A Review on Nanotechnology in Diagnosis and Treatment of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease is a progressive mental deterioration that can occur in middle or old age, due to generalized degeneration of the brain. It is the commonest cause of dementia. The currently available therapeutics for Alzheimer's disease only act to lower its symptoms. Therefore, the nanotechnology is advancing molecular detection, drug discovery, delivery and monitoring for a number of ever challenging human diseases, including cancer and neurodegenerative disorders. In this paper, we present the role of nanotechnology in the development and improvement of techniques for early diagnosis and effective treatment of Alzheimer's disease (AD). The nano diagnostic methods reported and compared in this paper include both of *in vitro* and *in vivo* approaches. The nano treatment methods for AD are numerous. They are categorized in this report under neuroprotective methods and nanocarriers for targeted drug delivery. Considering that the AD is a multi-factorial disease with several pathogenetic mechanisms and pathways, a multifunctional nanotechnology approach will be needed to target its main molecular culprits.

Keywords: Alzheimer's disease, Nanotechnology, *In vitro* and *In vivo* approaches.

INTRODUCTION

Alzheimer's disease is a neurodegenerative disease and most common form of dementia, a general term for memory loss and other intellectual abilities serious enough to interfere with daily life. Alzheimer disease is a major public health concern in the elderly. In the United States, it is the fourth leading cause of death and is the most common cause of dementia**.** Alzheimer's disease is quickly becoming one of the major universal healthcare problems[1]. Today, however, there are neither precise diagnostic approaches nor effective therapeutic agents available for Alzheimer's disease. The currently available therapeutics for Alzheimer's disease only act to lower its symptoms[2]. Therefore, whether the disease is diagnosed early enough or not, the conventional medical approaches are incapable of complete cessation or reversal of the disease progress. Now in the 21st century we have the technology to create a completely new form of drug, a drug using nanotechnology. The nanotechnology has been opening new chapters in many aspects of our lives, specially diagnosis and treatment of human diseases. Through nanotechnology, the controllable production of desired structures and least dimension in nanoscale (1-100 nm) is presently achievable. The nano scale particles have a greater surface area to weight ratio, due to this nanotechnological particles have a higher reactivity. The beauty of this technology is that there are so many different forms of nanoparticles. Nanotechnology is advancing molecular detection, drug discovery, delivery and monitoring for a number of ever challenging human diseases, including cancer and neurodegenerative disorders[3].

Causes of Alzheimer's

There is no sole cause of Alzheimer's disease but it is believed that the condition is linked to a person's health and lifestyle as well as age and genetics. One of the key factors is age, and nearly all the data suggests that the older you get the more likely you are to get Alzheimer's disease and hence dementia[4], seen in *Figure 1*. The probability of Alzheimer's in a person 65 years of age is about 1 in 1. This rises to 1 in 7 by the age of 80.
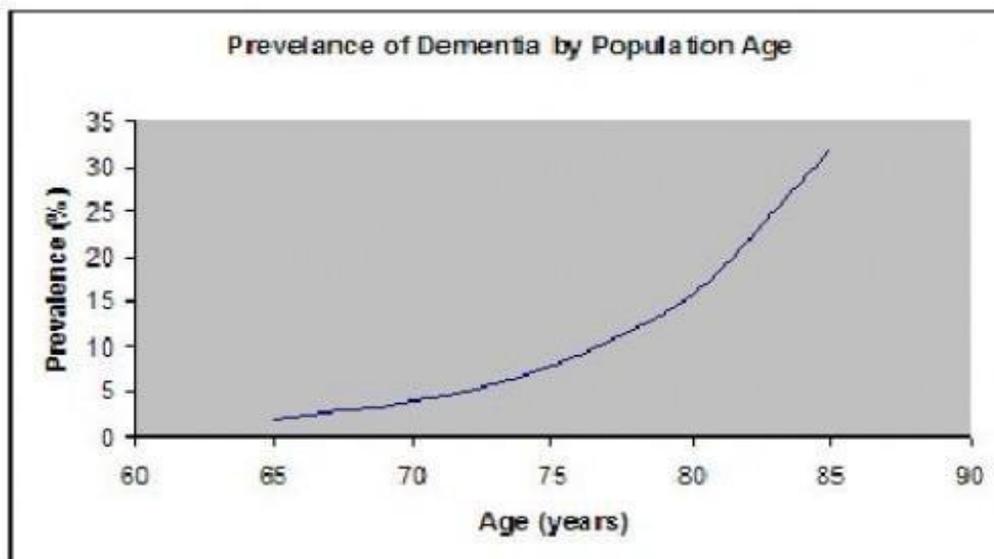
Figure 1: Doubling of Dementia Rates With Increasing Age

Figure 1: A graph comparing the prevalence of Dementia to age

The generics of Alzheimer's disease are somewhat subtle. Quite often conditions are inherited and passed down through the generations, this is not the case for Alzheimer's. Research has shown that if someone has a member of their immediate family with the disease then it only slightly increases the chance of developing the disease. The other contributing influence on this indisposition is a person's health and lifestyle. If an individual has a history of severe head injuries, scientists have shown that this increases their probability of developing the condition. Smoking and high serum cholesterol has also been shown to increase the probability of having the disease in later life.

Symptoms of Alzheimer's

Patients who suffer from Alzheimer's tend to suffer from dementia, mood swings and may lose the ability to speak. Alzheimer's disease is a progressive disease meaning it is slow and systematically gets worse. If the brain of an Alzheimer's sufferer was to be looked at, atrophie changes develop over time within the cerebral cortex and hippocampus. As the condition progresses more and more of the brain atrophies. In *Figure 2* it can be seen that the cerebral cortex and the hippocampus are diminishing in size as the disease progresses[5]. The hippocampus is the part of the brain used for memory and the cerebral cortex for language and problem solving and hence the consequences that are seen in dementia.
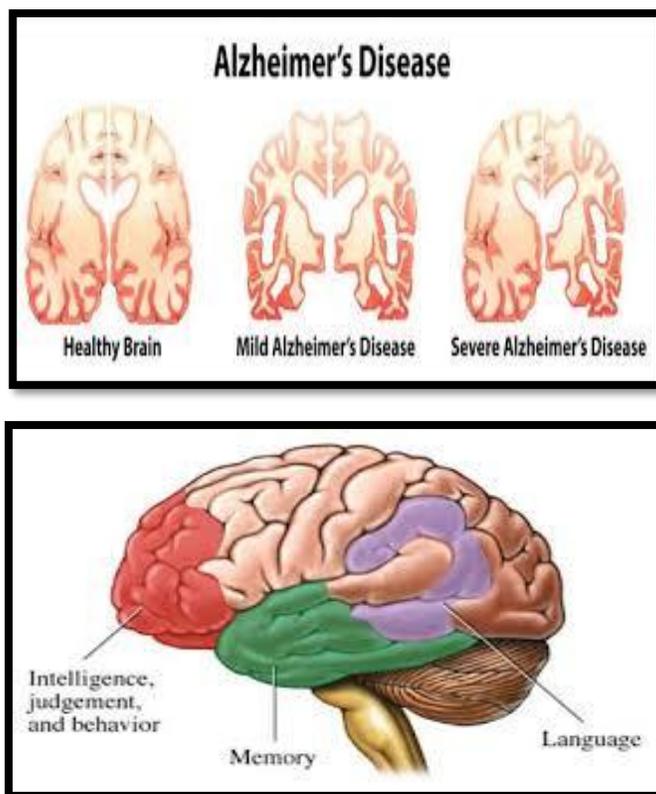
Figure-2: Stages of Alzheimer's in the brain

DIAGNOSIS OF ALZHEIMER'S DISEASE

The only method of definitively diagnosing AD is a brain autopsy. However, mental and behavioral tests and physical examinations allow physicians to make an accurate diagnosis of AD in 90 percent of cases[6]. The first step in finding a diagnosis is obtaining the patient history. During this time, the physician will determine what symptoms are present, when they began and how they have progressed over time. The family history of illness is also pertinent. The physician will perform a physical examination, including blood tests and urinalysis. This is done to rule out other potential causes of dementia, such as hormone imbalance, vitamin deficiency, and urinary tract infections. Brain scans may also be performed to exclude tumors, cerebrovascular accidents, traumatic brain injury, and infections. These scans are also helpful in identifying the characteristic tangles and plaques seen in AD. Structural imaging scans, including magnetic resonance imaging (MRI) and computed tomography (CT), provide information about the shape and volume of the brain. Functional imaging allows the physician to determine how effectively the brain cells are working. A functional MRI or positron emission tomography (PET) scan can be used[7].

Diagnostic Techniques:

Neuro imaging is a promising area of research for detecting AD. The imaging procedures that can be used to identify abnormalities in the brain include;

❖   Positron emission tomography (PET)

❖   Computed Tomography (CT) scan.

❖   Magnetic Resonance Image (MRI)

Positron emission tomography (PET) uses radiation signals to create a three-dimensional color image of the human body. The patient is injected with a radiotracer, composed of a radioactive medicine bound to a naturally occurring chemical. For the study of AD, the chemical is usually glucose. A PET scan has the capacity to detect changes in metabolism, blood flow, and cellular communication processes in the brain[8].

Computed Tomography (CT) scan takes a series of cross-sectional images of the body[9]. With the help of a computer, the individual scans are integrated into one detailed image. The CT scan provides the physician with information about the density of tissues in the body.

Magnetic resonance imaging (MRI) technique is first used in 1977, which create two or three-dimensional images of the body that can be used to diagnose injury and illness. The essential component of the MRI system is the superconducting magnet, which produces a large and stable magnetic field[10]. These magnets allow for different parts of the body to be scanned. MRI can effectively detect the structural changes and cellular death seen in the brain of an AD patient.
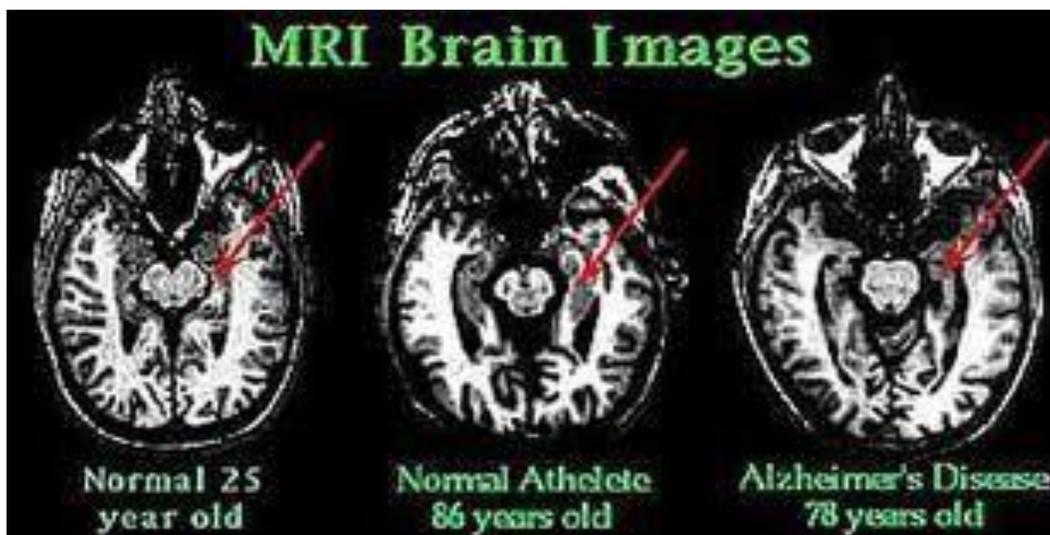

Figure 3: MRI scan of people with and without Alzheimer's disease

Nanotechnology-Based Diagnosis of Alzheimer's disease:

En route to very early diagnosis of a complex disease like AD we need to have an affordable, ultrasensitive and selective molecular detection method. The recently growing application of nanotechnology in molecular detection of biomarkers is promising for very early diagnosis of

Alzheimer's disease. From a practical point of view, one may perform a molecular detection process either inside the body (*in vivo*) or on the samples derived from the body (*in vitro*). Nanotechnology may help us to achieve early diagnosis of AD by providing us with a highly potent signal transduction approach. Signal transduction refers to the process through which a biological signal (a biomarker) transforms to a recordable signal, and is amplified enough to be recorded. This potential application of nanotechnology in molecular diagnosis is mainly based on the special physical (optical, electrical or magnetic), chemical and biological characteristics of certain multifunctional nanoparticles[11].

Table-1: Several Nanotechnology based biomarkers

Test Mode	Technology	Biomarker	Signal Detection	Signal transduction		Signal Documentation
				Signal transform	**Signal Amplification**	
In-vitro	Bio-barcode assay	ADDL	Sandwich assay (Monoclonal Anti ADDL Ab)	DNA barcode	Functionalized AuNP carrier for numerous DNA barcodes	Scanometric Recording
	Localized surface Plasmon resonance	ADDL	Sandwich assay (Monoclonal & Policlonal Anti-ADDL	Wavelength shift	Secondary Ab of Sandwich assay	Spectroscopy
	Scanning Tunneling Microscopy	Aβ(1-42)	Sandwich assay (Monoclonal Anti Aβ Ab)	Tunneling Current Change	Silver Staining of AuNPs	Frequency of pulse-like peaks
	Two Photon Rayleigh Scattering Assay	Tau protein	Monoclonal Anti-Tau Ab	TPRS intensity change	TPRS	TPRS Spectroscopy
In - vivo	μMRI	Aβ plaques	Aβ peptide	Magnetic heterogeneity	N/A	MRI scanner
	Optical (Fluorescent Imaging)	Aβ plaques	NIAD-4	Fluorescent excitation	Upon Binding Molecular Rigidification	multiphoton microscopy

In Vitro Nanodiagnostic Approaches:

A. DNA-Nanoparticle Conjugates (Bio-Barcode Assay)

DNA-Nanoparticle conjugates used for detection of protein biomarkers by an technique known as bio-barcode assay. Detection of amyloid β-derived diffusible ligand (ADDL) in cerebrospinal fluid (CSF) samples of AD patients through bio-barcode assay[12].

B. Nanoparticle Surface Plasmon Resonance

Recently a method for the detection of molecular biomarkers was examined for AD biomarkers which is said to be ultra-sensitive and inexpensive. It is called the localized surface plasmon resonance (LSPR) nano sensor and it is based on singular optical properties of triangular silver nanoparticles (AgNPs)[13].

C. Scanning Tunneling Microscopy System

Another recent development is a molecular detection system which was based on electrical detection using a scanning tunneling microscope (STM). This technique included immobilization of specific antibody fragments on gold (Au) substrate and Au nanoparticles (AuNP)[14].

D. Two-Photon-Rayleigh Spectroscopy

Recently, two-photon-Rayleigh scattering signal of AuNPs was examined as a transformed signal of an immune sensor for tau protein, one of AD biomarkers. The conjugates of AuNP with anti-tau antibody were used to detect tau proteins in a sample solution[15].

In vivo Nanodiagnostic Approaches:

A. Micro Magnetic Resonance Imaging (µMRI)

The usage of iron oxide nanoparticles as magnetic resonance imaging (MRI) contrast agents has been widely researched in the recent decades[16].

B. Optical Imaging

Another recently growing approach for *in vivo* detection of molecular biomarkers is optical imaging through special near-infrared (NIR) fluorescent contrast agents[17].

TREATMENT FOR ALZHEIMER'S DISEASE

Drug therapy

There is currently no cure for AD, however there are multiple drugs that have been proven to slow disease progression and treat symptoms. When initiating treatment for AD patients, physicians divide the symptoms into cognitive and behavioral and psychiatric categories. This enables treatment that is specific to the symptoms being experienced. Cognitive symptoms affect memory, language, judgment, and thought processes. Behavioral symptoms alter a patient's actions and emotions.

Table 2: Marketed available drugs for AD

Drug Name	Indication	Action	Dose
Donepezil (Aricept)	Mild to severe AD	Prevents the breakdown of acetylcholine (ACh)	5 mg/day
Galantamine (Razadyne)	Mild to moderate AD	Prevents the breakdown of acetylcholine	4 mg/day
Rivastigmine (Exelon)	Mild to moderate AD	Prevents the breakdown of acetylcholine	1.5 mg/day
Memantine (Namenda)	Moderate to severe AD	Blocks glutamatergic (NMDA) receptors	5 mg/day

Currently available therapeutics for AD, only act to lower its symptoms[18], However, significant amount of research have been focused on finding the neuroprotective agents, the therapeutics that could stop the disease progress by targeting special molecular mechanisms in the AD pathology process and also more futuristic approach, that could rebuild the damaged tissue, called as regenerative agents. These two (neuroprotective and neuroregenerative) approaches together are known as disease-modifying approaches.

Nanotechnology for Neuroprotective Potentials

The therapeutic potential of nanotechnology for AD includes both neuroprotective and neuroregenerative approaches. In addition, nanotechnology has shown promising applications in targeted drug delivery for AD, and several nanocarrier systems have been studied in recent years to increase the bioavailability and efficacy of different AD therapeutic agents[19].

Nanogel:

A nanogel is a nanoparticle composed of a hydrogel – a cross linked hydrophilic polymer network[20]. Nanogels are most often composed of synthetic polymers or biopolymers which are chemically or physically cross linked. Nanogels are usually in the tens to hundreds of nanometers in diameter. Like hydrogels, the pores in nanogels can be filled with small molecules or macromolecules[21].

Fullerene:

Fullerene (C60) and its derivatives could be the base of neuroprotective compounds[22]. The biological applications of fullerene, including its anti-oxidant and free radical scavenger potentials, are due to its kind of chemical structure that allows it to be linked (and to be functionalized) by several active chemical groups in a 3-dimensional orientation. Fullerene has also complete neuroprotective properties against NMDA receptor mediated neurotoxicity. NMDA receptor function is important to neuronal mechanisms of learning and memory.

Nano-ceria:

Cerium oxide (CeO2) nanoparticle (nano-ceria) is reported to have neuroprotective effects on AD in vitro models. In addition to the mentioned anti-oxidant properties, nano-ceria protects neurons from cytotoxic effects via modulating the intracellular signaling pathways involved in cellular death and neuroprotection.

Dendrimers:

Dendrimers, one of the polymeric nanotechnology building blocks, are macromolecular structures with globular shape and a densely packed surface. Their structure has offered them a number of biomedical potentials. Prevention from cytotoxic effects is another prospect of nanotechnology and application of modified dendrimers is a recent suggestion for this approach[23].

Gold Nanoparticles:

Gold nanoparticles (AuNPs) in weak microwave fields dissolve amyloid aggregates. Their design was based on dissolving those amyloid aggregates and prevention from further aggregations by providing local thermal energy at a molecular level. The nanometric size, high surface-to-volume ratio, biocompatibility and mobility of AuNPs make them suitable for providing a specific bond target with a selective supply of energy in a remotely controlled manner, and without any adverse effects on the proximity molecules[24].
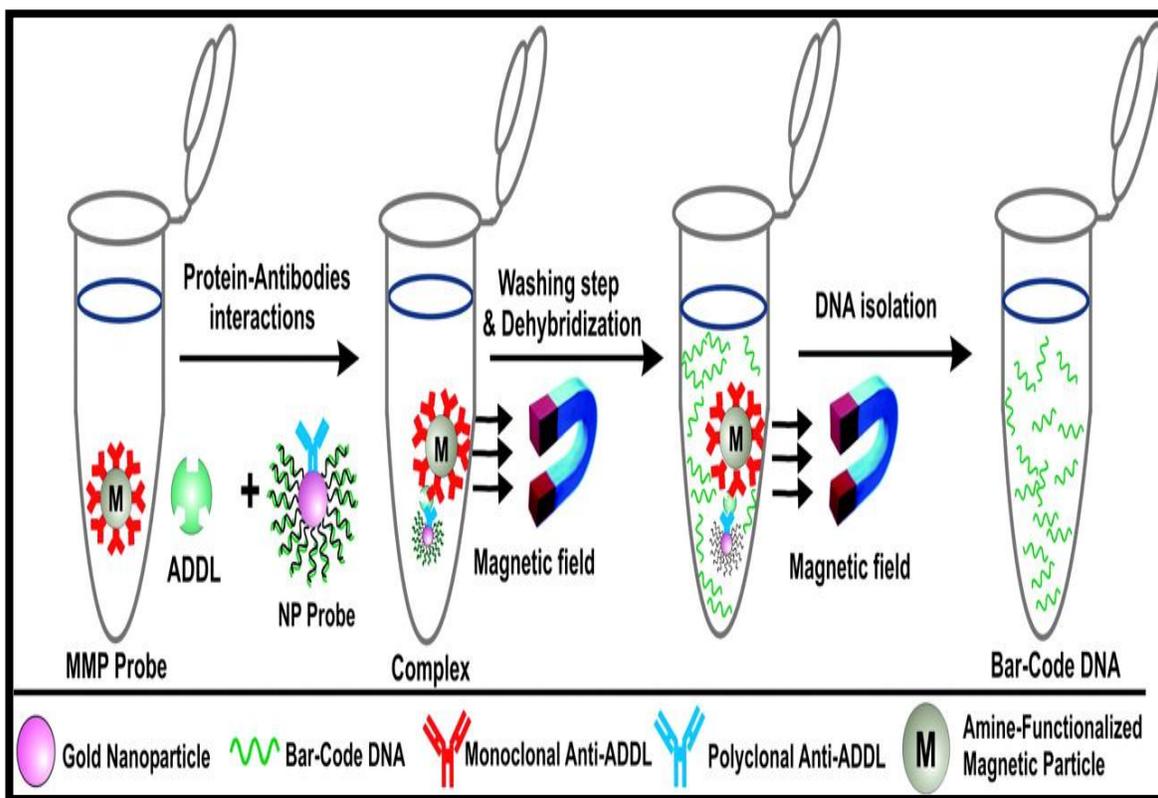

Figure 4: Gold Nanoparticles

Diamondoid Derivatives:

Diamondoids are in the category of most promising molecular building blocks in nanotechnology and specially nanomedicine[25]. Diamondoids and their derivatives are the basis of many varieties of anti-viral and anti-bacterial drugs, already in the market or in various developmental stages. A diamondoid-based drug (meantime), which is already in commercial use, slows down the progression of the Alzheimer's disease.

Table 3: Nanotechnology neuroprotective agents for treatment of AD

Nano system	Neuroprotective Function
Nanogels (Cholesterol bearing pullulan)	Aβ Anti-assembly (Incorporate Aβ monomers)
Fullerene (C_{60}):	Anti-oxidant
Carboxyfullerene,	Aβ Anti-Assembly
$C_{60}HyFn$, Fullerenol	Anti-oxidant / Maintenance of Ca^{2+} homeostasis
Dendrimers: Polyamidoamine (PAMAM))	Aβ Anti-Assembly
Nanoceria (CeO_2)	Anti-oxidant
Gold Nanoparticles (AuNP)	Aβ Anti-Assembly
Diamondoid Derivatives (Memantine) (1-amino-3, 5- dimethyladamantane)	NMDA receptor antagonism

Nano carriers:

The follows nanocarrier systems suggested for delivery of therapeutic agents for AD into the brain.

Curcuminoids Nanocarrier:

Curcumin is the active ingredient of turmeric, the yellow spice which has been recently discovered as a potential treatment for AD. This agent acts through several mechanisms including anti-amyloid assembly, anti-oxidant and anti- inflammatory. However, this agent is unstable due to rapid hydrolyzation or oxidization. Therefore, Poly n-but ylcyanoacrylate (PnBCA) nanocapsules have been used to carry these therapeutic agents through the BBB[26].

Acetylcholine Nanocarrier

Recently introduced a nanocarrier system for delivery of acetylcholine to brain of mice model of AD through low doses of nanotubes[27]

Cholinesterase Inhibitors Nanocarriers

Deficiency in the cholinergic neurotransmission is the principal neurochemical feature of AD, and the current main therapeutic agents against AD are targeting this deficiency. These drugs, known as acetylcholinesterase inhibitors (Rivastigmine and Tacrine, respectively) prepared as nanocapsules increase the transport through BBB[28].

Hormone Nanocarriers

Recent investigations have revealed that sex steroid hormones, especially estrogen and andorgens could have neuroprotective effects against several AD pathogenic mechanisms, including Aβ accumulation, cytotoxicity and neurotoxicity. Chitosan and poly (lactide-co-glycolide acid) (PLGA) nanoparticles are two nano carriers recently suggested and examined for delivery of sex steroid hormones (e.g. Estradiol) to the brain[29].

Gene Nanocarriers

The main part of gene therapy is gene delivery, through which the genetic material will be presented inside a cell. In order to protect the genetic material from biological obstacles, like cell membrane charge and enzymatic degradation, a carrier should accompany the genetic material. The usage of nanoparticles as nonviral gene carriers has significantly improved the efficacy of this method by minimizing the enzymatic degradation of genetic materials[30]. For example, polyethyleneimine is a polymeric nanoparticle, capable of forming stable complexes with nucleic acid and minimizing the enzymatic degradation of genetic materials.

Table 4: Applicable Nanocarrier Systems in AD Treatment

Nano carrier	BBB transport mechanism	Loaded Drug(s)
Pn BCA nanocapsule	LDL transport system	Rivastigmine Tacrine Curcumin
Chitosan	Unknown	Estradiol
PLGA	Unknown	Mifepristone Estradiol
Nanolipidic particle	Unknown	Polyphenol (EGCG)
Solid lipid nanoparticle (SLN)	Endocytosis, transcytosis or passive diffusion	Ferulic acid
Ormosil	N/a	DNA

CONCLUSION

The advanced nanotechnologies are beginning to exert a significant impact in neurology. These nanoparticulate approaches having high specificity for brain capillary endothelial cells, are currently being applied to early AD diagnosis and treatment. Nanoparticles could be used to detect biological markers that indicate Alzheimer's even at very low concentrations, which could lead to more conclusive screening to determine whether a patient has Alzheimer's. Drugs normally travel through the blood stream and then diffuse through into the brain. However, there is one thing that often stops drugs getting to the brain, the Blood Brain Barrier (BBB). The design of nanotechnology serves as transport vehicles for a variety of therapeutic agents across the BBB and delivers them to the amyloid ridden tissue. So, these nanotechnologies are expected increase the efficacy of AD drugs while reducing their systemic toxicity.

Nanotechnology provides tremendous potential for future diagnosis and medical treatment. The exclusive properties of nanotechnology give researchers the opportunity to experiment with new ideas to further our treatment of Alzheimer's Disease.

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