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Review Paper

Nanotechnology for Alzheimer's disease detection and treatment

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Abstract: 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). Since AD pathology is almost irreversible and present-day medications for AD only lower its associated symptoms, application of disease-modifying treatments could be successful only if AD early diagnosis is possible. The nanodiagnostic methods reported and compared in this paper include both of *in vitro* and *in vivo* nature. Of the *in vitro* approaches, the DNA-nanoparticle conjugates (bio-barcode assay), nanoparticle surface plasmon resonance, scanning tunneling microscopy, and two-photon Rayleigh spectroscopy are presented here. Of the *in vivo* methods, μMRI and optical imaging techniques are discussed here. The nanotreatment methods for AD are numerous. They are categorized in this report under neuroprotective methods from toxicity of amyloid-β peptide (Aβ) oligomers, oxidative stress of free radicals and nanocarriers for targeted drug delivery. The important agents for neuroprotection include nanogels, fullerene, nano-ceria, dendrimers, gold nanoparticles, and diamondoid derivatives. The major nanocarriers presented here include cholinesterase inhibitors nanocarriers, acetylcholine nanocarrier, metal chelator nanocarriers, (Iron chelators and copper chelators), curcuminoids nanocarrier, anti-oxidant nanocarriers, and gene nanocarriers. 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. These molecular targets must include, but not limited to, Aβ oligomers, reactive oxygen species (ROS), excessive metal ions, tau phosphorylating kinases and cell cycle proteins.

Keywords: Alzheimer's disease; Amyloid; nanocarrier; nanodiagnostic; nanoparticle; nanotechnology; nanotreatment; neuroprotection; tau protein; gene therapy; disease-modifying treatments.

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1. Introduction:

Alois Alzheimer defined "senile dementia" more than a century ago with a remarkable accuracy [1, 2]. It is now known as the Alzheimer's Disease (AD), and is the main cause of the dementia syndrome [3]. The incidence and prevalence of AD increase with age. Since the elderly population is growing worldwide, AD is quickly becoming one of the major universal healthcare problems [4]. Today, however, there are neither precise diagnostic approaches nor effective therapeutic agents available for Alzheimer's disease.

The degeneration of nervous tissue begins many years to even decades before the patient experiences any of Alzheimer's disease symptoms [5-7]. On the other hand, the currently available therapeutics for AD, only act to lower its symptoms [8]. 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.

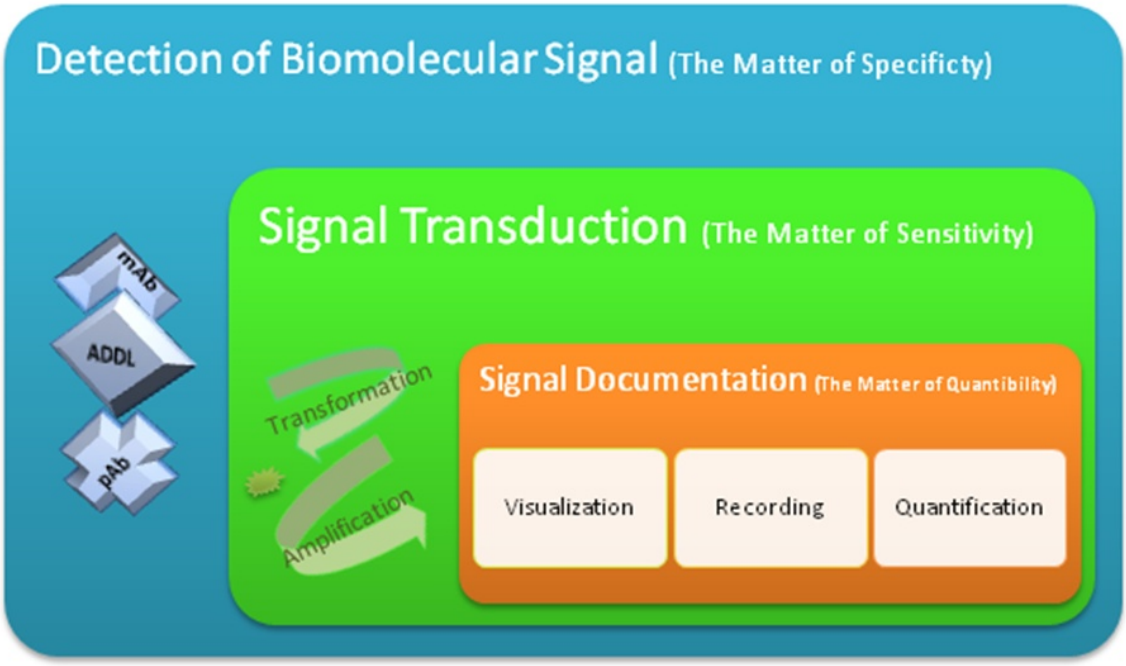
The uncovering potentials of nanotechnology have been opening new chapters in many aspects of our lives, specially diagnosis and treatment of human diseases [9-11]. Through nanotechnology, the controllable production of desired structures and devices with, at least, one dimension in nanoscale (1-100 nm) is presently achievable [12, 13]. Nanotechnology is advancing molecular detection, drug discovery, delivery and monitoring for a number of ever-challenging human diseases, including cancer and neurodegenerative disorders [10, 14-16].

The success of nanotechnology approaches towards diagnosis and treatment of AD presented in this paper, demonstrates the role of interdisciplinary research for the early diagnosis and possible cure for AD.

2. 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, ultra-sensitive 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 (see Figure 1). In what follows we present and compare a number of methods, which have aimed to address this need.

Figure 1- Different stages of a molecular diagnostic method. Stage 1(shown in Blue) is *Signal detection at molecular scale.* This stage determines the technical specificity of the molecular diagnostic tool. Stage 2 (shown in Green) is *Signal transduction*, through which the detected signal will become recordable. For this purpose, the signal must change into a recordable form which could appropriately be amplified. Diagnostic methods that are capable of making weak biological signals recordable are sensitive enough to detect those biomarkers. Stage 3 (shown in Orange) is *Signal documentation*. In this stage, the transformed and amplified signal is recorded, visualized and measured. This stage determines the quantifiability of the diagnostic tool.



2.1. Proposed In Vitro Nanodiagnostic Approaches

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

DNA-Nanoparticle conjugates are capable of the attomolar scale detection of protein biomarkers [17]. Through a technique known as bio-barcode assay, ultra-low concentrations of protein biomarkers may be detected, owing to carrier gold nanoparticles that match the specific antibody of the target biomarker with hundreds of DNA barcodes. Therefore, a single molecule of biomarker may be traced by hundreds of DNA barcodes (a biological signal transformation), which could be additionally amplified by the polymerase chain reaction (PCR) technique [17]. Investigators of this project claimed a highly sensitive detection of amyloid β-derived diffusible ligand (ADDL) in cerebrospinal fluid (CSF) samples of AD patients through bio-barcode assay [17]. The reported results of this study show a significant difference between concentrations of ADDL in AD diagnosed subjects and in age-matched healthy controls. The ADDL concentration medians of these two groups are reported as 1.7 fM and ca. 200 aM, respectively. This would prove a correlation between increased CSF concentrations of ADDL and affliction with AD.

2.1.2. Nanoparticle Surface Plasmon Resonance

Recently a method for the detection of molecular biomarkers was examined for AD biomarkers [18] which is said to be ultra-sensitive and inexpensive. It is called the localized surface plasmon resonance (LSPR) nanosensor and it is based on singular optical properties of triangular silver nanoparticles (AgNPs). In this method, any changes in the nanoparticle external environment lead to a change in the refractive index of the surrounding magnetic field. This change subsequently changes the AgNPs' λ max that could be detectable via spectroscopy. LSPR nanosensor is sensitive to different concentrations of target biomolecule (like ADDL) since the solution concentration directly changes the refractive index. Therefore, different wavelength shifts for different concentrations are detectable. Three applications for LSPR nanosensor are suggested which are: 1) To study oligomerization of the Aβ in ultra-low concentrations, similar to concentrations of *in vivo* conditions, 2) To screen patients for AD, and 3) To study the interactions of pharmaceutics with the target molecules in drug discovery.

2.1.3. Scanning Tunneling Microscopy System

Another recent development is a molecular detection system which was proposed based on electrical detection using a scanning tunneling microscope (STM) [19, 20]. The settings of this technique included immobilization of specific antibody fragments on gold (Au) substrate and Au nanoparticles (AuNP). After addition of the sample solution to the substrate, the antibody nanoparticle conjugates were added. The result was sandwich-type immune binding events that led to changes in tip-to–biosurface interval. These changes in the interval between the sample surface and the scanning tip of the STM affected the tunneling current (signal transformation). The tunneling current profile was then analyzed based on the frequency of the pulse-like peaks, which occurred every time the scanning tip passed over AuNPs. As it was reported, through this technique, an ultra-high sensitive detection of Aβ became possible at concentrations as low as 10 fg/ml [19, 20].

2.1.4. Two-Photon-Rayleigh Spectroscopy

Recently, two-photon-Rayleigh scattering signal of AuNPs was examined as a transformed signal of an immunosensor for tau protein, one of AD biomarkers [21]. In this study, conjugates of AuNP with anti-tau antibody was used to detect tau proteins in a sample solution. The basis of biomarker signal transformation was the ensuing aggregation of AuNP-antibody conjugates after addition of tau protein. It is claimed that through this technique tau protein could be detected at concentrations as low as 1pg/ml within 35 minutes. By using this technique, the investigators claimed to have introduced, for the first time, a fast, ultra-sensitive and specific nanosensor for detecting tau protein.

2.2. Proposed In vivo Nanodiagnostic Approaches

2.2.1. 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 [22, 23]. Two groups of investigators have reported the application of monocrystalline iron oxide nanoparticles (MION) and ultra-small superparamagnetic

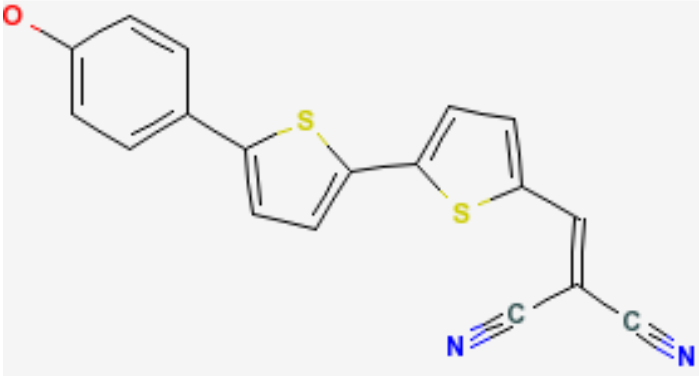
iron oxide (USPIO) nanoparticles as MRI contrast agents for *in vivo* detection of amyloid peptide plaques in the brain of transgenic mouse model of AD [24, 25]. In both of these studies Aβ was conjugated to the nanoparticle in order to detect amyloid plaques [23, 24]. This technique is claimed to be minimally invasive especially if the MRI enhancement agent (nanoparticles) is injected intravenously rather than intra-arterially [25]. However, since the amyloid plaques are targeted in the above-mentioned techniques, the technique could not be useful for very early diagnosis of AD. This is because the amyloid plaque formation appears in later, more advanced, stages of the disease. Therefore, applications of this technique in the detection of AD may be limited to confirmation of established AD pathology in dementia patients, and monitoring therapeutic efficacy for those drugs that are aimed to reduce amyloid plaques.

2.2.2. Optical Imaging

Another recently growing approach for *in vivo* detection of molecular biomarkers is optical imaging through special near-infrared (NIR) fluorescent contrast agents [26]. Due to the long wavelength, the scattered light from these contrast agents could penetrate through biological tissues. The common requirements for a molecular diagnostic probe for AD include the ability to cross the BBB and specifically target an AD related biomarker (e.g. Aβ). In addition to those, fluorescent contrast agents should have an appropriate absorption and emission wavelength interval (600-800 nm) and a strong rigidification [26]. Rigidification refers to a phenomenon through which the fluorescent molecule undergoes a significant conformational restriction upon binding with a molecular target. Such conformational restriction decreases the non-radiative decay rate (and therefore increases the quantum yield) by reducing the rotational and vibrational processes that couple the ground and excited state [27]. The practical result of this phenomenon is the substantial imaging contrast between bounded and unbounded fluorescent markers [26].

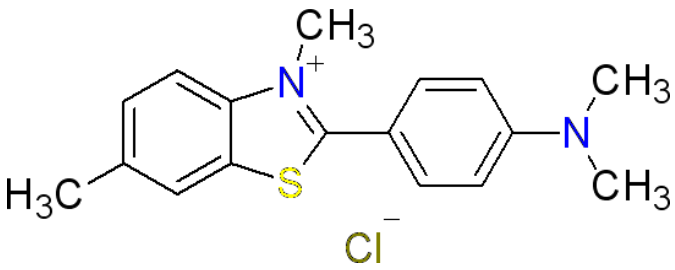
Nesterov *et al*. proposed a NIR Alzheimer's dye known as NIAD-4 with the chemical formula *[[5'-(4-Hydroxyphenyl)[2,2'-bithiophen]-5-yl]methylene]-propanedinitrile*, for *in vivo* molecular detection of Aβ.

Figure 2 - NIAD-4 [[5'-(4-Hydroxyphenyl)[2,2'-bithiophen]-5-yl]methylene]-propanedinitrile.



This molecule meets the above-mentioned requirements for a fluorescent molecular probe. Its specific structure and its rather low molecular weight (334 Da) make its rapid traverse through the BBB possible [26]. The structure of NIAD-4 core, somehow resembling that of Thioflavin T (see Figure 3), which is a well-known amyloid fibril detection agent, makes a highly specific binding with Aβ aggregates feasible.

Figure 3 - Thioflavin T



The NIAD-4 structural characteristics also make a quantum yield enhancement possible after binding. The *in vivo* tests of NIAD-4 in rat model of AD were promising. However, further work is needed to make this technique completely noninvasive by increasing the red shift and quantum yield [26].

Quantum dots are another group of nanotechnology-made fluorescent dyes [27-31]. These nanoscale semiconductor crystals have special fluorescent properties including minimal photo bleaching, optimal stability, high signal to noise ratio and broad absorption spectrum with very narrow but size-dependant tunable emission spectrum. These advantages over conventional fluorescent dyes [28] give QDs the potential for long-term tracking and simultaneous visualization of multiple physiological and pathological molecular events [29]. This simultaneous multiple labeling property is especially important for diagnosis of AD, since there are several biomarkers in the AD pathology, and not only their existence but also their proportion to each other may be helpful for ruling out other differential diagnoses [1].

However, due to the toxicity of the semiconductor materials presently used in QDs, like cadmium selenide (CdSe) and cadmium sulfate (CdS), their *in vivo* applications are questionable. Of course, some investigators have reported reduction in the toxicity of QDs when encapsulated in polymers (like phospholipids) [30] or coated by polyethylin glycol (PEG) [31]. Tokurako *et al.* recently developed a nanoprobe for amyloid-β aggregation and oligomerization using PEG coated QDs as $A\beta_{42}$ labels [32]. They examined the oligomerization behavior of $A\beta_{42}$ in solution and on intact cells and they compared the ingestion manner of microglia for $A\beta_{42}$ monomers and oligomers. However, in order to use this diagnostic technology to monitor $A\beta_{42}$ biochemical behavior *in vivo*, in addition to QD safety considerations, special attention should be paid to successful traverse of this QD-Aβ nanoprobes through the BBB. QDs conjugated with Transferrin were recently reported to be able to successfully transmigrate through an *in vitro* model of the BBB [33]. Further *in vitro* and *in vivo* research is required to evaluate similar approaches to transmigrate QD-based nanoprobes across the BBB [1].

In Table 1, we report all the available *in vitro* and *in vivo* AD diagnostic methods, in a comparative fashion based on the information available in the literature. Obviously, there is still a need for research to develop an affordable, ultra-sensitive and selective molecular AD detection method.

Table 1. Suggested *in vivo* and *in vitro* nanotechnology methods for AD diagnosis

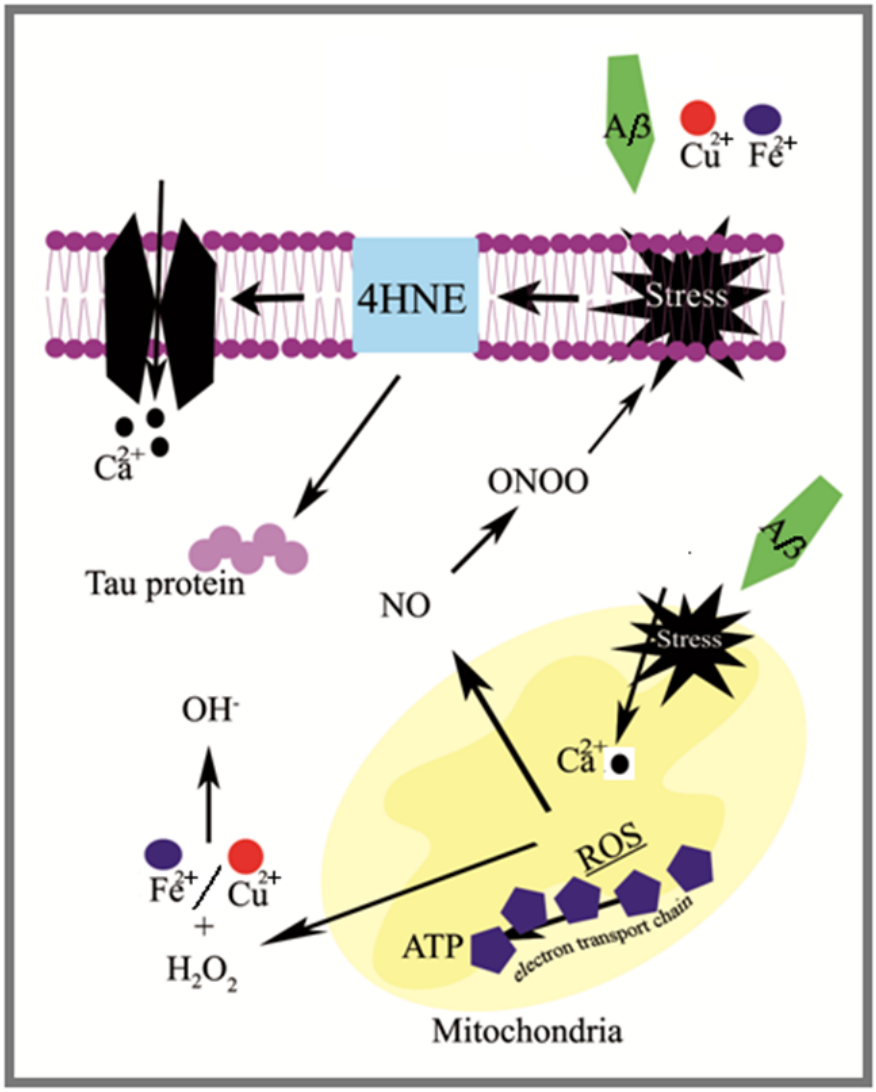
Test Mode	Technology	Biomarker	Signal Detection	Signal transduction		Signal Documentation	Sensitivity	Ref's
				Signal transform	Signal Amplification			
In-vitro	Bio-barcode assay	ADDL	Sandwich assay (Monoclonal Anti ADDL Ab)	DNA barcode	Functionalized AuNP carrier for numerous DNA barcodes	Scanometric Recording	aM	(17)
	Localized surface plasmon resonance	ADDL	Sandwich assay (Monoclonal & Policlonal Anti-ADDL Ab)	Wavelength shift	Secondary Ab of Sandwich assay	Spectroscopy	N/A	(18)
	Scanning Tunneling Microscopy	$A\beta_{(1-42)}$	Sandwich assay (Monoclonal Anti Aβ Ab)	Tunneling Current Change	Silver Staining of AuNPs	Frequency of pulse-like peaks	10fg/ml	(25)
	Two Photon Rayleigh Scattering Assay	Tau protein	Monoclonal Anti-Tau Ab	TPRS intensity change	TPRS	TPRS Spectroscopy	1pg/ml	(20)
In -vivo	µMRI	Aβ plaques	Aβ peptide	Magnetic heterogeneity	N/A	MRI scanner	N/A	(23, 24)
	Optical (Fluorescent) Imaging	Aβ plaques	NIAD-4	Fluorescent excitation	Upon Binding Molecular Rigidification	multiphoton microscopy	N/A	(26)

3. Nanotechnology-based Treatments for Alzheimer's Disease

As we discussed before, the currently available therapeutics for AD, only act to lower its symptoms [1, 8]. In recent years, however, significant amount of research have been focused on finding the so-called "neuroprotective agents", therapeutics that could stop the disease progress by targeting special molecular mechanisms in the AD pathology process [1, 34]. However, more futuristic are approaches that could rebuild the damaged tissue, called as "regenerative agents". These two (neuroprotective and neuroregenerative) approaches together are known as "disease-modifying approaches". They are distinguished from symptomatic approaches by the fact that in addition to ameliorating the symptoms they are aimed to stop the disease progress and restore the dysfunctional or dead tissue [34].

The therapeutic potential of nanotechnology for AD includes both neuroprotective and neuroregenerative approaches [1]. 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 [1].

Figure 4 - In this figure the interaction of Fe^{3+} and Cu^{2+} with Aβ leading to the production of oxidative stress is shown. Aβ can also oligomerize in the lipid bilayer of cell plasma membrane, leading to formation of membrane calcium channels [35]. These calcium channels cause an imbalance in calcium homeostasis [36], that ends in oxidative stress. In addition, the membrane integrated Aβ can chemically interact through (amino acid) Methionine 35 (not shown) with the membrane lipid molecules and the resultant lipid peroxidation produces 4-hydroxy-2-nonenal (4HNE) [37]. Such an interaction leads to membrane disruption and production of reactive oxygen species and finally oxidative stress in the involved brain tissue. The 4HNE and other reactive oxygen species (ROS) also lead to tau phosphorylation and aggregation. Moreover, intracellular aggregates of Aβ cause mitochondrial oxidative stress, and further imbalance in Ca^{2+} hemostasis. The resultant impairment of electron transfer chain leads to overproduction of superoxide anion, which is converted either to H_2O_2 or to peroxynitrite ONOO (following interaction with nitric oxide (NO)). The interaction of H_2O_2 with Fe^{2+} or Cu^{2+} produces the hydroxyl radical (OH^*), a strong ROS that induces membrane-associated oxidative stress [38]



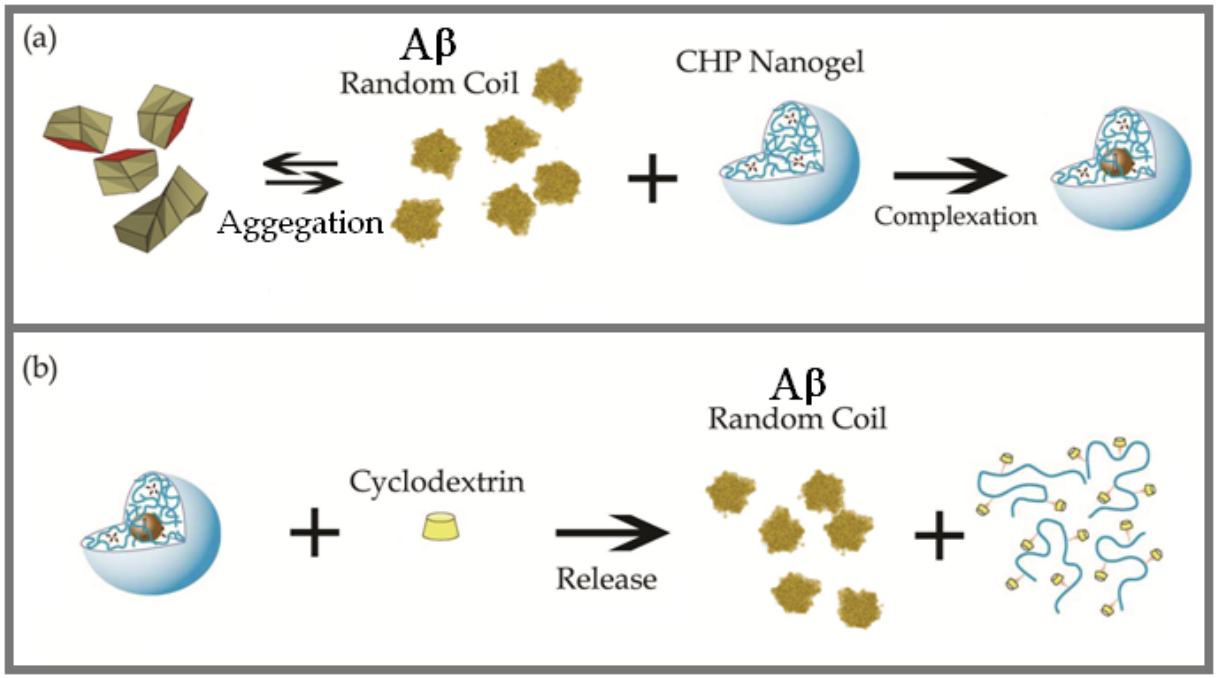
3.1. Nanotechnology Neuroprotective Potentials

The main two sources of neurotoxicity in AD pathogenesis are Aβ oligomers and free radicals (see Figure 4). Some of the nanotechnology-based approaches are capable of protecting neurons from Aβ toxicity by preventing from amyloid oligomerization (*anti-assembly* strategy) and/or accumulation of Aβ oligomeric species [1]. The other nanotechnology neuroprotective approaches include those that protect neurons from oxidative stress of free radicals [1].

3.1.1. Nanogels

The work of Ikeda *et al*. is an example for the Aβ anti-assembly strategy [39]. They designed an amphipathic nanogel that incorporates proteins and controls their folding and aggregation, similar to natural chaperones (proteins assisting the non-covalent folding and/or unfolding). In the case of Aβ, these nanogels would inhibit the amyloidogenesis process effectively through this mechanism (see Figure 5).

Figure 5 - (**a**). Schematic representation of the interactions between artificial nanoscale chaperone system and misfolded Aβ. (**b**). Refolded Aβ monomers are released after addition of cyclodextrin.



The nanogel (hydrogel nanoparticles) designed in this study was composed of cholesterol bearing pullulan (CHP). Pullulan is a natural water-soluble polysaccharide polymer consisting of maltotriose (a trisaccharide consisting of three glucose molecules linked with 1,4 glycosidic bonds) units. Inhibiting assembly at the monomer level, this technique prevents Aβ oligomerization and therefore reduces the concentration of toxic Aβ oligomeric species [1]. Recently, Boridy *et al*. demonstrated a significant reduction in $A\beta_{42}$ toxicity in the primary cortical cell culture and microglial cell culture after using CHP nanogels [40].

3.1.2. Fullerene

Fullerene (C_{60}) and its derivatives could be the base of neuroprotective compounds [41]. 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 [12, 14]. Dugan *et al.* demonstrated the effects of carboxyfullerenes (malonic acid derivative of C_{60}, $\{C_{63}[(COOH)_2]_3\}$) on $A\beta_{42}$ induced oxidative stress and neurotoxicity in cultured cortical neurons [41, 42]. Interestingly, the application of carboxyfullerenes blocked the $A\beta_{42}$ induced neuronal apoptotic death [42].

Podolski *et. al*. demonstrated anti-assembly effect of C_{60} hydrated fullerene (C_{60}HyFn) on the fibrillization of $A\beta_{25-35}$ fragment. It was demonstrated that injection of 3.6 nMol of C_{60}HyFn to each of the brain ventricles could prevent the cognitive impairment in rats previously injected with $A\beta_{25-35}$ fragment [43].

Fullerene has also complete neuroprotective properties against NMDA receptor mediated neurotoxicity according to the studies of Dugan *et al.* [44]. NMDA receptor function is important to neuronal mechanisms of learning and memory.

Fullerenols, which are water-soluble hydroxyl functionalized derivatives of fullerene, have shown neuroprotective properties against $A\beta_{42}$. Presumably, the neuroprotective effect of fullerenols is due to, both, anti-oxidant reactions and inhibition of $A\beta_{42}$-induced Ca^{2+} neurotoxicity. Huang *et al*. validated the latter finding in their investigation into the effect of fullerenol-1 upon Aβ-induced Ca^{++} influx in the cultured neurons [45].

Altogether, promising applications of functionalized fullerene derivatives including carboxyfullerene, hydroxyfullerene (*fullerenols)* and C_{60}HyFn are in discovery of new drugs for AD. However further research on their pharmacodynamic and pharmacokinetic properties is necessary [1].

3.1.3. Nano-Ceria

Cerium oxide (CeO_2) nanoparticle (nano-ceria) is reported to have neuroprotective effects on AD *in vitro* models [46]. This is mainly due to the anti-oxidant properties of nano-ceria, originating in the two oxidation-reduction (redox) states of cerium: Ce^{2+} and Ce^{4+} and the resultant oxygen vacancies [36, 47]. D'Angelo *et al*. showed that in addition to the mentioned anti-oxidant properties, nano-ceria protects neurons from cytotoxic effects of Aβ via modulating the intracellular signaling pathways involved in cellular death and neuroprotection [46].

3.1.4. Dendrimers

Dendrimers, one of the polymeric nanotechnology building blocks [48, 49], are macromolecular structures with globular shape and a densely packed surface [50]. Their structure has offered them a number of biomedical potentials [48, 49]. Recently, a multipurpose anti-amyloid strategy was suggested for dendrimers [50]. Aβ anti-assembly strategy of dendrimers could be performed either via their binding with peptide monomers or through blocking the end of protofibrils and fibrils. Prevention from cytotoxic effects of Aβ is another prospect of nanotechnology and application of modified dendrimers is a recent suggestion for this approach. Patel *et al*. demonstrated that dendrimers (both conjugated and unconjugated) could shield the cell membrane from Aβ membrane mediated

neurotoxicity, which is due to Aβ electrostatic interaction with the cell membrane [51]. In addition, dendrimers could sequester the Aβ toxic species and therefore block their pathological effects on the cell membrane. However, because of the probable toxic effect of dendrimers on cells, this method needs further investigation for *in vivo* application.

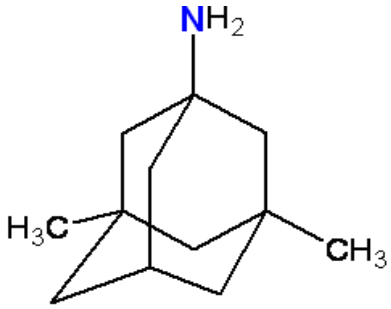
3.1.5. Gold Nanoparticles

Resolubilization of fibrillar amyloid species is another nanotechnology suggested anti-amyloid approach. Kogan *et al.* utilized gold nanoparticles (AuNPs) in weak microwave fields in order to dissolve amyloid aggregates [52]. Their design was based on dissolving Aβ aggregates and prevention from further Aβ aggregations by providing local thermal energy at a molecular level. The AuNPs, already attached to the specific target (i.e. Aβ), produce the thermal energy when a weak microwave field is surrounding. 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. Each AuNP provided a dissipating thermal energy of 10^{-14} J/s, capable of breaking a fibril (non-covalent) bond (10^{-20} J binding energy per bond) per microsecond without breaking the covalent bonds, which are two orders of magnitude stronger [52]. However, according to the cytotoxic effects of Aβ oligomeric species, the targeting must be arranged exclusively for these species. Otherwise, if the fibrillar species are targeted conversely, the toxic effects will increase due to an accumulation of Aβ oligomeric species, following the breakdown of the fibrillar species [1]. Therefore, the method of Kogan *et al.* seems to be advantageous for noninvasive investigation and manipulation of Aβ aggregates in AD, provided the appropriate amyloid species is targeted.

3.1.6. Diamondoid Derivatives

Diamondoids are in the category of most promising molecular building blocks in nanotechnology and specially nanomedicine [53]. 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 (memantine), which is already in commercial use, slows down the progression of the Alzheimer's disease [54]. Memantine [1-amino-3, 5-dimethyladamantane] with Namenda as its commercial name (Figure 6) is an FDA-approved drug for treatment of moderate to severe Alzheimer's disease. Memantine acts as a neuroprotective agent against excitotoxicity, an excessive exposure to the excitatory neurotransmitter, glutamate, or overstimulation of its membrane receptors, leading to neuronal injury or death. Excitotoxic neuronal cell death is mediated, in part, by overactivation of N-methyl-d-aspartate (NMDA)-type glutamate receptors. Nevertheless, NMDA receptor activity is also essential for normal neuronal function. This means that potential neuroprotective agents that block virtually all NMDA receptor activity will very likely have unacceptable clinical side effects. Memantine preferentially blocks excessive NMDA receptor activity without disrupting normal activity [55].

Figure 6 - Memantine (1-amino-3, 5- dimethyladamantane), commercially known as Namenda, a synthetic adamantane derivative with biological activity on the central nervous system.



Although memantine is already approved by FDA for Alzheimer's disease treatment, studies are underway for other diamondoid derivatives which could have stronger neuroprotective and possibly regenerative capability as well as their application for the treatment of diseases related to glutamatergic dysfunction.

In Table 2, we report a comparison of the above discussed six nanotechnology neuroprotective agents for treatment of AD, which include their neuroprotective functions, the mechanisms of their targeted AD pathology and their stage of study modes.

Table 2- Nanotechnology neuroprotective agents for treatment of AD

Nanosystem	Neuroprotective Function	Targeted AD pathology mechanism	Study Mode	Ref.s
Nanogels (Cholesterol bearing pullulan)	Aβ Anti-assembly (Incorporate Aβ monomers)	Oligomerization of Aβ	*In vitro* (Solution and Cell Culture)	(39)
Fullerene (C_{60}): Carboxyfullerene, C_{60}HyFn, Fullerenol	Anti-oxidant Aβ Anti-Assembly Anti-oxidant / Maintenance of Ca^{2+} homeostasis	Oxidative stress Oligomerization of Aβ Ca^{++} influx	*In vitro* (cell culture) *In vivo* (Rats)	(41-45)
Dendrimers: Polyamidoamine (PAMAM))	Aβ Anti-Assembly	Oligomerization of Aβ Aβ cell memberane toxicity	*In vitro*	(48, 49)
Nanoceria (CeO_2)	Anti-oxidant	Oxidative Stress	*In vitro*	(46, 47)
Gold Nanoparticles (AuNP)	Aβ Anti-Assembly	Oligomerization of Aβ	*In vitro*	(52)
Diamondoid Derivatives (Memantine) (1-amino-3, 5-dimethyladamantane)	NMDA receptor antagonism	Glutamate excitetoxicity	*In vivo* (FDA approved)	(54)

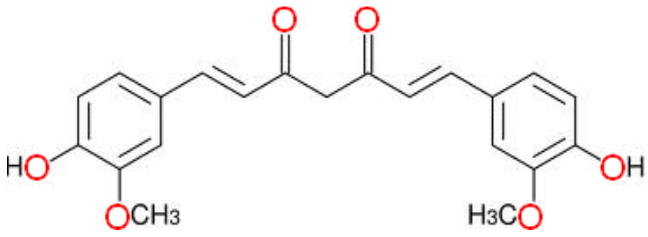
3.2. Nanocarriers

Targeted drug delivery is an important application of nanomedicine [56]. With regard to diseases of central nervous system (CNS) it is appreciably complicated due to the additional obstacle of the blood brain barrier (BBB) against the entry of a variety of molecules into the CNS tissue [57]. The use of biocompatible nanoparticles to facilitate the traverse of therapeutic agents across BBB has been extensively researched in the past decade [58]. In what follows, we discuss nanocarrier systems suggested for delivery of therapeutic agents for AD into the brain.

3.2.1. Curcuminoids Nanocarrier

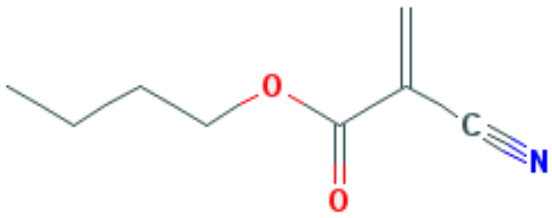
Curcumin, with the chemical formula reported in Figure 7, is the active ingredient of turmeric, the yellow spice which has been recently discovered as a potential treatment for AD [59].

Figure 7 – Curcumin



This agent acts through several mechanisms including anti-amyloid assembly, anti-oxidant and anti-inflammatory [59]. However, this agent is unstable due to rapid hydrolyzation or oxidization. Therefore, Poly n-butylcyanoacrylate (PnBCA) (Figure 8) nanocapsules have been used to carry these therapeutic agents through the BBB. These nanocapsules were coated by the protein ligand named apolipoprotein E3 (APoE-3), in order to use LDL receptors for the BBB crossover [60].

Figure 8 - Poly n-butylcyanoacrylate (PnBCA)



3.2.2. 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 (AChEIs), inhibit the enzyme that degrades the acetylcholine neurotransmitter (*i.e.* acetylcholinesterase). However, the efficacy of these drugs is significantly compromised by BBB, impeding their entrance into the brain. Therefore, higher doses, at which peripheral side effects occur, are needed. Recently some nanocarrier systems were suggested to transport these therapeutic agents beyond BBB [61]. Wilson et al. demonstrated 3.8 and 4 fold increase in the brain delivery of two of these AChEIs (Rivastigmine and Tacrine, respectively), by exploiting polysorbated 80-coated polymeric Poly n-butylcyanoacrylate (PnBCA) nanocapsules [62]. These PnBCA nanocarriers use the LDL receptors to transport through BBB.

3.2.3. Acetylcholine Nanocarrier

Yang *et al*. have recently introduced a nanocarrier system for delivery of acetylcholine to lysosomes in brain of mice model of AD through low doses of single-wall carbon nanotubes (SWCN) [63]. The advantage of SWCN is the possibility of their oral administration owing to their sufficient gastrointestinal absorption. Although no significant SWCN induced toxicity is reported at their low doses, considering their biologically non-degradable nature, further research on their safety is in demand [61].

3.2.4. 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 [64]. However, there are serious concerns about their side effects due to their uptake by other tissues. Targeted delivery of these potential drugs is what nanotechnology provides to minimize their complications. Chitosan and poly(lactide-co-glycolide acid) (PLGA) nanoparticles are two nanocarriers recently suggested and examined for delivery of sex steroid hormones (e.g. Estradiol) to the brain [65]. In the study of Wang *et al*. it was shown that intranasal administration of Estradiol loaded Chitosan nanoparticles could significantly increase the CSF concentration of the drug, with minimal increase in plasma concentrations, and therefore minimizing its peripheral side effects [66]. Two other studies have demonstrated a considerable increase in bioavailability of estradiol and mifepristone, after oral administration, when encapsulated in PLGA nanoparticles [61, 67]. Mifepristone (11β-[4-dimethylamino]phenyl-17β-hydroxy-17[propynyl]estra-4,9-dien-3-one) is an anti-progestrone compound which is reported to slow the cognitive impairment progress in AD patients [68].

3.2.5 Green Tea Polyphenol Nanocarrier

Recently, the green tea polyphenol, epigallocatechin-3-gallate (EGCG), was discovered to have therapeutic effects for AD [69]. In addition to anti-oxidant effects, EGCG is capable of reducing Aβ production. Aβ is known to originate from sequential cleavage of Aβ precursor protein (APP) by β-secretase and then γ-secretase [70]. APP could also be cleaved, instead of β-secretase, by another proteolytic enzyme termed as α-secretase. In this case, further cleavage of the remnant by γ- secretase results in a protein called P3 and not Aβ. It is reported that EGCG could promote α-secretase and thus deviate the APP cleavage process from production of Aβ [69]. However, due to its low bioavailability, EGCG alone could not be of therapeutic value if taken orally. In order to solve this problem, recently, Smith *et al*. co-solubilized EGCG with lipid nanocarrier and made EGCG:lipid complexes (nanolipidic particle) with a diameter of 30-80 nm [71]. With this new formulation, they showed an increase in bioavailability of EGCG and suggested a probable increase in EGCG brain uptake due to its very small size.

3.2.6. Chelator Nanocarriers

Brain normally contains a certain trace concentration of metal ions like Zn^{++}, Cu^{++} and Fe^{++}, which possess different physiological roles [72]. Of course, these trace metal ions are kept under strict homeostatic regulations and compartmentalization. Any disruption in the homeostasis of these trace elements leading to higher concentrations of these ions, may impose several proteins and membrane lipids to toxic effects of these trace elements and finally end in production of ROS [72-75] (see Figure 4).

Amyloid-β peptide (Aβ) has some selective metal binding sites, and it is actually a metalloprotein from this point of view. It is known that even trace amounts of Zn^{++} and Cu^{++} that may be released from synaptic terminals of brain cortical neurons could induce Aβ aggregation (and then precipitation) via interactions with Aβ histidine amino acid [72]. This precipitation is reversible by metal chelation before fibrillization happens. In addition, the interaction of Fe^{+++} and Cu^{++} with Aβ may lead to production of H_2O_2 due to double electron transfer to O_2 [72] (see Figure 4). The production of H_2O_2 is partly responsible for oxidative injury seen in AD. Furthermore, the interaction of Aβ with cell membrane is enhanced by Zn^{++} and Cu^{++} [82], and Cu^{++} could play an important role in the neurotoxicity of Aβ [77].

Metal chelation, which stands for the clearance of body tissues from excessive metal ions, could therefore be considered as one of the disease modifying agents for AD [72]. An efficient metal chelation strategy for AD may substantially decrease both the extracellular oxidative stress and Aβ aggregation, and therefore slow down the AD progress. For most current metal chelators, however, this is not the case, and there are some obstacles to an efficient therapeutic response. These obstacles include risk of non-specific metal chelation from other tissues, difficulty in passing through the BBB, and pooling of metal ions into amyloid plaques [72]. Nanotechnology approaches are recently used in some studies to engineer chelator therapeutic systems that overcome these obstacles [76]. Below we report the role of iron and copper chelators in AD modification.

3.2.6.1. Iron Chelators:

Liu *et al*. conjugated Desferrioxamine (also named as DFO-B, DFB, desferal, desferrioxamine B, or desferoxamine B), an FDA approved metal chelator, with nanoparticles (through an amido bond between a primary amino group in the chelator and a carboxyl group on the nanoparticle surface) to produce an efficient chelator nanoparticle system (CNPS) [79]. They reported that the chelation effect of metal chelator was retained after formation of CNPS. In addition to feasibility of carrying iron chelators across BBB, their work showed other advantages of nanoparticle technology. For instance, it was shown that the iron chelating CNPS was able to traverse the BBB in the reverse direction by preferential adsorption of Apo A-I and thereby removal through LDL transport system. Moreover, it was suggested that the inherent toxicity of the chelators are obviously decreased after conjugation with nanoparticles [80].

Later, Liu *et al*. suggested another synthesized nanoparticle-chelator conjugate (named Nano-N2PY), and demonstrated its *in vitro* ability to inhibit the cytotoxic effects of Aβ on human cortical neurons [81]. The nanoparticles used in this study were made of carboxyl functionalized polystyrene. The mechanism of such anti-cytotoxic effect of the Nano-N2PY was probably related to its preventive

effect on the Aβ aggregation. In addition, such a nanoparticle conjugate is capable of transporting metal chelators across the BBB, without disrupting the metal binding feature of the chelator. In addition to increasing the efficacy of metal chelating strategy, these nanoparticle conjugates were shown to reduce the toxicity of chelation strategy, by decreasing the lipophilicity of the chelators [81].

3.2.6.2. Copper Chelators:

In the study of Cui *et al*. [82] d-penicillamine (which is a degradation product of penicillin), an FDA approved drug for chelation of copper in Wilson's disease (an inherited disorder in which there is too much copper in the body's tissues), was examined as a metal chelator in AD. The conjugation of d-penicillamine with the nanoparticles containing, 1,2-Dioleoyl-snglycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], known as MPB-PE and pyridyldithio-propionylhosphoethanolamine known as PDP-PE enabled the traverse of d-penicillamine through the BBB in spite of its highly hydrophilic properties. This study showed the ability of d-penicillamine for chelating copper and desolubilizing $A\beta_{42}$. Cui *et al*. demonstrated in this study that, interestingly, the BBB integrity and permeability remained unchanged and no changes in the cerebral perfusion flow were evident. Therefore, it is suggested that the transport mechanism for this nanoparticle formulated carrier system is endocytosis, transcytosis or passive diffusion in the absence of barrier opening [82].

3.2.7. Anti-Oxidant Nanocarrier Agents

In addition to the above mentioned anti-oxidant nanotechnology building blocks, solid lipid nanoparticles (SLN) have been recently employed to improve the delivery of anti-oxidant agents [83, 84]. SLN is considered one of the safe nanoparticles suggested for drug delivery to the brain [58]. In the study of Bondi *et al*, SLN played the role of a nanocarrier for ferulic acid [3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid], a phenolic compound with a strong anti-oxidant activity [84]. The resultant nano-complex possesses a small colloidal surface with a highly negative surface charge when dispersed in water [83]. The effects of ferulic acid (FA), including reduced ROS production, normalized mitochondrial membrane potential and decreased cytochrom C release, all caused an increased cell viability in the experiment of exposure of neuroblastoma cells to $A\beta_{42}$ oligomers [83]. Enhancing the drug stability within biological fluids and making intracellular targeting feasible, the SLN nanocarrier improved the anti-oxidant effects of FA [83, 84].

3.2.8. Gene Nanocarriers

The main part of gene therapy is gene delivery, through which the genetic material will be presented inside a cell [85]. In order to protect the genetic material from biological obstacles, like cell membrane charge and enzymatic degradation, a carrier should accompany the genetic material. So far, most of the gene therapy studies for AD have employed viral vectors. However, due to the health risks associated with viral vectors, and also the limitations in size and number of genetic inserts through viral vectors, using alternative non-viral vectors have become the focus of attention in recent years [85]. On the other hand, usage of nanoparticles as nonviral gene carriers has significantly improved the efficacy of this method by minimizing the enzymatic degradation of genetic materials [85-87] In particular,

polymeric, ceramic and amino-terminated organically modified silica (ORMOSIL) nanoparticles have recently shown some promise in gene transfer to the CNS [85]. For example, polyethyleneimine is a polymeric nanoparticle, capable of forming stable complexes with nucleic acid and easily releasing from endosomes, both due to its protonable amino nitrogen atoms that make this cationic nanoparticle a "proton sponge" at any pH [88]. Boussif *et al*. reported the successful gene transfection to neurons and also a high efficiency of *in vivo* gene transfection through polyethyleneimine vector [88]. The more promising of all, ORMOSIL nanoparticles, was first examined for *in vivo* gene delivery to CNS by Bharali *et al*. [89]. In this study the ORMOSIL nanoparticles were functionalized with amino groups for DNA binding. Their experiment indicated that these nontoxic nanoparticles were appropriate gene vectors not only for *in vivo* brain therapy but also for therapeutic regulation of neural stem cells.

In Table 3, we compare the above discussed nanocarrier systems suggested for delivery of therapeutic agents for AD into the brain.

Table 3 - Summary of Applicable Nanocarrier Systems in AD Treatment

Nanocarrier	Structure	Size [nm]	BBB transport mechanism	Loaded Drug(s)	Therapeutic Effect/Category	Ref.
PnBCA nanocapsule	Polysorbated 80-coated polymeric Poly n-butylcyanoacrylate	40.5±0.6.9	LDL transport system	Rivastigmine Tacrine	Acetylcholinesterase inhibitors	(61, 62)
		178±0.59 to 197±2.3		Curcumin	Anti-amyloid assembly, Anti-oxidant and anti-inflammatory	(60)
SWCN	Single-wall carbon nanotubes	0.8–1.2 diameter and several microns length	Entering brain through nerve axons (cytoplasmic translocation)	acetylcholine	Neurotransmitter	(63)
Chitosan	linear polysaccharide formed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine	About 200 to 500	Unknown	Estradiol	Neuroprotection against Aβ	(66)
PLGA	poly(lactide-co-glycolide acid)	126.0±2.6	Unknown	Mifepristone Estradiol	Slowing the cognitive impairment progress in AD	(61, 67)
Nanolipidic particle	Lipid co-solubilized with drug	30-80	Unknown	Polyphenol (EGCG)	reducing Aβ production	(71)
Nano-N2PY	Carboxylic functionalized polystyrene nanoparticles Activated by N-cyclohexyl-N -(2-morpholinoethyl)carbodiimide methyl-ptoluensulfonate (CMC)	~240	Unknown	2-methyl-N-(2 -aminoethyl)-3-hydroxyl-4-pyridinone (MAEHP)	Iron chelator	(81)
MPB-PE/ PDP-PE	1,2-Dioleoyl-snglycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide] / pyridyldithio-propionyl-phosphoethanolamine	103±33 to 117±44	Endocytosis, transcytosis or passive diffusion	d-penicillamine	Chelating copper and desolubilizing $A\beta_{42}$	(82)
Solid lipid nanoparticle (SLN)	Solid hydrophobic core having a monolayer of phospholipid coating.	94-140	Endocytosis, transcytosis or passive diffusion	Ferulic acid	Anti-oxidant	(83, 84)
ORMOSIL	Ceramic and amino-terminated organically modified silica	~30	N/A	DNA	Gene therapy	(89)

4. Conclusions

Early diagnosis of AD would be a cost effective approach in order to prevent its irreversible and uncontrollable consequences. Early diagnosis need to be performed before the underlying pathology has become severe enough to present itself clinically. Several molecular biomarkers are recognized to be associated with the pathology of AD. However, the currently available laboratory methods are not sensitive enough for such an early diagnosis. Nanotechnology offers a number of highly sensitive molecular detection tools that may make this goal achievable. These nanodiagnostic tools utilize different nanoparticles/nanostructures, and are based on different physicochemical interactions that may be utilized either *in vitro* or *in vivo*.

Alzheimer's disease seems to be a multi-factorial disease with several pathogenetic mechanisms and pathways, which are not necessarily interrelated. Therefore, it would be a great step forward for Alzheimer's disease therapy if a multifunctional nanotechnology approach could be developed for designing therapeutic cocktails that simultaneously and specifically target the main molecular culprits involved in AD. These molecular targets include (but are not limited to) Aβ oligomers, ROS, excessive metal ions, tau phosphorylating kinases and cell cycle proteins.

Nevertheless, there are still many challenges regarding the biocompatibility of nanoparticles and nanodevices especially in a complex biological milieu like brain with a huge concentration of cells. Therefore, it seems that a long and puzzling path is ahead to make the envisioned nano-neurosurgical approaches of curing CNS diseases a practical technology and eventually a routine clinical practice [1, 11, 47, 90, 91, 92].

5. Glossary

Ab	antibody
AChEI	acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADDL	amyloid β-derived diffusible ligand
AgNP	silver nanoparticle
aM	attomolar (10^{-18} moles per liter)
amphipathic	Pertains to a particle having two ends with different characteristics and long enough so that each end shows its own solubility effect.
Apo A-I	Apolipoprotein A-I
APP	Aβ precursor protein
Au	gold
AuNP	gold nanoparticle
Aβ	amyloid-β peptide – A peptide generated from amyloid precursor protein, which is known in association with AD.
$A\beta_{42}$	One of the two (the other is $A\beta_{40}$) more common isoforms of amyloid-β protein
BBB	blood brain barrier
$C_{60}HyFn$	nanostructures of hydrated C_{60} fullerene

CdS	cadmium sulfate
CdSe	cadmium selenide
CeO_2	cerium oxide
CNPS	chelator nanoparticle system
CNS	central nervous system
CSF	cerebrospinal fluid
Da	Dalton = 1.6605×10^{-24} gram- The unified atomic mass unit
EGCG	epigallocatechin-3-gallate
FDA	Food and Drug Administration of the U.S. Government
FA	ferulic acid
fg	femtogram (10^{-15} g)
LDL	low-density lipoprotein
LSPR	localized surface plasmon resonance
Lysosomes	digestive organelles in animal cells
MAEHP	2-methyl-N-(2-aminoethyl)-3-hydroxyl-4-pyridinone
MION	monocrystalline iron oxide nanoparticles
MPB-PE	*1,2-Dioleoyl-snglycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide]*
MRI	Magnetic Resonance Imaging
μMRI	Micro Magnetic Resonance Imaging
N/A	not available
Nano-N2PY	nanoparticle-chelator conjugate
NIAD	near infrared Alzheimer's dye
NIAD-4	A NIAD with the chemical formula: *[[5'-(4-Hydroxyphenyl)[2,2'-bithiophen]-5-yl]methylene]-propanedinitrile*
NIR	near-infrared
NMDA	*N-Methyl-D-aspartic acid or N-Methyl-D-aspartate*
NP	nanoparticle
ORMOSIL	organically modified silica
PAMAM	poly(amido amine)
PCR	polymerase chain reaction
PDP-PE	*pyridyldithio-propionyl-phosphoethanolamine*
PEG	polyethylene glycol
pg	pictogram (10^{-12} g)
PLGA	poly(lactide-co-glycolide)
PnBCA	poly n-butylcyanoacrylate
QD	quantum dot
ROS	reactive oxygen species
SLN	solid lipid nanoparticles
STM	scanning tunneling microscopy
SWCN	single-wall carbon nanotubes
TPRS	two-photon Rayleigh scattering assay

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