

# Nanotechnology Solutions for Alzheimer's Disease: Advances in Research Tools, Diagnostic Methods and Therapeutic Agents

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**Abstract.** A century of research has passed since the discovery and definition of Alzheimer's disease (AD), the primary common dementing disorder worldwide. However, AD lacks definite diagnostic approaches and effective cure at the present. Moreover, the currently available diagnostic tools are not sufficient for an early screening of AD in order to start preventive approaches. Recently the emerging field of nanotechnology has promised new techniques to solve some of the AD challenges. Nanotechnology refers to the techniques of designing and manufacturing nanosize (1–100 nm) structures through controlled positional and/or self-assembly of atoms and molecules. In this report, we present the promises that nanotechnology brings in research on the AD diagnosis and therapy. They include its potential for the better understanding of the AD root cause molecular mechanisms, AD's early diagnoses, and effective treatment. The advances in AD research offered by the atomic force microscopy, single molecule fluorescence microscopy and NanoSIMS microscopy are examined here. In addition, the recently proposed applications of nanotechnology for the early diagnosis of AD including bio-barcode assay, localized surface plasmon resonance nanosensor, quantum dot and nanomechanical cantilever arrays are analyzed. Applications of nanotechnology in AD therapy including neuroprotections against oxidative stress and anti-amyloid therapeutics, neuroregeneration and drug delivery beyond the blood brain barrier (BBB) are discussed and analyzed. All of these applications could improve the treatment approach of AD and other neurodegenerative diseases. The complete cure of AD may become feasible by a combination of nanotechnology and some other novel approaches, like stem cell technology.

**Keywords:** Alzheimer's disease, nanotechnology, amyloid, tau protein, atomic force microscopy, nanodiagnostics, targeted drug delivery

## INTRODUCTION

Alzheimer's disease (AD), first defined a century ago [2], is the primary common cause of dementia in the geriatric population [83]. The incidence and prevalence of AD increases with age and as the life expectancy is rising worldwide, AD is quickly becoming one of the pressing universal healthcare problems [127]. Cur-

rently, there are more than twenty four million patients with dementia worldwide and this number is expected to increase to forty two millions by the year 2020 and to eighty one millions by 2040 [31]. At present, AD is one of the highly expensive health disorders both for the patient's family and for the society [31]. As an example, in the United States AD, which is presently the third most expensive disease, is costing the country more than \$100 billions per year [49] and it is predicted that this amount will near to \$500 billions by 2020 [97].

Patients with AD experience an impaired cognition ranging from an insidious impairment of episodic mem-

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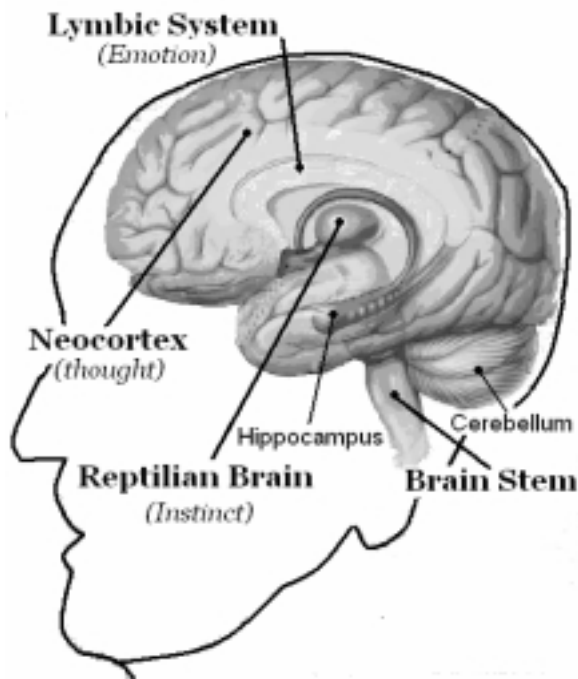


Fig. 1. During AD, neurons of some brain areas, especially neocortex and limbic system (hippocampus, amygdala and their associated cortices), gradually deteriorate and undergo death. Neocortex is responsible for processing the sensory information relayed to the brain, controlling voluntary movements, performing conscious thought and other mental activities. The Limbic system is mainly responsible for emotions and instinctive behavior. The hippocampus plays important roles in learning and short-term memory.

ory at the onset (also known as mild cognitive impairment (MCI)) to an eventual dementia syndrome [15]. The latter is a severe impairment or loss of intellectual capacity and function including deficits in attention, in memory, in thinking, in reasoning and in language skills [148]. Moreover, accompanied with the dementia syndrome is usually the inability of the patient to perform “motor functions” and “alterations of personality” [84,148]. On the average, the patient dies nine years after the AD diagnosis [16].

The pathology of AD is a progressive one in nature. In addition, the minimal repair and regeneration capacity of the brain tissue makes this progressive process almost irreversible [83]. This necessitates a quite early diagnosis and prevention of the disease. The earlier we stop the pathogenetic process, the less the patient will remain symptomatic. Therefore, undertaking precise diagnostic procedures for screening the AD high risk population is the current preferred approach against the disease [90]. However, at the present there is not any single diagnostic tool for precise screening or early and

accurate detection of the disease [33,108]; and only a probable diagnosis with an 80% confidence, on average, is possible based on clinical criteria (including laboratory tests, neuroimaging and neuropsychological assessment) [33]. Moreover, the commonly prescribed medications are only symptomatic and do not stop the progressive pathology of the disease [83]. Even the latest advanced medications currently available or aimed in some studies can only slow or cease the progress of the disease pathogenesis and are not able to restore the lost brain function. Therefore, AD is an incurable disease at the present [83,84].

Nanotechnology encompasses a recent and overwhelming group of atomic- and molecular-based techniques capable of arranging atoms and molecules in specially designed and controlled positions [79,80]. This capability has facilitated the production of new structures and devices with, at least, one dimension in nanoscale (1–100 nm) [79,80]. Richard P. Feynman, the Nobel laureate physicist and the pioneer of nanoscale sciences and technology [32], first envisioned the application of nanotechnology in medicine in 1961. Two decades later K. Eric Drexel, following the idea of molecular engineering and manipulation, postulated the possibility of direct interactions between devices of nanoscale and biological molecules [23]. These interactions have become the basis of new nanomethods for probing molecular changes within cells both for investigational and diagnostic purposes. Moreover, through nanotechnology it is now possible to target therapeutic agents to specific body cells, tissues and organs more effectively and with less adverse consequences.

The importance of medical applications of nanotechnology is more evident in diseases related to the central nervous system (CNS), mainly due to the highly elaborate structural and functional properties of the CNS [124]. Nanotechnology is promising a noninvasive access to this complex system that is not presently achievable by other approaches [56]. In this report, we present nanotechnology methods recently used for basic research on the AD molecular pathogenesis, as well as the potential and known nanotechnology approaches thus far suggested to combat AD, in the areas of diagnosis and therapy [48,109,118,123].

## ALZHEIMER'S DISEASE PATHOLOGY

According to the AD pathology, the neurons of some areas of the brain including neocortex and limbic struc-

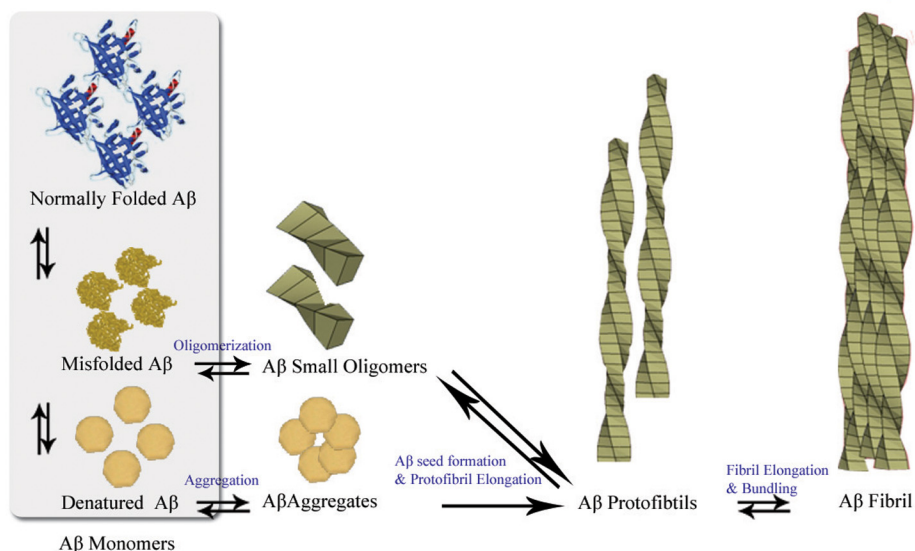


Fig. 2. The diagram of amyloidogenesis process: Sequential formation of *amyloid aggregates, protofibrils and fibrils*. In addition to accumulation of A $\beta$  monomers, some structural abnormalities can lead to misfolding or denaturation (unfolding) of A $\beta$  and make it prone to self-assembly, resulting in formation of A $\beta$  oligomers and aggregates. These are then converted to protofibrils, through A $\beta$  seed formation and elongation processes. Protofibrils are the penultimate species in the amyloidogenesis process. The final species are fibrils, consisting of bundles of 4–6 assembled protofibrils. Fibrils are considered to be the reservoir of all other species.

tures (hippocampus, amygdala and their associated cortices), (see Fig. 1) gradually deteriorate and undergo death [104].

Several hypotheses now exist for the molecular mechanisms of the AD pathogenesis [39,83]. Nevertheless, still there is no consensus on the root cause of the disease. According to the amyloid cascade hypothesis (see Fig. 2) [45] accumulation of the amyloid- $\beta$  peptide (A $\beta$ ) is the initial step in the AD pathogenesis.

A $\beta$  has six main alloforms composed of 38 to 43 amino acids. Among the six alloforms, A $\beta$ <sub>40</sub> peptide is the most abundant alloform in the normal brain tissue while A $\beta$ <sub>42</sub> is the most abundant alloform in the AD amyloid deposits [9]. A $\beta$  originates from cleavage of amyloid- $\beta$  protein precursor (A $\beta$ PP) in a specific pattern. A $\beta$ PP refers to a group of transmembrane protein isoforms ranging in size from 695 to 770 amino acids (counted from N-terminal as N1 to N770) [84,114]. There are specific sites on A $\beta$ PP for three proteolytic enzymes called  $\alpha$ -secretase,  $\beta$ -secretase (also known as BACE) and  $\gamma$ -secretase [115]. Sequential proteolytic effects of  $\beta$ -secretase and  $\gamma$ -secretase, cutting APP at N-terminus and C-terminus of A $\beta$  sequence respectively (as shown in Fig. 3), results in production of A $\beta$  [84, 114].

The accumulation of the A $\beta$  is due to an imbalance between its production and clearance mechanisms [83]. The abnormally higher production of A $\beta$  monomers than normal ranges is more associated with the familial type of AD and with early onset of AD in Down syndrome (trisomy 21) [115]. The root cause of this pathology is missense mutation in presenilin 1 and presenilin 2 genes in the familial AD and overexpression of A $\beta$ PP gene (on chromosome 21) due to an extra chromosome 21 in Down syndrome [114]. On the other hand, disruption of clearance mechanisms is another reason for accumulation of A $\beta$  [115]. This pathology is commonly responsible for the late onset sporadic AD (classic type) and it is controlled by special polymorphisms of a gene referred to as Apolipoprotein E4 on chromosome 19 [114]. Of course, Apolipoprotein E4 is not a pathological cause for AD, but it is a normal genetic polymorphism and considered only a risk factor for late onset AD [114].

In addition to accumulation, some structural abnormalities in A $\beta$  facilitate its oligomerization via enhancement of aggregation mechanisms [10,68,143]. These two pathogenetic factors (accumulation and aggregation) lead to amyloidogenesis process (see Fig. 2), through which the toxic amyloid species are formed. Among the different amyloid species, the final prod-

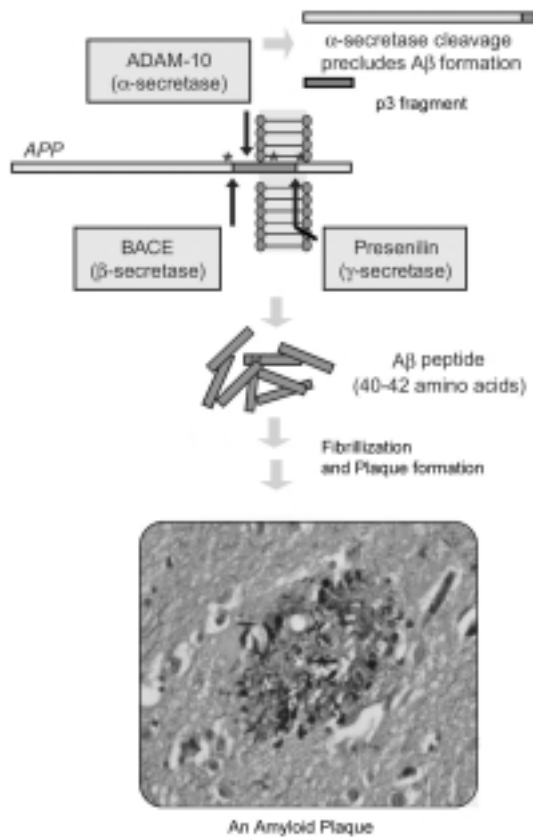


Fig. 3.  $A\beta$  monomers are originated from a specific proteolytic processing of amyloid- $\beta$  protein precursor ( $A\beta$ PP) in neurons.  $\alpha$ -secretase and  $\beta$ -secretase (also known as BACE) activity cut  $A\beta$ PP at N687 and N671 respectively (the amino acid numbers are not shown).  $\gamma$ -secretase truncates  $A\beta$ PP within the transmembrane (cell interior) domain. If  $A\beta$ PP has been already processed by  $\alpha$ -secretase,  $\gamma$ -secretase activity leads to the release of p3 fragment; meanwhile if  $A\beta$ PP has been already processed by  $\beta$ -secretase,  $\gamma$ -secretase activity leads to the release of  $A\beta$  (1-40/42).  $A\beta$  (1-42) is the major component of the senile plaques. (Figure adopted from <http://www.nia.nih.gov/Alzheimers/> and J. Götz, L.M. Ittner and N. Schonrock, *MJA* **185**(7), 2 Oct 2006).

uct of amyloidogenesis process, the amyloid fibrils (deposited in amyloid plaques), are commonly known as the main actors in the pathology of AD. However, recently some soluble oligomeric and protofibrillar species of amyloidogenesis process have been found to be neurotoxic, as well [17,37,67,139].

The damage to nerve cells due to oxidative stress is another suspected initial step in the pathogenesis of AD [96]. During the cellular respiration process in the mitochondria, semi-reduced oxygen species (e.g. super oxide and hydrogen peroxide) also referred to as reactive-oxygen species, are produced [145]. Physiologically, certain biochemical processes neutralize the reactive-oxygen (free radical oxygen) species. Ox-

idative stress occurs because of the imbalance due to the over-production or the slow neutralization of free-radical oxygen [43,133].

Nevertheless, the initial source of oxidative stress in AD is rather unknown. Of course, the assumption is that some vitamin deficiencies, including folic acid and vitamin E deficiencies, disrupt the neutralization mechanisms of the free radicals [133]. More interestingly, there are supporting evidences for a dual relation between  $A\beta$  formation and oxidative stress, i.e. beside the effect of  $A\beta$  on triggering oxidative stress, oxidative stress in turn mediates the aggregation of  $A\beta$ , thereby facilitating its oligomerization [145].

On the other hand, it is hypothesized that oxidative stress can lead to hyperphosphorylation of  $\tau$ -protein, "a microtubule associated protein". Therefore, we can consider oxidative stress as a link between amyloid toxicity and  $\tau$ -protein pathology in AD [21].

The hyperphosphorylation of  $\tau$ -protein, regardless of its root cause, is due to dysregulation in phosphorylation/dephosphorylation signaling pathways [14]. The main physiological role of  $\tau$ -protein is stabilization of microtubules [6]. Microtubules are the tube-shaped protein structures that form the cytoskeleton of eukaryotic cells in part, as shown in Fig. 4 [115]. Hyperphosphorylated  $\tau$ -protein results in destabilization of microtubules due to detachment of  $\tau$ -protein from microtubules [115]. Of course, there are some evidences against the exclusive association between  $\tau$ -protein hyperphosphorylation and microtubule reduction in AD. According to these evidences, microtubule reduction happens before  $\tau$ -protein hyperphosphorylation, and it is presumed to be a direct result of oxidative stress [13].

The subsequent intracellular events of  $\tau$ -protein hyperphosphorylation are not only due to  $\tau$ -protein dysfunction but also to its newly gained toxic properties [38]. Moreover, morphologic changes in synapses, because of cytoskeleton breakdown, will compromise the synaptic connections [6]. On the other hand, hyperphosphorylated  $\tau$ -protein is prone to polymerization, leading to the formation of paired helical filaments (PHF) deposited in the neurofibrillary tangles (NFT) (see Fig. 4). We discuss the structural properties of PHF, revealed by AFM, later in this report.

In addition to the above-mentioned mechanisms, there are other mechanisms in the AD pathogenesis [83,113–116], including disruption of neuronal ionic (e.g. iron, copper and calcium ions) homeostasis, disruption of cholesterol homeostasis, neuroinflammation, impairment in neurotransmission and apoptotic cell death [22,69]. Explanatory discussions over these mechanisms are beyond the scope of this report.



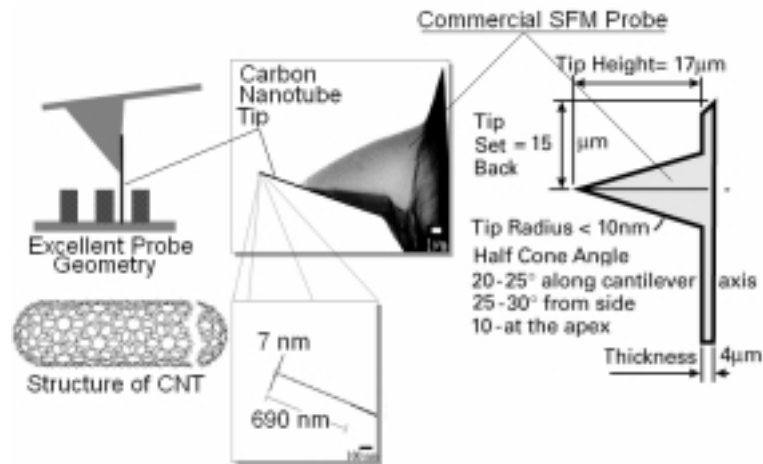


Fig. 5. A carbon nanotube (CNT) tip attached to a silicon cantilever tip assembly.

mines the feature resolution of images provided by AFM [142]. There are different kinds of probe tips. For example, commercial SFM probes made of “Oxide sharpened silicon tips” (see Fig. 5) are useful for scanning biological structures including cells. This application is because of the ability of these tips to combine high power with physical tolerance on a soft sample surface [14]. However, these tips cause major constraints on lateral resolution and their pyramidal shape makes it impossible to access narrow and deep features [14]. Recently, attaching carbon nanotubes (CNT) to the end of silicon tips has been a breakthrough in lateral resolution and imaging deep and narrow features (Fig. 5). In addition, MWNTs provide low tip-sample adhesion and therefore gentler imaging [14]. Wong S.S. et al. for the first time took advantage of this scanning probe technology for visualizing  $A\beta_{40}$  protofibril and fibrils. Their results show a 12–30 % increase in resolution when compared with the results using Si tips [142].

In almost all AFM studies completed so far for amyloid species, the tapping mode of operation of AFM is used [5,11]. Tapping mode is the choice mode for scanning single macromolecules including proteins [29]. In the contact mode, inevitable immobilization of the sample to a flat support may damage and inactivate the single molecule [29]. However, in tapping mode, taking advantage of an oscillating tip minimizes the lateral shear forces between the probe and the sample, and therefore provides high-resolution visualization of samples with loose substrate adsorption properties [1].

#### Review of the results

*In situ* AFM studies of prepared  $A\beta$  solutions on mica substrate have revealed sequential formation of *glob-*

*ular amyloid aggregates, protofibrils and fibrils* [5,58]. We have summarized the dimensions of these species, as reported in different AFM studies, in Table 1. The globular shape of  $A\beta$  aggregates on mica is a sign of its amphipathic character against a hydrophilic surface (such as mica). Lambert and coworkers [67] referred to these globular aggregates as amyloid derived diffusible ligands (ADDL). They described ADDL as a synthetic  $A\beta$  derivative produced from  $A\beta$  monomers under special conditions of inhibited or delayed fibrillization process [67]. They also reported on the high toxicity of ADDLs even at nanomolar concentrations [67]. However, there are still contradictions on the existence of ADDLs both in normal brains and in the brains of AD patients [37,67].

Shirahama et al. first described  $A\beta$  protofibrils in 1967 through an electron microscopic study [121]. The height differences between  $A\beta$  protofibrils and fibrils are more easily distinguishable in AFM studies than the studies using electron microscopy [47] (see Fig. 6 and Table 1). Amyloid protofibrils are transient intermediates of the  $A\beta$  fibrillization pathway; they are soluble and the dimensions of their earliest species are consistent with the dimensions of ADDLs [5], as discussed above. According to AFM studies, protofibril assembly has two steps: initiation and elongation. These steps are distinct and could be individually inhibited or observed [47]. Elongation is a bidirectional process, which is achievable by addition of monomers, aggregates and small protofibrils to the end of core protofibrils [11,47].

The different behavior of amyloid protofibrils in contact with hydrophilic mica and hydrophobic graphite, has confirmed the hydrophobic nature

Table 1  
Summary of AFM results for the structural properties of AD molecular hallmarks

Species	AFM substrate	Structure	Diameter/Width (nm)	Height (nm)	Length (nm)	Ref. No.s
$A\beta$ Aggregates	Mica	Globular	$4.4 \pm 0.4$	$\sim 5$	—	5, 65,
	Graphite	Elongated	—	1	—	65
ADDL	Mica	—	4.8 and 5.7	—	—	67
Protofibrils	Mica	—	$9.3 \pm 2.1$	$\sim 1.5$	$64.4 \pm 18.5 (< 100)$	5
Fibrils	Mica	nodular fibrils	$11.4 \pm 0.8$	11 (center) 5 (internodal spaces)	hundreds of nm to some $\mu\text{m}$	5
		smooth fibrils	$11.4 \pm 0.8$	5	hundreds of nm to some $\mu\text{m}$	
PHF twisted sheet	Mica	Double strand	10	20	8–12 (each unit)	88, 87

ADDL = Amyloid- $\beta$  derived diffusible ligand.

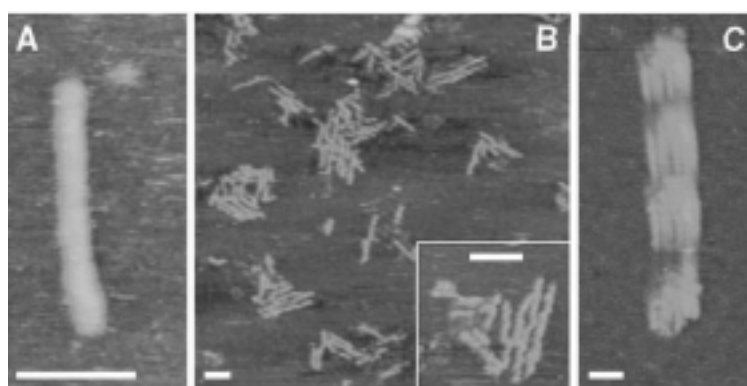


Fig. 6.  $A\beta_{42}$  protofibrils observed by AFM. **A)** Single protofibril and globular structures on mica **B)** Protofibril bundles on highly ordered pyrolytic graphite, **C)** Assembly of four protofibril bundles on mica. All images correspond to height AFM signal. Bar: 50 nm. Z scale: 6 nm (A, B), 5 nm (B, inset), and 30 nm (C). Figure is from [5].

of these species [5]. Observation of the behavior of amyloid protofibrils in an amphipathic (hydrophilic/hydrophobic) ambient is important for understanding of the probable reactions made by similar intermediates in similar *in vivo* conditions (e.g. the entrapments of oligomeric amyloid species within biological amphipathic environments such as lipoproteins and cell membranes) [5].

The final structural unit of the amyloidogenesis process is  $A\beta$  fibrils as shown in Fig. 2. By addition of a preformed *fibrillar* seed to a solution, containing monomers or protofibrils, the process of protofibril-fibril conversion can be initiated [47]. However, *ex situ* AFM studies of Ha et al. demonstrated the higher efficiency of  $A\beta_{42}$  oligomers in seeding the growth of amyloid fibrillar species, compared with the fresh amyloid monomeric or fibrillar (polymeric) seeds [40]. Therefore, we may conclude that the deposition of  $A\beta_{42}$  oligomers on the cellular membranes or the intercellular structures is the major initial event leading to amyloid plaque formation in the brain tissue [40].

According to AFM studies,  $A\beta$  fibrils consist of bundles of 4–6 assembled protofibrils. The bundles experience some internal rearrangements, ending in formation of nodular (bumpy) fibrils. The nodular fibrils continue conformational changes, ending in smooth fibrils [5]. Smooth fibrils have a height of 5 nm, however nodular fibrils are 11 nm in height at the center of nodules and 5 nm at the internodal spaces (see Table 1) [5].

AFM studies are also useful in order to understand the structural properties of paired helical filaments (PHF). PHF is the final structural unit defined for  $\tau$ -polypeptides. It is also the main building block of neurofibrillary tangles (NFT), one of the two important hallmarks of AD pathology [88,89,102]. By combining AFM and cryoelectron microscopy Moreno-Herrero et al. described the PHF structure by a model of twisted sheets with two strands of polymers separated by a narrow space, a model of two couple ribbons as shown in Figs 7 and 8 [89]. Moreno-Herrero et al. took advantage of an ultra sharp AFM cantilever tip with 5 nm in diameter to enable the high-resolution visualization of PHF structure. The application of such ultra sharp tips

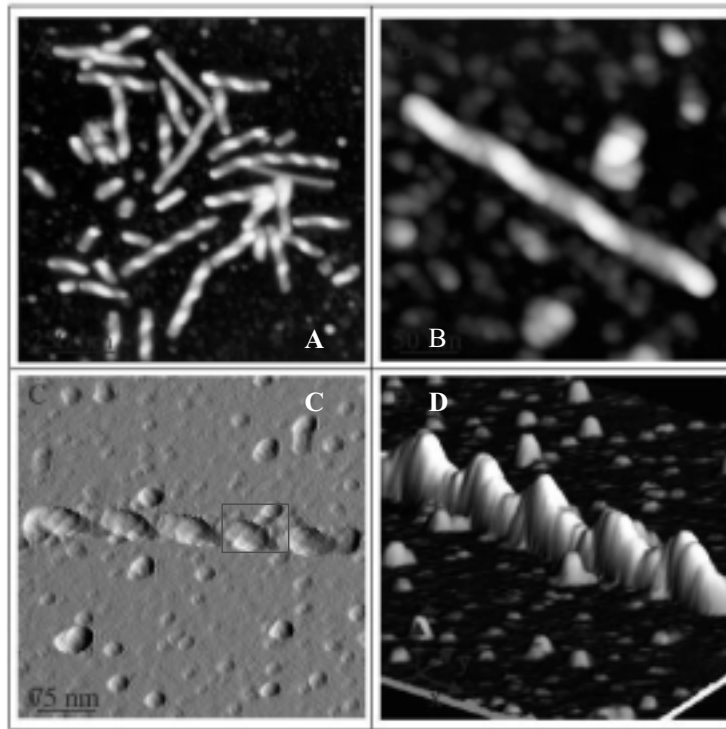


Fig. 7. Paired helical filaments (PHF) as observed by AFM on mica support in tapping mode. *A* and *B* are the topographic images obtained at different magnifications. *C* is a high magnification image visualizing a substructure with a period of 8–10 nm. *D* is the substructure in the 3D representation. Figure adapted from [89].

led to the observation of the two-strand twisted ribbons, a finding unachievable by standard AFM tips [89].

#### Single molecule fluorescence microscopy

Investigation into the molecular changes and intermolecular interactions of proteins is a challenging problem in protein misfolding diseases, such as AD. At present, we can perform measurement and study of molecular changes occurring in nanoscale by using a number of techniques. Fluorescence resonance energy transfer (FRET) microscopy is one such technique, which is applicable, both, for *in vitro* and *in vivo* systems [117]. FRET is in reference to an energy transfer mechanism between two chromophores. A chromophore is a region in a molecule where the energy difference between two of its different molecular orbitals falls within the range of the visible spectrum. In practice, FRET is the physical process of energy transfer from an excited donor fluorophore to a nearby acceptor fluorophore. The donor fluorophore could become excited by incident light [117]. This energy transfer depends on the distance between the donor and the ac-

ceptor fluorophores [112]. The mechanism of such an energy transfer is not radiation, but it is a result of intermolecular long-range dipole-dipole coupling [112]. Recently FRET microscopy became more applicable in biological investigations due to identification of some new fluorescence dyes, such as GFP (green fluorescent protein), which is an autofluorescent protein, and development of some novel optical methods [117,112]. Applications of single-molecule FRET methodology mainly include investigation into structural dynamics of an individual protein molecule, monitoring its behavior at different folding conformations and revealing the mechanism of its interactions with the neighboring molecules [136].

Recent AD applications of FRET include several diverse studies on abnormal protein species of AD pathogenesis. These studies uncovered several mechanistic relations between different molecular and sub-molecular actors in the AD pathogenesis. We analyze and report the results of two such studies below:

- (i) According to NMR studies,  $A\beta_{14-23}$  fragment assumes parallel  $\beta$ -sheet topology when incorporated in the polymerizing  $A\beta$  [8], although

inherently anti-parallel  $\beta$  sheet topology is preferred for this fragment. In a study reported by Shi et al. [120], the FRET method was used in exploration of the mentioned topology shift that occurs on the  $A\beta_{14-23}$  fragment when incorporated in longer fragments.  $A\beta_{14-23}$  fragment is referring to a fragment of  $A\beta$  between amino acids 14 and 23. The common form of  $A\beta$  has 40 amino acids.  $\beta$ -sheet is referred to a generally twisted-pleated sheet like conformation of proteins, known as the second form of regular secondary structure in proteins (the first form is the alpha helix). This structure may assume two different topologies known as parallel and anti-parallel  $\beta$ -sheet topologies, depending on the direction of its consisting  $\beta$ -strands.

In the studies of Shi et al. FRET was also used to reveal the role of specific amino acids in the aggregation of  $A\beta$ . This knowledge could help with the  $A\beta$  anti-aggregation drug discoveries [120].

- (ii) The FRET technique, in combination with a double immunofluorescence approach, was used by Kinoshita et al. in order to investigate the subcellular localization of  $A\beta$ PP and its interactions with human  $A\beta$ PP cleaving enzyme (known as  $\beta$ -secretase, or BACE) [60]. BACE is a membrane-anchored protease that produces "amyloid- $\beta$  protein precursor  $\beta$ " ( $A\beta$ PP $_{\beta}$ ) and a fragment of 99 amino acids through its proteolytic cleavage activity on  $A\beta$ PP. The studies of Kinoshita et al. put more emphasis on the interaction between  $A\beta$ PP and BACE on the cell surface. Furthermore, they suggested that FRET approach might be of use for screening the potential therapeutics targeted to block  $A\beta$ PP/BACE interaction [60].

The aforementioned studies are only a few examples of numerous FRET completed and ongoing studies, which are rapidly clarifying the way through which several different molecules cooperate to create a complex disorder like AD.

#### NanoSIMS microscopy

The effect of neurodegeneration in the brain tissue is not limited to morphological changes, but it also includes chemical elemental alterations [105]. These chemical elemental alterations in neurodegeneration are both in the total level of chemical elements and

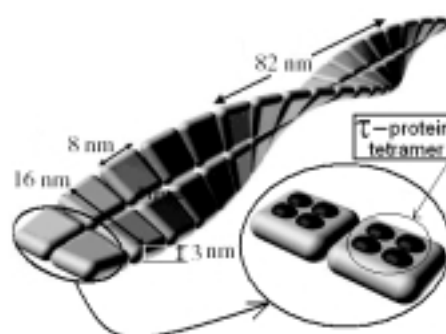


Fig. 8. The model of paired helical filaments (PHF) structure. Several AFM studies of PHF have revealed double strand structure for this polymer. A very fine gap separates the two strands from each other. The average pitch is calculated to be 82 nm. Each subunit has dimension of  $8 \times 8 \times 3$  nm. The subunits per se are composed of  $\tau$ -protein tetramers. Figure adapted from [89].

more importantly in the subcellular distribution of these elements [105]. The conventional histological microscopy methods including visible light microscopy and electron microscopy do not offer information about these chemical changes. NanoSIMS is a new type of "secondary ion mass spectroscopy" (SIMS), capable of highly sensitive and simultaneous detection of five elements at 50–200 nanometer spatial resolution (subcellular resolution) [105]. The five elements are *N*, *P*, *S*, *Fe* and *Ca*.

Quintana et al. [105] recently studied the usefulness of NanoSIMS microscopy for imaging the chemical as well as morphological alterations of pathological brain areas. Their studies also showed the distribution of iron, hemosidrin and ferritin in the hippocampus of brain of AD patients. In addition, NanoSIMS visualizes the senile plaques with higher resolution (in CN- and S-maps) than optical imaging as shown in Fig. 9 [105]. Quintana et al. also recorded the distribution of the five elements *N*, *P*, *S*, *Fe* and *Ca* in the senile plaques, glial cells and pyramidal neurons [105]. The contrast shown in the SIMS images presents the distribution of chemical elements. However, NanoSIMS microscopy does not measure the concentration of chemical elements directly [105].

#### NANODIAGNOSTICS FOR ALZHEIMER'S DISEASE

Presently, the prevailing common aim in the AD community is early detection for more timely and effective treatment of the disease. An ideal diagnostic tool for AD must have more than 80% sensitivity and

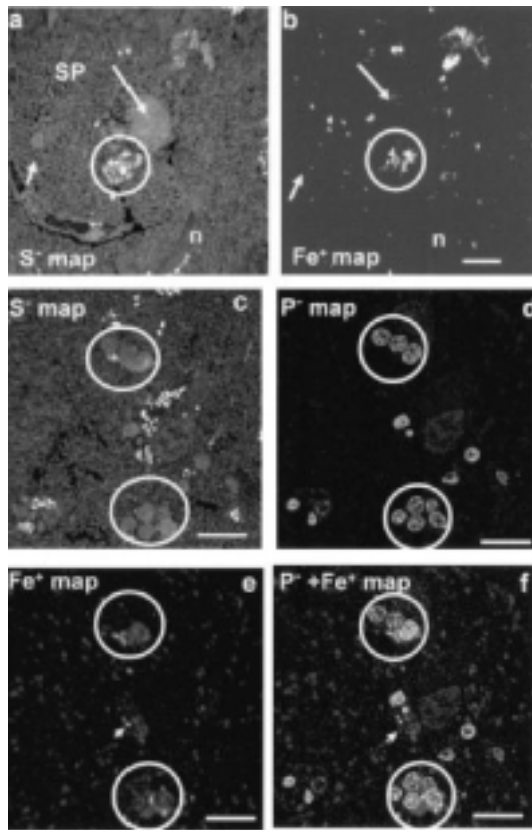


Fig. 9. The Iron distribution in the hippocampus of AD brain. (a & b) Senile plaque viewed by S and Fe maps. Iron is not identified in the core of senile plaque. (c–e) S, P and Fe maps of a brain tissue region containing glial cells and neurons with lipofusion granules. (f) digitally superimposed P and Fe maps demonstrating the presence of iron in the cytoplasm and nucleus of glial cells. *Figure taken from [105].*

specificity for early diagnosis of AD and ruling out other differential diagnosis. In addition, it must be reliable, reproducible, simple to perform, inexpensive and noninvasive [18,108,130].

The potential for early detection of AD is another important characteristic of an ideal diagnostic tool. This is because the neurodegeneration process due to AD begins well before AD becomes symptomatic [15,90,94]. Therefore, in order to prevent a progressing and complicated AD, it is fundamental to detect underlying AD pathology early before the symptoms or signs of the disease appear. Generally, for such an early detection of AD, a diagnostic tool with the following two characteristics is needed: 1. Independence from the disease severity [94]; 2. Independence from brain reserve [90]. The diagnostic tools without these two characteristics are not sensitive enough to detect *early* AD pathology since they can detect AD only after the progressive un-

derlying pathology has overcome the brain reserve and has reached the threshold in which the disease is severe enough to present itself clinically. The brain reserve here refers to the cognitive capacity of the brain that can mask the underlying disease pathology and its consequent brain functional loss [90]. For example, higher education and larger brain size are two important factors that increase the brain reserve and would postpone the symptomatic AD, if an underlying AD pathology existed [90].

Currently there are four general areas in the diagnosis of AD, which are as follows [94,101,111,130,133]: (i) Clinical assessments. (ii) Neuropsychological tests. (iii) Neuroimaging. (iv) Detection of special cerebral spinal fluid (CSF) biomarkers. The first two methods are dependent upon brain reserve and thereby are not sensitive enough to be used as screening tests [90]. The third method depends upon the severity of the disease [94]. The fourth method is presently less clinically common than the first three methods. This may be due to its invasiveness, making it an unacceptable test for screening and early detection of AD [94]. Altogether, none of these approaches is appropriate for very early detection of AD.

On the other hand, nanotechnology approaches seem to be minimally dependent upon the severity of the disease, because of their potential of detecting ultra low concentrations of AD biomarkers. In addition, by targeting the specific pathology related biomarkers, nanotechnology tools can diagnose underlying AD pathology early and independent from brain reserve. Moreover, the majority of nanotechnology diagnostic tools are capable of detecting multiple biomarkers simultaneously. This capability makes these tools favorite for AD diagnosis, since not only the elevated concentration of certain biomarkers, but also their concentration ratios are important for diagnosis of AD and ruling out other differential diagnoses [133].

Therefore, nanotechnology can be the basis of new tools for very early detection of AD. The idea of the application of nanotechnology in the diagnosis of AD was reinforced after publication of two articles in February 2005, each suggesting a different nanotechnology detection approach for AD biomarkers [36,41]. The bio-barcode assay and surface plasmon resonance (SPR) technology are the two detection approaches proposed.

#### *Bio-barcode assay*

Nanotechnology has recently reached an attomolar scale detection of protein biomarkers. Nam et

al. [92] developed a nanoparticle oligonucleotide bio-barcode assay capable of detecting concentrations of protein biomarkers several orders of magnitude lower than the concentrations detectable by conventional Enzyme-Linked ImmunoSorbent Assay (ELISA) technique [33].

This high sensitivity of the bio-barcode assay is due to using engineered gold nanoparticles that carry the specific antibody of the target biomarker and hundreds of DNA barcodes simultaneously. Therefore, a single molecule of biomarker can be traced by hundreds of DNA barcodes [36]. Moreover, these DNA barcodes can be additionally amplified by the polymerase chain reaction (PCR) technique (See Table 2 and Fig. 10) [58].

Furthermore, the bio-barcode assay approach is capable of simultaneous detection of several protein biomarkers, and therefore could be a sensitive technique for detection of a specific kind of dementia like AD among other differential diagnoses [58]. This potential of bio-barcode assay makes it also possible to determine the disease stage according to the concentration of different biomarkers.

Recently, Georganopoulou et al. reported a highly sensitive detection of ADDL in the CSF samples of AD patients through bio-barcode assay [36]. The 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 have been reported as 1.7 fM and ca. 200 aM respectively [36]. This proves a correlation between increased CSF concentrations of ADDL and affliction with AD.

In conclusion, bio-barcode assay could provide highly sensitive and specific detection of pathology related biomarkers of AD in the CSF [36]. In comparison with common biomarker detection techniques like ELISA, the bio-barcode assay is a million times more sensitive [33,54]. Moreover, when comparing with currently available imaging techniques for diagnosis of AD, bio-barcode assay is less expensive, faster and more efficacious [36]. However, it requires the invasive and unacceptable procedure of lumbar puncture, if CSF biomarkers are targeted.

Further studies are required to make the clinical usage of bio-barcode assay practical for screening and detection of AD. To achieve this it would be necessary to develop an accurate bio-barcode assay for detection of AD plasma biomarkers [53].

#### *Localized surface plasmon resonance (LSPR) nanosensor*

The principle behind localized surface plasmon resonance (LSPR) nanosensor is to use singular optical properties of triangular silver nanoparticles (SNPs) as shown in Fig. 11. The especial optical properties of triangular SNP include the ultra sensitivity of their peak extinction spectrum ( $\lambda_{\max}$ ) to nanoparticle size, shape and local external dielectric environment.

The sensitivity of silver nanoparticles  $\lambda_{\max}$  to their external nanoenvironment is the essence of signal transduction in the LSPR nanobiosensor [42]. In this method, any changes in the triangular SNPs external nanoenvironment will lead to a change in the local refractive index (caused by the adsorbed analyte, e.g. Anti-ADDL antibody). This change subsequently changes the SNPs'  $\lambda_{\max}$ , because of the sensitivity of triangular SNPs  $\lambda_{\max}$  to their external nanoenvironment. The change in SNPs  $\lambda_{\max}$  could be visualized through spectroscopy (see Fig. 11) [41]. Importantly, the LSPR nanobiosensor is sensitive to different concentrations of target biomolecule (ADDL in this case) since the solution concentration directly changes the local refractive index. Therefore, different wavelength shifts for different concentrations are detectable [41]. (see Table 2).

Beside to its high sensitivity and specificity [42], LSPR nanobiosensor has simple and inexpensive components [42]. In addition, LSPR nanobiosensor has *ex vivo* instrumentation; therefore, it does not intervene with the integrity of body tissues except for sampling the appropriate body fluids. In this way, the invasiveness of the test only depends on the invasiveness of the sampling procedure. CSF sampling makes the test an invasive diagnostic method, as it requires lumbar puncture procedure.

The designer team of this technology, Haes et al., undertook several experiments on different biomarkers as well as ADDL [41]. They suggested three applications for LSPR nanosensor in AD. These applications are as follows: 1) Studying the oligomerization of the A $\beta$  in ultra low concentrations, similar to concentrations of *in vivo* conditions, 2) Screening patients for AD, and 3) Studying the interactions between the pharmaceuticals and their target molecules in drug discovery [41].

#### *Other possible nanotechnology methods*

In addition to the two methods mentioned above, there are several other nanotechnology methods [54]

Table 2  
Currently available nanotechnology tools for AD diagnosis

Nanotech tool	Nanoparticle	Biomarker	Detection mechanism	Signal transduction basis	Ref. No.s
BCA (Bio-barcode assay)	Gold	ADDL	Sandwich immunoassay (monoclonal anti-ADDL antibody):	Simultaneous conjugation of Au NP with the specific Antibody and numerous DNA barcodes	58, 36, 54, 132
LSPR (Localized surface plasmon resonance)	Silver (Triangular)	ADDL	Sandwich immunoassay (monoclonal & polyclonal anti-ADDL antibody):	Sensitivity of silver nanoparticles $\lambda_{max}$ to their external nanoenvironment	42

ADDL = amyloid derived diffusible ligands; NP: nanoparticle;  $\lambda_{max}$ : wavelength of maximum extinction.

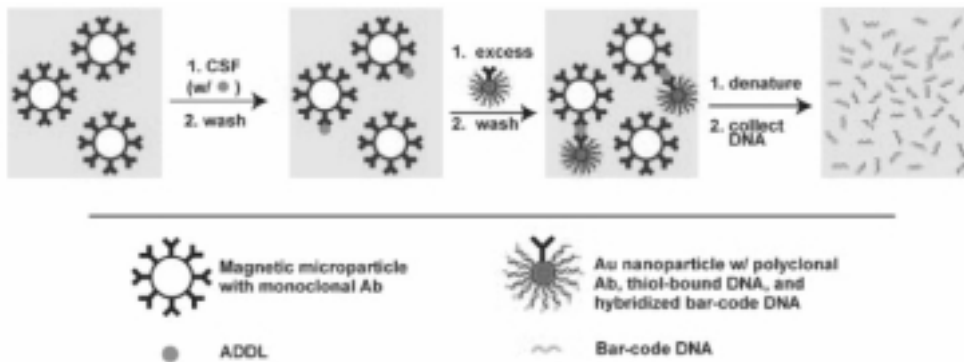


Fig. 10. Schematic representation of bio-barcode amplification assay for detection of ADDL in the solution: Magnetic microparticles that are conjugated with anti-ADDL monoclonal antibodies are used for primary detection of ADDLs in the solution. Afterwards, gold nanoparticle probes carrying anti-ADDL antibody and hundreds of bar-code DNA strands simultaneously are added to the solution. The result is a sandwich immunoassay. After a denaturation step hundreds of barcode DNA strands are collected for each antigen (ADDL) detection event. Figure is from [58].

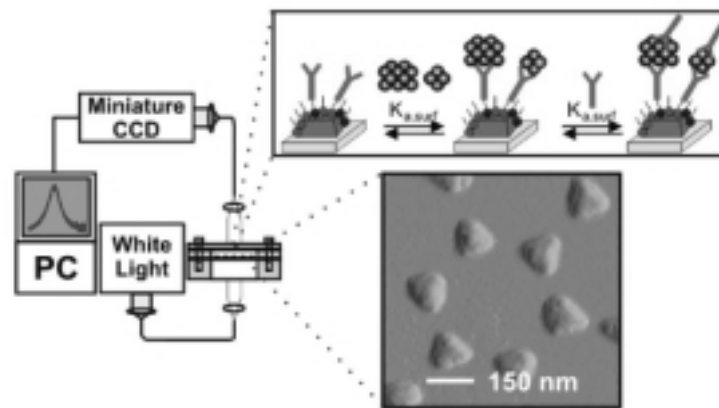


Fig. 11. Localized surface Plasmon resonance (LSPR) technology schematic representation. The top inset shows the sandwich immunoassay used for detection of ADDL biomarker. The bottom inset shows an AFM image of triangular Ag nanoparticles (NP height = 25 nm, NP width = 90 nm). Figure taken from [41].

that could have efficacious results in the route towards earlier and more sensitive and specific diagnosis of AD. Quantum dots, nanomechanical cantilever arrays, PEBBLE nanosensor, laser nanosensor, and nanoshell technology are the primary candidates of the alternative nanotechnology methods.

#### Quantum dots

Quantum dots (QD) are nanoscale semiconductor crystals with a fluorescent response to absorbed light. Their special fluorescent properties give them an advantage over using conventional fluorescent dyes [54]. Some of these special properties include: (i). Min-

imal photo bleaching; (ii). Optimal stability; (iii). High signal to noise ratio; (iv). Broad absorption spectrum with very narrow, but size-dependant, tunable emission spectrum; and (v). Proper surface for bio-functionalization [100]. Overall, these properties give QDs the potential for long-term tracking and simultaneous visualization of multiple molecular events (either physiological or pathological) both *in vitro* and *in vivo* [57].

The broad absorption spectrum and highly narrow emission spectrum of QDs enables the synchronic excitation of several QDs with different emission spectrums each specifically functionalized to track a certain biological process or mark a specific pathological biomarker [34]. This “simultaneous multiple labeling” property is especially important for diagnosis of AD, as discussed before.

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 the safety of QDs in efficacious, but extremely low concentrations for labeling applications [87], and others have shown insignificant biological activity and toxicity of QDs encapsulated in polymers (like phospholipids) [24]. Nevertheless, there is the question of whether the polymer coating of the QDs is sufficiently stable to prevent them to become toxic, especially for long term applications.

#### Nanomechanical Cantilever arrays

The original design of nanomechanical cantilever arrays was for the detection of oligonucleotides in biological samples. The micromechanical cantilever arrays have additional applications in proteomics.

Principle behind these sensors is due to their high sensitivity for detections of surface stress named as “nanomechanical detection”. In addition to high sensitivity, these cantilever arrays are fast in response and they are highly specific to analyte.

These nanosensors could be used for precise detection of several AD biomarkers in the CSF, including A $\beta$ 40, A $\beta$ 42,  $\tau$  and phosphorylated- $\tau$  proteins. More importantly, their application for detection of ultra low concentrations of AD biomarkers in plasma could be fruitful [53]. Since analysis of plasma for AD biomarkers only requires the minimally invasive procedure of blood sampling, it is preferred over CSF analysis. Altogether, one may envision the application of nanomechanical cantilever arrays for a synchronic, highly sensitive and specific evaluation of plasma biomarkers of AD. This could bring the accurate early detection and screening of the AD to reality.

## ALZHEIMER'S DISEASE THERAPY THROUGH NANOTECHNOLOGY

Presently there exist no therapeutic methods available for curing AD [84]. The cure for AD would require therapeutics that will cease the disease progress and will reverse its resultant damages. Today, common medications for AD are symptomatic and aim at the disrupted neurotransmission between the degenerated neurons. Examples of such medications are acetylcholine esterase inhibitors, including tacrine, donepezil, rivastigmine and galantamine [83].

With further research, mechanistic therapeutic approaches could gradually complement the above-mentioned medications. Design of each mechanistic therapeutic is for targeting a different stage of the AD pathogenetic process and therefore help to cease the progress of the disease [83].

Presently there are the following five molecular mechanistic therapeutic approaches under investigation [46,83]: (i). Inhibition of A $\beta$  production; (ii). Inhibition of A $\beta$  oligomerization, (iii). Anti-inflammation, (iv). Cholesterol homeostasis modulating; and (v). Metal chelation. Meanwhile, advances in nanotechnology are adding further opportunities for the AD therapy.

Generally, the focus of the nanotechnology therapeutic approaches for every disease have been on drug discovery and monitoring [55,78], controlled release of therapeutic agents [91,128], and targeted drug delivery. The later is the most researched one, and it is especially prerequisite for reaching stronger therapeutic effects with the least amount of side effects. These applications are quite remarkable and challenging in respect to diseases of the CNS and brain. The subject of targeted drug delivery, for example, is appreciably complicated for CNS, due to the additional obstacle of the blood brain barrier (BBB) against the entry of a variety of molecules into the CNS tissues. With respect to drug discovery and monitoring, the histological complexity of CNS is a restricting factor [126]. However, the potential capabilities of nanoparticles and nanodevices, including their controllable size and suspendability (based on modifiability of the nanoparticles outer layer), multi-functionality [85] and remote-controlled functionality [63] show promise in overcoming the CNS restrictions. Nevertheless, there are many challenges regarding the biocompatibility of nanoparticles and nanodevices especially in a complex biological milieu like brain with a huge concentration of cells and intercellular communications [126].

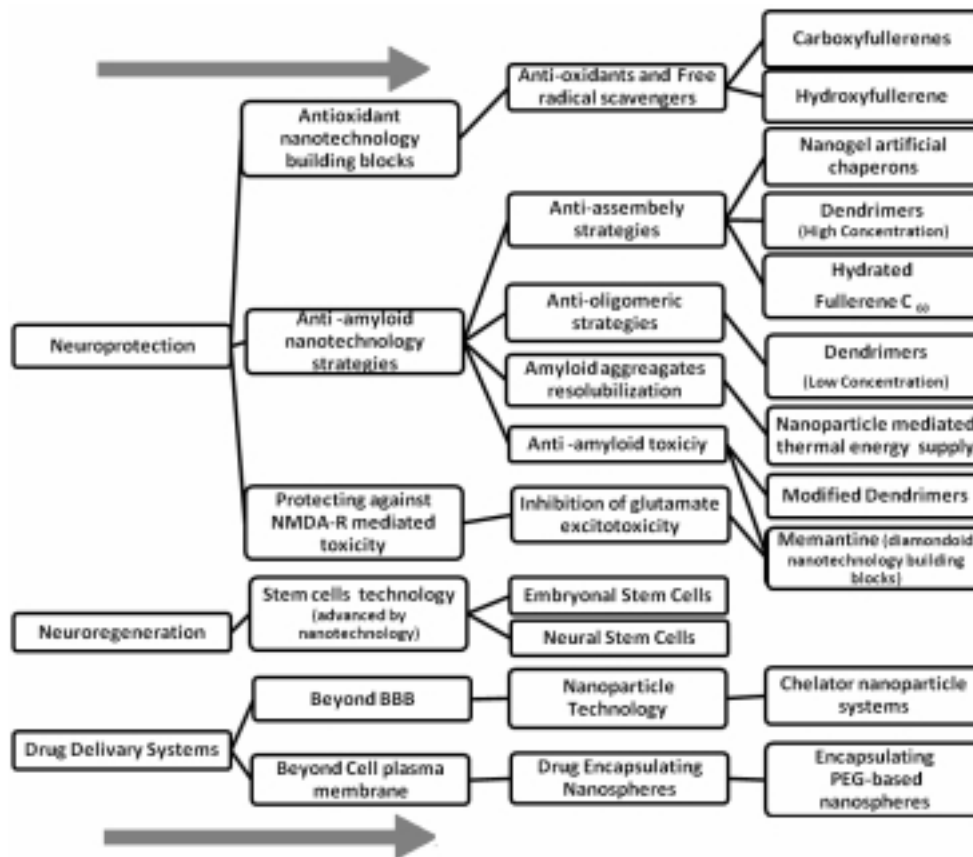


Fig. 12. The summary of applications of nanotechnology in the treatment of AD.

The current and envisioned applications of nanotechnology in neurology consist of neuroprotection, neuroregeneration and drug delivery beyond the BBB [124, 126]. All of these applications could improve the treatment approach of AD as a neurodegenerative disease (see Fig. 12). In the following sub-sections, we present each application separately.

### Neuroprotection

Designing therapeutic agents that protect neurons against cellular neurotoxicity is a rational and mechanistic approach for prevention and therapy of neurodegenerative disorders such as AD [83,147]. Oxidative stress and amyloid induced toxicity are the two basic toxicity processes in AD pathogenesis. Anti-oxidant and anti-amyloid therapeutics are the focus of current drug discoveries against these toxicity processes [83, 147].

### Protection against Oxidative Stress

Fullerene ( $C_{60}$ ), a nanotechnology building block [79,81,82], could be the base of neuroprotective compounds [26]. Fullerene molecule is composed of a three-dimensional array of carbon atoms, evenly spaced in a pattern similar to rhombuses on a soccer ball (Fig. 13) [124]. The biological applications of fullerene, including its antioxidant and free radical scavenger potentials, are due to its kind of chemical structure that allows it to be linked (to be functionalized) by several active chemical groups in a 3-dimensional orientation [55,79]. 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 [26,27]. Interestingly, the application of carboxyfullerenes blocked the A $\beta$ 42 induced neuronal apoptotic death [27].

Additionally, *fullerenols*, which are water-soluble hydroxyl functionalized derivatives of fullerene, have shown neuroprotective properties against A $\beta$ 42 [50].

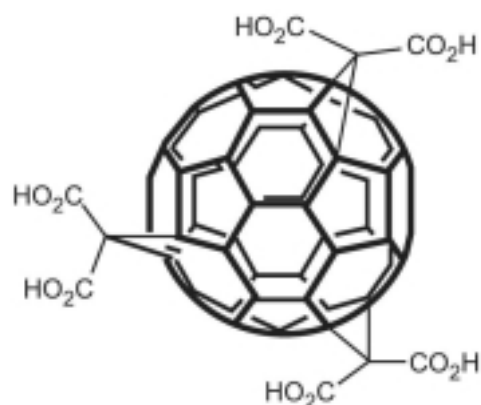


Fig. 13. Molecular structure of an antioxidant derivative of fullerene (C<sub>60</sub>): Functionalization of C<sub>60</sub> molecule with carboxyl groups, attached to cyclopropane carbons, accounts for its unusual antioxidant and free radical scavenger properties. Figure taken from [26].

Presumably, the neuroprotective effect of *fullerenols* is due to both antioxidant reactions and inhibition of A $\beta$ 42-induced Ca<sup>2+</sup> neurotoxicity [50]. Huang et al. validated the latter finding in their investigation into the effect of fullereneol-1 upon A $\beta$ -induced Ca<sup>2+</sup> influx in the cultured neurons [50]. Moreover, Dugan et al. have shown that fullerene poses complete neuroprotective properties against NMDA receptor mediated neurotoxicity [25]. NMDA receptor function is important to neuronal mechanisms of learning and memory. Altogether, applications of functionalized fullerene derivatives including carboxyfullerene and hydroxyfullerene (*fullerenols*), are promising in discovery of new drugs for AD; however further research on their pharmacodynamic and pharmacokinetic properties is necessary.

#### Anti-amyloid protections

Nanotechnology has recently offered some anti-amyloid neuroprotective approaches against the cellular and synaptic toxicity of oligomeric and fibrillar (polymeric) A $\beta$  species. The current ongoing nanotechnology research categories on anti-amyloid neuroprotective approaches are the following three: (1) Prevention from assembly of A $\beta$  monomers (2) Breaking and resolubilization of the oligomeric or fibrillar (polymeric) A $\beta$  species (3) Prevention from toxic effects of A $\beta$ .

An example for the *anti-assembly* strategy is the work of Ikeda et al. [51]. They designed an amphipathic nanogel to mimic the function of molecular chaperons. These nanogels, similar to natural chaperons, incorporate proteins and control their folding and aggregation [51]. In examining the described po-

tentials of nanogels against amyloidogenesis process, Ikeda et al. showed that nanogels could effectively incorporate A $\beta$  and inhibit the amyloidogenesis process (Fig. 14-C) [51]. The nanogel (hydrogel nanoparticles) designed by Ikeda et al. 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 [51]. CHP is formed from a backbone of polysaccharide and hydrophobic cholesteryl moieties (Fig. 14-A, B) [95]. It is noteworthy that the diameter of nanogels was 20–30 nanometers and each nanogel particle could incorporate 6–8 A $\beta$  molecules (Fig. 14-B, D) [51]. Generally, since this technique prevents assembly at the monomer level it prevents A $\beta$  from oligomerization. Therefore, anti-aggregation (i.e. anti-assembly) effect of CHP nanogels could be of therapeutic value for AD, by reducing the concentration of toxic A $\beta$  oligomeric species.

Podolski et al. demonstrated that the hydrated fullerene C<sub>60</sub> has anti-assembly effect on the fibrillization of A $\beta$ <sub>25–35</sub> fragment [103] by using transmission electron microscopy. The results of animal experiments for this effect of hydrated fullerene C<sub>60</sub> have suggested its potential to be used for producing new drugs for AD in human [103].

Recently, a multipurpose anti-amyloid strategy was suggested for dendrimers [62], which are considered as one of nanotechnology building blocks [79,81,82, 106]. Dendrimers are macromolecular structures with globular shape and a densely packed surface (Fig. 15-A) [62]. Their structure has offered them a number of biomedical potentials [82,93]. *Anti-assembly strategy* of dendrimers can be performed either via their binding with peptide monomers (Fig. 15-C1) or through blocking the end of protofibrils and fibrils (Fig. 15-C2) [62]. These anti-assembly effects of dendrimers take place at their higher concentrations. However, Klajnert and coworkers suggested that the higher concentrations of dendrimers will have toxic effect rather than therapeutic results [61]; because in higher concentrations, anti-assembly effect of dendrimers happens and prevents A $\beta$  fibrillization and thereby results in the accumulation of toxic low molecular weight A $\beta$  oligomers. Nevertheless, low concentrations of dendrimers are supposed to have therapeutic effects, according to their effect on lowering the oligomeric species lifetime. This is because low concentrations of dendrimers induce A $\beta$  oligomers to form less toxic fibrillar species [61].

Resolubilization of fibrillar amyloid species is the basis of some other nanotechnology research approach-

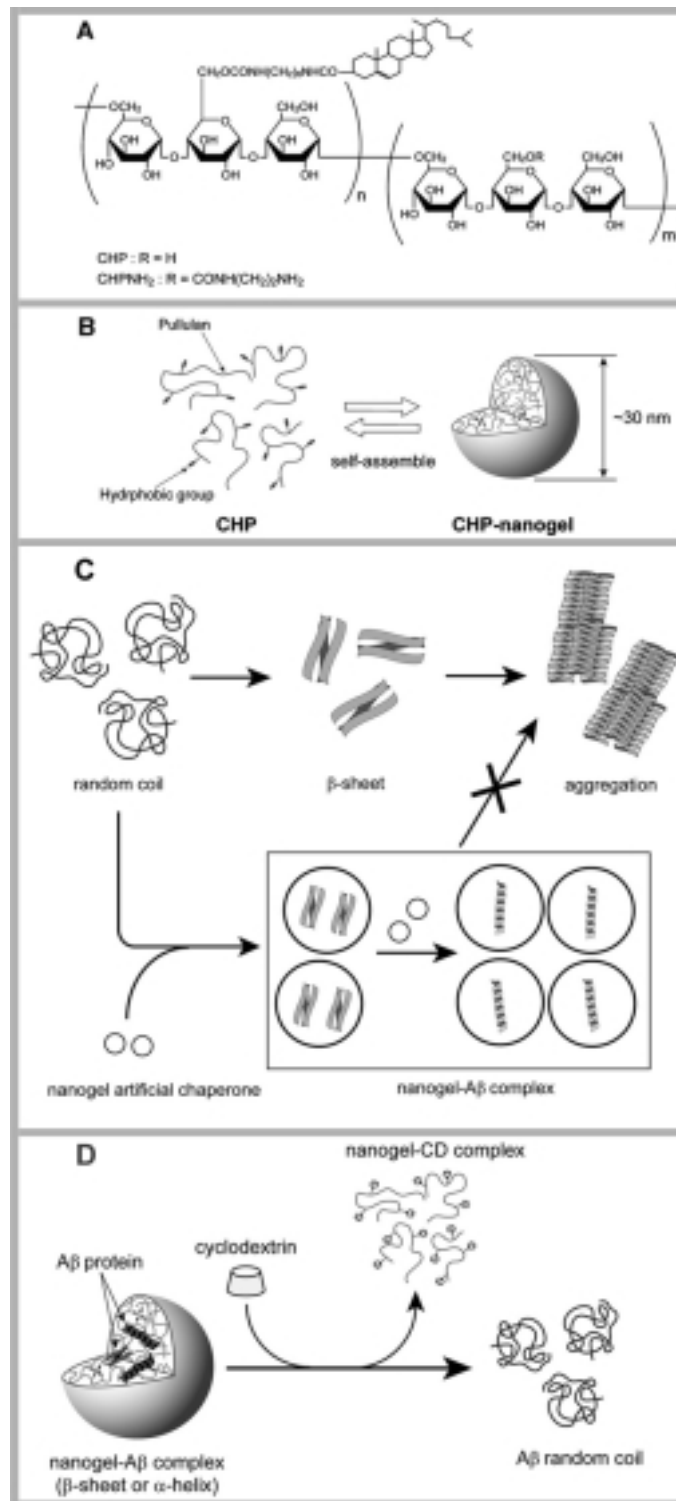


Fig. 14. Chemical structure of CHP and CHPNH<sub>2</sub>. (A) Schematic representation of nanogel formation. (B) Schematic diagram of the interactions between artificial nanoscale chaperon system and misfolded Aβ. (C) Refolded Aβ monomers are released after addition of MβCD. (D) Adapted from [51].

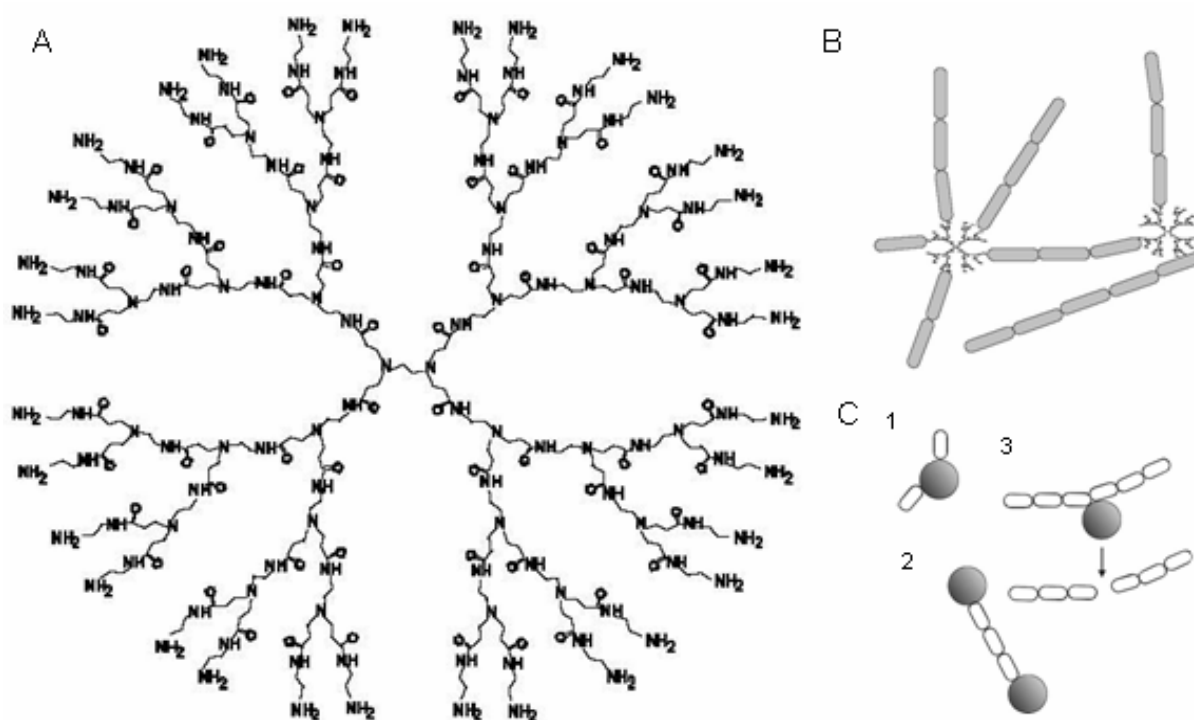


Fig. 15. Structure of generation 3 of polyamidoamine (PAMAM) dendrimers (A). Schematic presentation of interaction between peptides and low generation PAMAM dendrimers (B), the possible mechanisms of amyloidogenesis block by dendrimers (C): Inhibiting aggregation by binding to peptides (C-1); inhibiting elongation by blocking the free ends (C-2); breaking the fibrillar bonds (C-3). *A adapted from [61]; B and C [62].*

es on anti-amyloid protections. Recently, Kogan et al. utilized a combination of weak microwave fields and gold nanoparticles in their design for a remote amyloid dissolving technique. Their design was founded on dissolving  $A\beta$  aggregates and prevention from further aggregations through a highly concentrated thermal energy [63]. The thermal energy was produced from a low gigahertz electromagnetic energy source (microwave) by the gold nanoparticles, which are already attached to the specific target (i.e.  $A\beta$ ). Gold nanoparticles are selected for this experiment because of their nanometric size, high surface-to-volume ratio, biocompatibility, high electron density and mobility [63]. These properties make it feasible to provide a specific bond target with a selective supply of energy in a remotely controlled manner, and without any adverse effects on the molecular proximity. Each Au nanoparticle provides a dissipating power of  $10^{-14}$  J/s that allows them to break a fibril bond ( $10^{-20}$  J binding energy per bond) per microsecond without breaking covalent bonds, which are two orders of magnitude stronger [63]. However, careful consideration must be given to cytotoxic effects of  $A\beta$  oligomeric species. Therefore, 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  $A\beta$  oligomeric species following the breakdown of the fibrillar species. Considering the above-mentioned technical setting, the method of Kogan et al. seems to be advantageous for noninvasive investigation and manipulation of  $A\beta$  aggregates in AD.

Prevention from cytotoxic effects of  $A\beta$  is another prospect of nanotechnology for the *anti-amyloid approach*. Application of the modified dendrimers is a recent suggestion for this approach. Patel et al. demonstrated that dendrimers (both conjugated and unconjugated) could shield the cell membrane against  $A\beta$  membrane mediated neurotoxicity, which is due to  $A\beta$  electrostatic interaction with the cell membrane [99]. In addition, dendrimers can sequester the  $A\beta$  toxic species (Fig. 15-B) 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 [99].

Interestingly, Memantine, a newly FDA approved neuroprotective drug against AD pathogenesis [35, 83,107], is a derivative of adamantane (1-amino-3,5-dimethyladamantane), which is a diamondoid. Diamondoids are cage like saturated hydrocarbons, known as one of the nanotechnology molecular building blocks [79,81,82,106]. Nanotechnology molecular building blocks are used for building desired nano-objects with specific properties in a controlled and engineered manner [79,81,82,106]. Memantine, a derivative of adamantane, is beneficial for treatment of moderate to severe AD. Memantine could be prescribed if there is no therapeutic response to acetylcholine esterase inhibitors [35,107]. Presumably, the main mechanism of memantine is uncompetitive NMDA receptor antagonism, and therefore inhibition of excitotoxicity of excessive glutamate neurotransmissions [107].

### *Neuroregeneration*

The neuroprotection strategies cannot result in a complete cure for AD. This is because they are restricted to protecting neurons from further damage and degeneration. Therefore, only the progression of the disease lesion and symptoms will slow or ideally stop [12]. However, the degenerated nervous tissue remains and the resultant functional loss of the brain does not recover from these medications.

A very complex challenge of drug discovery for neurodegenerative diseases is designing methodologies for replacing the dysfunctional and degenerated nervous tissue with new and functional neurons. In order to restore the cognitive function of the AD patient's brain effective neuroregeneration must take place in the injured areas of the brain. In this regard, drug discovery in such disorders like AD must concern solutions for neuroregeneration as well [59].

Recent application of the stem cells technology in the problematic issue of neuroregeneration has suggested some solutions to reach a complete cure for AD [129, 141]. According to recent evidences, some specific regions of CNS possess neuronal regeneration potentials far more than the limited regeneration capacity of the other regions of the CNS tissue [137]. These regions (including the dentate gyrus of the hippocampus, the subventricular zone, the striatum and the substantia nigra) have a higher concentration of neuronal progenitor cells [137]. It is proposed that this potential could become the basis of neuroregeneration strategies in the treatment of neurodegenerative disorders [137]. However, the sufficiency of the innate neuronal progenitor

cells for treatment of neurodegenerative disorders is questionable [146]. On the other hand, replacing cells that are derived from "embryonic stem cells" have been suggested as a more reliable strategy for regeneration of the CNS tissue in senile neurologic disorders, such as AD and Parkinson's disease [146].

Currently, in the area of neuroregeneration, application of nanotechnology on axonal regeneration after traumatic insult is promising [28,124]. In addition, nanotechnology is helping to increase the performance of stem cell technology in several areas [3,125]. However, at present studies aimed at overcoming the challenges of stem cell therapy for degenerative disorders such as AD are rare. Attempts that take advantage of the potentials of nanotechnology may surmount the stem cell therapy challenges for AD, and therefore may bring us to a complete cure for this debilitating disease in the future.

### *Drug delivery systems*

Generally, penetration of drugs into the brain is strictly limited. Without exceptions, large pharmaceuticals are unable to cross blood brain barrier (BBB). More interestingly, only less than 2% of small molecules can cross BBB [97]. All therapeutics aimed to a target in the brain must have a group of specific characteristics for crossing BBB, including small size, lipophilicity, and compactness (measured from polar surface area) [89].

Nanotechnology can improve the efficacy of some potential therapeutic agents for CNS diseases including AD by facilitating their delivery across BBB. For instance, nanoparticle technology applies solid colloidal nanoparticles (ranging from 1nm to 1000nm in size) to disguise the limiting characteristics of potential therapeutic agents in order to enable their transport across BBB [75]. Moreover, nanoparticle technology retards drug release in the brain and decreases the peripheral toxicity [75].

Recently two studies have prepared a nanoparticle carrier for the model drugs thioflavin T and thioflavin S [48,123]. This nanoparticle carrier is composed of a polystyrene core and a PBCA [poly (butyl-2-cyanoacrylate)] shell with a diameter of 90–100 nm. The gradual enzymatic degradation of PBCA shell allows a controlled and long-term drug delivery in the brain [48,123]. The results show selective targeting of fibrillar A $\beta$  after intracerebral injections to APP/PS1 mice with age dependent  $\beta$ -amyloidosis. These studies could be suggestive of potential benefits of core-shell nanoparticles for prevention of A $\beta$  accumulation or for

acceleration of  $A\beta$  clearance, as well as sending  $A\beta$  sensitive detectors beyond BBB [48,123].

Some investigators have recently designed other nanoparticle carrier systems for metal chelation agents. Metal chelation therapy is a therapeutic approach for the AD that reduces the cellular oxidative stress [74]. This approach is based upon the effects of dis-homeostasis of metallic ions on triggering the oxidative stress in the AD pathogenesis. In studies undertaken by Liu et al. an efficient chelator nanoparticle system (CNPS) was prepared [74]. In these studies, Desferrioxamine, an FDA approved metal chelator, was used. Nanoparticles were conjugated with Desferrioxamine through an amido bond between a primary amino group in the chelator and a carboxyl group on the nanoparticle surface [74]. Liu et al. demonstrated that the chelation effect of metal chelator was retained after formation of CNPS. This work shows other advantages of nanoparticle technology in addition to feasibility of carrying iron chelators across BBB. For instance, it was suggested that one reasonable mechanism for nanoparticle mediated BBB transport could be the ability of nanoparticles to preferentially bind to Apo E [73]. On the other hand, it was shown that the iron chelated 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 [73]. Similarly, in the study of Cui et al. [20] d-penicillamine, an FDA approved drug for chelation of copper in Wilson's disease, was examined as a metal chelator in AD. They conjugated d-penicillamine with the nanoparticles containing MPB-PE and PDP-PE. This conjugation enabled the traverse of d-penicillamine through the BBB in spite of its highly hydrophilic properties. This study showed the ability of d-penicillamine and EDTA for chelating copper and disolubilizing  $A\beta_{42}$  [20]. Interestingly, it was demonstrated in this study that 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 [20].

As we reviewed before, a large portion of AD pathogenic mechanism happens inside the cells. These intracellular mechanisms mainly include oxidative stress, the formation of PHF and NFTs and intracellular oligomerization of  $A\beta$ . Generally, if a therapeutic

agent is designed to interrupt such intracellular pathologies in the brain tissue, it must be delivered into its target cells in addition to being delivered to the brain tissue. Nevertheless, there are a set of obstacles against the entry of a therapeutic agent into cytoplasm. Recently, B.T. Shea et al. have designed an especial nanosphere to deliver vitamin E to human neuroblastoma cultured cells, which have been already exposed, to  $A\beta$  [118]. In this study polyethylene glycol (PEG)-based nanosphere was used for encapsulation of vitamin E in order to facilitate its entrance to the cytosol. Results indicated an improvement of vitamin E efficacy against  $A\beta$  induced oxidative stress [118].

## CONCLUSIONS

AD is the primary common dementia syndrome world wide, with an astronomical devastating socio-economic burden on society. Solving the major problems of early diagnosis and effective cure for AD requires interdisciplinary research efforts. Research on the basic pathogenetic mechanisms of the disease has provided new insight for designing diagnostic and therapeutic methods. However, the need for further studies on the exact root causes of AD is still palpable.

### *Studies into molecular mechanisms*

Applications of nanotechnology methods in studies on the molecular mechanism of AD are promising:

- (1) Through AFM, studies on a number of direct *in vitro* observations of the main pathological structures of AD,  $A\beta$  species and  $\tau$ -protein are accomplished. Using the AFM tapping mode operation and applying multi-wall carbon nanotubes as the AFM tips has enabled high-resolution visualization of  $A\beta$  and  $\tau$  during their related chemical processes [142]. The results illuminate the mechanisms of these key processes in AD and the structural properties of their involved molecules.
- (2) Through single-molecule FRET (fluorescence resonance energy transfer), which is applicable both *in vivo* and *in vitro*, studies into the sub-molecular and intermolecular interactions of abnormal protein species in AD pathogenetic process [112,117] has been achieved.
- (3) In addition, using NanoSIMS microscopy has enabled investigation into subcellular chemical elemental changes during the AD pathogenesis [105].

However, few published *in vivo* studies have investigated the molecular processes of AD pathogenesis. It is expected that other nanotechnology methods will accelerate such studies in the future, for example through biologically stable markers (e.g. functionalized quantum dots) transcended beyond the BBB.

#### Early diagnoses

Development of a nanotechnology approach for the early diagnosis of AD is quite promising. This is because of the potential for development of multifunctional nano-sensors for simultaneous detection of ultra low concentrations of different biomarkers. The lack of one specific biomarker for AD and the ultra low concentrations of the group of AD biomarkers highlight the need for such multifunctional nano-sensor.

Lambert et al., performed *in situ* AFM studies on ADDL (amyloid derived diffusible ligands), a synthetic  $A\beta$  derivative produced from  $A\beta$  monomers. They emphasized on the possibility of *in vivo* existence of ADDL [67]. There is now a general consensus on the neurotoxicity of ADDLs [17,37,67,139], and it has become the choice biomarker in recent nanodiagnostic studies for AD [36,41].

The CSF (cerebral spinal fluid) analysis for the AD biomarkers has a higher degree of accuracy and can show brain tissue damages earlier than the known plasma biomarkers associated with the AD. However, the *in vitro* CSF analysis requires the invasive procedure of lumbar puncture for obtaining a sample. In this regard, a common deficiency of the proposed nanotechnology methods for detection of AD, thus far, is that they are limited to *in vitro* investigations of the CSF samples. Such nanotechnology methods include the bio-barcode assay and the LSPR techniques.

Nanotechnology has the potential to provide us with other tools that may be applicable for ultra sensitive detection of the AD biomarkers in plasma (e.g. nanomechanical cantilevers *in vitro* and quantum dots *in vivo*) or for *in vivo* investigations following parenteral administration, and thereby providing minimally invasive methods for early diagnosis of AD.

An ultimate goal for very early detection of an underlying AD pathology would be the development of a safe and implantable nanoscale biosensor for prolonged monitoring of AD biomarkers in the CSF. Such a sensor must be able to transmit any biomarker detection event to an external device that records the transmitted signals and reports an estimated amount for the concentration of AD biomarkers in the CSF. Of course, in order to send such biosensor to a place exposing with CSF, it is necessary to design noninvasive approaches.

#### Effective treatment

The current medical approach has been to lower the symptoms of the AD. Although, the resultant therapeutics are unable to resolve the root causes of the disease [49,86], they have offered many economic benefits. However, there are still possibilities for designing more potent but cost effective diagnostic and therapeutic approaches. These possible approaches could become more efficient if taking advantage of the nanotechnology potentials and the vast amount of information currently available about the molecular causation of the disease. Targeted drug delivery and prolonged drug release are the two major potential benefits of application of nanotechnology in designing more potent therapeutic agents for the AD.

Designing especial nanoparticles targeted to molecules with more initial role in AD pathogenesis is of high importance in effective drug discovery for AD (Fig. 12). Some recent studies have taken advantage of nanotechnology for a neuroprotective approach against AD pathology. For instance, neuroprotective effects of some simple nanomaterials including fullerenes, dendrimers and diamondoids (the root of FDA approved memantine) [25–27,61,103,106], and complex nanosystems like nanogels (as artificial chaperons) [51] have been evaluated and confirmed in these studies.

*En route* to making AD a curable disease, we must design drugs and methods for CNS regeneration. It must become possible to replace the dead nervous tissue in order to regain the brain function and completely cure the afflicted patients. We are still at the very early stages of such an ambitious project. However, the increasing abilities offered by the combination of nanotechnology and some other novel approaches like stem cell technology could bring about a promising cure for AD.

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## ABBREVIATIONS

aM	attoMolar ( $10^{-18}$ Molar)
AD	Alzheimer's disease
ADDLs	A $\beta$ -derived diffusible ligands
AFM	atomic force microscopy
Apo A-I	Apolipoprotein A-I is the main protein component of high-density lipoproteins
Apo E	ApolipoproteinE
A $\beta$ PP	amyloid- $\beta$ protein precursor
A $\beta$	Amyloid- $\beta$ peptide
A $\beta$ 40	the 40-residue form of Amyloid- $\beta$ peptide
A $\beta$ 42	the 42-residue form of Amyloid- $\beta$ peptide
BACE	$\beta$ -secretase
BBB	
BCA	bio-barcode assay
C <sub>60</sub>	fullerene
CdSe	cadmium selenide
CdS	cadmium sulfate
CHP	cholesterol bearing pullulan
CNPS	chelator nanoparticle system
CNS	central nervous system
CNT	carbon nanotubes
CSF	cerebrospinal fluid
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked ImmunoSorbent Assay
fM	femtoMolar ( $10^{-15}$ Molar)
FDA	Food and Drug Administration
FRET	Fluorescence resonance energy transfer
$\lambda_{\max}$	wavelength of maximum extinction in the spectrum of a given substance
LSPR	localized surface plasmon resonance
MCI	mild cognitive impairment
MMP	magnetic micro-particle
MPB-PE	1,2-Dioleoyl-snglycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide]
M $\beta$ CD	methyl- $\beta$ -cyclodextrin
nm	nanometer scale ( $10^{-9}$ meter)
NFT	Neurofibrillary tangles
NMDA	N-methyl d-aspartate
PBCA	poly(butyl-2-cyanoacrylate)
PCR	polymerase chain reaction
PDP-PE	pyridyldithio-propionyl-phosphoethanolamine
PHF	Paired Helical Filaments
QD	quantum dot
SFM	scanning force microscopy
SIMS	secondary ion mass spectroscopy
SNP	silver nanoparticle
STM	scanning tunnel microscopy

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