

NANOTECHNOLOGICAL ADVANCES TOWARDS ALZHEIMER'S DISEASE TREATMENT

Anurag Sangwan, Parth Malik*

Department of Biotechnology, Maharishi Markandeshwar University, Mullana, Ambala
(Haryana)

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***Correspondence for**

Author

Parth Malik

Department of Biotechnology,
Maharishi Markandeshwar
University, Mullana, Ambala
(Haryana).

ABSTRACT

Ever since the development of medical science, effective diagnosis and treatment of neurological disorders have been a prominent question of interest for medical specialists and research community all over the world. Alzheimer's disease (AD) is one of these troublesome disorders with complex pathophysiological background. Current treatment available enables a weak detection and prolongs only the appearance of related symptoms. This has considerably effected the severity of this disorder and consequently it has been presumed within the scientific brains across the world that death is the only destiny for sufferers. However, a ray of hope has been discovered in the better diagnosis and handling of this disease at the clinical stage by nanotechnologists

which not only enables the early detection, but also significantly improves the treatment methodologies involved. This requires the crossing of complex blood brain barriers. Nano-carriers such as nanoemulsion, nanosuspensions and dendrimer encapsulated with suitable drug formulations can be engineered to ensure targeted and sustained release at effected sites within the brain, so as to treat the disease in a better way. This article reviews various nanotechnological insights with an aim to cure the pathogenic impacts of AD along with a thorough understanding of molecular aspects associated with the occurrence of AD.

KEY WORDS: Alzheimer's disease (AD), neurodegenerative disease, nanotechnology, nanoemulsions, dendrimers.

INTRODUCTION

Alzheimer's disease (AD) is one of the most challenging disorders, with its source of origin in brain. It is a neurodegenerative disorder which gets complicated with advancing age and

begins to show concerned symptoms beyond 65 years as the most probable form of dementia^[1]. Due to rapid increase in elderly population worldwide, this disorder has further added curiosity to its rigorous search aimed at finding a reliable route that can facilitate its prevention^[2]. The disease is best characterized by memory loss although in progressive stages problems like hearing impairment, loss of abstract, logical thinking, listening power and overall body to brain coordination gets manifested. A number of studies focused on studying and evaluating the clinical pathobiology of AD have been carried out. In such a study, *Chandaket al* have explained that the causative factors of AD can be both biological and environmental^[3]. Till date most clearly established cause of the disease has been deposition of amyloid-(β)-peptide or its insufficient degradation from the brain. Although there are a number of other coordinated cellular pathways too that get blocked in AD as a consequence of improper or no signaling. A nice correlation exists for the occurrence of this neurological disorder, associated with the functioning of mitochondria. With advancing age, mitochondria gets degraded, besides its increasing biogenesis it is not sufficient to compensate the loss of already existing mitochondrial functions^[4-6]. Some other studies have correlated and extended the onset of AD with the events leading to dementia like symptoms. Dementia appears as a result of improper signaling networks across the cerebral neuronal circuits leading to gradual disappearance of neurons in entirety. An interesting fact correlated with the loss of neurons is their specific *in vivo* location within the brain. Rigorous analysis of clinical trials has led to the finding that this loss of neurons is exclusively in the locations of the brain that contributes or is responsible for memory features. These include hippocampus and adjacent cortex regions and are described by entorhinal, perirhinal and para-hippocampal regions of the brain. Movement of sensory impulse via neurons is mediated through a circular pathway starting from entorhinal cortex via dentate gyrus, through CA3 and CA1 neurons of the hippocampus to the subiculum and then finally at last back to the entorhinal cortex. This pathway of neuronal movements seems to be highly impaired in AD^[7]. In addition unusual mutations in the gene encoding amino acid residues of the neurodegenerative protein 'tau' are also significant. Most crucial and frequent histopathological observations through which AD is recognized are the development of extracellular amyloid plaques which are deposits of amyloid- β peptides and intracellular neurofibrillary aggregates which are deposits of hyper phosphorylated tau protein in the form of helical arrangements^[8-9].

Due to highly complex clinical pathogenesis of AD, its classification is equally diverse. Genetic analysis suggests three main genes responsible for pathogenesis of AD as gene for amyloid precursor protein (APP), presenilin 1 (PS-1) and presenilin 2 (PS-2). Normally AD is fractioned into early-onset (presenile) dementia and late-onset (senile) dementia. Numerous studies have reported mutations and aberrations in genes encoding amyloid precursor protein which get manifested in the form of continued deposition of amyloid- β peptides within the brain. However, classification of the disease manifestation is currently viewed under two distinguished categories, namely familial and sporadic. While most of the clinical studies till date have focused over the familial AD but sporadic AD, of late, has become an intense topic of debate because of its association with improper functioning of mitochondria. Increased deposition of amyloid- β peptides within the cerebral domains of brain result in adverse and non-relevant interference in the fission and fusion proteins of the mitochondria, resulting in mitochondrial dysfunction, fragmentation, disturbance in the redox balance of cellular environment and improper secretion of ATP's accompanied with inhibitory impacts in electron transport chains due to alteration in membrane potentials of the organelle^[10]. Current problems associated with the treatment of AD are numerous, varying from early onset of dementia like symptoms, its complex neurophysiological site of occurrence and finally difficult diagnostics due to highly evolving clinical features of occurrence and manifestation. As a result of which before finding a reliable solution through nanotechnological interventions for the disease treatment, efficient mechanisms for pinpointing accurate diagnosis of the disease needs to be developed. The characteristic focused on estimation of precise and accurate existence and prevalence of AD involves doing brain mapping through Magnetic Resonance Imaging (MRI).

Characterizing Features and Problems in Conventional Alzheimer's Disease Treatment

In order to gain an insight into the biochemical events leading to the onset of AD, a couple of characteristic physiological changes related to pathophysiological background of this disease need to be properly understood. For instance, an improper functioning of amyloid precursor protein (APP) has been reported which is responsible for the deposition and assembling of amyloid- β peptides in cerebrospinal fluid (CSF) regions of the brain. Because of the fact that amyloid precursor proteins acts as a precursor for the synthesis of amyloid tissues, the role of amyloid precursor proteins in the diagnosis of AD is of significance. Principally, if the APP metabolic activities are disturbed or negatively interfered, this feature can be used for the diagnosis of AD. Numerous studies have aimed at the assaying of APP, but have failed to

establish the fact as a precise and reliable inroad to be capitalized for screening of AD^[11-12]. However it has indeed been reported that CSF concentration of APP decreases with the severity of advancing demential symptoms, even though it remains fairly uniform in the surviving secreting precursors of neuronal APP^[13].

Formation of extracellular amyloid plaques, composed of amyloid- β peptides is one of the most common identifying features of this intriguing disease. This protein is a small 42 residue fragment, retrieved *via* proteolytic processing of a larger membranous protein already mentioned as amyloid precursor protein (APP) ^[14]. This APP is hallmark of AD and is a single-transmembrane protein with comparatively longer extracellular chain, composed of larger amino acid residues and a corresponding shorter cytoplasmic tail with lesser amino acid residues possessing intracellular trafficking signals ^[15]. Besides this, there is a further complexity and highly sensitive local specificity that is responsible for deposition of a protein with different sizes and number of amino acid residues within the CSF of brain. Studies have proved that A β protein with 42 residues gets deposited first inside the brain, resulting in the composition of senile plaques while those A β protein with residues ending at position or loci number 40 are deposited later ^[16]. This protein is the most significant key to break the jinx code for the efficient diagnosis of AD. On being spliced at locus 695, 751 and 770 the APP m-RNA gives rise to eight possible isoforms, three of which lie inside the brain^[17-18]. Quite interestingly, of all the normalized A β protein synthesized, those which end at residues numbered 40 contribute nearly 90% while those which end at 42 residues form the remaining 10% of the proteins. The relative proportions of these amyloid- β peptide fragments residing within brain is another vital factor responsible for causing AD. An interesting fact confirmed by several studies is that the number of amyloid- β peptides with end at position 42 is significantly decreased in patients suffering from AD^[19]. Corresponding plaques that are developed in AD are composed of multiple proteins with their cores chiefly comprised of amyloid- β peptide. This core protein is 39-42 amino acid long proteolytic fragments, derived basically from amyloid precursor protein. Another observation enlightens the differential existence of neurological protein tau. The molecular biology of this protein seems to be of significant interest in the blood brain barrier since this region is very sensitively linked and comparatively tougher to accomplish the specific medicated formulation; hence cerebrospinal fluid (CSF) is not extremely localized or independent in functioning from the intercellular space. Hence, to analyze the specific biochemical markers of AD, neuronal proteins linking the intercellular fragments with the CSF can be very resourceful. Tau protein is one of such

neuronal proteins. It is a routine neurological protein that is present in bound form in the microtubules regulating signals mediated through the axons. This protein is present in two distinct forms, namely truncated (t-tau) and phosphorylated (p-tau). In its phosphorylated form, this protein binds to the supporting protein microtubules and facilitates their requisite assembly in the neuronal axons which is very vital for correct signal transduction through the neurons and to other locations across the brain^[20]. The hallmark diagnostic indicator of AD has been an elevated level of this CSF tau protein^[21]. Several studies have proved this fact through the tagging of engineered antibodies to quantitatively screen the presence of different isoforms of this protein inside the body^[22-23]. In the form of neurofibrillary tangles (NFTs) and paired helical filaments (PHF) of the amyloid- β peptides during AD, tau protein has been known to be their most important and integral component. Till date, six different isoforms of this protein have known to exist in a hyper phosphorylated form, particularly in paired hexagonal filaments. *In vitro* studies with the hyper phosphorylated form of this protein have established the fact that in this state it can no longer interact properly with its cellular receptors microtubules. This leads to cellular dysfunction and ultimately to neuronal death^[24].

Third vital indicator for the prevalence of AD is presence of apolipoprotein E (ApoE). This is a glycoprotein composed of 299 amino acid residues with a relative molecular mass of 34,200 daltons^[25]. Three different allelic forms of single gene encoding for this protein exist, designated as E2, E3 and E4 respectively^[26]. Interestingly enough, the risk for carrying AD seems to be allele dependent. A study has revealed that individuals carrying two E4 alleles are at a higher risk and have an earlier onset of AD as compared to those who have either one or no E4 allele^[27]. There exist some very close fluctuations with the type of allele prevalent in the individuals and the possibility of being tested positive for AD. For instance, if apolipoprotein allele contributing for this protein is smaller unit E2, the effect is however much protective and against the occurrence of AD^[28-29]. Several related studies have proved that the prevalent genotype of ApoE is one of the most reliable genetic markers for screening the susceptibility towards AD^[27].

The complex and all time wavering *in vivo* biochemical changes mentioned above make the concrete diagnosis and containment of AD a reasonably tough task. The treatment strategies adopted are often rendered ineffectively due to various reasons. Most vital amongst these is comparatively the early onset of symptoms. Coupled with this is the use of conventional

screening procedures which can't screen biochemical imbalances prevailing within the blood brain barrier effectively. Nanotechnology can immensely improve this by improving diagnosis significantly through its excellent signal transduction and bio-sensitizing approaches. In the elderly persons who are the most likely suspects under the normal circumstances, there are many predictive measures to cure the disease and some strong but equally strange precautions to prevent it. For instance, the disease brings about the loss of memory and thinking regions of the brain located within hippocampus, therefore sufferers are normally encouraged to do intellectual activities such as solving Sudoku puzzles. In addition, the whole cycle of biochemical interactions in a localized specific manner is highly diversified and sensitive. Of significant note is the action of copper on the A β protein in AD patients through the intervening activities of APP. The gist of biochemical imbalance of the disease is malfunctioning of the mitochondria. Mitochondrial mechanisms are greatly affected in the sufferers of AD, whereby the electron transport chain intermediates are affected and the redox imbalance inside the cell gets perturbed. This leads to the generation of reactive oxygen species (ROS) which further degrades the tissues of CSF region of the brain and also other associated proteins by reactive oxidative free radical intermediates [30]. Another very vital aspect of the aggravation of appearance of AD symptoms is the presence of metal ions like those of Zn⁺², Fe⁺² and Cu⁺² in the brain for several different physiological roles resembling to the action of coenzymes in various neuro-signaling events. When these metallic ions reach an unfavorable concentration within the brain, they begin to show their reactive tendencies in an adverse manner. Homeostatic mechanisms regulating their normal concentration inside the brain are very delicate and if they increase their threshold cellular presence can give rise to the free radical activity. In AD, this effect has a special relevance because the amyloid- β peptide has some specified local sites which are prone to be bound by these ionic metal intermediates. Biochemical analysis of this amyloid- β peptide confirms that even a trace amount of zinc or copper ions (which may be released from synaptic terminals of neurons) is highly dangerous leading to the precipitation and accumulation of amyloid- β peptides locally through the interactions with the histidine amino acid present in the amyloid peptide [31]. Even though it is something beyond our will, but this precipitation can be reversible in case the metallic ions involved undergo or are subjected to chelation before they are fibrilized. Significant evidences enlighten the fact that on interaction with A β , the metallic ions may give rise to extreme concentrations of reactive oxygen species (ROS). In some cases there is severe damage to intracellular locations due to the generation of hydrogen peroxide (H₂O₂) which is highly reactive. To compound the risk, presence of zinc and copper

ions facilitate the interaction of A β with cell membranes, whereby cellular toxicity is further amplified [32-33]. Chelation of these metallic ions brings about a significant decrease in the precipitation of amyloid proteins and efforts in nanotechnology have enabled to amplify the efficiency of this chelation by stimulating the activity of chelators [31, 34].

Even after several rigorous attempts to investigate the concrete reason for the neuronal degradation, the exact mechanism underlying it remains poorly understood. The most probable aspects range from the degradation of NMDA receptors [35-36], disturbance in the Ca⁺² ion mediated channels of mitochondrial endoplasmic reticulum transport [37], abnormalities in APP metabolism, immature amyloid- β peptide being synthesized from APP [38-39] to several other cytotoxic malfunctions [40-41]. These have been depicted more clearly in Fig.2.

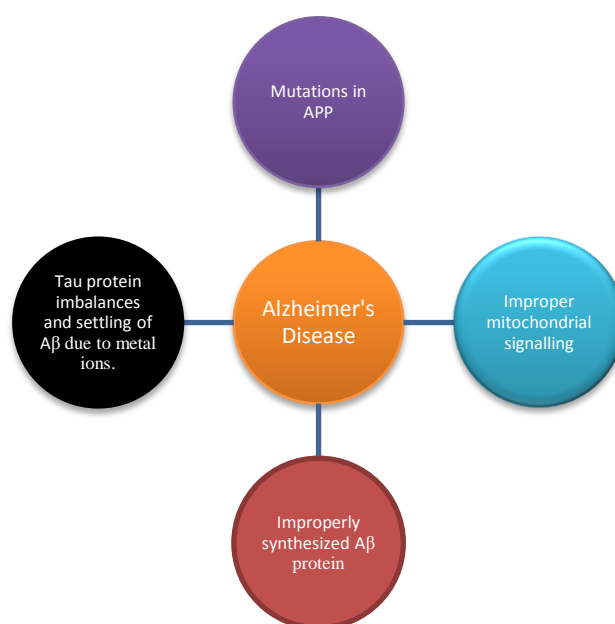


Fig. 2 The figure highlights the most probable metabolic inadequacies leading to the onset of Alzheimer's Disease (AD).

The following text describes the nanotechnology mediated diagnosis improvement of AD. The diagnosis of any disorder can be done in two broad approaches *In vitro* and *in vivo*. Nanotechnology enables improved diagnosis in both mechanisms through the use of nanoparticles which possess very good properties that enable their incorporation as sensors. Most important feature which nanotechnology can bring about in the diagnosis of AD is its detection well before the appearance of dreadful symptoms.

Nanotechnology enabled *In vitro* Approaches for Alzheimer's Disease Diagnosis

These approaches are based on the novel attributes of nanoparticles which make them different from the bulk matter and also enable them to improve the design, efficiency and accuracy of hybrid devices.

Nanoparticle Surface Plasmon Resonance Features

This approach enable us to use metal based nanoparticles for enhancing the efficacy of nanosensors employed to screen the presence of AD. This is basically a phenomenon by virtue of which any change in the surroundings of metal nanoparticles leads to a change in the refractive index of their surrounding magnetic field. As a result, the maximum wavelength at which silver nanoparticles can screen a particular associated biomolecule is increased and results far better screening, even ultra-low concentrations of associated precursors can be estimated. This approach is ultrasensitive and inexpensive in nature. The changes in detection of wavelength are important because the severity of disease is directly proportional to the concentration of a particular biomarker in the solution which further changes the refractive index of bio medium having corresponding affecting bio ingredients. The detection of A β is improved through LSPR via (i) Detection of oligomerization of A β in ultra-low concentrations (ii) Improved detection of pharmaceutical interventions with the desired target moieties and (iii) Overall efficacy of diagnosis getting improved ^[42].

Scanning Tunneling Microscopic Identification

This intervention allows us to take sharper images of the body tissues and intracellular locations to screen for amyloid- β peptide deposition. STM can be used in several variant forms and exploits different physical properties of the nanomaterials used to enhance its sensitivity. For instance, the use of quantum dots coupled with specific antibodies can screen the proteins even in the least quantities located within the body. The use of metal nanoparticles like those of silver and gold further add rich attributes to informative imaging of intracellular locations in which A β protein gets deposited. This technique estimates the problem by measuring the physical texture of body tissues. This is done through a scanner which scans over the tissue surface and reports the responses on a computer screen through the signal transformation technology. Wherever the tissue density is deviated from the normal texture an abrupt or interfering signal is displayed on the monitor. Using this procedure, studies have reported an ultra-sensitive detection of A β protein to the limit of 10 fg/ml ^[43].

Screening of Disease specific Proteins through Bionanoprobes

A number of biomarker proteins corresponding to the deficiency or disease caused is screened for the prevalence of the disease. Conventional techniques are often unable to screen quantities of the secreted proteins limited within microscale or perhaps even lesser. The biological screening performed using nanoparticles and other nanomaterials can improve the screening of associated proteins. This can enable diagnosis at much earlier time which will be a big boost for subsequent and timely treatment of AD. Nanoparticles can be used to improve the currently prevailing spectroscopic procedures. In one such study, silver nanoparticles have been used to identify the malfunctioned tau protein and estimate the concentration as low as 1pg/ml within 35 minutes. Similarly in another study nanoparticle conjugated DNA probe has been used to screen amyloid- β peptide derived diffusible ligand in CSF of the AD sufferers ^[44-45].

Nanotechnological Approaches for In vivo Diagnosis of Alzheimer's Disease

In some situations, taking out the affected tissue fluid does not give a reproducible estimate of the nature and extent of the damage persisting and thus results in the failed attempt of diagnosis. In such cases, it is urgent to screen the patient with *in vivo* treatments which enable us to take the pictures of the body tissues and they sometimes involve brain mapping tests. In AD, these are of special mention, as the disease encounters poor prognostics. Some vital procedures of this kind are as mentioned ahead.

Magnetic Resonance Imaging (MRI)

This involves scanning the brain to have an idea of the physiological environments prevailing inside it. Conventional magnetic resonance imaging has limited scope and extent for mapping the brain's activity. This can be significantly improved in terms of its scope by the use of nanotechnology. Of late, some studies have reported the use of iron oxide nanoparticles as couplers to improve the magnetic impulse and responses emanating from the brain^[46-47]. In particular while studying mouse models, two groups of study have reported the use of monocrystalline iron oxide nanoparticles (MION) and ultra small super paramagnetic iron oxide (USPIO) nanoparticles for the detection of amyloid sheath deposition in the brains of transgenic rodents^[48-49]. The study groups have also reported that this technique is least invasive if the MRI contrast improving agents such as nanoparticles are administered intravenously rather than intra-arterially^[49]. This technique however, has little significance in diagnosis of AD. That is so because in this nanoparticles are coupled with the amyloid- β peptide that is targeted to the amyloid plaques. This clearly explains that it can predict the

prevalence of disease only if amyloid plaques persist, which implies that the disease has already manifested in the concerned individual.

Optical Imaging Methods

These methods represent very versatile and interesting tools for catching hold on the progression of AD at an earlier stage. These include the fluorescence exhibiting special kind of dye molecules which have a near infra red wavelength. Because of such long wavelength, the light emitted from these molecules can travel through larger distances and easily penetrate the biological tissues. Critical requirements of these kinds of optical probe molecules is to cross the blood brain barrier which is crowded by tough physiological environments and to specifically bind a marker protein characterizing the AD, such as that of A β . Alongwith this, these probes should have suitable and requisite absorption-emission spectrum (within the range of 600-800 nm) and proper kind of sensing properties. It is due to these sensing properties that are collectively also termed as rigidification that these fluorescent molecules on binding with specific biomarkers undergo a change in their confirmation and show altered illumination properties. The overall analytical difference is observed in the final analysis in which there is a sharp contrast between the molecules binding the specific biomarkers and others which do not do so. This specific contrast exhibition is termed as rigidification^[50]. One such specific fluorescent biomarker for AD is the molecule NIAD-4 with the chemical formula [5'-(4-Hydroxyphenyl)-2,2'-bithiophen]-5-yl]methylene]-propanedinitrile. This molecule is a specific biomarker for screening of AD as it binds the A β protein and enables its detection *in vivo*.

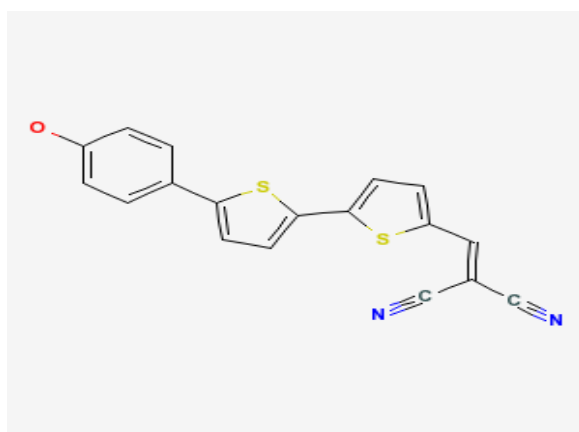


Fig. Showing structure of NIAD-4^[51]

The specific structure and low molecular weight of 334 daltons makes this compound makes it able to overcome the blood brain physiological boundaries. In addition, this compound also has a strong and close structural similarity with Thioflavin T, which is a very popular amyloid fibril detection agent. This feature facilitates the rapid and accurate binding of this compound with accumulated amyloid- β peptide and screens its presence in a precise manner.

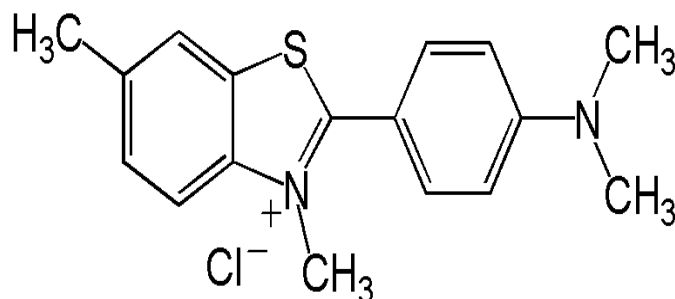


Fig.2.The structure of Thioflavin T^[52]

To improve this imaging of diseased and locally affected regions of the body, a number of studies have also been carried out using quantum dots, chiefly because of their remarkable electronic properties and the engineering of their performance properties as a function of their size. Semiconductor quantum dots in a hybrid composition such as those of CdSe possess a very good fluorescence character which can further improve this imaging. The application of quantum dots has provided some special advantages to improve the overall presence of A β aggregates in several ways. For instance, the versatile structure of quantum dots enables them to screen multiple areas of suspected locations corresponding to the disease by antibody tagging. Alongwith this, these structures exhibit extraordinary luminescence which makes them able to reach a number of *in vivo* locations in the body, a feature not even assumed to be accomplished through conventionally used fluorescent molecules. These findings have been obtained while screening of AD in the rodent models.

However, critical feature concerned with the use of quantum dots is sharply concerned with their *in vivo* toxicity and several interactions which they may initiate once they are administered into the body. In particular with the AD, a strong criterion affecting the incorporation of quantum dots for diagnosis relates with their entry into the blood brain barrier and subsequent release from the body once they perform the intended role. This problem has been resolved in some cases by incorporation of quantum dots with bio-friendly polymers or by the use of proteins opsonins which makes the escape of such structures from

body's immune system feasibly. Few studies have reported the safe evaluation of disease presence by the use of polymers such as poly ethylene glycol (PEG) [53-57].

Novel Nanotechnology Treatment Strategies to Confront Alzheimer's disease

For any disease, treatment strategies can be broadly of two types, **A. Corrective** and **B. Protective**. While preventive strategies aim at complete elimination of a disease from the individual's body, the corrective strategies on the other hand, aim at the reduction and effective management and tackling of the associated symptoms. To a great extent, the treatment in AD till date has been mostly rehabilitating, enabling the reduction of its symptoms [58-59]. Although active research is strongly aimed for making the treatment more and more effective as well as least invasive but late onset of disease and complex intricately linked factors associated with its clinical prevalence are strong challenges to find a reliable treatment. In this reference, nanotechnology provides a big boost for the treatment of AD as it highlights two different modes of disease correction, i.e. neuroprotective and neurodegenerative mechanisms. Of late, intensive efforts have contributed a strong neuroprotective treatment of AD. These approaches focus on the inhibition of specific molecular mechanisms involved in the onset of AD, so that the disease is taken care of even before its onset [58, 60]. However, the curative strategies are summed up all together under the neuroregenerative mechanisms. These approaches are adopted for the correction of tissue defects once the disease has inflicted its damage and form an important part of the study in the futuristic challenges. The most crucial area where nanotechnology has made a mark in the treatment of AD has been the targeted drug delivery so that the therapeutic preparation of the corresponding drug has a long lasting effect and more potent specificities [58]. Table 2 describes the salient features of different nanovehicles employed to improve the treatment of AD.

Table1: Depicts the several nanocarriers employed for drug delivery in Alzheimer's disease

Sr. No.	Formulation Employed	Target Receptors	Challenging Aspect	Ref.
1.	Nanoemulsions	Tau protein	Proper pH activation, polydispersity index, viscosity, zeta potential and lipidic emulsion formulation	[61]
2.	Biodegradable polymeric nanoparticles	Neurotransmitter Acetylcholine	Particle size, zeta potential, release kinetics.	[62-64]
3.	Dendrimers	A β peptide	Arrangement of 27 distinct terminal morpholine groups	[65]

4.	Carbon Nanotubes	Neurotransmitter Acetylcholine.	Maintaining their biocompatibility.	[66]
5.	Liposomes	A β peptide	Forming the nanoscale liposomal formulation of curcumin to improve its carrying content	[67]
6.	Nanosuspensions			[68]
7.	Nanofibers and other nanocarriers			[69-70]

Two major kinds of toxic intermediary events leading to AD are the presence of the beta-amyloid oligomers and free radicals. The delivery of medicinal preparations in the form of nanocarriers, nanovesicles, liposomes, polymeric nanoparticles, nanofibers and nanoemulsions has been of immense help as suggested further.

Nanocarriers mediated Hormonal Delivery

Recent studies have comprehensively shown an important role of sex hormones estrogens and androgens in the pathogenesis of AD and can very well arrest the accumulation of A β as well as its cytotoxicity and neurotoxicity. Even after the establishment of curative, therapeutic impacts and potentials of these hormones in the AD, one of the most interest sustaining issues has been their targeted delivery to the specifically affected areas of the organ. This is so as their delivery to elsewhere locations inside the body can interfere with biochemical pathways and give rise to serious side effects. Here, nanotechnology provides us very reliable solutions in a sense that the nanocarriers enable these hormones to be delivered only at the affected sites. *In vitro* trials of such delivery routes have established that chitosan and acidic copolymers of lactic and glycolic acid (PLGA) can enable the effective delivery of estrogen in the form of estradiol to the brain^[62]. To confirm the efficacy, Wang *et al*, in their comprehensive effort have shown that chitosan nanoparticles when loaded with estradiol and delivered through intranasal route increases the CSF concentration of encapsulated drug and this effect is highly localized without any side effects on other plasma tissues^[63]. Two other significant studies have also highlighted a significant increase in the bioavailabilities of estradiol and mifepristone through their encapsulation in the form of PLGA nanoparticles, when administered orally ^[71-72]. Mifepristone is an important hormone that arrests the cognitive impairment which leads to AD if left unattended^[73]. Most important factors for these models are the mechanisms which ensure that these will act only at the targeted sites and that too is accomplished using specific protein binding. The procedures are no doubt costly, but equally effective, less sensitizing and least time consuming in nature.

Nanocarriers mediated Antioxidant Delivery

It has been extensively claimed that binding of amyloid peptide by the iron and copper ions at specific locations gives rise to cytotoxic stress by stimulating the production of ROS via free radical generation. To mitigate this localized stress, antioxidants are very essential. A number of antioxidants including those of phenolic background have been used in this scenario. For instance, polyphenolic antioxidant in green tea, epigallocatechin-3-gallate (EGCG) has been analyzed for its anti-oxidant effects. Studies with its clinical trials have shown that along with anti-oxidative effect, this also helps to reduce the A β production. In a number of studies, it has been reported that EGCG can suppress the functioning of amylose precursor protein (APP) *via* inhibition of the proteolytic action of enzyme α -secretase. Studies report that through generation of this proteolytic enzyme, EGCG deviates the APP activity, thereby inhibiting the generation of A β . The crucial factor affecting the use of EGCG here is its inherent low bioavailability in the living tissues if administered orally. That's why it is been delivered through nanocarriers so as to facilitate its better, specific and more potent cellular uptake. It has been comprehensively shown by *Smith et al* that EGCG when delivered with nanolipidic carriers with the approximate diameter of EGCG: lipid complexes being in the range of (30-80) nm, significant increase in the bioavailability of EGCG was shown and its far better uptake by the brain was also observed, chiefly due to its smaller size^[74-75]. Similarly, curcumin, the yellow component of turmeric is another very potent natural phenolic antioxidant. Unfortunately, the studies with curcumin in rodent models has explained good anti-inflammatory and anticancer effects due to its extraordinary antioxidative nature but again it suffers from low bioavailability when taken orally^[76]. It remains poorly absorbed by body tissues and most of it is degraded in the liver and small intestine. To improve its biological absorption and specific targeting in blood brain barriers, it has been formulated with nanocapsules of poly n-butylcyanoacrylate (PnBCA) and coated with protein ligand apolipoprotein E3 so as to facilitate the traversing of overall formulation across the blood brain barrier^[77].

Nanocarrier mediated Ion Chelators

Metallic ions of iron and copper are required by the brain for some very specific and important physiological roles. As these can bind with the amyloid peptide due to local specific affinities, so these need to be cleared from the body tissues. Metal ion chelator systems have been used for the clearance of these metal ions from the brain components. Chelators are actually the molecules which reduce the reactive potential of metallic cations. These can be best exemplified through the solubilizing molecule EDTA. The bottleneck in

this remediation strategy is to administer the chelator systems across the blood brain barriers. After extensive trials, Liu et al have used Desferrioxamine as an ion chelator coupled with the nanoparticles for removal of Fe^{2+} ions from the blood brain barrier. This chelator system is also approved by FDA for clinical applications^[78]. In this way, efficient chelator nanoparticles systems (CNPS) have been reported to mitigate the onset of AD. These systems have been evaluated for their safety in living tissues through the analysis of their conjugations with nanoparticles^[79]. Same study also analyzed suggested another category of nano-chelators and also evaluated their *In vitro* ability to inhibit the cytotoxic effects of amyloid tissue deposition in the cortical neurons of the brain. The nanoparticles used in these chelators were made from polystyrene molecules functionalized with carboxyl group moieties. The main purpose of the intended use of these nano-chelator molecules is the fact that they help to deliver the metal binding chelators at the required sites through the blood brain barrier without any harm to any other sites in the brain. Coupling of these chelators with the nanoparticles significantly reduces their toxicity by decreasing the lipophilicity of the chelators^[80].

Miscellaneous Strategies(Gene Delivery)

In addition to all above nanocarriers, some other nanotechnology based vehicles have been used to improve the delivery of anti-amyloid factors across the blood brain barrier. For instance, the damaged cells or genes can be progressively treated by the intended use of gene delivery through nanoemulsions and nanofibers. Chitosan based nanoparticles have been used to incorporate rectified genes at the affected sites. It amplifies the remedial potency of gene therapy whereby either damaged tissues or cells are removed or are corrected following the treatment with medicinal formulations. Similarly, multifunctional molecules dendrimers have been used for the treatment of AD through the delivery of anti $\text{A}\beta$ protein which can be engineered not to bind with the cell membrane receptors and efficiently act on the specific sites through the electrostatic forces of attraction. The biggest advantage while using dendrimers for this purpose is the fact that these molecules are multi-functional in nature and possess multiple layers to impart greater versatility to the overall molecule^[81-82]. Likewise fullerenes and carbon nanotubes (in single walled configuration) have also been used in different ways to achieve better delivery of anti $\text{A}\beta$ protein regulating factors at the targeted sites within the brain. This is to ensure the improvement of their therapeutic efficiency. Fullerenes are well known antioxidants and free radical scavengers due to their highly excellent structure that enables a number of different kinds of molecules to be tagged

with them^[54,83]. In a study, Dugan *et al* have evaluated the neuroprotective functions of fullerenes by studying their effect on the NDMA receptors responsible for regulating mechanisms of memory and learning^[84]. In a similar manner, single walled carbon nanotubes have been used as nanocarrier molecules for the delivery of acetylcholine neurosignaling precursors. Through nanotubes, their specific binding to the damaged locations from AD is facilitated easily^[66].

Even though all these proposed nanocarriers present exciting outcomes for their bioavailability and pharmacodynamic performances, evaluation of their safety for their intended use in living beings and possible damage to cells *in vivo* needs to be studied in a detailed manner so that the harmful effects are minimized and the technology can be brought to the fore front at a commercial scale.

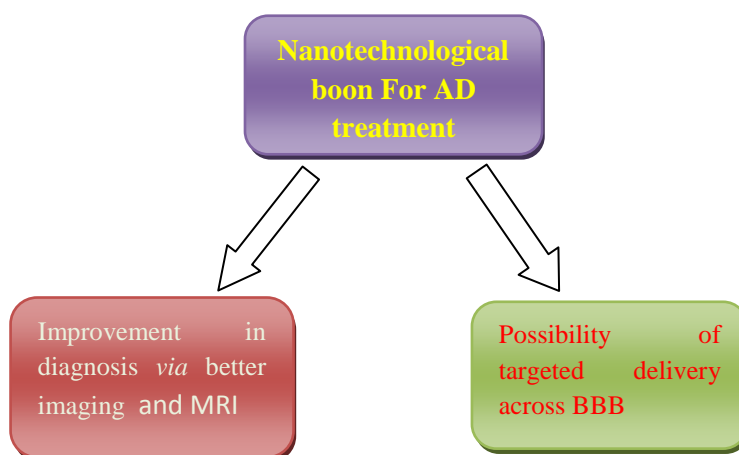


Fig.3.A summary of the nanoscale advantages towards Alzheimer's treatment

CONCLUSION AND FUTURE PROSPECTS

The pathophysiology of AD is very complex and dynamic in nature. Moreover, its site of occurrence and spread are most crucial factors affecting its timely diagnosis and treatment. Even though nanotechnological interventions exhibit significant potential and studies on animal models have reported success, much remains to be done as far as optimization and standardization of these routes is concerned. For instance, the ethical considerations of the fact that this technology is new, there are no core experts available and the toxic effects of nanoparticles and heterogeneous nanoscale assemblies like those of quantum dots are vital challenges that need to be properly addressed. Since the disease shows no vital treatment strategy through the conventional methods and the approval of FDA already prevailing with nanoscale trials for making nanomedicine more and more effective channel to invade the

physiologically tough locations in the body, the future of this technology argues very well for the treatment of AD.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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