Alzheimer’s disease (AD) is the most common progressive neurodegenerative disorder of aging. The characteristic features of AD begin as mild cognitive dysfunctions, which gradually progress to the fatal delirium through a total loss of cognition and executive motor functions (Pimprikar et al., 2010). Three decades later from now, more than 100 million people will suffer from AD worldwide by making it the most expensive disease (Prince et al., 2013; Bloom, 2014). The major pathological hallmarks of the AD is the extracellular amyloid-beta (Aβ) plaques deposition and the intracellular neurofibrillary tangle-aggregation of hyperphosphorylated tau-proteins. Despite the fact that Aβ and tau-phosphorylation is the primary etiology for the AD, the recent concern developed on anti-amyloid mechanisms, such as cholinergic dysfunction and reactive oxygen species (ROS) generation. The current most prevalent area of research is, treating amyloid or non-amyloid hypothesis individually. However, the intercorrelated nature of amyloid and non-amyloid hypothesis governs the need of the intervention of combined diagnostic approaches. The amyloid formation consists of two phases, such as nucleation and elongation. In the nucleation phase, monomers undergo a conformational change to form the oligomeric nuclei, whereas, in the elongation phase, nuclei rapidly propagate to form mature fibrils (Figure 1A) (Kumar and Walter, 2011). Mature Aβ plaque production leads to the synaptic and neuronal loss that enhance cognitive impairment. Aβ plaques forms as a result of the sequential proteolytic processes of amyloid precursor protein (APP) and the three proteases of α, β, and γ-secretases. The β-secretase activity governs the rate-limiting step of the Aβ aggregation process (Zhang et al., 2015). However, the intercorrelation nature of the Aβ cascade with other non-amyloid etiology leads to the severity and ambiguity of the disease.

Acetylcholine is an essential neurotransmitter in the central and peripheral nervous systems. In the mild AD conditions, the level of acetylcholinesterase enzyme, enhanced. As a causative, the acetylcholine and calcium-binding protein levels reduced by inducing the tangle formation and cholinergic dysfunction. The β-secretase is an essential enzyme that is participating in the detection of AD (Byun et al., 2017). Thus, the ROS, such as hydrogen peroxides, peroxide anions, nitric oxide, and hydroxyl radicals, cause to damage the neuronal lipid molecules, which ultimately result in the AD by destroying neurons. Moreover, Aβ deposition and ROS generation exhibit a synergistic equilibrium. The Aβ deposition enhances the level of ROS, whereas the APP expression upsurges when the brain attempts to repair the oxidative damage caused by ROS (Hettiarachchi et al., 2019b). Therefore, targeting the disease simultaneously in multifunctional pathological pathways, is crucially important to enhance the therapeutic efficacy.

The potential drugs used in current days have several limitations, such as poor drug solubility, lack of blood-brain barrier (BBB) penetration, short half-life, and enhanced cytotoxicity due to non-site-specificity. The nanoparticle (NP)-mediated deliveries became a highly popular alternative to oral and nasal drug administration. Nano platforms enhance the pharmacokinetics and pharmacodynamic properties by diminishing acute toxicity. In AD studies, NP-mediated single drug delivery systems are widely applied. For the Aβ and tau treatments, mostly the Aβ binding small peptides and siRNA (which knocks down the BACE1 gene) have been tested with NP-mediated deliveries. Tacrine and galantamine hydrobromide are the most popular acetylcholinesterase inhibitors used with nano-motifs. Magnetic NPs were used to identify the Cu 2+ and Zn 2+ ions, which knock down the BACE1 gene (hettiarachchi et al., 2019b). However, due to the ambiguity and broadness of the disease, individual pathologic treatments are far from the feasible remedy. Therefore, in this perspective article, we summarize a few targeted NP-mediated dual pathologic systems and their findings in recent AD studies.

NPs based dual-targeted deliveries in AD: NPs are 1–100 nm in particle size, except the liposomal NPs, which ranges from 1 nm to 1 μm. The most commonly used NPs in AD studies are natural chitopectic, synthetic polymeric, liposomal, metallic (gold and magnetic), carbon-based (single-walled carbon nanotubes, and carbon dots), and curcumin NPs. Metallic NPs were widely used in AD studies as colorimetric and fluorometric sensors. Chitosan, synthetic polymeric, liposomal NPs, and carbon nanotubes have used as single/dual drug cargos. Carbon dots based drug deliveries are highly prevalent in brain oncology studies, although the usage in AD studies are minimal. Currently, curcumin and its’ NPs are becoming attentional due to the ability of curcumin to provide neuroprotection and to inhibit the Aβ and tau related phosphorylation (Hettiarachchi et al., 2019b).

Characteristically, most of the NPs used in AD studies are non-toxic, non-immunogenic, non-lethal, and biocompatible except the single-walled carbon nanotubes which are toxic to human kidney cells, keratinocytes, and T-cells. Thus, the use of non-toxic, biocompatible NPs in AD studies is vitally essential while trying to implement dual pathogenesis targeted nano-deliveries. However, the use of NPs as dual etiology targets are still in the early stages. Thus, in the perspective article, we discuss three types of polymeric NPs that have applied in AD dual etiology targeted therapy.

Most of the AD-related NP studies have targeted only one pathogenesis at once. However, the ambiguity of the disease and the intercorrelated nature of the etiology revealed the need for multiple drug co-deliveries that enhances the drug efficacy by the synergistic effect. Liu et al. (2016) have introduced a dual-targeted therapy to inhibit Aβ plaque deposition and p-tau related fibril formation by using a synthetic polymer of poly-L-lysines (DGLs). The positively charged DGLs NPs were covalently conjugated with two peptides (Figure 1A). RVG29 and D-peptide. RVG29 is a 29 amino acid peptide derived from rabies virus glycoprotein, which facilitates the receptor-mediated endocytic BBB penetration by binding to the overexpressed n-acetylcholinesterase in the BBB. D-peptide is a 9 amino acid peptide (D-TLKVWGKKKC), which inhibits the tau-related fibril formation. The peptide conjugated DGLs NPs were also evaluated with BACE1 antisense shRNA (shBACE-As). shBACE1-As enabled the downregulation of the BACE1 enzyme to reduce the APP conversion into Aβ peptides. The in vitro BBB penetrability and the cytotoxicity of the conjugates were measured by a BBB model and SH-SY5Y cell lines. The in vivo studies were conducted with the APP/PS1 double transgenic mouse model. The zeta potential and the particle size of the DGLs-Peg-RVG29-D-peptide (shBACE1-As NPs were 7.72 ± 2.8 mV and 97 nm, respectively. The less cytotoxic nature of the DGLs-Peg-RVG29-D-peptide/shBACE1-As NPs was confirmed by the 80% cell viability at the highest concentration of 200 μM. Immunofluorescence and p-tau positive immunostaining studies revealed the ability of DGLs-Peg-RVG29-D-peptide/shBACE1-As NPs to downregulate the BACE1 gene and p-tau positive signals in the AD mice hippocampus region. Ex vivo and the in vivo images were displayed the higher accumulation of RVG29 conjugated NPs in the mouse brain compared to bare NPs, confirming the efficiency of BBB penetrability of RVG29 peptide. Therefore, DGLs-Peg-RVG29-D-peptide/shBACE1-As NPs were excellent candidates to target dual pathologies in AD.

Poly(lactide-co-glycolic acid) (PLGA) polymer NPs were used by Nag et al. (2013) to co-deliver S1 peptide and curcumin, along with iron-mimic cyclic peptide (CRT) (Figure 1A) (Kumar and Walter, 2011). The actual causality of the degeneration of basal forebrain cholinergic cells and Aβ deposition is far from clarity, the interrelationship between basal forebrain cholinergic neurotransmission and metabolism of the APP is evidenced in last decade (Schliebs, 2005). Therefore, finding treatments to combat jointly against cholinergic dysfunction, and deposition of Aβ peptides, would be essential to reduce the risk level of the disease.

Despite the mechanisms of the amyloid cascade, hyperphosphorylated tau-protein aggregation, and cholinergic dysfunction, the accumulation of neurotoxic ROS has been identified as another possible pathogenic mechanism that contributes to the severity of the AD. The brain consumes 20% more oxygen than other organs that have a higher tendency to produce elevated levels of ROS in the brain. Thus the ROS, such as hydrogen peroxides, peroxide anions, nitric oxide, and hydroxyl radicals, cause to damage the neuronal lipid molecules, which ultimately result in the AD by destroying neurons. Moreover, Aβ deposition and ROS generation exhibit a synergistic equilibrium. The Aβ deposition enhances the level of ROS, whereas the APP expression upsurges when the brain attempts to repair the oxidative damage caused by ROS. The nanoparticle (NP)-mediated deliveries became a highly popular alternative to oral and nasal drug administration. Nano platforms enhance the pharmacokinetics and pharmacodynamic properties by diminishing acute toxicity. In AD studies, NP-mediated single drug delivery systems are widely applied. For the Aβ and tau treatments, mostly the Aβ binding small peptides and siRNA (which knocks down the BACE1 gene) have been tested with NP-mediated deliveries. Tacrine and galantamine hydrobromide are the most popular acetylcholinesterase inhibitors used with nano-motifs. Magnetic NPs, such as FeO 2 , was used to identify the Cu 2+ and Zn 2+ ions, which tended to enhance the ROS production (Hettiarachchi et al., 2019b). However, due to the ambiguousness and broadness of the disease, individual pathologic treatments are far from the feasible remedy. Therefore, in this perspective article, we summarize a few targeted NP-mediated dual pathologic systems and their findings in recent AD studies.

Table 1

| BBB penetrability | Ex vivo | In vivo |
|------------------|---------|---------|
| DGLs-Peg-RVG29-D-peptide (shBACE1-As NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |
| Poly(lactide-co-glycolic acid) (PLGA) polymer NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |

Table 2

| BBB penetrability | Ex vivo | In vivo |
|------------------|---------|---------|
| DGLs-Peg-RVG29-D-peptide (shBACE1-As NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |
| Poly(lactide-co-glycolic acid) (PLGA) polymer NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |

Table 3

| BBB penetrability | Ex vivo | In vivo |
|------------------|---------|---------|
| DGLs-Peg-RVG29-D-peptide (shBACE1-As NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |
| Poly(lactide-co-glycolic acid) (PLGA) polymer NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |

Table 4

| BBB penetrability | Ex vivo | In vivo |
|------------------|---------|---------|
| DGLs-Peg-RVG29-D-peptide (shBACE1-As NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |
| Poly(lactide-co-glycolic acid) (PLGA) polymer NPs | 7.72 ± 2.8 mV and 97 nm | 80% cell viability at the highest concentration |
The 6 amino acid S1 peptide (POVGHL) promoted the Aβ inhibition process by binding to the cleavage site of β-secretase on APP. The polyphenolic compound, curcumin (Cur) used to reduce the neuroinflammation by diminishing the nitrogen oxide ROS related oxidative stress. CRT peptide (CRTGSPVC) is a BBB penetration facilitator which binds to the transferrin receptors on BBB. The average particle size of the CRT-NP-S1+Cur NPs is 139.8 nm, and the zeta potential is ~25.7 mV. The in vitro biodistribution and the in vivo BBB penetration of NPs were analyzed by brain microvascular bEnd.3 cell lines and transgenic mice, respectively. The in vitro studies revealed that the higher number of CRT-NP-S1+Cur NP were found in bEnd.3 cell lines by the displayed higher fluorescence intensity inside the cells. Compared to the bare PLGA NPs, the CRT-NP-S1+Cur NP were exhibited higher BBB penetration efficacy in the mouse brain (Figure 1B). The Aβ40 and 42 burden depletion were measured by using AD mice, and the results displayed a higher reduction of Aβ40 and 42 with CRT-NP-S1+Cur NPs compared to the NPs without S1 peptide (Figure 1C). Moreover, the elevated levels of cytokines tumor necrosis factor-α and IL-6 that release from activated microglia cells are common in ROS related AD brain. The excessive number of tumor necrosis factor-α and interleukin-6 leads to neuronal dysfunction and apoptosis. Therefore, Huang et al. (2017) further described that CRT-NP-S1+Cur NPs were able to decrease the amount of tumor necrosis factor-α significantly and IL-6, emphasizing the efficiency of curcumin loaded NPs than bare NPs.

Figure 1 | The schematic representation of the Aβ propagation and the experimental evidence for the reduction of Aβ plaques and the neuronal dysfunction by the curcumin (Cur) tethered poly (fascide-co-glycic acid) (PLGA) polymeric nanoparticles (NPs).

(A) The green sigmoidal curve indicates the kinetics of the formation of Aβ in the more prolonged lag phase and the rapid elongation phase. The red curve displayed the reduction of the lag phase and the enhancement of the rapid elongation with the addition of more monomer (Kumar and Walter, 2011). (B) The fluorescence intensity measurements of bEnd.3 cells lines with coumarin-6 labeled PLGA NPs (control), NP-S1+Cur, and CRT-NP-S1+Cur. (C) The time-dependent brain accumulation tracking of CRT-NP-S1-cur and NP-S1+Cur in nude mice. Adapted from Huang et al. (2019).

Conclusion and future perspectives: AD is the most common devastating neurodegenerative disease. Over the past decade, nanoparticle-mediated diagnostic systems have been widely investigated. However, due to the lack of explicative etiology, the viable cure is not yet found. Therefore, due to the ambiguities of the disease, targeting dual/variuous pathology at once would be highly promising. Thus far, only a few studies have performed in AD on NP-mediated multiple conjugations for the dual pathology treatments. Although the studies have shown the significant effects on dual pathology treatments, the particle size was >100 nm, which can be a burden for the BBB penetration. As described in this perspective article, most of the NPs used in AD studies are higher than 50 nm in size, and when PLGA-block-poly (ethylene glycol) (PLGA-PEG) polymeric NPs were used in Aβ and tau protein inhibition by Fan et al. (2018). PLGA-PEG NPs were conjugated with B6 peptide (CGHKAKGPRK) to facilitate the endocytic BBB penetration by binding to the transferrin receptors on BBB. The curcumin was loaded into NPs to analyze the potential inhibition of tau and Aβ fibrils. The zeta potential and the particle size of the PLGA-PEG/B6/ Cur NPs were 3.83 ± 0.89 mV and 150 nm, respectively. Less cytotoxic nature of the NPs was proved by the higher cell viability of HT22 cell lines at the concentration of 500 μg/mL. Higher cellular uptake was observed by PLGA-PEG-B6/Cur NPs compared to the bare curcumin. Compared to the bare curcumin NPs, the PLGA-PEG/B6/Cur NPs displayed prominent Aβ burden depletion when injected into APP/ PS1. Not only the Aβ inhibition, but Cur loaded NPs also showed a significant inhibitory effect in tau protein expressions. Therefore, Fan et al. (2018) have shown the dual synergistic efficacy of curcumin loaded polymeric NPs.

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