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Nanotechnology - an innovative approach to cope with the distinctive challenges linked with Autism Spectrum Disorder

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Abstract

The brain stands out as the most intricate organ in the human body, governing cognitive, behavioral, and emotional functions. It is susceptible to various diseases, ranging from injuries to cancers and neurodegenerative conditions, making brain disorders a significant cause of disability and mortality. Overcoming challenges such as delivery, specificity, and toxicity has been a persistent issue in developing drugs that enhance brain structure and function, especially those that can traverse the complex barriers surrounding the brain. Nanotechnology represents a groundbreaking approach to address the unique challenges associated with Autism Spectrum Disorder (ASD). One significant challenge in ASD is early and accurate diagnosis. Nanotechnology can contribute to the development of highly sensitive and specific diagnostic tools, enabling the identification of biomarkers associated with ASD at an earlier stage. These nanoscale devices may facilitate a more precise understanding of the underlying biological mechanisms, leading to improved diagnostic capabilities. In terms of intervention, nanotechnology can enhance drug delivery systems, allowing for the more targeted and efficient administration of medications. This targeted drug delivery can potentially mitigate side effects while maximizing the therapeutic impact, addressing some of the challenges in managing the diverse symptoms of ASD. Despite the promising potential of nanotechnology in addressing ASD challenges, it is essential to approach these innovations with ethical considerations, ensuring that the benefits are accessible and equitable for individuals with ASD. Ongoing research and collaboration between experts in nanotechnology and autism can lead to transformative advancements in understanding, diagnosing, and managing ASD. This review delves into the application of nanotechnology in diagnosing and treating ASD and shedding light on the promising role of nanoparticles.

Key Words –autism spectrum disorder; Diagnosis; Nanotechnology; Nanoparticles

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1. Introduction

The brain is the most intricate organ in the body and plays a central role in regulating cognitive, behavioral, and emotional functions [1]. However, it is also susceptible to a myriad of diseases and disorders, ranging from injuries to cancers and neurodegenerative conditions. Brain-related ailments are significant contributors to disability and rank among the leading causes of mortality [2]. Despite the potential demonstrated by various drugs in enhancing brain structure and function in animal models, researchers encounter numerous challenges, including issues related to delivery, specificity, and toxicity [3-5]. Addressing the long-standing challenge of developing drugs that can effectively traverse the physical blood-brain barrier (BBB), electrical, and chemical barriers of the brain to target specific regions with minimal adverse effects has been a persistent goal [3-5]. In recent times, nanotechnology has emerged as a crucial technique for modifying and manipulating various entities at the molecular level to achieve desired features. This technique has proven to be valuable in both the diagnosis and treatment of brain diseases and disorders, facilitating drug delivery and enhancing efficacy. Nanotechnology involves manipulating materials and devices at the nanoscale (typically between 1 and 100 nanometers) to achieve unique properties and functionalities. In recent years, treatments utilizing nanoparticles (NPs) have become a promising therapeutic approach for brain diseases and disorders. This is primarily attributed to their ability to easily traverse the blood-brain barrier, due to distinctive features such as small size, selectivity, low toxicity, biodegradability, and solubility [6-7]

In the field of medicine, Nanotechnology is utilized for the diagnosis and treatment of various diseases, such as metastatic cancers, brain tumors, and neurodegenerative disorders especially Autism Spectrum Disorder (ASD) [8]. ASD is a group of neurodevelopmental disorders characterized by early social interaction difficulties and the presence of repetitive behaviors and interests [9]. According to data from the Autism and Developmental Disabilities Monitoring Network of the Centers for Disease Control and Prevention, approximately 1 in 44 children are diagnosed with ASD [10]. The prevalence of autism is notably higher in boys, exceeding four times that in girls. Additionally, individuals with autism frequently experience co-occurring conditions, including epilepsy, depression, anxiety, and attention deficit hyperactivity disorder, along with challenging behaviors like sleep disturbances and self-harm [11] The onset of autism is influenced by a combination of genetic factors and early environmental exposures during development [12-13]. Genetic variations in certain genes significantly elevate the risk of ASD. While features of autism may be noticeable in early childhood, the actual diagnosis typically occurs later. Early identification requires a comprehensive multidisciplinary assessment, and while targeted behavioral interventions and pharmacological treatments can partially alleviate social impairment and aggression resulting from emotional instability, they do not provide a complete cure. Currently, there are no clearly defined neuropathological markers for autism that can serve as a basis for diagnostic criteria. The application of nanotechnology in diagnosing and treating Autism Spectrum Disorder (ASD) offers a potential advancement in the management of these intricate neurodevelopmental conditions. This review aims to spotlight the application of nanotechnology in diagnosing and treating ASD, emphasizing the potential of nanoparticles.

2. Drug Delivery Systems

Nanotechnology could be used to develop targeted drug delivery systems for medications used in autism treatment [14]. This could enhance the effectiveness of treatments while minimizing side effects. A multitude of drugs are accessible for treating central nervous system (CNS) disorders, yet challenges persist regarding issues like toxicity, specificity, and delivery [15-19]. The presence of challenging barriers, such as the BBB, poses a significant hurdle as it obstructs the passage of therapeutic drugs to their intended targets [20-21]. Researchers have been actively exploring strategies to facilitate drug permeation through the BBB, underscoring the imperative role of nanotechnology in modifying cellular processes to achieve desired outcomes [22-23]. Nanoparticles, emerge as a promising solution for bypassing the BBB, presenting an effective alternative for drug administration [24]. Nanotechnology holds the potential to revolutionize treatment and diagnostic approaches for CNS disorders, enabling efficient drug transfer [24]. Nanotechnology, operating at the nanoscale, offers promising solutions by employing materials like natural, synthetic/semisynthetic polymers, lipids, or metallic substances [26]. Nanoparticles play a crucial

role in targeted drug delivery, enhancing bioavailability, biodistribution, and therapeutic accumulation in specific disease areas. These colloidal systems act as stability enhancers, facilitating drug delivery to target sites, thereby improving therapeutic efficacy, minimizing toxicity, and reducing side effects [27]. Additionally, they protect drugs from biological degradation, enabling temporal and spatial control of therapeutics at the disease site. ASD treatment is one of the main challenges among neurodevelopmental diseases due to unclear pathogenesis research and limited efficiency in targeting brain lesions. Maternal immune activation stands out as a well-established model for ASD, and aspirin-triggered lipoxin A4, an anti-inflammatory mediator, plays a crucial role in resolving neuroinflammation associated with ASD [28]. Consequently, a novel cascade drug delivery system encapsulating aspirin has been developed. This system exhibits sequential targeting of the BBB and microglial cells, responding to the acidic microenvironment within lysosomes [29]. Nanoparticles encapsulated Aspirin effectively alleviates mitochondrial oxidative stress, DNA damage, and inflammation in microglial cells [29]. Treatment with encapsulated aspirin leads to significant improvement in social interaction and reduction in stereotype behavior and anxiety in ASD mice.

3. Nanotechnology-based Diagnostic Tools

Nanoscale materials and devices might be employed to create highly sensitive and specific diagnostic tools for early detection of ASD. Early diagnosis is crucial for effective intervention and support [30]. Nanotechnology involves the exploration and manipulation of matter at the molecular level. Molecular diagnostics based on nanotechnology hold significant promise in alleviating the suffering caused by various diseases, particularly neurological disorders, owing to the distinctive properties of nanomaterials [31]. Many neurological illnesses, often categorized as neurobehavioral disorders, are complex and multifactorial in nature. Conditions such as ASD could potentially benefit from improved treatment, diagnosis, prevention, and even cure facilitated by nanotechnology [32]. To enhance the quality of life for individuals affected by neurobehavioral disorders such as ASD, a diverse range of nanomaterials, including gold and silica nanoparticles, quantum dots, DNA, and various other forms of nanotechnology, have been under investigation for their utility in advancing molecular diagnostics [33]. Additionally, smaller-scale materials like viruses and proteins show promise as molecular diagnostic tools. Information obtained through nanotechnology-based diagnostics can be stored and manipulated using bioinformatics software [34]. This lays the groundwork for the development of more advanced nanotechnology-based diagnostic procedures, enabling the acquisition of greater proteomic and genomic knowledge, ultimately leading to more effective strategies in combating various diseases. Nanomaterials exhibit excellent potential for enhancing protein tests, contributing to improved diagnostic methods for various medical conditions, including neurological disorders [35].

Protein tests like enzyme-linked immunosorbent assays (ELISAs) a notable example capable of reliably detecting disease-associated antibodies and antigens. Nano-electrospray quadrupole time-of-flight mass spectrometry (TOFMS), has been successfully employed to determine endogenous peptide sequences up to 9 kDa in biological samples [36]. This method, coupled with liquid chromatography, allows for efficient decomplexation, enabling the accurate identification of proteins such as the human 8.6 kDa cerebrospinal fluid (CSF) ubiquitin-protein and the rat 5.0 kDa thymosin beta-4 and 4.3 kDa hypothalamic brain tissue proteins. Atomic force microscopy has provided insights at the single-molecule level, enabling the study of Soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) protein mechanics, including extension, rupture force, interaction energy, and spontaneous dissociation time. The utilization of fluorescent nanoparticles holds promise as an essential diagnostic tool fluorescent dye-doped nanoparticles have proven to be highly effective nanomaterials in protein microarrays and find versatile applications in various bioassay systems, including immunohistochemistry, immunocytochemistry, and fluorescent-linked immunosorbent assays. The combination of antibody-mediated specificity and the high-intensity luminescence of these nanoparticles has enabled ultrasensitive target detection [37-38].

Genetic diagnostics, such as DNA and RNA tests, play a crucial role in acquiring information about the molecular mechanisms of disease [39]. They are essential for treating, diagnosing, predicting the onset of diseases, and identifying carriers of genetic

disorders. Quantum dots, silica, and metal nanoparticles have been tested for their potential as genetic probes [40-41]. Other nanomaterials, including DNA, electrical, and optical biosensors, as well as smaller-scale materials like enzymes and viruses have also been explored as genetic probes [42-46]. These materials hold promise in the field of nanotechnology-based genetic tests for neurological disorders including ASD. Superparamagnetic iron oxide nanoparticles (SPIONs) linked with randomly sequenced oligodeoxynucleotides have been utilized as probes in magnetic resonance imaging (MRI) for tracing cerebral ischemia gene transcripts in living animals to target gene transcripts associated with acute neurological diseases [48] Experiments have demonstrated the effectiveness of fluorescent dye-doped nanoparticles (NPs) compared to quantum dots and other fluorescent labeling agents in detecting DNA and other biological molecules Fluorescent nanomaterials are excellent candidates for use as genetic probes[50] .

4. Neuroimaging and Monitoring

Nanoscale materials could be incorporated into imaging technologies to improve the resolution and sensitivity of brain imaging techniques [51]. This could aid in studying the neural underpinnings of autism and monitoring treatment responses. Neuro-imaging techniques are a preferred class of methods for diagnosing ASD among specialists. In the past decade, numerous investigations into ASD diagnosis have focused on neuroimaging data, including both structural and functional aspects. Analyzing the anatomy and structural connections of brain areas using structural neuroimaging, particularly magnetic resonance imaging (MRI) techniques, has become essential in studying structural brain disorders associated with ASD [52] Structural MRI images are employed to investigate cerebral anatomy, while diffusion tensor imaging MRI assesses anatomical connections [53]. Functional neuroimaging, which examines the activity and functional connections of brain areas is another valuable approach for studying ASD. Electroencephalography recording the electrical activity of the brain with high temporal resolution, has been a fundamental modality for functional neuroimaging in ASD diagnosis [54]. Functional MRI used in task-based (T-fMRI) or resting-state (rs-fMRI) applications, is a promising modality in functional brain disorders [54]. While fMRI offers high spatial resolution, its temporal resolution is limited due to the slow response of the brain's hemodynamic system, and it may not be ideal for capturing fast dynamics of brain activities [56]. Additionally, fMRI techniques are sensitive to motion artifacts. Functional MRI (fMRI) can detect blood oxygen level-dependent (BOLD) changes in MRI signals corresponding to changes in neuronal activity or brain state. External stimuli or tasks can evoke these signal changes, producing images related to the brain's neuronal activity. fMRI provides unique and valuable information for applications in clinical neurosciences, basic research, and translational studies [57]. In addition to EEG and fMRI, less prevalent modalities such as electrocorticography (ECoG), functional near-infrared spectroscopy (fNIRS), and Magnetoencephalography (MEG) have shown reasonable performance in ASD diagnosis [58]. An effective approach involves integrating machine-learning techniques with both functional and structural data to aid physicians in accurately assessing ASD [60].

Diffusion tensor imaging (DTI) is a quantitative MRI method that measures the microscopic movement of water diffusion in the brain. It is highly sensitive in providing information about the microstructural integrity of tissue, as well as the orientation and connectivity of white matter fibers in the brain. This method is widely used to investigate microstructural tissue integrity and structural connectivity in various pathological conditions, including ASD [61-62] Commonly utilized DTI metrics include fractional anisotropy (FA), which quantifies the strength of preferential direction of molecular diffusion of water along white matter tracts, and mean diffusivity (MD), which measures the magnitude of water molecule diffusion in tissue. DTI, when combined with behavioral and cognitive measures, has proven useful in understanding the relationship between the integrity of white matter pathways and the efficiency of cognitive and neural processing during brain development [61].

Various assessment tools, including screening and diagnostic methods, are employed to characterize ASD features from a very young age and measure the severity of ASD [63] . Developmental assessment tools are used to identify differences in various domains of brain development, such as cognition, receptive and expressive language, and gross and fine motor skills, between children with ASD and typically developing children. Several important assessment

tools for ASD are Modified Checklist for Autism in Toddlers-Revised/Follow-up (M-CHAT-R/F): it is a widely utilized screening tool with 20 items and provides information on whether the child is at no risk, medium risk, or high risk for ASD [64].

Childhood Autism Rating Scale (CARS): A behavior rating scale comprising two 15-item rating scales. It includes an unscored parent/caregiver questionnaire to assist in making ASD diagnoses and determining severity [65].

Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R): ADOS is a task-based tool with four modules adapted based on the child's ability. ADOS-2, published in 2012, includes updated norms, improved algorithms, and a new toddler module. ADI-R is a standardized parent interview-based questionnaire. Both tools aid in making ASD diagnoses and planning treatment and when used together, exhibit high accuracy, similar to the gold standard diagnostic procedure [66].

Mullen Scale of Early Learning (MSEL): A developmental assessment tool measuring cognitive development. Comprises fine and gross motor skills, visual reception, expressive and receptive language. The early learning composite score is considered equivalent to a traditional "IQ" score or developmental standard score for young children [67].

Social Communication Questionnaire (SCQ): It is widely used as a screener and is designed as a questionnaire version of the ADI-R [68].

5. Nanoparticles potential as neuroprotectants

Nanoparticles could potentially be designed to protect neural cells from damage, inflammation, or oxidative stress, which are factors associated with certain neurological conditions, including some forms of autism. Metallic nanoparticles, particularly magnetic, silver, and gold nanoparticles, exhibit enzyme-like antioxidant properties that effectively scavenge free radicals, reducing concentrations of reactive oxygen species (ROS) [69]. These metallic nanoparticles hold promise in the treatment and prevention of illnesses associated with excessive ROS production. The field of nanotechnology, combined with materials science, has made significant strides in minimizing free-radical generation during nanoparticle synthesis, resulting in the development of nano-antioxidants [70]. Nano-antioxidants are nonorganic nanoparticles either functionalized with antioxidants or antioxidant enzymes, serving as delivery systems for antioxidants, or possessing intrinsic antioxidant properties. Key antioxidant functionalities include superoxide dismutase, catalase, oxidase, and peroxidase-mimicking activities. The ability of metallic nanoparticles to transition between different oxidation states contributes to their significant antioxidative effects [71]. However, the specific mechanism underlying the antioxidant activity of nanoparticles remains unclear and warrants further exploration. The nano-antioxidant properties of nanoparticles heavily rely on the synthesis method employed, with various techniques such as the solvent displacement method, supercritical fluid technology, emulsion or solvent evaporation, templating technique, and nanoprecipitation technique being utilized. The antioxidant activity of novel metal nanoparticles, including silver, gold, copper oxide, and nickel oxide, has been extensively researched. Enhancing antioxidant activity is achieved by coupling or incorporating various phytochemicals into single or bimetallic combinational nanoparticles. The antioxidant characteristics are influenced by factors such as chemical composition, nature, stability, surface-to-volume ratio, size, surface coating, and surface charge [72]. Certain oxide nanoparticles, owing to their inherent physicochemical properties, can effectively scavenge reactive nitrogen and oxygen species, mimicking antioxidant molecules or antioxidant enzymes [73]. Another mechanism involves nanoparticles quenching free radicals by converting alkyl peroxyl radicals into hydroperoxides [74] Nanoparticle systems present a potentially straightforward and cost-effective avenue for preventing maternal autoantibody-related autism. Various nanoparticles, including those composed of gold, graphene, silica, and iron, have demonstrated versatile applications in targeted drug delivery. Iron oxide nanoparticles in particular, have gained prominence in biomedical applications due to their favorable physical characteristics, such as a high surface area-to-volume ratio and intrinsic magnetism. These attributes make these particles an excellent candidate for clinical applications, including serving as a contrast agent in magnetic resonance imaging and for the separation of environmental contaminants. Additionally, magnetic hyperthermia, involving the non-invasive heating of magnetic nanoparticles with alternating magnetic fields, holds promise for destroying tumor cells and could potentially be applied

to eliminate surface-bound pathological autoantibodies. Iron oxide nanoparticles exhibit other advantageous properties, including biocompatibility, stability, cost-effectiveness, and eco-friendliness [75]. It's worth noting that the Food and Drug Administration (FDA) has approved Iron oxide nanoparticles for use as MRI contrast agents and iron deficiency supplements [75].

6. Nanoparticle-Mediated Gene Therapy:

Exploration of nano-carriers for delivering therapeutic genes to specific brain regions, addressing genetic factors associated with ASD. Precision targeting of dysfunctional genetic pathways using nanotechnology to modulate gene expression and restore normal neurological function. While the application of nanotechnology in the diagnosis and treatment of ASD is a promising avenue, it is essential to address ethical considerations, safety concerns, and regulatory frameworks to ensure the responsible development and implementation of these innovative approaches. Ongoing research in this field has the potential to significantly impact the lives of individuals with ASD and contribute to advancements in neurodevelopmental healthcare. Moreover, gene-editing techniques hold the potential to repress gene expression or rectify mutations. Understanding the complexities of brain function has been significantly aided by emerging genomic engineering tools like transcription activator-like effector nucleases, zinc finger nucleases, and CRISPR-Cas9 [76]. Manipulating the genome offers opportunities to create models for understanding complex neurological disorders such as Autism Spectrum Disorder (ASD). Among these tools, CRISPR-Cas9 is considered the most extensive and effective, offering advantages like a low mutation rate, high target efficiency, and cost-effectiveness [77]. It has enabled the development of models replicating studies, researchers affixed the CRISPR/CAS 9 gene-editing technology to a nanoparticle to disrupt the gene responsible for mGluR5 receptors associated with the neurotransmitter glutamate, a factor implicated in conditions like fragile X syndrome and various forms of autism [77]. ASD's heterogeneous nature makes it challenging to pinpoint its exact cause, whether genetic or environmental. No standard medication has been established for treating ASD, except for a few drugs addressing specific symptoms. In vitro modeling of ASD has been beneficial for understanding the underlying mechanisms, although it has limitations such as high heterogeneity among induced pluripotent stem cell lines. CRISPR-Cas9 helps overcome these limitations by generating isogenic cell lines and enhancing experimental reproducibility. For deeper investigations into ASD pathogenesis, CRISPR enables successful genome editing in animals to create validated knockout (KO) and knock-in models. These models exhibit phenotypes related to ASD-associated genes, aiding in determining the condition's etiology and screening drugs to restore altered phenotypes. While CRISPR-Cas9 is not limited to modeling ASD, it has been instrumental in targeting and correcting mutated genes [77].

Despite these advancements, CRISPR-Cas9 has limitations, including off-target effects, delivery methods, immunogenicity, and associated risks, making its use challenging in clinical trials. Off-target effects, causing mutations in unintended genomic locations, pose a significant concern. Bioinformatics tools have emerged to mitigate these effects by predicting off-target modifications. Immunogenicity is another issue, as the introduction of Cas9 from Streptococcus pyogenes may trigger an immune response. Safety concerns include DNA double-strand breaks induced by CRISPR, which can lead to apoptosis and unnecessary deletions and rearrangements of sequences.

Considering these challenges, gene therapy using gene editing techniques remains a distant therapeutic approach. While studies using genomic editing tools have made progress in cultured cells or animal models, their application to clinical treatment for ASD requires cautious consideration, and the translation of preclinical study results to patient care demands careful attention [78].

7. Conclusion

Nanoparticles are being investigated for their potential role as neuroprotective agents, aiming to safeguard the nervous system, particularly neurons, from damage in various neurological disorders or injuries. Nanoparticles offer several benefits, including targeted drug delivery, the ability to cross the blood-brain barrier, inherent antioxidant and anti-inflammatory properties, and support for neuroregeneration. Additionally, nanoparticles can be employed for imaging and diagnostic purposes, aiding in early detection and monitoring of neurological

diseases. Despite these promising attributes, thorough research is ongoing to address safety concerns and optimize their effectiveness before widespread clinical implementation. It's important to note that while these possibilities are intriguing, the application of nanotechnology in autism is still an area of active research, and concrete therapeutic solutions have not yet been widely implemented. The ethical and safety considerations of nanotechnology in medical applications are also critical aspects that need careful examination.

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