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Nano Medicine Approach for Alzheimer's Disease

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Abstract:

Alzheimer's is a neurodegenerative disease that interferes with person's memory, thinking and behaviour. It is characterized by the cognitive inability of the affecter's brain, manifested due to the accumulation of β-amyloid, the formation of hyper phosphorylated neurofibrillary tangles, and a malfunctioned cholinergic system. Over 35 million people worldwide are affected by this ailment. Although, there is no conclusive treatment for humans yet, a promising approach is represented by nanotechnology. Nano medicine-based approaches have opened an area via theranostics of Alzheimer's disease, which are able to target and deliver drugs across the blood–brain barrier (BBB). This review article attempts to provide an insight on theranostic approaches of Alzheimer's disease, taking into account five different nanoparticles, namely, Gold, Cerium Oxide, Iron Oxide, PLGA and Selenium.

Keywords **—Alzheimer's disease, amyloid-β peptide, blood–brain barrier, theranostic approaches, nanoparticles**

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I. INTRODUCTION

Alzheimer's disease (AD) is one of the common neurodegenerative disorders, characterized by the accumulation of amyloid-beta (Aβ), the phosphorylation of tau and the formation of neurofibrillary tangles in brain. Amyloids are a group of proteins that are capable of forming amyloid fibrils, which are characterized by cross- β pattern by x-ray diffraction, Congo red birefringence by polarized light, and fluorescence staining by thioflavin probes.[7] Aβ peptide is considered as the major component of the senile plaques and its accumulation plays a vital role in the pathogenesis of AD and is one of the biomarkers of AD, detection of the concentration of Aβ in CSF can provide useful information of diagnosis of AD.[9]. While monomeric Aβ is inert, Aβ aggregates induce neurotoxicity, inhibit neuronal long-term potentiation, induce synapse loss, and disrupt memory and complex learned behavior. As a result, halting Aβ aggregation is one promising therapeutic strategy for AD. [5]

Numerous biomolecules and biological events are associated with the progress and pathogenesis of AD. Due to its complicated/different clinical, anatomic, and physiological features, multifactorial therapeutic approaches should be considered for AD. It is well-understood that multiple biochemical actors work together to survive neurons under adverse conditions.[14] Conventionally, Ab inhibitors were screened by staining with small dye molecules, such as thioflavin T. However, most of these protocols are complicated, time-consuming, labor intensive, and required specialized facilities,

especially for the purpose of large-scale, high-throughput screening. [8]

Metal ions promote Alzheimer's disease (AD) pathogenesis by accelerating amyloid-β (Aβ) aggregation and inducing formation of neurotoxic reactive oxygen species (ROS) such as hydrogen peroxide (H2O2). Although metal chelators can block these effects,[6] their therapeutic potential is marred by their inability to cross the blood-brain barrier (BBB) and by their non-specific interactions with metal ions necessary for normal cellular processes, which could result in adverse side effects. [6]

Metal nanoparticles have unique features that could contribute to the development of new therapies for these diseases. Nanoparticles have the capacity to carry several molecules of a drug; furthermore, their unique physico- chemical properties allow, for example, photothermal therapy to produce molecular surgery to destroy tumor cells and toxic structures.[13] Although new compounds are being developed for the treatment of disorders of the central nervous system, they are of limited use because of the presence of the blood brain barrier, a unique and highly selective fence that insulates the central nervous system from the other parts of the body, thus providing a stable environment for neuronal function. Currently, more than 98% of all small molecules and 100% of large-molecule pharmaceutics do not cross the blood brain barrier [13]. However, despite the protecting and isolating role of this barrier, the brain is not fully insulated; this organ needs essential nutrients and ions to maintain its integrity and to perform its functions. These basic nutrients are provided by endogenous influx mechanisms present on the capillaries that constitute the barrier. These Nanoparticles (NPs) have emerged as attractive therapeutic and diagnostic tools with applications in medical imaging, analytics, and drug delivery. [5] NPs can be synthesized from a wide range of materials including metals, polymers, and carbon-based molecules. Furthermore, the ease with which NP size, shape, and surface properties are controlled render NPs an ideal tunable platform for therapeutic applications.[5]

While a wide array of small molecules and peptides can disrupt Aβ aggregation [29, 30], none have been as effective as NPs at substoichiometric ratios, thus increasing their potential for delivery of therapeutically effective concentrations to the brain. While NP diameter and surface chemistry impact the extent of inhibition, electric charge determines the ability to influence aggregate morphology. In particular, smaller, anionic NPs are superior inhibitors, halting aggregation at substoichiometric ratios as low as 1:2,000,000 with the protein.[5]

Among the growing body of potential therapeutic applications for NPs is their ability to modulate amyloid protein aggregation [15–19]. Inhibition of Aβ aggregation, specifically, has been reported for NPs ranging in size from <10 nm to several hundred nanometers and exhibiting diverse surface chemistries.[5]

II. LITERATURE REVIEW

1. GOLD NANOPARTICLES

Amyloid plaques, comprised of aggregated amyloid-β (Aβ) protein and found through- out the cerebral cortex , are a pathological hallmark of AD. While monomeric Aβ is inert, Aβ aggregates induce neurotoxicity , inhibit neuronal long-term potentiation ,induce synapse loss, and disrupt memory and complex learned behaviour . As a result, halting Aβ aggregation is one promising therapeutic strategy for AD. [5]

In an experiment conducted by Liao et al, the relationship between AuNPs and protein aggregation was studied. Aβ monomer was treated and preformed fibrils with various concentrations of bare AuNPs and characterized A β fibrillization kinetics, species distribution, and species morphology by thioflavin T (ThT) assay, dynamic light scattering (DLS), and transmission electron microscopy (TEM).[7] Bare AuNPs were found to inhibit A β

fibrillization and redirect A β forming fragmented fibrils and spherical oligomers. Adding bare AuNPs to preformed fibrils resulted in smaller and ragged A β species and these AuNPs bound preferentially to A β fibrils but not amorphous aggregates. [7]The A β incubating with negatively charged AuNPs during A β fibrillization is less toxic and can be served as potential nanochaperones to inhibit and redirect A β fibrillization and leads to to potential applications for AD.[7]

AuNPs aggregation can be induced by inter-particle crosslinking such as DNA hybridization, antibody-antigen interactions, or peptide bridging with two binding tags.

Light scattering is often used to characterize protein aggregation. The AuNPs-based light scattering technique has been recently developed for the sensitive detection of DNA hybridization.[8,2]The remarkable enhancement of light scattering indicates that DNA hybridization can induce functionalized AuNPs aggregation. With addition of Ab peptide, the intensity of RRS is greatly enhanced indicating that Ab peptide can induce AuNPs aggregation.[8,2]

Protein aggregates can be photothermally ablated from surfaces using short irradiation times. Combining the efficiency of dot-blot immunoassay and high affinity of biotin and streptavidin, AuNP based dot-blot immunoassay has been developed for detecting Ab1–42 in complex biological samples. Compared with the established Ab1–42 detection methods, this approach has reasonable sensitivity combined with a large dynamic range .The experimental results also demonstrate that the AuNP based dot-blot immunoassay is readily transferable to analyze practical samples such as the quantitative determination of the biomarker (e.g. Ab) of neurodegenerative diseases, such as AD.[4]

The coexistence both Au nanoparticles for the immobilization of biomolecules and bare carbon for electrochemical detection on the electrode enables the electrochemical sensing with easy fabrication and low-cost.[11]. These advances in diagnosis of Alzheimer's help in early detection systems and more probability of the success of AD treatment.

The feasibility of gold nanoparticles (AuNPs) is identified by conjugating to the b-sheet breaker peptide LPFFD, modified with a Cys (C) residue at the N-terminus (CLPFFD) to promote chemisorption of the peptide onto gold surfaces, to recognize the b-amyloid toxic protein aggregates (Ab) (i.e. oligo- mers, protofibrils and fibrils) involved in AD. By means of irradiation with weak microwaves, in vitro Ab aggregates bound to the conjugate AuNP-CLPFFD are destroyed by local (nanometrically) dissipation of the absorbed energy.

The success of this approach as a type of molecular surgery to remove the toxic aggregates present in the brains of AD patients and thereby halt or slow down the progression of this disease require that the AuNPeCLPFFD conjugate overcome the blood brain barrier.[13]

The conjugation of CLPFFD to AuNPs may enhance the penetration of these particles to the brain through the following proposed ways: (a) the adsorption of specific plasma proteins, which could contribute to receptor-mediated trans- cytosis; (b) the enhancement in the amphipathic character of the functionalized particle, which could favor passive diffusion through the blood brain barrier; or (c) the decrease in the absolute value of zeta potential, which could contribute to a reduction in particle retention by the reticulum endothelial system

(RES), thus increasing the bioavailability of these particles .

The THR peptide is a potential shuttle for the AuNP-CLPFFD conjugate. Moreover, it is known that the THR sequence interacts with an area of the receptor that does not overlap with the native binding site of transferrin, thereby avoiding physiological effects on the protein function and consequently making this peptide very attractive from the therapeutic point of view. [13]

The AuNP-THR-CLPFFD conjugate exhibited the highest permeability across the in vitro cellular barrier.This result implies that the directing portion of the peptide continued to operate as a blood brain barrier shuttle. [13]

The nanoparticles could be cleared from the brain by passing from the brain parenchyma to the cerebrospinal fluid (CSF), and then being exported from the CNS to the venous circulation. The CSF moves only from the villus into the sinus. The AuNPs could be exported by vacuole-mediated transport of fluid and other elements (i.e. bacteria and blood cells) through the villus of the cells. As CSF traverses the villus, it moves along a pressure gradient from a point of higher pressure (the subarachnoid space) to a point of lower pressure (the venous sinus). [15]

A particle may have the capacity to carry several molecules of a drug, an improvement in AuNP delivery to the central nervous system would contribute to more efficient delivery of pharmaceutical agents to the brain. In addition, the conjugation approach may also enhance the number of AuNPs delivered selectively to toxic Ab aggregates, a crucial factor for the early diagnosis and therapy of neurodegenerative disorders such as Alzheimer's disease.[13]

2. CERIUM OXIDE NANOPARTICLES

As we know that, in Alzheimer disease, formation of senile plaques highly contribute to pathogenicity leading to Aβ cell apoptosis, cerium oxide plays a major role as a therapeutic agent. Due to its several unique properties, the most important being the ability to cross the blood brain barrier(BBB), it has been used in various experiments for AD treatment.

In one of the experiments, a novel double delivery platform based on an H2O2-responsive system for AD treatment is demonstrated. The design combined the anti-aggregation property of metal chelators and antioxidant property of CeO2NPs in one system. Compared with metal chelators or CeO2NPs alone, the two-in-one bifunctional nanoparticles can effectively inhibit Ab aggregate formation, decrease cellular ROS and protect cells from Ab-related toxicity.To demonstrate the H2O2 controlled actuation of the nanovalves, fluorescein was loaded as a guest molecule by soaking MSN-BA in phosphate buffered saline (PBS) solution. The pore was then capped by incubating with G-CeO2NP overnight in PBS buffer. The excess amount of molecules was removed by centrifugation and repeated washing with PBS. [18]

Similar Aβ aggregations were experimentally achieved, followed by gel electrophoresis and microscopic examinations. Then intercellular ROS determination is carried out. Cell toxicity assays are performed followed by scanning and staining. The dye used in the experiments, DCFH-DA is the most popularly used probe for detecting intracellular H_2O_2 and oxidative stress. It is also regarded as an easy and user friendly assay. Glucose coated CeO2NPs established as both capping and antioxidant agent for AD therapy.[64]

This inspires us to use G-CeO2NPs which exhibit enhanced stability and biocompatibility in biological matrix as pore blockers to prevent premature release of the encapsulated drug at unwanted organs or other part of an organism.[18]

GCeO2NP acted as an efficient cap for retention of guest molecules with negligible leakage. It worked well against Cu2+ ions which is regarded as best Aβ-aggregator. The advantage of this novel strategy is that metal chelator can only be released by the increased levels of H_2O_2, thus, it would not interfere with the healthy metal homeostasis and can overcome strong side effect of metal chelator after long□term use.

In some other sets of experiments, Western blotting and relative densitometric analysis were carried out.

3. IRON OXIDE NANOPARTICLES

In the study for novel theranostic agents for Alzheimer's disease, superparamagnetic iron oxide nanoparticles like Fe_3O_4 are synthesised by anchoring near infrared fluorescent framework. This near infrared framework allows fluorescent staining of magnetic nanoparticles to the Amyloid β plaques in transgenic mouse brains. These mice were altered to show Alzheimer's symptoms. Brain cells are quite sensitive to magnetic nanoparticles. After proper injection, *in vivo* near infrared imaging and MRI brain imaging was done. Results showed that Amyloid plaques were only detected by after injection with agent PH-1 (DPA-PEGylated), suggesting that PH-1 can penetrate the blood-brain barrier to detect Amyloid β plaques *in vivo.* [32]

The synthesis of superparamagnetic iron oxide nanoparticles coated with a DDNP-carboxyl derivative for in vitro magnetic resonance imaging of Alzheimer's disease concludes that DDNP-SPIONs binding to β-amyloid aggregates showed that the combination has induced the fluorescence enhancement of the DDNP-SPIONs. The relatively small size, monodisperse size distribution, and versatile surface chemistry open up many opportunities for the implementation of biologically specific SPIO nanoprobes for molecular diagnosis of Alzheimer's disease. [30]

In the experiment conducted to deduce the influence of the Physiochemical Properties of Superparamagnetic Iron Oxide Nanoparticles(SPIONs) on Amyloid β Protein Fibrillation in solution form, ultrasmall SPOINs with various surface characteristics were synthesised. This SPIONS were double coated with positive and/or negative charges. On evaluating with ThT fluorescence Assay, it was discovered that depending on surface charge, surface area dependent dual effect was observed. Lower concentration of SPIONs inhibited fibrillation while higher concentration enhanced the rate of Amyloid β fibrillation. This suggested that binding effects Amyloid β conformation which was mainly detected on positive charge suggesting that SPIONs designed medical imaging applications should consider using negative or uncharged surface coating. [36]

Alzheimer's is a neurodegenerative disease associated with old age. Experiment on age-dependent effects of microglial inhibition in vivo on Alzheimer's disease neuropathology using bioactive-conjugated iron oxide nanoparticles discussed that activated microglial cells have been reported to possess neuroprotective or neurotrophic effects *in vitro.*

These could secrete some neurotrophic factors such as NGF, NT-3 and BDNF which have been demonstrated to be neuroprotective. However, other studies have shown that inflammation plays a key role in the progression of neurodegenerative diseases such as Alzheimer's. Microglial cells can be an effective and perhaps necessary target for therapeutic strategies. Depending on the stage of the disease, it may be necessary to use a differential approach to microglial activation: increasing activation at early stages and reducing activation at later stages. This study has also shown the efficacy of γ-Fe_2O_3 nanoparticles in the stable delivery of fibrin $\gamma^{377-395}$ peptide to nervous tissue in vivo. This method can be used for the study of delivery of a number of substances to the brain, as well as monitoring their presence over time. [34]

4. PLGA NANOPARTICLES

PLGA loaded nano-particles have been in use for the treatment of various diseases because of its biocompatibility, biodegradability, toxicity levels and other features that have made it one of the most promising delivering carriers in the pharmaceutical industry. Here, we have mentioned how loading PLGA for drug delivery has had advantageous functioning in treating Alzheimer's disease (AD).

In most of papers that have been reviewed, use drug loaded PLGA NPs to go beyond the blood brain barrier to suppress the pathogen pathways of AD. These previously studied articles draw a conclusion by comparing the effects of administering free drugs and drugs loaded PLGA nano-particles and have put forth their benefits over invasive methods such as direct infusion and implantation of a drug.

Curcumin has many therapeutic effects and is also a low cost material which makes experimenting easier. Being coated on PLGA nanoparticles have been suggested to have increased neuronal targeting efficiency because of the multiple properties and showed no cytotoxicity. However, the usage of free curcumin is restricted because of its insolubility. By using PLGA water solubility was achieved and these also showed fluorescent properties along with being monodispersed in water.

Similarly, Memantine hydrochloride (MEM) is the only drug that has been approved in the Europe to treat moderate to severe AD, but has failed to show its effectiveness. Also, it needs to be administered on a daily basis, otherwise could have adverse effects. To solve these problems loading Memantine on PLGA NP was considered as a suitable candidate for treatment of AD. MEM–PEG–PLGA NPs as expected, contributed to a time-stable dose on the brain, prolonging drug release, reducing administration frequency and decreasing the adverse-side effects. They were non-cytotoxic on brain cell lines and were able to cross BBB both in vivo and in vitro.[43]

From these papers, we can measure the difference between the effectiveness of PLGA nano-particles when they are loaded and surface coated with different drugs and materials respectively.

5. SELENIUM NANOPARTICLES

Selenium Targeted nanoparticles have been proved useful to enhance the performance of therapies against AD in animal models. A better understanding of AD mechanisms will help the successful application of targeted nanoparticles for combined therapies.

One of the most important seleniumdependent detoxifying processes is associated with the

activity of GSH-Px enzyme. GSH-Px protein contains a selenocysteine (Se-Cys) moiety in its active site. GSH-Px, catalase and SOD enzymes have synergistic functions in the removal of H2O2 and organic peroxides [8]. GSH-Px catalyzes a reaction, in which 2 reduced monomeric glutathione (GSH) react with H2O2, and form oxidized glutathione (GS-SG) and H2O. GSH contains thiol groups in its structure.

GS-SG is reduced back to its thiol form (GSH) by the glutathione reductase enzyme [19]. Thioredoxins are small peptides in the cytosol and mitochondria and also play an important role in maintaining a reduced environment in the cells through thiol-disulfide exchange reactions and protects cells and tissues from oxidative stress [34].

Reduction of thioredoxin is catalyzed by thioredoxin reductase-1 (Trx1) and most radicals such as hydrogen peroxide (H2O2) and nitric oxide (NO) are scavenged by Trx1.

Oxidative nanoparticles decreased the activities of reactive oxygen species (ROS) scavenging enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase in the brain of rats and mice. However, Se-rich nanoparticles in small size (5-15 nm) depleted Aβ formation through decreasing ROS production. Reports on low levels of Se in blood and tissue samples and the low activities of GSH-Px, catalase and SOD enzymes in AD patients and animal models support the proposed crucial role of oxidative stress in the pathogenesis of AD.

III. MATERIALS AND METHODS

PAPER	MATERIALS	METHODS	REFERNCE
Influence of gold nanoparticle surface chemistry and diameter upon Alzheimer's disease amyloid-β protein aggregation	Aβ1–40, Gold (III) trichloride hydrate HAuCl4 · 3H2O, trisodium citrate, sodium borohydride (NaBH4), ascorbic acid, CTAB, ThT, Triton-X 100, XTT, and all cell culture media	Surface-coated gold NP synthesis and characterization, Toxicity of surface-coated gold NPs, Aβ1–40 monomer aggregation assays were performed, ThT detection of Aβ1–40 aggregates in the presence of surface-coated gold NPs	[5]
Design and Fabrication a Gold Nanoparticle-DNA Based Nanobiosensor for Detection of	Chloroauric acid, HAuCl4 3H2O, Trisodium citrate and NaCl, Synthetic Target miR-137 (5′ - TTATTGCTTAAGAA TACGCGTAG-3′), miR-137 with two mismatched bases,	HCR and Gel Electrophoresis, Preparation and Characterization of Gold Nanoparticles, microRNA Detection by the	[10]

microRNA Involved in Alzheimer's Disease	concentrated oligos stock solutions	Nanobiosensor, Sample Preparation for Atomic Force Microscopy	
Amyloid-β detection with saccharide immobilized gold nanoparticle on carbon electrode	Sodium L-ascorbate, HAuCl4·3H2O, Copper (II) sulfate, triethylene glycol, potassium carbonate, 3-bromopropyne, sodium hydrate, trichloroisocyanuric acid, iodomethane, 11-bromo1-undecene, 2,2′-azobis (isobutyronitrile) (AIBN), K3[Fe(CN)6], HCl, Na2HPO4, NaH2PO4, KOH, polyvinylalchol, NH4OH and dimetylsulfoxide, Amyloidbeta (Aβ) peptides (Aβ-(1-40), and Aβ-(1-42); trifluoroacetate	Electrodeposition and measurements, . Synthesis of the component molecules, Fabrication of the Aβ sensor, Detection of the Aβ	[11]
Delivery of gold nanoparticles to the brain by conjugation with a peptide that recognizes the transferrin receptor for Alzheimer's	Fmoc-Na-protected amino acids and resins, AG. DIEA, ninhydrin, and 2-mercaptoethanol, . Gold (III) chloride hydrate, sodium tribasic dihydrate, human serum (from human male AB plasma), 5(6)-carboxyfluorescein, collagen type IV, fibronectin, 8-(4-chlorophenylthio)-cAMP, NaHCO3, MEM non-essential amino acids, Lucifer yellow and HEPES	Synthesis of peptides and chromatography, The Kaiser colorimetric test assay, Initial conditioning of the resin, Fmoc group removal, Peptide elongation, Peptide desalting, Peptide characterization, Peptide stability in human serum, Bloodebrain barrier in vitro permeability assay, Transendothelial electrical resistance measurement (TEER)	[13]
Cerium oxide caged metal chelator	3-Glycidyloxypropyltrimethoxysilane (GLYMO, 98%), 4-carboxyphenylboronic acid (CBA), 3-aminopropyltrimethoxysilane (APTES), aqueous ammonia (30%), copper chloride hydrate (CuCl2), and 5-chloro-7-iodo-8-hydroxyquinoline (CQ) were purchased from Sigma-Aldrich.	Native polyacrylamide gel electrophoresis Native polyacrylamide gel electrophoresis (PAGE) was carried out using a 12% gel. Gels were run in a Tris/Tricine system aer which the gels were silver-stained. Transmission electron	[18]

	1,1,1,3,3,3-Hexauoro-2- propanol (HFIP) was obtained from Acros Organics. Cerium(III) nitrate hexahydrate was purchased Glucose was obtained. Deionized water (18.2 MU cm) uwas obtained from a Milli-Q system	microscopy Atomic force microscopy Intracellular determination of ROS Cell toxicity assays	
Synthesis of superparamagnetic iron oxide nanoparticles coated with a DDNP-carboxyl derivative for in vitro magnetic resonance imaging of Alzheimer's disease	Iron(III) acetylacetonate, oleic acid, oleylamine, phenyl ether, malononitrile, sodium metabisulfite, 4-dimethylamiopryidine(DMAP), 1,2-hexadecanediol, 2- acetyl-6-methoxynaphthalene, succinic anhydride, triethylamine, ethanol, ethyl acetate and hexane, Sodium dihydrogen phosphate and disodium hydrogen are some common chemicals used in the in vitro binding experiment.	high temperature thermal decomposition method was used for Synthesis of superparamagnetic iron oxide nanoparticles (SPIONs). Synthesis of 1,1-dicyano-2-[6-(dimethylamino)naphthalene-2-yl]- propene carboxyl derivative. Synthesis of superparamagnetic nanoparticles coated with1, 1-dicyano-2-[6-(dimethylamino)naphthalene-2-yl]propene carboxyl derivative (DDNP-SPIONs) at room temperature for 24 h under nitrogen environment. Magnetic resonance imaging of nanoparticles at 37 °C in combination with a knee radio frequency coil for excitation and signal reception. Binding of DDNP-SPIONs to Aβ(1–40) fibril in vitro	[30]
Ultrasmall superparamagnetic iron oxide nanoparticles-bound NIR dyes: Novel theranostic	Monomeric Aβ1-42, Transgenic mice (APPswe/PSEN1dE9, 9 months old, male) and male C57BL/6, multi-mode spectrophotometer for the fluorescence properties.	Synthesis of superparamagnetic iron oxide nanoparticles anchored near-infrared fluorescent framework using fluorescent probes-NHS and Fe_3O_4-	[32]

agents for Alzheimer's disease		DPA-PEG-NH_2. Synthesis of superparamagnetic iron oxide nanoparticles PH-1 and PH-2 Fluorescent staining of MNPs to Aβ plaques in transgenic mouse brain In vivo near-infrared imaging In vivo MRI brain imaging	
Age-dependent effects of microglial inhibition in vivo on Alzheimer's disease neuropathology using bioactive-conjugated iron oxide nanoparticles	ferrous chloride tetrahydrate, hydrochloric acid (1 M), sodium hydroxide (1 M standard solution), sodium chloride, sodium nitrite, gelatin from porcine skin, divinyl sulfone (DVS), triethylamine (TEA), fibrinogen-derived $\gamma^{377-395}$ peptide, Bicarbonate buffer (BB, 0.1 M, pH 8.4) and phosphate-buffered saline (PBS free of Ca^{+2} and Mg^{+2}, 0.1M, pH 7.4), midi MACS magnetic columns, Elgastat Spectrum reverse osmosis system anaesthesia ketamine:xylazine lethal dose of thiopental sodium solution and were perfused intracardially with isotonic PBS (pH 7.3) followed by 4% paraformaldehyde solution in 0.1M PBS for tissue preparation. Lectin staining, Iron staining, Immunohistochemistry	Synthesis of bioactive γ-Fe_2O_3 nanoparticles Prepare Animal model rTg4510 mice Intracranial injections administered Tissue preparation Staining	[34]
Influence of the Physiochemical Properties of Superparamagnetic Iron Oxide Nanoparticles on	Analytical grade iron salts (iron chloride) and sodium hydroxide (NaOH), Dextran, dimethyl sulfoxide, sodium periodate, potassium cyanide, and ammonium persulfate, Carboxylated-dextran	Synthesis of the Ultrasmall SPIONs with Various Surface Characteristics. Preparation of Double-Coated SPIONs with Various	[36]

Amyloid β Protein Fibrillation in Solution	and aminodextran.	Surface Characteristics. Amyloid beta peptide Aβ(1−42) was synthesized. Thioflavin T Fluorescence Assay for evaluation of the fibrillogenes process	
Cellular uptake of PLGA nanoparticles targeted with anti-amyloid and anti-transferrin receptor antibodies for Alzheimer's disease treatment	- PLGA, Resomer® - -EDC - methanol -EDTA - Traut's reagent -BSA -phosphate buffer saline (PBS) -diammonium salt (ABTS), -mABs: OX26 (anti-transferrin) and DE2B4 (anti Aβ)	-To work with the BBB, the porcine brain capillary endothelial cells (PBCECs) were collected and cultivated and maintained at 37 degree Celsius in an humidified incubator. - The PEG reagent, Malemide-PEG-Amine was coupled with PLGA. This process required number of mixtures and centrifugations with several reagents. -PLGA nano-particles were formed by nano-precipitation with previously prepared PLGA-peglytated mixture. -NPs were modified. First, $iA\beta_5$ has to loaded on to the NP followed by mAbs. - Characterization of PLGA NPs were done with two mABs and results were recorded.	[47]
Development and evaluation of polymer nanoparticles for oral delivery of estradiol to rat brain in a model of Alzheimer's pathology	-PLGA Resomer RG 50:50 H -DMAB -Estradiol -ELISA kits -Animal groups were purchased under Animals (Scientific Procedures) Act 1986.	-Preparation of PLGA nano-particle -Amount of coating determined by quantitative tests. -Stability of coatings were tested	[52]

		-Experimentation on rats such as behavioural tests, elevated pulse maze test, and open field test. -Further segmentations, characterizations and analysis was done	
Vitamin D-binding protein-loaded PLGA nanoparticles suppress Alzheimer's disease-related pathology in 5XFAD mice	-DBP -T-80 -PBS and FBS(buffer) -Thioflavin-S -RPMI-1640 Medium -Dimethyl sulfoxide (DMSO) -Fluoroshield™ -PLGA	-DBP was loaded onto PLGA NPs by diffusion method. -Characterization of the NPs (size, xeta-potential, amount of DBP in NP) -Administration by intravenous injections or into the tail vein. -Brain tissue preparation -Quantification and analysis	[51]
Design of PLGA-functionalized quercetin nanoparticles for potential use in Alzheimer's disease	-Synthetic Aβ proteins -PLGA (50:50 monomer ratio) -Quercetin -PBS (buffer) -Thioflavin T -Other reagents	-PLGA NPs were synthesized and modifications were done. -Characterization -Peptide preparation -ThT based fluorometric assay to identify fibrils - In vitro drug release -Cytotoxic assay -Further test and analysis was done	[44]
Memantine loaded PLGA PEGylated nanoparticles for Alzheimer's	-PLGA-PEG Resomer RGP d5055 -Memantine -Millipore MilliQ system	- MEM loaded NPs were synthisized by double emulsion method	[43]

disease: in vitro and in vivo characterization		-Characterization (size, quantity) -Efficiency was calculated -Design of Experiments used to optimize formulation -In vitro drug release -Cell culture -In vitro transport across BBB -Further analysis was done	
Preparation and *in vitro* evaluation of multi-target-directed selenium-chondroitin sulfate nanoparticles in protecting against the Alzheimer's disease	Thioflavin T (ThT) and okadaic acid (OA) Sodium selenite (Na_2SeO_3), Congo red, Hoechst 33342, 1-anilino-8-naphthalene sulfate (ANS) and L-cysteine (L-cys)	Physicochemical characterization of the CS@Se nanoparticles Congo red binding assay ANS fluorescence measurements Preparation of the $A\beta_{1-42}$ peptides ThT fluorescence spectroscopy measurements	[54]

IV. DISCUSSION

1. GOLD NANOPARTICLES

Amyloid peptide (A) is found in the brain and blood of both healthy and diseased individuals alike. However, upon secondary structure transformation to a -sheet dominated conformation, the protein aggregates. These aggregates accumulate to form neuritic plaques that are implicated in the pathogenesis of Alzheimer's disease.[17]

The use of the simple SPR method for the detection of beta-amyloid demonstrates a poor sensitivity, as antigen binding does not bring significant mass change due to it small size.[16] As there is not much change in mass on the resonant surface of detection, it affects both the SPR angle change and detection limit of -amyloid—which shows little effect for SPR angle change. Gold (Au) nanoparticles have been known to provide a significant shift in the angle of plasmon resonance.[16]

Gold is selected as the NP core material because gold NPs are readily synthesized, easily functionalized, and highly stable against

oxidative dissolution . Examination of four NP surface chemistries as well as three different NP diameters revealed that electric charge, surface chemistry, and size all modulate the ability of gold nanospheres to inhibit Aβ aggregation.[4].Gold nanoparticles (AuNPs) are the most biocompatible and have been explored in applications for biomolecule sensing, bioimaging, and cancer thermal therapy. [7] Recently, bare AuNPs have been demonstrated to induce co-aggregation of lysozyme and AuNPs and folding and fibril formation of the α -helical coiled-coil based model peptides.[7]

2. CERIUM OXIDE NANOPARTICLES

Despite advances in understanding the causative reasons of NDs ,no current treatments have yielded critical outcomes. [19]

Cerium oxide nanoparticles (CeONPs) have as of late rose as therapeutics for the treatment of NDs because of their cell reinforcement properties. Interest in CeONPs as a potential nanomedicine for NDs has increased due to: their ability to alter signaling pathways, small diameter allowing passage through the blood–brain barrier and scavenging of reactive oxygen species. Due to these properties, CeONPs could eventually revolutionize existing treatments for NDs. [19]

Alzheimer's disease(AD) is a reformist neurodegenerative pathology that is the most widely recognized reason for dementia[18]. Oxidative stress and free radical production are found to be associated with Alzheimers disease.[22] The cell reinforcement properties of cerium oxide nanoparticles show guarantee in the treatment of AD. Studies show that CeO2 nanoparticles do not act as mere anti-oxidant agents, but they seems to affect, directly or indirectly, signal transduction pathways involved in neuronal death and neuroprotection, raising the possibility of their use as therapeutic tools for neurodegenerative diseases.[23] Cerium oxide nps may also serve as starting materials for developing potential AD theranostic agents, as they can also inhibit some of the processes thought to be responsible for AD etiopathology or detect AD lesions such as Aβ amyloid plaques[28].

3. IRON OXIDE NANOPARTICLES

In the recent years, nanotechnology and the use of iron oxide nanoparticles have been integrated into the development and improvement of medical techniques used for early diagnosis and more effective treatment of Alzheimer's disease (AD) [31]. Back in the 1950s, abnormal concentration of iron was associated with this disease. Since then, researches have been conducted to associate iron concentration with various neurodegenerative conditions. In recent studies, Fe^{2+} valence state of iron has been discovered to be existing in tissue samples that are affected by AD pathology. This high concentration is seen in tissue section due to accumulation of Ferrosoferric oxide (Fe_3O_4). This has been a significant discovery, as ferrosoferric oxide is the only stable iron oxide with Fe^{2+} valence state. As a result, this can be exploited as a biomarker for early diagnosis of AD through iron based MRI [39].

Nanodiagnostic approaches involve both *in vivo* and *in vitro* methods [31]. The first step towards early *in vivo* diagnosis of AD is the advance of molecular imaging probes in order to target senile plaques as markers for AD. Ferrosoferric oxide, being a superparamagnetic nanoparticle, is an invested strategy to recognise unique molecular markers of this disease [30]. Other factors that contribute to Fe_3O_4 as a promising biomaterial are its high biocompatibility, strong T2 effects, high sensibility, and capacity for use as multimodal contrast agents. Brain cells are quite sensitive to magnetic nanoparticles (MNPs).

Under the exploit of low radio-frequency field, moderate amount of heat emanated from the MNPs can progress blood-brain barrier (BBB) penetrability without even distressing other brain cells [32].

4. PLGA NANOPARTICLES

The main contributors of Alzheimer's disease are the beta-amyloid plaques formed between neurons that cause disruption of usual CNS processes and collection of excess amount of iron that gets delivered by transferrin, which causes oxidative stress. To reach the targeted area of the for inhibiting of the above mentioned abnormalities the device to be used has to cross the blood he blood-brain-barrier. BBB is highly selectively semi-permeable bounds of endothelial cells. From these papers, we can measure the difference between the effectiveness of PLGA nano-particles when they are loaded and surface coated with different drugs and materials respectively.

Experiments mainly focus on inhibiting and disassembling the amyloid fibrils and metal ions, like iron. Out of many possible drugs and coatings used some of them have shown promising changes and efficiencies in their treatment process. Loading specific monoclonal antibodies onto the PLGA NPs has made it easier to get iAβ5, a beta sheet breaker, across the blood-brain barrier that would work on the abnormalities, like the beta amyloid plaques and the excessive aggregation of iron [47].

Memantine has also been incorporated with these nano-technology to study the changes in its effects. Memantine is the only drug approved both in Europe and in the United States for moderate to severe degrees of AD. However, it has it shown much significant effectiveness and to overcome this issue, this study works on how memantine could be considered as a candidate for drug loading onto PLGA NPs [43].

The conclusion drawn from most the papers that we have chosen to review show the success of PLGA NPs when they are modified with antibodies, drugs and other components that have previously showed some effect on inhibiting the aggregation of beta-amyloid protein, making them promising candidates for the treatment of AD.

5. SELENIUM NANOPARTICLES

Selenium is an essential trace element in our body. [55]One of the most important seleniumdependent detoxifying processes is associated with the activity of GSH-Px enzyme. GSH-Px protein contains a selenocysteine (Se-Cys) moiety in its active site. GSH-Px, catalase and SOD enzymes have synergistic functions in the removal of H2O2 and organic peroxides [8]. GSH-Px catalyzes a reaction, in which 2 reduced monomeric glutathione (GSH) react with H2O2, and form oxidized glutathione (GS-SG) and H2O. GSH contains thiol groups in its structure. GS-SG is reduced back to its thiol form (GSH) by the glutathione reductase enzyme [19]. Thioredoxins are small peptides in the cytosol and mitochondria and also play an important role in maintaining a reduced environment in the cells through thiol-disulfide exchange reactions and protects cells and tissues from oxidative stress [34]. Reduction of thioredoxin is catalyzed by thioredoxin reductase-1 (Trx1) and most radicals such as hydrogen peroxide (H2O2) and nitric oxide (NO) are scavenged by Trx1.

Oxidative nanoparticles decreased the activities of reactive oxygen species (ROS) scavenging enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase in the brain of rats and mice. However, Se-rich nanoparticles in small size (5-15 nm) depleted

Aβ formation through decreasing ROS production. Reports on low levels of Se in blood and tissue samples and the low activities of GSH-Px, catalase and SOD enzymes in AD patients and animal models support the proposed crucial role of oxidative stress in the pathogenesis of AD.

At present, the FDA-approved drugs for treating AD mainly include acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists, but these drugs can only slightly relieve the symptoms of AD [57]. Pharmaceutical companies and academic scientists have conducted many studies to search for disease-modifying drugs for AD. Many potential drugs (such as , bapineuzumab [58], avagacestat [59], gantenerumab [60], verubecestat [61], idalopirdine [62]) have entered phase II and phase III clinical trials, but the results were almost generally disappointing, especially in the late-stage clinical trials of treatment for amyloid metabolism.

However, the results from a recent phase III trial of GV-971 (Sodium Oligo-mannurarate) have offered new hope. The phase III clinical trial data indicated that GV-971 possessed statistical and clinical significance in terms of improving the cognitive function of AD patients. GV-971 is an oligosaccharide extracted from brown algae. [63

CONCLUSION

Individually AuNP diameter and surface chemistry are established to control the extent of aggregation, while AuNP electric charge influenced aggregate morphology. Theoretical calculations suggest that this low stoichiometry could arise from altered solution conditions near the AuNP surface. Specifically, local solution pH and charge density are congruent with conditions that influence aggregation. The potential of surface-coated gold nanospheres serve as tunable therapeutic agents for the process of inhibition of Aβ aggregation.

Gold NPs toxicity depends upon their size, shape, and surface coating. They are very biocompatible. Biocompatiblity is determined by probing them as therapeutic agents , and their potential of eliciting a toxic response is monitored.

Interaction of gold NPs with Aβ fibrils, and subsequent exposure to weak microwave fields, results in a local rise of temperature and dissolution of the fibrils. Gold NPs have the potential to slow down or stop the progression of AD, while these NPs cause no harmful effects in the brain .In a recent study, novel gold NPs conjugated with two compounds interfering with Aβ fibrils were used. These multifunctional 22 nm gold NPs were able to decrease the cytotoxicity of Aβ fibrils and Aβ-mediated peroxidase activity *in vitro* (Gao et al., 2015). Thus, these results indicate that inorganic multifunctional NPs have potential for the treatment of AD.

Positively charged AuNPs could attach to Aβ stronger than the AuNPs with negative charge. The stronger interactions between AuNPs and Aβ leads to fewer β-sheets and more random coil structures. AuNPs could act as an efficient vehicles for drug delivery across the BBB.

The biggest limitation of using a AuNP is that they are very expensive to work with.

It established that CeO2 nanoparticles protects cell viability and cell morphology from Aβ injury suggesting a neurotrophic role for these nanoparticles. It has been previously reported that oligomeric Aβ is more toxic than fibrils, especially concerning the induction of neuroinflammation and of subtle and early neuronal damage.

Some studies indicate that Alzheimer's disease is somehow related to mitochondrial dysfunction. Abnormal ROS production decreases mitochondrial maintenance systems.

The drawback that can be seen in this is that, the ceria NPs therapy to mitochondrial dysfunction is achieved in vitro; the in vivo disease model study of ceria NPs on mitochondria has not been done yet.

The use of nanoparticles as theragnostic agents, to inhibit fibril formation, could be a potential treatment for such diseases. In this case, previous reports have showed that nanoparticles of various surface chemistries and sizes could both accelerate and inhibit Aβ fibrillation in solution. Among various types of nanoparticles, MNPs such as SPIONs, are recognized as promising nanoparticles due to their multi task capabilities like drug delivery, hyperthermia, and imaging within the same nano-system [67].

Designing nanoparticles to pass through the BBB is even more challenging and complex than conventional drug delivery. However, as mentioned in the study for novel theranostic agents for Alzheimer's disease, PH-1 can penetrate the blood-brain barrier to detect Amyloid β plaques *in vivo*. In fact, the olfactory pathway provides a non-invasive route for nanoparticles to enter the Central Nervous System [66].

In terms of biocompatibility, Fe_3O_4 NPs synthesized using the safe-by-design approach showed no adverse effect on cells, as assessed by cytotoxicity assays and cell cycle analysis in MCF-7 cells. As a result, these are a very good candidate as a carrier for targeted drug delivery [72].

We can establish that the non-reactivity between PLGA and approved drugs helps in conserving the natural properties of both of the compounds as it has shown increased efficiency with drugs such as Curcumin, Memantine, Quercetin, and specific proteins as well.

Compared with other nano-carriers, such as liposomes, micelles and dendrimers, the advantages of polymeric NPs include improved stability, high loading capacity, sustained drug release, non-immunogenic property, reduced drug toxicity, improved bioavailability, enhanced therapeutic efficacy of the entrapped drug and versatile surface modification [68]. PLGA and PLA are commonly used polymers for nano-delivery because of their biocompatibility, biodegradation and well-studied degradation kinetics; PLGA and PLA have also been approved for pharmaceutical application by the US Food and Drug Administration [68]. Brain delivery can be achieved by well-designed polymeric NPs fabricated from polymers such as PLGA and PLA of different molecular weights, with surfactants and other surface modifications that confer the surface characteristics (i.e., zeta potential and hydrophilicity) desired for improved BBB uptake via adsorptive transcytosis [69].

Synthetic polymers have the advantage of high purity and reproducibility over natural polymers. PLGA has a wide range of erosion time [70] and also its mechanical properties can be tuned.

There are few limitations of using this polymer based nano-particles and these are very specific problems that are being worked upon. For example, a limitation on the use of PLGA NPs in drug delivery is, however, their fast uptake and clearance from the reticuloendothelial system (RES). To overcome the RES clearance, surface coating of NPs with poly (ethylene glycol) (PEG) has been recommended, an approach that has demonstrated to reduce NPs' clearance signifcantly in vivo. The bare PLGA nanoparticles have major drawbacks such as

hydrophobic surface, rapid phagocytic clearance and initial burst release. They have acidic nature which may not be suitable for certain drugs [71].

Some of the effects seen using Selenium oxide nanoparticles have been mentioned below:

- Selenium Oxide Nanoparticles represses Aβ total and shields SH-SY5Y cells from Aβ1–42-prompted cytotoxicity.
- Selenium Oxide Nanoparticles ensures the cytoskeleton structures and diminishes the cytoskeleton shakiness that is prompted by okadaic corrosive.
- Selenium Oxide Nanoparticles smothers the oxidative pressure initiated by Aβ1–42 in SH-SY5Y cells.
- Selenium Oxide Nanoparticles weakens the hyperphosphorylation of tau at Ser396 and Ser404 locales by controlling the statement of GSK-3β.[54]

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