Brain and Quantum Dots: Benefits of Nanotechnology for Healthy and Diseased Brain

Yuri N. Utkin*

Laboratory of Molecular Toxinology, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia; National University of Science and Technology MISIS, 119049 Moscow, Russia

Abstract: Introduction: The brain is the most complicated organ in a vertebrate's organism. In a human, it contains about two hundred billions of neurons and non-neuronal cells. To understand the mechanisms of the brain functions is the great challenge for the researchers. Much is already done on this way; however, it remains a lot to do still, and to get deeper knowledge, new approaches should be developed. One of this is to use benefits that nanotechnology brings in this area. Nanotechnology opens up unique opportunities, not only for material science research, but also for biology, medicine, and many other disciplines. There are several kinds of nanoparticles that can be applied in brain studies, Quantum Dots (QD) being so far most often used. QD are semiconductor light emitting nanocrystals with nanometer-sized structures of unique optical properties. They have bright fluorescence, are resistant to bleaching and able of emitting fluorescent light of different wavelengths. These properties make QD perfect tools for visualization of brain structures and mechanisms underlying its functions. Due to unique QD properties, even single molecules under study can be observed. Moreover QDs can be used for brain-targeted drug delivery.

Conclusion: In this review, the application of quantum dots for the brain research is considered and benefits that it can bring are discussed.

Keywords: Brain, conjugates, drugs, fluorescence, quantum dots, visualization.

1. INTRODUCTION

Nanotechnology is a modern and rapidly developing technology, which has found application in many spheres of human activity. It handles materials possessing nanometer size in at least one dimension. Nanomaterials cover a heterogeneous range of materials and include inorganic non-metallic nanomaterials (e.g., synthetic amorphous silica, aluminium oxide, titanium dioxide, cadmium selenide), carbon based nanomaterials (e.g., carbon dots, carbon nanotubes), metal nanoparticles (e.g., nanosilver) and organic, macromolecular or polymeric particulate materials (e.g., dendrimers) [1]. The reduction in size gives new properties to ordinary bulk materials, making nanomaterials very promising for novel applications in various fields. Thus, they have been applied for magnetic and fluorescent bioimaging, as carriers for drugs, and as drugs themselves. Among the great number of different nanoparticles, semiconductor nanocrystals or so-called Quantum Dots (QDs) occupy a special place. QDs fluorescence when excited with a light source. They possess excellent optical properties, including high brightness and resistance to photobleaching. These properties can be utilized for visualization of complex biological processes occurring in the organism.

In a vertebrate's organism, the most complex processes take place in the brain and the brain is the most complicated organ. In a human, it contains about two hundred billions of neurons and non-neuronal cells [2]. To understand the mechanisms of the brain functions is the great challenge for modern science. Much is already done in this way; however, it remains a lot to be done. To get deeper knowledge, new approaches should be developed. Using the advantages of QDs can significantly help in addressing this problem. Due to their unique optical properties, QDs have been widely used in neuroscience, including basic neurological studies and diagnosis or therapy for neurological disorders.

It should be mentioned that the brain is protected by the Blood-Brain Barrier (BBB), and to penetrate into the brain, any material should overcome this barrier. One of the QD applications is their use to enhance and to monitor the drug delivery to brain. QDs are efficient fluorescent probes and nanovectors, which can be utilized to transverse across the BBB and visualize drug delivery inside the brain [3]. With the aid of QDs of high specificity and multifunctionality, therapeutics, imaging agents and diagnostic molecules can be delivered to the brain across BBB, enabling considerable
progress in the understanding, diagnosis, and treatment of brain diseases [4].

QDs either alone or conjugated with biomolecules can accumulate in the brain and visualize different structures ranging from brain vasculature [5] to single receptor molecule [6]. In addition, such tools will lead to a more complete understanding of the mechanism of tumor development and help in the elaboration of new methods for their treatment. So, for surgical removal of brain tumors, it is necessary to accurately determine the boundaries of tumor tissue in the brain. Fluorescent nanoparticles targeted to tumors can be detected using optical imaging, thus allowing the surgeon to see the tumor in real time during resection and biopsy of brain tumors [7, 8].

To improve the detection and to diminish QD toxicity, several novel types of QDs were recently developed. This especially concerns Near-Infrared (NIR) fluorescent imaging which is a powerful tool for the non-invasive visualization. Several types of advanced NIR emitting QDs have been recently designed [9, 10]. Nitrogen-doped carbon dots, which exhibited a negligible cytotoxicity and could be applied as efficient nanoprobe for real-time imaging of live cells were synthesized [11]. Despite all the advantages of QDs there have been great concerns regarding their toxicity in living organisms and the toxicity for neural cells in particular [12, 13]. These effects still are not well studied and require further exploration.

In this review, the application of QDs in neuroscience, including basic neurological studies and diagnosis or therapy for neurological disorders is considered, and the recent advances that QDs contribute to the development in research of the brain function and the treatment of diseased brain are summarized. It should be mentioned that this is a very challenging and fast expanding area; therefore, new important data might appear during the publication of this review.

2. QUANTUM DOTS PENETRATION THROUGH BRAIN-BLOOD BARRIER

The QDs, modern versatile tools for in vivo molecular and cellular imaging, have also been explored as therapeutic carriers to deliver drugs to different organs. The brain is a difficult organ for drug delivery, because the BBB functions as a complex physiological interface that restricts the free diffusion of circulating molecules from the blood into the Central Nervous System (CNS). Delivery of imaging agents and drugs to the brain is highly important for the diagnosis and treatment of CNS diseases, as well as the understanding of their pathophysiology. Therefore, development of novel methodologies to address this challenge is crucial for both the diagnosis and treatment of brain diseases.

To transverse across the BBB, QDs can be utilized in several ways. In general, to penetrate the BBB, QDs should be conjugated with some molecules enhancing their barrier crossing capacity. However, there are some examples when fluorescent nanoparticles pass BBB without conjugation; for example, these are so called carbon Dots (cQDs). With a number of advantages over traditional semiconductor QDs, cQDs have become more widespread recently [14]. Thus, carbon dots (cQD-Asp) synthesized via pyrolysis of D-glucose and L-aspartic acid exhibit significant capability of targeting C6 glioma cells within the brain without the aid of any extra targeting molecules [15]. In vivo fluorescence images showed a much stronger fluorescent signal of cQD-Asp in the glioma site than that in normal brain 15 min after tail vein injection. This data indicates that cQD-Asp are able to freely penetrate the BBB and precisely target glioma tissue. Other example is nitrogen-doped cQDs which were synthesized using a one-pot hydrothermal treatment of citric acid in the presence of polyethyleneimine [11]. The nitrogen-doped cQDs demonstrated an insignificant cytotoxicity and the good ability to cross BBB in a concentration-dependent manner on an in vitro BBB model. Recently, it has been shown [16] that because of the small size graphene quantum dots possessing low cytotoxicity and great biocompatibility may cross the BBB.

In contrast to these few examples to penetrate the BBB, traditional semiconductor QDs have to be combined with biocompatible stuffs targeting their delivery to the brain. These stuffs differ in chemical nature ranging from low molecular peptides to high molecular weight polymers. It was reported that ultra-small (3.1 nm) multifunctional CdS: Mn/ZnS core-shell semiconductor QDs conjugated with a cell penetrating peptide TAT were able to cross without manipulating the BBB, migrated beyond the endothelial cell line and reach the brain parenchyma following intra-arterial administration [17, 18]. QDs without TAT did not label the brain tissue confirming the fact that TAT peptide was necessary to overcome the BBB. In other study [19], tripeptide asparagyl-glycyl-arginine (NGR) specifically targeting the enzyme aminopeptidase N (also known as CD13) which is associated with the growth of different human cancers was used to synthesize a novel nanoprobe. Biotinylated NGR peptide was conjugated to avidin-polyethylene glycol (PEG)-coated CdSe/ZnS QDs, which at non-toxic concentrations could cross the BBB and target CD13-overexpressing glioma and tumour vasculature in vitro and in vivo.

In several works Transferrin (Tf) or Transferrin Receptor (TfR) were used to deliver QDs to the brain. TfR transports iron to each cell that needs it. It is a transmembrane protein highly expressed on brain endothelial cells and has been proposed to undergo transcytosis at the BBB to allow entry of iron-bound Tf by constitutive endocytosis. To cross the BBB, QDs were conjugated with monoclonal antibodies Ri7 targeting the murine TfR [20]. After intravenous injection in mice, Ri7-QDs exhibited a fourfold higher volume of distribution in brain tissues as compared to controls. The Ri7-QDs were massively internalized by brain capillary endothelial cells and most QDs within brain capillary endothelial cells were observed in small vesicles, with a smaller proportion detected in tubular structures or in multivesicular bodies. At the same time parenchymal penetration of Ri7-QDs was extremely low and comparable to control immunoglobulin.

To investigate crossing the BBB with Tf conjugated carbon QDs, Li and coworkers [21] employed zebrafish as a model animal. Easy culturing conditions of this species, transparent body and fast formation of the BBB make it very good biological model for studying QD penetration through the BBB using TfR mediated endocytosis. To examine if
QDs alone or the QDs conjugated with Tf could cross the BBB. QDs and the conjugate were intravascularly injected into the zebrafish heart. Fluorescence spectroscopy revealed the QD conjugate in the CNS, which suggested that QDs could cross the BBB by TR-mediated endocytosis. In contrast, the QDs alone were not able to penetrate through BBB [21].

To enhance the delivery of QDs into the brain, Gao and coworkers [22] designed a new imaging platform, in which QDs were encapsulated into PEG-poly(lactic acid) nanoparticles functionalized with wheat germ agglutinin. The resulting nanoparticles, with high payload capacity were delivered into the brain via nasal application and showed excellent and safe brain targeting and imaging properties.

The matrix-degrading metalloproteinase MMP-9 is involved in the neuroinflammation processes leading to disrupting of BBB. Recently, QDs complexed with MMP-9-siRNA (nanoplex) were used for downregulating the expression of MMP-9 gene in Brain Microvascular Endothelial Cells (BMVEC) that constitute the BBB [23]. The data obtained by different methods demonstrated the significant decrease in MMP-9 expression in BMVECs mediated by nanoplex. Thus QDs act as non-viral gene delivery vectors and have great potential for therapeutic applications.

The cQDs with high quantum yield (51%) and a size of around 2.6 nm were prepared by hydrothermal synthesis using citric acid and polyethyleneimine as the carbon precursor and as the surface passivation agent, respectively [11]. The cQDs showed negligible cytotoxicity for 293T cells. To study the BBB-penetration ability of cQDs, a biomimetic BBB model consisting of rat BMVEC and astrocytes were employed. The measurement of the fluorescence intensity of cQDs accumulated in the lower chamber of a BBB-biomimetic transwell indicated a concentration and time-dependent manner in crossing the BBB by cQDs, which was explained by their small size and cationic polyethyleneimine on the surface.

The data discussed above show that the QDs can be selectively delivered to the brain. However, in some cases the BBB penetration by QDs is not desirable effects. Thus it was shown [24] that captopril-conjugated QDs following intraperitoneal injection into male ICR mice as a model system were delivered via systemic blood circulation into liver, spleen, kidney and brain at 6 h after injection and were located predominantly inside the blood vessels in these organs. Only a few were distributed in the parenchyma, especially noteworthy in the brain. These results suggest that careful studies on acute as well as chronic toxicity of QDs in the brain are required prior to their clinical application.

3. VISUALIZATION OF BRAIN STRUCTURES WITH QUANTUM DOTS

The extremely complex structure of the brain and the great variety of the processes ongoing there require application of special investigation methods that produce minimal influence on the object; i.e. noninvasive imaging techniques are needed. Such approach is granted by fluorescent imaging in vivo. Application of QDs allows to greatly extend the opportunities for this method. The main advantages of QDs are high absorption cross-sections and quantum yields (over 50%), detection down to the single nanoparticle level, large excitation/emission Stokes shift (up to 300 nm), the possibility for time resolved detection of fluorescence, extreme photostability, and narrow, tunable emission bands across the visible spectrum. Their application makes possible visualization of complex structures, cells, and single molecules at significantly greater depth in vivo, as compared to that achieved by organic fluorophores.

3.1. VISUALIZATION OF BRAIN TISSUES

The imaging of the brain’s complex organization and interconnections is crucial for understanding its functions in healthy organisms and in neurodegenerative diseases. Optical imaging methods are fast and provide good resolution. For example, exogenous dyes (sodium fluorescein and Evans blue) are injected intravenously for observation of blood vessels. They act as tracers and contrast the blood vessels against the surrounding tissues. QDs have a good potential to enhance the capability of fluorescent imaging in vivo. Application of NIR emitting QDs may even more substantially increase their power for the non-invasive visualization of the inner brain structures.

Thus, lead sulfide (PbS) QDs were used for in vivo NIR fluorescence imaging of cerebral venous thrombosis in septic mice [10]. PbS QDs have a 1100 nm emission peak (second optical window) which offers better spatial resolution as compared to conventional NIR fluorescence imaging at 700-900 nm (first optical window). To make these QDs soluble in water, they were coated with mercaptoundecanoic acid and injected into a caudal vein of living mice. Non-invasive NIR fluorescence imaging of cerebral blood vessels through the scalp and skull was achieved by this way. NIR fluorescence imaging of cerebral venous thrombosis in septic mice induced by the administration of lipopolysaccharide was also performed. It was found that the number of thrombi significantly decreased after the administration of heparin, an inhibitor of blood coagulation. These results show that NIR fluorescence imaging with PbS QDs may be used for the observation of cerebral blood vessels and the evaluation of their pathological state.

It should be kept in mind that the composition and structure of organic coating of nanoparticles are crucial for their application in imaging in vivo [25]. Thus, encapsulation of QDs in crosslinked dendrimers resulted in low cytotoxicity and increased biocompatibility. These nanoparticles did not affect blood pressure and heart rate, and did not induce vasoconstriction to vasoconstriction. The conjugation of silica-shelled QDs with PEG 1100 increased their stability and half-life in the circulation without significant enhancement of their size. Using these QDs, it was possible to visualize capillaries, which makes them appropriate probe for investigation of vasoconstriction, vasodilatation, and brain circulation in intact animals in vivo [25].

To achieve more controlled delivery of QDs to the brain, special tags are used. So, CdS:Mn/ZnS QDs were conjugated with a cell penetrating peptide TAT [17]. The conjugate was rapidly delivered to rat brain, migrated beyond the endothelial cell line and reached the brain parenchyma. Fluorescent visualization of the whole rat brain was possible using a low
power hand-held UV lamp. QDs without TAT did not label the brain tissue.

Even within brain QDs can be selectively delivered to different cell types. So, depending on the QD coating cell-membrane-penetrating chaperone lipopeptide JB577 (WGdapol(Palmitoyl)VKIKKP9GGH6) can be delivered to individual cells in neonatal rat hippocampal slices [26]. The preferential uptake in neurons and the lack of uptake in glia is strongly associated with having a region of greater negative charge on the QD coating. Moreover, a positively charged PEG coating promoted the uptake in oligodendrocytes.

Agarwal and coworkers [27] conjugated CdSe/ZnS QDs with the palmitolyated peptide WGdapol(Palmitoyl)VKIKKP9GGH6, previously shown to uniquely facilitate endosomal discharge. Conjugated QDs were microinjected into the embryonic chick spinal cord canal at embryo day 4 (E4). The labeling of spinal cord extension into the ventricles, migratory neuroblasts, maturing brain cells, the choroid plexus was subsequently observed. QD intensity extended throughout the brain, and peaked between E8 and E11 when fluorescence was concentrated in the choroid plexus before declining to hatching (E21/P0) [27]. These findings indicated that QDs can be used to identify and track neural stem cells as they migrate, and that QDs can deliver drugs and peptides to the developing brain.

To validate the therapeutic benefits of cell transplantation therapy for central nervous system disorders, NIR-emitting QDs were used to visualize the Bone Marrow Stromal Cells (BMSC) transplanted into the infarct brain in rats [28]. Rat BMSCs were labeled with QDs and stereotactically transplanted into the ipsilateral striatum of the rats subjected to permanent middle cerebral artery occlusion 7 days after the insult. Using the fluorescence imaging technique, NIR fluorescence emitted from the transplanted BMSCs engrafted in the peri-infarct neocortex through the scalp was detected up to 8 weeks after transplantation. These results suggest that NIR fluorescence imaging can be used to track the BMSCs transplanted into the brain.

Zhang and coworkers [29] have recently identified an antibody (scFvA) that could target an endocytosing BBB receptor in a rat brain endothelial cell line RBE4. A biotinylated scFvA was used to create scFv tetramers and to target streptavidin-coated QDs for BBB endothelial cell internalization. The scFvA-QDs clearly immunolabeled brain capillaries in rat brain tissue sections. These promising results indicate the potential application of scFvA for delivery of therapeutic molecules or particles to brain endothelial cells.

Recently basing on an indium arsenide QD cores, improved core-shell-shell QDs that span the entire sensitivity range of modern short-wavelength infrared cameras (900-1,600 nm), exhibit quantum yields up to 82%, and show drastically improved probe stability were developed [5]. The longer imaging wavelengths promise increased spatiotemporal resolution, penetration depths and unprecedented sensitivity. To ensure colloidal stability and brightness, QDs were incorporated into PEG-phospholipid micelles. The micelle solution was injected into an anesthetized mouse (C57BL/6J) via the tail vein and the vasculature of the mouse brain was imaged through the intact skin and skull using diffuse 808 nm excitation. The images were acquired at different wavelength: it was found that the longer imaging wavelengths lead to enhanced resolution of the vascular structure of the mouse brain and improved image contrast.

3.2. Visualization of Single Molecules

Unique properties of QDs have made it possible to visualize individual molecules. Thus, they have been used to track the receptors and ion channels in neuronal synapses. However, QD conjugates with ligand or antibody to the targeted protein should be prepared for this purpose.

The lateral mobility of individual nicotinic acetylcholine receptors of a7 type in live rat cultured hippocampal interneurons was monitored by single particle tracking [30]. The specific receptor marker α-bungarotoxin linked to QDs was used for labeling. It was found that the lateral diffusion of receptors was dependent on their subcellular localization and their cell surface dynamics was modulated by changes in neuronal activity.

A specific antibody against an extracellular epitope of ether-a-go-go-1 (Eag1) ion channels conjugated to QDs was used to monitor lateral mobility of the channels [31]. State of the art single-particle-tracking techniques was applied to demonstrate that Eag1 (Kv10.1) rapidly entered and exited synapses by laterally diffusing in the plasma membrane of cultured rat hippocampal neurons. It was also shown that Eag1 channels exhibited Brownian diffusion extrasynaptically, but got transiently trapped when they diffused inside synapses. Furthermore, the mobility of Eag1 channels was highly regulated specifically inside synapses by actin filaments, microtubules and electrical activity [31]. These data suggest an important role for the mobility of voltage-gated ion channels in synaptic activity.

This approach was further developed and a fluorescence hyperspectral imaging platform to simultaneously track different subtypes of individual neurotransmitter receptors trafficking in and out of synapses was designed [32]. A simultaneous image acquisition of at least five fluorescent markers in living neurons with a high-spatial resolution was made possible with this platform. QDs emitting at different wavelengths and functionalized to specifically bind to single receptors were used. Five different synaptic proteins, including subtypes of glutamate receptors (mGluR and AMPAR) and postsynaptic signaling proteins were simultaneously monitored. The platform permitted the quantification of their mobility after treatments with various pharmacological agents. The proposed method may accelerate the screening of effective compounds for treatment of CNS disorders [32].

The major glutamate transporter for clearing synaptic glutamate is the astrocytic protein GLT-1. QD labeled antibodies were used to study the surface trafficking of this transporter in subcellular domains of the astrocyte membrane [33]. Single particle tracking using QDs revealed that clustered GLT-1 was more stable than diffuse GLT-1 and that glutamate increased GLT-1 surface diffusion. GLT-1 surface mobility increased in proximity to activated synapses and alterations of neuronal activity modulated the dynamics of the transporter. Authors suggested that astrocytic GLT-1 surface mobility was modulated during neuronal firing, be-
ing a key process for shaping glutamate clearance and glutamatergic synaptic transmission [33].

Confocal laser scanning microscopy in combination with QDs of different sizes was applied to obtain three-dimensional images of the subcellular localization of pituitary hormones and their mRNAs in a pituitary cell [34]. QDs 655 conjugated with anti-rabbit antibodies and QDs 605 conjugated with streptavidin were used for staining of hormones and mRNA, respectively. It was found that Growth Hormone (GH) was more abundant than Prolactin (PRL), and GH was localized in the vicinity of its mRNA, whereas PRL was less associated with PRL mRNA. These data suggest more rapid transportation of PRL to the plasma membrane and secretion as compared to GH. Thus, the application of QDs facilitated the visualization of the processes of transcription, translation, transport, and secretion of pituitary hormones [34].

Brain-Derived Neurotrophic Factor (BDNF) is known to modulate synapse development and plasticity. Endogenously synthesized BDNF is normally stored and transported in dense core vesicles and secreted at synapses in response to activity. To study intracellular trafficking dynamics and molecular mechanisms regulating BDNF release, its conjugates with QDs were used. QD-BDNF conjugates were prepared by high-affinity binding of biotinylated BDNF to streptavidin-QDs [35]. It was found that QD-BDNF bound to TrkB receptors with high specificity, activated TrkB downstream signaling, and allowed single QD tracking capability for long recording durations deep within the soma of live neurons. Real time measurements demonstrated that individual QD-BDNF complexes underwent internalization, recycling, and intracellular transport. These trafficking showed little synchronicity and instead possessed widely heterogeneous dynamics, including extended durations of sustained rapid transport as well as immobility (30-120 s). Moreover, the path trajectories of individual BDNF complexes showed no apparent end destination. This diversity of BDNF trafficking dynamics contrasted previously reported linear axonal transport data and called for models that surpass generally limited concepts of primarily nuclear-directed transport in the soma [35].

Using a microfluidic chamber, the axonal transport of QD-BDNF was investigated in primary rat E18 hippocampal neurons with single molecule sensitivity, in real-time and with spatial and temporal resolutions [36]. The QD brightness and excellent photostability makes it possible to perform long-term tracking of BDNF transport. It was found that QD-BDNF moved essentially retrogradely, at a moving velocity of around 1.06 μm/sec. However, some molecules travelled anterogradely and those moving in both directions paused during the movement. Stationary QD-BDNFs not moving during 100 sec were also observed. This finding supports the observation of Vermehren-Schmaedick and coworkers [35].

Secreted BDNF may be endocytosed by neurons and transported within neuronal cytoplasm in the form of endosomes. Fluorescence imaging showed that the endocytosed BDNF-QDs were preferentially localized to postsynaptic sites in the dendrite of cultured hippocampal neurons and could be released in response to synaptic activity [37]. This synaptic release of endocytic BDNF requires a synaptotagmin isoform distinct from that, which regulates the secretion of dense core vesicles, and may serve as a source for activity-dependent secretion of synaptic BDNF.

The up-regulation of Epidermal Growth Factor Receptor (EGFR) during tumorigenesis makes it a promising therapeutic target. In the absence of the EGF, propranolol treatment leads to internalization of empty/inactive receptors. To understand the molecular events involved in this endocytosis, the effects of propranolol on the mobility of single quantum-dot labeled EGFRs were studied [38]. For this purpose, the isolated Fab fragments of the anti-EGFR antibody was coupled to biotin and then conjugated with streptavidin-QDs. Using total internal reflection fluorescence microscopy, it was observed that the single receptors showed a clear stop-and-go motion; their diffusive tracks were continuously interrupted by subsecond stalling events. The presence of propranolol resulted in a significant reduction of the diffusion rate, however no increase of the effective membrane tension was found by atomic force microscopy suggesting that clustering of the receptor was the likely mechanism for its reduced mobility. The propranolol eventually induced stalling of the receptor for multiple seconds, which may signal the first step of the internalization process [38].

4. APPLICATION OF QUANTUM DOTS FOR THE TREATMENT OF BRAIN DISORDERS
4.1. Visualization of Brain Tumors

As discussed above, QDs allowed visualization of tiny mechanisms underlying the brain functions, however they also substantially enhanced the identification of neoplastic tissue within normal brain during biopsy and tumor resection. The most prevalent brain tumor responsible for about 60% of brain tumors is glioma, originating from various glial cells. The glioma diagnostics is usually based on the data of magnetic resonance tomography and morphological examination of tumor tissues. The common methods for glioma treatment are surgical removal, radiation therapy, stereotactic radiosurgery and chemotherapy. However, very often it is difficult to identify tumor margins during brain surgery due to its inherent infiltrative character. Staining of the tumors with brightly fluorescent QDs may greatly enhance their detection and resection.

To stain the gliomas, the QDs conjugated with proteins that specifically target the tumor are usually used. Thus, it has been found that EGFR expression is upregulated in great number of gliomas. QD-labeled antibodies were used for visualization of EGFR expression in human brain tumor cells and in surgical frozen section slides of glioma tissue [39]. Streptavidin-coated QDs were conjugated to anti-EGFR antibodies and human glioma tumor cell lines with elevated levels of EGFR expression (SKMG-3, U87) as well as frozen tissue sections of glioblastoma multiforme and of oligodendroglioma were stained with these conjugates. The conjugated QDs were bound selectively to brain tumor cells and tumor specimens expressing EGFR [39]. In other study QDs were coupled to EGF and/or monoclonal antibodies against EGFR [40]. For labeling experiments, glioma cell lines, mouse orthotopic tumors and ex vivo human biopsy material were used. It was observed that conjugated QDs produced highly specific labeling of all malignant samples including
native human glioma biopsies that could be distinguished from normal brain tissue down to the single cell level. It was possible to identify even low-grade glioma biopsies [40].

The constitutively active mutant of the EGF receptor EGFRvIII is identified in a high percentage of brain cancers. NIR QDs (QD800) were conjugated to an anti-EGFRvIII single domain antibody, made of the variable region with an extra cysteine for site-specific conjugation (QD800-EG2-Cys) [41]. This conjugate was internalized more strongly in U87MG-EGFRvIII cells in vitro as compared to QD800 conjugated with the Fc region of the antibody or unconjugated QDs. The contrast in NIR imaging of mice bearing orthotopic glioblastoma was improved by QD800-EG2-Cys application. Its increased accumulation was confirmed by fluorescence microscopy of brain sections [41].

To detect cytosolic EFRG, QDs were functionalized with a monoclonal biotinylated antibody which specifically recognized an intracellular epitope of EFRG. To enable targeted cytosolic delivery of these QDs into human brain tumor-derived cells, Sendai virus-based liposomes were utilized [42]. The Sendai virus was safely used for over two decades to deliver molecules into numerous cell types. Recent studies have demonstrated the significance of intracellular markers for identification of neoplastic stem-like populations [43]. The intracellular delivery of QDs by chimeric fusions between the Sendai virus and cationic liposomes incorporating QD conjugates with antibody was studied on medulloblastoma and glioblastoma cell lines using fluorescence microscopy and transmission electron microscopy. It was found that these virus-based liposomes decreased the amount of non-specifically endocytosed nanoparticles by 50% in both cell lines studied. The more important is that the targeted binding of QDs to cytosolic EGFR within cultured cells has been significantly improved. This is essential for the early detection and characterization of malignant brain tumors [42].

To target QDs to glioma, aptamer 32 (A32) was conjugated to the QDs surface [44]. A32 is a single-stranded DNA capable of binding to the EGFRvIII specifically expressed in glioma cells. The conjugate obtained was nontoxic in vivo and in vitro. The investigation of labelling glioma cell lines and human brain glioma tissues showed that conjugate specifically bound to the U87-EGFRvIII glioma cells and human glioma tissues in vitro. In experiments in vivo, QD-A32 could penetrate the BBB in orthotopic glioma model mice bearing U87-EGFRvIII and selectively accumulated in the tumors, generating a strong fluorescence. Thus, the margins of gliomas were visualized clearly [44].

As discussed above TfRs are overexpressed in various cancers. To obtain information about TfRs internalization, quantification and distribution on cell surface, QDs were conjugated to Tf by covalent coupling and obtained QDs-Tf conjugates were applied to quantify and evaluate the distribution of TfRs in two human glioblastoma cells lines, U87 and DBTRG-05MG, as well as in HeLa cells by using flow cytometry and confocal microscopy [8]. It was found that the cells were labeled by QDs-Tf with high specificity. HeLa and DBTRG-05MG cells showed practically the same TfR labeling profile by QDs-Tf, while U87 cells were labeled less intensively. DBTRG-05MG cells were more efficient in recycling the TfR than the other two cell types [8].

To maximize the accuracy of glioma surgical resection, a dual-targeting nanoprobe capable to cross the BBB, target the glioblastoma, and then function as a simultaneous magnetic resonance/NIR upconversion luminescence bimodal imaging agent was developed [45]. Upconversion nanoparticles produce high energy visible radiations from low energy NIR radiation via multiphoton absorption. To prepare the bimodal imaging Gd-doped upconversion nanoparticles, angiopet-2 (ANG, TFFYGGSRGRNFKTEEY) was used as a dual-targeting ligand able to bind specifically to the low density lipoprotein receptor related protein, which is overexpressed on both BBB and glioblastoma cells. Cell line and experimental animal studies proved that these nanoparticles could traverse through the BBB by receptor-mediated transcytosis and achieve glioblastoma cell targeting efficiently. These probes showed a great potential in preoperative diagnosing and intraoperative positioning the brain tumors by magnetic resonance and NIR upconversion luminescence imaging. They had a good biocompatibility, manifesting a negligible in vivo toxicity.

To target the brain tumor effectively, tumor-homing NGR motif was used [19]. This peptide binds type II transmembrane glycoprotein CD13 upregulated in many solid tumors and promoting tumor adhesion, invasion, migration, chemoresistance and angiogenesis. Biotinylated NIR peptide was conjugated to avidin-PEG-coated CdSe/ZnS QDs producing nanoparticles of less than 100 nm in size and stable over pH range from 4.0 to 8.0. These nanomaterials were able to cross the BBB and target CD13-overexpressing glioma and tumor vasculature in vitro and in vivo, contributing to fluorescence imaging of this brain malignancy and facilitating glioma diagnosis and excision under an operative fluorescence microscope [19].

Several studies have shown that even non-conjugated QDs can be used for identification and visualization of tumors. Thus, a variety of nanoparticles, including QDs, is phagocytized by macrophages in vivo and may be used as an optical aid in the surgical resection or biopsy of brain tumors. To prove this hypothesis, male Fisher rats were implanted intracranially with C6 gliosarcoma cell lines to establish tumors [46]. After tumor development, QDs were injected via the tail vein, and the tissues of sacrificed animal were examined 24 hours after the injection of QDs. It was found that at low doses, the majority of QDs are sequestered in the liver, spleen, and lymph nodes; at higher doses, increasing quantities of quantum dots are noted within the experimental brain tumors. Under excitation with blue or ultraviolet light, a deep red QD fluorescence detectable with charge-coupled device cameras, optical spectroscopy units, and in dark-field fluorescence microscopy was observed. Macrophages and microglia carrying QDs colocalized with glioma cells and thereby optically outlined the tumor [46].

To make brain tumor detection more versatile, a dual-modality nanoprobe (Gd-Ag2S) was designed, which integrated sophisticated chelating reagent loaded with Gd for magnetic resonance imaging and Ag2S QDs emitting with high intensity in the second NIR window [47]. In a mouse model, after intravenous injection of Gd-Ag2S nanoprobe a brain tumor (U87MG) was outlined by Gd-based magnetic resonance imaging. Assisted with Ag2S QD NIR fluoro-
cence imaging, the chirurgical resection of the tumor was precisely accomplished [47]. The high biocompatibility of the nanoprobe was confirmed by the absence of histologic changes in the main organs of the mouse for 1 month after administration of Gd-Ag2S nanoprobe.

Recently an indium-arsenide-based QDs emitting in the short-wavelength infrared region (1000–2000 nm) and providing general lack of autofluorescence, low light absorption by blood and tissue, as well as reduced scattering were introduced [48]. These QDs bearing CdS or CdS/ZnS shell exhibit narrow and size-tunable emission and a dramatically higher emission quantum yield than previously described probes. The surface of QDs was functionalized to produce QD nanosomes, QD phospholipid or QD composite particles; the latter were used to perform angiography in the brain of a mouse, directly identifying arteries and veins. Moreover, QD composite particles allowed quantifying blood flow in the vasculature of the brain by tracking individual particles during intravital microscopy, which enabled the visualization of the dramatic differences between blood flow in healthy vasculature and in vessels at the tumor margin [48].

Being more biocompatible as compared to metal based QDs, cQDs were used for brain imaging as well. Through a pyrolysis of L-aspartic acid and D-glucose, cCDs were synthesized as a self-targeting material for non-invasive diagnosis of brain cancer cells [14]. The cQDs thus obtained efficiently reached the glioma after injection from the tail veins of glioma-bearing mice, while cQDs synthesized from D-glucose, L-aspartic acid alone or D-glucose and L-glutamic acid have no or low selectivity for glioma. The authors suggested that cQDs obtained from L-aspartic acid and D-glucose crossed the BBB with the assistance of transport proteins such as glucose transporter GLUT1 and glutamine transporter ACST2 via carrier-mediated transport [14].

In recent years, sorcin toxin chlorotoxin (CTX) found in the Israeli Leirusquinquestris’s venom has been explored as candidate for glioma diagnosis and therapy [49]. CTX binds preferentially to a matrix metalloproteinase-2 (MMP-2) receptor-associated chloride channel and a glioma-specific chloride channel. Upon binding of CTX, the MMP-2 complex and glioma-specific chloride channel are internalized into the cell. This phenomenon can be used for imaging and therapy of gliomas [50].

A novel magnetofluorescent nanoprobe combining CTX with near-infrared fluorophore and iron oxide particles coated with a biocompatible PEG-modified chitosan was developed [51]. This nanoprobe was able to cross the blood-brain barrier, capable of mainly targeting brain tumor cells, showed persistent contrast enhancement for 5 days and revealed no toxic properties. It has good prospects for application in preoperative diagnostics, tumor resection, as well as postoperative assessment with either magnetic resonance or optical imaging.

The upconversion nanoprobe based on PEG-coated hexagonal-phase NaYF(4):Yb, Er/Ce nanoparticles were prepared and conjugated with recombinant CTX [52]. The probe displayed good biocompatibility in cell and animal toxicity studies. After intravenous injection, the nanoparticles were visualized by laser scanning upconversion fluorescence microscopy. The fluorescence imaging of xenograft glioma tumors in Balb-c nude mice in vivo and ex vivo demonstrated highly specific tumor binding and direct tumor visualization with bright red fluorescence under 980 nm near-infrared irradiation.

Cadmium-free silver-indium-sulfide (Ag-In-S or AIS) QDs and their core-shell structures (AIS/ZnS QDs) were prepared using thermal decomposition and loaded into the core of PLGA-PEG (5kDa:5kDa) based micelles to form the AIS/ZnS QD-micelles [53]. The QD-micelles were conjugated with CTX, a ligand that specifically binds to U-87 brain tumor cells. Cellular imaging studies showed that the QD-micelles conjugated with CTX were specifically internalized into the brain tumor cells.

The data presented above show how the application of QDs may facilitate the use of tumor-targeted fluorescence imaging for the diagnosis, surgical resection, and postoperative examination of glioma.

4.2. Targeted Delivery of Drugs to Brain

Emergence of nanomaterials substantially affected the development of brain-specific drug delivery. Application of nanocarrier systems of different chemical nature and functionality allowed efficient delivery of therapeutics to the brain across the BBB. Bioconjugated QDs being excellent fluorescent probes and nanovectors have been explored as therapeutic carriers to transverse across the BBB and visualize drug delivery inside the brain.

Thus, QDs were used to deliver siRNA for silencing the gene of metalloproteinase MMP-9 in BMVEC [23]. Earlier in vitro studies showed that the inhibition of MMP-9 expression resulted in the enhanced expression of extracellular matrix proteins including collagens I, IV, and V, and hence a decrease in endothelial permeability, which can potentially fortify the BBB against invasion of inflammatory cells [23].

As discussed above, QDs are used to visualize different structures including tumors within the brain. QDs specifically interacting with brain tumors may be loaded with anticancer drugs for their targeted delivery. Such approach combining the target detection and therapy is known as theranostics, and nanotechnology is very important player on this field. Nanotheranostics based on advantages of nanomaterials can diagnose brain cancer at early stages, initiate and monitor therapy. In brain nanotheranostics, therapeutic and diagnostic agents are loaded in a single nanoplatform, which can be further developed as a clinical formulation for targeting various modes of brain cancer [54].

For targeted co-delivery of anticancer docetaxel (DTX) and QDs into brain cancer cells, Tf conjugated theranostic liposomes were developed [55]. To achieve this goal, acid functionalized D-a-tocopheryl PEG 1000 succinate (TPGS) was conjugated covalently to the amino groups of Tf (TPGS-Tf). This conjugate TPGS-Tf was assembled on the surface of the liposomes and a therapeutic DTX and imaging agent QDs were encapsulated in the lipophilic portion of the liposomes by solvent injection method. The theranostic liposomes without Tf were prepared as well. Drug encapsulation efficiency was about 71%. The drug release from Tf conjugated liposomes was sustained for more than 72 h with 70% of drug release. To prove the transport efficiency of DTX...
and QDs across the BBB and targeted delivery into the brain, liposomes were tested in vivo at intravenous administration. In comparison to the non-targeted liposomes and marketed Docet(TM), targeted theranostic liposomes demonstrated significantly higher delivery of DTX and QDs into brain tissue. Targeted liposomes were about 9 times more efficient as compared to Docet(TM) after 2 h treatment [55].

In other work of this group tripeptide arginine-glycine-aspartic acid (RGD) was coupled to TPGS instead of Tf. Then liposomes containing this formulation were loaded with DTX and QDs for brain targeted delivery [56]. RGD peptide specifically binds integrin of alphavbeta3 type overexpressed in angiogenic endothelial cells and/or cancer cells, therefore RGD-coupled theranostic liposomes can be internalized via receptor-mediated endocytosis. In addition, positively charged RGD-containing liposomes may improve the transcytosis of drug though the negatively charged BBB. In this case, about 70% of drug encapsulation efficiency was achieved with liposomes. The drug release from RGD-TPGS decorated liposomes was sustained for more than 72 h with 80% of drug release. RGD-targeted liposomes were about 7 times more efficient as compared to Docet(TM) after 4 h treatment. RGD-containing nanoparticles did not show any signs of brain damage or edema [56].

It should be noted that at present, with increasing life expectancy, the problem of treating neurodegenerative diseases, including Alzheimer's and Parkinson's, is becoming more and more acute. Nanotheranostics can also contribute to the solution of this problem.

Thus, QDs and apomorphine used in advanced Parkinson's disease intermittent hypermobility were incorporated into liposomes to eliminate uptake by the liver and enhance brain targeting [57]. QDs were completely encapsulated by the vesicles and about 80% of apomorphine were enclosed. Liposomal incorporation of QDs greatly increased fluorescence intensity in mouse brains as compared to free QDs, while QD uptake by the heart and liver was reduced. Ex vivo imaging of the organs confirmed these results. Apomorphine accumulation in the brain increased by 2.4-fold after incorporation in liposomes. Liposomes were efficiently endocytosed into mouse brain endothelial hBEND3 cells by clathrin- and caveola-mediated endocytosis [57]. The developed liposomes provide a novel perspective for the treatment of Parkinson's disease.

To deliver QD therapeutics to the brain, TfR can be used as well. Thus, Mahajan and coworkers [58] stably incorporated the antiretroviral drug, Amprenavir, within a Tf-conjugated double-shelled CdSe/CdS/ZnS QDs and shown that bioconjugation of Tf to the QDs can facilitate transfer across the BBB. The CdSe/CdS/ZnS QDs were transferred in aqueous media by ligand exchange with short thiol chain, mercaptosuccinic acid. Following the synthesis of the QDs terminated with carboxyl groups, they are covalently bound with Amprenavir and Tf molecules using simple chemical strategy. It was demonstrated that a Tf-QD-Amprenavir nanoplex transversed the in vitro model of the human BBB and significantly inhibited HIV-1 replication in HIV-1-infected monocytes in vitro.

Despite the encouraging result on using metal based QDs in theranostic systems, serious concerns remain about their potential toxicity in vivo due to toxic nature of the semiconductor materials themselves. Different strategies have been implemented to reduce QD adverse effects, including the application of QDs based on silicon or carbon materials. General application of cQDs is described in the recent review by Zhou and coworkers [59]. Here are some very recent examples of cQD use in theranostic systems.

Thus, effective anticancer drug doxorubicin (Dox) and human Tf targeted to TfR overexpressed on the BBB and cancer cells were covalently attached to cQDs synthesized from raw carbon powder [60]. The successful conjugation of Dox and Tf with cCDS was confirmed by different spectroscopic techniques. To examine the cellular uptake of the conjugate, glioblastoma S1G8M2 cells were treated with 500 nM conjugate or Dox for 18 hours under serum-free conditions. Approximately 5-fold increase in Dox concentration was observed for the conjugate as compared to Dox alone. The efficacy of conjugate was investigated in four pediatric cell lines derived from tumor specimen. It was found that 10 nM of conjugate solution possessed the highest lethality to all the pediatric tumor cells reducing viability by 14-45% [60].

To develop an approach for the treatment of Alzheimer disease (AD), neuroprotective peptide glycine-proline-glutamate was conjugated to graphene QDs [61]. It was found that conjugate obtained inhibited the aggregation of amyloid beta 1-42 peptide in vitro. To observe the therapeutic effect, the graphene QDs conjugate was administrated to APP/PS1 transgenic mice by intravenous injection, and Morris water maze was performed to examine learning and memory capacity of the mice. The results obtained indicated enhancement of learning and memory capacity in the experimental mouse group. Moreover, the conjugate reduced the inflammatory response and increased amounts of dendritic spine, thus protecting the synapse and promoting the neurogenesis [61].

The preparation of cQD conjugate with Tf to deliver the cQDs across the BBB in a zebrafish model [20] was discussed above. The applicability of this conjugate and cQDs alone for AD treatment were investigated in vitro using experimental and computational methods [62]. Molecular dynamics simulations showed that the hydrophilic surface of cQDs might promote inhibition of amyloid beta fibrillation. cQDs were also able to inhibit the active site of beta-secretases 1 enzyme and delay the formation of beta-amyloid toxic species in vitro. It was shown that cQD-Tf conjugate was targeted mostly to forebrain of zebrafish as compared to the dorsal and ventral brain sections. In addition, the cQD-Tf conjugates retained the activity in deactivating beta-secretases 1 and retarding amyloid fibrillation [62]. These results suggest a novel route for AD treatments.

5. MAJOR CHALLENGES AND FUTURE DIRECTIONS

The QDs have numerous applications and possess great advantages over the traditional organic fluorophores. The main applications of QDs in brain research are, but are not limited to, bioimaging and targeted drug delivery. Significant progress is achieved in this area, and with a high degree of reliability QDs allow vascular imaging, tracking of single
molecules labeled with QDs, and tumor imaging. However, as research deepens, problems that need to be addressed became evident. They include potential adverse effects of nanomaterials, efficient QD penetration through BBB, specificity of drug delivery to the afflicted brain area etc.

5.1. Tissue Distribution and Degradation of Quantum Dots

One of the main problems in the use of nanoparticles and QDs in particular is the uncertainty of their toxicity in vivo and the long-term consequences of their application. It should be noted that the nanomaterials, including QDs, with sizes in the range of 20-200nm can avoid renal filtration. Therefore, in the body, QDs can accumulate in various organs, where they may remain intact or be subjected to modification or metabolism. Analysis of the data available revealed that accumulation, metabolism and excretion of QDs depended on many parameters, including their size, chemical nature of surface modification, route of administration and others. However, a few general conclusions may be done [63]. Thus in general, administered QDs are completely and rapidly cleared from the bloodstream. Then, QDs injected intravenously are accumulated in the liver and spleen and to a lesser extent, in kidneys, lymph nodes, and bone marrow. However, when QDs are injected either subcutaneously, intradermally, or directly into animal tumor tissues, their distribution in organs is different.

The distribution and stability of orally administered (CdSe)ZnS QDs in digestive tract of Wistar rats were investigated by fluorescence spectroscopy [64]. The QDs were detected in the organs of the digestive system and did not eliminate from the organism neither with urine nor excrements. No traces of QD were detected in liver, pancreas and spleen. The author suggested that QDs were degraded in the digestive system of animals and the liberation of Cd²⁺ might result in their toxicity [64].

Anti-HER2 antibody conjugated CdSe/ZnS QDs were intravenously injected in Wistar rats and their toxicity in vivo was investigated [65] in comparison to non-conjugated QDs. Practically no toxic effects were observed for antibody conjugated QDs, while significant changes in complete blood count, biochemistry panel assay and comet assay were found for non-conjugated QDs. Moreover, cadmium deposition was confirmed in the rats treated with non-conjugated QDs. The data obtained suggest that the antibody coating assists in controlling possible adverse effect of QDs. The similar results were obtained in a pilot study on non-human primates [66]. Rhesus macaques were intravenously injected with phospholipid micelle encapsulated CdSe/CdS/ZnS quantum dots. Blood and biochemical markers were normal, and histology of major organs after 90 days showed no abnormalities. However, at that time chemical analysis revealed that most of the initial dose of cadmium remained in the liver, spleen and kidneys. The continued evaluation for one year showed no ill effects. Thus, the phospholipid micelle-encapsulated QDs had very low toxicity.

In the work by Zhao et al., [67] after subcutaneous injection, CdTe/ZnS QDs entered nearby lymph nodes and were distributed in a ring pattern, encircling the lymph node capsule. At the cellular level QDs showed spotty distribution in the cytoplasm of the four cell lines without penetration into the nucleus. Cells containing QDs completed mitosis normally and the distribution of QDs involved the vesicular transport system, including vesicles, endoplasmic reticulum and lysosomes [67]. The similar distribution of carboxyl-coated CdSe/ZnS QDs was observed in NIH3T3, MCF-7, and HepG2 cells [68]. After penetration the cell membrane and redistribution in the cytoplasm, diverse intracellular vesicles in the range of approximately 0.5-8 µm in diameter containing QDs were observed, but none were found in the nucleus.

To address the potential toxicity of QDs on immune cells, the immunotoxicity of CdSe/ZnS QDs using the in vitro model in macrophages and lymphocytes was investigated [69]. The effects of QDs on the macrophages and the lymphocytes were quite different. The macrophages treated with QDs exhibited decreased cell viability, increased levels of Reactive Oxygen Species (ROS), elevated apoptotic events, altered phagocytic ability, and decreased release of TNF-α and IL-6 upon subsequent stimulation. In contrast, lymphocytes exposed to QDs exhibited enhanced cell viability, increased release of TNF-α and IL-6 and decreased transformation ability. The authors suggested that exposures to CdSe/ZnS QDs could suppress immune defense against foreign stimuli.

To address the problems of elimination and metabolism of QDs in the individual cells, mouse embryonic stem cells (ESCs) and mouse embryonic fibroblasts were labeled with QDs [70]. A quick loss of QDs in ESCs was observed within 48 hours, which was not prevented by inhibition of cell proliferation. Supernatants collected from labeled ESCs in culture were used to label cells again, indicating that some QDs were excreted from cells. It was well documented that phagocytic cells (e.g., neutrophils and monocytes) as well as macrophages accumulated QDs and other nanoparticles in vivo. The phagocytes are capable of generating hypochlorous acid (HOCI) at concentrations enough to degrade polymer-encapsulated QDs [71]. It was shown that HOCI and hydrogen peroxide resulted in fluorescence quenching and chemical degradation of polymer-encapsulated QDs by an oxidative mechanism. This process caused fluorescence quenching and produced soluble metal (e.g., cadmium and zinc) and chalcogenide (e.g., sulfur and selenium) species which may lead to the potential toxicity of semiconductor nanocrystals.

Thus, QDs may have toxicity because of being composed of toxic elements such as cadmium, selenium, tellurium etc [72] and due to generation of ROS. Different strategies have been applied to minimize QDs toxicity, the most common of which are use of non-toxic materials and surface coatings with biocompatible molecules.

5.2. New Fluorescent Nanomaterials

To solve the problem of the creation of QDs that do not contain toxic chemical elements, biodegradable luminescent porous silicon nanoparticles were designed [73]. These nanoparticles injected intravenously accumulated mainly in mononuclear phagocytic system organs and were degraded in vivo into apparently non-toxic products within a few days and removed from the body through renal clearance.
As discussed in the above sections, carbon-based QDs are effective means towards a drug delivery system into diseased brain. Unlike metal-based QDs, cQDs are nontoxic and possess functional groups similar in number and quantity to that of polymers commonly used for functionalization. With the recent achievement in the synthesis of new advanced carbon or graphene QDs [74], they can leave behind the traditional semiconductor QDs.

Other recently emerged fluorescent nanomaterials in future may outstrip currently used QDs. The so-called two-dimensional inorganic materials-based QDs demonstrate high chemical stability, good aqueous dispersibility, excellent optical property, good biocompatibility and easy functionalization [75]. These QDs based on phosphorene, silicene, carbides, nitrides, transition metal dichalcogenides, transition metal oxides, MXenes etc. make a rapid progress in bioimaging and cancer therapy. The interest to these QDs is constantly growing that is reflected in the increasing number of publication on this topic. Quite recently a new type of polymer fluorescent single-chain nanoparticles (SCNPs) of very small tunable size (as small as 3 nm) has been developed [76]. Several methods for their preparation were elaborated, which allowed synthesis of SCNPs with different optical properties. However future studies in this emergent area are necessary to result in the use of fluorescent SCNPs for in vitro and in vivo optical imaging as new fluorescent probes with ultra-small size, higher brightness, and better photostability than previous systems.

5.3. New Drug Delivery Routes

The BBB function is to prevent the brain damage. However, this barrier hinders the delivery of drugs for the treatment of CNS diseases. Although current approaches allow transferring drugs through the BBB, they undoubtedly require further enhancement. New drug delivery routes that may more precisely target the brain are under development [77]. Thus, non-cationic and amphiphatic indololezepinone-constrained oligomers have been synthesized as new vectors for intracellular delivery [78]. The compounds synthesized were tested in an in vitro BBB permeation assay. One of the oligomers showed significant permeation in the in vitro cell-based human model of the BBB, suggesting an active mechanism of cell penetration. Recently a new BBB-translocating peptide PepNeg (SGTQQEY) with unique properties has been identified [79]. PepNeg is an efficient BBB translocator able to carry a large cargo, while maintaining BBB integrity. This anionic trans-BBB peptide opens a way for the development of new delivery systems to the CNS. A while ago, it became evident that even well-known compounds can be used to deliver drugs to the brain. For example, borneol, a naturally occurring compound in a class of “orifice-opening” agents, can improve drug delivery to the brain [80]. The borneol effect is reversible, characterized by rapid and transient penetration of the BBB and highly specific brain regional distribution. Several processes are involved in the enhancement of the BBB permeability, including the modulation of multiple ATP-binding cassette transporters, P-glycoprotein; tight junction proteins; and some others. Systemic co-administration of drug with borneol improves their delivery to the brain in a region-, dose- and time-dependent manner [80].

The more sophisticated systems for brain directed drug delivery were suggested, i.e. tandem nanomicelles co-functionalized with brain tumor-targeting and cell-penetrating peptides, Angiopep-2 and TAT [81]. These nanomicelles with markedly enhanced BBB permeation, glioma accumulation and penetration, and glioma cell uptake. They provide a novel and effective strategy for targeted glioma therapy.

The coating of QDs with biocompatible polymer plays an important role in targeted delivery to the brain as well. The influence of the chemical nature and structure of the organic/bioorganic shells of quantum dots on their application for in vivo diagnostic imaging was studied [25]. It was shown that QDs coated with non-crosslinked dendrimers were cytotoxic, while QDs encapsulated in crosslinked dendrimers had low cytotoxicity and were biocompatible. The conjugation of silica-shelled QDs with PEG1100 increased their stability and half-life in the circulation. These data demonstrated that the type and structure of organic/bioorganic shells of QDs affected the colloidal stability, solubility in physiological fluids, the basic physiological parameters, and cytotoxicity of QDs [25].

The application of CTX isolated from scorpion venom for QD delivery to glioma was discussed above. However animal venoms are rich source of toxins selectively interacting with different biological target and possessing good prospects for application in nanotechnology [82]. Recently the conjugates of QDs with alpha-cobratoxin, a specific marker of nicotinic acetylcholine receptor of alpha7 and muscle types, were prepared and applied for visualization of alpha7 receptors [83]. These conjugates can be used to target the tumors overexpressing nicotinic acetylcholine receptors.

So, the further development in this area will result in functional imaging and therapeutic approaches for the diagnosis and treatment of several brain diseases such as tumour, stroke, neurodegenerative disorders and some others.

CONCLUSION

In this review, the recent progress in application of QDs as agents for brain bioimaging and targeted drug delivery to the brain is discussed. It provides the current information about the approaches for imaging and delivery of therapeutics based on QDs. By integrating other materials with QDs, multimodal imaging can be achieved in one platform. Target-selective drug delivery can be greatly improved by their conjugation with QDs allowing simultaneous imaging and therapy. However, despite the great progress in using QDs for bioimaging and drug delivery across the BBB, there remains a serious concern about the toxicity of the QDs in vivo. The solution of this problem will open a new page in the treatment of brain diseases.

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AD</td>
<td>Alzheimer Disease</td>
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<tr>
<td>BBB</td>
<td>Blood-Brain Barrier</td>
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<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
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<td>BMSC</td>
<td>Brain Marrow Stromal Cells</td>
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Brain and Quantum Dots: Benefits of Nanotechnology

BMVEC = Brain Microvascular Endothelial Cells
CNS = Central Nervous System
cQDs = Carbon Dots
CTX = Chlorotoxin
Dox = Doxorubicin
DTX = Docetaxel
Eag1 = Ether-a-go-go 1
EGFR = Epidermal Growth Factor Receptor
ESCs = Embryonic Stem Cells
GH = Growth Hormone
HOCI = Hypochlorous Acid
MMP-2 = Metalloproteinase-2
NGR = Asparagyl-Glycyl-Arginine
NIR = Near-Infrared
PEG = Polyethylene Glycol
PRL = Prolactin
QDs = Quantum Dots
RGD = Arginine-Glycine-Aspartic Acid
ROS = Reactive Oxygen Species
SCNPs = Single-Chain Nanoparticles
Tf = Transferrin
TfR = Transferrin Receptor
TPGS = D-a-tocopheryl PEG 1000 Succinate

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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