curative treatments and advancing current diagnostic techniques in order to achieve early detection and accurate diagnosis of AD.

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Appendix

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