APPLICATION FOR NANOTECHNOLOGY FOR THE TREATMENT OF BRAIN DISEASES AND DISORDERS, 2nd Edition

EDITED BY: Bingyang Shi, William Banks, Miqin Zhang, Pu Chun Ke, Meng Zheng and Yan Zou

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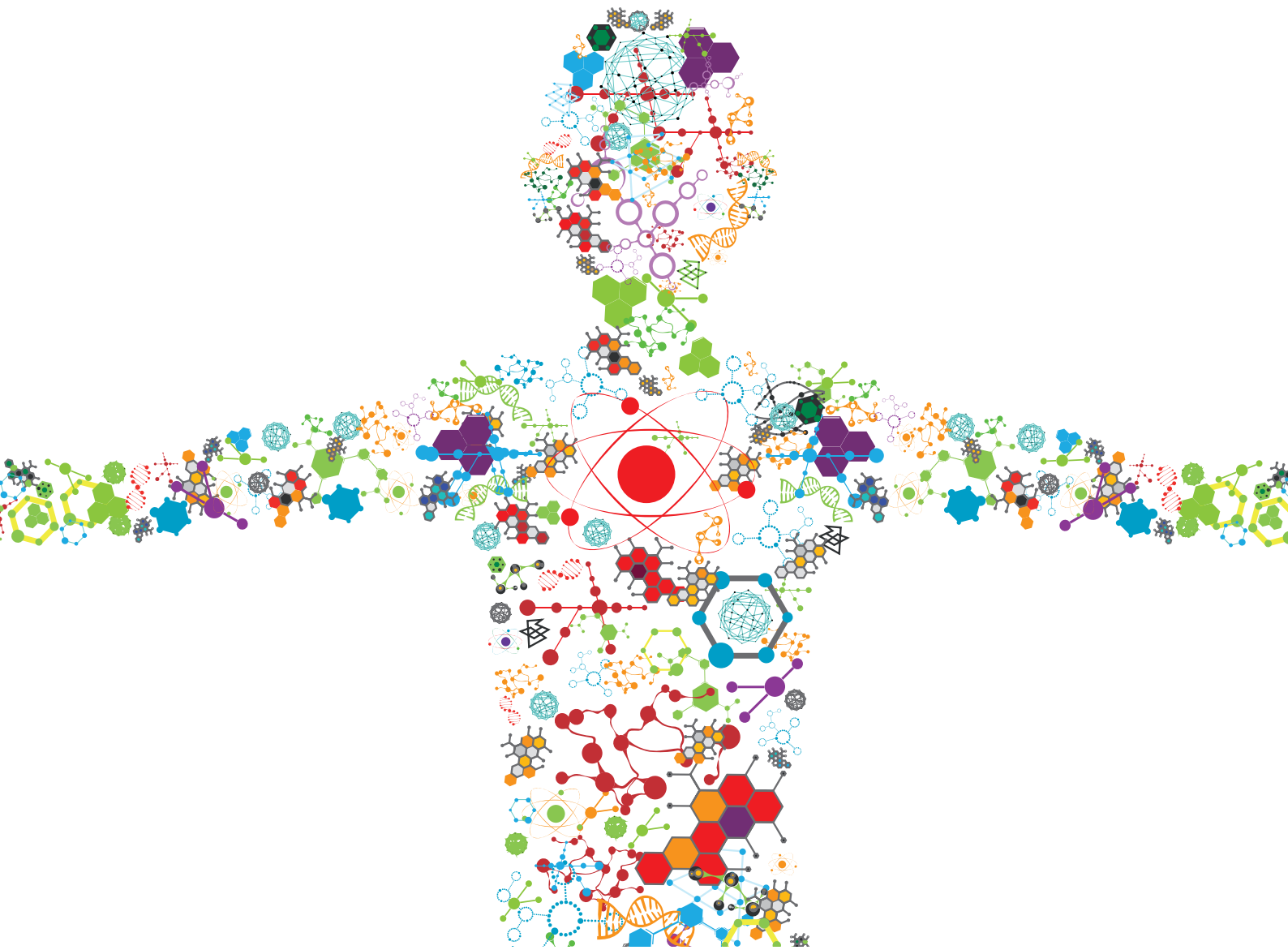

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APPLICATION FOR NANOTECHNOLOGY FOR THE TREATMENT OF BRAIN DISEASES AND DISORDERS, 2nd Edition

Topic Editors:
Bingyang Shi, Macquarie University, Australia
William Banks, University of Washington, United States
Miqin Zhang, University of Washington, United States
Pu Chun Ke, The GBA National Institute for Nanotechnology Innovation, China
Meng Zheng, Henan University, China
Yan Zou, Henan University, China

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EDITORIAL
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Editorial: Application for Nanotechnology for the Treatment of Brain Diseases and Disorders

Jia Li[1], *Meng Zheng*[2], *Yan Zou*[1,2], *Pu Chun Ke*[3,4], *Miqin Zhang*[5], *William A. Banks*[6,7] *and Bingyang Shi*[1,2]*

[1]Centre for Motor Neuron Disease, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia, [2]Henan-Macquarie Uni Joint Centre for Biomedical Innovation, School of Life Sciences, Henan University, Kaifeng, China, [3]Nanomedicine Innovation Division, The GBA National Institute for Nanotechnology Innovation, Guangzhou, China, [4]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia, [5]Departments of Materials Science and Engineering, University of Washington, Seattle, WA, United States, [6]Division of Gerontology and Geriatric Medicine, University of Washington, Seattle, WA, United States, [7]Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care Center, Seattle, WA, United States

Keywords: nanotechnology, blood brain barrier, brain disease therapy, drug delivery, brain imaging

Editorial on the Research Topic

Application for Nanotechnology for the Treatment of Brain Diseases and Disorders

Most brain diseases are fatal with no effective therapeutic solutions currently available, contributing to major health issues globally. One of the major obstacles in overcoming the pathologies of these diseases is the existence of the blood-brain barrier (BBB), which physically separates the brain and the bloodstream (Profaci et al., 2020). The BBB blocks nearly all drugs from entering the brain and reaching the diseased cells and tissues at dosages sufficient to fulfil their therapeutic potential (Srikanth and Kessler, 2012).

In the past decades, various strategies including drug modification, novel delivery systems, transiently opening the BBB by physical or chemical methods and bypassing the BBB through intracranial or intranasal delivery (Li et al., 2021) have been actively explored to increase the brain uptake of therapeutics. Among them, nanoparticles-based delivery has emerged as a promising strategy owning to its simplicity of preparation, non-invasiveness, high efficiency and low toxicity (Li et al., 2021). Nevertheless, development in this area still lags far behind clinical requirements. Further improvement in the efficiency of nanoparticle BBB penetration faces considerable challenges–the fundamental mechanisms underlying the regulation of BBB integrity (Sweeney et al., 2016; Nation et al., 2019; Li et al., 2020) and the penetration of nanoparticles through the BBB are still unclear, hence smart nanoparticles that circumvent the selectivity of the BBB have yet to be developed.

This Frontiers Research Topic brings together contributions in new advancements in the mechanisms of the BBB regulation, the development of novel nanoparticles with the capability to be traced *in vitro* and *in vivo*, and the application of nanoparticles for treating various brain diseases.

Brain diseases are often associated with a dysfunctional often disrupted, BBB (Sweeney et al., 2018). Therefore, understanding the mechanisms of BBB regulation is critical to the development of novel therapeutics for treating brain diseases. In this special issue, Wang et al showed that microRNAs (miRNA) could either inhibit or enhance the expression of tight junction molecules, thereby directly regulating the BBB. In addition, miRNAs affected the structure and function of brain endothelial cells, including the cytoskeleton, channels and transporters of brain endothelial cells. Furthermore, miRNAs also targeted inflammation molecules and other molecules often used in the crosstalk between brain endothelial cells and the other cells of the neurovascular unit. The profound

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Hasan Uludag,
University of Alberta, Canada

***Correspondence:**
Bingyang Shi
bingyang.shi@mq.edu.au

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effect of miRNAs on BBB function and integrity makes them a promising target for treatment of brain diseases. Additionally, improvement of brain vascular function may benefit the treatment of brain diseases. In the article by Zhu et al, the authors developed amorphous selenium nanoparticles (A-SeQDs) for treating chronic isocarbophos poisoning through the protection of endothelial function. They showed that A-SeQDs inhibited inflammation while increasing oxygen saturation, leading to sodium hydrogen exchanger 1-dependent reduction of endothelial apoptosis. Consequently, isocarbophos-induced vascular dysfunction was inhibited.

The development of nanoparticles with the capability for imaging is critical to disease diagnosis and *in vivo* drug monitoring. Chung and Zhang developed a novel colloidal stable and non-toxic fluorescent probe, an iron oxide and carbon dot-based nanoparticle, to deliver chemotherapeutics for killing of cancer cells. This new type of nanoparticle was low in toxicity and able to respond rapidly for quantitative imaging. Transparent cranial implants provided a possibility for chronic brain imaging, thereby facilitating brain research. Halaney et al analyzed the optical properties of a ceramic, nanocrystalline Yttria-Stabilized Zirconia (nc-YSZ)-based transparent cranial implant. The optical properties of the implant were critical to the design of optical systems for imaging the brain and for interpreting imaging outcomes.

Nanoparticles have been employed to transport therapeutic agents to the brain, providing a safe and effective approach to improve brain drug delivery (Srikanth and Kessler, 2012). Ngowi et al summarized the unique properties of nanoparticles for diagnosis and treatment of brain diseases, including brain tumor, ischemic stroke, amnesia, and amyotrophic lateral sclerosis. The small size of nanoparticles, usually less than 100 nm, enables them to cross the BBB for delivering therapeutics and diagnostic probes to the brain parenchyma. In addition, nanoparticles can be modified for improved solubility, bioavailability and specificity of conventional drugs. To accelerate clinical translation, the authors highlighted the importance of determining the toxicity and bioaccumulation of nanoparticles in clinical settings. Khan et al discussed the application of various nanoparticles in treating Alzheimer's disease (AD). They focused on the formulation of nanoparticles employed for this purpose, including organic, lipid-based, as well as metallic nanoparticles. The authors contended that the development of nanoparticles with multi-therapeutic capacities would be the research direction of future AD nanomedicine. Finally, in this special issue Li et al summarized the application of nanomedicine in AD and Parkinson's disease by focusing on the pathogenic targets of nanoparticles, such as oxidative stress, protein fibrillation, and inflammation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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The Application of Nanotechnology for the Diagnosis and Treatment of Brain Diseases and Disorders

Ebenezeri Erasto Ngowi[1,2,3†], Yi-Zhen Wang[1†], Lei Qian[1†], Yasmeen Ahmed Saleheldin Hassan Helmy[1,2], Bright Anyomi[4], Tao Li[1], Meng Zheng[5], En-She Jiang[1,6], Shao-Feng Duan[1,7], Jian-She Wei[1,4], Dong-Dong Wu[1,8] and Xin-Ying Ji[1,9*]*

[1] Henan International Joint Laboratory for Nuclear Protein Regulation, School of Basic Medical Sciences, Henan University, Kaifeng, China, [2] Kaifeng Municipal Key Laboratory of Cell Signal Transduction, Henan Provincial Engineering Centre for Tumor Molecular Medicine, Henan University, Kaifeng, China, [3] Department of Biological Sciences, Faculty of Science, Dar es Salaam University College of Education, Dar es Salaam, Tanzania, [4] Brain Research Laboratory, School of Life Sciences, Henan University, Kaifeng, China, [5] International Joint Center for Biomedical Innovation, School of Life Sciences, Henan University, Kaifeng, China, [6] School of Nursing and Health, Institutes of Nursing and Health, Henan University, Kaifeng, China, [7] School of Pharmacy, Institute for Innovative Drug Design and Evaluation, Henan University, Kaifeng, China, [8] School of Stomatology, Henan University, Kaifeng, China, [9] Kaifeng Key Laboratory of Infection and Biological Safety, School of Basic Medical Sciences, Henan University, Kaifeng, China

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Edited by:
Roberto Molinaro,
University of Urbino Carlo Bo, Italy

Reviewed by:
Jianbo Jia,
Guangzhou University, China
Chiara Martinelli,
Italian Institute of Technology (IIT), Italy

***Correspondence:**
Dong-Dong Wu
ddwubiomed2010@163.com
Xin-Ying Ji
10190096@vip.henu.edu.cn

† These authors have contributed equally to this work

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Brain is by far the most complex organ in the body. It is involved in the regulation of cognitive, behavioral, and emotional activities. The organ is also a target for many diseases and disorders ranging from injuries to cancers and neurodegenerative diseases. Brain diseases are the main causes of disability and one of the leading causes of deaths. Several drugs that have shown potential in improving brain structure and functioning in animal models face many challenges including the delivery, specificity, and toxicity. For many years, researchers have been facing challenge of developing drugs that can cross the physical (blood–brain barrier), electrical, and chemical barriers of the brain and target the desired region with few adverse events. In recent years, nanotechnology emerged as an important technique for modifying and manipulating different objects at the molecular level to obtain desired features. The technique has proven to be useful in diagnosis as well as treatments of brain diseases and disorders by facilitating the delivery of drugs and improving their efficacy. As the subject is still hot, and new research findings are emerging, it is clear that nanotechnology could upgrade health care systems by providing easy and highly efficient diagnostic and treatment methods. In this review, we will focus on the application of nanotechnology in the diagnosis and treatment of brain diseases and disorders by illuminating the potential of nanoparticles.

Keywords: nanotechnology, brain diseases and disorders, diagnosis, treatment, nanoparticles

INTRODUCTION

Brain diseases and disorders refer to a large group of health conditions affecting the brain including injuries, infections, tumors, and neurological disorders. Based on the 2015 statistics, brain diseases and disorders are the main cause of disabilities and the second leading cause of mortality with more than 250.7 million disability-adjusted life-years and 9.4 million

deaths (GBD 2015 Neurological Disorders Collaborator Group, 2017). By definition, the term "brain diseases" encompasses a group of medical conditions that are usually transmittable and commonly caused by external forces such as viruses, bacteria, and so on (Ghosh and Higgins, 2018), whereas "brain disorders" include non-transmittable but commonly inheritable medical conditions caused by the disruption of the normal body structure and functioning as a result of birth defects or genetic malfunctions (Borsche et al., 2020). Brain diseases include viruses/bacteria/fungi/parasite-caused brain infections (BIs), whereas disorders include conditions such as multiple sclerosis (MS), autism spectrum disorder (ASD), and Alzheimer disease (AD). Despite their differences, the two terms are regularly used interchangeably. The most notable features of brain diseases and disorders include deterioration of cognitive, motor, and behavioral functions resulting from the impairment of neurological activities. The treatment of these conditions has been hindered by the complexity and sensitivity of the organ. Some of the diseases including bacterial and fungal BI can be cured by specific antibiotics if discovered in initial states, or vaccines can be applied to prevent their onset (Baccarini et al., 2020); however, others such as neurodegenerative disorders have no exact cures. The physical, chemical, and electric barriers prevent the entrance of materials including most drugs into the brain (Janzer and Raff, 1987; Butt and Jones, 1992; Boulton et al., 2002). Previously, potential drugs used to be dissolved in the solvents that could disrupt the blood–brain barrier (BBB) such as ethanol, polysorbate 80 (PS-80), and dimethyl sulfoxide in order to increase their penetration and sensitivity (Hanig et al., 1972; Azmin et al., 1985; Butt and Jones, 1992). In recent years, nanoparticles (NPs)–based treatments have emerged as the potential therapy for brain diseases and disorders due to easy transportability across the BBB, a credit of their unique features such as small size, selectivity, less toxicity, biodegradability, and solubility (Broadwell et al., 1982; Rabanel et al., 2020).

NPs refer to smallest particles usually within the size range of 1–100, at most less than 1,000 nm (Narayanan and Sakthivel, 2011). The particles are formed from natural or artificial manipulation of compounds or metals. So far, different kinds of NPs have been produced such as metal and metal oxides, liposomal, polymeric, fullerenes, nanoemulsions, solid–lipid (SL), polylactide-co-glycoside (PLGA) NPs, and so on, with varying physical and chemical properties (**Figure 1**; Narayanan and Sakthivel, 2011; Djordjevic et al., 2015; Ding et al., 2016; Lee et al., 2016; Dong et al., 2018; Rasouli and Tabrizian, 2019; Matsuno et al., 2020). The synthesized NPs have been applied in different fields such as cosmetics, agriculture, and medicines (Lu et al., 2015; Karny et al., 2018; Hydbring and Du, 2019; Katebi et al., 2019). In medicines, NPs are used in the diagnosis and treatments of different diseases especially the ones that are deeply seated such as metastatic cancers, brain tumor, and neurodegenerative disorders (**Figure 2**; Zhang et al., 2016; Li et al., 2019a). Because of the challenges facing the effective and

Abbreviations: BBB, blood–brain barrier; NPs, nanoparticles; P-gp, p-glycoproteins; SL, solid lipid; CS, chitosan; PEG, polyethylene glycol; IO, iron oxide; Ag, silver; STPP, sodium tripolyphosphate; Au, gold; Aβ, amyloid β; MI, molecular imaging; PS-80, polysorbate 80; RE, rare-earth; UCH-L1, ubiquitin-C-terminal hydrolase-L; EGFR, epidermal growth factor receptor; CNS, central nervous system; ROS, reactive oxygen species; Se, selenium; CuO, copper oxide; ZnO, zinc oxide; CeO_2, cerium oxide; BI, brain infections; TBI, traumatic brain injury; CSF, cerebral spinal fluid; IS, ischemic stroke; SC, scopolamine; ASD, autism spectrum disorder; TiO_2, titanium dioxide; ALS, amyotrophic lateral sclerosis; AD, Alzheimer disease; GSK-3, glycogen synthase kinase-3; HDAC, histamine deacetylase; PD, Parkinson disease; LB, Lewy bodies; Na-K, sodium–potassium; PBCA, poly(butyl cyanoacrylate).

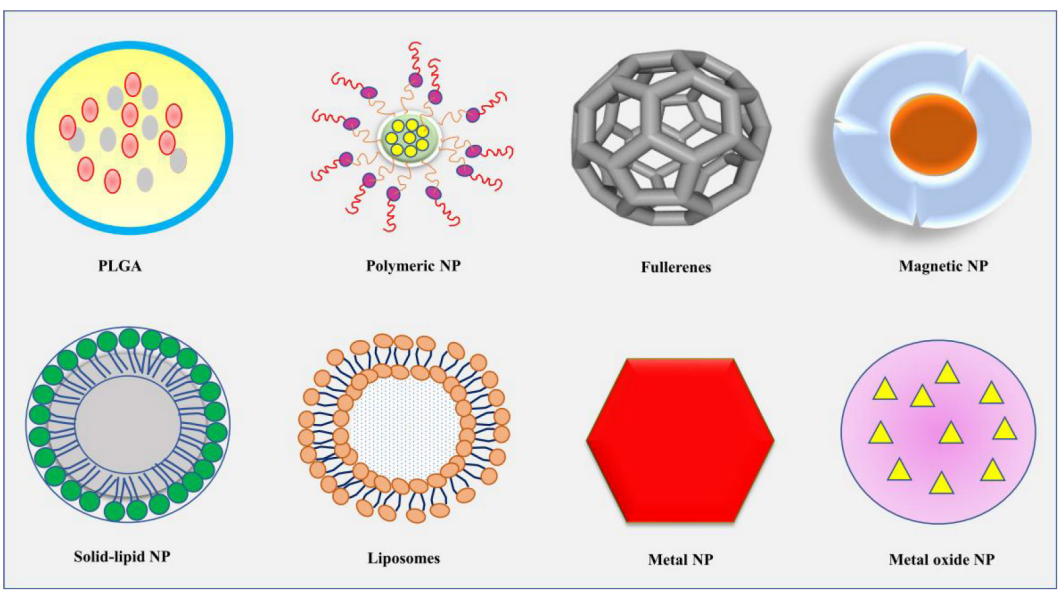

FIGURE 1 | The diagrammatic presentation of some of the common shapes of NPs, including PLGA, polymeric NP, fullerenes, magnetic NP, solid-lipid NP, liposomes, metal NP, and metal oxide NP. NP, nanoparticle; PLGA, polylactide-co-glycoside.

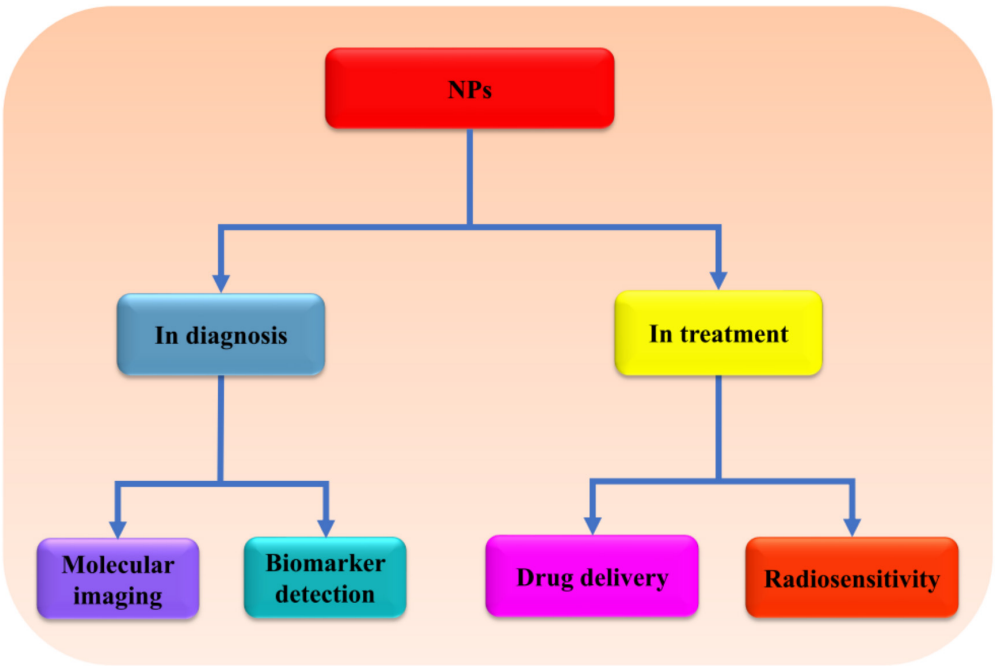

FIGURE 2 | The possible application of NPs in brain therapy. NPs can be used in diagnosis as well as treatment of brain diseases and disorders due to their high sensitivity, specificity, and ability to cross BBB. NPs, nanoparticles; BBB, blood–brain barrier.

efficient delivery of drugs in such conditions, NPs appear to be an important discovery that may enhance the effectiveness and efficiency of potential drugs. In response to the current rise in number of NPs that have shown enormous potential in the treatment of brain diseases and disorders, this review will summarize the application of NPs in the treatment of brain diseases and disorders, as well as the challenges facing this novel discovery. Hereby, the therapeutic potential of several NPs including metal, lipid, polymeric, coffee, SL, chitosan (CS), magnetic, rare-earth (RE), fullerenes, poly(butyl cyanoacrylate) (PBCA), PLGA, betulinic, and liposomal NPs will be discussed.

THE ROLE OF BBB AND ITS INFLUENCE ON THERAPY FEASIBILITY

BBB is a physical barrier formed by endothelial cells (ECs) with the main role of maintaining and regulating the movement of nutrients and other essential materials to the brain, thereby protecting its integrity. The ECs are located on the outer and inner sides of the closely packaged tight junctions that touch the outer EC membranes and prevent easy penetration of materials (Reese and Karnovsky, 1967). Some of the main functions of the BBB include the regulation of the flow of materials in and out of the brain, ionic balance, and protection from the diffusion of circulating agents, neurotransmitters, xenobiotics, and other substances that can affect the integrity of the brain (Abbott et al., 2010). Studies show that poor permeability of molecules across the BBB is significantly associated with high electrical and chemical [P-glycoproteins (P-gp)] resistance (Crone and Christensen, 1981; Butt et al., 1990; Li et al., 2018). Some of the vital regulators of tight junctions' activities identified are cyclic adenosine monophosphate and astrocytes (Rubin et al., 1991; Hurst and Clark, 1998). In brain diseases and disorders, the BBB is highly disrupted, resulting into unregulated diffusion of molecules, leading to further brain damage (Dallasta et al., 1999; Algotsson and Winblad, 2007). Because the BBB prevents the entrance of materials basing on their size and solubility, most of the potential drugs fail to penetrate because they do not meet the required criteria (Pardridge, 2012). One of the common techniques used to improve the transportation of drugs across the barrier is the temporal disruption using focused ultrasound (McDannold et al., 2012), although the mechanism involved and the effect of the technique on an already disrupted barrier are yet to be elucidated. Otherwise, the search for a non-disruptive technique for transportation of drugs to the brain has also been given a high priority, and in recent times, NPs have proven to be efficient in fulfilling the role.

ADVANTAGES OF NPS FOR BRAIN THERAPY

NPs Have Small Particle Size That Facilitates Their Penetration Across the BBB

Crossing the BBB and blood–cerebrospinal fluid (CSF) barrier has been the main challenges hindering the treatment of brain diseases and disorders. The efflux of materials across BBB

is carefully mediated by P-gp; hence, its downregulation is implicated with the progression of neurodegenerative disorders and tumor (Henson et al., 1992; van Assema et al., 2012; Jeynes and Provias, 2013). The inhibition of P-gp improves the penetration of drugs across the BBB and their subsequent effects (Jablonski et al., 2014). NPs of PBCA have been reported to suppress P-gp-mediated phenytoin resistance in rats (Fang et al., 2016). Moreover, a recent study shows that encapsulation of andrographolide (a neuroprotective drug) into SL NPs increases its permeability to the BBB compared to free drug (Graverini et al., 2018). In summary, the above data indicate that NPs can enhance the penetration of potential drugs and increase their target ability by regulating p-gp (**Figure 3**).

NPs Have Low Toxicity and Can Be Used to Improve the Toxicity of Conventional Drugs in Their Targeted Cells

The therapeutic efficacy of most drugs is affected by their cytotoxicity. The brain toxicity induced by NPs is much less compared to conventional therapy. For example, in cerebral ischemia/reperfusion model, intranasal administration of PLGA NPs is reported to be highly effective in transporting a mitoNEET ligand inhibitor NL-1 with no toxicity (Saralkar et al., 2020). Further studies have also shown that the encapsulation of cytotoxic drug such as amphotericin B (antifungal drug), thioridazine (antipsychotic drug), and sorafenib (anticancer drug) into NPs markedly improves their toxicity index by enhancing drug solubility, bioavailability, and sustained release (Tang et al., 2015; Vibe et al., 2016; Li et al., 2020).

Alternatively, NPs can also increase the cytotoxicity of conventional drugs in their targeted area, e.g., tumor cells. In a recent study, treatment with polyethylene glycol (PEG)-modified silica (Si) NPs has been shown to increase the cytotoxicity of anticancer drug, 3N-cyclopropylmethyl-7-phenyl-pyrrolo-quinolinone as compared to free drug in an *in vitro* model (Morillas-Becerril et al., 2020). Despite the observed potential of NPs, some of these compounds can also result in cytotoxicity, including Si NPs whose effect is shown to be influenced by the porosity and size of the particles (Mohammadpour et al., 2019). Apart from the use of particle sizes and shapes that show less toxicity, another method that can be used to improve the efficiency of NPs is the addition of PEG also known as PEGylation (Mendonça et al., 2016; Abakumov et al., 2018). In summary, the evidences above indicate significant reduction in drug toxicity when loaded into some NPs as compared to when administered freely; although in some cases the encapsulation can result in increased cytotoxicity, the event can be reduced with PEGylation or alteration of particle size and porosity.

NPs Improve the Solubility and Bioavailability of Conventional Drugs

Other parameters that are essential in determining the efficacy of a drug is solubility and bioavailability. Solubility is the ability of the drug to dissolve, whereas bioavailability is the extent to which the drug can reach the systemic blood circulation and subsequently the targeted site (Chow, 2014; Alany, 2017). Unlike solubility, the factors affecting drug bioavailability can be drug-related or body-related. Some of them include age, sex, gut pH, genetics, drug dosage, and formulation. Because of the importance of these parameters, improving both of them can lead to better drug efficacy and ultimately treatment of the disease. It has been reported that silver (Ag) NPs can significantly enhance the solubility of methane and ethane in water, with the solubility shown to increase with NPs mass loading (Rahmati-Abkenar and Manteghian, 2020). A recent study indicates that the loading of hydrophobic drug, carvedilol, into CS-sodium tripolyphosphate (STPP) NPs increases its bioavailability and promotes slow and

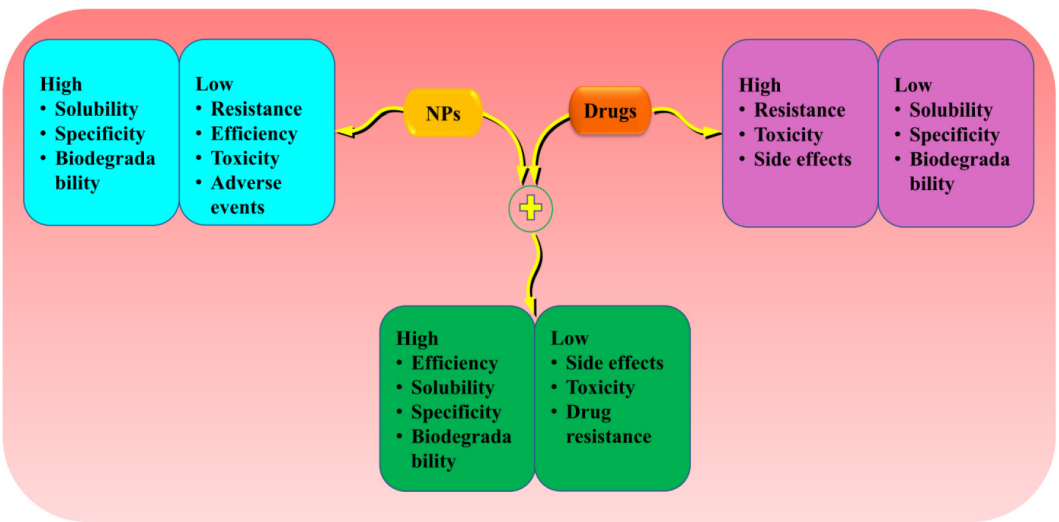

FIGURE 3 | The advantage of loading drugs into the NPs compared to individual treatments. Encapsulation of potential drugs offers advantages into the NPs and drugs features to provide high effectiveness and efficiency. NPs, nanoparticles.

sustained release of the drug (Sharma et al., 2019). Similarly, the oral bioavailability and solubility of curcumin (a polyphenol and turmeric compound) can be improved by loading into PEGylated SL NPs (Ban et al., 2020). The bioavailability and solubility of many other potential drugs such as astilbin, sorafenib, apigenin, and astaxanthin also have been reported to be improved following the encapsulation into NPs (Huang et al., 2019; Liu et al., 2019; Park et al., 2019; Zheng and Zhang, 2019). Together, the data above suggest that NPs can potentially increase the solubility and bioavailability of less-soluble drugs, therefore improving their efficacy.

NPs Improve the Specificity and Biocompatibility of Conventional Drugs

The specificity and biocompatibility of the drug ensure effective delivery to the targeted site. Incorporating drugs into NPs help to substantially enhance these parameters. A recent evidence shows that chimeric antigen receptor T-cell membrane–encapsulated NPs have high specificity in targeting tumor cells by recognizing glycan-3 proteins, which are highly expressed in hepatocellular carcinoma cells with good biocompatibility and safety in normal cells (Ma et al., 2020). Biomimetic gold (Au) NPs stabilized by seaweed extracts have also been reported to be lethal in breast cancer cells MDA-MB-231 at the dose of less than 45 μg/mL while showing no effects on human embryonic kidney cells at 150 μg/mL, which confirmed the high biocompatibility and selectivity of the NPs (Jeyarani et al., 2020). In addition, specific antibody-loaded iron oxide (IO) NPs have shown high sensitivity and specificity, greater than 95 and 90%, respectively, in capturing amyloid β (Aβ) and Tau proteins in the serum and CSF-mimicking samples and about 80–90% in human whole blood samples as compared to the common antibody-conjugated magnetic micron beads, which show approximately only 20% specificity and sensitivity, suggesting the potential of the technique as a biomarker for dementias (Li et al., 2019b). Overall, the above data imply that NPs are highly specific and biocompatible and therefore can be used to deliver drugs to the targeted sites more efficiently.

NANOTECHNOLOGY IN BRAIN DISEASES AND DISORDERS

Molecular Imaging (MI)

MI is an important field in biomedical science associated with the analysis of pathogenesis or body functioning at the molecular level. The imaging techniques provide easy visualization, characterization, and quantification of activity of interest in the body with high sensitivity and specificity (Weissleder and Mahmood, 2001). It involves the use of advanced techniques of different capabilities including microscopy, bioluminescence imaging, ultrasound, X-ray radiography, magnetic resonance imaging, positron emission tomography, and single-photon emission computed tomography. MI techniques have proven to be useful in analysis and characterization of different brain diseases ranging from infections to brain tumors and neurological disorders (Mankoff, 2007; Aldossary et al., 2019; Bocan et al., 2019). The specificity of MI is enhanced by the use of contrast beacons known as probes (Schocke et al., 2002). Probes that bind to specific targets are called targetable probes, whereas the ones that react with specific indicators on their targets to produce a visible signal are termed as activatable probes. A previous study suggests that oligopeptides NPs can act as activatable probes because of their ability to produce fluorescence as a result of the stimulation by low pH of tumor microenvironment (Massoud and Gambhir, 2003). Besides, it has been shown that PS-80–coated PBCA dextran polymeric NPs can be used to transport targetable probes across the BBB, thereby facilitating the visualization of Aβ plaques in AD model (Zhao et al., 2014). A recent study also reports that sulfated dextran-coated IO NPs can effectively improve bioimaging of the activated microglia-induced brain inflammation by binding to the highly expressed class A scavenger receptors (Tang et al., 2018). In addition, it has also been shown that RE-doped NPs can be used in fluorescence imaging to facilitate the emission of short-wave infrared light after binding to integrin α Vβ3 (Naczynski et al., 2018). In summary, the information above indicates that NPs can be used to improve MI by delivering bioimaging probes or acting as probes themselves, confirming their importance in diagnosis of deep-seated tumor and brain diseases.

Biomarker Detection

Biomarker is simply a detectable substance/indicator that is directly associated with a certain condition or state. The effectiveness of the biomarker to differentiate between healthy and unhealthy individuals and its specificity in characterization of the disease stage are key in management of diseases. Different biomarkers have been identified in brain diseases and disorders; however, their application is hindered by the lack of suitable techniques. NPs have shown to be useful in detecting key biomarkers of brain diseases and disorders with great efficiency. In traumatic brain injury (TBI) patients, plasma levels of ubiquitin-C-terminal hydrolase-L1 (UCH-L1) have been identified to be significantly elevated as compared to healthy individuals and therefore could serve as a potential biomarker for the condition (Posti et al., 2016). A recent study demonstrates that a novel method involving surface plasmon resonance of Au NPs can rapidly and effectively detect UCH-L1 biomarker in TBI patients with 100% sensitivity and specificity (Singh et al., 2018). Besides, Aβ levels have been markedly correlated with dementia and associated diseases (van Steenoven et al., 2019). Studies have shown that modified magnetic NPs can effectively and safely detect Aβ plaques in the mouse model of AD (Cheng et al., 2015; Zeng et al., 2018). Anticholesterol antibody-bound magnetic NPs have further been shown to be effective in detecting elevated cholesterol levels, which is also a key marker for AD (Fernández-Cabada and Ramos-Gómez, 2019). Moreover, a recent study indicates fluorescent NPs can be used to detect AD biomarkers including Aβ, inflammatory cytokines, and Tau proteins (Sun et al., 2021). Collectively, these data suggest that NPs are quick and effective in detecting biomarkers for brain diseases and disorders.

Delivery of Drugs

Delivery of the potential drugs in the brain is one of the main challenges facing the treatment of brain diseases and disorders. The drug is supposed to be able to cross the BBB and reach the designated target without causing serious short- or long-term damage into the brain. The size and number of hydrogen bonds are among the factors preventing the transportation of the drugs across the BBB (Pardridge and Mietus, 1979; Pardridge, 2012). In recent years, NPs have gained a lot of attention due to their ability to cross the BBB and serve as a carrier for potential drugs. NPs show enhanced BBB penetrating capabilities and can be loaded with potential brain-targeting drugs (Lin et al., 2016; He et al., 2019; Sadegh Malvajerd et al., 2019). In addition, Au NPs have also been shown to be effective in delivering antibody across the BBB by binding to transferrin receptors, although the effect depends on the affinity and valency of the conjugated antibody (Johnsen et al., 2018). Intranasal delivery of huperzine A with lactoferrin-conjugated N-trimethylated CS-modified PLGA increases its bioavailability and retention time in the mouse model of AD (Meng et al., 2018). Together, these data imply that NPs can effectively and efficiently deliver different drugs across the BBB.

Radiosensitization

The resistance to potential drugs is one of the main challenges in treatment of chronic and progressive diseases. However, radiosensitization offers a promising solution to the situation. It involves the sensitization of the tissues/cells to the radiation by physical, chemical, or pharmacological means (Gallez, 2015). It has been reported that the treatment of glioblastoma mouse model with a folate-targeted NP-mediated kringle 1 domain of hepatocyte growth factor gene can significantly induce antitumor effects and improve the sensitivity to ionizing radiation by promoting checkpoint kinase-1–induced cell cycle arrest and inhibiting the activation of tyrosine kinase receptors, ataxia telangiectasia mutated—checkpoint kinase-2 pathway, and Ki-67 expression (**Figure 4**; Zhang et al., 2018). Similarly, NPs made of lipid–poly(hypoxic radiosensitized polyprodrug), IO conjugated with epidermal growth factor receptor (EGFR), and RE have also been reported to enhance the sensitivity of radiotherapy in glioma cells by increasing the oxidative stress (Bouras et al., 2015; Hua et al., 2018; Lu et al., 2019). The cotreatment of PEGylated-Au NPs with radiation improve sensitivity, resulting in enhanced DNA damage (Joh et al., 2013). However, compared to Au NPs, Ag NPs have more sensitization

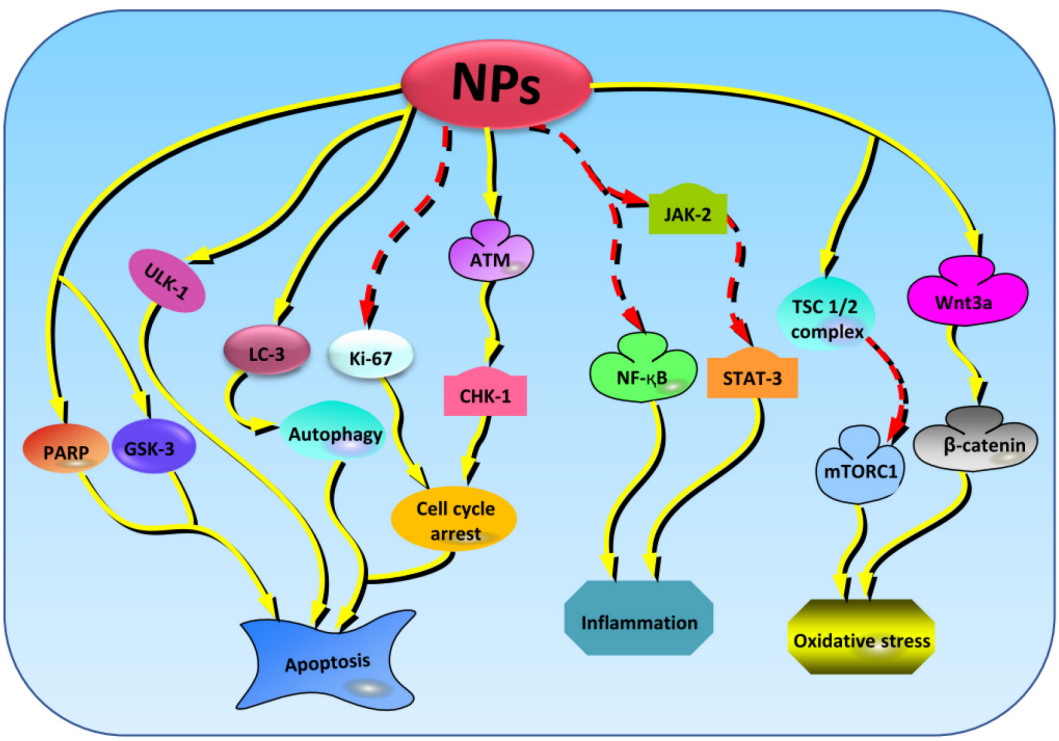

FIGURE 4 | The key cellular markers targeted by NPs in regulating the oxidative stress, inflammation, and apoptosis activities. From the left to right; NPs stabilize the levels of PARP, GSK-3, and ULK-1 to regulate apoptosis. Next, NPs amplifies the expression of LC-3 to induce protective autophagy and regulate apoptosis. NPs also suppress the expressions of Ki-67 and promotes the activation of ATM/CHK-1 cascades resulting in promotion of apoptosis. NPs can also enhance the inhibition of NF-κB and JAK-2/STAT-3 signaling pathways to regulate inflammation. Besides, NPs elevate the levels of TSC1/2 complex and Wnt3a, thereby promoting the inhibition and activation of mTORC1 and β-catenin, respectively, and resulting in the stimulation of antioxidant activities. NPs, nanoparticles; PARP, poly(ADP-ribose) polymerase; GSK-3, glycogen synthase kinase-3; ULK-1, unc-51 like autophagy activating kinase-1; ATM/CHK-1, ataxia telangiectasia mutated/checkpoint kinase-1; NF-κB, nuclear factor κB; JAK-2/STAT-3, janus kinase 2/signal transducer and activator of transcription 3; TSC1/2, tuberous sclerosis protein complex 1/2; mTORC1, mammalian target of rapamycin complex 1.

effect mediated through the promotion of autophagy (Liu et al., 2016). In brief, NPs enhance the radiosensitivity of brain cells by promoting autophagy and hypoxia-induced oxidative stress, suggesting that this combination could be effective in treatment of brain diseases.

The Application of NPs in the Treatment of Brain Diseases and Disorders

Brain Tumor

Brain tumor involves malignant and benign types of tumor that affect the brain. Because of the complexity of the brain, not only the metastatic but also the growth of benign tumor can have detrimental outcomes. According to the 2018 report, there were more than 298,000 new cases of brain tumor worldwide (Bray et al., 2018). The progression of the disease is associated with cognitive dysfunction (Cramer et al., 2019). The pathology of the disease is not well classified, and its treatment is still uncertain. However, several studies have reported the therapeutic advantage of NPs in delivering potential antitumor drugs (Nance et al., 2014; Feng et al., 2017; Chen et al., 2018). The delivery of small interfering RNA by targeting several genes including sodium–potassium (Na-K)–chloride cotransporter 1, yes-associated protein 1, roundabout homolog 1, EGFR, and survivin using polymeric NPs can significantly reduce the growth and migration of glioblastoma cells in a selective manner (Kozielski et al., 2019). It has been shown that modified polymeric NPs loaded with herpes simplex virus type 1 thymidine kinase combined with ganciclovir can markedly reduce the viability of glioma cells and increase the survival of tumor-bearing mice (Mangraviti et al., 2015). The above data confirm the use of NPs for gene and drug delivery to target brain tumors.

BIs

BIs consist of rare but deadly infections caused by microorganisms such as bacteria, viruses, fungi, and parasites that trigger inflammation in the brain or surrounding tissues (Sarrazin et al., 2012). Bacterial and viral infections are the most common. These infections are characterized by acute to chronic inflammations, oxidative stress, and subsequently neuronal impairment. A report shows that treatment with nerolidol-loaded NPs can efficiently improve memory defects and stabilize the levels of reactive oxygen species (ROS) and activities of Na-K ATPase and acetylcholine esterase previously altered by *Trypanosoma evansi* infection in mice (Baldissera et al., 2017). Also, the encapsulation of elvitegravir drug into PLGA NPs improves its inhibitory effects on human immunodeficiency virus 1 (HIV-1)–infected human monocyte-derived microglia-like cells and mouse model without affecting the integrity of the BBB (Gong et al., 2020). The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9/gRNA-loaded magnetoelectric NPs have also been reported to inhibit HIV-1 infection in microglia cells, indicating the potential of NPs in transporting gene therapy (Kaushik et al., 2019). Studies also show that NPs can increase the bioavailability of anti-HIV drugs such as darunavir, indinavir, and efavirenz, thereby improving their ability to cross BBB and targetability of viral infections in the brain (Desai and Thakkar, 2018; Karami et al., 2019; Martins et al., 2019). Besides, cobalt phosphate and hydroxide NPs have also shown potential inhibitory effects on parasite-induced toxicity of granulomatous amoebic encephalitis caused by *Acanthamoeba castellanii* belonging to the T4 genotype (Anwar et al., 2019a). The conjugation of the antidiabetic drugs glimepiride, repaglinide, and vildagliptin with Ag NPs can significantly inhibit the *A. castellanii*–mediated BI by preventing encystation and cytotoxicity (Anwar et al., 2019b). Further studies indicate that the treatment with Au and zinc oxide (ZnO) NPs can effectively improve oxidant/antioxidant status and neuronal impairment by regulating different genes altered following *Schistosoma mansoni* infection in mice (Dkhil et al., 2015; Bauomy, 2020). Similarly, Au NPs have been shown to reduce herpes simplex virus-1 infection–associated neurological defects by attenuating Aβ peptides aggregation and β-secretase activities (I et al., 2020). Otherwise, drug delivery with NPs has also shown enormous potential in treating cerebral tuberculosis, encephalitis virus infection, and amoebic meningoencephalitis infections in mouse models (Marcianes et al., 2017; Rajendran et al., 2017; LaBauve et al., 2018). Overall, the use of NPs in BI could reduce the cytotoxic effects and improve the efficiency of the treatments.

TBI

TBI is the leading cause of trauma-associated disabilities and deaths worldwide, estimated to affect more than 69 million people each year (Dewan et al., 2018). Accidents, sports, and gunshot are among the common causes of the TBI. The condition is associated with elevated levels of melanin in CSF that results in the promotion of oxidative stress and metabolic defects (Seifman et al., 2008). TBI patients have high levels of inflammatory markers in the CSF, which can be correlated with the severity of their condition (Kerr et al., 2018). TBI has been identified to be among the risk factors for neurodegenerative diseases including AD and Parkinson disease (PD) (Guo et al., 2000; Fleminger et al., 2003). Encapsulation of brain-delivered neutrophic factor, stromal cell–derived factor-1, cerebrolysin, and ROS-reactive agents into the NPs have been reported to be effective in improving neurological impairments resulting from TBI in mouse models (Ruozi et al., 2015; Khalin et al., 2016; Yoo et al., 2017; Zamproni et al., 2017). A recent study reports that treatment of TBI rat model with cerium oxide (CeO_2) NPs can significantly reduce brain damage by restoring the cognitive abilities and promoting antioxidant properties (Bailey et al., 2020). It has been reported that the administration of immunomodulatory NPs can markedly improve motor impairments and reduce inflammatory and edema in TBI mouse model (Sharma et al., 2020). A risk analysis study reports that consumption of coffee in midlife reduces the risk of development of AD later in life, indicating that coffee has neuroprotective properties (Eskelinen et al., 2009). The NPs of coffee can be synthesized by boiling the coffee in water, filtrating to remove oil and large particles, followed by sonication (Ratliff et al., 2019). In TBI mouse model, treatment with nano-coffee can effectively improve behavioral characteristics and stabilize the levels of glycogen synthase kinase-3 (GSK-3) and poly(ADP-ribose) polymerase, the key biomarkers for apoptosis and

cellular damage (Ratliff et al., 2019). In summary, NPs offer a crucial option for delivering drugs and reduce TBI-associated neurological damage by inhibiting apoptosis, inflammation, and oxidative stress.

Ischemic Stroke (IS)

Stroke is one of the primary causes of death and disability worldwide. IS is the most common type of stroke accounting for more than 79% of all stroke cases reported in 2017 (Virani et al., 2020). Almost 53% of new cases of IS reported in 2016 occurred in people between the ages of 44 and 70 years (Lindsay et al., 2019). The condition is characterized by the blockage of blood vessel as a result of blood clot or fat deposition. The progression of the disease is associated with several mechanisms including excitotoxicity, oxidative stress, and inflammation, which causes damage to cells and tissues (Castillo et al., 1999; Suwanwela et al., 2006; Kelly et al., 2008; Chehaibi et al., 2016). A recent study indicates that treatment of murine models of IS with selenium (Se) NPs can efficiently suppress neurodegenerative properties by regulating autophagy, inflammation, and oxidative stress through the upregulation of Unc-51–like autophagy activating kinase-1 and Wnt3a and suppression of Jack2/Stat3 and mTORC1 signaling cascades (Amani et al., 2019). Another study demonstrates that betulinic NPs improve the transportation of glyburide in BBB, resulting in enhanced antiedema and antioxidant properties in IS mice (Deng et al., 2019). Moreover, AMB3100-conjugated, size-shrinkable NPs have also been reported to facilitate the delivery efficiency of glyburide and reduce its toxicity (Guo et al., 2018). Besides, it has also been shown that the administration of polyhydroxylated fullerene NPs can significantly suppress brain damage by alleviating antioxidant status and nitric contents in IS mouse model (Vani et al., 2016). The protective effect of fullerene NPs is also linked with the downregulation of aquaporin-1 protein resulting in inhibition of edema (Darabi and Mohammadi, 2017). It has also been reported that treatment of IS rat model with melanin NPs can significantly inhibit the ROS and reactive nitrogen species–induced brain damage (Liu et al., 2017). So et al. (2019) showed that the administration of acetate-loaded liposomal NPs can markedly reduce microglial stimulation and chronic inflammation without affecting oxidative stress, apoptosis, and neurogenesis processes. Overall, the above information suggests that NPs can help in treatment of IS by acting as a delivery vehicle for drugs and by directly affecting the mechanism leading to the progression of the disease.

Amnesia

Amnesia is a medical condition characterized by memory loss, which results from either brain diseases or injury. Most common risk factors for the disease are substance abuse, toxicity, brain diseases, head injury, and blood loss (Langer, 2019). Currently, there are no specific drug treatments for the disease. Similar to other brain diseases, targeting amnesia is challenged by the drugs-associated protection mechanisms of the brain. A previous study reports a significant increase in memory recovery rate following the treatment with PS-80–coated rivastigmine CS NPs in mouse model of scopolamine (SC)–induced amnesia (Nagpal et al., 2013b). Similarly, nerve growth factor–loaded PBCA NPs modified with PS-80 reduce amnesic activities and improve cognitive functions in amnesia rat models (Basel et al., 2005). Besides, treatment with galantamine-loaded thiolated CS NPs can also restore memory defects in amnesia animal model (Sunena et al., 2019). In contrast, the gallic acid–loaded CS NPs coated with PS-80 and ZnO NPs show no significant improvement in memory loss induced by SC treatment in mice as compared to the administration of their corresponding pure drugs; however, these NPs can be used to enhance brain delivery of potential drugs (Nagpal et al., 2013a; Yadav et al., 2018). Collectively, NPs have shown improved drug-delivery efficiency; however, more studies are needed to investigate their effects in the treatment of amnesia.

ASD

ASD is a developmental disorder characterized by behavioral and communication difficulties. Individuals with ASD have poor communication skills as well as limited and repetitive behavioral patterns and interests (Bejerot and Nordin, 2014). The symptoms are usually seen in early childhood and progress to adulthood; however, with proper support and interventions, some difficulties can be camouflaged (American Psychiatric Association, 2013). The prevalence of the disorder in United States is 1/59 for 8 year-old children (Baio et al., 2018). In Norway, the prevalence of the ASD has been reported to increase, with more effect observed in preschool children $\leq$ 5 years old compared to school children aged 6–16 years (Özerk and Cardinal, 2020). Despite the lack of global data, there is a need for developing novel treatment options for the disorder. In valproic-induced ASD rats, treatment with nano-hesperetin could restore behavioral defects and inhibit inflammation and oxidative stress activities (Khalaj et al., 2018). Alternatively, prenatal exposure of mice with titanium dioxide (TiO_2) NPs induces ASD-like behavioral impairment in offspring; however, the compound has no physiological effects (Notter et al., 2018). In brief, the above information indicates that NPs have potential in delivering drugs; however, more studies are needed to assess the side effects of the NPs.

Amyotrophic Lateral Sclerosis (ALS)

ALS is a group of progressive neurons-targeting degenerative diseases that affect the central nervous system (CNS), resulting in motor malfunctions and paralysis. The disease mostly affects children from 2 to 5 years, and in most cases, the death occurs within 5 years as a result of respiratory paralysis (Brown and Al-Chalabi, 2017). Currently, there are no cures for the disease. Compared to healthy individuals, ALS patients have a high rate of oxidative stress (Chico et al., 2018), neurotoxicity (Lam et al., 2016), and inflammation (Keizman et al., 2009). It has been reported that treatment with CeO_2 NPs can effectively improve muscle activities and survival of ALS-induced mouse models by reducing oxidative stress–induced damage (DeCoteau et al., 2016). Similarly, treatment with Au NPs loaded with an inhibitor of hypoxia-inducible factor FM19G11 has been shown to promote the differentiation and proliferation of epidermal stem progenitor cells by elevating the associated genes in ALS mouse model (Marcuzzo et al., 2019). Moreover, treatment with adapalene-loaded poly(lactic acid)–poly(ethylene glycol)

NPs induces neuroprotection and improves survival and motor functioning in ALS mice by stimulating retinoid signaling pathway (Medina et al., 2020). Together, these data imply that NPs have a great potential in improving the efficiency and transporting ASL drugs.

AD

AD is a common type of dementia characterized by aging-related progressive degeneration of neurons resulting in reduced cognitive ability and other neuropathological features. According to the 2016 data, at least one person develops the disease after each 66 seconds in America, and the number is expected to increase abruptly in the coming years (Alzheimer's Association, 2016). The accumulation of Tau proteins is among the pathological features associated with the progression of neurodegenerative diseases including AD (Nam et al., 2020; Tagai et al., 2020). A recent study shows that protein-capped cadmium sulfide and IO NPs can effectively inhibit the polymerization and fibrillization of Tau proteins with the inhibition rates of 63 and 49%, respectively (Sonawane et al., 2019). Another pathological feature of AD is the accumulation of Aβ, which results into the reduced Aβ-binding capacity and formation of plaques (Hansson et al., 2009; Esparza et al., 2013). GSK-3, a serine/threonine kinase, has been shown to participate in the production of Aβ and hyperphosphorylation of Tau proteins and subsequently the progression of AD (Qu et al., 2014). Further evidences indicate that GSK-3 works with histamine deacetylase (HDAC) proteins to regulate neuronal activities (Chen et al., 2010; Bardai et al., 2012). Correspondingly, the inhibitors of GSK-3 and HDAC have been reported to be effective in suppressing AD (Green et al., 2008; De Simone et al., 2019; Soares Romeiro et al., 2019). The loading of nicotinamide, an HDAC inhibitor into the SL NPs, can significantly reduce cognitive impairment associated with AD by reducing the phosphorylation of Tau proteins in rat model (Vakilinezhad et al., 2018). Alternatively, the treatment of 5XFAD mice with vitamin D–binding protein–loaded PLGA NPs attenuates cognitive defects by inhibiting Aβ binding and accumulation (Jeon et al., 2019). Au NPs have also been reported to induce cytoprotective effects in AD rat model by promoting anti-inflammatory responses and improving antioxidant status (Dos Santos Tramontin et al., 2020). Furthermore, surface-coated Au NPs have also been shown to reduce Aβ aggregation, with the effect varying with the diameter and surface chemistry of the NPs (Moore et al., 2017). It has been reported that Au NPs with negative surface potential significantly reduce Aβ fibrillization and associated neurotoxicity in AD model (Liao et al., 2012). Furthermore, a recent study suggests that Au NPs with smaller size are more effective in suppressing Aβ fibrillization compared to the larger ones (Gao et al., 2017). Collectively, the data above signify that NPs can be used to deliver drugs targeting peptides dysregulated in AD such as Aβ more efficiently and effectively.

PD

PD is one of the most common types of neurodegenerative disorder with high prevalence in adults older than 50 years. Data show that more than 6.1 million people had PD in 2016, which is an increase of 2.4-fold from 1990 (GBD 2016 Parkinson's Disease Collaborators, 2018). The disease is characterized by the loss of substantia nigra dopaminergic neurons and formation of Lewy bodies (LBs) and symptomized by motor and non-motor defects (Forno, 1996). Both genetics and environmental factors play a crucial role in the progression of the disease. The LBs contain α-synuclein aggregates (Spillantini et al., 1997, 1998), which contribute to the progression of the disease by facilitating neuronal loss and sensitivity to stresses (Cooper et al., 2018). α-Synuclein further participates in the promotion of apoptosis (Lee et al., 2001), inflammation (Chatterjee et al., 2020), and suppression of neuronal stem cell differentiation (Oliveira et al., 2015). A recent study indicates that α-synuclein is highly expressed in plasma and serum of PD patients compared to healthy individuals and suggests the possibility of using the protein as a diagnosis indicator for the disease (Chang et al., 2020). With respect to NPs, it has been reported that treatment with α-synuclein short-hairpin RNA-loaded magnetic IO NPs coated with oleic acid can efficiently improve motor dysfunction in PD mouse model by reversing α-synuclein–mediated elevation of apoptotic markers Bcl-2–associated X protein and p53 and suppression of B-cell lymphoma 2 (Niu et al., 2017). Study also shows that microRNA-124–loaded polymeric NPs are effective in repairing motor defects and alleviating PD symptoms (Saraiva et al., 2016). Alternatively, ceria NPs can also reduce ROS levels in PD mouse model (Kwon et al., 2018). Besides, treatment with iron (Fe) chelation NPs modified with zwitterionic poly(2-methacryloyloxyethyl phosphorylcholine) and HIV-1–transactivating transcriptor to delay its saturation in blood and increase its *in vivo* lifetime can reverse PD symptoms more effectively compared to individual treatments (Wang et al., 2017). Further study reveals that treatment of alkaline reserpine–induced PD mouse model with Au NPs can significantly reverse behavioral defects and improve antioxidant status and neuronal survival (da Silva Córneo et al., 2020). Moreover, the treatment of PD-induced mouse model with nanodopamine drugs also improves motor defects with low toxicity as compared to pure levodopa, a primary drug used for the treatment of PD (Vong et al., 2020). Likewise, metformin-loaded polydopamine NPs promote anti-inflammatory, antiapoptotic, and antioxidative properties associated with the proteolytic degradation of phosphorylated serine 129 of α-synuclein protein induced by targeting a histone-lysine N-methyltransferase enzyme known as the enhancer of zeste homolog 2 (Sardoiwala et al., 2020). Other NPs and nanodrugs that have been reported to have significant potential in the treatment of PD by regulating oxidative stress and inflammation including vitamin E–loaded naringenin nanoemulsions (Gaba et al., 2019), selegiline CS NPs (Sridhar et al., 2018), borneol and lactoferrin comodified NPs (Tang et al., 2019), resveratrol NPs (Palle and Neerati, 2018), and Cerium NPs (Hegazy et al., 2017). In summary, NPs and nanodrugs have great potential in treatment of PD because of their role in the regulation of inflammation, oxidative stress, apoptosis, α-synuclein activities, and the downstream effects in motor and non-motor dysfunctions.

Huntington Disease (HD)

HD is a progressive neurodegenerative disease of autosomal dominant origin characterized by motor, cognitive, and psychiatric impairments. Genetically, the disease occurs because of the mutation in huntingtin gene indicated by the extension of polyglutamate repeats in exon-1, the event that leads to the posttranslational-mediated functional defects of its downstream protein (Langbehn et al., 2004). High rate of tryptophan metabolism, inflammation, oxidative stress, excitotoxicity, and gene dysregulation has been established as key molecular processes associated with the progression of the disease in patients and animal model (Augood et al., 1997; Shin et al., 2005; Stoy et al., 2005; Sánchez-López et al., 2012; Hsiao et al., 2013). The analyses of brain autopsy from HD patients indicate a significant reduction of Se, an essential metal with protective properties against cytotoxicity and redox imbalance (Lu et al., 2014). Alternatively, a recent study reports that Se, iron, and chromium are among the essential elements that are considerably elevated in the blood samples of HD patients compared to normal individuals (Squadrone et al., 2020). In *Caenorhabditis elegans*, treatment with low doses of Se NPs reverses brain condition by improving oxidative status and inhibiting the aggregation of huntingtin proteins, suggesting the potential of the compound in the treatment of HD (Cong et al., 2019). Similarly, evidence shows that TiO_2 NPs have the ability to catalyze the oxidation of methionine on the N-terminal domain of the mutant huntingtin protein, thereby forming a sulfoxide and preventing the aggregation of the protein (Ceccon et al., 2019). It has also been shown that the loading of thymoquinone into the SL NPs markedly suppresses the progression of HD by increasing the activity of ATPase enzymes and reducing the production of inflammatory markers and the nuclear translocation of phosphorylated nuclear factor κB in rat model (Ramachandran and Thangarajan, 2018). Moreover, the encapsulation of peptide-based polyglutamate aggregation inhibitors into PLGA NPs can enhance their protective effects in Neuro 2A and PC12 cellular models as well as its biocompatibility in *Drosophila* model of HD (Joshi et al., 2019). In both neuronal cell and mouse model, poly(trehalose) NPs have also been reported to be extremely efficient in inhibiting the progression HD by suppressing the accumulation of mutant huntingtin protein (Debnath et al., 2017). The alteration of cholesterol metabolism has also been reported in the animal model of the disease (Marullo et al., 2012). Specifically, HD is linked with the alteration in the levels of 24S-hydroxycholesterol, a vital cholesterol metabolite produced by the hydroxylation reaction catalyzed by cholesterol-24 hydrolase (Leoni et al., 2013). A recent study shows that the elevation of the enzyme is crucial for the treatment of the disease as it facilitates the proteasomal and autophagy-mediated clearance of mutant huntingtin aggregates (Kacher et al., 2019). Besides, evidence shows that treatment with cholesterol-loaded glycopeptide-modified polymeric NPs can reverse behavioral and cognitive defects in HD mice (Valenza et al., 2015). In analyzing the nose to brain delivery, Passoni et al. (2020) reveal that liposomal NPs are effective in delivering cholesterol via this route in HD mouse model, confirming its potential in the treatment of HD. In brief, these above evidences confirm the neuroprotective role of NPs and their potential in the treatment of HD by targeting key mechanisms involved in the progression of the disease.

MS

MS is a neurological disorder of the CNS and a common cause of disability in young adults. The disease affects more than 2.2 million people worldwide, and its prevalence has increased significantly in many regions (GBD 2016 Multiple Sclerosis Collaborators, 2019). Currently, there are no effective cures for the disease; however, several drugs are used to treat/reduce the symptoms of the disease especially in initial stages. The main features of the disease reported to occur in early patients include cortical demyelination and meningeal inflammation (Bø et al., 2003; Lucchinetti et al., 2011). Recent studies demonstrate that SL NPs and CS NPs can potentially increase the bioavailability and neuroprotective effects of a relapsing-MS drug dimethyl fumarate in rat model (Ojha and Kumar, 2018; Ojha et al., 2019). Another study also suggests that glucocorticoids and inorganic–organic hybrid NPs can also be used to treat MS (Montes-Cobos et al., 2017). Moreover, the encapsulation of chondroitinase ABC 1 into porous silicon NPs counteracts the neuronal damage by facilitating remyelination in MS mouse model (Rezaei et al., 2020). Together, these data suggest that NPs can be used to improve the efficiency and bioavailability of potential MS drugs.

TABLE 1 | Clinical trials for NPs-based treatments for brain diseases and disorders.

Type of NPs	Medical conditions	Effects	Mechanisms	References
Magnetic IO NPs + reduced radiotherapy	Glioblastoma multiforme	Improves patients' overall survival	Suppression of tumor growth by increasing Caspase-3, heat shock protein, and programmed death ligand 1 levels	Maier-Hauff et al., 2011; Grauer et al., 2019
Nano-curcumin + ω-3 fatty acids	Migraine	Reduces recurrent headaches	Suppression of gene expressions of pro-inflammatory TNF-α, intercellular adhesion molecule 1, and cyclooxygenase-2/inducible nitric acid	Abdolahi et al., 2017, 2019; Soveyd et al., 2018
Ag NPs	Acute occlusive hydrocephalus	Improves patients' status	Prevents catheter-associated ventriculitis	Lackner et al., 2008
Ultrasmall magnetic IO	IS	Enhances inflammatory cytokines targetability	Promotes macrophage infiltration	Saleh et al., 2007

NPs, nanoparticles; Ag, silver; IO, iron oxide; IS, ischemic stroke.

Epilepsy

According to the International League Against Epilepsy, epilepsy is regarded as a brain disease characterized by (i) two unprovoked or reflex seizures occurring over 24 h apart; (ii) one unprovoked/reflex seizure and at least 60% probability of further seizures to occur over the next 10 years, after two unprovoked seizures; and (iii) the diagnosis of epilepsy syndromes (Fisher et al., 2014). The disease can occur at all ages; however, it is common in children and adults. The activation of astrocytes and microglia plays a key role in the progression of the disease (Shapiro et al., 2008; Najjar et al., 2011; Bedner et al., 2015). The treatment of epilepsy has been hindered by the low bioavailability and the delivery of the drugs to the brain. Curcumin has proven to be potential in the treatment of epilepsy because of its ability to suppress cognitive deficit and glial activation and promote antioxidant and anti-inflammatory properties (Kaur et al., 2015). In a mouse model of chronic epilepsy, the incorporation of curcumin with CS-alginate STPP NPs significantly increases its corresponding effects on cell death, cognitive defects, and glial activation, consistently with the solubility of the compound (Hashemian et al., 2017). Curcumin-loaded SL NPs induce neuroprotective effect by reducing apoptosis via upregulation of erythropoietin and klotho, reduction of tumor necrosis factor-α (TNF-α), and the subsequent activation of P38 MAPK pathways (Mansoor et al., 2018; Huang et al., 2020). It has also been shown that treatment with piperine-loaded CS-STPP NPs strongly inhibits the progression of epileptic symptoms by suppressing cell death and astrocyte stimulation compared to non-loaded piperine-treated mice (Anissian et al., 2018). A previous study also reports that treatment of epileptic rat model with pluronic P85-coated PBCA NPs alleviates the effects of P-gp in phenyltoin resistance and increases its bioavailability (Fang et al., 2016). The loading of carbamazepine (an anticonvulsant drug used to treat epilepsy) into the poloxamer 188–coated PLGA NPs also improves the drug effect in isoniazid-induced epilepsy rat model compared to the administration of free drug (Zybina et al., 2018). Further evidence suggests that the treatment with quercetin-conjugated IO-β-cyclodextrin NPs can markedly enhance the therapeutic effect of quercetin in epileptic mouse model (Hashemian et al., 2019). Together, the data above suggest that the incorporation of potential epilepsy drugs into NPs improve their sensitivity and efficiency.

APPROVED NANODRUGS AND ONGOING CLINICAL TRIALS

The success of NPs in clinical trials is evidenced by more than 250 US Food and Drug Administration–approved nanodrugs available on the market. Some of the interesting drugs include Doxil (doxorubicin HCL liposome injection), Invega Sustenna (paliperidone palmitate), DepoCyt (liposomal cytarabine), and Plegridy (PEGylated interferon β-1a) used for the treatment of multiple myeloma, schizophrenia, lymphomatous meningitis, and MS, respectively (Ventola, 2017). Liposomal formulation is the most common nanodrug available in the market so far, implicating more than 33% of drugs (D'Mello et al., 2017).

TABLE 2 | Possible side effects associated with metal NPs.

Type of NPs	Animal models	Effects	References
Carbon black	Female pregnant mice	Impairs neurons development on infants and causes irreversible brain damage on mother	Zhang et al., 2019
ZnO	Male Wistar rats	Impairs neuronal mitochondria functions by ROS production	Liu et al., 2020a
	Swiss albino mice	Induces motor defects	Yaqub et al., 2020
Aluminum oxide	Male Wistar rats	Increases inflammatory responses and oxidative stress	Liu et al., 2020b
IO	Mice	Impairs motor and behavioral functions	Manickam et al., 2019
TiO_2	Mice	Damages dopaminergic neurons	Heidari et al., 2019
CuO	Mice	Reduces motor, behavioral, and locomotor activities	Ouni et al., 2020
Ag	Rats	Induces myelin sheath damage due to ROS	Dąbrowska-Bouta et al., 2019

ZnO, zinc oxide; IO, iron oxide; TiO_2, titanium oxide; CuO, copper oxide; Ag, silver; ROS, reactive oxygen species.

In addition, numerous clinical trials have been conducted to identify the applicability of NMs in clinical settings. Previous study reports that glioblastoma patients treated with magnetic NPs and reduced radiotherapy have improved overall survival compared to conventional therapy–treated counterparts (Maier-Hauff et al., 2011). Besides, NPs also help to reduce toxicity caused by conventional drug (Eckes et al., 2011). It is worth noting that magnetic NPs also show 70% chemotherapy (temozolomide)–delivering capability and distribution in intracranial tumor region in pet dogs (Young et al., 2018). Moreover, recent studies reveal that treatment with omega-3 fatty acids and curcumin NPs significantly reduces inflammation in migraine patients by suppressing the expressions of TNF-α, intercellular adhesive molecule 1, and cooxygenase-2/inducible nitric oxide synthase (Abdolahi et al., 2017, 2019; Soveyd et al., 2018; **Table 1**). Together, the above evidences indicate that NPs have enormous potential in brain diseases by facilitating drug delivery, inducing synergistic effects, and reducing drug toxicity.

CONCLUSION AND FUTURE DIRECTION

The incorporation of nanotechnology in medical field helps to improve the diagnosis and treatment of different diseases by increasing the sensitivity of equipment and different parameters of the drugs, thereby enhancing their efficacy. Because of the ability of NPs to cross the BBB, these compounds provide a potential option for diagnosing and treating the brain diseases and disorders, which have proven to be challenging

for many years. However, to ensure the effectiveness and efficiency of these particles, further studies are needed to determine their toxicity and bioaccumulation in clinical settings. Some of these NPs cause deterioration of the brain functioning and increase oxidative stress (**Table 2**). Therefore, the use the NPs with therapeutic usefulness and low toxicity should be prioritized to achieve high outcome and prevent further damage to the brain. In addition, it is important to enhance their sensitivity to target specific biomarkers by improving the formulation with specific antibodies. After further exploration, the potential of nanotechnology in the treatment of brain diseases and disorders will be limitless.

AUTHOR CONTRIBUTIONS

EN, Y-ZW, LQ, D-DW, S-FD, and X-YJ: conceptualization. EN, Y-ZW, and LQ: data curation. X-YJ and D-DW: funding acquisition. EN, Y-ZW, LQ, YH, BA, TL, MZ, and E-SJ: writing–original draft. S-FD, J-SW, D-DW, and X-YJ: visualization and supervision. EN, Y-ZW, LQ, and D-DW: editing. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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Group Refractive Index of Nanocrystalline Yttria-Stabilized Zirconia Transparent Cranial Implants

David L. Halaney[1], Nitesh Katta[2], Hamidreza Fallah[3], Guillermo Aguilar[1] and Thomas E. Milner[2]*

[1] Laboratory of Guillermo Aguilar, Department of Mechanical Engineering, University of California, Riverside, Riverside, CA, United States, [2] Laboratory of Thomas Milner, Department of Biomedical Engineering, University of Texas, Austin, TX, United States, [3] Department of Physics, University of Isfahan, Isfahan, Iran

Transparent "Window to the Brain" (WttB) cranial implants made from a biocompatible ceramic, nanocrystalline Yttria-Stabilized Zirconia (nc-YSZ), were recently reported. These reports demonstrated chronic brain imaging across the implants in mice using optical coherence tomography (OCT) and laser speckle imaging. However, optical properties of these transparent cranial implants are neither completely characterized nor completely understood. In this study, we measure optical properties of the implant using a swept source OCT system with a spectral range of 136 nm centered at 1,300 nm to characterize the group refractive index of the nc-YSZ window, over a narrow range of temperatures at which the implant may be used during imaging or therapy (20–43°C). Group refractive index was found to be 2.1–2.2 for OCT imaging over this temperature range. Chromatic dispersion for this spectral range was observed to vary over the sample, sometimes flipping signs between normal and anomalous dispersion. These properties of nc-YSZ should be considered when designing optical systems and procedures that propagate light through the window, and when interpreting OCT brain images acquired across the window.

Keywords: brain, chromatic dispersion, cranial implant, group refractive index, imaging, optical coherence tomography, window to the brain

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Xue Xia,
Henan University, China

****Correspondence:***
Guillermo Aguilar
gaguilar@engr.ucr.edu

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INTRODUCTION

Neurosurgeries often involve craniectomy (removal of a portion of the cranial bone) to gain access to the brain for therapy, followed by the placement of a cranial implant to replace the excised bone. Cranial implants are normally made from a variety of materials including metals, polymers, and ceramics, and provide mechanical protection to the underlying brain tissue (Bonda et al., 2015). To our knowledge, current cranial implants available to patients lack optical transparency which could allow for brain optical imaging or therapy without implant removal or additional open skull procedures. We recently introduced a novel optically transparent cranial implant made from a biocompatible ceramic, nanocrystalline Yttria-Stabilized Zirconia (nc-YSZ), which we refer to as the "Window to the Brain" (WttB) implant (Davoodzadeh et al., 2018, 2019a,b; Cano-Velázquez et al., 2019). We have demonstrated chronic brain imaging across this implant *in vivo* using

optical coherence tomography (OCT) (Halaney et al., 2020). OCT is an imaging technique based on broadband near-infrared light which can penetrate into scattering media such as brain tissue underlying the WttB implant. However, optical properties of these transparent cranial implants are neither completely characterized nor completely understood. Fundamental optical properties of the implant such as the group refractive index and chromatic dispersion are important to consider when planning or designing time-based and/or multispectral imaging strategies across the window, and for correct interpretation of recorded brain images. The group refractive index describes the speed at which a light wavepacket travels through the window, and is important to consider when focusing light across the window as well as when interpreting OCT images, where on-axis dimensions of reconstructed images are determined by time-of-flight of the OCT wavepacket. Chromatic dispersion also effects the quality of images recorded with broadband light sources like the ones used to record OCT images. Characterizing variation of group refractive index and chromatic dispersion will allow development of dispersion compensation techniques for obtaining higher quality OCT images. Furthermore, design of other multispectral imaging strategies that utilize cranial implant windows will be impacted by the group refractive index and chromatic dispersion.

Methods to measure refractive index include non-interferometric methods and interferometric methods. Non-interferometric methods include the use of index matching liquids, and refractometers. Index matching liquids are not ideal for determining the refractive index of nc-YSZ, which has lower transparency in the visible range and enhanced transparency in the near-infrared, because these liquids are typically designed for visible wavelengths and require switching the liquid until a near match is found, giving a rough estimate of the index of nc-YSZ. Refractometers require the thickness to be known at each location under interrogation, and due to the thin nature of our samples, this thickness measurement must be very precise (i.e., micron-scale) to yield an accurate index measurement. Because the samples we are measuring do not have perfect flatness nor perfectly uniform thickness, we needed a method which can simultaneously measure thickness and group refractive index at each location under interrogation on the sample. Of the interferometric methods available to measure refractive index, OCT is the most appropriate technique for our samples. Other interferometric methods are appropriate for highly transparent materials, whereas materials like transparent nc-YSZ exhibit some light scattering. While this scattering is enough to introduce artifacts in other methodologies, OCT has been used to measure the group refractive index of human skin, muscle and adipose tissue (Tearney et al., 1995), which is highly scattering, and thus is capable of accurately assessing the group refractive index of nc-YSZ. There are no other methods for these materials as precise as OCT, but because OCT is a broadband technique, we are able to assess group delay (GD) and group refractive index rather than phase index. Because OCT brain imaging is one of the most applicable imaging techniques for the Window to the Brain implant, group index over OCT wavelengths is highly applicable to envisioned clinical applications for the implant.

In this study, we measured optical properties of the transparent cranial implants using a swept source OCT system to characterize the group refractive index of the nc-YSZ window, over a narrow range of temperatures at which the implant may be used during imaging or therapy, ranging from room temperature (RT) to the point where thermal tissue damage begins to occur (20–43°C) (Yarmolenko et al., 2011). Additionally, chromatic dispersion of the OCT pulse was assessed.

MATERIALS AND METHODS

Implant Fabrication and Preparation

The transparent 8 mol% $YO_{1.5}$ nc-YSZ WttB implant used in this study was fabricated from a precursor yttria-stabilized zirconia nanopowder (Tosoh USA, Inc., Grove City, OH, United States) densified into a bulk ceramic *via* current-activated pressure-assisted densification (CAPAD) as described previously (Garay, 2010). The resulting ceramic disk was 19 mm in diameter and 1 mm thick. The thickness was reduced further by polishing with 30 μm diamond slurry on an automatic polisher (Pace Technologies, Tucson, AZ, United States). The two faces were then polished using progressively finer abrasives (from 30 μm diamond slurry down to 0.2 μm colloidal silica slurry) to reduce light scattering by the implant surfaces and thus increase transparency. A photograph of the nc-YSZ overlying the words "transparent nc-YSZ" with back lighting is shown in **Figure 1A**.

OCT Imaging

The OCT system used in this study utilized a swept-source, mode-locked laser (Axsun, Billerica, MA, United States) with central wavelength emission at 1,300 nm, 136 nm sweep and an A-scan-rate of 100 kHz (**Figure 1B**). The swept source output was coupled into a fiber-optic (SMF-28) Mach–Zehnder interferometer with pathlength and dispersion matched sample and reference arms. Sample path light was collimated (RC04, Thorlabs) and directed onto two galvanometer mirrors (GVS012, Thor Labs Inc.) positioned in a telecentric configuration with an aspheric ZnSe scanning lens (AR112-ZC-XWL-25-25, ISP Optics). An identical ZnSe lens was used in the reference path for dispersion compensation. Light backscattered from the sample and reflected from the reference mirror interfered and directed onto balanced detectors (BD) to obtain one-dimensional interferogram A-scans. Each one-dimensional scan (or A-line) collected contained 1,472 points (or pixels) to complete the interferogram. A pixel in the depth dimension was determined to correspond to a real thickness in air of 6.19 μm. Orthogonal scanning galvanometer mirrors in the scan head allowed for recording two-dimensional images (or B-scans where each B-scan consisted of 512 A-lines). **Figure 1C** shows an example of OCT angiography of mouse cerebral vasculature through a square nc-YSZ implant within a craniectomy,

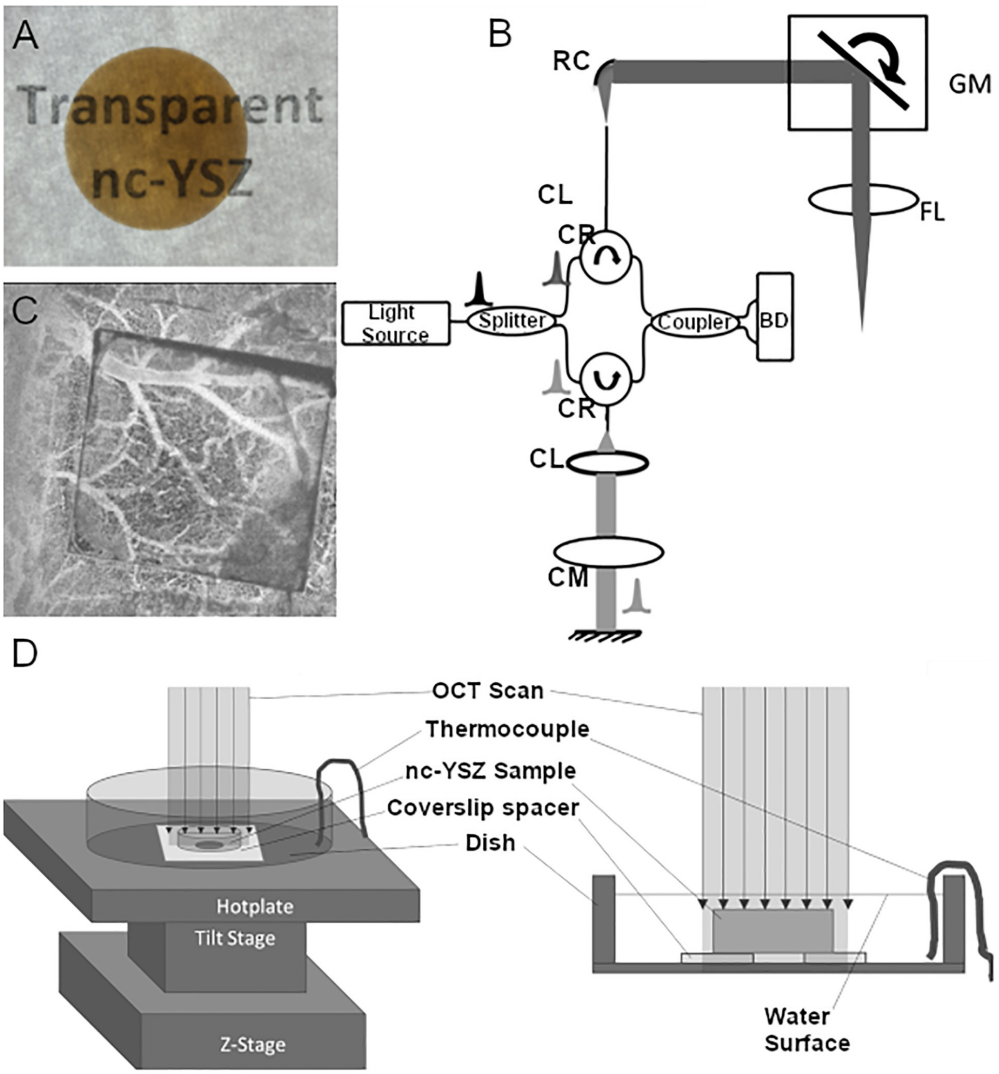

FIGURE 1 | (A) A photograph of the nc-YSZ overlying the words "Transparent nc-YSZ" with backlighting. **(B)** Schematic of bench-top OCT system. Intensity OCT uses a Mach–Zehnder interferometer with circulators in the sample and reference paths (CR) using balanced detection (BD) and dispersion compensation (CM). Sample path delivery fiber APC; and reference path uses a reflective collimator (RC). Sample path uses galvo-mirrors (GM) placed at the back focal plane of the scanning lens (FL). **(C)** OCT angiography of mouse cerebral vasculature through a square nc-YSZ implant within a craniectomy, demonstrating the improved imaging across the implant compared to surrounding cranial bone. **(D)** Experimental setup for temperature-dependent measurements.

demonstrating the improved imaging across the implant compared to surrounding cranial bone.

Experimental Setup for Temperature-Dependent Measurements

The OCT imaging head was placed over a glass dish containing a 100 μm coverslip spacer with hole in the center and secured in place with epoxy. The refractive index of the coverslip spacer (made of borosilicate glass) is 1.504 at central wavelength of OCT at 1.31 μm. The glass dish was placed atop a temperature adjustable plate on a tilt and *z*-stage, allowing for the dish to be positioned with normal incidence to the OCT beam (**Figure 1D**). A baseline image was acquired, showing the vertical height of the dish and spacer when imaged in air (**Figure 2A**). In **Figure 2A**, the dish surface appears displaced when imaged through the coverslip spacer compared to when imaged through the hole in the center of the spacer, due to the refraction of light in the coverslip spacer (red arrows), although this surface is in fact continuous and flat. Next, the nc-YSZ sample was carefully placed atop the spacer, and water was slowly added to submerge the sample. A thermocouple was used to measure the water temperature as 20.8°C. A RT image was acquired, showing the apparent displacement of the spacer and dish due to non-unity group refractive index of water (at locations F and G) or due to the nc-YSZ and water (at locations A, B, C, D, and E) (**Figure 2B**). Weight of the water also caused a real downward displacement of the dish due to compression of the temperature adjustable

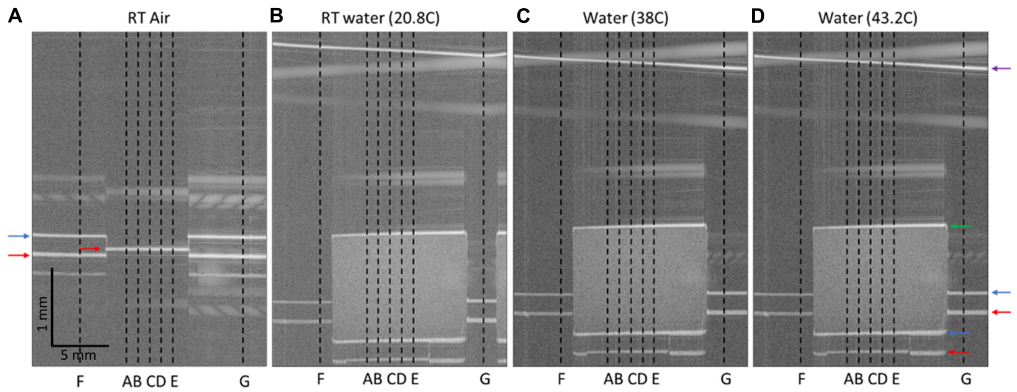

FIGURE 2 | OCT B-Scans of nc-YSZ sample, with locations of interest identified. **(A)** Spacer and dish imaged in air at room temperature, **(B)** nc-YSZ sample imaged atop spacer and dish in water at room temperature (20.8°C), **(C)** nc-YSZ imaged in water at 38°C, **(D)** nc-YSZ imaged in water at 43.2°C. Locations A, B, C, D, and E are used to analyze nc-YSZ optical properties, while F and G are used to measure the real vertical displacement of the experimental setup within the imaging field. Horizontal arrows highlight relevant surfaces (red = dish; blue = spacer top/nc-YSZ bottom; green = nc-YSZ top; purple = water surface).

plate. The water temperature was increased by heating the temperature adjustable plate to 38°C (near body temperature). After the temperature stabilized, an OCT image was acquired. Displacement in the image was observed to change relative to the RT image due to several factors. The increased temperature changes the group refractive index of the water, and potentially of the nc-YSZ. Evaporation of the water during the heating to 38°C decreased the height of the air-water interface as well as decreasing the real displacement of the temperature adjustable plate *via* compression. The temperature adjustable plate also undergoes thermal expansion during heating, translating the dish upward in the image. Finally, the temperature was increased further, to 43.2°C. This temperature is near the upper limit with which the implant should be used *in vivo*. Higher temperatures were not attempted, as turbulence of the water became a confounding factor at temperatures above 45°C. Similar to the case with 38°C, the displacement in the image was observed to change relative to images recorded at lower temperatures due to the same factors discussed above.

Group Refractive Index of Water

Refractive index of water was calculated using reference data (Thormählen et al., 1985), and assuming 1.0221 bar atmospheric pressure. Refractive index values were calculated for several wavelengths near the central OCT wavelength of 1,300 nm for each temperature of interest, and converted to group refractive index using Equation 1 (Paschotta, 2021):

$$n_g = n - \lambda_0 \cdot (dn/d\lambda_0) \quad (1)$$

where n_g is the group refractive index, n is the phase index of wavelength λ, and λ_0 is the central OCT wavelength of 1,300 nm. Calculated group refractive index values of water are 1.337, 1.339, and 1.340 at 20.8, 38, and 43.2°C, respectively.

TABLE 1 | Group refractive index of nc-YSZ measured at five locations and three temperatures, using an image-based approach.

ng_{nc-YSZ}	20.8°C	38°C	43.2°C
A	2.159	2.159	2.168
B	2.164	2.150	2.165
C	2.176	2.164	2.159
D	2.176	2.174	2.159
E	2.181	2.173	2.176
Average	2.171	2.164	2.165
St. Dev	0.009	0.010	0.007

TABLE 2 | Real thickness of nc-YSZ sample measured at the five locations and three temperatures, using an image-based approach.

d_{nc-YSZ}	20.8°C	38°C	43.2°C
A	668 μm	668 μm	665 μm
B	669 μm	671 μm	663 μm
C	663 μm	669 μm	671 μm
D	666 μm	666 μm	674 μm
E	667 μm	669 μm	666 μm

TABLE 3 | Group delay of nc-YSZ sample measured at the five locations and three temperatures.

GD	20.8°C	38°C	43.2°C
A	6.059e-11 s	6.034e-11 s	6.178e-11 s
B	6.059e-11 s	6.207e-11 s	6.116e-11 s
C	6.059e-11 s	6.126e-11 s	6.208e-11 s
D	6.125e-11 s	6.126e-11 s	6.118e-11 s
E	5.998e-11 s	6.147e-11 s	6.144e-11 s

Method 1: Image-Based Analysis (Group Refractive Index and Sample Thickness)

Using ImageJ, distances within the image were quantified (in microns and pixels). At locations of interest (A, B, C, D,

E, F, and G in **Figure 2**), distances were measured from the top of the image to the following features: top surface of spacer, bottom of dish, top and bottom surfaces of nc-YSZ sample, and surface of water (see **Figure 2**). From these measurements, and group refractive index values of water at 20.8, 38, and 43.2°C, it is possible to separate the total displacement in the image Δ_{total} into apparent displacement $\Delta_{apparent}$ of the dish bottom and/or spacer (due to non-unity group refractive index of water and nc-YSZ) and the real displacement Δ_{real} of the setup (due to compression and/or expansion of the temperature adjustable plate), using Equations 2, 3:

$$\triangle_{\text{total}} = \triangle_{\text{apparent}} + \triangle_{\text{real}} \tag{2}$$

$$\triangle_{\text{apparent}} = T - d = T - \frac{T}{ng} \tag{3}$$

Where T = optical thickness, d = real thickness, and ng = group refractive index. First, the real displacement Δ_{real} of the setup at each temperature is found using the total displacement Δ_{total} of the spacer relative to the baseline image at locations F and G, where $\Delta_{apparent}$ is caused by ng_{water} only. Combining Equations 2, 3, Equation 4:

$$\triangle_{\text{real}} = \triangle_{\text{total}} - (T_{water} - \frac{T_{water}}{ng_{water}}) \tag{4}$$

The RT image had a real downward displacement Δ_{real} = 4 px (∼25 μm) relative to the baseline image in air. This is due to the weight of the water compressing the temperature adjustable plate. The 38°C image had a real upward displacement Δ_{real} = 2.5 px (∼15 μm) relative to the baseline image in air. This is due to thermal expansion of the temperature adjustable plate and experimental setup, as well as evaporation of some of the water compared to the RT image. The 43.2°C image had a real upward displacement Δ_{real} = 4 px (∼25 μm) relative to the baseline image in air, due to thermal expansion of the temperature adjustable plate and experimental setup, as well as water evaporation compared to the RT and 38°C images.

Subtracting these real displacements from the total displacement Δ_{total} in the images (using Equation 2) yields the apparent displacement within the image. To calculate the group refractive index of nc-YSZ from the apparent displacement, the method is the same as that used to compute real displacement Δ_{real} above, except it is applied at locations A, B, C, D, and E, where the apparent displacement $\Delta_{apparent}$ is due to non-unity group refractive index of water and nc-YSZ, using Equation 5:

$$\begin{aligned}\triangle_{\text{apparent}} &= \left(T_{\text{water}} - d_{\text{water}}\right) + \left(T_{nc-YSZ} - d_{nc-YSZ}\right) \\ &= \left(T_{\text{water}} - \frac{T_{water}}{ng_{water}}\right) + \left(T_{nc-YSZ} - \frac{T_{nc-YSZ}}{ng_{nc-YSZ}}\right)\end{aligned} \tag{5}$$

TABLE 4 | Group refractive index of nc-YSZ sample calculated from group delay and sample thickness.

ng_{nc-YSZ}	20.8°C	38°C	43.2°C
A	2.165	2.156	2.218
B	2.162	2.208	2.202
C	2.182	2.186	2.209
D	2.196	2.196	2.167
E	2.147	2.194	2.202
Average	2.170	2.188	2.200
St. Dev	0.019	0.019	0.019

TABLE 5 | Group delay dispersion of nc-YSZ sample measured at the five locations and three temperatures.

GDD	20.8°C	38°C	43.2°C
A	−4.83e-27 s^2	−3.16e-27 s^2	1.40e-25 s^2
B	−7.74e-27 s^2	1.61e-25 s^2	7.18e-26 s^2
C	−4.71e-27 s^2	8.25e-26 s^2	1.59e-25 s^2
D	8.02e-26 s^2	8.26e-26 s^2	8.01e-26 s^2
E	−2.91e-25 s^2	−1.26e-25 s^2	7.89e-26 s^2

TABLE 6 | β-parameter of nc-YSZ sample calculated from GDD and sample thickness.

β	20.8°C	38°C	43.2°C
A	−3.61e-24 s^2/m	−2.36e-24 s^2/m	1.05e-22 s^2/m
B	−5.79e-24 s^2/m	1.20e-22 s^2/m	5.41e-23 s^2/m
C	−3.55e-24 s^2/m	6.16e-23 s^2/m	1.18e-22 s^2/m
D	6.02e-23 s^2/m	6.20e-23 s^2/m	5.94e-23 s^2/m
E	−2.18e-22 s^2/m	−9.40e-23 s^2/m	5.92e-23 s^2/m

Method 2: Spectral Phase Function Based Analysis (Group Delay and Group Delay Dispersion)

The second method of analysis uses the spectral phase function to calculate group refractive index and chromatic dispersion (Walmsley et al., 2001). The spectral phase function describes the relationship between the optical frequencies in the OCT pulse and the difference in phase for each frequency returning from the top and bottom surfaces of the nc-YSZ sample. The spectral phase function may be written as a Taylor series expanded about the central OCT optical frequency, Equation 6:

$$\begin{aligned}\Phi(\nu) = {} & \Phi^{(0)} + \Phi^{(1)}(\nu_0)(\nu - \nu_0)' + \frac{1}{2}\Phi^{(2)}(\nu_0)(\nu - \nu_0)'' \\ & + \frac{1}{6}\Phi^{(3)}(\nu_0)(\nu - \nu_0)'''.\end{aligned} \tag{6}$$

Where Φ is phase, ν is optical frequency, ν_0 is the central OCT optical frequency, $\Phi^{(0)}$ is a common phase shift, $\Phi^{(1)}(\nu_0)$ is the GD, $\Phi^{(2)}(\nu_0)$ is the group delay dispersion (GDD), and higher order terms are higher order dispersion. Single A-lines were analyzed from the five locations of interest at the three temperatures. Each A-line was filtered using a Hilbert transform/narrowband phase-invariant spectral filter (Baumann et al., 2007) to isolate photons returning from the top and bottom surfaces of the sample (defined by full-width-half-maximum, FWHM, of the intensity peaks at the surfaces).

These two phase functions correspond to the spectral phase functions of light returned from the sample's top and bottom edges. Fitting this data with a polynomial curve can approximate the spectral phase function (Equation 6). 4th order polynomial fits of the optical phase vs. frequency data were performed in MATLAB (Mathworks, 2020). The curve fits were weighted by the normalized intensity spectrum of the OCT light source, and the high and low tails of the spectrum were trimmed by 200 pixels prior to the curve fitting to eliminate regions of low SNR and reduce the impact of noise. Root-mean-squared-error, RMSE, values were less than unity for all fits. The 1st order coefficient (Equation 6) of the spectral phase function approximation is the GD. The GD of each interface corresponds to the actual optical path length difference between the interface and the reference arm. A subtraction of these GDs for the top and bottom interfaces corresponds to the distance the light

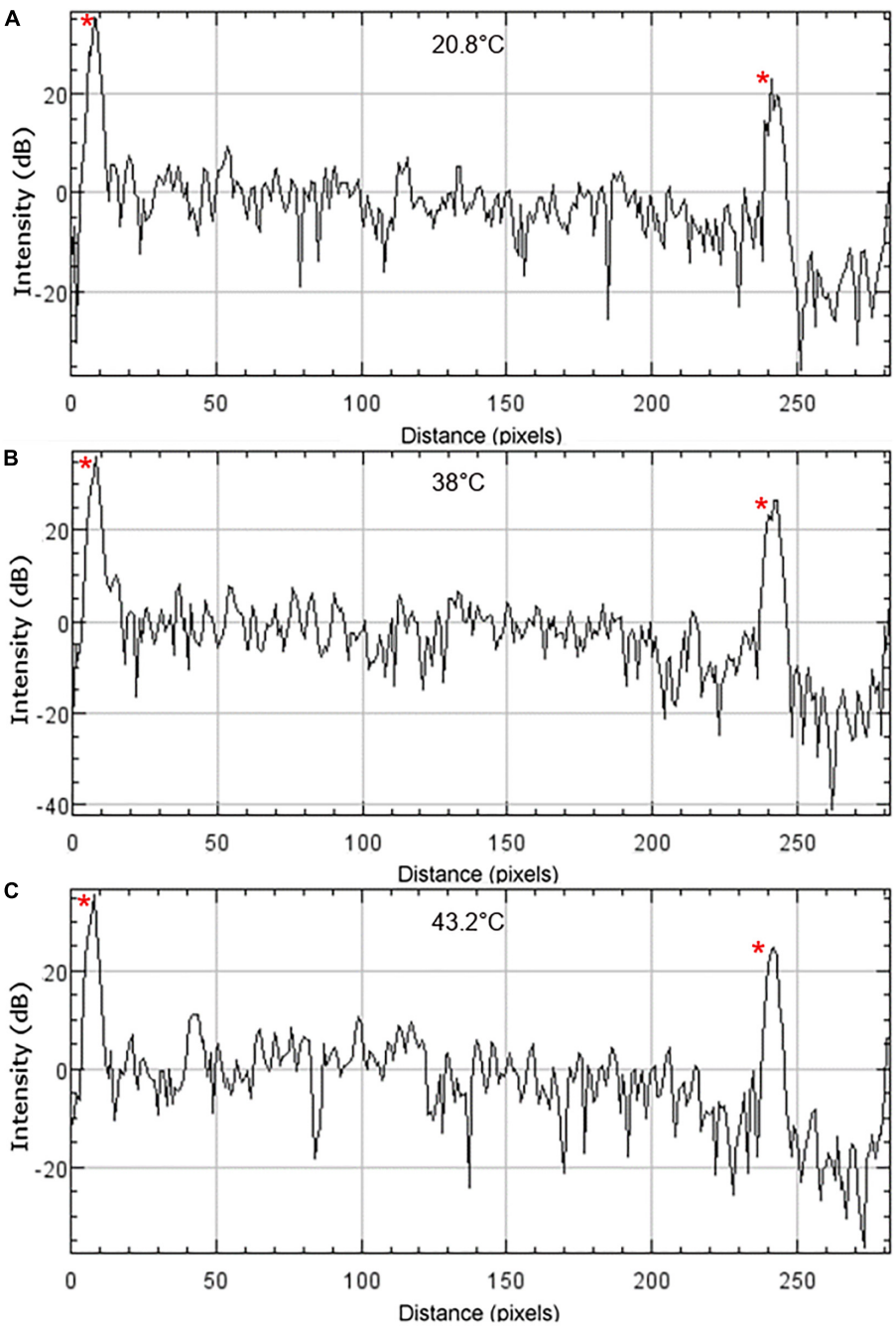

FIGURE 3 | OCT intensity profiles of nc-YSZ sample at location E at **(A)** 20.8°C RT, **(B)** 38°C, and **(C)** 43.2°C. The upper and lower surfaces of the sample are visible as intensity spikes on the left and right side of the profiles, respectively, and are identified with red asterisks.

experienced within the sample ($T = ng \times d$, Equation 2). The 2nd order coefficient (Equation 6) of the spectral phase function of the bottom surface is the approximation of half of the GDD the light experienced within the sample (from the top surface to the bottom surface of the sample).

RESULTS

Group refractive index of nc-YSZ was determined at each location and each temperature using the image-based analysis (Method 1) described above (**Table 1**). This method also allowed for determination of real sample thickness d_{nc-YSZ} at each location and temperature, using the optical thickness T divided by the group refractive index ng (Equation 2) along with a calibration factor of 6.19 microns per pixel in the OCT image (**Table 2**). This method is based on whole pixels, and an error analysis shows that the measurement has a 2% error.

Next, GD of the nc-YSZ sample was calculated using the spectral phase function approach (Method 2) described above. The 1st order coefficient of the spectral phase function approximation (Equation 6) is the GD. The GD for each location and temperature are shown in **Table 3**. From GD, group refractive index ng can be calculated using Equation 7 and sample thickness values d_{nc-YSZ} obtained from Method 1 (**Table 2**):

$$ng = \frac{GD \cdot c}{4\pi \cdot d} \tag{7}$$

Where c = 2.998e+8 m/s. Group refractive index values calculated using Equation 7 are shown in **Table 4**. Because this method uses sample thickness values determined from Method 1, it also has a 2% error in the calculated values of group refractive index, or an error of ∼0.043.

The 2nd order coefficient of the spectral phase function approximation (Equation 6) is half of the GDD. The GDD for each location and temperature are provided in **Table 5**. Dividing the GDD by twice the sample thickness d_{nc-YSZ} (round trip), the β-parameter of dispersion measured for the sample is shown in **Table 6**. Dispersion values varied between locations in the sample and between temperatures at a single location, even flipping sign between normal and anomalous dispersion. These values were reproducible by sequential A-lines at each location and temperature, causing us to believe this variability is not due to recording method or analysis.

DISCUSSION

Group refractive index of the nc-YSZ window is important to consider when interpreting OCT images of underlying brain tissue, like those we reported previously (Halaney et al., 2020). Because OCT is a time-based imaging technique, on-axis dimensions in reconstructed OCT images depend on time-of-flight of the OCT pulse. When imaging across the WttB implant with a group refractive index of ∼2.1, the implant appears 2.1 times of its mechanical thickness, and displaces other features beneath the window downward in the image. This effect can be visualized in **Figures 2B–D**, where the coverslip spacer beneath the nc-YSZ implant is displaced downward and appears discontinuous with the parts of the coverslip spacer on either side of the implant (blue arrows). Knowing the group refractive index is also important for focusing light through the window onto underlying tissue. Changes in group refractive index between the different temperatures investigated in this study (20–43°C) were ≤0.036, and within the 2% uncertainty of the sample thickness measurement. This temperature stability is not surprising, since YSZ has high thermal stability (Ghosh et al., 2009) and stable broadband IR reflection at much greater temperatures than those used in this study (i.e., >1400°C) (Leib et al., 2016).

Chromatic dispersion values were found to vary across different sample locations and different temperatures at fixed locations, even flipping sign between normal and anomalous dispersion (**Tables 5**, **6**). Despite this variability, individual measurements were reproducible by repeated A-lines at each fixed location and temperature. Thus, this variability may be a material property and not an artifact of data recording or analysis. The observed dispersion variation may be due to light scattering within the bulk of the sample at grain boundaries. Scattering within the sample is apparent in recorded OCT images shown in **Figure 2**, and is quantified in **Figure 3** at location E for each temperature. This chromatic dispersion should be studied further, and will be an important consideration for any multispectral optical approaches that propagate light through the window where precise pulse duration needs to be maintained.

There were several limitations to the current study. As explained in the introduction, an additional method to validate the OCT findings was not conducted, due to the scattering properties of our samples and the need for simultaneous determination of the sample thickness at each location being interrogated. Additionally, while this measurement covered the wavelength range and temperature range anticipated for clinical applications of the implant with OCT brain imaging, wavelengths, and temperature effects outside of this range were not assessed. The values of group refractive index reported here are applicable to light with a spectral range of 136 nm and centered at 1,300 nm only.

CONCLUSION

Nc-YSZ cranial implant windows have a group refractive index of 2.1–2.2 for OCT imaging with a spectral range of 136 nm centered at 1,300 nm at normal working implant temperatures (20–43°C). Chromatic dispersion for this spectral range was observed to vary over the sample, sometimes flipping signs between normal and anomalous dispersion. These properties of nc-YSZ should be considered when designing optical systems and procedures that propagate light through the window.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee, University of Texas at Austin.

AUTHOR CONTRIBUTIONS

DH, NK, and HF performed the OCT imaging and analysis. TM and GA provided guidance on study design and data interpretation. All authors contributed to manuscript revision, read, and approved the submitted version.

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REVIEW
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Nanomedicine: A Promising Way to Manage Alzheimer's Disease

Nazeer Hussain Khan[1], Maria Mir[2], Ebenezeri Erasto Ngowi[1,3], Ujala Zafar[4], Muhammad Mahtab Aslam Khan Khakwani[1], Saadullah Khattak[1], Yuan-Kun Zhai[1,5], En-She Jiang[1,6], Meng Zheng[7], Shao-Feng Duan[1,8], Jian-She Wei[1,9], Dong-Dong Wu[1,5] and Xin-Ying Ji[1,10]**

[1] Henan International Joint Laboratory for Nuclear Protein Regulation, School of Basic Medical Sciences, Henan University, Kaifeng, China, [2] Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, [3] Department of Biological Sciences, Faculty of Sciences, Dar es Salaam University College of Education, Dar es Salaam, Tanzania, [4] School of Natural Sciences, National University of Sciences and Technology, Islamabad, Pakistan, [5] School of Stomatology, Henan University, Kaifeng, China, [6] Institutes of Nursing and Health, School of Nursing and Health, Henan University, Kaifeng, China, [7] International Joint Center for Biomedical Innovation, School of Life Sciences, Henan University, Kaifeng, China, [8] Institute for Innovative Drug Design and Evaluation, School of Pharmacy, Henan University, Kaifeng, China, [9] Brain Research Laboratory, School of Life Sciences, Henan University, Kaifeng, China, [10] Kaifeng Key Laboratory of Infection and Biological Safety, School of Basic Medical Sciences, Henan University, Kaifeng, China

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Edited by:
Sílvia Pujals, Institute for Bioengineering of Catalonia (IBEC), Spain

Reviewed by:
Jesús M. De La Fuente, Institute of Materials Science of Aragon (ICMA), Spain
Carlotta Pucci, Center for Micro-BioRobotics, Italian Institute of Technology (IIT), Italy

***Correspondence:**
Dong-Dong Wu
ddwubiomed2010@163.com
Xin-Ying Ji
10190096@vip.henu.edu.cn

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Alzheimer's disease (AD) is a devastating disease of the aging population characterized by the progressive and slow brain decay due to the formation of extracellular plaques in the hippocampus. AD cells encompass tangles of twisted strands of aggregated microtubule binding proteins surrounded by plaques. Delivering corresponding drugs in the brain to deal with these clinical pathologies, we face a naturally built strong, protective barrier between circulating blood and brain cells called the blood–brain barrier (BBB). Nanomedicines provide state-of-the-art alternative approaches to overcome the challenges in drug transport across the BBB. The current review presents the advances in the roles of nanomedicines in both the diagnosis and treatment of AD. We intend to provide an overview of how nanotechnology has revolutionized the approaches used to manage AD and highlight the current key bottlenecks and future perspective in this field. Furthermore, the emerging nanomedicines for managing brain diseases like AD could promote the booming growth of research and their clinical availability.

Keywords: Alzheheimer's disease, pathogenesis, blood brain barrier, nanomedicines, cellular transport, nanoparticles, drug delivery, theranostic

INTRODUCTION

Alzheimer's disease (AD) is a devastating condition of the aging population characterized by the progressive and slow brain decay due to plaque formation in the hippocampus (Querfurth and LaFerla, 2010). It has been found that the formation of those plaques starts up about 20 years before the onset of clinical symptoms, which makes the exact trajectory of pathologies associated with AD unclear (Jack and Holtzman, 2013; Fagan et al., 2014). The incidence of AD is rising worldwide.

Abbreviations: AD, Alzheimer's disease; BBB, blood–brain barrier; CNS, central nervous system; NPs, nanoparticles; T2DM, diabetes mellitus type 2; RAGE, receptor for advanced glycation end products; LRP1, lipoprotein receptor related protein 1; JAMs, junctional adhesion molecules; FDA: Food and Drug Administration; PLGA-NPs, polylactide-co-glycolide-nanoparticles; PBCA, polybutyl cyanoacrylate; TPP, triphenylphosphonium; EGCG, epigallocatechin-3-gallate; TAT, *trans*-activating transduction; PEG, polyethylene glycol; RES, reticuloendothelial system.

More than 50 million people were affected with AD in 2019, and its burden is continuously rising, which may lead to an enormous effect on both the world economy and manpower (Patterson, 2018). It is estimated that by the middle of the current century, 13.8 million Americans of age 65 and older may have AD symptoms. AD is the sixth leading cause of death in the United States, where the death rate has increased to 146.2% in 2018 and 122,019 people died (Alzheimer's Association, 2020).

There are several limitations to deal with the pathology of AD. The drugs used to cure the cognitive impairments of the brain in AD are based on the neurotransmitters or enzyme modulation with the intranasal route for their delivery to the brain (Sood et al., 2014). However, with the use of these drugs, frequent therapy failures are being reported due to their less absorption in neuronal cell membranes, instability, brain toxicity, and other pharmacokinetic and pharmacodynamic parameters (Arias, 2014; Suri et al., 2015). Engineered nanoparticles (NPs) with unique physicochemical properties and the capability to cross the blood–brain barrier (BBB) may be a promising strategy to solve these biomedical and pharmacological problems in the treatment of brain diseases like AD (Mukherjee et al., 2020). The delivery of drugs to the brain through these NPs could enhance the pharmacokinetic and pharmacodynamic profiles of the drugs with minimal toxicity (Orive et al., 2003; Gupta et al., 2019). The therapeutic potential of the drugs can be increased by employing nanotechnology-based drug delivery approaches (Nazem and Mansoori, 2008; Brambilla et al., 2011). The most essential advantage of nanomedicines for the treatment of brain diseases like AD is the controlled release of drugs at a particular site (Betancourt et al., 2009; Safari and Zarnegar, 2014). The synthesis of these NPs for drug delivery is an emerging approach, a multidisciplinary field that provides new understandings and opens avenues to manipulate materials, tissues, cells, and DNA with at least one dimension sized from 1 to 150 nm (Li S. et al., 2018; Cheng et al., 2019; Ovais et al., 2019). Nanomedicine has made considerable achievements in many fields including medicines, pharmacy, chemical/biological detection, and optics (Binda et al., 2020; Chen K. et al., 2020; Pan et al., 2020). The present study intends to provide an overview of how nanotechnology has revolutionized AD treatment/imaging and the understanding of cellular function by mainly focusing on the state-of-the-art nanomedicine-based approaches used for AD. The current key bottlenecks and future perspective in this field are also highlighted.

In this review, we depict the advances and merits of nanomedicines in the treatment of AD, believing that the emerging nanomedicines could promote the booming growth of research in this field and become clinically available for the diagnosis and monitoring of therapeutic interventions for AD and other similar central nervous system (CNS) disorders in the future.

PATHOGENESIS OF AD

For a better understanding of the pathogenesis of AD, it is essential to identify the targets for direct therapy and intervention at the earlier stages when the changes are reversible. The prominent features in AD onset are the appearance of intracellular neurofibrillary tangles and extracellular amyloid plaque formation in the brain. The histopathologic traits include hippocampal neuronal loss, synaptic degeneration, and aneuploidy. Moreover, neuroinflammation, oxidative stress, microbial infection, mitochondrial dysfunction, and a compromised brain lymphatic system have also been recognized as early pathophysiological modifications in the course of AD (Swerdlow, 2007; Khoury and Grossberg, 2020). There are several physiological factors involved in the onset of AD (Harilal et al., 2019), which are summarized in **Figure 1**. The lifestyle- and age-related factors can aggravate the progression of AD. The following subsections describe these factors to better understand the pathogenesis of AD.

Hypertension

The most associated, age-dependent factor that contributes to the development of AD in an aged population is hypertension. Although the mechanisms of this association are complex, it is considered that hypertension can influence AD through the association of cerebrovascular pathology (Diaz-Ruiz et al., 2009; Nehls, 2016). Hypertension–high blood pressure may also contribute to the pathology of AD as the investigation indicates that hypertension leads to the increased plaque formation in the brain (Petrovitch et al., 2000; Hoffman et al., 2009).

Diabetes Mellitus

Recent findings have revealed the associations between high sugar level and AD development. It has been found that MA-[D-leu-4]-OB3 used for the treatment of obesity and diabetes can relieve AD pathologies (Anderson et al., 2019). About the association of AD and diabetes mellitus type 2 (T2DM), a study has reported that the depletion of caveolin-1 in T2DM can induce AD (Bonds et al., 2019). Recently, a theoretic support is depicted on the association of diabetes and AD with new targets to prevent the diabetic patients from AD (Sun et al., 2020). It has also been observed that preventive measurements against diabetes like weight–diet balance, sports, and other physical activities could relieve AD (Kang et al., 2006; Stampfer, 2006).

Homocysteine

Homocysteine, a sulfur-containing amino acid, plays an essential role in AD-related pathologies and participates in the elevation of beta amyloid (Aβ) plaque formation (Refsum et al., 2004; Pacheco-Quinto et al., 2006). Additionally, homocysteine may contribute in raising the oxidative stress in the brain that leads to the progression of AD pathologies (Selley, 2004).

Inflammatory Biomarkers

Inflammatory biomarkers including C-reactive proteins, interleulin-6, fibrinogen, alpha-1-antichymotrypsin, and other lipoprotein-associated phospholipase A2 have a strong association with total dementia risk (Irizarry, 2004; Mrak and Griffin, 2005). However, more findings need further evaluations.

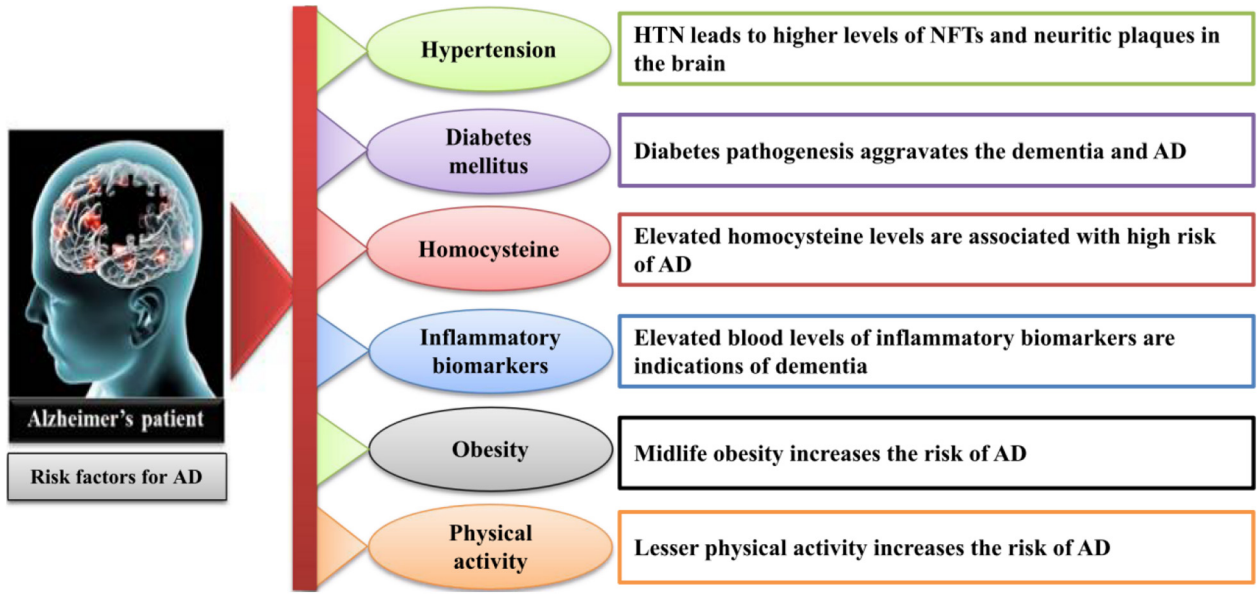

FIGURE 1 | The lifestyle- and age-related factors involved in the onset of AD. AD, Alzheimer's disease; HTN, hypertension; NFT, neurofibrillary tangles.

Obesity

As aforementioned, there is lack of literature for better evaluation of the risk factors involved in the onset of AD, but there are suggestions from different studies on the relation of midlife obesity and increased AD risk. Nevertheless, with some non-conformities, the studies based on neuroimaging, adiposity, and cognitive decline support the association of obesity and AD development (Atti et al., 2008; Beydoun et al., 2008).

Physical Activity

Many investigations have verified the notion that the progression of AD is associated with the physical activity of a person. Healthy physical activities could retard the cognitive decline in older adults (Abbott et al., 2004; Akbaraly et al., 2009). Similarly, other studies have shown that the brain is more active during exercises such as meditation and yoga (Streeter et al., 2007; Gard et al., 2014). It has also been reported that physical activity is significantly correlated with gut microbiota that play roles in the prevention and development of AD (Schlegel et al., 2019). Another study has proved the benefit of regular exercise for AD, suggesting that exercise exerts anti-inflammatory effects and provides other numerous benefits through different pathways that might help in preventing the progression of AD (Valenzuela et al., 2020). However, the inverse correlation of physical activity and cognitive decline needs further investigations.

Role of BBB in the Pathogenesis of AD

The human CNS is a complex system that is well distinguished by two specialized barriers in the form of cerebrospinal fluid barrier (CSFB) and BBB (Pathan et al., 2009). BBB plays a crucial role in the pathogenesis of AD. In AD, cerebrovascular dysfunction results in cognitive impairment and dementia, which may lead to cerebral amyloid angiopathy. It also mediates the accumulation of Aβ peptides in the brain. The BBB is important for the regulation of Aβ transport to brain via two primary receptors, namely, (1) receptor for advanced glycation end products (RAGE) and (2) the low density of lipoprotein receptor related protein 1 (LRP1). The faulty clearance of Aβ via the deregulated RAGE/LRP1 receptors, arterial dysfunction, and impaired angiogenesis may commence Aβ accumulation, neurovascular uncoupling, brain hypoperfusion, cerebrovascular regression, and neurovascular inflammation. Eventually, these events result in compromised BBB and subsequent neuronal and synaptic impairment (Deane and Zlokovic, 2007).

BBB: A LIMITING FACTOR IN DRUG DELIVERY TO BRAIN

Blood–brain barrier plays a pivotal role in shuttling biomolecules in and out of the brain neuronal system. Therefore, understanding the structural and functional characteristics of BBB is essential for improving the drug delivery to the brain. This protective unit factor helps to prevent shuttling of molecules between blood and brain composed of vascular endothelial cell layers bound back by tight junctions and other supportive structures (Ballabh et al., 2004). The endothelial cells are surrounded by a basement membrane covered by astrocyte end-feet and continuously monitored by surveying microglial cells (Abbott et al., 2010). Cohesive domains, bound to endothelial cells, provide perseverance for the selective transport of small molecules across the BBB (Ballabh et al., 2004; Abbott et al., 2010). To meet the requirements of proteins and peptides for brain homeostasis, a controlled intracellular transport occurs via transcytosis. Depending on the nature of the molecules (hydrophilic and hydrophobic), endothelial cells

with the help of various special transporting proteins could facilitate the transport. To cure brain illness like AD, different nanocarriers have been reported in preclinical studies. These carriers encapsulate the drugs against AD as cargo and cross the BBB. For a detailed view of the transcytosis and nanocarrier transport across the BBB, readers are referred to recent reviews on these topics (Villaseñor et al., 2019; Mulvihill et al., 2020).

NOSE-TO-BRAIN DRUG DELIVERY AND LIMITATIONS

The delivery of drugs to the brain via the nasal route generally starts from the respiratory epithelium to the olfactory region with the help of the trigeminal nerve and olfactory nerve cells. This drug delivery route is supposed to transport the drug molecules to different parts of the brain including the frontal cortex, olfactory bulb, cerebrum, and brain stem (Illum, 2000; Dhuria et al., 2010). To deal with AD, the mechanism of drug transport into the brain can be classified in two ways, namely, intracellular drug transport and extracellular drug transport.

Intracellular drug delivery is the intraneuronal route of drug transport. This route of transport is relatively slow and takes around 24 h to reach from the nasal cavity to brain cells (Kristensson and Olsson, 1971). After entering the nasal cavity, exploiting the mechanism of endocytosis, the drugs could move to olfactory sensory neurons and peripheral trigeminal neurons via the olfactory and respiratory epithelium, respectively. Further from nerve cells, intracellular and transcellular transports mediate the movement of drug to different parts of the brain. The intracellular route delivers the drug to the olfactory lobe from the olfactory nerve and to the brain stem from trigeminal nerves. At the same time, the transcellular route provides the drug to the lamina propria, which further enters the brain through different ways. This type of transport facilitates the delivery of those drugs coated with lipophilic molecules that can adopt the passive diffusion or active transport/receptor-mediated transcytosis (Lochhead and Thorne, 2012). Extracellular transport facilitates the transport of hydrophilic drug substances, various proteins, and peptides. This is the sharp route of drug delivery from the nose to the brain and it is further divided into slow and fast extracellular transport.

Extracellular transport of drug is the fastest route of drug delivery from the nose to the brain. In this route, drug molecules use the intercellular clefts in the olfactory and respiratory epithelium and extracellular transport along the olfactory and trigeminal neural pathway to reach the spinal fluid and brain. Once the drug reaches the lamina propria, there are different options including (i) getting into systemic circulation via being absorbed in olfactory blood vessels, (ii) it may enter nasal lymphatic vessels, (iii) or it may enter the cranial compartment associated with olfactory nerve bundles by extracellular diffusion (Lochhead and Thorne, 2012). The challenges encountered in this route are mostly associated with the physicochemical properties of drugs, including molecular weight, lipophilicity, characteristics of the drug formulation, and the presence of specific receptors on the mentioned routes (Illum, 2000). The drug in the nasal route may be eliminated by mucociliary clearance or through CSF into blood (Harush-Frenkel et al., 2008). It is also reported that the surface charge, type, and concentration of the nanomedicine carrier also influence the drug delivery to the brain (Jones, 2008). In other limitations, sometimes due to the nature of the NPs used, e.g., phospholipid complexes target inflammatory sites and the reticuloendothelial system by themselves (Khan et al., 2013). Some other functionalized NPs tend to adsorb an arbitrary biological entity and form a protein sheath, referred to as a "protein corona," which leads to the non-targeted interaction of drugs and also random deposits/accumulation of the carrier substance in biological systems (Salvati et al., 2013; Zanganeh et al., 2016). In order to achieve a sufficient therapeutic level in the target region of human brain, there has been a continuous struggle to improve the uptake of those drug-encapsulated nanocarriers and to explore the safest transport mechanisms after absorption. Various pathways for the delivery of therapeutic moieties from the nasal route to the brain are illustrated in **Figure 2**.

NANOMEDICINES: A PROMISING APPROACH FOR DRUG DELIVERY THROUGH BBB

For the safe and effective delivery of U.S. Food and Drug Administration (FDA)-approved (FDA, 2019), commercially available drugs, several small-sized nanocarriers have been adopted to cure brain illnesses including brain cancer and AD. These nanocarriers with specific drugs belong to nanomedicines. While there is no cure for AD, **Figure 3** depicts the function of currently FDA-approved drugs to treat the symptoms of AD. The current anti-AD drugs can improve the clinical symptoms rather reversing or preventing the progression of the disease. To deliver these recommended drugs to the affected part of the AD brain, NP-functionalized nanomedicine is considered as the most useful applicable approach. Nanomedicines have a set of unique properties that enable them to deliver the anti-AD drugs at target sites in the brain. Nanomedicines have the advantages of reduced dimensions and increased biocompatibility that facilitate easy transport of therapeutic substances into the brain (Spuch et al., 2012; Fakhoury et al., 2015; Leszek et al., 2017). Small-size (approximately 100–10,000 times smaller than a human cell) nanomedicines can easily interact with the proteins and molecules on the cell surface as well as inside the cell (Kim et al., 2012). NP-functionalized nanomedicines have central core structures that ensure the encapsulation or conjugation of drugs and provide the protection and prolonged circulation in the blood (Knop et al., 2010; Li Y. et al., 2018). Nanomedicines are also specialized to target cells or even an intracellular compartment like Aβ in cells and thus can deliver the drug at a predetermined dosage directly to the pathological site (Gao and Jiang, 2006). Nanomedicines can minimize the dose and frequency and then improve patient compliance (Altinoglu and Adali, 2020). Regardless of some clinical issues, nanomedicines have potential advantages of favorability to the brain, greater stability, biocompatibility

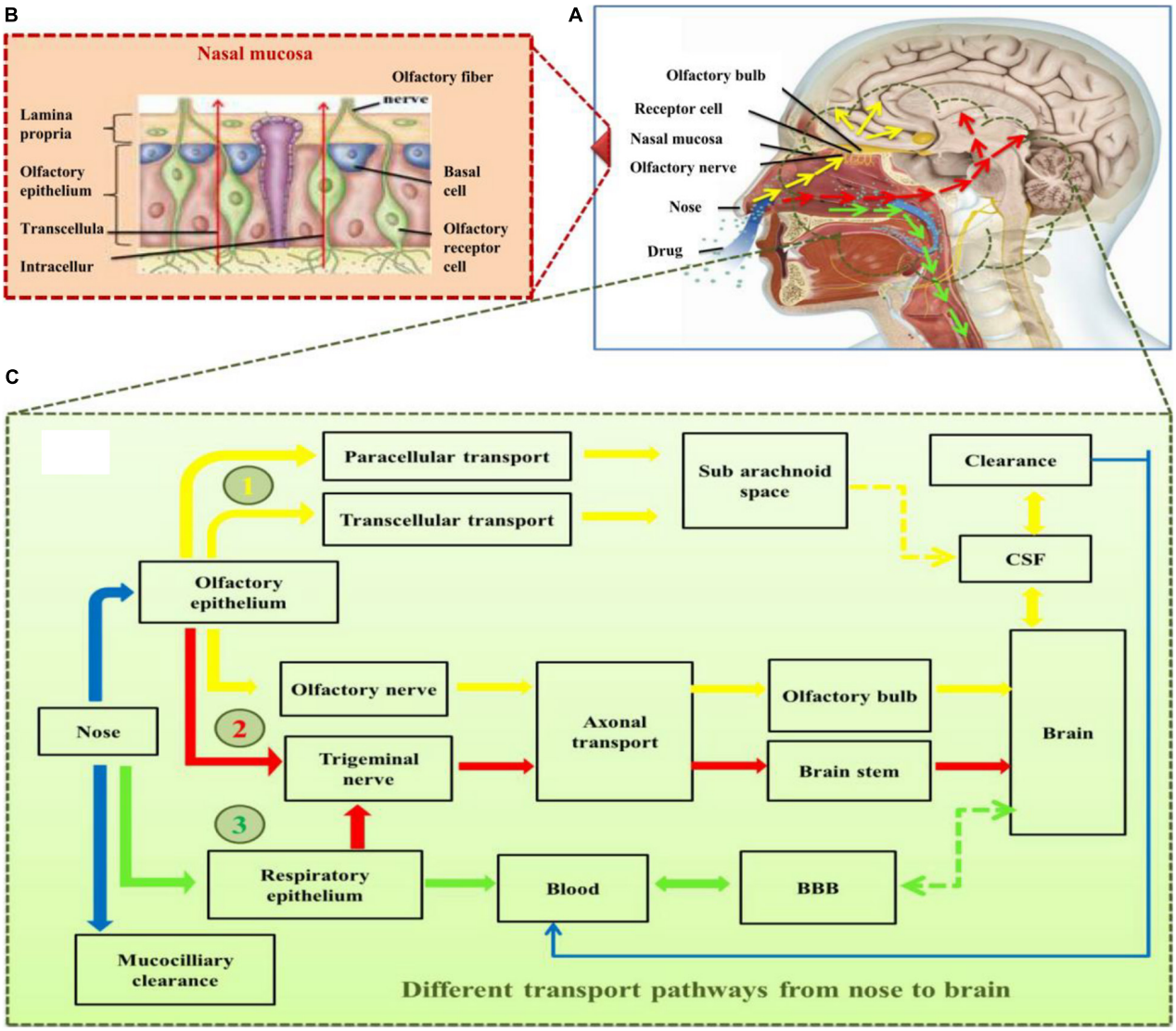

FIGURE 2 | (A) Skull structure presenting major components of nose and brain involved in drug transport to the brain, **(B)** structural components of nasal mucosa, **(C)** the various pathways (highlighted in different colors; yellow, red, and green) for the delivery of drugs into the brain through intranasal route. Yellow arrows present the major direct pathway involving the olfactory nerve, red arrows present the pathway primarily involving the germinal nerve route, and green arrows illustrate the minor pathway involving blood–brain barrier (BBB) following the systemic absorption of the drug molecules through respiratory epithelium. BBB, blood–brain barrier; CSF, cerebrospinal fluid.

and biodegradability, protection from enzymatic degradation, increased half-life, improved bioavailability, and controlled release over other conventional ways of drug delivery to the brain to cure AD (Altinoglu and Adali, 2020). **Figure 4** demonstrates how functionalized NPs have been employed to overcome the BBB, exploiting different transport pathways to achieve anti-AD effects of the delivered cargoes.

NANOMEDICINES TO MANAGE AD

Nanomaterials are being widely explored to manage the pathologies of AD. The following sections describe how nanostructure-based delivery systems are being employed for the diagnosis and treatment of AD. **Table 1** presents the most recent applications of functionalized nanomaterials as carriers for delivering therapeutic moieties and imaging agents to brain for managing AD-related pathologies. Here, we present an updated view of nanomedicines as nanocarriers for delivering therapeutic moieties.

Organic Nanostructures

Polymeric-Based NPs

Polymeric biodegradable NPs functionalized with PEG and antibody have been successfully designed and tested in transgenic

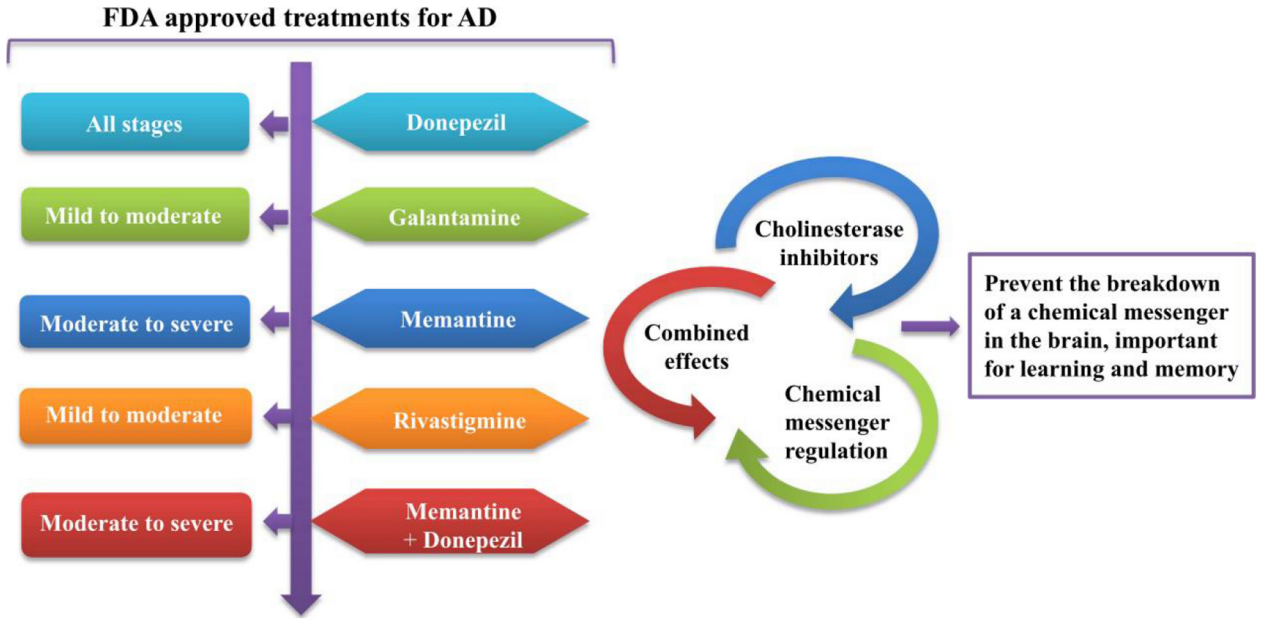

FIGURE 3 | The functioning of currently FDA-approved drugs to treat the symptoms of AD. FDA, Food and Drug Administration; AD, Alzheimer's disease.

AD mice. A recent study reveals that PEGylated NPs' exposure can lead to the correction of memory defect and a significant reduction in Aβ soluble peptides. Thus, the designed formulation can be used to cure AD illness (Carradori et al., 2018).

To enhance the efficacy of memantine against AD, a study has been conducted in which memantine is loaded into biodegradable polymeric NPs synthesized by double emulsion method. Targeting the AD brain with memantine-loaded NPs can lead to a significant reduction in Aβ plaques and AD-associated inflammation (Sánchez-López et al., 2018). Similarly, vitamin D binding protein, a therapeutic candidate to relieve AD symptoms, has been loaded in biocompatible and degradable poly lactic-co-glycolic acid (PLGA) NPs to treat AD. It has been shown that significant reductions in Aβ accumulation, neuroinflammation, neuronal loss, and cognitive dysfunction in transgenic AD mice model have been observed (Jeon et al., 2019). Targeting the brain with zinc-loaded polymeric NPs can also lead to the decrease in amyloid plaque size and mitigate other neuronal dysfunctions in AD mice (Vilella et al., 2018). The polysaccharides in NP formulations have several advantages in biological systems, including being highly stable, being non-toxic, being biodegradable, and having a hydrophilic nature (Liu et al., 2008; Venkatesan et al., 2016; Jhaveri et al., 2021). It has been investigated that sitagliptin (SIT) [a dipeptidyl peptidase-4 (DPP-4) inhibitor]-loaded NPs show effective therapy against AD symptoms in an animal model (Wilson et al., 2020). To deliver huperzine A, an acetylcholinesterase inhibitor, the mucoadhesive and target PLGA-NPs with surface modified by lactoferrin-conjugated N-trimethylated chitosan have been adopted. The formulation has demonstrated a good sustained-release effect and target ability against AD pathologies (Meng et al., 2018). A special nanocarrier system of alginate-chitosan NPs can act as an excellent transporter to deliver the SpBMP-9, a short peptide derived from bone morphogenetic protein (BMP-9), across the BBB to AD brain. SpBMP-9 is known as a candidate drug for AD for its function in promoting the differentiation of cholinergic neurons and inhibiting GSK3β (Elnaggar et al., 2015; Beauvais et al., 2016; Lauzon et al., 2018). Similarly, with the delivery of another AD drug, Perlerin, CS-NPs have shown significant effective results with no brain toxicity and improved cognitive skills in AD Wistar rats (Hanafy et al., 2015). Trimethyl chitosan-PLGA NPs provide excellent transport of coenzyme Q10 into the brain of transgenic mice where it protects the brain from AD pathologies (Wang et al., 2010).

Curcumin is a naturally occurring antioxidant with low toxic nature and a free radical scavenger phytochemical (Kim et al., 2008; Mishra and Palanivelu, 2008; Shen and Yu, 2008). Curcumin provision to brain against tau protein aggregation in AD is considered the most attractive approach in AD treatment where it binds with tau protein-based amyloid and shows anti-amyloid properties in the micromolar concentration range (Yang et al., 2005; Garcia-Alloza et al., 2007; Mohorko et al., 2010; Re et al., 2010; Yanagisawa et al., 2010). Furthermore, there is an inhibitive effect of curcumin on the hyperphosphorylation of tau proteins (Park et al., 2008). Curcumin delivery to the brain faces the issues of poor stability and bioavailability that lead to less brain uptake (Anand et al., 2007). To deal with these issues, use of nanocarriers is preferred due to its safety, as well as higher and prolonged exposure to the brain. Various specialized NPs have been designed that encapsulate the curcumin and deliver it to the brain via transcytosis across the BBBs. Due to the friendly nature of the biological system, use of PLGA NPs is a common practice. Curcumin loaded with PLGA NPs can permeate through the BBB and reach the AD regions where it shows protective effect against

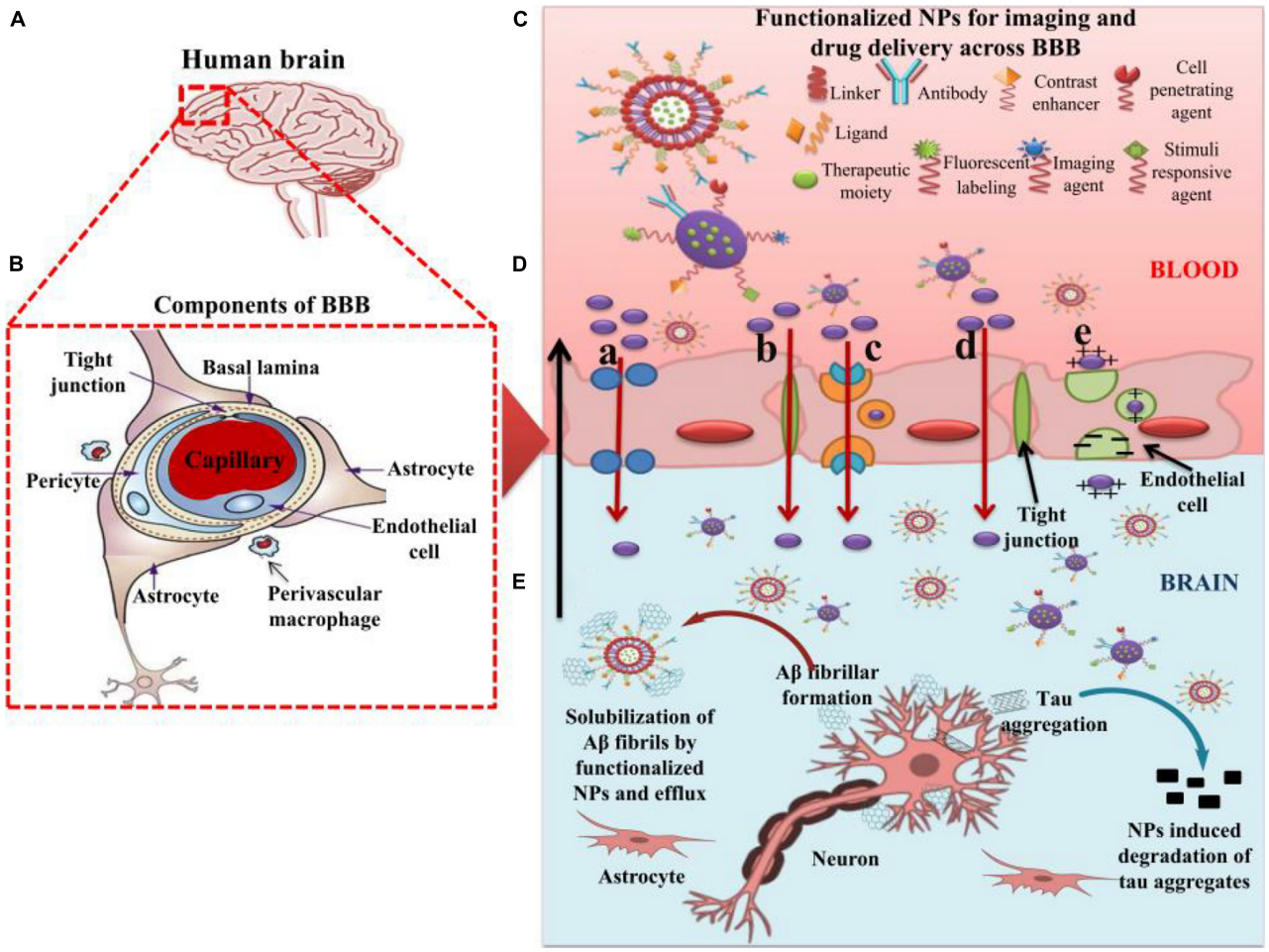

FIGURE 4 | The role of nanoparticles in overcoming the BBB for efficient delivery of therapeutic moieties to treat AD. **(A)** Image of human brain. **(B)** Components of the BBB. **(C)** Functionalized nanoparticles (NPs) for imaging and targeted drug delivery to the AD brain. **(D)** Different pathways of transport **(a–e)** across BBB utilized by functionalized NPs. **(a)** Transport of NPs through cellular transport proteins; **(b)** transport of NPs through tight junctions; **(c)** transport of NPs via receptor-mediated transcytosis; **(d)** transport of NPs via transcellular pathway following diffusion, specifically adopted by gold NPs; **(e)** transport of cationic NPs and liposomes via adsorption-mediated transcytosis. **(E)** Effect of functionalized NPs in treating AD via the degradation of tau aggregates and efflux of Aβ fibrils after getting solubilized by the NPs. AD: Alzheimer's disease; NPs: Nanoparticles; BBB: Blood–brain barrier.

the beta amyloid accumulation (Joseph et al., 2018). A stabilized and sustained curcumin delivery across the BBB is also achieved by using a nanoemulsion of red blood cell membrane-coated PLGA particles with embedded T807 molecules on the red blood cell membrane surface (T807/RPCNP) loaded with curcumin. Mutual effects of T807, 807/RPCNP exhibit strong inhibitory effects against tau-associated pathogenesis (Gao C. et al., 2020). In another study, hydroxypropyl-cyclodextrin-encapsulated curcumin complexes (CUR/HP-CD inclusion complexes) emulsion shows greater cellular uptake of curcumin across the BBB and can be considered as a better carrier system to deliver curcumin to the brain for AD therapy (Zhang et al., 2020). Furthermore, curcumin loaded with chitosan and bovine serum albumin NPs increase the drug permeation and accelerate the phagocytosis of the Aβ peptide to relieve AD symptoms (Yang R. et al., 2018). Other biological advantages of curcumin-based nanomedicines against brain diseases include its neuroprotective role by activating the transcription factor Nrf2, which is known as a master regulator of antioxidant response (Yang et al., 2009) and protects neuronal cells from dopaminergic toxicity (Szwed and Miłowska, 2012). In the Aβ-induced rat model, curcuminoids regulate the proliferation of neuronal stem cells via different kinase pathways (Ahmed et al., 2010; Liao et al., 2012). The use of curcumin for AD therapy could improve the neuronal cognition in the rat model (Xu et al., 2007; Dong et al., 2012). It plays important roles in neurogenesis, synaptogenesis, and migration of progenitor cells (Kang et al., 2006). Furthermore, curcumin-containing PLGA NPs are involved in the expression of genes that lead to neuronal cell proliferation and differentiation (Tiwari et al., 2014).

Nanomicellar

A nanomicellar water-soluble formulation of coenzyme Q10 (UbisolQ10) is applied to double transgenic AD mice in the

TABLE 1 | The applications of functionalized nanomaterials for delivering therapeutic moieties and imaging agents to brain for managing AD related pathologies.

Nanomaterials	TM/IG/FA	Delivery route	Particle size	Roles of materials in the enhancement of drug delivery	Effects on AD	References
Biomimetic nano-system comprising of RVG29 and TPP attached RVG/TPP-MASLNs	GS as TM RVG29 and TPP as FA	IV	<140 nm	MA membranes were used as a bioactive material to camouflage SLNs in order to bypass RES to prolong the systemic circulation of the nano-system. RVG29 and TPP act as targeting moieties to facilitate the transport across BBB and subsequently, internalization into neuronal mitochondria.	Results of *in vivo* studies demonstrated that both RVG/TPP-MASLNs-GS and MASLNs-GS reached in systemic circulation in higher concentrations in comparison to the un-modified SLNs-GS and free GS solution. A remarkable neuroprotective effect with efficient BBB permeability and mitochondria targeting of the biomimetic nano-system in both Aβ-damaged HT22 neuronal cells and AD model mice was obtained.	Han et al., 2021
EO-SLNs	EPO as TM	IP	219 nm	SLNs can enhance the bioavailability of EPO by overcoming the P-gp efflux and first pass effect. Additionally, changes in EPO from hydrophilic nature to lipophilic particle can improve the permeability to brain. Moreover, small size can facilitate the penetration into the target cells.	EPO-SLN prevented Aβ1-42-induced impairment of spatial recognition memory in AD model. Additionally, the EPO-SLN showed the anti-oxidant properties, prevented the Aβ plaque deposition, and decreased the ADP/ATP ratio, suggesting the suitability of the developed system for AD treatment.	Dara et al., 2019
PCL	Busulfan and Etoposide as TM	ICV	37–138 nm	Encapsulation into the self-assembly NPs and administration through ICV injection can improve NPs penetration in AD brain parenchyma and internalization in microglia cells.	Developed nano-system has demonstrated higher drug loading efficiency and controlled release of drugs in the targeted microglia cells, brain immature myeloid cells, in comparison to un-encapsulated drug, showing lesser side effects.	Peviani et al., 2019
USPIONs	Iron oxide as multi modal contrast agent PH-1 and PH-2 as theranostic agents	IV	12 nm	DPA-PEGylated USPIONs labeled with PH-1 and PH-2 (Phenothiazine-based small molecules), where, PEG was chosen as a linker, owing to its ability to reduce protein absorption and increase the circulation time of NPs. PH-1 and PH-2 have potential to inhibit β-amyloid aggregation and to be used as NIR imaging probes for amyloid plaques in AD.	The established nano-system has simultaneously performed *in vivo* MRI and NIR based enhanced fluorescence of Aβ plaques in the brain of double transgenic mice, prevented Aβ aggregation, disaggregated the already formed Aβ fibrils and demonstrated a protective effect against the toxicity of human neuroblastoma cells induced by $A\beta_{1-42}$.	Cai et al., 2020

(Continued)

TABLE 1 | Continued

Nanomaterials	TM/IG/FA	Delivery route	Particle size	Roles of materials in the enhancement of drug delivery	Effects on AD	References
PC-Fe_3O_4 and CdS-NPs	Fe_3O_4 and CdS as tau aggregation inhibitors	—	10–20 nm	The primary feature of the NPs developed is their complete biological synthesis using two fungal species; *Fusarium oxysporum* and *Verticillium* sp. The magnetite NPs were capped with hydrolytic proteins from fungi, while the CdS NPs were capped with four different kinds of proteins belonging to the group of sulfate-reducing enzymes. CPs avoid the aggregation of NPs.	The designed formulations have not affected the viability of neuroblastoma cells. Furthermore, PC-CdS NPs showed dual properties of disaggregation and inhibition of Tau. Hence, the NPs could be used as potent Tau aggregation inhibitors and can be subjected to several modifications for specific drug delivery owing to their very small size.	Sonawane et al., 2019
PBNPs	FAM, TM	—	50 nm	Based on florescence quenching ability of PBNPs and that DNA can adsorb on PBNPs surface via binding of phosphate skeleton in DNA to Fe^{2+}/Fe^{3+}, FAM-AptAβ@PBNPs-based florescent aptasensor to detect the $Aβ_{40}O$ was established.	Results indicated the sensitivity, selectivity, simplicity and applicability of the designed aptasensor for early diagnosis of AD. AD patients can be distinguished from healthy persons using this approach to detect $Aβ_{40}O$ levels in clinical samples of cerebrospinal fluid.	Chen W. et al., 2020
Lipid based NPs decorated with multi-target directed ligands	SHPs (SHP-2-Bn and SHP-2-R) as antioxidents and AChE and BChE inhibitors	Intranasal route	100 nm	Delivery system based on L-α-phosphatidylcholine and two SHP were developed as a multi-target treatment of AD. Insoluble SHP-2-Bn was chosen for modification of phospholipid membrane to prevent oxidation and membrane denaturation, whereas, water-soluble SHP-2-16 was chosen as an AChE inhibitor.	Results indicated that established nano-system has significantly reduced the scopolamine-induced AD-like dementia in rats. Moreover, the multi-target nano-system displayed the highest antioxidant activity with no toxicity and significant inhibition of brain AChE.	Burilova et al., 2020
DTNPs	Dopamine Tryptophan	Intra-ventricular	140 nm	Dopamine and tryptophan have been known to exhibit excellent anti-aggregation properties, neuroprotective effects, and anti-amyloid and fibril disaggregation activity, along with inherent fluorescent property. Tryptophan can cross the BBB via LAT1, thus, its presence in the nano-system can facilitate the delivery system to cross the BBB efficiently.	*In vitro* and *in vivo* investigations displayed the neuroprotective effects in neuroblastoma cells and anti-aggregation efficacy of DTNPs in both FF derived amyloid fibrils and preformed Aβ-peptide fiber, along with improved cognitive impairment in ICV-STZ induced animal model of dementia. Moreover, DTNPs also showed fluorescent properties and lighted up the cytoplasm of neuroblastoma cells illustrating their capacity to be used as an-intracellular bio-imaging agent.	Sharma et al., 2020

(Continued)

TABLE 1 | Continued

Nanomaterials	TM/IG/FA	Delivery route	Particle size	Roles of materials in the enhancement of drug delivery	Effects on AD	References
Solid lipid nanoparticles SLN and NLC	Quercetin as TM Transferrin as FA	—	200 nm	Quercetin exhibits strong neuroprotective effects in AD. Lipid NPs can prevent the photodecomposition, and degradation of quercetin. NPs were decorated with transferrin to help the transport of NPs across BBB via transferrin receptors expressed on brain endothelial cells.	Permeability studies across cell monolayers displayed that NLC can effectively permeate the BBB, and amyloid-β studies showed NPs potential to inhibit fibril formation. The developed system proved to be efficient in site-specific delivery of quercetin in brain cells.	Pinheiro et al., 2020a
RVG29- NPs	Quercetin as TM RVG29 as FA	—	250 nm	Lipid NPs were decorated with the RVG29 to improve the brain targeting via nicotinic acetylcholine receptors. Quercetin was encapsulated into NPs owing to its neuroprotective effects.	NPs did not show any cytotoxicity in hCMEC/D3 cell line and RVG29- NPs have increased the permeability up to 1.5 folds across the BBB in comparison with non-functionalized NPs. Finally, NPs showed the inhibition of amyloid-beta aggregation illustrating the neuroprotective potential of the formulation.	Pinheiro et al., 2020b
Amyloid-β oligomer-targeted gadolinium-based NIR/MR multi-modal theranostic nanoprobe	F-SLOH as targeting and imaging agent	IV	50 nm Aβ oligomer-selective cyanine dye	F-SLOH is an Aβ oligomer-specific cyanine dye that can facilitate target specific imaging. Based on biocompatibility, tunable bio-distribution, and multimodal imaging potentials, Gd3+-based NPs have been chosen to develop multimodal targeted theranostic probe.	The nanoprobe displayed the excellent diagnostic capabilities, along with inhibitory effect on Aβ fibrillation and aggregation, and strong neuroprotection against Aβ-induced toxicity. Moreover, NPs were demonstrated to be cell membrane permeable, biocompatible, Aβ-targeted and BBB-penetrable, suggesting their potential to be used as an excellent theranostic for AD.	Gao W. et al., 2020

IG, imaging agent; FA, functionalizing agent; TM, therapeutic moiety; GS, genistein; RVG29, rabies virus glycoprotein 29; TPP, triphenylphosphine; NPs, nanoparticles; MA, macrophage membrane; AD, Alzheimer's disease; FAM, carboxyl fluorescein; IV, intravenous; EPO, erythropoietin; EO-SLNs, erythropoietin loaded solid lipid nanoparticles; MASLNs, macrophage membrane (MA)-coated solid lipid nanoparticles; RES, reticuloendothelial system; PCL, self-assembly poly-caprolactone; ICV, intra-cerebro-ventricular; PEG, polyethylene glycol; IP, intraperitoneal; ATP, adenosine triphosphate; ADP, adenosine diphosphate; P-gp, P-glycoprotein; Fe_3O_4, iron oxide; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; LAT1, L-type amino acid transporter; NIR, near-infrared; USPIONs, ultrasmall superparamagnetic iron oxide nanoparticles; MRI, magnetic resonance imaging; PC, protein-capped; CdS, cadmium sulfide; FF, fibril formation; PBNPs, Prussian blue NPs; FAM, carboxyl fluorescein; BBB, blood brain barrier; SHP, sterically hindered phenols; DTNPs, dopamine tryptophan-nanocomposites; SLN, solid lipid nanoparticles; NLC, nanostructured lipid carriers.

form of drinking water. The results reveal that it improves the long-term memories and inhibits the levels of circulated Aβ plaques (Muthukumaran et al., 2018). It has been observed that combined micelles with Tween-80 to formulate curcumin micelles can increase the availability and efficacy of curcumin in the treatment of AD symptoms (Hagl et al., 2015). Recently, the effects of PEG ceramide nanomicelles on neuronal N2 cells have been investigated. It has been found that applied nanomicelles effectively mediate the degradation of tau proteins and induce autophagy in target cells (Gao J. et al., 2020). Another study shows a significant inhibition of the amyloidogenesis in AD mice by employing curcumin-loaded polymeric nanomicelles as a targeted therapeutic delivery system through the glycation method of bovine serum albumin in the presence of phosphate-buffered saline (Mirzaie et al., 2019).

Dendrimers

Dendrimers are considered as promising materials for the treatment of AD (Aliev et al., 2019). A novel finding has been achieved by coupling the lactoferrin and low-generation dendrimers for brain-targeted delivery of memantine in AD-induced mice. A recent study has reported the significant impact on the memory aspects in target mice (Gothwal et al., 2019). To increase the efficacy of drug-related CNS disorders such as AD and PAMA, dendrimers with ethylenediamine core, generation 4.0 and 4.5, are commonly used to enhance the drug solubility and bioavailabity for greater permeation across the BBB to target the damaged parts in brain (Igartúa et al., 2018). The dendrimers with poly(propylene imine) core and maltose-histidine shell (G4HisMal) have been successfully designed and could exhibit substantial improvement in AD symptoms including memory impairment (Aso et al., 2019; Igartúa et al., 2020). Co-administration of tacrine with both generation 4.0 and polyamidoamine dendrimers as nanocomposites has also been used to enhance the biocompatibility and reduce the toxicity of drugs used for the therapy of AD (Igartúa et al., 2020). Furthermore, the nanocomposites of poly(amidoamine) dendrimer and gold NPs have been used to design the disposable immunoplatforms for simultaneous determination of the biomarkers for AD (Serafín et al., 2020).

Nanogels

Currently, nanogels possess the ability to hold active molecules, macromolecules, and drugs together, which are considered promising drug delivery vehicles and have been exploited in many challenges associated with different kinds of pathologies including AD (Aderibigbe and Naki, 2018). A recent study reveals that the delivery of deferoxamine in the form of nanogels using the chitosan and tripolyphosphate via ionotropic method could be one of the effective therapies against AD (Ashrafi et al., 2020). Artificial chaperones in the form of polysaccharide pullulan backbones with cholesterol moieties have shown a significant effect on relieving AD pathologies by inhibiting the formation of Aβ amyloids (Ikeda et al., 2006). In a preclinical study in mice, it has been evaluated that the nose-to-brain delivery of insulin, a candidate drug for AD, can be enhanced by using nanogels as carrier (Picone et al., 2018).

Lipid-Based NPs for AD Therapy

Many studies indicate the extraordinary importance of lipid-based nanocarriers for their usage in the drug delivery systems to cure CNS diseases like AD. Lipid NPs have remarkable potentials in delivering anti-AD drugs via nasal routes to manage AD (Akel et al., 2020).

Solid Lipid NPs

Solid lipid NPs are considered as excellent carriers for a-bisabolol in AD brain. The formulation has shown significant inhibitory effects against amyloid aggregation (Sathya et al., 2020). Recently, a novel approach is introduced to induce the expression of p-glycoprotein and breast cancer resistance protein transporters on brain endothelial cells via targeting the MC11 ligands. The transferrin-functionalized nanostructured lipid carriers could induce the expression of these proteins, which can be considered as a potential strategy toward AD therapy (Arduino et al., 2020). In both *in vivo* and *in vitro* experiments, the formulation of solid lipid NPs loaded with donepezil has the potential to enhance the drug delivery to the brain through the intranasal route (Yasir et al., 2018b). In another study, solid lipid NPs and donepezil formulation can be prepared by the solvent emulsification diffusion technique. The result exhibits a promising improvement in drug efficacy as compared to other formulations (Yasir et al., 2018a). Recently, the curcumin-loaded lipid-core nanocapsules have been successfully designed. The curcumin nanocapsules have shown significant neuroprotective effects against Aβ 1-42-induced behavioral and neurochemical changes in AD mice model (Yavarpour-Bali et al., 2019). Lipid carriers with curcumin nanostructures were used to treat oxidative stress parameters in AD brain to improve and recover memory conditions. These nanostructures have the potential of suppressing the hallmarks of Aβ in AD (Sadegh Malvajerd et al., 2018). Similarly, in another study, both nanostructure lipid carriers and solid lipid NPs loaded with curcumin exhibit significant neuroprotective effects against AD pathologies with greater bioavailability of curcumin to the brain (Sadegh Malvajerd et al., 2019).

Liposomes

The nanosized vesicular liposomes possess self-assembling and amphiphilic properties and have been extensively used as nanocarriers to deliver drugs to brain tissues (Micheli et al., 2012). Liposomes can be readily functionalized and surface modulated using several polyether, functional proteins and cell-penetrating peptides (CPPs) that aid in target-specific drug transport across the BBB (Rocha, 2013). For instance, polyethylene glycol (PEG)-coated liposomes are reported to successfully evade the opsonization of RES. In addition, glutathione-PEGylated liposomes are also reported to efficiently enhance the cellular uptake of the drug across endothelial BBB (Wong et al., 2012; Rip et al., 2014). Curcumin-loaded liposomes can significantly enhance the delivery of drugs to CNS via corresponding receptors on BBB cells (Mourtas et al., 2014; Lajoie and Shusta, 2015). To deliver apolipoprotein E ($ApoE_2$) in AD brain, the liposome carrier system altered with surface containing mannose ligand and CPPs have been applied. The results indicate that

functionalized liposomes are safe and compitable and can deliver substantial concentration of genes to the target tissues in AD therapy (Arora et al., 2020). Due to the protective effects for hippocampus neurons and anti-Aβ properties, osthole (Ost) is considered as an anti-AD compound. An Ost-liposomes carrier system has been developed for its bioavailability and the exposure to target sites in the AD mice brain (Kong et al., 2020).

Niosomes

Administration of a niosome–lipid nanocarrier system loaded with artemisia-absinthium against amyloid aggregation shows significant effects on AD pathologies. Thus, the formulation can be used to preclude the development of amyloid for AD therapy (Ansari and Eslami, 2020). In order to enhance the drug exposure to AD brain via the intranasal route, pentamide-loaded chitosan glutamate-coated niosomes have been developed. The results exhibit a significant increase in pentamide efficacy across the BBB (Rinaldi et al., 2018). Low blood level of folates is considered as the primary cause of AD. To deal with the hindrance of folic acid transport across the BBB, formulation with different concentrations of folic acid-niosomes has been prepared. It has been found that niosomes with span 60 and cholesterol in the ratio of 1:1 (50 mg:50 mg) show higher entrapment efficacy with more exposure to the affected parts of the brain (Ravouru et al., 2013). Rivastigmine is an acetylcholine esterase inhibitor and can improve brain functions in CNS disorders like AD. A niosome formulation is prepared using sorbitan esters and cholesterol by film hydration technique. The formulation exhibits amazing results in improving drug efficacy to target brain tissues (Estabragh et al., 2018).

Nanoemulsion

Nanoemulsion formulations maximize the efficacy of anti-AD drugs and make them specific against specific target sites in the brain (Nirale et al., 2020). A nanoemulsion using homogenization and ultrasonication has been used to load memantine for intranasal delivery to bypass the BBB for AD therapy. Both *in vivo* and *in vitro* experiments reveal the promising effects of emulsion against AD pathologies (Kaur et al., 2020). In order to improve the clinical usage with enhanced efficacy, naringerin nanoemulsion is further prepared. The result reveals that nanoemulsion from naringerin could be a potential approach to overcome Aβ neurotoxicity and amyloidogenesis (Md et al., 2018).

Cubosomes

Cubosomes are another lipid-based NPs that may have the potential biomedical application for drug delivery to the brain (Gaballa et al., 2020). The results of donepezil-HCL delivery through cubosomal mucoadhesive *in situ* nasal gel show that formulated gel could be considered as a promising carrier for drug delivery to target the affected parts of the brain (Patil et al., 2019).

Amylolipid Nanovesicles

To achieve the maximum drug concentration across the BBB for rapid and greater impact of drugs on brain cells, a novel lipid-based nanocarrier system has been developed. It is a self-assembled lipid-modified starch hybrid system. A study demonstrates that intranasal administration of curcumin loaded in amylolipid nanovesicles has greater tendency to cross the BBB and shows significant effect against AD pathologies. Thus, the findings prove that this carrier system is a promising carrier for drug delivery to AD brain tissues (Sintov, 2020).

Metallic NPs

In nanomedicine-based approaches to AD, the use of metallic NPs is considered a potential research area for targeted drug delivery across the BBB. Due to the utilization of chemistry-based techniques in their synthesis, metallic NPs have some limitations, but some of the metallic NPs like cerium, selenium, gold, and iron are known to exhibit significant anti-AD properties. Currently, researchers are oriented toward the use of green chemistry-based approaches for designing biologically friendly NPs.

Selenium NPs

As aforementioned, reducing ROS level in the brain is a key strategy to relieve AD. There are many trace elements such as selenium (II), sodium selenite (VI), and sodium selenite (IV) known as active ROS inhibitors. Being important micronutrients of the human body and gifted with biomedical application of selenium nanoformulation, selenium- and selenite-containing NPs play roles in lowering the oxidative stress and inhibiting the cytotoxicity of cells. Therefore, they have the potential to be used in curing neurodegenerative diseases like AD (Fernandes and Gandin, 2015; Rajeshkumar et al., 2019). It has been found that the modified selenium NPs with sialic acid can cross the BBB and their exposure can inhibit the Aβ aggregation reactions (Yin et al., 2015). Similarly, sialic acid-modified selenium NPs coated with high BBB permeability peptide-B6 and epigallocation-3gallate (EGCG) could inhibit the Aβ aggregation (Zhang et al., 2014). In a transgenic AD mouse model, a novel modified nanoformulation of selenium NPs encapsulated into PLGA nanospheres with curcumin has exhibited strong inhibitory effects against Aβ aggregation, which can be considered as a valued delivery system for targeted drug delivery in the treatment of AD (Huo et al., 2019).

Cerium NPs

Cerium oxide NPs could protect vital neuronal function against high ROS levels in an AD patient. It has been evaluated that CeONPs have no negative effects and are extremely useful for the treatment of AD. The success of AD treatment with ceria can be attributed to greater uptake across the BBB and no unwanted accumulation in other biological sites (Rzigalinski et al., 2017; Wahle et al., 2020). In a preclinical AD mouse model, ceria NPs coupling with triphenylphosphonium (TPP) localize in the mitochondria and prevent neuronal death (Kwon et al., 2016). In another study, γ-FeO3/CeOx@PEG2,000 NPs could effectively scavenge radicals and decrease the oxidative stress (Horák et al., 2020).

Gold NPs

Gold NPs play important roles in drug delivery across the BBB to the brain for the treatment of neurodegenerative

diseases. There are various AuNP formulations that are used for the diagnostic and therapeutic strategies in the treatment of AD (Sivanesan and Rajeshkumar, 2019). In the AD mice model, D-glutathione-stabilized gold NPs can cross the BBB following intravenous administration and show strong inhibitory effects against Aβ42 aggregation with no neurotoxicity (Hou et al., 2020). Another study reveals that treatment with gold NP formulation via intrahippocampal and intraperitoneal injections could improve the acquisition and retention of spatial learning and memory (Sanati et al., 2019). To enhance the neuroprotective efficacy of dietary polyphenolic compounds like anthocyanin, conjugation of anthocyanin with gold NPs has been developed. It has been demonstrated that treatment with anthocyanin-loaded PEG-AuNPs in an amyloid beta mouse model of AD is a promising strategy to prevent the age-associated neurodegenerative disease (Ali et al., 2017). A recent study indicates that administration of maize tetrapeptide-anchored gold NPs can improve central cholinergic system function and reduce the level of acetylcholinesterase, suggesting that novel tetrapeptide can be used as a neuroprotective agent to prevent AD (Zhang et al., 2021). Another study has reported that treatment with AuNPs in AD animals has significantly reversed the symptoms of AD by reducing neuroinflammation and modulating mitochondrial functions (Dos Santos Tramontin et al., 2020).

Iron NPs

Iron oxide NPs have been widely used in biomedical studies. It has been investigated that ultrasmall superparamagnetic iron oxide NPs coupled with phenothiazine-based near-infrared (NIR) fluorescent dye can act as novel theranostic agents for AD. The particles have the ability to perform NIR fluorescence and magnetic resonance imaging of Aβ plaques and prevent their aggregation in the brain of AD mice (Cai et al., 2020). In addition, treatment with protein-capped (PC) Fe_3O_4 and PC-cadmium NPs can act as potent tau aggregation inhibitors in AD cells, which may provide a novel strategy to design anti-tau aggregation drugs for AD patients (Sonawane et al., 2019). Furthermore, the iron oxide NP formulations may possess potential applications in the diagnosis and treatment of neurodegenerative diseases such as AD (Luo et al., 2020).

NP-Chelation-Based AD Therapy

A major pathology in AD is neuronal degeneration, and it has been found that oxidative stress is one of the leading risk factors that initiate and promote neurodegeneration. Compared to normal brain, AD brain shows the dysregulation of metal level, such as iron, aluminum, zinc, and copper, which may facilitate oxidative stress, toxic radical formation, and disruption in DNA functioning, and mediate the onset of AD symptoms (Lovell et al., 1998).

To deal with the metal accumulation and their resultant oxidative stress in the brain, nanotechnology contributes effectively to the form of chelation therapy in the inhibition of oxidative stress. To reduce the levels of corresponding metals in AD brain, NPs of Fe and Cu metals in the form of chelators have been employed. These chelators are designed to be safe in delivery and have minimal neurotoxicity to healthy brain tissues (Mandel et al., 2007). Chelation therapy shows significant effects on the solubilization of Aβ plaques in AD brain. In metal chelation, copper is the most vital trace element and 54 copper binding proteins have been found in human proteomes (Liu et al., 2009b; Blockhuys et al., 2017). It has been found that copper ions are involved in the modulation of the expression of amyloid precursor proteins, and their chelators can reduce Aβ accumulation up to 50% in AD transgenic mice. The Cu-conjugated NP formulation in the form of chelator clioquinol (CQ) has the potential to reverse the metal precipitation of amyloid protein in AD patients (Barnham and Bush, 2008). NP–chelator conjugates exhibit inhibitory effects against Aβ aggregation and protect neurons from neurotoxicity without affecting their proliferation (Liu et al., 2009b). For better intake and higher concentration, NP–iron chelator conjugates are designed with the coating of polysorbate 80 that mimic the low-density lipid (LDL) receptors on brain cells and facilitate the entry across BBB (Cherny et al., 2001; Kreuter, 2001; Kreuter et al., 2002; Cui et al., 2005).

In *in vitro* experiments, 8-hydroxyquinoline derivatives especially compound-5b chelation have shown significant inhibitory effects against the self-induced Aβ aggregation in AD. Furthermore, they exhibit no toxic effects and possess excellent penetrative tendency across the BBB (Yang X. et al., 2018). Similarly, xanthone derivatives in the chelation form also exhibit a selective inhibitory effect against a neuronal enzyme, namely, acetylcholinesterase. Based on the inhibition of acetylcholine and antioxidant activity, these derivatives have the potential to be used in the treatment of AD (Kou et al., 2020). In another study, deferasirox and tacrine chelators are designed, and their supportive roles in the treatment of AD are further evaluated. The compounds have good multifunctional activities in the inhibition of acetylcholinesterase (Wang et al., 2019). Nano-N2PY, a NP–chelator conjugate, plays a significant role in protecting cortical neurons from Aβ-related toxicity (Liu et al., 2009a). Similarly, other metal chelators such as ethylene diamine tetra acetic acid (EDTA), iodochlorhydroxyquin (clioquinol), and deferoxamine are being used against AD pathologies and exhibit promising results in AD treatment (Ritchie et al., 2003).

Nanomedicine–Theranostics Formulations

Gold (Au) NPs

The mechanism involved in the treatment of Aβ fibrils with gold-containing NPs (AuNPs) is the same as treating cancer cells with metallic NPs (Kabanov and Gendelman, 2007). The molecular docking, system biology, and time course simulation analysis confirm and validate the synergistic role of AuNPs in inhibiting Aβ formation in the brain (Kabanov and Gendelman, 2007). AuNPs play a promising role in diagnosing the AD both in bare form and in conjugation with other compounds. Based on the antioxidant and anti-inflammatory characters, AuNP exposure can relieve the brain damage in the AD model (Kaushik

et al., 2019). AuNPs in combination with reduced graphene or coated with anti-tau antibodies can act as neuro-probes and detect the Tau-441 target proteins both in serum fluid and cerebrospinal fluid (Neely et al., 2009; Karaboga and Sezgintürk, 2020). AuNPs conjugated with Co^{2+} can be used to investigate the Aβ peptides' aggregation kinetics and their self-assembly stages in MRI images (Sparks, 2008). In the diagnosis of AD pathologies, AuNPs follow the biosensory strategies. For example, AuNPs are used in the development of an electrochemical immunosensor to detect the tau proteins (Razzino et al., 2020). Furthermore, it has been found that chiral recognition of stable AuNPs could enhance their ability to prevent Aβ aggregation (Hou et al., 2020).

Protein-Coated NPs

In biomedical sciences, the usage of protein-coated NPs in multifunctional therapeutic approaches has great importance in the treatment of AD (Hong et al., 2020). It has been shown that serum albumin (SA)-NP formulation increases the efficacy of R-flurbiprofen (an anti-AD drug) in reducing Aβ peptide toxicity in the brain (Wong and Ho, 2018). Similar to R-flurbiprofen, SA-NPs are also used in the transport of tacrine and can stabilize the bioavailability with minimum hepatotoxicity (Luppi et al., 2011). In addition, the NPs coupled with BSA and sialic acid have been used to detect the early Aβ formation in the onset of AD (Zhao et al., 2015). Furthermore, delivered protein-based NPs serve as contrast agents in the brain and facilitate in providing better imaging to study the Aβ plaques (Zhao et al., 2015).

Antibody (Ab)-Decorated NPs

To cure AD, delivering immunotherapy doses against amyloid bodies imposes serious side effects in the form of meningoencephalitis (Gelinas et al., 2004; Moretto et al., 2007). To minimize the side effects of immunotherapy, the usage of NPs coated with antibodies for specific target proteins can be the best alternative to detect and dissolve the protein aggregations in brain cells. Using the secondary ion mass spectrometry, antibodies coated with metal oxide NPs are adopted for the imaging of AD-associated proteins in the brain (Moon et al., 2020). Chitosan-based smart nano-vehicles coated with modified Ab fragments have been used to target the Aβ amyloids in AD cells. For greater uptakes across BBB and better diagnostic approach, NP-Ab formulation is coupled with contrast agents such as FITC and Alexa Fluor (Agyare et al., 2008). Superparamagnetic iron oxide NPs conjugated with Aβ oligomer-specific scFv-AbW20 and class A scavenger receptor activator XD4 (W20/XD4-SPIONs) exhibit promising results in the therapeutic benefits for AD (Liu et al., 2020a). In another study, multifunctional superparamagnetic iron oxide NPs conjugated with Aβ oligomer-specific scFv-antibody and class A scavenger receptor activator show promising early diagnostic potential for AD (Liu et al., 2020b). PEG-NPs coated with specific Abs are used to degrade Aβ-42 (Gao W. et al., 2020). Decorating the PLGA NPs surface with 83-14 monoclonal Ab could effectively reduce the neurotoxicity induced by Aβ fibrils in AD brain (Kuo and Tsai, 2018).

CONCLUSION REMARKS AND FUTURE PERSPECTIVE

This review has provided an outlook of the recent advances in nanomedicines employed to cure AD. Considering the ultimate goals of nanomedicines, much advancement will be achieved in the treatment of AD. The nanomedicines have tailored and transformed both diagnostic and therapeutic approaches for AD. For the promising future of nanomedicines used in AD, we suggest the revision of the current practices to consider the neglected factors at the nano–bio interface in order to minimize the risk of misinterpretations of the outcomes. Furthermore, the adoption of multipurpose NPs with multi-therapeutic capacities (e.g., delivering a variety of therapeutic moieties to manage inflammation, tau phosphorylation, oxidative stress, and mitochondrial dysfunctionality) is also recommended. Moreover, the challenges in the large-scale production of reproducible NPs need to be addressed. Considering the key targets of the current drugs involving tau proteins, neuroinflammation, and Aβ proteins, there is an urgent need to develop the drugs with new targets that can not only treat the symptoms but also prevent the progression of the disease at an early stage, which can ultimately lead to a better quality of life.

AUTHOR CONTRIBUTIONS

NK, D-DW, and X-YJ: conceptualization. NK, MM, and EN: data curation. X-YJ and D-DW: funding acquisition. NK, MM, EN, UZ, MK, SK, Y-KZ, E-SJ, MZ, S-FD, and J-SW: writing—original draft. D-DW and X-YJ: visualization and supervision. NK, D-DW, and X-YJ: editing. All authors: contributed to the article and approved the submitted version.

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MINI REVIEW
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Emerging Nanotechnology for Treatment of Alzheimer's and Parkinson's Disease

Amanda Li[1†], Joel Tyson[2†], Shivni Patel[3,4†], Meer Patel[3,4], Sruthi Katakam[3,4], Xiaobo Mao[3,4] and Weiwei He[5*]*

[1] Washington University School of Medicine, St. Louis, MO, United States, [2] Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, Baltimore, MD, United States, [3] Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [4] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [5] Key Laboratory of Micro-Nano Materials for Energy Storage and Conversion of Henan Province, Henan Joint International Research Laboratory of Nanomaterials for Energy and Catalysis, College of Chemical and Materials Engineering, Institute of Surface Micro and Nano Materials, Xuchang University, Xuchang, China

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Institute of High Energy Physics, Chinese Academy of Sciences, China

***Correspondence:**
Xiaobo Mao
xmao4@jhmi.edu
Weiwei He
heweiweixcu@gmail.com

[†] These authors have contributed equally to this work

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The prevalence of the two most common neurodegenerative diseases, Parkinson's disease (PD) and Alzheimer's Disease (AD), are expected to rise alongside the progressive aging of society. Both PD and AD are classified as proteinopathies with misfolded proteins α-synuclein, amyloid-β, and tau. Emerging evidence suggests that these misfolded aggregates are prion-like proteins that induce pathological cell-to-cell spreading, which is a major driver in pathogenesis. Additional factors that can further affect pathology spreading include oxidative stress, mitochondrial damage, inflammation, and cell death. Nanomaterials present advantages over traditional chemical or biological therapeutic approaches at targeting these specific mechanisms. They can have intrinsic properties that lead to a decrease in oxidative stress or an ability to bind and disaggregate fibrils. Additionally, nanomaterials enhance transportation across the blood-brain barrier, are easily functionalized, increase drug half-lives, protect cargo from immune detection, and provide a physical structure that can support cell growth. This review highlights emergent nanomaterials with these advantages that target oxidative stress, the fibrillization process, inflammation, and aid in regenerative medicine for both PD and AD.

Keywords: Parkinson's disease, Alzheimer's disease, nanotechnology/nanomaterials, oxidative stress, nanozymes

INTRODUCTION

Protein aggregation is a typical histopathological hallmark in Parkinson's disease (PD) and Alzheimer's Disease (AD). PD is characterized by the aggregation of misfolded α-synuclein (α-syn) protein in inclusions called Lewy bodies (LB) in dopaminergic neurons, resulting in severe motor dysfunction. Alzheimer's Disease is characterized by abnormal accumulation of amyloid-β (Aβ) plaque and tau neurofibrillary tangles, resulting in brain damage affecting critical cognitive processes. Emerging clinical and experimental results support the hypothesis that pathological α-syn, Aβ, and tau are prion-like peptides/proteins that can induce the propagation of endogenous

monomers, and cause proteinopathy spreading from cell-to-cell (Braak et al., 2003; Kordower et al., 2008; Li et al., 2008; Luk et al., 2012; Guo et al., 2016; Mao et al., 2016; Tyson et al., 2016; He et al., 2018; Kam et al., 2018; Kim et al., 2019).

Native α-syn undergoes a misfolding process from soluble and random conformation to the insoluble and fibrillar form in pathological conditions. When misfolded α-syn aggregates, it localizes in the mitochondria, inducing mitochondrial fragmentation and decreased membrane potential (Li et al., 2007; Devi et al., 2008; Ganjam et al., 2019). The aggregation of Aβ peptide is thought to be a result of dysfunctional mitochondrial reactive oxygen species (ROS) production and dyshomeostasis of metals from oxidative stress (Tönnies and Trushina, 2017; Gupta et al., 2019; Poulson et al., 2019). In AD, microtubule-associated tau protein is known to undergo abnormal hyperphosphorylation, leading to tau tangles with prion-like activity. Though the mechanism and effects of tau tangles are not yet well understood, there is some promise that tau is an effective therapeutic target (Binder et al., 2005; Gong and Iqbal, 2008; Iqbal et al., 2016; Takeda, 2019).

Similar pathological mechanisms in both diseases cause enhanced production of ROS leading to a cascade of oxidative stress (Li et al., 2007; Devi et al., 2008; Goedert, 2015; Tönnies and Trushina, 2017; Hassanzadeh and Rahimmi, 2018; Ganjam et al., 2019; Gupta et al., 2019). With increased cell stress, microglial reactions, and increased expression of inflammatory cytokines, both diseases significantly increase neuronal inflammation, which is thought to promote cell death and further protein/peptide aggregation (Tufekci et al., 2012; Lema Tomé et al., 2013; Rivest, 2015; Gupta et al., 2019). The biological mechanisms that affect both PD and AD as described are misfolded protein aggregation, oxidative stress, inflammation, and cell death (de Bem et al., 2021).

Despite decades of clinical trials using traditional therapeutics, highly successful treatment of both oxidative stress and protein misfolding in neurodegenerative diseases has been elusive (Querfurth and LaFerla, 2010). Combating amyloidosis in both AD and PD with small molecules, peptides, and monoclonal antibodies especially, has resulted in little success. This leaves the door open for nanomaterials with appealing physicochemical properties, tenability, and multifunctionality to improve understanding and treatment of the diseases (Andrikopoulos et al., 2020; Chen P. et al., 2020; Ke et al., 2020; Pichla et al., 2020; Kakinen et al., 2021).

Nanotechnologies are increasingly being used in biomedical applications, and more treatments for neurodegenerative disorders are expected to emerge. Nanomaterial formulations have shown the ability to alleviate oxidative stress and inflammation directly (Wang et al., 2019; Eleftheriadou et al., 2020), and to overcome barriers in passage across the blood brain barrier (BBB) (Ulbrich and Lamprecht, 2010; Leyva-Gómez et al., 2015), although the specific mechanism for delivery across the BBB varies and has not yet been fully elucidated. Traditional therapeutic drugs are also likely to have off-target effects. Nanomaterials demonstrate the ability to improve localized targeted delivery of disease therapeutics by enhancing the dosing efficacy of delivered drugs, controlling cargo release profiles, and by being functionalized for the specific biological target of interest (Su and Kang, 2020). Additionally, composite nanomaterials are being developed to improve regenerative medicine techniques and encourage new cell growth in PD and AD (Bordoni et al., 2020).

Nanoparticles (NPs) can enhance the transport of therapeutics across the BBB during pathological conditions in PD and AD. Characteristics of disease-afflicted BBB include greater vascular permeability, decreased expression of tight junctions and BBB transporters, and the build-up of blood-derived debris and cells into perivascular spaces (Sweeney et al., 2018; Huang et al., 2020). Such pathological conditions impair concentration gradient-driven diffusion, decreasing the function of carrier-mediated transport (CMT) and receptor-mediated transport (RMT) (Sweeney et al., 2018). These conditions pose additional concerns due to the increased chance of agents getting trapped in the enlarged perivascular spaces (Wang et al., 2018). Although CMT has been a difficult transport route for NP systems due to the high selectivity of carrier proteins (Curley and Cady, 2018), RMT has been found to be more conducive to NP systems and is currently the most common type of transport for NP entry into the brain (Saraiva et al., 2016). To use RMT for transport into the brain parenchyma for NP systems, NPs can be coated with ligands (such as insulin, transferrin, lactoferrin) or surfactants (such as polysorbate 80) capable of undergoing RMT (Saraiva et al., 2016; Lopalco et al., 2018). Nanoparticles functionalized by cationic substances such as albumin can cross the BBB by adsorptive transcytosis (AMT) (Lu et al., 2012), or travel across the BBB via cell-mediated transcytosis, a form of transport that relies on immune cell phagocytosis of the NPs (Chen and Liu, 2012).

In this mini-review article, we will explore the current field of nanomaterials for therapeutic application in PD and AD and highlight emerging trends and materials that appear to be forging a path toward a multifaceted approach to the similar pathologies of the diseases. The present review is divided into major sections describing important therapeutic trends: addressing oxidative stress and mitochondrial damage, prevention of α-syn, Aβ, and tau aggregation and cell-to-cell spreading, addressing inflammation, and aiding cellular regeneration (**Table 1**).

NANOMATERIAL APPROACHES TO RELIEVING OXIDATIVE STRESS IN PD AND AD

Direct Antioxidant Nanoparticles

This section focuses on metal nanomaterials that directly relieve oxidative stress by serving as ROS and nitric oxide (NO) scavengers, mimicking the major antioxidant enzymes involved in oxidative-stress response including metal oxides, and redox nanozymes that mimic redox enzymes catalase, superoxide dismutase (SOD), and other antioxidants (Ambani et al., 1975; Riederer et al., 1989; Abraham et al., 2005; Li et al., 2020).

Nanozymes are nanomaterials with enzyme-like properties, tunable catalytic activity, high stability, and often the ability to simultaneously mimic multiple enzymes (Liang and Yan, 2019).

TABLE 1 | Nanotechnology addressing mechanisms of PD and AD.

Biological target	Therapeutic approach		References
Oxidative stress	ROS scavengers	Antioxidant mimicking metal oxide NPs and other nanozymes, enhancing oxidative stress tolerance	Singh et al., 2017; Liu et al., 2020; Ma et al., 2020; Ruotolo et al., 2020; Yu et al., 2020
	Antioxidant drug delivery	Antioxidant liposomes (resveratrol, baicalein, curcumin, EGCG), lipoic acid capped gold, tunable hydrogels, catalase exosomes	Wang et al., 2011; Elnaggar et al., 2015; Haney et al., 2015; Ethemoglu et al., 2017; Rajput et al., 2018; Huang et al., 2019; Lai et al., 2019; Adnet et al., 2020; Chen W. et al., 2020; Piersimoni et al., 2020; Kuo et al., 2021
	Nanoemulsions	Intranasal delivery of vitamin E, coenzyme Q10 through nasal mucosa	Gupta et al., 2018; Gaba et al., 2019
Protein Aggregation	Protein degradation	Graphene quantum dots, gold NPs, light sensitive nanoassemblies	Lee et al., 2018; Tanimoto et al., 2012; Gao et al., 2016, 2019; Zhang et al., 2016; Chung et al., 2017; Kim et al., 2018; Javed et al., 2019; Zhang H. et al., 2020
	Fibrillization inhibition	Metal oxide NPs, solid lipid NPs, cerium oxide NPs, siRANA exosomes, hydrogels	Hossain and Mukherjee, 2013; Cooper et al., 2014; Jiang et al., 2016, 2018; Vakilinezhad et al., 2018; Yau and Tycko, 2018; Ma et al., 2020; Mahapatra et al., 2020; Ruotolo et al., 2020; Simpson et al., 2020
Inflammation	Lipoic acid capped gold NPs, ibuprofen nanoemulsion, loaded NPs, mitochondria targeting nanozymes		Testa et al., 2014; Mandal et al., 2016; Ganesan et al., 2019; Piersimoni et al., 2020; Zhang L. et al., 2020
Regenerative Medicine	Neutrophic factor particles and hydrogel scaffolds, self-assembling peptides		Yu et al., 2014; Cui et al., 2016; Adil et al., 2017; Moriarty et al., 2017; Struzyna et al., 2018; Moriarty et al., 2019; Bordoni et al., 2020

Reaction mechanisms of nanozymes may vary based on their specific composition. Non-metallic nanozymes, such as carbon-based nanozymes, contain an aromatic ring that facilitates electron transfer, imitating the function of the porphyrin ring present in natural enzymes (Gao et al., 2020). Metal oxide nanozymes contain metal sites that imitate the metal catalytic active site of natural metalloenzymes. Metal oxide nanozymes tend to exhibit peroxidase-like activity, catalyzing the oxidation of a chromogenic substrate in the presence of hydrogen peroxide (Gao et al., 2020). Certain metal oxide nanoparticle systems such as cerium oxide can mimic multiple enzymes at once, and display a mechanism resembling that of redox enzymes due to their ability to switch between oxidation states (Yang et al., 2016; Hegazy et al., 2017). Metal-based nanozymes can be optimized by forming bimetallic nanoparticles, such as the PtCu system discussed below. Bimetallic nanozymes can mimic single or multiple enzymes simultaneously, and the catalytic activity of a bimetallic nanozyme system can be controlled by adjusting the ratio of the metals (He et al., 2017; Liu et al., 2020).

Cellular PD models demonstrate the potential of nanomaterials to reduce oxidative stress. A study by Ruotolo et al. examined the effects of cerium oxide NPs (CeO_2 NPs) on a yeast cell model overexpressing human α-syn. Results showed that CeO_2 NPs significantly reduced α-syn cytotoxicity in a dose-dependent manner through inhibition of α-syn cytoplasmic inclusion formation, and counteracted α-syn-induced mitochondrial damage. Upon treatment with CeO_2 NPs, α-syn-expressing yeast cells possessed lower levels of mitochondrial fragmentation, considerably higher amounts of actively functioning mitochondria, and a significantly smaller pool of free radicals (Ruotolo et al., 2020). Hao et al. (2019) demonstrated the ability of copper-based NPs (particularly Cu_2O and CuO) to eliminate ROS in a neuronal cell model of PD induced by 1-methy-4-phenylpyridinium (MPP+). Hao continues the study with mice, inducing PD with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The study results showed that Cu_xO nanoclusters mimic the activity of peroxidase, superoxide dismutase, catalase, and glutathione peroxidase, thus inhibiting neurotoxicity (Hao et al., 2019). In another cellular study using a PD model induced by MPP+, Singh et al. (2017) found that Mn_3O_4 nanozymes effectively mimic SOD, catalase, and glutathione peroxidase, which are three major antioxidant enzymes with normally cytoprotective roles that are hampered in PD. The ability of Mn_3O_4 nanozymes to simultaneously mimic all three major antioxidant enzymes is significant, as each antioxidant enzyme serves a different role in combating oxidative stress. Moreover, simultaneous expression of all three major antioxidant enzymes has been found to enhance tolerance oxidative stress in plant-based models (Lee et al., 2007; Sharma et al., 2012). Liu et al. (2020) injected preformed fibrils (PFF) of α-syn to create a sporadic PD model in neuronal cells and mice. PtCu bimetallic nanoalloys (NAs) were formulated and their antioxidant capacity was quantified using standard radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Liu et al. (2021) demonstrated that PtCu NAs display peroxidase, catalase, and SOD-like activity, and can scavenge DPPH, making them promising antioxidants. Results of their study showed that the PtCu nanozyme is significantly efficient at preventing prion-like α-syn spreading in PD (Liu et al., 2020; Figure 1E). The study demonstrated by proof of concept that redox nanozymes can be promising therapeutic strategies against the pathological spread of α-syn. Further study into the optimization of nanozymes against prion-like propagation would

be worthwhile, as nanozyme therapy may serve as an effective strategy against PD and other prion-like proteinopathies.

In recent studies with transgenic mice, metal oxides are used as antioxidant foundations of nanozymes, functionalized with Aβ targeting molecules. Ma and colleagues encapsulate CuO NPs in erythrocyte membranes incubated with a construct made from peptides, PEG, and phospholipid. The assembly was shown to have SOD-like activity and H_2O_2 and superoxide removal capacity in a 3xTg-AD mouse model (Ma et al., 2020; Figure 1C). Yu et al. (2020) also used the 3xTg-AD mouse model to test metal oxide frameworks to relieve AD-related oxidative stress. Yu took cerium oxide NPs and retinoic acid (RA) and enclosed them in MIL-100 (Fe) frameworks made from iron and trimesic acid. The framework preferentially breaks down in inflammatory environments with H_2O_2 allowing RA to upregulate neurogenesis genes while the ceria particles act as H_2O_2 antioxidants (Yu et al., 2020; Figure 1F). Carbon-based nanomaterials hold some promise for use as antioxidants, neuroprotectants and radical species scavengers. Like NPs, carbon based nano assemblies can pass through the BBB. These include carbon nanotubes, PEG functionalized carbon clusters, and fullerenes (Eleftheriadou et al., 2020). For example, Dal Bosco found PEGylated single walled carbon nanotubes induce a delayed antioxidant response in rat hippocampus without any lasting effects on memory or locomotion (Dal Bosco et al., 2015). However, some CNT formulations have been found to have opposite effects depending on their structural features, such as ROS formation or antioxidant depletion (van Berlo et al., 2012).

Antioxidant Drug Delivery Nanomaterials

In this section, we focus on nanomaterials that are indirectly targeting oxidative stress and mitochondrial damage by the delivery of antioxidants. Materials discussed include NPs, nanoemulsions, liposomes, and exosomes. The materials improve localized delivery to the brain through better BBB penetration, functionalization to better target α-syn, or by escaping immune detection.

Nanoparticles (NPs) have potential for use as effective carriers of therapeutics to improve BBB penetration. The potential of NPs to increase the efficacy of PD therapies is exemplified by the case of lipoic acid, a molecule naturally present in the mitochondria with powerful anti-inflammatory and antioxidant properties capable of attenuating oxidative stress (Moura et al., 2015; Molz and Schröder, 2017; Andreeva-Gateva et al., 2020). The administration of lipoic acid is challenging due to the molecule's short-half life and limited bioavailability caused by hepatic degradation (Teichert et al., 2003; Salehi et al., 2019). NPs can enhance the yield of intracellular lipoic acid delivery. Piersimoni et al. found that lipoic acid capped gold NPs (GNPs-LA) were biocompatible, capable of easily entering cells, and increased the efficacy of drug-delivery *in vitro*. GNPs-LA restored intracellular physiological conditions by preventing ROS formation and restoring normal mitochondrial function (Piersimoni et al., 2020). However, further study is still necessary to elucidate the exact molecular mechanism behind the GNPs-LA system.

In addition to NPs, nanoemulsions are a colloidal particulate system that can improve delivery across the BBB through noninvasive and direct delivery of lipophilic drug either through the mucosa intranasally or by improving the solubility and oral bioavailability of lipophilic drugs. Antioxidants, vitamin E, and coenzyme Q10, have both been incorporated in nanoemulsions for intranasal and oral delivery, respectively (Gupta et al., 2018; Gaba et al., 2019). Both were shown to improve behavior effects and reduce oxidative stress in PD rat models, with no signs of ciliotoxicity.

Additional carriers for antioxidants include nanoliposomes and exosomes, which can enhance BBB penetration. Antioxidants resveratrol (RES), baicalein, and epigallocatechin gallate (EGCG) have additionally been found to inhibit α-syn aggregation (Stojanoviæ et al., 2001; Wu et al., 2011; Xu et al., 2016; Aliakbari et al., 2018). Resveratrol-loaded liposomes performed better than free RES in protection from oxidative stress in rat models, and similarly, baicalein-loaded nanoliposomes exhibited increased delivery, stability, and internalization of baicalein *in vitro*. Liposomes have also been used to deliver drugs in combination, including curcumin paired with RES and EGCG paired with RES (Huang et al., 2019; Kuo et al., 2021). Functionalization of the RES-EGCG loaded liposomes with leptin and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate (PA) increased BBB penetration and α-syn targeting through binding to the leptin receptor and PA binding to α-syn, with increased delivery, reduced apoptosis and oxidative stress, and neuronal rescue (Kuo et al., 2021). Exosomes additionally can be embedded with adhesive proteins and avoid phagocytosis by the immune system. An antioxidant catalase loaded exosome was developed by Haney et al. (2015), which resulted in high loading efficiency, sustained release, and catalase preservation against protease degradation. These exosomes were readily taken up by neuronal cells *in vitro* and were substantially detected in PD mouse brains after intranasal administration with significant neuroprotective effects (Haney et al., 2015).

There have been recent studies that use hydrogel-nanomaterial composites or nanogels to improve antioxidant drug delivery in AD. Rajput et al. (2018) developed xanthan and gellan gum gels containing nanostructured lipid formations to solubilize RES for treatment of AD using a nasal route. In the work of Elnaggar et al., chitosan nanogels were shown to aid in the delivery of a neuroprotectant used to treat AD, piperine, intranasally. The chitosan gels have mucoadhesive properties which allowed effective, non-invasive piperine delivery with a fraction of the oral dose in AD rat models (Elnaggar et al., 2015). Cardia and colleagues use chitosan hydrogel NPs to improve the delivery of neuroprotective progesterone and found increased progesterone concentration in rats that took the hydrogels intranasally (Lai et al., 2019).

Enhanced nasal delivery could allow the use of normally minimally bioavailable anti-AD drugs such as Timosaponin BII. Chen administered timosaponin BII to mice intranasally in a formulation of in-situ gellan gum hydrogels (Chen W. et al., 2020). Salatin et al. use a surfactant (F-127) hydrogel to embed NPs made from Eudragit RL-100 polymer. The hydrogel acts as a thermoreversible delivery system that enables

controlled, intranasal delivery of the AD drug, Rivastigmine (Salatin et al., 2020).

NANOTECHNOLOGY APPROACHES TARGETING FIBRILLIZATION PROCESSES

Nanomaterials Approaches Targeting α-Synuclein Aggregation in PD

Strategies to decrease α-syn aggregation involve targeting the misfolded α-syn fibrils themselves or preventing increased expression of them. Here we discuss graphene quantum dots and cerium oxide NPs, which bind to α-syn and disaggregate fibrils directly, as well as a modified exosome that targets the central nervous system to deliver α-syn siRNA and prevent α-syn translation.

Graphene quantum dots (GQDs) are NPs composed of layers of graphene and are approximately 100 nm in size. Kim et al. (2018) found that GQDs successfully pass through the BBB and bind to α-syn fibrils, a process which inhibits α-syn fibrillization and disaggregates fibrillated α-syn in a time-dependent manner. GQDs also inhibited transmission of α-syn PFFs. Kim et al. additionally demonstrated that GQDs display neuroprotective properties. After treatment of α-syn PFFs induced synaptic dysfunction and mitochondrial damage, GQDs were found to restore reduced synaptic protein levels, relieve the effects of α-syn-induced mitochondrial damage, and reduce the formation of Lewy body/neurites. Additionally, GQDs did not cause any significant long-term toxicity *in vitro* or *in vivo* (Kim et al., 2018). Although studies have demonstrated the protective effect of GQDs, further studies are needed to understand the mechanisms.

Additionally, the cerium oxide NPs, as previously discussed in section 2.1, have been shown to fit best into the active site of α-syn and disaggregate fibrillar α-syn *in vivo* compared to other biomaterials such as gold and superparamagnetic iron-oxide NPs through recent molecular docking studies (Kaushik et al., 2018; Zand et al., 2019). In Ruotolo's study, CeO_2 NP treatment on a yeast model with cells overexpressing human α-syn significantly reduced α-syn cytotoxicity in a dose-dependent manner through inhibition of α-syn cytoplasmic inclusion formation and decreased mitochondrial damage as discussed (Ruotolo et al., 2020).

Altering the gene expression of α-syn is an additional approach to decreasing misfolded α-syn levels. Exosomes can be modified to enhance delivery as Cooper et al. did with the central nervous system-specific rabies virus glycoprotein peptide (RVG) to deliver siRNA to reduce α-syn expression. The modified RVG-exosome has therapeutic potential in delivery of α-syn siRNA to delay and reverse alpha synucleinopathies (Cooper et al., 2014).

Nanomaterials Approaches Targeting Aβ and Tau Aggregation in AD

In older studies, water soluble gold NPs (AuNP) synthesized in the presence of sodium citrate and functionalized with a peptide to form AuNP-Cys-Leu-Pro-Phe-Phe-AspNH_2 were found to block amyloid fibril growth in a gel media when irradiated in a magnetic field. When the surfaces of the gold particles were heated using inductive coupling in a magnetic field, they transmitted their heat to surrounding tissues, consequently disrupting amyloid deposits (Kogan et al., 2006).

B-casein (βCas) proteins have chaperone-like activity, stemming from their lack of tertiary structure, existence as heterogeneous oligomers, and ability to bind to a range of partially folded proteins thus preventing their aggregation (Thorn et al., 2009). Coating βCas with AuNPs and delivering them intracardially has been found to control the toxicity of $A\beta_{42}$ when induced in the brain of zebrafish larvae and adults. βCas alone does not exhibit any controlling activity on $A\beta_{42}$ activity, suggesting that AuNPs are critical in delivering βCas to $A\beta_{42}$ aggregate regions in the brain (Javed et al., 2019). AuNPs serve as the foundation for the design of a polyoxometalate-based nanozyme with a multifaceted approach to AD mitigation (Gao et al., 2016). Gao and colleagues functionalized AuNPs with a serine protease-like complex consisting of polyoxometalate conjugated to an octapeptide motif which was found to simultaneously target Aβ fibrilization, ROS, and metal ion accumulation through protease-like activity, Cu scavenging, and metal chelation, respectively (2016).

The CuO-based nanozymes of Ma and colleagues were functionalized with a KLVFF-modified PEG with phospholipid to embed the construct into erythrocyte membrane. The KLVFF motif binds to Aβ in blood circulation and the biomimetic construct was found to improve peripheral Aβ clearance when injected into mice (Ma et al., 2020). Recent cellular and mouse model study shows some promise that selenium-chondroitin sulfate NPs could be a multifunctional agent for AD treatment, inhibiting both Aβ aggregation and attenuating the hyperphosphorylation of tau at Ser396 and Ser404 by regulating glycogen synthase kinase 3 β (Guo et al., 2016; He et al., 2018).

Protein-capped (PC) metal NPs were synthesized by Sonawane et al. (2019) by exposing the fungal species *F. oxysporum* and *Verticillium* spores to various metal solutions to create different types of NPs. Aqueous mixtures of ferricyanide or ferrocyanide were used to create magnetite NPs, and Cd^{2+} and SO_4^{2-} were used to create CdS NPs. PC-metal NPs inhibit tau fibrillization by reducing hyperphosphorylation. Sonawane et al.'s study showed the direct effect of PC-metal NPs on tau aggregates, as they were quantifiably inhibited, and mature fibrils readily dissolved. Uncapped CdS NPs are toxic to bacterial and HeLa cells, due to oxidative stress caused by increased concentrations of reactive oxygen species (Hossain and Mukherjee, 2013). However, capping the NP makes them far more biocompatible and represents a viable therapeutic route.

Vakilinezhad et al. (2018) found that nanoformulations of histone deacetylase (HDAC) inhibitor, nicotinamide also inhibits tau hyperphosphorylation. While large doses of nicotinamide may be hepatotoxic (Knip et al., 2000), the researchers used solid lipid NPs (SLN) to provide localized, controlled release of nicotinamide by making them in different sizes. The SLNs were made from the physiological lipids, phosphatidylserine, and phosphatidic acid. Inhibition of hyperphosphorylation of tau in

a rat model was confirmed by an enzyme-linked immunosorbent assay, making it a possible candidate for AD treatment.

Photosensitizing materials are a recent area of study in the development of anti-Aβ therapies. These materials can have high affinity for Aβ and generate oxidative stress in response to light, thereby impeding Aβ aggregates (Hirabayashi et al., 2014; Mangione et al., 2015). Several nanoassemblies are being developed as light-responsive anti-Aβ agents. Fullerene is known to block Aβ aggregation. Tanimoto et al. (2012) hybridized fullerene with hydrophilic moieties to increase solubility while lowering cytotoxicity. When exposed to UV, the fullerene denatures $A\beta_{42}$ monomers and oligomers (Tanimoto et al., 2012). Chung et al. (2017) used 10 nm, carbon nanodots functionalized with polyethyleneimine to inhibit the aggregation of $A\beta_{42}$ using only visible light. Graphitic carbon nitride nanosheets have been used with visible light to suppress amyloidosis through the generation of reactive oxygen species from photochemical reactions. These reactive oxygen species then oxidize $A\beta_{42}$ (Chung et al., 2016). Zhang and colleagues use photodynamic micelles functionalized with light-sensitive chlorin e6 to inhibit and degrade Aβ in 655 nm light (Zhang et al., 2016). In recent work, iron copper selenide NPs have had success preventing amyloidopathy in mouse models with near-infrared illumination (Zhang H. et al., 2020).

Common hydrogels made from collagen, agarose, HA, and PEG were all found to have neuroprotective effects in the presence of Aβ. However, Simpson et al. (2020) found that non-functionalized hydrogels thermodynamically favor Aβ aggregation due to confinement effects. In the work of Jiang, hydrogels made from conjugated hyaluronic acid (HA) and curcumin were observed to slow down Aβ aggregation through opposing forces caused by its hydrophobic binding of curcumin and electrostatic repulsion of HA (Jiang et al., 2016, 2018). Yau and colleagues seeded Aβ fibril fragments in *N*,*N*'−methylene−*bis*−acrylamide and *bis*−acryloylcystamine hydrogels and found it successfully sequestrated excess Aβ in solution (Yau and Tycko, 2018).

NANOTECHNOLOGY TARGETING INFLAMMATION

Anti-inflammatory drugs have been used to treat both PD and AD, but nanoemulsions and NPs can improve delivery through direct delivery from nasal mucosa or improved BBB penetrance.

Lipophilic anti-inflammatory medication, ibuprofen, has neuroprotective effects, and to improve its delivery to the brain, Mandal et al. (2016) loaded ibuprofen into sodium hyaluronate based mucoadhesive nanoemulsion (MNEI). This intranasal nanoemulsion can more directly deliver ibuprofen to the brain through nasal mucosa than traditional oral routes. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice models were treated with ibuprofen for two weeks as an intranasal plain drug solution and with the nanoemulsion. Nanoemulsion delivery of ibuprofen significantly reduced MPTP-mediated dopamine depletion (Mandal et al., 2016).

For the treatment of AD, NPs of anti-inflammatory molecule quercetin encapsulated in β-cyclodextrin-dodecylcarbonate were shown to have increased anti-inflammatory effects compared to free quercetin *in vitro* with the goal of improving permeation and bioavailability (Testa et al., 2014). Curcumin loaded solid lipid NPs showed improved bioavailability, a greater inhibition of NO production and reduced inflammatory markers compared to conventional curcumin in a dose-dependent manner *in vitro* (Ganesan et al., 2019). A modified Poly(lactic-co-glycolic acid) (PLGA) NP was conjugated with CD47 extracellular domain via ROS-responsive phenylborate ester bond, which acts as a "do not eat me" signal, and a BBB penetrating peptide (CRT) encapsulating a microglia modulation agent necrostatin-1 (Nec-1). The NP efficiently increased the half-life of Nec-1 via prevention of phagocyte engulfment as well as increased brain distribution. The encapsulated Nec-1s are released as the ROS-sensitive bond between CD47 and the NPs are broken in AD mouse brains, allowing microglia engulfment of Nec-1 to modulate pathogenic microglia, reducing neuroinflammation (Zhang L. et al., 2020). Additionally, mitochondria-targeted quantum dot nanozymes were designed to switch microglia from proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype, which can mitigate Aβ aggregate-mediated neurotoxicity. These nanozymes effectively crossed the BBB, escaped from lysosomes, targeted the mitochondria, and prevented spontaneous neuroinflammation through regulation of proinflammatory mediators *in vitro* and in AD mouse models (Ren et al., 2020).

NANOTECHNOLOGY FOR REGENERATIVE MEDICINE

Currently, there is no cure for PD or AD, but efforts in regenerative medicine are aimed at promoting neuronal growth and axonal extension to repair cell damage of both diseases. To stimulate guidance of axonal growth and cellular survival, neurotrophic factors can be delivered via nanomaterials to extend their half-life and enhance delivery to the brain. Additionally, scaffolds not only provide a vehicle to deliver neurotrophic factors but are also an ideal physical structure with optimal microenvironment to promote cell growth of host neurons or implanted neurons.

Glial cell derived neurotrophic factor (GDNF) has a short *in vivo* half-life, but delivery in PLGA microparticles increased half-life, improved motor function and dopaminergic neuron restoration, with no adverse effects on immunogenicity, cerebellar degeneration, or weight loss *in vivo* (Garbayo et al., 2016). GDNFs delivered in hydrogel scaffolds have additional physical support and promote cell survival and axonal growth *in vivo* and *in vitro* (Wang et al., 2016; Ucar and Humpel, 2019). In addition to collagen hydrogel scaffolds, cryogels and microcontact printing also showed enhancement of axonal fiber growth *in vitro* (Ucar et al., 2021). As hydrogel scaffolds provide favorable environments for neuronal growth and protection from host immune responses, the encapsulation of dopaminergic neurons in scaffolds for transplantation present improved graft

survival in PD. The hydrogel can be functionalized with RGD cell adhesion peptide and heparin to secure cells and immobilize growth factors for *in vitro* maturation of neurons (Adil et al., 2017). Hydrogel scaffolds can additionally be structured to mimic the nigrostriatal pathway architecture with micro-columns of dopaminergic neurons as in the work of Struzyna et al. (2018).

Self-assembling peptides have the potential to improve the benefits of neural stem cell (NSC) transplantation to treat AD (Bordoni et al., 2020). Alternating amino acid sequences can be designed to create a nanofibril scaffold that can support neuronal growth and differentiation. Guohong Cui and colleagues designed a self-assembling peptide to successfully treat AD in a mouse model. Cui observed improved NSC survival, improved NSC differentiation, and lowered Aβ levels using peptides modeled after laminin (Cui et al., 2016). Yu et al. (2014) used lactoferrin peptides in a polymersome (PEG-PLGA) assembly to enhance delivery of humanin, a peptide known to inhibit AD-related cell death caused by Aβ.

Dongqin Yu used the previously mentioned metal oxide frameworked nanozymes functionalized with siRNA to downregulate SOX9, known to cause gliogenesis (2020). At the same time, RA within the framework was released to upregulate neuronal genes, creating a vital balance for neurogenesis without excessive glial response (Yu et al., 2020).

CONCLUSION AND FUTURE PERSPECTIVES

Amid contemporary challenges in targeted drug delivery and barriers to transport of drugs across the BBB, nanomaterials are emerging as promising approaches for the treatment of neurodegenerative disorders. This review provides insights into the role nanomaterials can play in improving the delivery of therapeutics to patients of PD and AD, the two most common neurodegenerative diseases, by targeting the pathological mechanisms of oxidative stress or mitochondrial damage, protein fibrillization, inflammation, and cell death (**Table 1**).

Current and emerging challenges in NP research include concerns regarding the toxicity of NPs. Research has shown that the very properties that account for the benefits of NPs may also contribute to toxic effects (Aillon et al., 2009; Song et al., 2016; Mohammadi and Nikkhah, 2017). Generally, neurotoxicity from NPs stems from their production of reactive oxygen species, causing oxidative stress (Teleanu et al., 2018). However, some NP biocompatibility improves with certain modifications. Looking forward, NP toxicity must be researched and thoroughly addressed, and efforts must be made to reduce or eliminate any toxic effects during the development phase.

The effect of NPs on pathological protein aggregation are found to vary depending on their composition, size, shape, and charge. Further study is necessary in identifying how NPs act under various combinations of these factors. *In vitro* and *in vivo* studies are both crucial to the study and identification of the best NP-based treatment for neurodegenerative diseases.

Additionally, the recent innovations in nanozymes present exciting new possibilities of addressing oxidative stress and inflammation in AD and PD, but more work needs to be done for safe clinical use. Further research on the catalytic mechanisms of nanozymes will be necessary to have the understanding to optimize the structure, function, and regulation of catalytic activity. Additionally, the biocompatibility and nano-bio interactions of nanozymes should be further explored to ensure safety and efficacy of nanozyme treatment (Wang et al., 2018; Tian et al., 2020).

The modifiability and freedom in design of various nanomaterials present a wide variety of delivery strategies for medications by enhancing transport across the BBB and enabling targeted delivery or additional functions while accommodating drug chemistries and solubilities and avoiding immune system detection. In regenerative medicine, which aims to reverse the damage of neurodegeneration, materials, such as hydrogel scaffolds, could play a crucial role in creating an optimal physical and microenvironment to support neuronal growth and axonal extension. However, the most ideal microenvironments and drug combinations must be better understood.

Nanomaterials represent an ever-growing field to approach neurodegenerative disease treatment, both in transforming the delivery of therapeutic agents and in creating an entirely new class of therapies targeting canonically challenging disorders.

AUTHOR CONTRIBUTIONS

MP and SK completed initial nanoparticle literature review for AD. SP completed the initial nanoparticle literature review for PD. AL completed nanomaterial literature review outside of nanoparticles for PD. JT completed nanomaterial literature review for AD. XM and WH supervised the manuscript preparation and writing. All authors contributed to the article and approved the submitted version.

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Targeting microRNAs to Regulate the Integrity of the Blood–Brain Barrier

Juntao Wang[1,2], Fang Xu[1,2], Xiaoming Zhu[1,2]*, Xianghua Li[3], Yankun Li[3,4] and Jia Li[5]**

[1] School of Nuclear Technology and Chemistry and Biology, Hubei University of Science and Technology, Xianning, China, [2] Hubei Key Laboratory of Radiation Chemistry and Functional Materials, Hubei University of Science and Technology, Xianning, China, [3] School of Pharmacy, Hubei University of Science and Technology, Xianning, China, [4] Hubei Key Laboratory of Cardiovascular, Cerebrovascular, and Metabolic Disorders, Hubei University of Science and Technology, Xianning, China, [5] Centre for Motor Neuron Disease, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

The blood–brain barrier (BBB) is a highly specialized neurovascular unit that protects the brain from potentially harmful substances. In addition, the BBB also engages in the exchange of essential nutrients between the vasculature and brain parenchyma, which is critical for brain homeostasis. Brain diseases, including neurological disorders and cerebrovascular diseases, are often associated with disrupted BBB integrity, evidenced by increased permeability. Therefore, defining the mechanisms underlying the regulation of BBB integrity is crucial for the development of novel therapeutics targeting brain diseases. MicroRNAs (miRNA), a type of small non-coding RNAs, are emerging as an important regulator of BBB integrity. Here we review recent developments related to the role of miRNAs in regulating BBB integrity.

Keywords: blood–brain barrier, regulate, targeting, microRNA, nanobiotechnology

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Yan Zou,
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Axel Montagne,
University of Edinburgh, United Kingdom
Virginia Pensabene,
University of Leeds, United Kingdom

****Correspondence:***
Juntao Wang
juntao-wang@hotmail.com
Xiaoming Zhu
495072782@qq.com
Jia Li
jia.li1@mq.edu.au

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INTRODUCTION

The blood–brain barrier (BBB) is a multicellular neurovascular complex, mainly consisting of brain endothelial cells and supporting cells, such as pericytes and astrocytes (Profaci et al., 2020). In addition to its key role in preventing neurotoxic agents from entering the brain, the BBB also regulates the exchange of essential nutrition between the brain and the blood (Banks, 2016). The function of the BBB depends on its intact structure, or BBB integrity (Banks, 2016). Disrupted BBB integrity has been shown to contribute to the onset and progression of diseases in the brain, including neurodegenerative diseases and cerebrovascular diseases (Sweeney et al., 2018).

A large body of studies has focused on understanding how BBB integrity is regulated (Sweeney et al., 2016; Nation et al., 2019; Li et al., 2020). These efforts have led to the finding of several key molecules and singling pathways, in both brain endothelial cells and supporting cells, which are critical for the maintenance of BBB integrity, including microRNAs (miRNAs), endothelial junction molecules (e.g., VE-cadherin, claudin-5), fatty acid transporter (e.g., mfsd2a), platelet-derived growth factor (PDGF) signaling, and Wnt/β-catenin signaling (Ben-Zvi et al., 2014; Chakraborty et al., 2020; Li et al., 2020).

MicroRNAs are a class of endogenous small non-coding RNAs (20–25 nucleotides) that regulate genes at the post-transcriptional stage through either cleavage of mRNA or inhibition of translation (Chakraborty et al., 2020). In humans, approximately 2,500 mature miRNAs have been identified to regulate more than 30% of all proteins expressed in humans, suggesting the profound role of miRNAs in human physiology and pathology (Kozomara et al., 2019). Recently, the role of miRNAs

in the modulation of BBB integrity has drawn great attention (Toyama et al., 2018; Chakraborty et al., 2020). In this review, we attempt to outline the involvement of miRNAs in the regulation of BBB integrity. We first briefly summarize the biogenesis and function of miRNAs, followed by a discussion of how miRNAs regulate BBB integrity by targeting different units in the BBB complex with an emphasis on the brain endothelial cells. We end by highlighting the challenges of developing efficient miRNA-based therapeutics targeting the disrupted BBB.

BIOGENESIS AND FUNCTION OF miRNAs

MicroRNA genes, which can be either intergenic or intronic, are first transcribed by RNA polymerase II to pri-miRNAs, followed by being processed into pre-miRNAs in the nucleus (Treiber et al., 2019). Pre-miRNAs are then exported to the cytoplasm to be further processed into imperfect double-stranded RNA duplex including guide strand (miRNA) and passenger strand (miRNA*). After being loaded to the RNA-induced silencing complex (RISC), the passenger strand is quickly excluded from RISC and degraded rapidly, leading to a strong preference toward the guide stand, the form of a mature miRNA (Gebert and MacRae, 2019).

MicroRNA is a posttranscriptional regulator of gene expression that contributes to diverse cellular processes, such as development, proliferation, differentiation, and apoptosis. MiRNAs recognize and bind to their target mRNAs via the seed region, a sequence of six contiguous nucleotides found from position 2–7 at the 5′-end of the molecule through direct Watson-Crick base-pairing (Gebert and MacRae, 2019). Once loaded into the RISC, the mature miRNA acts to guide the RISC to bind to partially complementary sequences within the 3′ untranslated region (UTR) of target mRNAs, resulting in the destabilization of mRNA or/and inhibition of translation (Treiber et al., 2019). For example, the first miRNA, *lin-4*, negatively regulates its target, lin-14, by repressing translation. In comparison, miR-27a regulates its target VE-cadherin at both mRNA and protein level (Young et al., 2013). It has been proposed that ancillary nucleotides at the 3′-end of the miRNA also play an important role in target recognition (Li et al., 2016).

MiRNAs IN REGULATING BBB INTEGRITY

The integrity of the BBB is mainly determined by brain endothelial cells, which are the fundamental unit of the BBB (Greene et al., 2020). Changes in tight junctions between brain endothelial cells and transcytosis in these cells have a significant effect on BBB integrity (Ayloo and Gu, 2019). In addition, crosstalk between endothelial cells and supporting cells forming the neurovascular unit (NVU), such as pericytes, astrocytes, immune cells, and other cells in the brain also contributes to the maintenance of BBB integrity (Profaci et al., 2020). Despite the fact that miRNAs are involved in brain diseases through various pathways (Juzwik et al., 2019; Ludwig et al., 2019; Sonoda et al., 2019; Starhof et al., 2019; Qian et al., 2020), the majority of miRNAs found to regulate BBB integrity exert their impact by targeting brain endothelial cells (Chakraborty et al., 2020). MiRNAs can either directly target endothelial junction molecules or modulate inflammation, endothelial cell survival, apoptosis, actin cytoskeleton, and other pathways to indirectly influence tight junctions in the BBB, leading to change in BBB integrity. Additionally, miRNAs may also have an impact on the crosstalk between brain endothelial cells and supporting cells, which is critical for the maintenance of BBB integrity (**Figure 1**).

MiRNAs and Tight Junctions

One of the key features of the BBB is the existence of extremely tight junctions between brain endothelial cells (Vanlandewijck et al., 2018), which are controlled by a range of junction molecules, including tight junction molecules [e.g., *zona occludens* (ZO), occludin, and claudin-5] and adherens junction molecules (e.g., VE-cadherin) (Cristante et al., 2013). MiRNAs have been shown to directly target these junction molecules, leading to a change in BBB integrity (Ma et al., 2017, 2020; Zuo et al., 2019; **Table 1**).

During ischemic stroke, BBB integrity is compromised as shown by increased immune cell infiltration and solute leak, eventually leading to neuronal loss. In ischemic stroke, miR-15a/16-1 cluster was significantly upregulated to mediate BBB breakdown by direct downregulation of claudin-5. The specific depletion of the miR-15a/16-1 cluster in endothelial cells enhanced brain claudin-5 expression after transient ischemia in mice, resulting in the restoration of BBB integrity with smaller brain infarcts and decreased neuroinflammation (Ma et al., 2020). ZO-1 is another highly expressed tight junction molecule in the BBB Toyama et al. (2018). Identified miR-501-3p mediated inflammation-induced BBB breakdown via directly targeting ZO-1. Using a mouse model of vascular cognitive impairment with increased inflammation, they further showed that the expression of miR-501-3p and its target ZO1 were inversely correlated. Inhibition of miR-501-3p with a specific inhibitor rescued ZO-1 gene expression, leading to restoration of BBB integrity within the white matter and amelioration of working memory deficits.

The regulation of tight junctions by miRNA can also be indirect. For example, miRNAs can control the transcription factor of tight junction molecules to regulate their expression. One of the transcription factors of claudin-5 is forkhead box protein O1 (FoxO1), which positively regulates claudin-5 expression (Taddei et al., 2008). By directly targeting FoxO1, miR-182 negatively regulates claudin-5 expression and tight junctions of brain endothelial cells, while inhibition of miR-182 protects BBB integrity (Zhang et al., 2020). MiR-107 was identified to directly target endophilin-1 (Liu et al., 2016), which regulates ZO-1 and occludin expression via the epidermal growth factor receptor (EGFR)-extracellular signal-regulated protein kinase (ERK)1/2 pathway (Liu et al., 2014). In addition, miR-143 was shown to contribute to methamphetamine-induced BBB disruption by targeting p53 unregulated modulator of apoptosis (PUMA), which leads to a decrease of tight junction molecules, such as claudin-5, occludin, and ZO-1 (Bai et al., 2016).

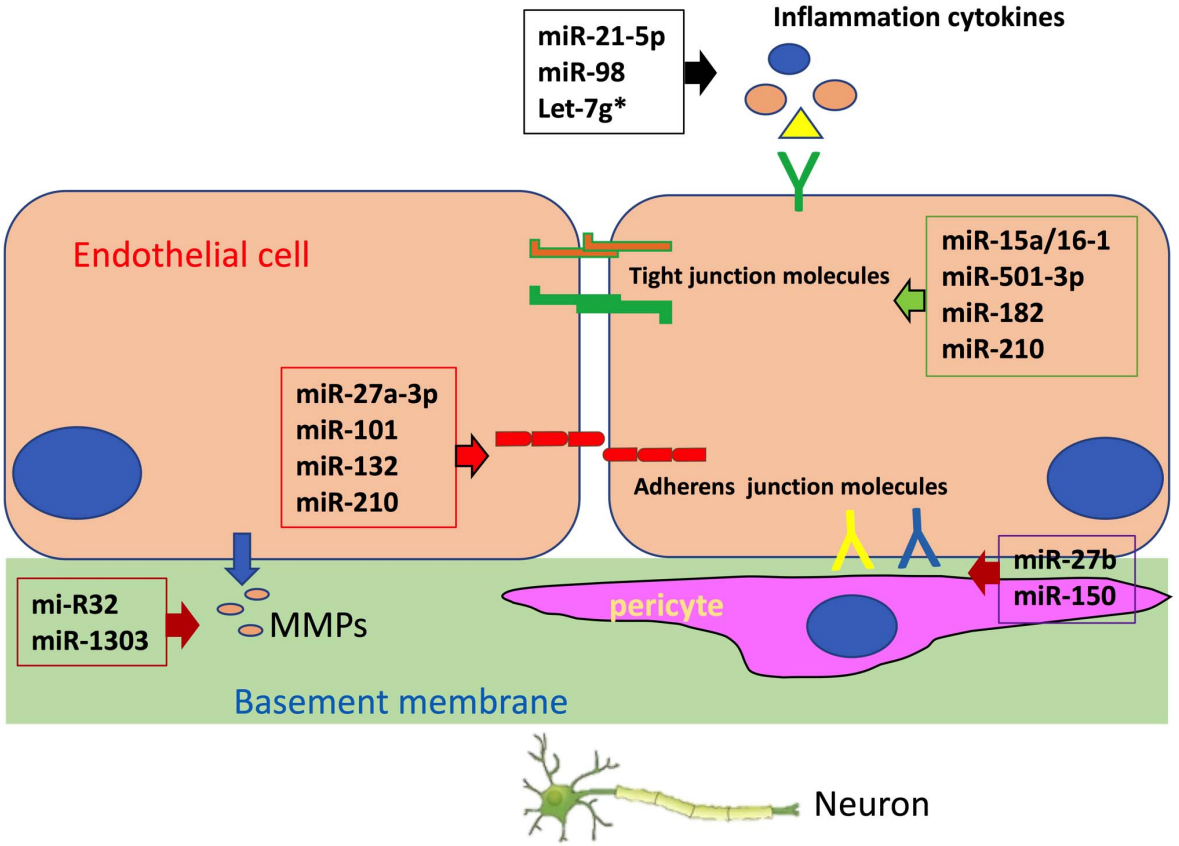

FIGURE 1 | MicroRNAs (miRNAs) regulate blood–brain barrier (BBB) integrity by targeting endothelial tight junction, basement membrane, pericyte coverage, and inflammation.

Adherens junctions form before tight junctions and are also a major regulator in vascular integrity (Taddei et al., 2008; Lampugnani et al., 2018). VE-cadherin, a key adherens junction molecule, was shown to be decreased in cerebral vascular diseases (Li et al., 2020) and neurodegenerative diseases (Li et al., 2018). MiR-27a directly regulates VE-cadherin (Young et al., 2013). In cerebral cavernous malformation, where BBB is disrupted, blockage of the interaction between miR-27a and VE-cadherin by a specific target site blocker CD5-2 restored BBB integrity and reduced severity of diseases (Li et al., 2020). VE-cadherin was also shown to be regulated by miR-101 (Mishra and Singh, 2013). In HIV-1-infected human brain microvascular endothelial cells, miR-101 mediated the disruptive effect of infection on endothelial barrier integrity by downregulating VE-cadherin. MiRNA may also indirectly regulate VE-cadherin. For example, miR-132 directly targets eukaryotic elongation factor 2 kinase (EEF2K), which inhibits VE-cadherin by phosphorylation of eukaryotic elongation factor 2 (eEF2) (Xu et al., 2017). In this interesting study, neurons secreted miR-132-containing exosomes to brain endothelial cells, leading to an increase in functional and mature miR-132 expression in brain endothelial cells. Consequently, VE-cadherin was upregulated, and BBB integrity was enhanced. When miR-132 was antagonized by specific miR-132 morpholino antisense oligonucleotides, severe intracranial hemorrhage and disrupted BBB integrity was exhibited. Although VE-cadherin is an upstream regulator of claudin-5 and tight junctions (Taddei et al., 2008), in this study, changes in tight junction molecules such as claudin-5, occludin, ZO-1, were not observed, suggesting VE-cadherin can modulate BBB integrity via modulating adherens junctions without altering tight junctions.

Some miRNAs regulate BBB integrity by targeting both tight and adherens junction molecules. For example, miR-210 was shown to directly regulate tight junction molecule occludin and adherens junction molecule β-catenin (Ma et al., 2017). During ischemia, miR-210 expression was significantly upregulated in the brain. Inhibition of miR-210 with its complementary locked nucleic acid oligonucleotides (miR-210-LNA) reduced BBB leakiness by increasing expression of both occludin and β-catenin in the BBB.

MiRNAs and Inflammation

Inflammation is often associated with disrupted BBB (Haruwaka et al., 2019). On the one hand, miRNAs can mediate inflammation-induced BBB permeability; on the other hand, miRNAs may act as an upstream of inflammation by targeting inflammation molecules and pathways to regulate BBB integrity (**Table 1**).

TABLE 1 | The effect of microRNAs (miRNAs) on blood–brain barrier (BBB) integrity.

Modulation of the BBB	miRNA	Target	Function	References
Tight junction	miR-15a/16-1	Claudin-5	BBB destructive	Ma et al., 2020
	miR-501-3p	ZO1	BBB destructive	Toyama et al., 2018
	miR-182	FoxO1	BBB destructive	Taddei et al., 2008
	miRNA-107	Endophilin-1	BBB destructive	Zhang et al., 2020
	miR-143	PUMA	BBB destructive	Bai et al., 2016
	miR-27a-3p	VE-cadherin	BBB destructive	Li et al., 2020
	miR-101	VE-cadherin	BBB destructive	Mishra and Singh, 2013
	miR-132	EEF2K	BBB protective	Xu et al., 2017
	miR-210	Occludin/β-catenin	BBB destructive	Ma et al., 2017
Inflammation	miR-125a-5p	N/A	BBB protective	Reijerkerk et al., 2013
	miR-155	N/A	BBB destructive	Lopez-Ramirez et al., 2014
	miR-21-5p	TNF-α/IL-6	BBB protective	Ge et al., 2016
	miR-126-3p	VCAM-1	BBB protective	Fu et al., 2019
	miR-98 and let-7g*	CCL2 and CCL5	BBB protective	Rom et al., 2015
	miR-1303	MMP9	BBB protective	Lampugnani et al., 2018
	miR-132	MMP9	BBB protective	Li et al., 2018
Supporting cells coverage	miR-27b	SEMA6A/D	BBB protective	Demolli et al., 2017
	miR-150	Tie-2	BBB destructive	Fang et al., 2016
Apoptosis/cell cycle	miR-182	mTOR	BBB protective	Zhang et al., 2020
	miR-285	Yki/Mask	BBB protective	Li et al., 2017
Actin cytoskeleton	miRNA-181c	PDPK1	BBB destructive	Tominaga et al., 2015
Channel and receptor	miR-30a	ZnT4	BBB destructive	Wang et al., 2021
	miR-27a-3p	AQP11	BBB protective	Xi et al., 2018

In brain endothelial cells treated with the proinflammatory mediator tumor necrosis factor α/interferon γ (TNFα/IFNγ), 107 miRNAs were significantly changed (Reijerkerk et al., 2013), among which miR-125a-5p was downregulated. Consistently, in the inflamed blood vessels of patients with multiple sclerosis (MS), there was also significantly less miR-125a-5p than in its expression in non-inflamed blood vessels (Reijerkerk et al., 2013), suggesting a positive correlation between miR-125a-5p and BBB integrity during inflammation. In contrast, miR-155 was shown to negatively affect BBB integrity during inflammation (Lopez-Ramirez et al., 2014). It was upregulated in disrupted BBB of MS human patients and animals—in experimental allergic encephalomyelitis (EAE), a model of MS. When miR-155 was knocked out in EAE mice, BBB leakage was reduced by 50% compared with wild-type mice. Inhibition of miR-155 also reduced TNFα/IFNγ-induced endothelial permeability *in vitro*. The putative targets of miR-155 included focal adhesion molecules and junctional complex, suggesting miR-155 may function by targeting these molecules to modulate BBB integrity.

MicroRNAs can also target inflammatory cytokines or markers to regulate BBB integrity, as exemplified by miR-21-5p, which regulates the BBB by targeting pro-inflammatory cytokines TNF-α, interleukin 6 (IL-6), and nuclear factor kappa B (NF-κB) signaling (Ge et al., 2016). Another miRNA, miR-126, was shown to attenuate intracerebral hemorrhage-induced leukocyte adhesion and BBB disruption by targeting vascular cell adhesion molecule-1 (VCAM-1), a classic inflammation marker critical for leukocyte adhesion to blood vessels (Fu et al., 2019). Glycogen synthase kinase 3β (GSK3β) was shown to protect BBB under neuro-inflammation conditions. MiR-98 and let-7g*, both of which belong to the highly conserved let-7 family, mediated the BBB-protective effect of GSK3β by targeting inflammatory molecules CCL2 and CCL5 (Rom et al., 2015). Overexpression of let-7g* and miR-98 reduced neuro-inflammation-induced BBB leakiness. Matrix metalloproteinase-9 (MMP9) contributes to inflammation-induced BBB breakdown (Shigemori et al., 2006; Turner and Sharp, 2016). Several miRNAs, including miR-1303 and miR-132, have been shown to target MMP9 and play a protective role in BBB integrity under inflammation conditions (Song et al., 2018; Zuo et al., 2019).

MiRNAs and Crosstalk Between Brain Endothelial Cells and Supporting Cells

Pericyte coverage on brain endothelial cells is another key indicator of BBB integrity (Ting et al., 2019). When pericyte coverage is reduced, BBB permeability is increased. MiRNAs can modulate the integrity of the BBB by regulating pericyte coverage-associated molecules. One of the examples is miR-27. There are two miR-27s, miR-27a and miR-27b, which differ from each other by one nucleotide outside the seed region (Young et al., 2013). While miR-27a targets VE-cadherin to compromise BBB integrity, miR-27b promotes the interaction of endothelial cells with pericytes by targeting semaphorin 6A/D (SEMA6A/D), leading to enhancement of endothelial barrier function (Demolli et al., 2017; **Table 1**). This opposite role of miRNAs with identical seed regions is not rare, as evidenced by the difference in

miR-23a and miR-23b (Li et al., 2016), reflecting the complexity of miRNA regulation.

The recruitment of pericytes to the endothelium can also be mediated by angiopoietins/Tie-2 signaling. MiR-150 was shown to target Tie-2 (Fang et al., 2016), leading to inhibition of claudin-5 expression and endothelial cell survival. It will be interesting to see whether miR-150 also has an effect on pericyte coverage to the BBB. Astrocytes, another type of BBB supporting cells, can release factors to strengthen BBB function via regulating miRNAs, one of which is mir-125a-5p, in the brain endothelial cells. How these miRNAs mediate crosstalk between astrocytes and brain endothelial cells remains to be defined (Reijerkerk et al., 2013).

MiRNA and Other BBB-Relevant Pathways

In addition to the aforementioned endothelial tight junctions, inflammation and supporting cell coverage, other pathways regulating endothelial function, such as cell survival, cytoskeleton, and ion channels also contribute to the regulation of BBB integrity (**Table 1**).

Endothelial cell survival is critical for its function. MiR-182 was shown to mediate BBB breakdown in cerebral ischemia, a disease associated with massive BBB damage. Further studies suggested that miR-182 directly targeted mTOR, which is anti-apoptotic, suggesting miR-182 may regulate BBB integrity by regulating apoptosis of brain endothelial cells after ischemia. Mir-285 is another mRNA shown to affect BBB integrity through regulating apoptosis (Li et al., 2017). By targeting Yorkie (Yki in *Drosophila*, or YAP and TAZ in mammals), miR-285 inhibits cell proliferation and induces apoptosis, leading to regulation of BBB integrity.

The actin cytoskeleton is associated with endothelial barrier functions (Nag, 1995), through intercellular connections with tight and adherens junctions between endothelial cells. MiR-181C was shown to target 3-phosphoinositide-dependent protein kinase-1 (PDPK1), which delocalized actin fiber to destruct the BBB integrity. Intriguingly, unlike BBB-protective neuron-secreted miR-132, miR181c-containing extracellular vesicles can be secreted from brain metastatic cancer cells to break the BBB, leading to brain metastasis (Tominaga et al., 2015).

Several miRNAs have been shown to regulate BBB integrity by targeting channels and transporters in brain endothelial cells. MiR-30a targets zinc transporter ZnT4, leading to reduced intracellular free zinc in endothelial cells and an increase in BBB permeability in both cellular and animal models of ischemic stroke (Wang et al., 2021). In contrast, in intracerebral hemorrhage, miR-27a-3p protects against BBB disruption by targeting endothelial aquaporin-11 (AQP11), a functional water channel that permeates both water and glycerol with a possible role in the pathophysiology of brain edema (Xi et al., 2018). This is a conflict with the reports showing miR-27a-3p is disruptive for endothelial barrier function (Young et al., 2013; Li et al., 2020), indicating that a specific miRNA may function differently via regulating different targets.

CHALLENGES AND PERSPECTIVE

The effect of miRNAs on BBB integrity makes them a promising target to transiently open the BBB for brain-targeted drug delivery and to restore BBB integrity for disease treatment. However, several key challenges remain to be overcome before translating BBB-targeted miRNA-based therapeutics into the clinic (Rupaimoole and Slack, 2017).

The first challenge is the identification of miRNA targets. Through the miRNA array, the change in miRNA expressions caused by disrupted BBB can be measured (Reijerkerk et al., 2013). However, how these significantly regulated miRNAs contribute to BBB disruption remains unclear. This requires the identification of the targets of these miRNAs. This process often includes experiments to measure mRNA or protein levels of possible targets after modulating endogenous miRNA expression. To further identify whether the regulation is direct or indirect, luciferase reporter assays have been commonly used (Li et al., 2016). Because of the time for cloning and generation of mutants, these methods are quite time-consuming and only feasible for the identification of a small number of targets. In addition, it is becoming apparent that miRNAs may shift their targets in different types of cells and biological environments, further complicating the strategies for target validation.

The second challenge is the specificity of miRNA-based therapeutics. Each miRNA has dozens if not hundreds of potential targets. The ability of miRNAs to regulate a wide range of mRNAs gives them a unique advantage to regulate complex biological processes. However, it also raises possible side effects when miRNA expression is modulated. To overcome these drawbacks, antisense oligonucleotides (ASOs) that specifically block miRNA interaction with a mRNA of interest have been developed (Young et al., 2013; Zhao et al., 2017). Instead of modulating miRNA expression, these ASOs, named miR-Mask, target site blocker (TSB), or BlockmiRs, bind to the miRNA binding sites in the 3′UTR of the target mRNA through full complementarity (Sonneville et al., 2017; Natarelli et al., 2018). Consequently, they prevent miRNA from regulating a specific mRNA. This can be highly useful in identifying the importance of a specific miRNA:mRNA interaction or developing miRNA therapeutics for validated drug targets. However, the design of TSB remains a challenge as the principles of the design are not fully understood. Further understanding and improvement in the design principles are required to improve the success rate.

The third challenge is the delivery of miRNA-based therapeutics to the brain. Current modifications of miRNA modulators, including mimics and inhibitors, have successfully increased their retention time in the circulation system (Rupaimoole and Slack, 2017). However, the majority of naked miRNA modulators are accumulated in the liver and kidneys. It is important to increase miRNA accumulation in the brain to enhance their effect targeting the BBB. Viral and non-viral delivery systems with specificity to brain endothelial cells have been successfully employed to deliver nucleotide-based drugs into the brain (Lee et al., 2019; Marcos-Contreras et al., 2020). However, the potential immunostimulatory effects and

toxicity of these delivery systems may hinder clinical translation (Mitchell et al., 2021). Identification of high-affinity ligands targeting BBB-specific receptors and development of biocompatible delivery materials are required to improve the specificity and efficiency of BBB-targeted delivery systems.

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JW and JL contributed to the conception and design of the review and wrote the first draft of the manuscript. FX, XZ, XL, and YL critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Microwave-Assisted Synthesis of Carbon Dot – Iron Oxide Nanoparticles for Fluorescence Imaging and Therapy

*Seokhwan Chung and Miqin Zhang**

Department of Materials Science and Engineering, University of Washington, Seattle, WA, United States

Fluorescence microscopy is commonly used to image specific parts of a biological system, and is applicable for early diagnosis of cancer. Current fluorescent probes, such as organic dyes and quantum dots, suffer from poor solubility and high toxicity, respectively, demonstrating a need for a colloidal stable and non-toxic fluorescent probe. Here we present an iron oxide and carbon dot (CD) based nanoparticle (CNPCP) that displays optical properties similar to those of conventional fluorescent probe and also exhibits good biocompatibility. Fluorescent CDs were synthesized from glucosamine onto chitosan – polyethylene glycol (PEG) graft copolymer using microwave irradiation. These NPs were monodispersed in aqueous environments and displayed excitation-dependent fluorescence; they demonstrated good size stability and fluorescence intensity in biological media. *In vitro* evaluation of CNP as fluorescent probes in cancer cell lines showed that these NPs caused little toxicity, and allowed fast and quantitative imaging. Model therapeutic doxorubicin (DOX) was conjugated onto the NPs (CNPCP-DOX) to demonstrate the multifunctionality of the NPs, and *in vitro* studies showed that CNPCP-DOX was able to kill cancer cells in a dose dependent manner. These results indicate the potential of using CNPCPs as fluorescent probes capable of delivering chemotherapeutics.

Keywords: carbon dots, fluorescence imaging, iron oxide nanoparticles, microwave-assisted synthesis, chemotherapy, drug delivery

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Sun Yat-sen University, China

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Sun Yat-sen University, China
Li Wu,
Nantong University, China

***Correspondence:**
Miqin Zhang
mzhang@uw.edu

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INTRODUCTION

Fluorescence microscopy is a powerful tool used for selective imaging of tissues, cells, and organelles. Combined with an excitable, stable fluorophore, it can be used for early diagnosis of diseases such as cancer (Jie et al., 2014; Pak et al., 2015; Wolfbeis, 2015). An ideal fluorophore should demonstrate good biocompatibility so that it does not interfere with any cellular processes, and exhibit fluorescence intensity sufficiently higher than any autofluorescence within the biological system for a more sensitive imaging system. The current state of the art fluorophores are organic dyes and metallic or semiconductor-based quantum dots (QDs) (Wegner and Hildebrandt, 2015; Yu et al., 2015; McHugh et al., 2018). However, many organic dyes contain hydrophobic functional groups, and hence suffer from poor solubility in aqueous environments. This requires them to be

conjugated onto the surface of a biocompatible, water soluble carrier particle; they are also known to have poor photostability and are easily photo-quenched, making long-term imaging difficult (Wang et al., 2010; Grimm et al., 2015). On the other hand, QDs typically consist of heavy metals or semiconductors, which typically lead to severe levels of both short and long term toxicity (Bottrill and Green, 2011; Tavares et al., 2011; Jensen, 2012; Das and Snee, 2016).

Carbon-based nanomaterials have emerged as an alternative to heavy metal semiconductor QDs. Carbon dots (CDs) exhibit tunable optical properties, while lacking the toxicity associated with heavy metals and semiconductors in biological systems (Yang et al., 2009; Luo et al., 2014; Schroeder et al., 2016; Winkless, 2016; Iannazzo et al., 2017). These CDs consist of an amorphous carbon matrix core that consists of mainly sp3 hybridized carbons, but also contains sp2 domains and a passivated surface. The delocalized electronic states in the sp2 domains and the nature of the passivation on the surface can affect the photoluminescence of CDs (Li and Dong, 2018). There has been a number of reported methods for synthesis of CDs having various optical properties (Pan et al., 2010; Fan et al., 2013; Ahirwar et al., 2017). Compared to single- and multi-layered carbon nanotubes (SW/MWCNT), CDs are easier to synthesize, and their photoluminescence can be easily tuned, which has fueled the efforts at developing CD-based optical imaging systems for biological applications (Hu et al., 1999; Lin and Zhang, 2012).

Recent studies have focused on synthesis of CDs from relatively simple carbon sources, such as glucose, through microwave-assisted hydrothermal reactions. These methods use an aqueous solution of carbon-containing precursors that is irradiated with microwave radiation, and various separation techniques, such as centrifugation and column chromatography, are used to purify the CDs that display the desired properties (Tang et al., 2012, 2014). The microwave irradiation allows quick and uniform heating of the solution, which improves the homogeneity in physical and chemical properties. Furthermore, the aqueous nature of the reaction leads to water-soluble CDs that do not require further surface passivation due to the presence of hydrophilic functional surface groups. CDs can also be synthesized through other methods such as electrolysis and hydro/solvothermal treatment. However, these reactions do not allow precise control over physical and optical properties, and require strong oxidizing reagents as part of the synthesis process. Compared to these methods, the microwave irradiation yields more uniform products, and is a safer and faster approach to synthesis of CDs. Though the chemical reaction is complex and not yet fully understood, many reports suggest that glucose-based CDs are an ideal alternative to fluorophores (Agbenyega, 2012).

Iron oxide nanoparticles (IONP) are a versatile platform for a variety of biomedical applications due to their favorable properties such as good biocompatibility, versatile surface chemistry, and superparamagnetism. A typical IONP system consists of an iron oxide core that is coated by molecules such as polymers (Stephen et al., 2014, 2016), proteins (Huy et al., 2015), and small macromolecules such as glucose (Sun et al., 2009). These coatings provide various chemical functional groups on the surface of the IONPs, which improves their solubility, while providing sites for conjugation of targeting ligands (Sun et al., 2008), therapeutic drugs (Hwu et al., 2009; Kievit et al., 2011), and nucleic acids (Jiang et al., 2013; Stephen et al., 2016). In addition to the biochemical properties, IONPs with magnetite (Fe_3O_4) cores have superparamagnetic properties and have been developed as contrast agents for magnetic resonance imaging (MRI) (Laurent et al., 2008; Lee and Hyeon, 2012). For these reasons, IONPs have been widely investigated in cancer research as multifunctional nanomaterials, with applications such as tumor imaging (Ji et al., 2019) and drug delivery (Mansouri et al., 2017).

Here, we report a method for microwave-assisted synthesis of fluorescent CDs onto chitosan-PEG copolymer, which is then used to stabilize IONPs through a co-precipitation synthesis method to produce a dual imaging contrast agent. While IONPs are widely used as MRI contrast agents, they do not have inherent fluorescent property, limiting their utility to a single imaging modality. Previous reports of IONPs for multimodal imaging probes have utilized organic dyes on surface of core-shell nanoparticles. Significant fluorescence quenching of the dyes was observed in such designs (Jang et al., 2014). In this design, modification of chitosan-PEG with CDs allows the IONPs to be used as multimodal imaging agents, and no fluorescence quenching of the CDs was observed. Chitosan is well-known to be biocompatible, and contains functional groups which would allow conjugation of therapeutic molecules or ligands onto the surface of CNPCP. Previously, iron oxide nanoparticle coated with biocompatible chitosan-PEG (NPCP) without the CDs was reported to be a T_2 MRI contrast agent (Sun et al., 2008; Stephen et al., 2019). CDs of small size are rapidly cleared by the body; the IONP as their host would greatly increase the residence time of the CDs *in vivo*, allowing long-term fluorescence imaging. The short reaction times and the availability of the precursors in the synthesis method make the method easy to scale up mass production. In this method, an aqueous solution of chitosan-PEG and glucosamine was irradiated inside a conventional microwave oven in presence of ammonia as a catalyst. The resulting polymer consisted of CDs formed from glucosamine on the chitosan-PEG, and fluorescent IONPs were synthesized through addition of ammonia into the solution of the modified polymer and ferrous and ferric ions. The physicochemical properties were analyzed using transmission electron microscopy, dynamic light scattering (DLS), and Fourier transform infrared spectroscopy. The optical properties of the NPs were optimized by varying the reaction conditions in the microwave and were analyzed using fluorescence spectroscopy and UV-Vis absorbance spectroscopy. Finally, *in vitro* experiments were performed to evaluate the NPs as fluorescence probes for cellular imaging. Toxicity, stability in biological media, and nanoparticle uptake in human glioma cells were assessed, and confocal fluorescence microscopy was used to evaluate the capability of NPs as fluorescence imaging probes. Furthermore, to demonstrate the utility of the design, DOX was conjugated onto the surface of the NPs as a model therapeutic. The therapeutic effect of the resultant NP-DOX conjugate was evaluated through assessment of cell viability after incubation with various doses.

MATERIALS AND METHODS

Materials

All reagents were obtained from Sigma Aldrich (St. Louis, MO, United States) unless noted otherwise. Chitosan (MW 3900) was obtained from Acmey Industrial (Shanghai, China). Cell culture reagents including Dulbecco's Modified Eagle Media (DMEM) and antibiotic-antimycotic solution were purchased from Invitrogen (Carlsbad, CA, United States). Fetal bovine serum (FBS) was purchased from Atlanta Biologicals. DOX was purchased from LC Laboratories (Woburn, MA, United States).

Preparation Fluorescent Iron Oxide Nanoparticles (CNPCP)

Chitosan-PEG coated iron oxide nanoparticles with CD incorporated (CNPCP) were synthesized via co-precipitation as previously reported (Veiseh et al., 2009). Briefly, purified chitosan (3.9 kDa) and aldehyde-activated methoxy PEG were reacted via reductive amination to produce a PEG-grafted chitosan polymer (CP). To synthesize the CD-CP complex, purified CP (150 mg) and glucosamine hydrochloride (25 mg) was dissolved in deionized water. Diluted ammonia was added at 12.5 mg/ml. The solution was then transferred to a capped glass vial, and placed inside a conventional microwave. The vial was irradiated at 300W for varied duration. The resulting fluorescent CD-CP was purified through size exclusion chromatography in S-200 resin (GE Healthcare, Piscataway, NJ, United States) equilibrated with deionized water.

To synthesize the CNPCP, polymer mixture consisting of varied ratios of CP and CD-CP (150 mg total), iron (II) chloride (9 mg), and iron (III) chloride (15 mg) were dissolved in degassed deionized water (2.18 ml). Ammonia solution (36%) was titrated into the solution while being sonicated and stirred vigorously for 25 min under a nitrogen atmosphere. The ammonia was evaporated from the solution by continuing the sonication and stirring for additional 20 min to continue the growth of the nanoparticles. The resulting CNPCP were purified using S-200 resin equilibrated with deionized water.

Characterization of CNPCP

Aqueous solution of CNPCP was diluted into a 50 mM HEPES buffer solution (pH 7.4), and hydrodynamic size and zeta potential of CNPCP were measured using a Zetasizer system. Electron micrographs of the samples were taken using a FEI Tecnai G2 F20 transmission electron microscope (TEM) (FEI, Hillsboro, OR, United States) operating at a voltage of 200 kV. The samples for TEM imaging was prepared by depositing 10 μL of the CNPCP solution onto a carbon-coated Cu 300 mesh grid. Fourier Transform InfraRed (FTIR) spectra of the samples were obtained with a Nicolet 6700 FTIR Spectrometer (ThermoFisher, Waltham, MA, United States). To minimize the interference from the iron oxide core, the core was dissolved with hydrochloric acid, and separated from the coating using a 3 k MWCO spin filter. The samples were then lyophilized and mixed into a KBr pellet at 0.2 wt%. Optical characterization was performed using fluorescence spectroscopy and UV-Vis absorbance spectroscopy. The fluorescence of the CNPCP solutions at various excitation wavelengths were recorded using a Horiba FL3-21tau Fluorescence Spectrophotometer (Kyoto, Japan). UV-Vis absorbance spectra of the CNPCP solutions were acquired using a UV-vis Spectrometer (Agilent Technologies, Santa Clara, CA, United States).

Assessment of Stability in Biological Media

To assess the stability of CNPCP in biological media, CNPCPs were dispersed in DMEM with 10% FBS and 1% Penicillin-streptomycin, and incubated in water bath at 37°C for 14 days, during which DLS and fluorescence measurements were made every 2 days.

Cell Viability Evaluation Through Alamar Blue Assay

SF763 human glioblastoma cells were cultured in DMEM supplemented with 10% FBS and 1% Penicillin-streptomycin. The cells were incubated at 37°C with 5% CO_2 atmosphere. The effect of CNPCPs on viability of SF763 cells was determined using the alamarBlue assay following the manufacturer's protocol (Life Technologies, Carlsbad, CA, United States). Briefly, cells were plated and treated with CNPCP as described. After treatment, cells were washed with phosphate buffered saline (PBS) three times before adding 10% alamarBlue solution in DMEM medium to the well. Cells were incubated for 24 h, then the alamarBlue solution was transferred to a 96-well plate, and the fluorescent emission at an excitation wavelength of 560 nm and an emission wavelength of 590 nm was read with a microplate reader.

Evaluation of Cellular Uptake of CNPCP

SF763 human glioblastoma cells were seeded at 30,000 cells per well in a 24 well plate. Various concentrations of CNPCP were added to the wells. After 4 h of incubation time, the cells were collected, washed with PBS, and fixed in 4% formaldehyde (Polysciences, Inc., Warrington, PA, United States) for 30 min. Cells were then washed three times with PBS, and the cellular uptake was analyzed using the fluorescence of the CNPCPs through flow cytometry.

Evaluation of CNPCP as Fluorescent Probes Through Confocal Microscopy

SF763 cells (50,000) were plated on a 24 mm glass coverslip and allowed to attach for 24 h. CNPCP solution was added to the cells, which were then incubated for 4 h. Afterward, cells were washed with PBS and fixed in 4% formaldehyde for 30 min. Cells were then washed three times with PBS, and coverslips were mounted on microscope slides. Images were acquired on an LSM 510 Meta confocal fluorescence microscope (Carl Zeiss, Inc., Peabody, MA, United States) with the appropriate filters.

Preparation of CNPCP-DOX

To conjugate DOX onto CNPCP, DOX (1 mg) and succinimidyl iodoacetate (SIA) (0.57 mg) was reacted in dimethyl sulfoxide

(DMSO) for 2 h. To activate the amine groups on CNPCP, 2-iminothiolane (1.18 mg) was added to 1.5 mg/ml of CNPCP in 0.1 M sodium bicarbonate, 5 mM EDTA buffer solution (pH 8.0) and reacted for 2 h. Activated CNPCP and DOX were mixed and reacted overnight at room temperature. Unreacted reagents were purified through size exclusion column chromatography using S-200 resin. Drug loading capacity was calculated by measuring the difference in absorption of CNPCP-DOX and CNPCP at 494 nm using a UV-vis Spectrometer (Agilent Technologies, Santa Clara, CA, United States).

Evaluation of Therapeutic Effects of CNPCP-DOX

SF763 cells were seeded at 10,000 cells per well in a 96 well plate. Various concentrations of CNPCP-DOX were added to the wells. After treatment for 24 and 48 h, cells were washed with phosphate buffered saline (PBS) three times before adding 10% AB solution in DMEM medium to the well. Cells were incubated for 4 h, then the AB solution was transferred to a 96-well plate, and the fluorescent emission at an excitation wavelength of 560 nm and an emission wavelength of 590 nm was read with a microplate reader.

RESULTS

Physiochemical Characterization of CNPCP

Iron oxide nanoparticles coated with chitosan-PEG copolymer and carbon dots (CNPCP) were synthesized using a coprecipitation method. The chitosan-PEG coating provided the initial solubility in aqueous environments, and the steric stability imparted by the PEG kept the CNPCP well-dispersed without aggregation. The mechanism for the synthesis of CNPCP is illustrated in **Figure 1A**. The synthesized c-dots formed on the chitosan-PEG coating of the nanoparticle. The resulting solution was dark brown and highly fluorescent (**Figure 1B**).

The effect of varying parameters of the microwave-assisted reaction on the fluorescent properties of the resulting CNPCPs was investigated. The duration of irradiation in the microwave oven was first investigated. The concentration of reagents, as well as the incident power of the microwave irradiation was kept constant. The observed fluorescence spectra of CNPCP shows the fluorescence intensity increasing with longer times up to 40 s, after which the intensity slightly decreases (**Figure 2A**). Also observed with 45 s of microwave irradiation was a darker solution, followed by aggregation of the polymer after resting. At reaction times shorter than 25 s, no significant fluorescence was observed (data not shown), and at reaction times longer than 45 s, the solution was overheated and evaporated from the vial. This result shows that there is a critical temperature that the reaction must reach before formation of CDs on the polymer. However, prolonged irradiation leads to degradation of fluorescence and instability of the final product, likely due to uncontrolled carbonization of the reactants. Chitosan-PEG irradiated for 40 s was used for the remainder of the experiments.

The amount of glucosamine was also varied in the precursor solution, and all other parameters were kept constant. The resulting spectra from the samples shows that the fluorescence generally increased as the concentration of glucosamine was increased from 5 to 25 mg/ml (**Figure 2B**). A clear correlation was observed between the concentration of glucosamine and fluorescence intensity, with a sudden increase between 5 and 10 mg/ml, and between 20 and 25 mg/ml, indicating non-linear kinetics of CD growth with respect to the concentration of the

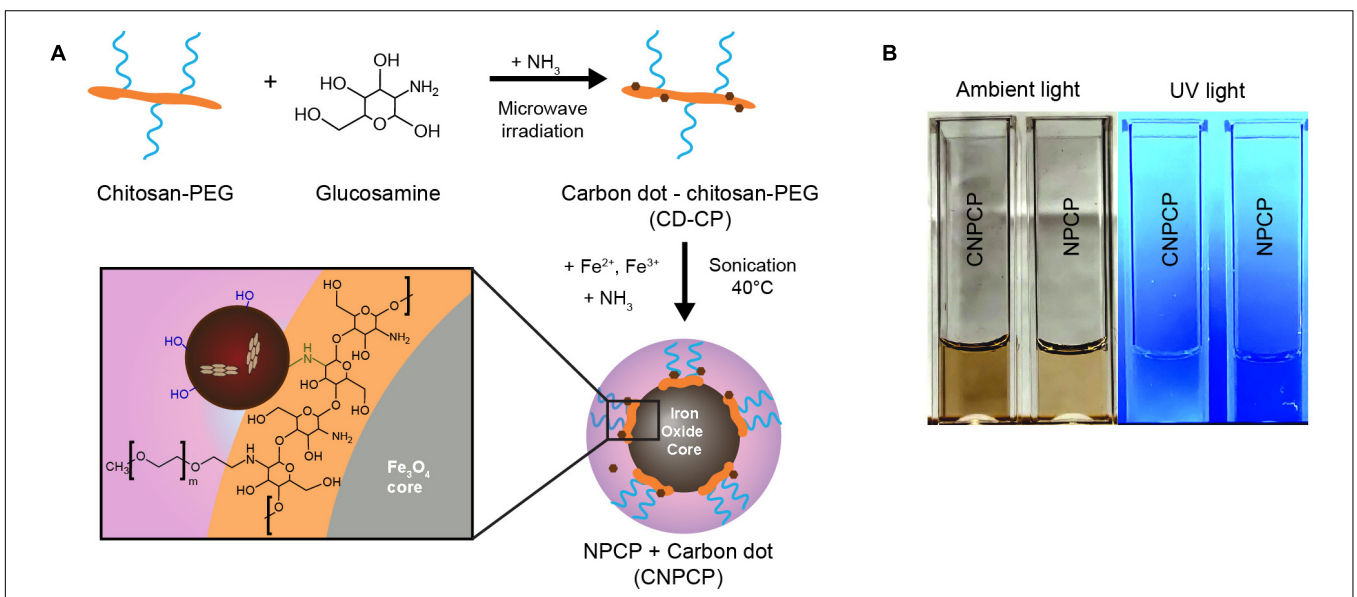

FIGURE 1 | Overview of synthesis of CNPCP. **(A)** Schematic representation of synthesis of carbon dot-chitosan-PEG (CD-CP) and fluorescent iron oxide nanoparticle (CNPCP). **(B)** Images of aqueous solutions of CNPCP and NPCP.

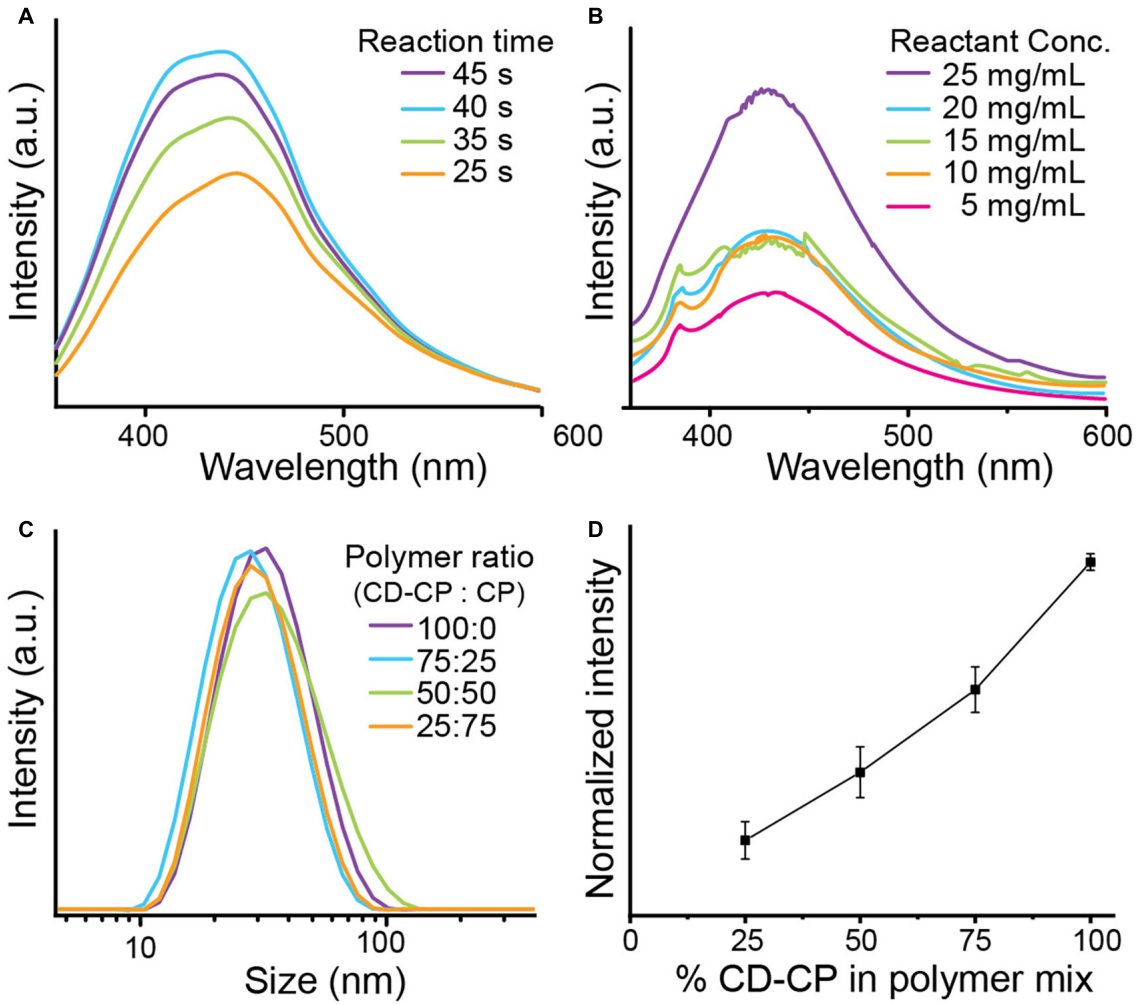

FIGURE 2 | Optimization of CNPCP synthesis parameters. **(A)** CD-CP reacted for various lengths of time, and **(B)** CD-CP reacted with varied concentration of glucosamine. **(C)** Hydrodynamic size distribution of CNPCP made with varying ratio of CD-CP and CP. **(D)** Maximum fluorescence intensity with increasing CD-CP content in polymer mix.

precursor molecules. In contrast to the intensities, the window of the fluorescence emissions is shown to be largely unchanged with the variation of the reaction conditions.

To test whether using a mix of chitosan-PEG modified with CD (CD-CP) and unmodified chitosan-PEG (CP) would affect the stability of the synthesized CNPCPs, the hydrodynamic size of each batch of CNPCP made with varied ratio between CD-CP and CP was measured using DLS. As shown in **Figure 2C**, no significant differences were observed in the size distribution of the CNPCPs made with different ratio of CD-CP to CP. To investigate the effect of the polymer ratio on the optical properties of the resultant CNPCP, fluorescence intensities of CNPCP made with each polymer ratios were measured. While the trend shows that increasing the amount of CD-CP increases the fluorescence of the CNPCP, it can be seen that CNPCP synthesized with CD-CP only exhibits fluorescence most efficiently (**Figure 2D**). Using 75, 50, and 25% of CD-CP in the polymer mix yielded CNPCP that displayed 63.9, 40.6, and 21.3% of the fluorescence intensity exhibited by CNPCP made with 100% CD-CP.

The parameters were optimized to synthesize stable and fluorescent CNPCPs, and transmission electron microscopy was used to observe the effect of modification of CP on the morphology and size of the iron oxide core. **Figures 3A,B** show the diameter of the iron oxide core to be 8.38 ± 2.32 nm; the CDs in the CNPCP are shown to be near the core of the nanoparticle with diameter 1.61 ± 0.31 nm (2b, inset). The hydrodynamic size of CNPCP was measured through DLS and was 38 nm, which was larger than the hydrodynamic size of 28 nm of NPCP prepared with the unmodified CP, but still monodisperse and smaller than 100 nm (**Figure 3C**). The monodispersity demonstrated by the CNPCPs indicates that while the CDs formed on the chitosan-PEG coating, the presence of the CD did not significantly affect the ability of the chitosan-PEG copolymer to form iron oxide nanoparticles in solution, and provide steric stability to the

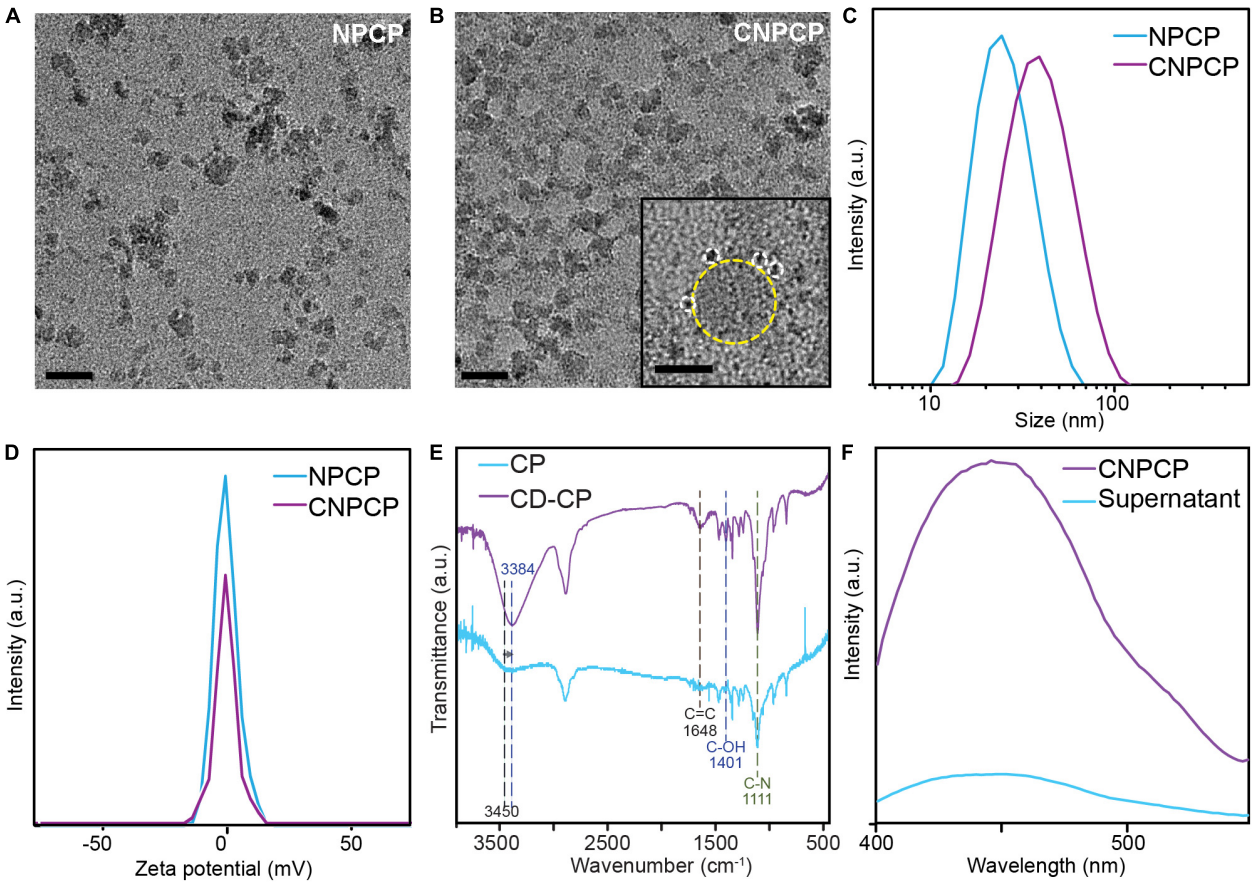

FIGURE 3 | Physicochemical characterization of NPCP and CNPCPs. TEM images of **(A)** NPCP (synthesized without CD-CP), and **(B)** CNPCPs. CDs (circled with white dashed circle) can be found surrounding the iron oxide core of CNPCP (yellow) (inset). Scale bar = 20 nm, 5 nm in inset) **(C)** Hydrodynamic sizes and **(D)** zeta potentials of NPCP and CNPCP. **(E)** FTIR spectra of CD-CP and CP. **(F)** Fluorescence emission of CNPCP solution before (CNPCP) and after (supernatant) magnetic separation of CNPCP from the solution.

nanoparticle. The zeta potential measurements (**Figure 3D**) show that there was a decrease in the zeta potential from −1.01 to −1.24 mV after the reaction, indicating the presence of hydroxyl and carboxylic groups on the surface as a result of the decomposition of the glucosamine molecules. The presence of the functional groups on the surface of CNPCPs leads to high solubility in aqueous solutions, as well as provides sites for further conjugation with therapeutics and targeting ligands.

The chemical structure of the polymer coating and the CDs was investigated using FTIR spectroscopy, with the FTIR transmittance spectrum of CP as reference. Transmittance peaks at 1,648 cm^{-1} and 1,401 cm^{-1} correspond to C = C bonds and C–OH bonds, respectively, and are only present on the spectrum of the CNPCPs, indicating the formation of CDs. The structure of the chitosan-PEG copolymer does not contain any C = C bonds, and while chitosan contains hydroxyl groups, the peak at 1,401 cm^{-1} peak is observed in graphene-like structures, indicating the presence of a chemical structure that is different from the chitosan-PEG copolymer. The intensity of the 1,111 cm^{-1} peak that corresponds to C–N bonds is also greater in the CNPCP, indicating the formation of additional C–N bonds between the CDs and the chitosan backbone, as well as within the CDs. The shift of the peak from 3,450 to 3,384 cm^{-1} after the reaction also indicates the change in the amount of N–H and O–H bonds as the CDs are formed (**Figure 3E**).

The strong bond between the CD and the CP-coated nanoparticle was also investigated. A solution of CNPCP was placed above a strong magnet to separate the magnetic CNPCP from the solution, and the fluorescence of the resulting supernatant was measured to investigate the strength of the association between the CNPCPs and the CDs. As shown in **Figure 3F**, the supernatant demonstrated significantly lower fluorescence than that of the CNPCP solution. Since the CD are the primary source of fluorescence in CNPCP, this result indicates that much of the CDs were associated with the CNPCPs that were separated from the solution. While some CDs remained in the supernatant, as shown by the peak observed in the fluorescent spectrum of the supernatant, this shows that the CDs are strongly bound to the surface of the nanoparticles, as opposed to being loosely associated with the nanoparticles. This highlights the stability of the CNPCPs, which is important to the application of CNPCPs as imaging probes by ensuring strong, undiffuse signal.

Optical Properties of CNPCP

The fluorescence of CNPCP was measured using various excitation wavelengths. **Figure 4A** shows the excitation-dependent fluorescence emission of CNPCPs. This wavelength-dependent tunable fluorescence is characteristic of many QD-based nanoparticle systems. The maximum fluorescence intensity was observed when an excitation wavelength of 360 nm was used (**Figure 4A**), which produced an emission spectrum with a peak around 450 nm (**Figure 4B**). As the iron oxide core contributes to the background absorbance in the UV-Vis spectrum, the absorbance spectra of the modified and unmodified polymers were recorded. The UV-Vis absorbance spectrum of the CD-CP contains a peak at 340 nm that is not present in that of CP, demonstrating a change in the optical properties with the formation of CDs in the CNPCPs (**Figure 4C**).

To investigate the origin of the fluorescence demonstrated by CNPCPs, CP, glucosamine, and a mixture of CP and glucosamine were reacted in the microwave. The resulting fluorescence spectra is shown in **Figure 4D**. The solution containing CP and glucosamine and glucosamine only displayed much greater fluorescence than that of the solution containing CP only. The combination of CP and glucosamine in the initial solution resulted in stronger fluorescence than the reaction of glucosamine by itself.

As the structure of CD contains labile protons, the pH of the aqueous environment and the subsequent protonation/deprotonation of these functional groups could lead to changes in the fluorescence of the CNPCP. To investigate this effect, CNPCP were placed in buffers of various pH. No significant photo-quenching was observed above pH 6; however, at low pH below 6, the fluorescence was quenched drastically (**Figure 4E**). It is important the CNPCPs be fluorescent between pH 6–8, as many physiologically relevant pHs fall within these values, and CNPCPs can be used as fluorescent probes in biological systems. The fluorescence of CNPCPs following incubation in cell culture media at 37°C was measured over time to assess the stability of the fluorescent CDs in biological conditions. Fluorescence intensity of the CNPCP solution in cell culture media was stable for up to 10 day, after which slight decrease in the fluorescence intensity was observed (**Figure 4F**).

Cell Viability and Uptake of CNPCP

AlamarBlue assay was used to assess the cell viability of SF763 cell line treated with CNPCPs. Though CNPCP demonstrated good water solubility and stability in cell culture media, it is important to assess potential toxicity of the nanoparticles for use in biological systems. The cells were treated at various concentrations of CNPCPs from 0 to 100 μg/ml. As can be seen

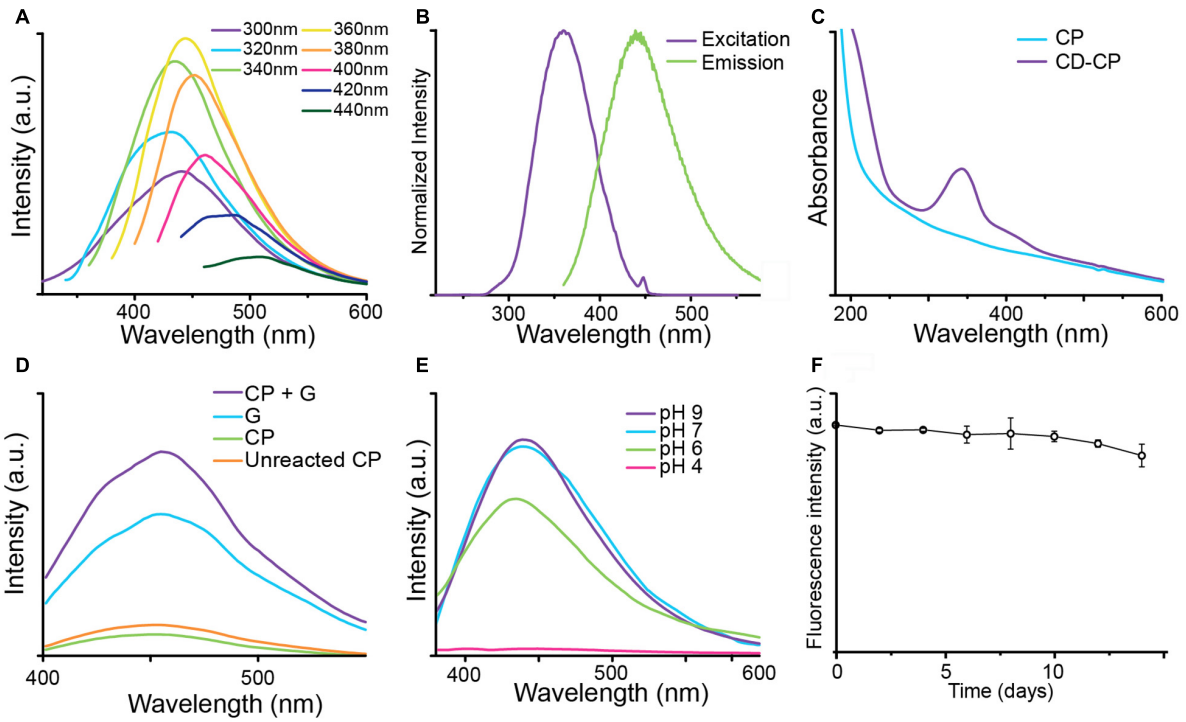

FIGURE 4 | Optical properties of CNPCP. **(A)** Fluorescence emission spectra generated under different excitation wavelengths. The CNPCP display a strong fluorescence in the green-blue range of the spectrum. **(B)** Fluorescence excitation (purple) and emission (green) profiles of CNPCP. **(C)** UV-Vis absorbance spectra of CP and CD-CP. The CD-CP shows an absorbance peak around 340 nm, indicating the presence of CDs on the NPs. **(D)** Fluorescence emission from microwaved solutions of chitosan-PEG and glucosamine (CP + G), glucosamine (G), chitosan-PEG (CP), and unmodified chitosan-PEG (Unreacted CP). **(E)** Fluorescence spectra of CNPCP in aqueous solutions of various pH values. **(F)** Fluorescence intensity of CNPCP in DMEM incubated at 37°C over time.

in **Figure 5A**, the viability of the cells treated for 24 and 72 h both show minimal toxicity. As glucosamine, iron oxide, and chitosan are all known to be biocompatible, little to no cell killing was expected from these NPs. This design of chitosan-PEG coated IONP previously reported by our group was reported to be non-toxic, and many studies on CDs show that CDs cause little to no cytotoxicity *in vitro*. This further demonstrates that the CNPCPs are suitable as bioimaging probes, as they do not display high levels of cytotoxicity on their own.

The extent of cellular uptake of CNPCP was assessed through flow cytometry following incubation with varied concentrations of CNPCPs. The innate fluorescence of the CNPCPs allowed measurement of uptake without the need to conjugate additional molecular fluorescent dyes onto the nanoparticles. The SF763 cells exhibited high uptake of CNPCPs – incubation in 200 μg/ml of CNPCPs showed that 95.6% of the cells had internalized the nanoparticles (**Figure 5B**). At lower concentrations, as shown in **Figure 5C**, the mean value of the fluorescence observed from the cells correlated with the concentration of the CNPCPs. These results indicate that CNPCPs exhibit stability in biological media, do not elicit significant cytotoxicity, and are taken up by glioblastoma cells in a dose-dependent manner.

To demonstrate the capability of CNPCPs as fluorescence imaging probes, confocal images of SF763 cells treated with CNPCPs show that the CNPCPs have been internalized, with the greatest intensity observed around the nucleus (**Figures 5D–F**). Due to their small size and near-neutral surface charge, the CNPCPs were able to readily penetrate the cell membrane. No NPs were observed in the interior of the cell nuclei. NP systems commonly used to target the cell nucleus are either conjugated with targeting peptides or display highly positive surface charge to penetrate the nuclear membrane. Since there were no additional conjugation onto the CNPCPs, and zeta potential was measured to be near neutral, the NPs were expected to remain outside the nucleus. The intensity of the fluorescence from the CNPCP shows correlation with the nanoparticle uptake data, which indicates that the fluorescence of the CNPCPs were not affected by interaction with the intracellular components.

CNPCP-Mediated *in vitro* Delivery of DOX

In addition to their fluorescence, CNPCPs also contain functional groups which allow conjugation of therapeutic molecules. Doxorubicin (DOX) was conjugated onto the amine groups of the chitosan backbone (CNPCP-DOX) to demonstrate the utility of CNPCP in therapeutic applications (**Figure 6A**). The drug

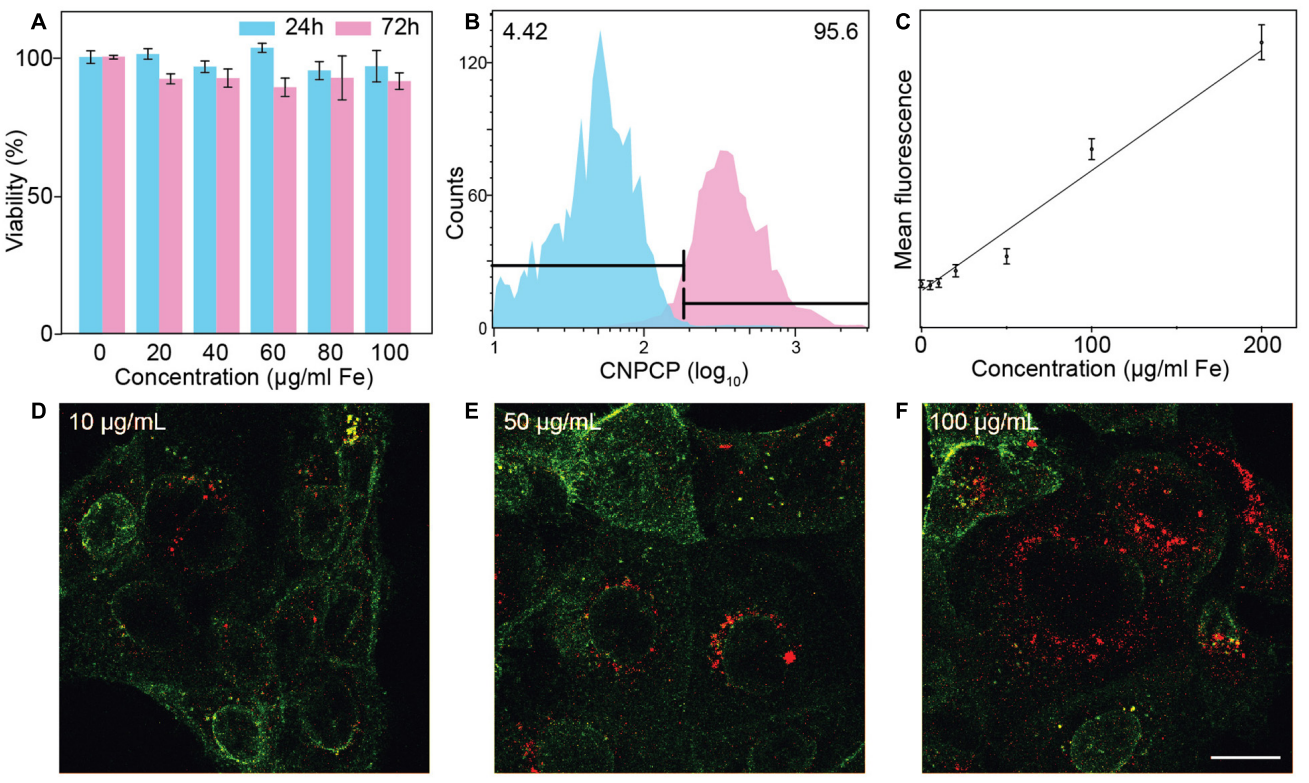

FIGURE 5 | *In vitro* evaluation of CNPCP as fluorescence imaging probe. **(A)** Cell viability of SF763 cells evaluated via alamarBlue assay. **(B)** Representative histogram of distribution of cells treated (pink) with CNPCP and untreated (blue) cells. **(C)** Comparison of mean fluorescence and nanoparticle measured through flow cytometry. **(D–F)** Confocal fluorescence microscopic images of SF763 cells treated with NPCPs at 10, 50, and 100 μg/ml. WGA-AF647 membrane stain will show in green and fluorescence from CNPCP is shown in red. Scale bar = 20 μm.

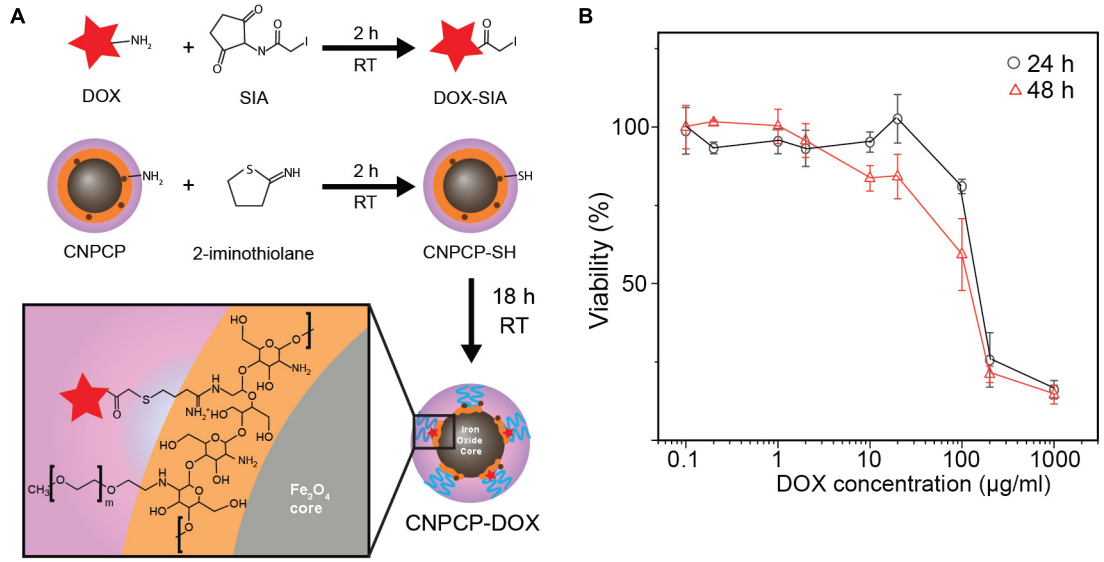

FIGURE 6 | Chemotherapeutic delivery by CNPCP. **(A)** Schematic representation of conjugation of DOX onto CNPCP. **(B)** Cytotoxicity curve of CNPCP-DOX on SF763 after 24 and 48 h incubation.

loading capacity was calculated to be 13.3% by measuring the absorption at 494 nm. The CNPCP-DOX was then added to SF763 cells which were incubated for 24 and 48 h. Viability of the cells was evaluated using alamarBlue assay, which showed that at concentrations above 100 μg/ml DOX, the proportion of healthy cells was much lower (**Figure 6B**). Since the CNPCPs were shown to be non-toxic, the therapeutic effect of CNPCP-DOX can be attributed to the conjugated DOX only. This result highlights the versatility and utility of the CNPCP design, which can be used in both imaging and therapeutic applications.

DISCUSSION

Physiochemical Characterization of CNPCP

Stable and monodisperse fluorescent iron oxide nanoparticles coated with biocompatible chitosan-PEG polymer was synthesized using a coprecipitation method. To confer fluorescent properties to iron oxide nanoparticles which are not inherently fluorescent, the chitosan-PEG polymer (CP) was mixed with glucosamine and ammonia, and irradiated in a conventional microwave. Microwave irradiation onto various carbon-based precursor molecules have shown to produce CDs more rapidly than conventional means of synthesis (Choi et al., 2017; Xiao et al., 2017). The resulting complex of CD and CP (CD-CP) was used to stabilize the iron oxide nanoparticles during coprecipitation. Comparison of FTIR spectra (**Figure 3E**) and UV-Vis absorbance spectra (**Figure 4C**) between CD-CP and CP shows the presence of carbon double bonds and delocalized electrons in CD-CP, confirming the synthesis of CD through microwave irradiation. Though many studies have shown microwave-assisted synthesis of carbon-based materials, this unique design utilized CP as a substrate in the reaction, and used the resulting complex of CD-CP to stabilize iron oxide nanoparticles. The effect of the microwave-assisted modification of the CP on size and stability of the as-synthesized CNPCPs was also investigated.

Size and solubility are of utmost importance in the evaluation of nanoparticle for biological applications; NPs with sizes outside of a range of 10–100 nm will often be cleared out by the liver and the kidney, and NPs with poor solubility lead to aggregation, causing further complications (Shilo et al., 2015; Wang and Liu, 2018). As the CNPCPs have a hydrodynamic size within this range, they are expected to have minimal initial clearance in biological systems. The zeta potential can indicate the stability of the nanoparticle in the dispersion medium. Though a higher magnitude of zeta potential is associated with greater stability due to electrostatic repulsion between the nanoparticles, the CNPCPs show good stability in aqueous environments due to the PEG grafted onto the chitosan coating. The PEG provides steric stabilization of the CNPCPs, allowing them to have a small value of zeta potential and inhibiting the aggregation of the nanoparticles. Furthermore, nanoparticles with high zeta potential have been shown to cause acute cytotoxicity. As the zeta potential of the CNPCPs are close to neutral, they are not expected to cause significant cytotoxicity. These characteristics are conducive to applications of CNPCPs in biological aqueous environments.

Optical Properties of CNPCP

Chitosan is known to exhibit fluorescence, which has been utilized in various applications such as biosensors and probes (Geng et al., 2015; Lee et al., 2017). To distinguish the inherent fluorescence of chitosan from that of the CDs in CNPCP, fluorescence intensities of solutions of CP, glucosamine,

and a mixture of CP and glucosamine that were irradiated with microwave were compared to fluorescence intensity of unreacted CP (**Figure 4D**). While fluorescence intensities of the solutions containing glucosamine were increased, the solution that contained both CP and glucosamine displayed the greatest fluorescence intensity. This can be attributed to the heterogeneous nucleation of CDs on the CP, which requires less energy than the homogeneous nucleation in the solution containing glucosamine only. As a result, greater amount of CDs were formed in the solution containing CP and glucosamine. The CP copolymer serves not as a source of fluorescence, but as a substrate for the growth of CDs. Interestingly, the fluorescence of CP was observed to be diminished after the treatment in the microwave, likely due to quenching of the fluorescence at higher temperatures (Baker, 2005).

The CNPCPs display optical properties similar to those observed in CD systems, such as the excitation wavelength dependence of fluorescence emission spectra, and the characteristic absorbance peak at 340 nm. The fluorescence spectra displayed by the CNPCPs can be attributed to several factors including size effects, edge states, and functional groups (Sangam et al., 2018). While similarities in fluorescence emission of various carbon-based nanostructures have been observed, various explanations for the origin of fluorescence in CD structures have been proposed (Demchenko, 2019). One possible explanation of the origin of the fluorescence properties of the CDs on the CNPCPs could be that each CD acts as a quantum emitter, while the variation in size, surface properties, and composition between individual carbon nanostructure leads to a mixture of fluorescence emissions from the individual CDs (Demchenko and Dekaliuk, 2016). One of the important factors in determining the emission window of the fluorescence of carbon nanostructures is the composition of the precursors (Dey et al., 2014; Hasan et al., 2018). As demonstrated in the optimization of the reaction parameters, the chemical composition of the reagents remained unchanged in these experiments and no significant shift in the general fluorescence emission was observed.

To further investigate the fluorescence behavior of the CNPCPs, the fluorescence was measured in aqueous solutions of various pHs. The results showed that there were no significant photo-quenching observed above pH 6 (**Figure 4E**). According to previous reports, the PL mechanism of CDs are affected by proton concentration, as the edge functional groups can be protonated (Sangam et al., 2018). Deprotonation of these sites at low pH renders the photoluminescence of CDs inactive. Conversely, at high pH, the edge functional groups are protonated, and the PL of CDs is restored. It is important the CNPCPs be fluorescent between pH 6–8, as many physiologically relevant pHs fall within these values, and CNPCPs can be used as fluorescent probes in biological systems. While the fluorescence is quenched below pH 6, it was shown to be restored at higher pH, allowing the CNPCPs to be used as a pH sensitive on/off probe.

The UV-Vis absorbance spectrum of CNPCPs shows a peak at 340 nm, attributed to the π–π^* transitions in the C = C bonds within the CDs (**Figure 4C**). Furthermore, a shoulder-peak is observed around 400 nm, indicating the n-π^* of the C = O bonds. These absorbance peaks imply that the electronic transitions within the CDs provide delocalized π states in the basal plane, and the carbonyl or carboxylic groups produce the PL behavior observed in the CNPCPs.

CNPCP as Multifunctional Imaging and Therapeutic Delivery System

The evaluation of cellular uptake of CNPCPs in SF763 highlighted the utility of the CNPCPs, as no fluorescent dye had to be conjugated onto the NP, and the inherent fluorescence of CNPCPs was used to analyze the population of treated cells. A linear trend is observed between the concentration of the CNPCP and the mean fluorescence of the treated cell, showing that the uptake and the resulting fluorescence of the cell is dose-dependent, and that the cellular uptake mechanism was not exhausted. Confocal fluorescence images of SF763 cells treated with CNPCP (**Figures 5D–F**) show that the regions with CNPCP can be distinctly identified in the images. The CNPCPs displayed consistent hydrodynamic size during incubation in 37°C, exhibited negligible toxicity, and was utilized as a fluorescent imaging probe.

The presence of functional groups on the chitosan presents opportunities to conjugate various therapeutics or targeting ligands onto the CNPCP to confer multifunctionality. To evaluate the effectiveness of CNPCP in therapeutic applications, doxorubicin (DOX) was chosen as the model therapeutic drug. DOX has been used previously to treat various cancer types; however, in the case of glioblastoma, it has seen limited use due to the high dosage required for systemic injection, and its inability to cross the blood-brain barrier. CNPCP-DOX induced toxicity at high concentrations and was able to kill tumor cells. In addition to delivery of DOX to glioma cells, the CNPCP was also able to aid in increasing the solubility of DOX in aqueous solution. In our previously reported study, iron oxide NP synthesized through co-precipitation and coated with chitosan-PEG was modified with chlorotoxin, a targeting peptide for glioma cells, was able to cross the blood-brain barrier (Veiseh et al., 2009). Because the CNPCP is synthesized from a similar design and materials, it is possible that CNPCP would also allow crossing of the blood-brain barrier for therapeutics that previously was limited by the this biological barrier.

In this study, chitosan-PEG co-polymer was modified with glucosamine via a microwave reaction to form carbon quantum dots in the polymer, and was used to synthesize fluorescent iron oxide nanoparticles (CNPCPs) that can be utilized as a fluorescent probe in biological systems. The NPs were small (under 100 nm) and showed good stability in cell culture media and minimal cytotoxicity *in vitro*. The NPs also showed great optical properties, as measured through fluorescence spectroscopy and UV-Vis absorbance spectroscopy. Cell viability results suggested that the CNPCPs were biocompatible and non-toxic, as no significant changes in cell viability was found both 24 and 72 h after the cells were treated with the CNPCPs. The CNPCPs were shown to be internalized within the cytoplasm in cells by fluorescence imaging, and the

greatest intensity (i.e., the highest NP accumulation) was observed around the nucleus. These results show that the CNPCPs are capable of being used as a fluorescence imaging probe and could potentially be used for nuclear targeting with conjugation of targeting ligands. The versatility of CNPCPs was evaluated through conjugation of DOX as a model therapeutic. CNPCP-DOX was able to kill SF763 glioma cells at concentrations above 100 μg/ml. Through this study, we demonstrated a simple and yet effective nanoparticle synthesis approach using a conventional microwave to produce a fluorescence imaging probe. This synthesis approach holds a great potential at developing nanoparticle systems that could be used for not only biological imaging, but also therapeutic delivery and biosensing.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SC and MZ designed the project. SC prepared the materials, performed the measurements, and analyzed the data. Both authors have read and agreed to the published version of the manuscript.

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