Multidisciplinary Role of Mesoporous Silica Nanoparticles in Brain Regeneration and Cancers: From Crossing the Blood–Brain Barrier to Treatment

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Mesoporous silica nanoparticles (MSNs) have gained wide attention for their role in biomedicine and as drug delivery vehicles. Their structural tunability, high surface area, and easy functionalization impart significant advantages over conventional materials. In this Review, recent advances in the synthesis, drug delivery, and therapeutic roles of MSNs in the treatment of various neurodegenerative and neuroinflammatory diseases are presented. The intention is to understand how MSN formulations that are capable of encapsulating drug molecules can enhance drug delivery by overcoming the blood–brain barrier (BBB) mediated by specific transport processes. The composition and characteristics of the BBB, and how alterations are observed in neurodegenerative diseases including Alzheimer’s, epilepsy, and intracerebral hemorrhage are reviewed. Finally, the factors affecting efficient delivery of MSNs into the brain are summarized, and their most promising functional outcomes are discussed.

1. Introduction

Improvements in life expectancy of the majority of citizens worldwide has led the World Health Organization (WHO) to predict that by 2040, neurodegenerative diseases will surpass cancer as the second reason of death after cardiovascular diseases in such aged populations,[1,2] As per a 2017 report, neurological diseases cost the United States of America about $800 billion annually and this number is expected to increase even further over the coming years as the percentage of aged citizens increases.[3]

Unlike most other major diseases, the pace of drug development and delivery in case of neurodegenerative diseases has been stationary, partially due to lack of biomarkers that can diagnose such diseases long before the neurological symptoms arise and the challenges associated with identification of targets for drugs that can terminate or minimize neurodegeneration. Most importantly, delivering new cerebral therapeutic agents is impeded by the extensive and robust blood–brain barrier (BBB) which prevents the vast majority of drugs from crossing to the brain after systemic administration. During brain infections, intracerebral hemorrhage, or in neurodegenerative disorders, the BBB is altered in such a way that it allows easy access of inflammatory inducing molecules that may cause adverse neuronal damage.[4] Given the importance of early diagnosis and treatment of neurodegenerative diseases, new drug delivery technologies have appeared in the last decade.

As shown in Scheme 1, unique nanomaterials have been reported and several nanosized drug delivery systems including liposomes, dendrimers, carbon nanotubes (CNTs), inorganic and hybrid nanoparticles, and polymeric micelles have gained attention and have been tested for targeted drug delivery not only for neurodegenerative diseases but also for several other ailments.[5–10] The discovery of MCM-41 was recognized as a major breakthrough in materials science and since then mesoporous silica nanoparticles (MSNs), thanks to their superior physiochemical properties such as large porosity, high surface areas, low toxicity, controllable sizes, and wide range of morphologies compared to conventional nanoparticles (NPs) have emerged as favorable tools in biomedical applications as nanocarriers for encapsulation and delivery of therapeutics.[11–14] Designing biocompatible MSNs and their multifunctional derivatives for drug transport as well as theranostics is one of the hottest areas of research in the field of nanobiotechnology and nanomedicine.[15–19] High loading capacity, acceptable biocompatibility, and limitless possibilities of surface functionalization for specific cellular recognition...
undoubtedly make MSNs also suitable for detection, diagnosis, and treatment of neurodegenerative disorders, brain cancers, and in brain regeneration. To the best of our knowledge, this is a first review article focusing on the use of mesoporous silica nanoparticles for drug transport and therapy in degenerative and inflammatory diseases of the central nervous system (CNS).

In this review, we have discussed the synthesis, biocompatibility, ability, and mechanisms by which MSNs cross BBB. We have provided a detailed account of application of MSNs (and of silica nanoparticles if appropriate) and their BBB pathology in glioblastoma, Alzheimer’s disease (AD), epilepsy, nerve agent detoxification, reduction of reactive oxygen species, and in intracerebral hemorrhage. In addition, a brief account of the synthetic strategies for preparing target MSNs including soft and hard template method, self-assembly, aerogel techniques, and Stober method is given. In addition, we have discussed mechanisms of toxicity, and the factors governing the biocompatibility of functionalized particles. Here, the composition of BBB is discussed and specific examples have been provided to see the influence of endogenous stimuli such as pH, redox, and H2O2. At the same time, we have laid emphasis on functionalization strategies keeping in mind the passage mechanism (enzyme-mediated, receptors-mediated, stem-cell mediated). We have summarized current challenges that need to be dealt with to promote these potential particles to practical use. We believe that this review will be useful for a wide audience working in the field of neuroscience and brain regeneration.

2. Synthesis and Characterization of MSNs

First successful synthesis of nanosized mesoporous silica nanoparticles was reported by Cai et al., Fowler et al., and Nooney et al.[20–22] This was followed by the work of Lai et al. who popularized the term “MSNs” signified by mesoporous silica nanospheres.[23] Thanks to the work of organic chemists on surfactants, templating strategy particularly is useful in synthesizing tailored mesoporous silicas with exotic shapes (hexagonal, cubic, worm-hole like, and lamellar), sizes (nanometer to several centimeters), and morphologies (tubules, gyroids, fibers, spheres, and hollow spheres). Several researchers including Wu et al. and Tang et al. have provided detailed account of synthetic methodologies in preparing well-dispersed MSNs and their hollow counterparts that can be size tuned and morphologies of which can be easily altered.[24–26] Here we provide a brief account of the synthetic strategies for preparing target MSNs using rapid self-assembly, soft and hard template method, Stober method, colloidal templating, and aerogel techniques (Scheme 2). MSNs synthesized using one or more of these methods are promising materials in several applications such as drug release and theranostics.

Several experimental factors are responsible for regulating the interactions between the template and silica core, silica condensation rate, reaction kinetics, nucleation, and development rates. Rate of silane hydrolysis and condensation of Si–O–Si bonds are strongly dependent on charge states. Isoelectric point of silica is 2.0; below this point, the silica moieties are negatively charged. Electrostatic and weak hydrogen bonding interactions appear between cationic surfactants and negatively charged silicates or between silicates and neutral polymers; these conditions allow the development of silica-surfactant nuclei without precipitation.[27] Condensation rate increases beyond the isoelectric point due to favored nucleophilic attack and becomes maximum around pH = 7, then declines beyond this point. This method allows size and shape control and materials synthesized are catalytically active MSNs.

Stable colloidal solutions can be prepared by controlling the aggregation between particles using high dilution methods or perhaps by using binary surfactant mixtures (structure directing agents (SDA) and neutral triblock polymers).[21] Pluronic F127 is one example of this kind of polymer used by Suzuki et al., and some examples of capping agents (surface protectants) include, poly(ethylene glycol) (PEG), l-lysine, and triethanolamine (TEA).[28] Since the first discovery of monodisperse silica nanoparticles by Stober in 1968, several uniform MSNs have been developed by modifying the method using different surfactants, changing the solvent system and pH, altering the solvent–water ratio and reaction time, and including homologs...
of quaternary ammonium surfactants or silica precursors with an aim to form special MSNs from few nanometers to sub-micrometer levels. Small changes can exhibit significant differences, for example, to impart basicity to the reaction mixture; if TEA is used as a substitute to ammonia, MSNs with a diameter of 20–150 nm are obtained; however if the same reaction mixture is diluted significantly, then MSNs with a diameter of ≈20 nm can be obtained. Yamada et al. showed that even the chain length of tetraalkoxysilane precursors \((\text{Si(OR)}_4)\) significantly influences the size of the particles.

Scheme 1. Various nanoparticles reported for treatment of neurodegenerative diseases.

Scheme 2. Schematics comparing different templating approaches to synthesize mesoporous silica nanoparticles.
Several efforts have been directed toward varying the orientation of nanochannels in MSNs. For example, Yano et al. showed that altering the methanol–water ratio was capable of achieving nonaggregated MSNs with radially aligned mesopores.\textsuperscript{[12]} Seed incorporation has also been widely accepted toward obtaining radially aligned or hexagonally arranged nanochannels and there are few examples in literature that have used seeds including MCM-48-like and MCM-41.\textsuperscript{[33,34]} While aerosol-assisted surfactant method of self-assembly is also useful in forming exotic nanochannels, the control of pore size becomes equally important sometimes,\textsuperscript{[35]} especially, at useful in forming exotic nanochannels, the control of pore size becomes equally important sometimes,\textsuperscript{[35]} especially, at instances when large protein molecules, DNA, or biopolymers need to be accommodated. Researchers have investigated the use of swelled agents including N,N-dimethylhexadecylamine (DMHA), 1,3,5-trimethylbenzene (TMB), and TMB to form pore enhanced MSNs to incorporate large protein molecules and for gene delivery.\textsuperscript{[16]} Additionally, a combination of nonionic triblock copolymers such as Pluronic P65 and P123 and a cationic fluorinated surfactant such as FC-4 is particularly helpful for controlling the pore sizes of MSNs.\textsuperscript{[37]}

Hollow silica nanoparticles (HSNs) with high pore volume are important in biomedicine for drug release, functionalized encapsulation, biosensing, and nanovector injection applications. Their synthesis using soft templates including micelles, vesicular structures, and microemulsion droplets have been reported.\textsuperscript{[38–40]} There have been studies on micelle generation using single micelle templating silica method that is carried out by combining triblock copolymers with organosilica precursors.\textsuperscript{[41]} However, small size of micelles using this strategy, restricts the size of HSNs to below 20 nm. On the other hand, uniform MSNs in the size range of 25–100 nm can be synthesized from vesicle-templating strategy that incorporates a mixture of silanes and silicates as silica source, as well as a mixture of cationic and anion surfactants to minimize the curvature of mesostructured templates. This strategy allows the formation of a variety of interesting structures such as spheres, rods, cylinders, or lamellar/sheets.\textsuperscript{[42]} When it comes to core–shell structures, microemulsion templating is a well-known method where hydrolyzed silica precursors are treated and are diffused with o/w microemulsions prepared from a reaction mixture of water, oil, and surfactants with alkaline solutions. Several researchers also like to include hard templating strategies like polymer-beads templating, and metal-oxide MSNs during their synthesis as they provide pure phased and narrow particle size distribution.

3. Biocompatibility of MSNs

Mesoporous silica nanoparticles have gained significant attention in bioapplications owing to their excellent biocompatibility, well-defined structures, and controllable functionalities. Their exceptionally high surface areas, tunable porosity, and large pore volumes impart greater drug loading capacity and possibility of surface functionalization.\textsuperscript{[43]} They are capable of protecting the host and guest molecules from degradation and can regulate their movement (guests) in and out of the pores. The guest molecules remain entrapped until a specific stimulus triggers their release.\textsuperscript{[44]} The silicon oxide matrix remains stable under the biological environment, and is composed of a hexagonal array with several mesopores. For in vivo biomedicine applications, such particles should be efficient, specific, biocompatible, and nontoxic.

Although silica is generally considered less toxic, the physicochemical properties of nanomaterials like morphology, structure, shape, particle size, porosity, and surface area have been proven to play vital roles in the particles’ biocompatibility and biotranslocation.\textsuperscript{[25]}

Currently, there is limited data regarding the safety and biocompatibility of MSNs in brains and related neurodegenerative and neuroinflammatory diseases. Thankfully, biocompatibility of MSNs has been extensively studied in other biological systems. The extent of noncovalent interactions including electrostatic interactions and hydrogen bonding occurring with cell membranes on surface functionalization of MSNs bilayer of lipids or polymers significantly reduces due to the intrinsic porosity of the MSNs. Additionally, the limited condensation of siloxane framework and high surface area promote large dissolution into nontoxic, soluble silicic acid species. High drug capacity of these special particles further reduces the required dosages in comparison to conventional nanocarriers leading to mitigation of potential toxicity.

Generally, the toxicity of any functionalized particle in a given situation is dependent on the dose. Any possible toxicity arising as a result of using MSNs can mainly be due to the following two mechanisms: A) electrostatic interactions between MSNs and tetraalkylammonium-based phospholipids generating surface silanolates that lead to membranolysis and, B) cell death caused by reactive oxygen species (ROS) via necrosis or apoptosis. Sadeghnia et al. have thoroughly investigated the influence of MSNs and surface functionalized MSNs on cell viability and markers of oxidative stress that mainly included formation of intracellular ROS. They studied oxidative DNA damage in vitro in rat pheochromocytoma PC12 cells.\textsuperscript{[45]} Even after one day of exposing PC12 cells to MSNs (1.95–1000\(\mu\)g mL\(^{-1}\)), no significant reduction of cell viability was noticed in comparison to control. Additionally, even at high concentrations or with prolonged exposures, ROS formation and oxidative DNA damage were not significantly affected by these nanoparticles, proving the fact that both MSNs and functionalized MSNs were safe and remarkably had nontoxic properties toward PC12 cells. While, Brinker and coworkers showed that high-temperature treatments can produce strained three-membered siloxane rings from which ROS emerge and settle on the surface of amorphous silica.\textsuperscript{[46]} a strong relationship is observed between particle charge and ROS species, and enhancement in the ROS-led toxicity follows this order: cationic > anionic > neutral.\textsuperscript{[47]} At a particular instance, it is unlikely that all silica particles have similar levels of toxicity, since the appearance of siloxanes and different types of silanols on the surface of MSNs depend mainly on the synthetic factors and porosity of these particles.

Interesting results have appeared in case of mesoporous silica nanoparticles and nanorods during the in vivo biocompatibility investigation which indicated negligible in vivo toxicity with possible induction of glomerular filtration and biliary excretion dysfunction. MSNs modified with PEG exhibited higher accumulation in the lung and were excreted in the feces and urine. However, the particle shape determined their
clearance rate from the body; experimentally short-rod MSNs were excreted faster than long-rod MSNs.\[48\] Similarly, He et al. demonstrated that MSNs and PEG–MSNs of varying sizes were generally detected in liver, spleen, and to a lesser extent in lungs. Other organs such as heart and kidneys also showed small amounts of the nanoparticles. PEG modification of MSNs was effective in reducing the uptake of nanoparticles from blood stream by liver, spleen, and lungs. The degradation products of both types of nanoparticles were excreted in urine for a period of up to 1 month post administration of the nanoparticles.\[49\] Also, Dogra et al. demonstrated that MSNs are excreted through the urinary and fecal routes using an in vivo model of healthy female rats.\[50\] It was also found that silica nanoparticles were excreted mainly through the urinary and fecal routes after administration using different routes; this may happen due to kidney and hepatobiliary excretion processes.\[51\]

Biotranslocation of MSNs has been shown to depend largely on the size of the particles and Mou and coworkers have proved through their findings that the extent of cellular uptake is size dependent. The 50 nm nanoparticles showed a higher cellular uptake (2.5 times) in comparison to 30 nm particles; in general the extent of cellular uptake followed the order 50 nm > 30 nm > 110 nm > 280 nm > 170 nm.\[52\] Even the cytotoxicity has been shown to be size dependent by He et al. in case of spherical MSNs. Their results revealed that 190 and 420 nm MSNs had a higher cytotoxicity at concentrations more than 25 mg mL\(^{-1}\) in comparison to 1220 nm sized particles which may be attributed to decreased endocytosis at larger sizes.\[53\]

Biocompatibility of nanoparticles also depends on the surface properties of these particles. Functionalized MSNs play a vital role in changing the surface reactivity, enhancing biocompatibility, altering biodistribution and excretion, and elongating in vivo circulation time. Although neutral charge particles preferentially exhibit better interstitial transport in tumors and longer circulation times, cationic particles are considered to induce higher immune response, hence higher cytotoxicity.\[53,54\] Even when exposed surface silanol moieties constitute only 6% of the total particles, they can effectively interact with biomolecules including membrane lipids and protein and are capable of damaging the inherent structure of these molecules.\[55\] Common surface modification strategies include: 1) PEGylation, where a hydrophilic layer is formed around the particles causing high dispersion, and can significantly enhance half-life time by adjourning opsonization,\[56\] 2) functionalization with groups with negative, neutral, or positive zeta potentials such as carboxyl (-COOH), amino (-NH\(_2\)), methyl phosphonates (-PO\(_3\)-), and aromatic/phenyl (-Ph) groups, finally 3) modification of MSNs using lipid layers. PEGylated layer acts as a protection in masking reactive surface silanol groups; it prevents additional silanol formation from entering distorted pores. While MSNs on PEGylation exhibit decreased distribution in reticuloendothelial system (RES) tissues of liver and spleen after systemic administration, their circulation lifetime is enhanced, excretion rate decreases, and they can also enhance hemolytic activity.\[49,57-59\] On the contrary, lipid coating of the MSNs has been shown to increase biocompatibility, performance, suspendability, and specific binding in some cases.\[60-62\]

In the past few years, research is being carried out in different groups across the world on exploring the effect of shape on performance, nanotoxicity, and biodistribution of MSNs. So far, much work done in this field is theoretical or focusses on in vitro cellular uptake.\[63,64\] Dissipative particle dynamics (DPD) reveal that the initial orientation of particles and shape anisotropy determine the interactions between the particles and the lipid bilayers of cell membranes.\[65\] Chauhan et al. and Decuzzi et al. have provided some results showing shape effect on cell–nanoparticle interactions, in vivo biotranslocation of MSNs, and in silica coated-CdSe quantum dots.\[66\] Figure 1 shows studies carried out by Huang et al. where prominent shape effect is observed; they found that short-rod MSNs were trapped in liver, while long-rod MSNs were distributed in spleen.\[48\] Additionally, short-rod MSNs were cleared rapidly from the body than their long counterparts.

Although most work done in this area is theoretical and in vitro, more efforts are required to explore this promising field of research as it can be beneficial for the development of nanoscale delivery systems and characterization of their biodistribution.

Physical properties such as particle size, surface properties, structure, and shape of the MSNs can significantly affect their biocompatibility. However, seemingly simple MSN drug carriers actually may show inconsistent results at time due to the complex interplay between these properties. For instance, nanoparticles with large surface area and several silanol groups may generate higher number of ROS, therefore they are generally considered toxic. However, there are many cases where porous MSNs with high surface areas have been proved to have lower cytotoxicity and hemolytic activity, because the true region of interaction with biomolecules, also called the “cell contactable surface area” is less in MSNs. To go in depth about the parameters affecting the biocompatibility of the MSNs is beyond the scope of this review. For further information, the readers are advised to check some reviews on this topic provided by Tang et al., Tarn et al., and Asefa and Tao.\[65,67\]

4. Crossing the Blood–Brain Barrier

Central nervous system is composed of several protective barriers like blood–spinal cord, blood–retinal, blood–cerebrospinal fluid, and BBB, and among them BBB is the most expansive and important one.\[68\] It is critically located between neural tissues and circulating blood, and consists of closely bound brain capillary endothelial cells and disjointed films of pericytes. Its unique cellular architecture regulates the movement of selective species and controls brain homeostasis. Neuroinflammation and degradation are consequences of malfunctioning of the neural circuits and breakdown of special multicellular components in the cerebral cortex. In several neurodegenerative diseases including Parkinson’s, Alzheimer’s, and epilepsy, the BBB is altered and even allows permeation of species that can cause inflammation or severe neuronal damage.\[69\] Treatment of these diseases currently involve strategies that are symptomatic and are unable to reverse or even halt the damage. Moreover, drug delivery in the brain is hampered by the extensive BBB which prevents the majority of drugs from passing to the brain after systemic administration. To avoid the difficulties associated with crossing the BBB, some researchers including...
Lungare et al. have exploited strategies where they could deliver the drugs by passing the BBB using nose-to-brain delivery via olfactory pathway.\cite{70} Although challenging, several research groups reported on the entrapment and translocation therapies involving encapsulation of therapeutic drugs in the pores of nanoporous particles and their delivery across the brain vasculature with an aim for brain regeneration and restoration of normal functions as discussed in this review.

It is vital to understand the composition of blood–brain barrier and the mechanism of permeation before developing NPs, because the type and surface modification of NPs will determine which mechanism will allow passage across the BBB. This knowledge will also enable the development and delivery of neuroprotective and regenerative medicine. As shown in Scheme 3, the endothelial cells in brain in order to maintain cohesiveness of the barrier are linked to each other through tight junctions (TJs) and adherens junctions (AJs), where TJs are composed of cytoplasmic and transmembrane proteins like zonula occludens (ZO), junction adhesion molecules (JAMs), claudins, occludins, and accessory proteins.\cite{68} Well packed cellular arrangement of endothelium still allows certain selective cells and molecules to move in and out of this zone; their passage occurs in three major ways: paracellular (mobility between endothelial cells), transcellular (mobility through/ across endothelial cells), and cellular invaginal.\cite{71, 72} Paracellular transport is responsible for movement of ions, solutes, and maintaining concentration gradient, while transcellular transport is responsible for passage of some gases, lipids, and proteins due to the presence of certain specific receptors. Finally, the third kind of transport occurs via development of cellular invaginations called caveolae, which form vesicles around species letting them move in and out of the brain. The paracellular and transcellular barriers are key regions which are coordinated to present a dynamic interface to protect the brain from harmful agents through synergistic enzymatic, metabolic, and physical mechanisms, unfortunately preventing even the best drugs from reaching their target sites and making the treatment largely dependent on the drug delivery shortcomings.

Colloidal NPs used for regenerative medicine include either polymeric or inorganic NPs. Biocompatible polymeric particles reported so far have been synthesized using polylactic acid (PLA), poly(ethyleneimine) (PEI), poly(e-caprolactone) (PCL), poly(amicidamino) dendrimers (PAMAM), poly(lactic-co-glycolic acid) (PLGA), and poly(alkylcyanoacrylates), or they occur naturally in the form of polysaccharides (chitosan and alginate), and proteins (albumin and gelatin).\cite{73–76} Although this category of particles has an advantage of being easily recognized by the biological receptors located on endothelium, they suffer from poor detection by imaging techniques, difficulty of surface modification, and reproducibility. Some inorganic NPs also reported for delivery across BBB include gold, silica, and mesoporous silica nanoparticles. While, these can be advantageous in terms of easy regulation of size, shape, synthesis, and functionalization, and easy detection using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and magnetic resonance imaging (MRI) techniques, but some of these particles suffer from biocompatibility, biodegradation, and cytotoxicity.

In general, nanoparticles can cross the BBB through one of these transport mechanisms compiled by Saraiva et al.\cite{77} i) NPs expand tight junctions present between endothelial cells or impart local toxicity allowing permeation of the BBB, giving access to the drug either bound to NPs or in a free form; ii) NPs pass endothelium by transcytosis; iii) or they are transported by endocytosis, they release the drug in cell cytoplasm and finally undergo exocytosis in the endothelium abluminal side; or iv) the mechanism may follow one or in combination with several other reported above, and mechanisms (ii), (iii), and (iv) are considered dominant mechanisms of transport.\cite{78–81} In this
review, we will focus on the entrapment and transport of therapeutic drugs using mesoporous silica nanoparticles. We will review alterations of the BBB in pathological conditions, especially in Alzheimer’s, epilepsy, Parkinson’s, and in reduction of reactive oxygen species.

4.1. Mesoporous Silica Nanoparticles and Blood–Brain Barrier

Given the fact, that MSNs have been recognized for their role in delivering therapeutic drugs for various diseases, in the year 2016, Baghirov et al. investigated the uptake, transport, and cytotoxicity of MSN based drug nanocarriers functionalized with PEG–PEI block copolymer using in vitro models of the BBB.[82] They used RBE4 rat brain endothelial cells, as well as Madin–Darby canine kidney epithelial cells for their experiments, as given in Figure 2. They evaluated the effect of shape and functionalization of MSNs on the BBB, for which they used spherical and rod-shaped MSNs and tested them as bare and as surface functionalized particles. The transport studies revealed that nonfunctionalized MSNs had low permeability, the cellular uptake experiments showed enhanced uptake of PEG–PEI functionalized MSNs. Neither bare MSNs, nor functionalized MSNs showed significant toxic effects on the cell viability indicating their potential safety for therapeutic purposes. Although the shape effect was negligible, but uptake studies observed using real-time surface plasmon resonance (SPR) measurements, indicated that PEG–PEI coating of copolymers significantly contributed to enhanced uptake of MSNs, especially in case of rod-shaped particles. Two-photon in vivo imaging in the brain vasculature of the copolymer-coated rod-shaped MSNs was carried out. These specialized MSNs were noticeably visualized after systemic injection and did not cause damage to the BBB, thus proving the fact that judiciously designed MSNs are potential materials for delivering drugs in the brain via transcellular transport mechanism.

Preclinical successes have been observed for some NP formulations other than MSNs targeting brain microvessel endothelial cells (BMECs) using transferrin, lactoferrin, insulin, and low-density lipoprotein receptors.[83,84] Interestingly, endothelial cell membranes of the BBB have transferrin receptors present in high density, that is why most research has been carried out targeting these receptors. For example, the rat R7 antimouse TfR antibodies were seen to largely occupy BMECs via transferrin receptors. It is not surprising, if antibodies are researched intensively, applied, and characterized in terms of developing BBB targeting MSN based nanocarriers.[85,86]

In the year 2016, Song et al. demonstrated a drug delivery system to the brain by conjugating lactoferrin (Lf) molecules on the surface of silica nanoparticles (Figure 3).[87] Lf is an iron-binding cationic glycoprotein that is considered a potential targeting candidate owing to its acceptable biocompatibility, excellent receptor-mediated transport efficacy, and low cost.[88,89] The Lf receptors exist in vascular endothelial cells of the BBB and may be beneficial in Lf-mediated transcytosis, and are considered to be even better than transferrin ligands widely used owing to the lower endogenous concentration of Lf.[90–93] Similar to Baghirov et al., here the surface of nanoparticles was functionalized with PEG to minimize protein surface adsorption, and avoid clearance...
by the RES. Here they used three kinds of cells including endocytes, pericytes, and astrocytes for studying the transport efficiency of Lf treated silica NPs in in vitro BBB model. They observed relocation of functionalized NPs from the apical side to the basolateral side according to the size of particles, indicating size dependent high transport efficiency of Lf functionalized particles across the BBB, with maximum efficiency observed for the 25 nm particles. This receptor-mediated transcytosis of silica NPs through the endothelial cells, is a good example that may be utilized to deliver therapeutic and imaging agents to the brain.

In the same year, Bouchoucha et al. reported the receptor-mediated uptake of antibody-conjugated MSN based nanocarriers by brain microvessel endothelial cells, where they evaluated the effects of size and bioconjugation of MSNs on their targeting ability toward the brain microvessel endothelial cells. As shown in Figure 4, here, MSNs with two different diameters, 50 and 160 nm were simultaneously functionalized with rat Ri7 antibodies via PEG linkers, MRI contrast agent gadolinium chelate (Gd-DTPA, DTPA = diethylenetriaminepentaacetic dianhydride), and a fluorescent moiety. These MSN preparations had high colloidal stability in suspension and their in vitro studies showed that the Ri7 antibodies conjugated to MSNs exhibited high binding affinity and specificity. Cells incubated with Ri7-MSNs (Gd incorporated) exhibited prominent MRI positive contrast enhancement signals, indicating their use as promising materials for theranostic applications. Quantitative cellular uptake assays were carried out to investigate the affinity of Ri7-MSNs for the brain neuronal and endothelial cells. It was found that endocytosis of nanoparticles was mediated via transferrin receptors, and just like the findings of Song et al., the Ri7-MSNs cellular uptake was seen to be size- and time-dependent. However, in this case the highest uptake was observed for 50 nm particles and upon systemic administration, the particles accumulated in the brain microvessel endothelial cells, demonstrating the strong potential of antibody-treated MSNs for in vivo brain microvessel endothelial cells targeting.

Recently, Tamba et al. reported on the synthesis of glucose (Glu) and glucose-poly(ethylene glycol) methyl ether amine (Glu-PEG) functionalized spherical silica NPs (50 nm) using microemulsion method incorporating ruthenium centers as fluorescent moieties (Ru@SNPs) and investigated their capability to infiltrate the BBB in rodent brains. The combination of glucose and PEG-amino groups on the surface of such particles was responsible for promoting their uptake by neuronal cells. They characterized the functionalized nanoparticles using field emission scanning electron microscopy, dynamic light scattering, and Fourier-transform infrared spectroscopy - attenuated total reflection studies. Tailored particles were examined for their biodistribution and penetration of BBB in mice brain using confocal microscopy, flow cytometry, and TEM.
revealed that Glu-SNPs and Glu-PEG functionalized silica NPs administered systemically, reached the vascular endothelial cells via penetrating the BBB. According to the authors, the mechanisms for uptake of these particles seems to be a combination of receptor-mediated endocytosis by the brain capillary endothelial cells, and the subsequent transcytosis using support of various transporters such as apolipoprotein E (apoE), glucose transporter (GLUT), and low-density lipoproteins. The results of these in vivo experiments are promising and prove that the functionalized derivatives of silica nanocarriers are capable of crossing the BBB. Such results will help in the rational engineering of multifunctional nanoparticles and understanding the BBB permeability.

Other than the MSNs, there are bifunctional contrast agents made of nanodots that may be used to image brain tumors using MRI.\[96\]

5. Applications of MSNs

5.1. Carriers for Drug Delivery for the Brain Glioblastoma

Gliomas are tumors originating mostly from the brain glial cells that include the glioblastoma multiforme (GBM) (WHO grade IV gliomas) as well as other low-grade gliomas. GBMs are the most frequent, malignant and aggressive subtypes
of glioma, with exceedingly high relapse and poor prognosis, because GBM progenitor cells with tumor-precursors are resistant to radiation and chemotherapy.\cite{97–102} Devastating glioma cells show fast infiltration and hide in inaccessible areas of brain, which rules out the possibility of surgery, and radiation methods.\cite{103,104} Chemotherapy fails in most of the GBM patients since most drugs are incapable of crossing the BBB and arriving at tumor tissues.\cite{4,105} Vasculogenic mimicking capacity of glioma cells gives more challenges to the treatment when tumor cells make vascular channels which supply nutrients to the cells of glioma in the preliminary stages of cancer and reappearance of tumors even after cancer therapy.\cite{106–109} There is an urgent need to develop novel nano drugs that can target and recognize glioma, while they simultaneously have permeation properties to cross the BBB. Targeting strategies could enhance the recognition and cellular uptake of MSNs in cancer cells that can taper the therapeutic doses and unwanted off-target side effects. Generally, conjugation has been carried out on the surface of MSNs using targeted molecules such as RGD (arginine–glycine–aspartic acid), transferrin, folic acid, and mannose.\cite{110–112}

Mo et al. in the year 2016 reported on well-tailored MSNs loaded with antineoplastic doxorubicin (DOX) and functionalized with cancer-targeting polymer (PEI-cRGD) to increase the antiglioblastoma properties. Figure 5 shows design and synthesis of size tuned DOX@MSNs, 40 nm particles exhibited the highest antiglioblastoma properties and highest cellular uptake owing to their rapid infiltration into cancer cells and their hard excretion out of cells.\cite{113} These nanovehicles were capable of permeating the BBB, disrupting the VM-ability of GMB cells.

![Figure 5](image-url)
depriving them of necessary nutrition for their growth, expansion, and regeneration, and antagonizing cancerous cells with high selectivity and precision. Finally, they were successful in achieving adequate anti-glioblastoma efficiency and minimizing undesirable toxic side effects. The high efficiency and selectivity of these functionalized particles compared to free DOX were attributed to their ability to selectively recognize and bind with U87 cells and show high expression of ανβ3 integrin receptors on cell membranes, which could sequentially enhance cellular uptake and cause apoptosis of glioma cells by triggering ROS overproduction.[114,115]

The RGD peptide is an effective targeted moiety which is considered to interact with ανβ3 integrin receptors overexpressed on surface of different cancers cells. Owing to the its well-known structure and small molecular weight, this peptide is an ideal targeting molecule suitable for conjugation with polymers, liposomes, and inorganic nanoparticles.[116,117] You et al., reported on the synthesis of MSN nanosystems conjugated to RGD peptide and tested as a delivery platform for organic selenium compound (BSeC), an anticancer agent to treat human brain glioma (Figure 6).[118] This BSeC@MSNs-RGD nanosystem was able to effectively cross the BBB and showed enhanced cellular uptake in tumor cells. On internalization, these functionalized particles stimulated mitochondrial dysfunction and intracellular ROS generation that subsequently activated p53 and MAPKs pathways. Through in vivo models, the authors observed that this delivery platform was able to inhibit the growth of U87 tumors, prolonged circulation time of the drug and was effective in reducing in vivo toxicity.

If nanomaterials were to enter cells by endocytosis and exit by exocytosis, this would lead to random drug delivery. Therefore, specialized nanovehicles are required that can selectively bind to the tumor cells that overexpress certain receptors, and demonstrate receptor-mediated internalization. In the year 2014, He et al. had performed interesting studies where they reported on MSN-based fluorescent theranostic drug delivery system that was used to monitor the cellular uptake and localization of such delivery system in cancer cells. Moreover, they investigated RGD peptide conjugated MSNs which incorporated fluorescent ruthenium polypyridyl complexes (RuPOP@MSNs). Functionalization using RGD peptide was shown to increase the uptake of nanoparticles by target cells through receptor-mediated endocytosis, and displayed increased selectivity for cancer rather than normal cells. RuPOP@MSNs exhibited substantially high cytotoxicity toward cancer cells that overexpress the integrin receptors. The extrinsic pathway leading to cell apoptosis was corroborated by enhanced expression levels of Fas and TNFR-2 death receptors, activated caspase-8 and truncated Bid. Free RuPOP complexes were released in the cytoplasm after the functionalized NPs were internalized, and these RuPOPs altered the phosphorylation of p53, AKT, and MAPKs pathways leading to apoptosis of cells. Furthermore, fluorescence of RuPOPs allows to trace drugs, and encompasses the control of theranostics to subcellular level.[115]

In recent years, stem cells are becoming very popular and some studies have shown that neural stem cells (NSCs) have intrinsic tumor-tropic migration potential which can be beneficial in targeted delivery of anticancer drugs to tumor sites and

![Figure 6.](image)
they have been studied by various groups as carriers in xenograft models of human glioblastoma.\(^{119–123}\) Although there are reports on using mesenchymal stem cells as platform for delivering drug-loaded NPs through intratumoral administration, but there is only one report so far provided by Cheng et al. on using neural stem cells as a medium for delivering antitumor drug in an orthotopic human glioma xenograft model.\(^{124–127}\) Cheng et al. developed 80 nm MSNs as carriers for the delivery of DOX loaded MSNs, where doxorubicin was considered to have higher antitumor efficiency than temozolomide (TMZ) a well-known antitumor drug.\(^{127}\) These DOX molecules were conjugated to MSNs through a pH-sensitive hydrozone bond, theMSN-dox particles were further treated with trimethylammonium (TA) groups to enhance the surface charges under neutral pH conditions and to enhance cellular uptake. Moreover, they used the FDA-approved HB1.F3.CD neural cell line as a mean to deliver the doxorubicin loaded MSNs (MSN-DOX). Such potent combination was found to promote self-destruction of HB1/F3.CD vehicles that infiltrated glioma cells. This cell vehicle was loaded efficiently with MSN-DOX and the DOX release which was regulated by pH delayed the drug-induced toxicity against the loaded cells, this release also caused significant toxicity against U87 glioma cells. The tumor-tropic migration of their MSN-DOX loaded stem cells in the intracranial U87 xenograft mouse model showed apoptosis of tumor cells leading to high survival rate of animals. The authors observed that both intratumoral and contralateral injections were adequate to deliver significant amounts of MSN-DOX-loaded stem cells in tumors compared to MSN-DOX alone.

Liu et al. reported the synthesis of anticancer drug formulation depending on the near infrared (NIR) stimulated release of doxorubicin from mesoporous silica coated nanoparticles. This was achieved by incorporating “photomechanical” azobenzene groups in the pores of the mesoporous silica coating that may play a role as “stirrers” to expel doxorubicin out of the nanoparticles under the influence of the emitted UV/vis photons by the nanoparticles after absorbing the NIR waves, which will transform the azobenzene groups into the cis and trans forms reversibly. This allows the release of doxorubicin in a controlled way.\(^{128}\) Moreover, the same group extended their study to detect the amount of doxorubicin release from their nanoparticles in vivo in a real-time fashion using simultaneous upconverted luminescence and MRI. They coated a gadolinium-based coating (NaGdF\(_4\) shell) on the nanoparticles to impart contrast to MRI and strengthen the upconverted luminescence signal. They used a zebrafish model to test their nanoparticles and showed that they were able to establish correlations between doxorubicin release and the upconverted luminescence and MRI signal changes.\(^{129}\) There is a recent report demonstrating the synthesis of gadolinium-based contrast agent for MRI to detect the tumor vascular microenvironment based on the lattice oxygen vacancies on the surface of contrast agent.\(^{130}\)

The use of MSNs for drug delivery and medical imaging applications may be advantageous compared to other forms of nanoparticles due to diverse reasons. For the synthesis of MSNs, silica and silicon compounds are available in wide range of forms, and are considered safe, biocompatible, and biodegradable.\(^{131}\) We may easily control the pore size and structure, surface functionalization and morphology of MSNs.\(^{132}\) Compared to other forms of nanoparticles such as dendrimers, liposomes and niosomes, MSNs have higher stability regarding their degradation and resistance to external stress due to their stable Si–O bonds.\(^{12}\) With respect to cancer, MSNs may improve the outcomes of conventional therapies due to several reasons. MSNs have relatively high drug loading capacities, can transfer the loaded drugs to target tissues without leakage, improve the bioavailability of poorly soluble drugs, provide protection to loaded drugs from gastrointestinal fluids and acids.\(^{131,133}\) MSNs may be used as an effective combination therapy vehicle (drug/siRNA or drug/drug) to overcome multidrug resistant cancers.\(^{134,135}\) Moreover, MSNs can be designed to attain multifunctional approaches for cancer therapy, such as surface modification to escape from the immune system of host, targeting the tumor sites, enhanced uptake by cells, drug release in the appropriate sites with appropriate rates, and finally safe excretion from the body.\(^{131,136–138}\)

On the other side, the use of MSNs in clinical practice is still lagging. This is due to several challenges and drawbacks of MSNs, including the lack of detailed studies concerned with the immunogenicity and toxicity of different forms of MSNs, their pharmacodynamics and pharmacokinetics properties, and their biodistribution and clearance from human body. Also, it is not an easy job to scale up the production of MSNs on an industrial level while keeping strict control on their properties and reproducibility.\(^{139}\) Furthermore, depending on the surface properties of MSNs, they may interact with the phospholipids of red blood cell membranes causing hemolysis.\(^{140}\) In addition, it was reported that MSNs may reduce the amounts of endogenous ROS leading to enhanced malignant melanoma growth.\(^{141}\) Also, accumulation of MSNs in normal body organs, specially the liver and spleen is an issue that needs to be addressed before further translation of MSNs into clinical practice.\(^{142}\)

### 5.2. Treatment of Alzheimer’s Disease

AD is a neurodegenerative disorder that progress with time. This common form of dementia features accumulation of extracellular amyloid-beta protein (A\(_\beta\)) plaques due to elevated levels of trace metal ions (Fe\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\)), synaptic and neuronal loss, reticular formation, and appearance of tangling neurofibrils due to abnormal phosphorylation. Constant reduced activation of N-methyl-D-aspartate (NMDA) receptors can also lead to excitotoxicity.\(^{143}\) \(\alpha\) aggregation is perceived as the main process leading to Alzheimer’s and metal ions play a vital role in its aggregation as abnormal interactions occurring between the \(\beta\)-amyloids (A\(_\beta\)42) and Fe\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\) can cause peptide aggregation and oxidation, and the formation of ROS leading to neurotoxicity.\(^{144–146}\) Therefore, much emphasis is laid on chelation therapy in this disease and quite a few metal chelators have been explored for this purpose including cludioquin (CQ) and 8-hydroxyquinoline derivative (PBT2).\(^{147}\) However, these chelators have difficulty crossing the BBB or are incapable of segregating and wisely recognizing normal biological metal ions from those involved in toxic A\(_\beta\) plaques, and can disturb the normal physiological functions and cause adverse side effects like myelooptic neuropathy.
These shortcomings have inspired the researchers to develop new NP-based drug delivery systems that can contain bifunctional molecules capable of targeting both amyloid plaques as well as unwanted metal ions causing AD. This approach could enhance the efficacy of the transporters and reduce the toxicity associated with chelation therapy. Compared to nonporous NPs, MSNs having large surface area and high porosity allow considerable loading of drugs. The diffusional release of therapeutics from the well-ordered mesopores of MSNs is tunable and protects drugs from premature release as well as activates target area with biogenic local concentration, thereby reducing overall dose and preventing any acute or chronic complications.

The formation of ROS by Aβ-Cu complex is a mechanism of AD pathogenesis. ROS causes oxidative stress triggering a series of devastating effects on the cellular components including DNA, proteins, and lipids. Under reducing environment, the Aβ-Cu complex is thought to react with O2 to form hydrogen peroxide. There have been ongoing efforts in detecting oxidative stress using surface chemistry of cellular oxidants like H2O2. In this context, Geng et al. in the year 2012 reported on a biocompatible drug delivery platform using H2O2-responsive controlled release system loaded with AD therapeutic metal chelator based on MSNs functionalized with arylboronic acids. Given the property of phenylboronic acid that it can form stable cyclic esters with saccharides using their diol groups, it was envisaged that glycoproteins could possibly be linked to MSNs by forming boronate ester bonds. For their experiments, they selected human immunoglobulin G (IgG ≈ 150 kD) antibodies with an 11 nm hydrodynamic diameter as a nanoscopic cap. The mechanism of delivering the entrapped metal chelator guest molecules is based on a redox reaction that takes place only when H2O2 is present, and where arylboronic esters are oxidized to phenols. This leads to the removal of the IgG cap and release of the metal chelator guest molecules resulting in chelation of Cu2+ and disassembly of Aβ-Cu complex plaques (Figure 7).

In the year 2016, Yang et al. reported on the synthesis of gold capped MSNs with a H2O2-responsive controlled release system to deliver metal chelator CQ targeting toxic Aβ plaques involved in Alzheimer’s disease. Here CQ is released in high H2O2 environment that is created in the presence of Aβ plaques, therefore it is unlikely to interfere with the normal metal homeostasis and can bypass adverse side effects of metal chelators. They showed that incorporation of AuNPs in MSNs (Au-MSN-CQ) made these particles more efficient in decreasing Aβ self-assembly, and inhibiting Cu2+ induced Aβ40 aggregation in comparison to bare MSN-CQs. Benefits of using Au-MSN-CQs also include minimized ROS-mediated apoptosis caused from Aβ40 -Cu2+ complexes, cell membrane disruption, and microtubular defects. This example successfully demonstrates the application of these functionalized MSNs in efficient BBB penetration, suppression of Aβ aggregation, and excellent biocompatibility making them promising vehicles in pharmaceutical industry.

Another method for the treatment of AD is by delivering drugs than can prevent the metabolism of certain enzymes in the brain. For example, rivastigmine hydroginate tartrate (RT) is a therapeutic drug which is a carbamate derivative and it reversibly prevents the metabolism of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the CNS. However, the delivery of RT to the brain is very challenging as it restricts permit to the brain due to its hydrophilic nature, requires repeated dosing, and causes serious cholinergic side effects such as bradycardia, anorexia, dyspepsia etc. Therefore, it becomes imperative to develop drug vehicles that can cross biological barriers. In the year 2017, Karimzadeh et al. developed high porosity MSNs (P1-MSNs), which they functionalized using succinic anhydride (S-P1-MSN) and 3-aminopropyltriethoxysilane (APTES) forming AP-CO-P1-MSNs. Nanoparticles were characterized using multiple analysis methods, and they compared maximum entrapment efficiency and RT loading percentages for both nonfunctionalized and functionalized sample. Entrapment efficiency of P1-MSN, AP-CO-P1-MSN and S-P1-MSN was found to be 21.26%, 41.5%, and 11.9%, while RT loading percentages were 25.5%, 49.8%, and 14.28% for 24 h. In the mimicked gastric and body fluids, the rate of release of RT loaded functionalized particles was lower than that nonfunctionalized nanoparticles, however when orally administered, the sustained release of RT was observed in functionalized particles. Fortunately, cytotoxicity associated with bare MSNs was negligible, but the cells treated with functionalized MSNs showed a reduction in SY5Y cell viability, which may be attributed to easy access and accumulation of these particles in multiple cell components as corroborated by TEM. It was also reported that certain types of nonsilica based nanoprobes have the capabilities of capturing and detecting Cu2+ which can be used for the treatment and diagnosis of Alzheimer’s disease.

5.3. Treatment of Epilepsy

An average human brain consists of 86 billion neurons and our cognitive abilities arise from the well-endowed interconnected synapses that are contact points for electrochemical communication. Neural circuits created from assembly of synaptic...
connectivity are involved in information processing in the brain through the excitatory or inhibitory response of neurons, and normally these contrasting responses are well balanced through a variety of homeostatic mechanisms.[162]

Neural circuit dysfunction or spontaneous seizures happening in epilepsy can arise from any region of the cerebral cortex due to excessive electrical activity in the brain and due to numerous brain pathologies. Temporal lobe epilepsy (TLE) is a common refractory type of epilepsy which is drug-resistant and characterized by complex seizures.[163] There is a demand to implement new administration techniques with an aim to achieve best possible outcomes. Methods for controlled release of drugs are crucial in the pharmaceutical industry in terms of dose advantages.

In the year 2006, microentrapped using SBA-15 ordered microporous silica particles impregnated with valproic acid and sodic phenytoin was reported by Lopez et al. and the biocompatibility of such reservoirs was evaluated and the neurohistopathology studies of their effects on brain tissues were illustrated.[163] Their TEM imaging revealed that the nanocylindrical morphology of the SBA-15 was not significantly altered after loading 31.7% valproic acid by weight. In order to investigate neurohistopathology, rats were stereotactically implanted with reservoir capsules, and were sacrificed and analyzed after six months. From the histological section at the hippocampus, it was evident that the implants did not cause inflammation or necrosis. Stained sections of the neurons in proximity to the reservoirs did not show damage or pathological effects. To the best of our knowledge, this is the only example so far showing animal experiments using loaded MSNs targeted to epilepsy, we believe more efforts are required to explore this interesting area.

In the year 2010, Thomas et al. had reported on the synthesis of highly ordered MSNs prepared from triblock copolymers with high poly(alkylene oxide).[164] Loading efficiency of these high surface area particles was then assessed by passive loading of three important antiepileptic drugs namely, carbamazepine (CBZ), oxcarbazepine (OXC), and rufinamide (RFN) and was found to vary between 17–25%. The adsorption of these compounds was analyzed using DSC and powder X-ray diffraction (PXRD) and showed good loading efficiency. Dissolution studies of these particles showed rapid liberation profiles within the first 3 h and showed sustained release particularly in case of RFN. The presence of MSNs did not affect the viability of 3T3 endothelial cells which provided evidence of low cytotoxicity.

In the following year, Lopez et al. reported on using template technique for synthesizing mesoporous silica and titania nanotubes.[165] They incorporated the antiepilepsy drug phenytoin (PH) in ordered MSNs (SBA-15) and titania nanotubes. In this study they mainly carried out material analysis and evaluated the extent of incorporation using multiple techniques such as PXRD, FTIR, TEM, SEM, and N2 adsorption–desorption at 77 K, however there was no evidence of performing vivo or in vitro studies. Loading and release was studied using UV–vis spectroscopy and was found to be better in case of TiO2 nanotubes. We hope this interesting area of research can be explored in the future as it will be beneficial to the pharmaceutical industry.

5.4. Nerve Agent Detoxification/Rapid Cerebral Drug Delivery

The threat of chemical warfare agents has motivated the researchers to find means for their easy detection, demolition of their stockpiles, and decontamination of affected regions for humanitarian purposes. In addition to chemical warfare agents, potentially lethal organophosphorus compounds are being used as pesticides in farms and insecticides in agricultural products.[166,167] Within a short time, these NAs are able to penetrate the CNS, and exposure to even slight amounts of NAs can cause irreversible inhibition of acetylcholinesterase (AChE) in the brain leading to dangerous situations like seizures, breathing difficulties, or even immediate death.[168] Unfortunately, the existing brain-targeted nanodrugs (reactivators) in this situation are capable of only reactivating the AChE, but are not able to cross the BBB.[169,170] This requires researchers across the world to develop novel brain-targeted nanodrugs capable of adsorbing and rapidly detoxifying the poisonous NAs. It would be even better, if they can successfully repurpose a well-known nanomaterial to acquire a therapeutic effect in case of an emergency that cannot be achieved using existing drugs.

In the year 2012, Wilmsmeyer et al., showed that common stimulant, an organophosphorus compound, namely DMMP (dimethyl methylphophonate) bound hydrophilic silica more significantly than DMCP (dimethyl chlorophosphate), indicating that the sarin phosphorus-halogen bond affects the surface chemistry of the agent in a complicated way.[171] Then in the year 2014, Davis et al. studied the energetics and mechanisms that regulated the hydrogen-bonding interactions when soman and sarin adsorb on silica.[172] In the same year, Li and coworkers evaluated the effect of MSNs on detoxification of toxic DMMP and according to them, MSNs is an ideal promising adsorbent agent owing to their high specific surface area, dense Si–OH surface groups, and large pore volume.[173] They carried out their work using a micro-gravimetric detecting platform depending on resonant cantilever. Gravimetric detecting curves allowed calculation of the increased mass of MSNs on treatment with organophosphorus vapors. Thermodynamic data obtained from isotherms of temperature-varying measurements further helped to study the interactions between MSNs and the toxic vapors. The results were further corroborated using GC-MS analyses, solid state NMR, and FT-IR. Results clarified the substitution mechanism of binding of Si–OH surface groups of MSNs to organophosphorus compounds.[173]

Interestingly in 2016, Yang et al. investigated a special brain-targeting nanodrug against NA toxicity that was capable of penetrating the BBB and could rapidly release the drug molecules as shown in Figure 8.[174] They envisaged that NPs with a sponge-like porous structure could meet the delivery demands related to the releasing time and loading capacity. It would be possible to store sufficient amount of drugs in several inner pores and release them via appropriate channels speedily without any surface polymer barriers. MSNs belong to a family of classic sponge-like nanomaterials that can be potential decontaminating agents or NAs destroyers owing to their good biocompatibility and rapidly absorbing nature.[172,175,176] Yang et al. in their studies presented a detoxification system (DS) composed of MSNs functionalized with a brain-targeted protein coatings that were endowed with HI-6 reactivators for
effective targeting of soman-inhibited AChE in the brain. So the MSN cores were functionalized with transferrin (TF) shells as the TF receptors are largely expressed on the BBB surface and presumably these particles were capable of crossing the BBB via receptor-mediated transport mechanism.

The AChE reactivator HI-6 that is not capable of entering the brain by itself was loaded on the TF-MSNs and was released rapidly from the particle matrix into the site of damage to antagonize toxic soman, thereby restoring acetylcholinesterase degradation. The brain-targeting effect using TF-MSNs was examined in vivo using a zebrafish model that has a BBB structure resembling mammals. On injecting with TPNPs with fluorescein isothiocyanate (FITC), the zebrafish showed a significant fluorescence signal in the brain. On the contrary, on injecting with MSNs without TF modification, weak fluorescence signal was observed. When the functionalized TF-MSNs were injected intravenously in Kunming mice, they were clearly seen in the hippocampal neurons using TEM, indicating that transferrin is successful in targeting the MSNs to the brain. In this case, the HI-6 significantly reactivated the soman-inhibited cerebral AChE, imparting neuroprotection and reducing the mortality of soman-poisoned mice. From these results, it is evident that HI-6-loaded TF-MSNs show substantial antidote effect against soman, which can be attributed to the speedy release of the incorporated drug and their potential to cross the BBB. Importantly, these functionalized MSNs were safe for use as 92.4% particles were excreted from the brain one week after administering and the remaining MSNs caused no significant side effects.

5.5. Reduction of ROS

ROS are chemically reactive species containing oxygen (superoxides, peroxides, singlet oxygen, and hydroxyl radicals) that are produced naturally through normal metabolism of oxygen and constitute integral roles in cell signaling and homeostasis. However, excess intracellular ROS can destroy various essential biomolecules and cell components, disrupting the delicate redox balance of cells, resulting in DNA damage as well as cell apoptosis through several signaling pathways. Alteration in normal redox states arising from either increased production of ROS or impairment of the antioxidant system triggers oxidative stress in the body. Given the fact that, oxygen demand of the brain is
high and it consists of large amounts of peroxidation-susceptible lipid cells, effects of ROS inevitably influence the brain. Literature survey of the pathophysiology of neurodegenerative diseases like Parkinson’s and Alzheimer’s diseases, reveals that oxidative stress has a vital role in these diseases. Cellular impairment, damage of the DNA repair system, as well as mitochondrial dysfunction are all signs of accelerating aging process arising as a result of cumulative oxidative stress.\(^{[181–183]}\)

Cellular reactive oxygen species are produced from either exogenous sources like ultraviolet (UV), environmental toxins, ionizing radiation, and some drugs, or from endogenous sources. Endogenous sources include mitochondrial and non-mitochondrial ROS-generating enzymes like the nicotinamide adenine dinucleotide phosphate oxidase (NADPH), xanthine oxidase (XO), cytochrome P450, flavin oxidases, mitochondrial respiratory chain, and Nox systems. Neuronal death is possible from superoxides (\(\cdot\text{O}^2\)) and nitric oxides (\(\cdot\text{NO}\)) released from activated microglia and NADH oxidase/nitric oxide synthase.\(^{[184]}\)

In general, neurodegenerative diseases can be treated or prevented using antioxidant therapies, however their efficiency is debatable due to contradictory results.\(^{[185]}\) Antioxidant therapies can ameliorate the disastrous effects of oxidative stress considerably. Resveratrol (RSV), is a well-known antioxidant which also plays a powerful role as antiinflammatory, cardioprotective, and neuroprotective agent.\(^{[186,187]}\) It is reported that RSV is also effective in removing excess ROS or reactive nitrogen species in the CNS, but its systemic delivery in the brain is inefficient as it has low solubility and very low bioavailability due to rapid metabolism in liver and intestines.\(^{[188]}\) Shen et al., reported on PLA-coated MSNs as nanovehicles for delivering RSVs.\(^{[189]}\) PLA-MSNs were decorated with ligand peptides of low-density lipoprotein receptors (LDLRs) to improve their transcytosis across the BBB. Biodegradable aliphatic polyester (PLA) coating is incorporated as a gatekeeping layer that can control the release of drug. These PLA coatings have been used in the past under intestinal conditions to delay the release of venlafaxine from the mesoporous silica nanospheres.\(^{[190]}\) Additionally, it has been observed that ROS can hasten the degradation of such PLA coatings,\(^{[191]}\) that is why Shen at al used PLA as a ROS-responsive gate keeper for MSNs to secure effective drug release in high oxidative stress conditions.\(^{[189]}\) Resveratrol was loaded into 200 nm MSNs with an average pore size of 4 nm at 16 \(\mu\text{g mg}^{-1}\) (w/w). As a gatekeeping layer, the PLA coating hampered the RSV burst release, whereas the ROS triggered the drug release by enhancing PLA degradation. To evaluate the RSV delivery across BBB, an in vitro BBB model was established using a coculture of rat brain microvascular endothelial cells (RBECs) and microglia cells. The binding of low-density lipoprotein receptors significantly enhanced the migration of MSNs across the RBEC monolayers. Here RSV, was used as a model drug to diminish the detrimental effects of oxidative stress in activated microglia. After the release of RSV from MSNs, the inflammation of activated microglia was reduced and the generation of superoxides and nitric oxides also decreased. The combined effect of LDLR peptides and PLA gatekeeper as well as its ROS responsiveness these special MSNs were promising materials for ROS responsive RSV delivery to alleviate oxidative stress in CNS.

Another example of antioxidant therapy includes the one reported by Elle et al. where antioxidants such as caffeic acid (MSN-CAF) and rutin (MSN-RUT) were used along with MSNs to minimize the effects of oxidative stress developed after transfection of cells.\(^{[192]}\) Here they used two cell lines for the delivery of MSNs in the body, the intestinal Caco-2 and epidermal HaCaT cells. Rutin showed better results in terms of reduction of ROS levels and cellular toxicity reduction after 24 h incubation of cells treated with functionalized MSNs and its performance was more significant in HaCaT than in Caco-2 cells, exhibiting certain cellular specificity. To understand the Nrf2 response deeply, stably transfected HaCaT cells consisting of repeats of the antioxidant response element (ARE) along with a luciferase reporter gene was produced. Surprisingly, in these transfected cells, both tert-BHQ and quercetin (Nrf2 agonists) were able to induce the luciferase response in a dose-dependent manner which was not observed in case of rutin. Interestingly, at elevated concentrations, MSN-RUTs were able to stimulate a significant Nrf2 protective response in HaCaT cells. However, these responses were less prominent in Caco-2 cells. According to these results Elle et al. claimed that in keratinocyte cell line, rutin bound MSNs were beneficial for significantly reducing reactive oxygen species, maintaining cellular viability, and in producing defensive nature via activation of the Nrf2 antioxidant pathway.\(^{[192]}\)

### 5.6. Neuron Growth

Mystery of the human brain generating new neurons is a topic of immense scientific interest. To harness the brain’s regenerative capacity in order to promote cognition and ameliorate the loss of neural functions in case of injury or disease has been a priority for people in this domain. There is a great demand for therapeutic strategies to boost nerve cell growth and trigger nerve fiber reconnections.\(^{[193]}\) Conventional methods use injection of nerve growth factors (NGF), however quick clearance, short half-life, and degradation of these NGFs is a setback to the healing process and leads to a reduced effective concentration reaching the damaged nerves.\(^{[194–196]}\)

Nanoparticle platforms for delivering NGFs provide several advantages due to their ability to bind and protect NGFs from degradation and their ability to perform targeted delivery to nerve cells.\(^{[197]}\) Inorganic and polymer nanoparticles, and hydrogels are well known platforms for NGF delivery.\(^{[198–202]}\) In the year 2016, Sun et al. reported on the synthesis of MSNs with a diameter of 65 nm and had conjugated NGFs onto the surface of MSNs as an effective therapy to stimulate nerve healing.\(^{[201]}\) They compared the differentiation and proliferation of neurites arising from PC12 cells reacted with MSN-NGFs with PC12 cells treated with only free NGFs and with free MSNs, they performed MTS assay and evaluated their viability after exposing for 72 h. According to the authors, the PC12 neurosecretory cell line is known for its uniformity and reproducibility when used as a neuronal differentiation model.\(^{[193,197]}\) Their studies revealed that MSN-NGFs were able to significantly promote PC12 generation and neurite growth compared to free NGFs alone.\(^{[203]}\) The lengths of neurites were significantly elongated when PC12 cells were exposed to NGF loaded silica nanoparticles. Soluble NGF also increased the length of neurite, but the extent was less.
Regenerative medicine approach is a promising field which provides lineage alteration of somatic cells by reprogramming where mature cells are converted into a plethora of different types of cells. The direct production of functional neurons from fibroblasts was reported by using a mixture of small-molecule compounds to modulate specific signals and trigger gene expression in cells. In such studies the neurogenesis inducer Isoxazole 9 (ISX-9), played a pivotal role in activating the neuron-specific genes and promoted the neuronal differentiation of neural stem cells.\[204,205\] Therefore, it was considered a good approach to combine ISX-9 and plasmids containing other reprogramming factors for the direct conversion and formation of neuron cells. However, using viral vectors to introduce the reprogramming factors to cells in humans is generally unwanted and carries major risks of tumorigenesis, therefore, using nonviral carriers for the delivery of reprogramming factors is highly desirable. In the year 2018, Chang et al. reported on using MSNs in regenerative medicine, where MSNs were used as nanocarriers for transduction of three key reprogramming factors (Ascl1 plasmid DNA (pAscl1), Brn2 plasmid DNA (pBrn2), and Myt1l plasmid DNA (pMyt1l)) along with ISX-9 to convert mouse fibroblasts into functional dopaminergic neuronal-like cells (fDA-neurons).\[206\] As shown in Figure 9, they used ISX-9 which was delivered along with three key reprogramming factors using MSNs (pABM-I@M) to induce the direct conversion of neuronal-like cells. The satisfactory transfection efficiency of plasmid@MSN made it possible to repeat the dosing to achieve high exogenous gene expression. The dopaminergic activity and the electrophysiological properties of fDA-neurons were also validated. ELISA assay revealed significant levels of released-dopamine in the conditioned medium and surprisingly, Day 22 showed the presence of rich Na\(^{+}/K\(^{+}\)-channels in the fDA-neurons.

5.7. Theragnosis of Intracerebral Hemorrhage

Theranostic probes combine the properties of targeted therapy and medical imaging.\[207\] Intracerebral hemorrhage (ICH) arising due to the sudden rupture of a blood vessel in the brain, is a devastating subcategory of stroke for which there is no effective treatment available at this time.\[208–210\] Several research attempts aiming to either remove the hematoma or to reduce its size have been made, but none were successful due to either deep seated location of hematoma or due to surgical complications.\[211,212\] Intense inflammatory reactions occurring in the perihematomal spaces after this stroke are more damaging than the hematoma itself, thus lead to the production of ROS, finally causing oxidative stress, brain edema and neurological deterioration.\[213\]

Cha et al. recently reported a first nanobiomaterial for successful theragnosis of ICH using lipid-coated mesoporous silica nanoparticles (LMCs) as nanocarriers for ceria nanoparticles (CeNPs) for effective scavenging of ROS, and iron oxide nanoparticles (FeNPs) for MRI as shown in Figure 10.\[214\] These particles exhibited potent antiinflammatory effects via scavenging ROS and increased the contrast of MRI in the perihematomal area. These LMCs were composed of mesoporous silica nanoparticle-supported lipid bilayers and on loading with CeNPs, they showed strong antiinflammatory and antioxidative effects in vitro. In their rat ICH model, intracerebrally injected LMCs migrated to the perihematomal areas, were uptaken by macrophages and clearly visualized using MRI of the brain. Interestingly, these LMCs diminished the inflammatory macrophage infiltration, and strongly reduced the brain edema. Finally, the LMC treatment significantly improved the neurological outcomes of the rats with ICH.

6. Elimination of Degradable MSNs

Solid drug delivery systems such as MSNs are generally thought to keep the loaded drugs from premature release. However, such nanoparticle formulations may be difficult to degrade and hence will be eliminated with difficulty from human body.\[215\] Generally, positively charged MSNs are uptaken by liver cells and excreted in the feces through the hepatobiliary excretion pathway. Whereas, negatively charged MSNs accumulate in the Kupffer cells of liver without metabolism resulting in hepatotoxicity.\[216,217\] Under physiological conditions, many forms of
MSNs can dissolve into soluble silica, which can be excreted in urine as silicic acid or oligomeric silica compounds.\[218,219\]

On the cellular level, Zhai et al. showed that hollow MSNs can be degraded in the cytoplasm and lysosomes of human umbilical vein endothelial cells (HUVEC), then the MSNs degradation products were excreted outside cells. However, the metabolic pathway and degradation mechanism were not provided in this study.\[215\] He et al. showed that the degradation of mesoporous silica in simulated body fluid occurs in three stages which are fast bulk degradation (takes hours), followed by decelerated degradation due to formation of calcium/magnesium silicate layer over the mesoporous silica, and finally a slow diffusion stage.\[220\]

It is an important requirement to design MSNs with biodegradable properties. Kong et al. developed biodegradable hollow MSNs loaded with doxorubicin, all-trans retinoic acid and interleukin-2 (IL-2) to provide synergistic anticancer effects with less side effects. They showed that their nanoparticles can be degraded naturally in physiological conditions of 37 °C in phosphate buffered saline (PBS).\[221\] In 2018, Seré et al. reported the synthesis of MSNs using different procedures and studied the consequent effects on their degradation kinetics in PBS. They showed that degradation of MSNs increases with high concentration of MSNs, and low amount of catalyst and low temperatures used for the synthesis of MSNs. They also showed that amino group surface functionalization increased the degradation rate compared to the carboxylated and nonfunctionalized MSNs.\[222\]

In 2019, Zhang et al. constructed organic-inorganic hybrid MSNs loaded with doxorubicin and siRNA as a nanocarrier platform for synergistic anticancer therapies. They enhanced the biodegradability of their nanoparticles by using two mixtures of silicon in their synthesis (TEOS and BTES in 5:3 ratio). They tested the degradation of their nanoparticles in simulated tumor microenvironment fluids and found that the nanoparticles showed early signs of degradation by becoming irregular after 7 days, and started to disintegrate and became fragmented after 14 days.\[223\]

7. Conclusion

Mesoporous silica nanoparticles are the third generation of Si-based nanomaterials which are promising materials for biomedical applications. With growing research interest in the use of these nanoparticles for diagnosis and imaging, in this review we have discussed proper delivery and functionalization strategies necessary to cross the vital blood–brain barrier in treatment of several neurodegenerative diseases. We have studied how MSN formulations are capable of encapsulating drug molecules to target specific transport processes in the brain and are able to enhance drug delivery in ischemic disorders. We have reviewed composition and characteristics of BBB, and how variations are observed these diseases and examined the diverse nature of surface modification with peptides, antibodies, and stem cells that significantly affect the outcome of delivery and therapy. The delivery of therapeutics for repairing inflammation and recovery of damaged cells demands judicious methods for tuning the bioavailability and stability of bioactive species while they simultaneously augment the regeneration process. A thorough examination of the dynamism of in vivo environment and compatibility of these particles as well as their competitive and interfering interactions with other cell components remains to be explored and should provide valuable knowledge related to the criteria of MSN design.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

drug delivery systems, esoporous silica nanoparticles, glioblastoma, neurodegenerative diseases

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