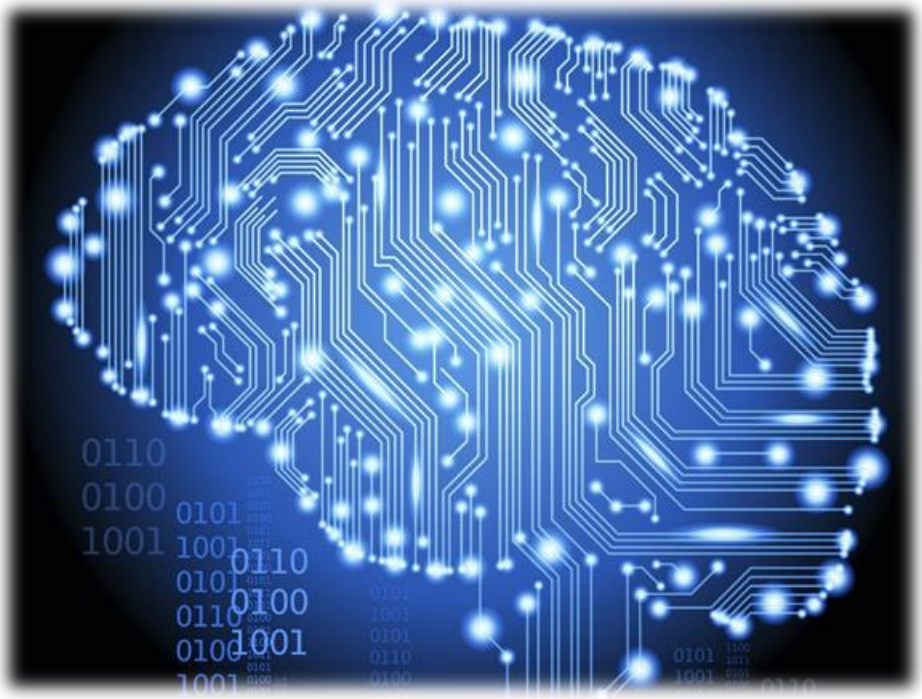
NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS AND ALZHEIMER'S DISEASE



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1. ABSTRACT

With an ever increasing occurrence of Alzheimer's disease (AD) in an equally growing elderly populace, AD has become a general health problem. With currently only conventional drugs being prescribed for symptom management instead of treatment, a quest for new approaches has the scientific world captivated. One of these new approaches is the application of nanotechnology. Despite extensive research in nanotechnology based drug delivery systems (NTDDS) it's application in clinical activities is lacking compared to other diseases such as cancer. With this manuscript I want to highlight several different NTDDS, their advantages and disadvantages, their possible usability within AD and I also highlight various means of traversing the blood-brain barrier. Lastly I will briefly mention promising therapeutic treatments for AD.

2. INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia within the elderly populace and continues to increase. AD is a progressive neurodegenerative disease. It's most noticeable symptom is memory loss however, impaired communication, behavioural changes, thinking disorders and ill orientation and communication are also included symptoms with AD [1]. Not only does it affect the patients but also family and caretakers. This all stresses the healthcare economics to the maximum with expensive treatments and increasing financial burdens for caregivers or hospitalized cases. This all goes along with a yearly increase in AD patients [2], therefore it is vital to subdue the prevalence of AD.

With the onset of AD being able to occur 20 years or more prior before the indication of the first symptoms, it is known as a slow and gradual disease [3]. AD can be inherited, which is defined as familial AD, which makes up 5-10% of all AD patients. A sporadic occurrence of AD defines 70% of the total AD patients. While familial AD can be inherited trough mutations in amyloid precursor protein (APP), Presenilin 1 (PSEN1) and Presenilin 2 (PSEN2), sporadic AD is a combination of factors e.g. environmental factors and choice in lifestyle [4]–[6]. There are currently two molecular pathological hypotheses to AD.

2.1. ALZHEIMER'S DISEASE: TWO MOLECULAR PATHOLOGICAL HYPOTHESIS

2.1.2. AMYLOID CASCADE HYPOTHESIS

APP is a transmembrane glycoprotein that is present in a multitude of cell types. The APP proteolysis is being regulated by 3 secretases, α-secretase, β-secretase and γ-secretase [7]. The amyloid cascade hypothesis consists of the β-secretase cutting the APP resulting in the

formation of amyloid-β (Aβ) $_{1\text{-}40/1\text{-}42}$ proteins. These Aβ proteins will form oligomers and tend to aggregate into plaques. The accumulation of oligomers and plaques is neurotoxic, inducing neurodegeneration and eventually leading to AD[7]–[9]. In vivo evidence also supports a role of Aβ oligomers who induce a loss of long-term potentiation (LTP) and disruption of synaptic plasticity within the hippocampal region. However with treatment of γ-secretase inhibitors the formation of oligomers is fully prohibited [9]–[11]. Further evidence also indicates Aβ might support a role in the formation of microtubule associated protein (MAP) tau tangles and/or vice versa [12]–[15].

2.1.3. TAU HYPOTHESIS

As just stated, MAP tau can form tangles. MAP tau consists of 3 different proteins: MAP1A, MAP1B and MAP2 and are responsible for the assembly and stability of microtubules [16]. The biological activation of tau is regulated trough phosphorylation by tau protein kinases. With AD tau is hyper-phosphorylated which impairs its functions and causes microtubule instability and tau oligomer formation, leading to an accumulation of neurofibrillary tangles (NFT) and eventually to neurodegeneration [16]–[18].

2.2. SYNAPTIC CONSEQUENCES

As previously stated Aβ induce a loss in LTP and causes a disruption in synaptic plasticity. Aβ stays in equilibrium between oligomers and plaques. The Aβ oligomers bind to several cell-membrane receptors (e.g. PrP(C), NMDA) and can induce a blockage of LTP where as the role of plaques is currently debatable [9], [11], [19]–[21]. The Blockage leads to dendritic retraction of pyramid cells which leads to an impaired spatial memory [9], [10].

Aβ also interacts with the N-methyl-D-aspartate (NMDA) receptor, which is essential for synaptic plasticity and memory. Some studies suggest a major role of GluN2B in LTD and loss of neuroplasticity [22], [23]. A prolonged exposure of the NMDAR to Aβ significantly decreases the influx of Ca^{2+} through the NMDAR. The reduction of Ca^{2+} influx causes mitochondrial dysfunction and together with a decrease in NMDAR activity an association with the production of reactive oxygen species (ROS) was determined [24]–[26]. ROS eventually leads to apoptosis of the neurons.

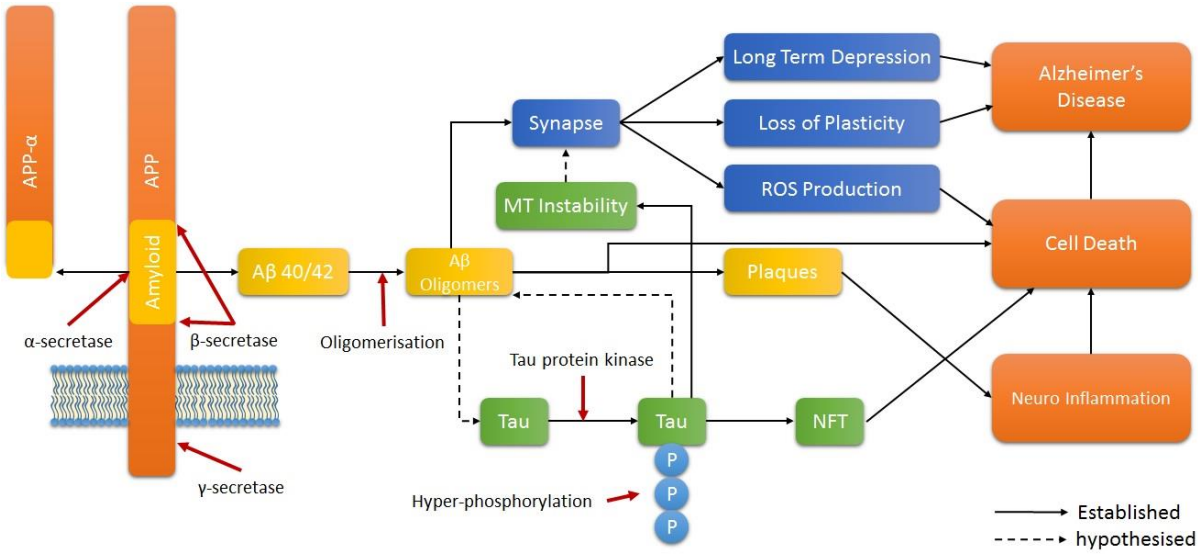

Figure 1. Schematic overview of established and hypothesized AD progression. APP= Amyloid precursor protein, Aβ = amyloid-beta, MT = Microtubule, ROS = Reactive oxygen species, NFT = neurofibrillary tangles.

3. Blood-Brain Barrier

As stated above, all symptoms and progress of AD act in the central nervous system (CNS). Currently there are several drugs (e.g. rivastigmine, donepezil, galantamine) that have impact on AD progression and symptoms. These drugs are usually prescribed as oral formulations and for the drugs to reach the central brain they have to be given in high doses, otherwise its functionality is obsolete. There are several factors to overcome (oral absorption, hepatic metabolism, distribution) and the biggest challenge is to traverse the blood brain barrier (BBB) to obtain a therapeutic significance. Due to the high doses given to obtain this therapeutic significance a high occurrence of adverse effects have been established. This is due to the side effects the drugs have on the peripheral tissues [27], [28]. Lowering the doses increases the quality of life but decreases the therapeutic effects thus new ways to easily bypass the BBB are needed. The BBB consists of endothelial cells (EC), astrocytes, tight junctions (Tj's) and adhesive junctions (Aj's). The BBB is the regulator of all transport between blood and CSN and has a surface area of 12-18 m^2 in adult brains. Aj's hold the EC together by giving structural support to the tissue and are essential for the formation of Tj's who form the barrier of the para-cellular pathway [29]–[31]. The para-cellular pathway usually facilitates the transport of macro- and polar charged molecules however due to the Tj's this is impossible [30]. A wide range of lipid soluble molecules can diffuse passively trough the BBB however this also occurs in peripheral tissue [32] so for a lipid soluble drug an equilibrium must be found between BBB and peripheral diffusion. To fully optimize the drugs effect in the CNS and lower the occurrence of side effects, a possible new approach has been found in nanotechnology-based drug delivery systems (NTDDS).

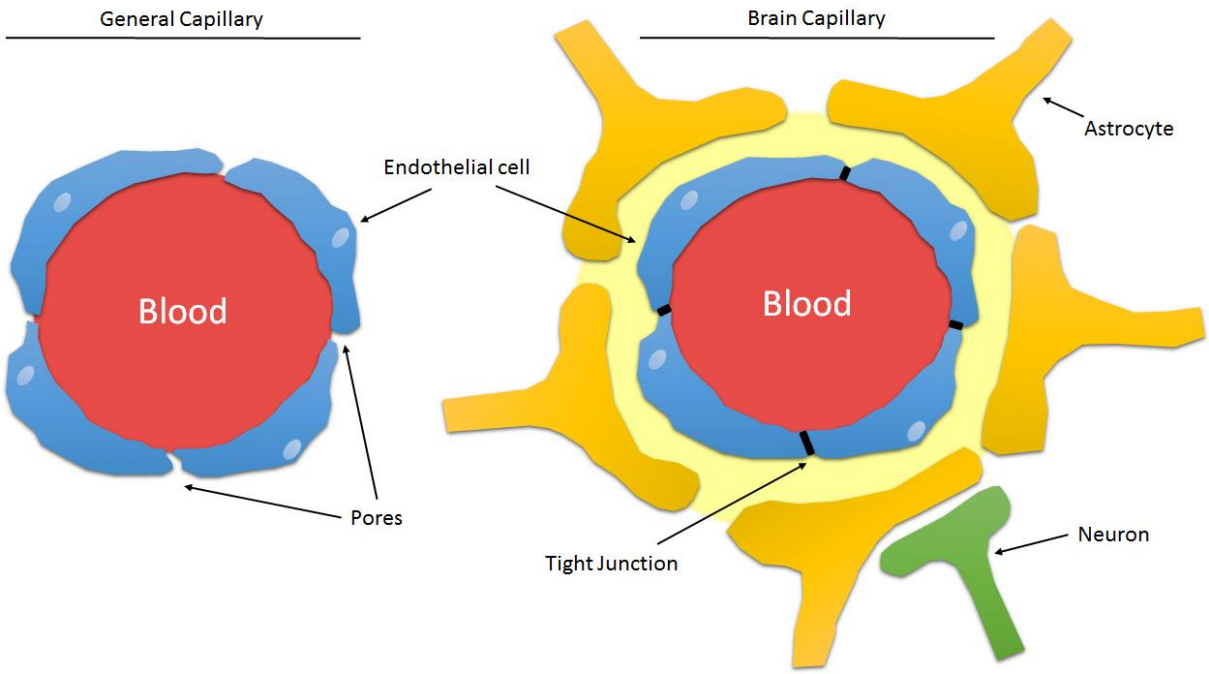

Figure 2. The cell association of a general capillary (GC) vs BBB.

4. NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS

4.1. LIPOSOMES

Phospholipids are part hydrophilic and part hydrophobic so when in contact with water, the interactions with the hydrophobic, hydrophilic sites and the water will initiate a self-assembly of lipids which will often create the form of a liposome. Liposomes have an aqueous core, which is surrounded by a bi-layer of phospholipids. The name liposome comes from the Greek words “lipos” and “soma” which mean fat and body respectively [33], [34].

When first discovered, solely natural lipids were used in the process of creating liposomes. As of present day, a combination of natural and synthetic lipids can be created and with the ability to attach surfactants, specific targeting liposomes can be created. The size of the liposomes can vary but to have a therapeutic effect a range of 50-450 nm is recommended [35], [36].

Since the first discovery, liposomes have always been recognized as drug-delivery vesicles, this due to their nature of being biocompatible and biodegradable [37]. Aside from their biocompatibility, liposomes can incorporate both hydrophilic and hydrophobic drugs [38]. With the encapsulation of the biologically active drugs, they are protected from any form of degradation or inactivation. Therefore the use of liposomes lowers the required dosage of drugs while still maintaining therapeutic significance and decreases the occurrence of adverse effects [39].

There are a multitude of liposomes created to administer medicine in the central brain. Asmari et al. created a poly ethylene glycol (PEG)-ylated liposome containing Donepezil [40],

liposomes coated with cell penetrating peptides (CPP) containing rivastigmine were generated by Yang et al [41], Li et al. produced propylene glycol (PG) coated flexible liposomes containing galantamine [42] and Mourtas et al. created curcumin conjugated nanoliposomes [43] to give a few examples. While PEG, CPP and PG coated liposomes were created for a longer half-life, BBB penetration and drug delivery, curcumin conjugated nanoliposomes was created to function as a drug [40]–[43]. All studies were performed *in vitro*.

4.2. SOLID LIPID NANOPARTICLES

Solid lipid nanoparticles (SLN) are nanoparticles (NPs) with a solid lipid core and with a mono-layered phospholipid exterior. This core is solid at both room and body temperature. Depending on the composition if the SLN, it could exhibit a low toxicity while maintaining the same properties as other NPs (e.g. Drug carrying, controlled drug release, targeting) [44]. A disadvantage is that only lipophilic drugs can be incorporated due to the lipid core however SLN provide a better colloidal stability and thus can create an opportunity for a sustainable drug release system [45]. There are currently several methods to prepare SLN however the warm micro emulsion technique is favourable. This technique focusses on the interaction between lipids and water which allows a great flexibility in design of the characteristics of the finalized product [46]–[48].

Bondi et al. reported that SLN overcome the BBB trough endocytosis and accumulate in the CNS due to their lipophilic structure and that due to their small size, the SLN can be injected intravenously bypassing not only oral adsorption but also avoiding macrophage uptake [49]. While Gobbi et al. suggested an imaging probe or Aβ targeting molecule application for SLN [50], Misra et al. created a SLN that incorporated galantamine which demonstrated a significant memory restoration after oral administration *in vivo* [51].

4.3. POLYMERIC NANOPARTICLES

Polymeric nanoparticles (PNP) are carriers into which drugs can be incorporated in a solid state or in solution through covalently binding or absorbed and linked to the surface [52]. There are several different techniques to create polymeric nanoparticles where some substances require a polymerisation step while others can directly form a macromolecule or polymer [53]. Depending on the composition of the polymer and drug features, difference in half-life time, passing the BBB and difference in size can be made.

Several studies created poly lactic-co-glycolic acid (PLGA) polymers which had a high incorporation efficiency and a stable drug release of galantamine or donepezil while the fabrication of the NPs different techniques were used (solvent emulsification diffusion-evaporation, nano-emulsion templating techniques, resp.) [54], [55]. Khalil et al. created PEG-coated PLGA in combination with PLGA NPs using another technique (single-emulsion solvent-evaporation technique) and determined that PEG could increase curcumin release efficiency [56]. Joshi et al. fabricated a PLGA-poly butyl cyanoacrylate (PBCA) polymer by nano-emulsion and emulsion polymerisation techniques respectively to create a PLGA-PBCA PNP with a

sustainable release of rivastigmine [57]. Chaudhari et al. created a PEGylated PBCA polymer however they concluded that the need for an easy and reliable method of PEGylating PBCA is required in order become a promising PNP [58].

4.4. DENDRIMERS

Dendrimers (dendron = tree, meros = part) are tree-like molecules, which have a single core that branches out multiple times. There are two main fabrication methods for dendrimers, the diverging and the converging methods. With the diverging method a core molecule is being branched out by branching units. Depending on the variegation of branching units the molecule can be built up until steric hindrance prevents further attachments. With the converging method works in an opposite manner. First the end groups are fabricated and then the skeleton is constructed towards the core [59], [60]. As previously stated the oligomerisation of Aβ is the onset of AD with NFT and plaque formation. What would be a way of action against AD development is to prevent the oligomerisation of Aβ trough molecules that disrupt the structure.

Sorokina et al. generated cationic pyridylphenylene dendrimers and showed that these dendrimers partially disaggregated Aβ and disrupt further Aβ aggregation [61]. Klajnert et al. created 3rd, 4th and 5th generation polyamidoamine (PAMAM) dendrimers in which they showed that the higher generation (more layers is higher generation) had a larger level of inhibition compared to lower generations. They also showed that the higher generation were more effective at disrupting existing fibrils [62]. Benseney-Cases et al. also created PAMAMs and showed inhibition of Aβ aggregation and other prion diseases. However due to the cationic charge of the PAMAM they tend to be toxic so Klajnert et al. fabricated biocompatible glycodendrimers which showed similar anti-amyloidogenic properties compared to PAMAM [62]. It has been established that Aβ binds with a relatively high affinity to sialic acid clusters on cell membrane and that removal of these clusters decreases Aβ toxicity [63], [64]. Thus Patel et al. manufactured sialic acid conjugated dendrimers and proved that an increase in sialic acid could trap more Aβ but also increased the toxicity of the dendrimer [65].

4.5. MICRO-DRUGS

Some drugs are able to penetrate the BBB without the need for NPs. Lithium (Li) salts for instance, are administered for treatment of psychiatric disorders [66]. Evidence has shown that Li increases neuronal viability through several mechanisms (e.g. inhibition of apoptosis, synthesis of neurotropic factors, regulation of autophagy and that Li attenuates Tau tangle formation and prevents cell death and neurotoxicity due to Aβ oligomer exposure [67]–[69]. Further evidence also suggests that Li reduces glycogen synthase kinase 3B (GSK3B) enzyme, which leads to a reduction of GSK3B degradation. An increase of GSK3B degradation leads to pathological AD symptoms and eventually to Aβ accumulation and Tau hyper-phosphorylation [67], [70], [71]. However several clinical studies suggest that Li has no significant effect on AD patients cognitive abilities or even increase the risk of dementia [72]–[75].

Cannabidiol (CBD) is a substance, found in marijuana, which has several positive properties (e.g. anti-epileptic, anti-inflammatory, anti-psychotic) and evidence also supports a neuro protective role of CBD[76], [77]. Furthermore CBD reduces the Aβ induced neuro-inflammation and promotes neurogenesis and APP ubiquination through PPAR involvement [78]–[80]. However further in depth research is necessary in order for CBD to become a potential AD drug.

Non-steroidal anti-inflammatory drugs (NSAIDs) are immune system modulators (e.g. Ibuprofen, Aspirin), which could potentially reduce the risk of Aβ induced neuro-inflammation [81]. NSAIDs action is mediated trough the inhibitory effects on cyclooxygenase (COX) activity [82]. Apolipoprotein E (APOE) is linked to AD, specifically APOE ε4, and is susceptible for COX2 though, NSAIDs and selective COX2 inhibitors did not significantly reduced the dementia [83], [84]. Interestingly however is that with the use of a non-selective COX inhibitor protection of AD onset is revealed [84]. Several studies suggested that long-term usage of NSAIDs reduced the occurrence of AD [85]–[89], however no apparent advantage between Aβ lowering and non Aβ lowering NSAIDs were found [88], [89].

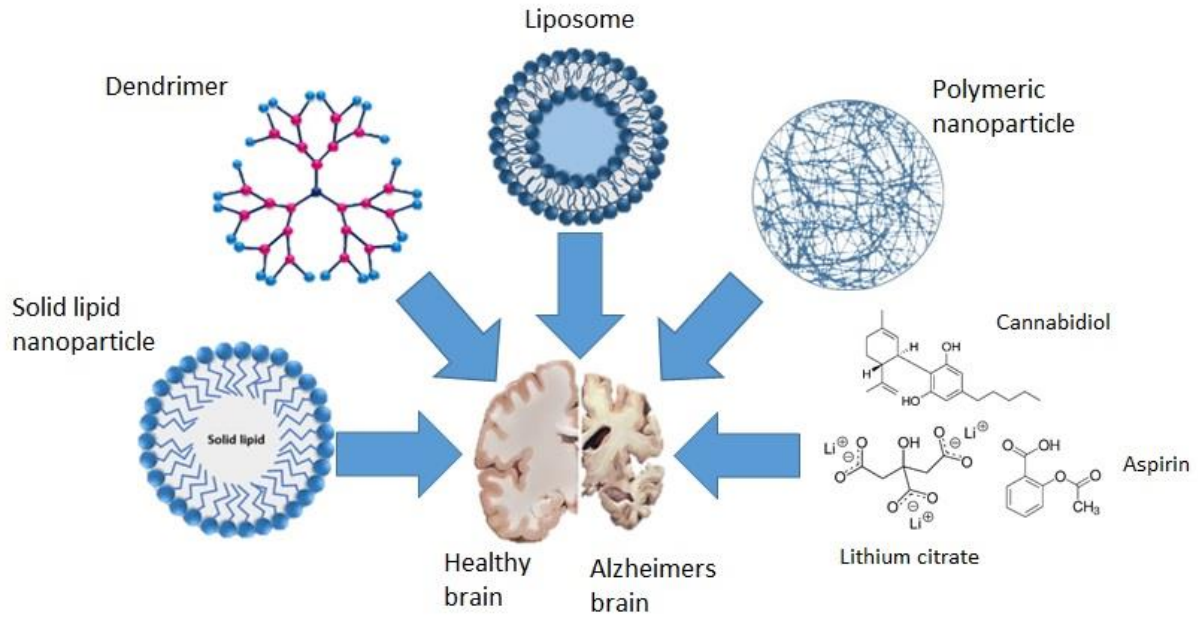

Figure 3. Representation of different types of nanoparticles. Partially derived from Wen et al. [90] *and modified.*

5. ADVANTAGES AND DISADVANTAGES OF THE DRUG DELIVERY SYSTEMS

Nanoparticle	*Advantages*	*Disadvantages*
Liposomes	good loading of lipophilic and lipophobic drugs	poor *in vivo* stability
	Bio-compatible and non-toxic due to phospholipid nature	production could create cytotoxic side-products
	protects incorporated drugs from degradation	production is a secure procedure
	simple preparation with a high entrapment yield	
	save and scalable techniques for industrial production	
Solid lipid nanoparticles	good loading of lipophilic drugs	poor loading of lipophobic drugs
	low toxicity, controlled drug release	Drug incorporation is limited to lipophilic drugs, lipid type, surface properties and production method
	protects incorporated drugs from degradation	Metal contamination may occur upon using ultrasound
	easy and low costs with production by ultrasound	production by emulsification-evaporation may result in toxicity
	easy surface modification	
	lipophilic nature facilitates endocytosis trough BBB	
Polymeric nanoparticles	Good stability	polymers may be toxic and have low degradability
	Bio-compatible, biodegradable, low toxicity and immunogenic response	difficult in modification and handling
	controlled drug release	in absence of surface modification, PNPs have low BBB penetration
	PNPs can be easily produced	
	PNPs of lipophilic nature have long half-time	
Dendrimers	High drug loading capacity	Polymers may have possible toxicity
	offers capability in imaging and drug delivery	
	size, molecular composition and properties can be easily controlled	
Micro drugs	Very drug specific	Very drug specific

Table 1. An overview of the advantages and disadvantages of nanoparticles.

6. Crossing the Blood-Brain Barrier

6.1. Solute carrier transport

Because of tight junctions, many necessary polar nutrients (e.g. glucose, amino acids) cannot traverse the BBB via para-cellular diffusion thus a different approach has to be made. This approach is the solute carrier (SLC) transport. The brain EC expresses a variety of transport proteins for solutes and nutrients. Some of these proteins are polarized and when expressed, are either on the luminal or abluminal membrane while some are on both sides of the EC [91]–[93]. The orientation of these transporters results in a preferential transport of solutes and nutrients form blood to brain and/or vice versa.

GLUT-1 is a glucose SLC transporter, which is present on both luminal and abluminal membranes and thus facilitates glucose transport from blood to brain and vice versa. The LAT2 transporter is different. LAT2 transports a few amino acids facilitative and bi-directional but several amino acids are sodium-dependent when transported from brain to EC. Other transporters are sodium-dependent when transporting from the blood into the EC [29].

6.2. ATP-binding cassette transport

Comparing lipophilicity of solutes and drugs with BBB penetrance a lower rate of penetrance is detected than expected. These lipophilic substances are being transported from the blood to the brain and vice versa via ATP-binding cassette transporters (ABC) [94]. Increasing the lipid solubility of drugs and NPs may provide better diffusion from blood to brain however, if the drug or NPs become to lipophilic they might function as ABC efflux substrates [94], [95]. The ABC transporter family contain 48 members and on basis of homology are grouped into 7 sub-families (ABC A-G) [96]. The main function of these ABC transporters is as ATP dependent efflux pumps for lipophilic substances from the brain and CNS into the bloodstream. This also functions as a neuroprotective role due to the efflux of possible neurotoxic and xenobiotic molecules [97]. A ABC transporter is P-glycoprotein (Pgp). Pgp is found in several excretory capillaries (e.g. BBB, kidney, liver) and is responsible for excretion of bile and urine and acts as a barrier for xenobiotics [98]. Study has shown that a defect in Pgp, an accumulation of Aβ disposition occurs however, upon restoration of the Pgp a decrease in Aβ was established [99], [100]. Several other ABC transporters (e.g. ABCA1, ABCB2, ABCG2) are found to be associated with Aβ excretion [101]. Recent studies provided that ABCA7 is up regulated in AD patients and Holton et al. found that the minor allele of ABCA7 is associated with age of onset and the duration of AD thus can possibly provide further insights in AD progression [102], [103].

6.3. Transcytosis

There are two ways of transcytosis that allow macromolecules to pass the BBB, receptor mediated (RMT) and adsorptive mediated transcytosis (AMT). With RMT macromolecules binds to specific receptors on the EC which then triggers an endocytotic effect. This effect causes the internalization of the molecule and receptor and forms a vesicle. This vesicle is then routed across the cytoplasm and exocytosed into the opposite side [104], [105]. The

transferrin receptor (TfR) is a receptor, which can be implemented in transcytosis of nanoparticles. Coating these NP with either transferrin of transferrin receptor antibodies allows the transcytosis of these NP [105]–[109]. There are several other protein receptors capable of RMT thus extensive research is required to possibly optimize NP uptake trough RMT [110].

AMT requires an excess of positive charge on a molecule, which makes it cationic, in order to achieve transcytosis. Interaction of the cationic molecule with cell surface binding sites initiate endocytosis and subsequently transcytosis [111]. EC have a natural negative charge barrier on their surface due to a physiological pH provided by the glycocalyx. Evidence provides that both RMT and AMT uptake is concentration- and time dependent and that both processes require energy [112].

6.4. PARA-CELLULAR PATHWAY

As previously stated, a para cellular pathway is no option due to the Tj's. However there are several methods to open up the Tj's. Bradykinin is an inflammatory mediator which dilates capillaries, lowering blood pressure, and evidence shows bradykinin also increases permeability of the BBB [113]. Bradykinin binds to the B_2 receptor and a second messenger system is activated. This causes a relaxation, trough cellular Ca^{2+} uptake, of the Tj's [114]. Unfortunately Bradykinin only has a half-life of several seconds however, there is an agonist labradimil that has significant longer half-life only a lower affinity to B_2 receptors however, labradimil proofs to be more potent in *in vivo* and *in vitro* studies [113], [115], [116]. When the concentration of labradimil declines a recovery of the Tj's within minutes is seen and thus suggests that labradimil is a great temporary BBB opener [113].

There is also a mechanical way to open the BBB. Focused ultrasound (FUS) can be used to open the BBB in highly targeted areas. However there were severe side effects, Coagulation, gas formation and even haemorrhage [117]. In a recent study a low power ultrasound and a micro bubble contrast agent were used. When these micro bubbles pass through the ultrasound field, they oscillate at the exact same vibration, resulting in a stable expansion and retraction of the micro bubbles. This is a stimulus to the BBB and opens up the BBB. Because the micro bubbles concentrate the ultrasound energy, lower energy is needed, resulting in a significant lower risk of the previously mentioned side effects [117], [118].

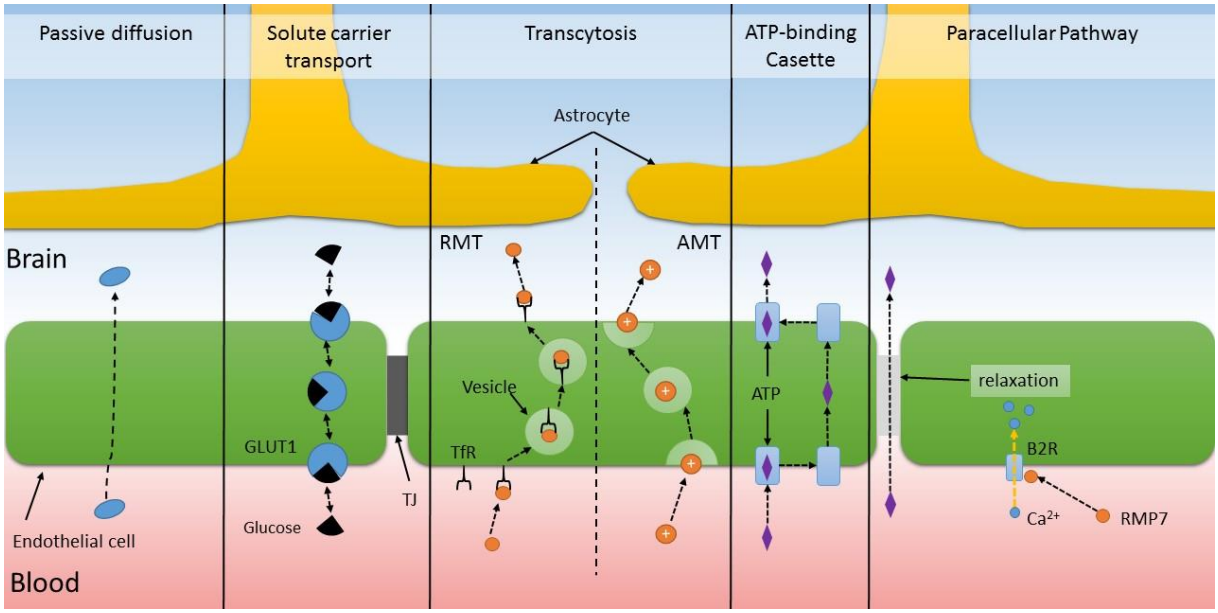

Figure 4. Overview of different BBB pathways. GLUT1 = Glucose transporter 1, TJ = tight junction, RMT = receptor mediated transport, TfR = Transferrin receptor, AMT = Adsorptive mediated transport, ATP = Adenosine triphosphate, B2R = B2 receptor, RMP7 = Labradimil.

7. Clinical Trials

Current treatment of AD is mainly based on galantamine, rivastigmine and donepezil. These drugs are mostly given as an oral formula as tablets, capsules, solutions, etc. Rivastigmine is also prescribed as transdermal patch to extend the release [27]. As previously described is that the efficiency of these drugs and other drugs is significantly reduced due to the BBB. Thus pharmaceutical research into bypassing the BBB is needed. Several studies already showed that NTDDS into the brain have high efficiency and accuracy [119]**.** However there are several questions; can the NP be internalized through the BBB? How safe is long-term usage? What are the side effects? Most studies of NTDDS have been tested on rat, mice and rabbit models, thus there is a long road to travel in clinical stage trials in order to assess potential medicinal value and toxicity of NTDDS.

7.1. Promising therapeutic treatments

Several studies support a significant improvement of cognitive function and memory with mild to moderate AD when treated with rivastigmine, galantamine and/or donepezil [28], [51], [57]. Unfortunately, upon till now, no relevant clinical trials of NTDDS with these drugs are on-going, this probably due to the high costs of manufacturing the NP's. However the usage of NTDDS are not to be overlooked, especially due to the many advantages (e.g. multi-functionality, high efficacy, low toxicity, specific targeting).

It is noteworthy to mention that there are currently clinical trials in passive and active immunisation of Aβ and Tau through vaccination which are in either phase 1 or phase 2 [120].

Table 2. Nanotechnology based drug delivery systems. Recent studies on NP delivering drugs.

Carrier type	*Drug*	*Carrier material*	*Adm.*	*Ref.*
Liposomes	Donepezil	PC/CH/PEG	Intranasal	40
	Rivastigmine	PE/PEG/CPP	Intranasal	41
	Galantamine	PC/GH/PG/CH/PG	Intranasal	42
	Curcumin	PC/DPPG/CH/Curcumin	*In vitro*	43
Solid Lipid Nanoparticle	Ferulic acid	Multiple SLN	*In vitro*	49
	-	PA/CL	*In vitro*	50
	Galantamine	GB	Oral	51
Polymeric Nanoparticle	Donepezil	PLGA	Intravenous	55
	Curcumin	PEG/PLGA	Oral	56
	Rivastigmine	PLGA/PBCA	Parenteral	57
	docetaxel	PEG/PBCA	Intravenous	58
Dendrimers	-	cationic pyridylphenylene	*In vitro*	61
	-	3rd, 4th ,5th gen PAMAM	*In vitro*	62
	-	Cationic PAMAM	*In vitro*	63
	-	glycodendrimers	*In vitro*	64
	-	sialic acid / PAMAM	*In vitro*	65

PC = Phosphatidylcholine, PEG = poly ethylene glycol, PE = Phosphatidylethanolamine, CPP = Cell penetrating protein, GH = , CH = cholesterol, PG = diphosphatidylglycerol, DPPG = Phosphorylglycerol acylated with palmitic acid, FA = , PA = dimyristoylphosphatidic acid, CL = cardiolipin, GB = glyceryl behnate, PLGA = poly lactic-co-glycolic acid, PBCA = poly butylcyano acrylate, PAMAM = polyaminoamide.

8. CONCLUSION/DISCUSSION

AD is one of the major health problems, impacting both economic and the community, and is currently facing an ever-increasing number of patients only aggravating the situation. Current administered drugs have low BBB penetration and thus have been given in high dosages, which not only increases the occurrence of side effects but also costs of manufacturing in ever increasing amounts. An alternative to higher dosage is to employ nanotechnology to easily bypass the BBB. With a lot of research going on in the employment of NP's in drug delivery, a potential new method of drug delivery is offered. These NTDDS vary in shape and function, however they share a similar purpose, delivering drugs in a controlled manner through the BBB and reduce the prevalence of side effects.

Liposomes are good carriers of different types of drugs, a poor *in vivo* stability and a possibility of cytotoxic side-products put liposomes under a scrutinizing eye in terms of a possible usage as a NTDDS. While liposomes are good carriers of lipophilic and lipophobic drugs, SLN usually only carry lipophilic drugs due to the lipid centre however, this lipophilic nature allows for easy BBB penetrance. The toughest challenge with SLN is manufacturing despite the easy and low cost production. Toxic side effects such as heavy metal contamination or increased toxicity of the SLN are perceived. The manufacturing of PNP doesn't present toxic side effects however, it poses difficulties with modification and handling of the particles and in order to pass the BBB surface modification is needed. Dendrimers pose several different ways in which it can

be used, such as a nano carrier or nano probe for imaging purposes. The size and composition can be easily controlled and has a high loading capacity for both lipophilic as lipophobic drugs. Because of the lack of in-depth study, dendrimers could possess toxic properties. With the usage of micro-drugs that can penetrate the BBB Cannabidiol poses a viable option however, further in-depth research is necessary in order for Cannabidiol to become a potential AD drug.

With several means of transport across the BBB, there are several options for the NP to traverse the BBB. NP with a polar surface (Glucose) can cross the BBB via uptake trough SLC. This however is a slow process. Another method is ABC. This form of transportation is dependent on the lipid solubility of the substrates thus increasing lipid solubility of a NP provides better uptake however, it might also react in a reversed manner by the NP functioning as a efflux substrate. Transcytosis is a relative easy way of transport trough the BBB because of receptor-substrate interactions. There is the possibility of selectively passing the BBB via usage of the correct corresponding substrates on the surface of the NP. With the usage of labradimil crossing the BBB is no longer an obstacle because of the created permeability of the BBB via opening the tight junctions. Using ultrasound is also an option only this has severe side effects.

Maybe the next generation of NTDDS employ motor-proteins as surface molecules to decrease the time of crossing the BBB, or are using cell specific antibodies to specifically target EC of the BBB or incorporate a primary release of labradimil before passing the BBB and deliver a secondary drug.

The search for a medication or preventative method for AD is an on-going process, which may have been found in immunization, however the risk of generating an immune response against self-proteins might result in autoimmune disease or can have other severe side effects.

Nanotechnology is thought to have great potential in future development of medicine however the potential dangers and toxicity are far less researched that efficacy. Thus in-depth research is needed before nanotechnology is a valid and safe option for drug delivery.

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