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Review Article

MESOPOROUS SILICA NANOPARTICLES AS DELIVERY SYSTEM AGAINST DISEASES

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ABSTRACT

Despite insolubility, non-specificity and high toxicity of therapeutics associated with biological obstacles such as drug resistance, blood-brain barrier and systemic enzymatic degradation, patients have to take their high dosages to attain the expected therapeutic efficacy for the disease-treatment. To overcome these complications, various drug carriers are being investigated in the pharmaceuticals to supply their therapeutics to the specific sites in the body. Nowadays, mesoporous silica nanoparticles (MSNPs) have emerged as promising candidate because they can overcome all the barriers maximally by producing their biological effectiveness in a controlled and sustained manner to the diseased site. As the mesoporous silica materials have the excellent favorable physicochemical features, the multi-functionalized MSNPs are capable to target and release their cargos into the diseased cells according to the requirement upon exposition to external or internal stimuli. This review demonstrates the state of knowledge for the consideration MSNPs as delivery system against various diseases.

INTRODUCTION

Many people suffer from acute and chronic microbial infections, biofilm development, asthma, bronchitis, tumorigenesis and neurodegenerative diseases throughout the world. When the body is exposed by virulent microorganisms and / or various potent chemicals, the disease is induced, promoted, and progressed. The invasions or administration of microorganisms or chemicals are counteracted by body defense mechanisms such as innate and acquired immune responses [1] and antioxidant defense actions [2,3] for their protection from infection or any disorder. The disease is generally initiated when the body defense mechanisms cannot overcome against the biological inductions of infectious agents. As conventional therapy has so many limitations to target active compounds to specific site of interest with least toxic effect to surrounding healthy cells, it is needed to design a delivery system which not only can act as antimicrobial and anti-carcinogenic agent but also can act as delivery vehicle to target lead compounds to specific sites with insignificant side effect in the biological system.

In the last decades, the investigations regarding active components transport technologies have been modified appreciably as the complex human body is generally dependent on the developed disorder to be treated while the procedures to administer different lead compounds to cells are quite different. Additionally, as drugs-toxicity can affect the healthy cells of the body, it has given emphasized to target and control the therapeutics into the diseased cells specifically with a sustained liberation by overcoming mostly all the biological barriers to avoid secondary adverse side effects. In this context, nanotechnology has restructured the delivery and targeting processes by changing the perspective of the pharmaceutical industries as nanoparticles may have extent below 100 nm, get dissolved, and may entrap, encapsulate or anchor lead molecules, ligands by their functionalizations affecting biodistribution, bioavailability to target specific diseased sites as efficient and potent delivery carriers. MSNPs among other nanoparticles, have emerged as a decisive and innovative delivery carrier owing to their exclusive mesoporous configuration to preserve chemical stability, biocompatibility, surface functionality by ensuring the regulated components-liberation and targeted contents-delivery of various active lead compounds [4,5].

The superior textual characteristics such as good biocompatibility and stability, high surface area, tunable pore diameter, large pore volume, narrow and ordered pore size distribution, high component-loading capacity, facile surface chemistry, and easy silanol-containing surface functionalizations associated with capping / or coating / or coupling of targeting ligands through chemical linking with other therapeutic molecules make MSNPs suitable for targeting

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lead compounds to a particular site in a controlled and sustained liberation manner [4,6-12]. In general, MSNPs interact with microbials and diseased cells to disrupt them through penetration of the cell membrane or by producing reactive oxygen and / or nitrogen species to cause cellular oxidative damages such as lipid peroxidation, mitochondrial dysfunction, aberrent aggregation of nucleoplasmic proteins and DNA destructions through different signalings e.g. apoptosis and necrosis [13-23]. This review demonstrates different surface modified MSNPs to consider as a potent delivery system in bio / nanomedicine for therapeutic applications against several diseases and for biofilm eradication.

Synthesis of mesoporous silica nanoparticles

MSNPs-synthesis is performed at a low concentration of surfactant as a structure-directing agent dependent upon the interactions between the cationic surfactant and the growing anionic oligomers of orthosilicic acid to produce their smaller sizes [4,24]. By hydrogen bonding and electrostatic interactions, the silica precursors such as tetrakis (2-hydroxyethyl) orthosilicate, alkoxides and pure alkoxysilanes, orthosilicic acid, glycerol-obtained polyol-based silanes, sodium metasilicate, tetramethoxysilane (TMOS) and tetraethyl orthosilicate are concentrated at the hydrophilic interfaces to form mesoporous amorphous silica [4,25] while the residual surfactants can be removed by extraction and calcinations method [26]. The synthesis procedures of MSNPs are mainly followed by microwave, hydrothermal, sol-gel and template methods [27-32].

Microwave synthesis method

Microwave, a kind of electromagnetic wave (frequency 300 MHZ-300 GHZ), is operated under the action of electromagnetic field with both outside and inside rapid heating, energy efficient and time saving, while MSNPs are synthesized by microwave irradiation within 6 h, resulting ordered pore size with good crystallinity [33-36].

Hydrothermal synthesis

For hydrothermal synthesis, surfactant as a template agent and alkali or acid as a catalyst are used, and then an inorganic substance is slowly added to the mixed solution for obtaining a hydrogel which is transferred to the autoclave to obtain high pressure and temperature for the reaction precursors for their separation with the removal of organic matter [37].

Sol-gel method

Sol-gel method provides controlled functionalization on the synthesized MSNPs-surface by their formation in the solution of glue body suspension with a subsequent three-dimensional network of polymer gel chain exhibiting simple equipment controlled operation, low temperature environment and high pure yield [38].

Template synthesis method

Template synthesis for surface activity in the solvents with alkaline or acidic conditions for forming micelles, inorganic precursors to micelles react slowly as template and subsequently burn back for forming ordered MSNPs [39-42]. The soft template method relates the non covalent bond between the inorganic reactants and the surface active agents, while the yield varies 10-1000 nm. The hard template method is used for filling an object with its template pores ranging 2-50 nm for obtaining ordered MSNPs (2-50 nm) after the removal of the template.

In brief, pyrogenic amorphous silica nanoparticles are synthesized in closed reactors by the alkyl chlorosilanes (CH_3SiCl_3, $HSiCl_3$, $SiCl_4$) hydrolysis in a hydrogen / oxygen flame at 1200°C-1600°C. Proto and subsequent primary particles of SiO_2 are generated by nucleation, condensation and coagulation process following their aggregation and agglomeration. Precipitated silica gel and silica nanoparticles consisting of haphazardly attached spherical polymerized primary particles are generally produced by the acidification of sodium silicate with sulphuric acid. The precipitate is then filtered, cleansed, dehydrated and milled with a controlled way to yield MSNPs having average 2-50 nm pore diameters. These uniform pores may be achieved by the reaction of tetraethylorthosilicate (TEOS) and template of surfactant amphiphilic polymers under acidic or alkaline condition following evacuation by calcinations or washing with a solvent [43,44]. Colloidal silica nanoparticles are synthesized through the partial neutralization of an alkali-silicate solution by ion exchange, electro-dialysis or acidification to form silica nuclei (1-5 nm) and then either fusion form in chains resulting silica gel by reducing pH <7 or adding salt or the gradual colloidal growth of the separated subunits by keeping pH slightly on the neutral alkaline side. The emerging colloidal suspension is then stabilized by the inclusion (upto 10%) of HCl, NH_3, NaOH or KOH or by substitution of electrostatic Si atoms such as Al to get higher negative charge keeping pH below the neutral point. The resulting suspension is then concentrated by liquid phase evaporation while hydrogen ions from colloidal silica surface incline to segregate in aqueous solution forming negative charges. Spherical colloidal silica nanoparticles having controlled uniform porosity and size are also synthesized by the alkyl silicates hydrolysis and consequent silicic acid condensation in ethanol and ammonia [45].

Functionalization of mesoporous silica nanoparticles

As the pore walls and the outer surface of the MSNPs possess silicon hydroxyl groups, their functionalization can alter the pore hydrophobicity / hydrophilicity [46], pore size and acidity [47], and adjust the chemical properties of the MSNPs-surface [48]. In this aspect, MSNPs modified by the amino group can effectively enhance the loading amount and prolong the sustained release owing to the strong interactions between the carboxyl and amino groups of the IBU. As the hydrophilicity of the amino groups is better than that of the silanol groups, the surface functionalization of the MSNPs can significantly improve the hydrophilicity of MSNPs by enhancing the hydrophilicity of the molecular loaded amount [49].

Co-condensation approach for creating bi-functional MSNPs was performed where organosilane (R-TES or R-TMS) (R, organic functional groups, TES, triethoxysilane, TMS, trimethoxysilane) is adjoined into a reaction solution containing tetraethylorthosilicate (TEOS), or instantly after TEOS-adjoining to afford incorporation of organosilanes in the final substance [50].

Surface functionalization with inorganic and organic substances can provide various functionalities of MSNPs for regulating diffusion and liberation of cargos and cell surface

recognitions among others. For biomedical applications, MSNPs have been designed to attach their biocompatible external surfaces with molecular or macromolecular moieties for providing tunable interactions with the biological environment for making the delivery system highly controllable [51-53]. The triggered cargo release from MSNPs may be performed through gating concepts where pore gating systems consisted of nanoparticles or bulky molecular groups such as gold, iron oxide -nanoparticles, proteins block the pore entrances to seal the interior mesoporous environment [48,54,55] while the macromolecular structures generally become degradable or anchored to the MSNPs-surface through linkers cleaved by stimuli exposure [56,57]. Better pore sealing can be achieved by a complete MSNPs coating of oligonucleotides, polymers and lipids [58-67] while competitive displacement or phase transitions reactions may lead to pore openings and efficient cargos delivery [68,69]. The other strategy for controlled component release relates component attachment in the porous system of the silica nano-vehicles where covalent or coordinative bonds may be cleaved by stimuli such as reducing agents or competitively binding molecules for activating components liberation [70-72].

Characterization of mesoporous silica nanoparticles

The morphology and particle size of MSNPs may be detected by Field Enission Scanning Electron Microscopy. The homogeneity and porous structure of the MSNPs may be identified by the High-Resolution Transmission Scanning Electron Microscopy. The particle size distribution and the zeta potential of the MSNPs may be determined by using Dynamic Light Scattering. The textural properties may be analyzed by nitrogen adsorption isotherm utilizing Micromeritics ASAP Porosimeter following the Brunauer-Emmet-Teller method where the pore size and the specific surface area (S_{BET}) are calculated from the desorption curve, and the average pore diameter is worked out from the pore volume presuming cylindrical pore and S_{BET} determined by BET surface area.

Mechanism of action of silica nanoparticles

Silica nanoparticles upon exposure to cells can generate endocytosis- dependent or independent silicon-based free radicals such as **Si**$^{\cdot}$ SiO$^{\cdot}$ and SiOO$^{\cdot}$ owing to nanoparticles' oxidation [73,74]. These radicals, inturn, induce reactive nitrogen species (RNS) and reactive oxygen species (ROS) generations directly or by cell-activation. In this aspect, $O_2^{\cdot-}$, followed by H_2O_2 and $^{\cdot}OH$ generations derived by the NADH oxidase and NADPH dehydrogenase activities, and NO/ONOO- generation from activated NO synthase, and silicon radicals, by binding proteins, lipids, nucleic acids through thiol, carboxylic, azide, amine and phosphate groups, can produce cellular oxidative damage supported by oxidative stress-induced membrane disruption, DNA destruction or aberrant aggregation of nucleoplasmic proteins, cell cycle arrest, apoptosis and necrosis [75-77]. The involvement of mitochondria for the generation of ROS relates the leakage of electron transport chain i.e. its pathway damage where cellular apoptotic death takes place by mitochondrial intrinsic pathway and receptor-mediated extrinsic pathway [78], and cellular necrosis occurs due to the massive cell membrane damage leading to rapid intracellular ATP levels reduction and cellular osmotic balance loss [79]. Silica nanoparticles may also be adsorbed to the cell-surface due to electrostatic interaction affinity resulting membrane damage by abrasion and binding to other sub-cellular organelles to deactivate their normal functions.

Mesoporous silica nanoparticles as delivery system

Drug delivery

MSNPs display unique features as ideal nanocarriers to host for protecting them through transportation of cargos to the target site for their feasibility to integrate targeting components in the external surface for directing to the affected tissues with increasing specificity and diminishing toxicity. In this context, their pore entrances may be capped by utilizing stimuli-responsive gatekeepers to maintain and operate pore opening for cargo-release to the target.

For selective passive targeting, MSNPs can extravasate through enlarged pores of capillary endothelium to reach tumor site by enhanced permeation and retention activity [80,81], whereas for active targeting, surface functionalizations of MSNPs with molecules make them capable in targeting for selective interactions with specific membrane receptors possessed in tumor cells. The surface modification of MSNPs with targeting ligands directs them for their affinity towards the blood vessel to irrigate solid tumor while tumor destruction takes place by disrupting nutrients and oxygen supply [82]. Recently MSNPs functionalized with folic acid and triphenylphospine to bind to folate receptor and mitochondrial membrane respectively for cancer cells have been developed for their targeting as efficient antitumor therapies [83]. As MSNPs have poor penetration ability on diffusion within tumor mass owing to the residence of collagen-rich extracellular matrix, these nanocarrier systems have been proposed to design a pH-sensitive collagenase attached with coating of radical polymerization of acrylamide as the structural monomer, 2-aminoethylmethacrylate for providing amino groups to attach to nanoparticles' surface, and ethylene glycol dimethacrylate as the pH-partible cross-linker [84]. Under acidic pH of solid tumor environment, functionalized MSNPs break triggering the collagenase-liberation for digestion the extracellular matrix leading to their improved penetration to the tumor site. In this concern, as human mesenchymal stem cells (MSCs) have also the capability to migrate towards tumors, MSCs and doxorubicin loaded-MSNPs have been applied as a tool for efficient cancer cell -death both *in vivo* and *in vitro* [85,86].

Internal stimuli such as pH, enzymes and redox potential, and external stimuli such as light, ultrasound and magnetic fields may be accomplished through attaching pore blocking caps throughout linkers to cleave and liberate cargo to the target site. The capping agents such as polymers, macromolecules or inorganic nanoparticles can seal the mesopore entrances by hindering premature components release. The coatings of inorganic and organic chemicals may also be used as blocking caps and capable to degrade under stimulation allowing pore uncapping and cargo release. The incorporation of super paramagnetic iron oxide or other metals within MSNPs allows the employ of alternating magnetic fields and physicochemical changes by triggering temperature increase while gatekeeper moieties become capable to provoke pore opening and drug release to target area upon exposure [87-92]. Mechanophores i.e. chemical bonds e.g. of 2-tetrahydropyranyl methacrylate (hydrophobic monomer) may be cleaved and transformed to

hydrophilic methacrylic acid under ultrasound stimulus while this moiety is used for MSNPs drug delivery as mesopore gatekeeper [93-96]. MSNPs are also decorated with porphyrin nanocaps attached through ROS-cleavable linkages while porphyrin blocking caps upon visible light exposure induce singlet oxygen molecules to split the sensitive linker and activate the mesopores-openings to liberate cargo to the target site [97].

Gene delivery

Nucleic acids, including DNAs such as plasmid DNAs (pDNAs) which act through deficient gene re-expression in diseased tissues, endogenous micro RNAs (miRNAs) and exogenous small interfering RNAs (siRNAs) which are capable of recognizing endogenous target mRNAs via sequence complementarity to control gene expression via repressing translation or inducting degradation by RNA interference (RNAi), and antisense oligonucleotides (ASOs), the single-stranded nucleic acids and complementary to endogenous mRNAs, which can attach with to control mis-spliced mRNAs through their inactivations, are all potent gene regulators as therapeutic drug candidates [98-100]. To protect nucleic acids from poor membrane permeability and serum unstability, nucleic acid-MSNPs complexes have been prepared through electrostatic and hydrophobic interactions as simple nucleic acids loading onto MSNPs and their rapid liberation upon delivery into cells. Several investigators showed their nanoparticles' encapsulation and delivery in different ways where amino molecules utilized to functionalize MSNPs-surface for pDNA loading [101,102], polyethyleneimine coating on MSNPs-surface used to anchor DNA and siRNA [103,104], poly-arginine and poly-lysine coated on MSNPs-surface to condense siRNA [105,106], which may be associated with different cargos-loading inside the MSNPs-pores [107] to assist their deliveries into cells [108]. MSNPs may also be functionalized with various targeting ligands through basic chemical reactions such as maleimide / thiol ally, condensation reactions between isothiocyanates and amines or between amines and carboxylic acids to deliver DNAs through sugar receptor-mediated, siRNA through folate receptor-mediated specific cells [109-112]. In this context, nucleic acid-guided therapy implies intracellular delivery of nucleic acid-MSNPs complex while they are endocytosed into cells via receptor or non-specific -conciliated cellular accumulation followed by their disappearance from the endosome / lysosome, and nucleic acids liberation from the complexes into the cytoplasm to employ their biological activities [113]. PEI-overlay of MSNPs on the DNA delivery efficacy [103] and miR-34a encapsulated MSNPs conjugated with an antibody which targets the cell surface antigen disialoganglioside GD2 for selective delivery were investigated by some researchers [114]. siRNA human epidermal growth factor receptor 2 (HER2), anti HER2 and siRNA vascular endothelial growth factor (VEGF), were loaded separately onto MSNPs to deliver them into HER2-positive breast cancer cells and angiogenic tumor cells respectively [115-118]. In this aspect, P-glycoprotein siRNA and doxorubicin loaded in MSNPs showed their inhibitory delivery efficiency against drug-resistant breast cancer cells [119]. Upon small molecule-ASO-MSNPs complexes delivery into the cells, small molecules inside the nanoparticles-pore could not restrict miRNA in the beginning owing to the capping of the pore with ASOs from entry of small molecules to cytoplasm but the anchoring of ASOs with endogenous miRNAs exposed the pore and liberated the encapsulated cargos into the cytoplasm to realize dual-restriction of miRNAs such as miR-122 and miR-21 expressed in hepatocellular and ovarian cancer cells respectively [120-121].

Biodistribution and elimination of mesoporous silica nanoparticles

Biodistribution of MSNPs in animal differs depending on their administration route such as intravenous, intra-peritoneal, oral and subcutaneous associated with the particles' shape, size, surface modifications, charge and concentration. It was investigated that MSNPs were accumulated more in tumor site, and the highest accumulation of the particles was monitored in the kidney and lungs, and lower uptakes in liver, heart, intestine and spleen [122]. Folate-anchored MSNPs showed enhanced tumor accumulation for their specific targeting to cancer cells. In this concern, MSNPs composed of -Si-O-bonds become susceptible in an aqueous medium to nucleophilic attack by hydrolytic water hydroxide into orthosilicic acid ($Si(OH)_4$) from siloxane (Si-O-Si) which is biocompatible and eliminated easily through the activity of the complement system or urination [123-126]. Another study demonstrates that positively charged nanoparticles have been eliminated from the liver through gastro-billiard system in the feces, while negatively charged particles have been sequestered within the liver [50].

Immunotoxicity of silica nanoparticles

MSNPs, upon entering the body, probably interact with immune cells monitored in various cell lines. THP-1-derived macrophages were exposed with 100 μg/mL silica nanoparticles (30 nm) for 6 h, while a significant enhancement of interleukin-1-beta (IL-1β) via phagocytic uptake of nanoparticles was observed [127]. Larger sized silica nanoparticles (150-200 nm) phagocytosed by microglial cells after 24 h exposure to various concentrations (0.0728-7.28 μg/mL), also showed a significant increment of IL-1β [128]. Silica nanoparticles (10 and 50 nm) showed dose-dependent (6.25-100 μg/mL) enhancement and decrement of TNF-α and IL-6 productions respectively in RAW.264.7 macrophages, while their amino surface-functionalized counterparts showed insignificant toxicity [129].

CONCLUSIONS AND FUTURE PERSPECTIVES

MSNPs appear as promising delivery device as they combine their constituents' stability and biocompatibility with versatile chemistry on the control of dimensions, morphology and surface properties, and are capable of encapsulation or grafting with various active components to release them in a sustained manner to target site overcoming the biological barriers to destroy microbes, infected cells, drug resistant cells and biofilms. Immune system upon the exposure of MSNPs, induces cascades of events such as the release of endosomal substances, productions of ROS, RNS, cytokines and chemokines resulting inflammation though the responses are regulated by the designing of the size, shape, surface area, surface charge and porosity of the nanoparticles. Therefore, it is needed to coat cargos-encapsulated MSNPs with ligand to minimize any toxicity and to target components to specific site of interest for proper biomedical application against different

diseases. In this concern, thorough in vivo study regarding their biodistribution, biocompatibility, degradability, elimination, pharmacokinetics and toxicity should be investigated systematically before their clinical translation.

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