Silica based-matrices: State of the art and new perspectives for therapeutic drug delivery

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Abstract

Colloidal carriers based on silica matrices are an innovative approach within the context of therapeutic drug delivery systems. These carriers are emerging as a great promise for diagnosis and treatment of a wide range of injuries, particularly in cancer and infection diseases. **In addition, bioencapsulation** for biosensing and cell therapy in silica sol-gel allows the survival of enzymes and cells for a long period of time. Due to their porosity, large surface area and high capability of functionalization, silica nanoparticles have been considered an attractive option for several bioanalysis applications, such as selective bioseparation, imaging and drug and gene delivery. However, although great advances are achieved in the biomedical fields, some toxicity effects can be associated to the use of silica nanoparticles. The present article aims to give a comprehensive review of recent technological advances for silica matrices in biomedical applications, as well the potential impact of silica-based materials to **human** health and environment.

Keywords: Amphiphilic materials, biosensors, biomedical, microcarriers, silica, cell encapsulation, drug delivery, biosensor, imaging, toxicity

1. Introduction

Extensive efforts have been conducted in the development and design of better, intelligent therapeutic agents and devices for clinical diagnosis and drug delivery and targeting. Among the different "smart biomaterials" developed by biological and biomedical purposes, silica-based matrices offer new possibilities for bioimaging, bioseparation, biosensing, cell and enzyme encapsulation, as well as for gene and drug delivery [1, 2].

Amorphous silica-based scaffolds can be synthesized by sol-gel technology and show important characteristics that make them successful matrices for cells and enzymes immobilization, such as (i) the preservation of chemical and function of entrapped biomolecules or cells; (ii) control of shape, size and porous, providing a suitable local for host cell viability with diffusion of nutrient and oxygen; (iii) the silica degradation can be carried out by chemical process and (iv) silica matrices are resistant to microbial attack [3-5]. **Silica matrices can be loaded with biomolecules, as well as with biosensing molecules, which given their optical properties, improve the signal transducers and thus may be exploited for diagnostics.** The sol-gel technology has also been used for developing silica and silica derivatives as scaffold for drug delivery, allowing better control over drug loading and release.

After the successful discovery of the mesoporous materials as drug delivery systems, mesoporous silica nanoparticles (MSNs) have attracted special attention in biotechnology and pharmaceutical sciences [4, 6], presenting many interesting features for drug encapsulation, such as: (i) high porosity that can accommodate large amounts of pharmaceutical agents, (ii) high surface area that allows better contact with the drug and (iii) biocompatibility that implies their use without toxicity or inflammatory effects [7].

In the nanotechnology field, the pH-responsive drug delivery systems have attracted great interest in regulate the drug release at targeting desired sites. In particular, silica nanoparticles have demonstrated to release large amounts of chemotherapeutical agents (e.g., doxorubicin and paclitaxel) in tumor cells, resulting in high selectively for cancer cells, thus avoiding accumulation in health tissues [8].

The high capability of silica nanoparticles functionalization allows the attachment of molecules with various reactive functional groups (e.g., carboxyl, amine or thiol) that can even improve the stability of colloidal system in aqueous medium [9], reducing nonspecific binding by inhibiting the biomolecules adsorption [10] and act as scaffolds for the grafting of

biomolecules entities, such as antibodies, DNA, proteins or peptides [11]. Also, for cell detection, fluorescent silica nanoparticles have been widely explored due to high photostability compared to single dye molecules [12]. In addition, silica coated-magnetic nanoparticles are able to separate traces amount of target DNA/RNA molecules from a complex mixture with high specificity, being an important technique to identify even oncogenes [13].

Advances in molecular biology in the treatment of a wide range of diseases have enabled the engineering of an innovative strategy based on multifunctional silica nanoparticles with precise delivery of therapeutic and diagnostic agents mainly for cancer and infection diseases [14]. With one structure, **it** is possible to combine diagnosis and therapeutic treatment (theragnostic) to obtain faster and precise diagnostic assays, allowing improved biomedical and bioengineering applications.

Considering their wide range of applications, the impact of silica nanoparticles on human health and on the environment is of great interest. In the toxicology field, nanoparticles can be taken up by different types of cells via either active or passive mechanisms **(i.e. diffusion)**, depending on their physicochemical properties, such as size, shape, surface charge and coating [15]. Microscopic analysis demonstrated the presence of normal cell morphology after the MSNs uptake [16]. However, many studies suggest that the physicochemical properties of silica nanoparticles might provide potential risk to human health, causing toxic events especially in the liver [17-20]. **This review reports the recent technological advances for silica matrices in biomedical applications, discussing the potential impact of silica-based systems in human health and environment.**

2. Amorphous silica-based scaffolds

2.1. Properties of gels and drug release

Sol-gel technology has been widely employed for preparing silica matrices. This technology provides several possibilities to synthesize various types of gels, which structure depends on many parameters, such as type and concentration of precursors, water to precursor ratio, nature and concentration of catalyst agent, solvent, temperature, as well as aging and dry processes [21-24]. Sol-gel technology involves the synthesis of colloidal particles by the hydrolysis and condensation of alkoxysilanes precursors under acidic or basic environments.

Silica gels obtained by sol-gel process are usually classified according to the dispersion medium used in the gel preparation. Hydrogel consists in hydrophilic polymer networks with three dimension configuration enabling high amounts of water biological fluids [25, 26]. In contrast, an alcogel is a colloidal gel which alcohol is the dispersion medium. After gel preparation, gel aging and drying can lead to the production of a xerogel or an aerogel. A xerogel is based on a solid from gel by drying at ambient pressure. This kind of drying usually results in shrinkage of the network, expulsing the liquid from the pores. The shrinkage mechanism is generally attributed to the new bond formation through condensation reaction.

Alternatively, when solvent removal occurs under supercritical conditions, the surface tension between liquid and vapor can be avoided and an aerogel is produced without porous collapse [27]. Aerogels consist of pearl-necklace –like network of particles which 99% of their bulk volume is empty [28].

Although, sol-gel based silica xerogels and aerogels have been exhaustively investigated as drug delivery carriers [29, 30] the release mechanisms of drug from the gels have not been elucidated. Data demonstrate that the control release can be based on simple diffusion or a combination of the diffusion and erosion process [31, 32]. These processes are affected by several aspects, such as, molecular size and chemical characteristics of the drug, the matrix size, pH, interactions between the drug and the matrix and the texture of the gel produced [33]. The hydrolysis and condensation reactions can be influenced by water/alkoxide increasing the surface area that can control and sustain the release of encapsulated compounds [34].

Recently, an interesting pH responsive sol-gel hybrid hydro-xerogel for drug release of doxorubicin was reported by Angelopoulou and co-workers [35]. For this purpose, the authors used a Si/Ca or Mg based-xerogel with a dextran hydrogel. The hybrid showed good apatite deposition properties. For doxorubicin, the release is more pronounced under low pH, reaching ~70% of drug released after 180 h. In contrast, at neutral pH, only 20% of doxorubicin is released. The authors envisage that such observations can be used in bone cancer therapy.

2.2. Sol-gel for biological species immobilization

Enzyme-based electrode has been widely studied for developing sensitive and selective biosensors. The traditional method of enzyme immobilization consists in non-covalent or covalent attachment [36]. However, biosensor development for enzyme immobilization may be limited due to the lack of simple and generic protocols. Thus, a simple and low cost method to immobilize and stabilize enzymes is required.

Sol-gel technology has been extensively used for encapsulating a large number of proteins and cells. The produced sol-gel glasses can protect the biomolecules from denaturation, maintaining their activities, as well as can permit the growth and proliferation of entrapped cells. However, several factors could affect the bio species encapsulation in sol-gel matrices [37, 38]. The hydrolysis and condensation of silicon alkoxides lead to the release of alcohol molecules as byproduct, being a potential obstacle for enzyme and cell encapsulation. Many approaches have been adopted to avoid the presence of alcohol. One way, is the alcohol removal via evaporation under vacuum. The alcohol-free route has been used for encapsulating horseradish perosidase (HRP), hydroxynitrile lyase (HbHNL) or allophycocyanin (APC) [39]. However, the removal of alcohol is not complete, since its release can proceed for prolonged periods. Thus, aqueous sol-gel route using sodium silicate as precursors has been developed to avoid the generation of alcohol. The encapsulation is carried out at room-temperature and neutral pH, minimizing the degradation of biomolecules. Horseradish peroxidase (HRP) and glucose-6-phosphate dehydrogenase (G6PDH) were successfully encapsulated in this aqueous route processing [40]. The use of biocompatible alcohols such as polyol-based silanes can also avoid denaturation by alcohol. The stability of entrapped enzymes is improved, since glycerol is produced under hydrolysis reaction. Recently, the use of diglyceroxysilane (DGS) as precursor and poly(ethylene glycol) (PEG) as stabilizer agent has been employed for HRP immobilization in **monodispersed** spherical silica nanoparticles, which large PEG molecular weights lead to better steric stabilization and more monodisperse particles [41].

Glucose biosensor analysis was developed by combining immobilized glucose oxidase in silica gel with an oxygen sensing film using sol-gel technology [42]. Even in the presence of high concentrations of ascorbic acid or chloride ion, no interference was detected and the activity of enzyme was preserved after 2 months of storage. In addition, the evaluation of glucose concentrations was conducted using a 96-well plate and a fluorescence plate reader t

hat permit an optimization of the tests in a laboratory scale.

Table 1 summarizes the various enzymes entrapped in silica sol-gel matrices for biosensor **applications**.

[Please insert Table 1 about here]

As described above, another exciting research area is the development of sol-gel based matrices for tissue derived cell growth aiming at cell therapy and at living organism immobilization. However, active cell immobilization still remains a challenge. Cells are more sensitive than biomolecules, requiring material with high biocompatibility, as well appropriate immobilization methods. In addition, nutrients, oxygen and degradation products should be easily diffused by the material porous [49]. **Thus**, sol-gel matrices can also act as template allowing the growth and proliferation of the host cells and can be remodeled in a living tissue, as well as promote successful immobilization of numerous microorganisms as shown in Table 2. Although the harmful of alcohol release during the silica-gel preparation using alcoxides precursor has been exhaustively described, encapsulated cells by alcoholic route demonstrated high survival and long-term viability. These advantages can be attributed to the better mechanical, toughness and transparency properties of the gel prepared by alcoholic route [50, 51].

[Please insert Table 2 about here]

3. Silica at the nanoscale level

3.1. Stöber method

Although some works have performed the production of nanoparticles under acidic conditions [57], Stöber **method** has been widely applied for the preparation of colloidal monodispersed silica spheres between 50 and 2000 nm size, and it is represented as follows:

The method is based on hydrolysis and condensation of TEOS under alkaline conditions in a water-alcohol solution [58]. The formation of nanometer silica particles highly depends on the reaction parameters. By optimizing the concentration of TEOS, ammonia, water and alcohol, Park et al. [59] synthesized ultra-fine silica particles with a mean size of 13.7 ± 4.5 nm. The effect of these factors on the resulting particle size and distribution of silica particles has been extensively studied. The particle size can increase with the increase of TEOS and ammonia concentrations. This phenomenon is attributed to the increase of primary particles at the induction period leading to secondary particles and consequently aggregation. The presence of ammonia increases of hydrolysis and condensation rate of TEOS, resulting in the increase of silica nanoparticles [60]. However, some works have demonstrated with increasing the ammonia concentration smaller particles were produced [61]. In the presence of higher water concentration a high nucleation rate and formation of sub-particles during a shot period occurs. Moreover, hydrogen bond of produced sub-particles is stronger at higher water concentration due to excess of water, causing agglomeration and formation of larger particles [61]. In contrast, some studies relate that at extreme water concentration, the particle growth can be inhibited due to the incomplete hydrolysis and condensation reactions [62]. Although, conventional Stöber **method** has been widely used to synthesize silica nanoparticles, this method presents some disadvantages, such as the lack of size control of particles and the incompatible with biomolecules stability, due the presence of high alcohol concentrations, as well as the extreme alkaline pH.

3.2. Microemulsion technique (polymerization)

Several techniques have been developed for producing silica nanoparticles with controlled size using the compartmentalization of sol-gel solution into nano droplets that work as nano-reactors. To synthesize particles with dimensions comparable to these nano-reactors, two approaches have been employed, such as spray-drying and emulsion polymerization.

Spray-drying process consists in a production of silica microspheres by atomization of pre-hydrolyzed sol-gel solution into a heated reactor, evaporating the fluid within the droplets. The droplets size is usually controlled by many parameters, including sol-gel viscosity and surface tension as well as by flow rate and atomizer characteristics [3]. Although this process can produce controlled release microspheres, several limitations can result from this method.

Since the spray-dried produced particles are non-pores, the release rate is controlled by dissolution of the silica matrix. This phenomenon can lead to a bust release in the sol-gel isoelectric point and when using less water-soluble drugs and coagulation of the droplets inside the reactor due to increasing the water to alkoxide ratio [63, 64]. In addition, the particles synthesized by spray-drying are limited to the micrometer range, and thus, they cannot be applied in intravenous administration. Finally, the right temperature used during the processing prevents its application for encapsulating biomolecules such as, enzymes and other proteins.

The combination of water-in-oil emulsion (W/O) with alkoxide hydrolysis contributes to better assembling of these materials and retains the release control of sol-gel technology. The technique is based on the immiscibility between apolar organic solvent and polar sol-gel solution leading to a compartmentalization of sol-gel within water droplets stabilized by a surfactant. The water droplet size is directly controlled by the emulsion parameters, including the used solvent, nature of surfactant and water/surfactant ratio [65].

In recent years, some sol-gel/emulsion methods have been adopted to synthesize silica particles [66-69]. By combining sol-gel technology with emulsion polymerization, Barbé et al. [70], demonstrated that silica micro-and nanoparticles can preserve the control of the release rate of encapsulated active agents when in comparison to xerogel monolits. However, this process is limited to small hydrophilic molecules, and the presence of solvents and surfactants may be harmful for biomolecules encapsulation. Therefore, this technique is always associated to extensively centrifugation and washing cycles to remove the surfactant and solvent molecules before the contact of biological species.

4. Silica matrices as versatile nanomedicine scaffolds

4.1. Cell Imaging

Cellular imaging using fluorescent optical microscopy is an important technique in biomedical application to monitor several tissues, and thus, to recognize defect cells. Quantum Dots (QD), gold nanoparticles, organic dyes and phosphors have been extensively used for tumor detection [71]. However, the high production cost of gold nanoparticles and phosphors, as well as the decomposition, short lifetime and accumulation of heavy metal ions in the body, in the case of QD [72, 73]. Therefore, their application in life science is limited.

Very recently, Wang and co-workers [74] developed a new strategy for synthesis of near infrared (NIR) PbS-QD functionalized with a silica-polymers dual layer, leading to an improvement of PbS-QD stability, as well as prevention of PbS-QD ionization in Pb^{2+}, eliminating, thus the cytotoxicity events.

Silica nanoparticles can also act as carriers for dye molecules, being a great potential for bioimaging. Compared to a single dye molecule, dye-doped silica nanoparticles demonstrated a highly signal quality with greater sensitivity and photostability without cytotoxic effects [75-78].

Several organic dye molecules can be doped into silica nanoparticles. Recently, Accomasso and co-workers [79] reported the development of fluorescent silica nanoparticles using cyanine dyes prepared *via* microemulsion method. Cyanine-doped silica nanoparticles easily penetrate into stem cells and did not affect the cell viability and growth which are essential properties for cell tracking agent.

4.2. Biomolecular and cell separation

Magnetic nanoparticles of iron oxide have been widely developed for many applications in, for example, drug and gene delivery [80], magnetic resonance imaging (MRI) [81], magnetic hyperthermia for tumor cells [82], biolabelling and biomolecular separation [13, 83]. Magnetic separation has the ability to separate biological molecules and cells due to the external magnetic field exerted in magnetic nanoparticles. However, these nanoparticles tend to aggregate and can lead to the potential toxicity to the biological systems. To circumvent such issues, silica-coated magnetic nanoparticles have been successfully used in bioseparation of nuclei acids, cells and peptides.

In 2003, Zhao and co-workers developed the first bioseparation in a functionalized silica surface [84]. The authors created genomagnetic nanocapturers (GMNC) for collection and detection of DNA/mRNA molecules by hybridization events followed by magnetic separation. GMNC is constructed by bioconjugation of DNA probe molecules at magnetic nanoparticles surface via avidin-biotin linkage.

Silica-coated magnetic nanoparticles have been also developed to detect the presence of DNA sequences related to human immunodeficiency virus (HIV) [85]. This strategy is based on the hybridization reaction between Ag/SiO2 core-shell nanoparticles-based RAMAN tags functionalized with oligonucleotides (detection probe) and the amino groups functionalized

silica-coated magnetic nanoparticles (capture probe). The silica coating demonstrated signal amplification of hybridization assay, allowing better DNA detection sequences by RAMAN spectroscopy.

4.3. Drug Delivery

After the discovery of mesoporous materials for drug delivery, ordered MSNs have attracted considerable attention on biomedical application. Mobil Crystalline Materials (MCM-41, MCM-48) and Santa Barbara Amorphous (SBA-15) are examples of mesoporous silica that consist in a honeycomb-like porous structure with mesopores (2-10 nm) suitable for encapsulate large amounts of drug [86].

MCM-41 is the most type of MSNs used for controlled drug release. Generally, a cationic surfactant is employed as template in the synthesis of MCM-41, such as cetyltrimethyl-ammonium bromide (CTAB). When CTAB concentration is above the critical micelle concentration (CMC), the surfactant can self-aggregate into micelles. Thus, the silica precursor is hydrolyzed at the polar region of micelles, leading to the silica matrix around the micelle surface. The removal of CTAB results in MCM-41.

Unlike MCM-41, MCM-48 possesses bicontinuous channels, which can confer more suitable drug loading and release [87]. However, the MCM-48 synthesis under cationic or anionic surfactant as templates and high temperatures may produce particles in micrometer dimension. Methods for circumventing this problem have been suggested, such as utilizing modified Stöber method at room temperature employing pluronic F127 as surfactant to synthesize monodispersed spherical MCM-48 nanoparticles [88].

As alternative to the use of surfactants, amphiphilic triblock and diblock copolymers under acidic media have been extensively used as template for synthesizing silica mesoporous, such as SBA-15, which possesses a 2D hexagonal symmetry (p6mm) [89, 90]. In comparison to MCM-41, SBA-15 possesses larger sizes (~200 nm), thicker pore walls and wider pore sizes ranging of 5 to 30 nm.

Due to the easy of introducing of several organic groups onto MSNs surface by covalent bounding or electrostatic interaction, various interesting hybrid structures have also been investigated. The preparation of single colloidal mesoporous silica nanoparticles coated with an intact supported lipid bilayer using three different kinds of lipid has been reported. The colloidal mesoporous silica nanoparticles were synthesized by sol-gel method using TEOS as

silica source and the surfactant CTAB as pore template. The resulting nanoparticles were then suspended in the lipid solution. The hybrid system loaded with the anticancer drug colchicines are readily taken up by cells and lead to the depolymerization of microtubules with remarkably enhanced efficiency as compared to the same dose of drug in solution. The potential use of the hybrid was demonstrated by delivery of colchicine into HuH7 liver cancer cells. The diffuse release of the drug inside the living cells inhibits, after 120 min, the microtubule polymerization and hence induces cell death, increasing drug efficiency. In addition, this novel drug delivery system presents the advantage of providing a stable colloidal suspension in aqueous media, carrying large amounts of drug inside the mesoporous [91].

Recently, several studies have used PEG coating on the surface of various nanoparticulate systems make them water-soluble and/or biocompatible [92, 93]. PEG is an important polymer used for drug delivery due to its water solubility and its ability to prevent opsonization, consequently increasing circulation lifetime of the drugs or vehicles [94]. In order to study the potential application as intravenous drug delivery vehicle, PEGylated silica spheres have been successfully synthesized. Immobilization of bovine serum albumin was carried out to evaluate the protein adsorption after PEGylation. The addition of PEG onto silica surface can avoid the RES compared to bare silica. The influence of PEG's chain length was also analyzed, demonstrating that PEG concentrations above 300 g/mol maintain constant the reduction of protein adsorption [95].

The oral absorption of peptides is hindered by several difficulties, such as their high molecular weight and hydrophilicity [96], low pH of gastric medium and presence of enzymes, leading to low oral bioavailability [97]. Thus, parenteral administration is the principal route for these therapeutic drugs. To overcome such barriers, the development of protein delivery systems that maintain its stability, as well as release the drug near the site of absorption and prolong its residence time through the small intestine is needed. However, there are few studies based on silica nanoparticles for protein oral delivery.

Liposomes coated with silica have been explored as protein delivery, improving its stability as well as its encapsulation efficiency. Silica coating was synthesized by polymerization reaction of silica source under acid catalysis at room temperature. The anionic silanol groups interact with cationic phosphatidylcholine (PC) leading to silica coat over insulin-encapsulated PC vesicles. The acidic catalyzed polymerization reaction can improve the formulation stability, as well as it prevents the insulin denaturation. In addition, *in vivo*

studies demonstrated that insulin remains biologically active, reducing, thus, the glucose levels in comparison to standard insulin by parental route [98].

Mesoporous silica structures with different pore sizes have also been investigated for insulin delivery. Tozuka and co-workers demonstrated **that adsorption of insulin** on silica mesoporous **could be achieved** by freeze-thaw method [99]. Concerning the pore size, the desired insulin release profile **can be designed since e.g. smaller pore sizes usually** lead to **faster** release of insulin. However, although this investigation published the application of mesoporous as a carrier for insulin, do not provide any information about the protein stability. Other drugs have also been incorporated in SiNP (Table 3).

[Please insert Table 3 about here]

4.3. Gene Therapy

Gene therapy has gained more attention as a promising strategy for the treatment of several disorders, such as cancer and infections' diseases. The most challenge in gene delivery processes is the optimization of gene carriers that confers not only gene transfection efficiency but also low toxicity.

The successful plasmid DNA (pDNA) gene delivery was achieved *in vivo* rat Achilles tendon using MSNs [106]. The release of pDNA entrapped in MSNs was sustained for at least 2 weeks with gradual decrease of the luciferase activity. Plasmid encoded Platelet Derived Growth Factor (PDGF) gene in MSNs demonstrated high gene transfection in injured tendon, resulting in a strong organization of collagen fibers with no inflammation or necrosis signs.

He and co-workers [107] reported the development of a hybrid for non-viral gene delivery. The authors synthesized PEI-SiNP via Michael addition followed by crosslinking of silaxanes. The hybrid nanoparticles complexed-DNA showed higher internalization in COS-7 cells in comparison to PEI-DNA complexes. Also, PEI-SiNP demonstrated serum-resistant properties with low cytotoxicity.

Another example involves *in vitro* and *in vivo* SiRNA delivery by an expansion of amine functionalized-MSNs porous with an average of 23 nm [108]. The expansion of silica pores allowed high loading capacity of SiRNA, as well as maintained the chemical stability of the nucleic acid upon exposures to nucleases. Fluorescence microscope images indicated the

suppression of blood vessel by down-regulation of vascular endothelial growth factor (VEGF).

5. Multifunctional silica nanoparticles: A new approach in theragnostic nanomedicine

Theragnostic systems are a recent approach that associates diagnostic and therapeutic agents in one single structure. This approach has the ability to integrate imaging and therapy aiming to improve the response to the treatment by means of optimizing imaging information, drug selection and safety. In this context, multifunctional silica nanoparticles have been developed for simultaneous diagnostic and therapeutic purposes.

Wang and co-workers [109] developed a multifunctional system based on mesoporous silica nanocages for imaging, drug delivery and photodynamic therapy. Doxorubicin was loaded into silica porous and released in a sustained behavior at low pH. Fluorescein isothiocyanate (FITC) was used as dye molecules for fluorescent imaging and hematoporphyrin molecules were also covalently attached in the nanocages for photodynamic therapy.

A versatile multifunctional hybrid designed for drug delivery and MR imaging was reported by Hyeon and co-workers [110, 111]. The authors synthesized two kinds of systems using MSNs coated with Fe_2O_3 nanocrystals nanoparticles or core-shell MSNs using Fe_2O_3 nanocrystals as core. The multifunctional properties of both systems were demonstrated by fluorescence imaging with FITC or rodhamine B isothiocyanate (RITC) dye molecules, MR imaging allowed by Fe_2O_3 nanocrystals, and drug delivery with the incorporation of doxorubicin.

Another example developed for multifunctional applications is described by Zhang and co-workers [112]. Fe_2O_3 coated with mesoporous silica nanocapsules were functionalized with PEI for fluorescent imaging and magnetically guided siRNA delivery. Fluorescent dye (e.g., RITC) was incorporated into Fe_3O_4 nanoparticles to obtain fluorescence imaging. PEI was used as cationic polymer for siRNA attachment by electrostatic interactions between PEI amino groups and phosphate groups of siRNA. In comparison with Lipofectamine 2000, a commercial transfection agent, PEI- Fe_2O_3-SiO_2 demonstrated better siRNA delivery.

These recent evolutions in the nanotechnology field can open the door for new biomedical applications in cancer diagnosis and therapy.

6. Biocompatibility and toxicological concerns

The concept of nanotoxicology has emerged recently, after the rapid development and introduction of a significant number of nanomaterials in the industrial processes and consequently in the market [113].

Recently, several studies have shown the cytotoxicity effects of silica nanoparticles, including reduction of cell viability and oxidative damage in cellular membrane [114, 115]. For example, it is confirmed that silica nanoparticles could induce oxidative stress, proinflammatory responses and clusters of topoisomerase I in the cellular nucleoplams, as well as, they could promote fibrogenesis in Wistar rats [116, 117]. In addition, long term exposure to silica nanoparticles could also accumulate in embryonic cells [118]. Ye and co-workers [119] investigated the effect of silica nanoparticles on myocardial H9c2 (2-1) cells at different doses for various periods of time. Results showed that silica nanoparticles produced cytotoxicities in size, dose and time exposure dependent manners. The particles caused oxidative stress, induced G1 phase arrest and up-regulated levels of p53 and p21.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a widely used test for determining the potential cytotoxicity of various agents and pharmaceutical molecules in cell cultures. MTT is reduced in living cells by mitochondrial system or by membrane enzymes of endosome/lysosome complex [120]. Mesoporous silica nanoparticles can affect the **exocytosis** of MTT, decreasing the MMT reduction. This observation can be attributed to uptake of silica nanoparticles by cells. The mechanism by which the particles enhance amount of MTT crystals on external cell surface membrane is not still elucidated. **The stimulation of MTT formazan exocytosis has been attributed to perturbation of intracellular vesicle trafficking by uptake of mesoporous silica nanoparticles.** However, these systems are endocytosed efficiently by cells and can alter endo-lysosomal vesicles trafficking [121], and thus, can affect the *in vitro* toxicity results.

Although numerous *in vitro* studies are being investigated, the prediction *in vivo* toxicity of silica matrices still remains to be demonstrated. *In vivo* testing of the silica nanoparticles has been performed in mice and rats. Studies with ORMOSIL have demonstrated high distribution of nanoparticles in liver, spleen, and stomach, being their excretion via the hepatobiliary route without any adverse effect or injuries in the tissues [122]. The charge type and density can control the residence time of nanoparticles inside the body. Studies with positively and negatively charged MSNs at physiological pH 7.4 have shown that particles

with positive charge are rapid uptake and eliminated via liver while particles with negative charge possess high uptake and retention in liver [123].

Recently, a systematically study described by Fu and co-workers [124] related the use of MSNs with the average size of 110 nm for the investigation of absorption, distribution, excretion and toxicity of SiNP in mice after different administration routes. Absorption in short time is achieved after oral and intravenous administration. Histological studies demonstrated that Si content is excreted manly through renal system without any morphological abnormalities of the kidney. The toxicology assessment of SiNP administrated by different routes showed that some inflammation events were observed at the injection local after the hypodermic and intramuscular administration of SiNP. These findings highlight the importance of the interaction between nanoparticles and biological structures in order to select better administration routes for nanoparticle-mediated drug delivery systems.

Although many studies have been related the interaction between nanoparticles and biological systems, the molecular mechanism of cytotoxicity induced by nanoparticles is not fully understood and the investigation of their influence on cells is still ongoing [125-128].

7. Conclusions and Perspectives

Over the past decades, silica nanoparticles have been studied for a variety of biomedical applications such as cell tracking, contrast imaging, biosensing, targeted drug delivery, and as scaffolds for tissue engineering. The versatility of these systems in the design and functioning has made them alternative choices in additional biological and biomedical purposes. Recent developments of silica matrices as tool for biomedical applications were reviewed in this article. The advantages of silica-based materials able to carry drugs or diagnostic **dyes** have been highlighted. Such systems allow bioimaging of cells, detection of damage tissues, biomolecule separation, cell and enzyme encapsulation and efficient delivery of drugs and genes at a desired site. However, significant limitations still need to be overcome. The progression of silica matrices **towards** clinical practice is hindered by several difficulties. Firstly, the lack of stability of colloidal systems can lead to the nanoparticles agglomeration. Second, the entrapment of sufficient drugs with controlled release or dyes with high stability needs strongly investigation. Finally, toxicological studies involving both short and long-term nanostructures are still in their early stage of development. Several works have reported the interaction of silica nanoparticles with reagents for evaluating cell viability.

Therefore, the validation of bioassay in nanotoxicology field is needed to demonstrate the impact of these **innovative** nanomaterials **in** the environment and **in human** health.

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Table 1: Relevant achievements in biosensing from silica sol-gel matrices

Sensor preparation	Transducer	Detection range	Sensitivity (μA/mM)	Response time (s)	*Km* (mM)	Reference
Glucose oxidase in chitosan-SiO_2 gel dropped on Pt/MWCNT nanoparticles	Amperometric	1 μM to 23 mM	5.89	5	14.4	[43]
L-lactate oxidase in silica-sol gel film on Pt/MWCNT nanoparticles	Amperometric	0.2–2.0 mM	6.36	5	-	[44]
Cholesterol oxidase + cholesterol esterase in chitosan-SiO_2/MWCNT bionanocomposite film	Differential pulse voltammetry	0.15-7.68 mM	3.8	10	0.052	[45]
Glucose oxidase in silica sol–gel film onto Prussian Blue modified electrode	Amperometric	0 to 4.75 mM	-	12	6.7	[46]
HRP in sol-gel chitosan –APDMOS film	Amperometric	5.0×10^{-9}–1.0×10^{-7}M	-	2	1.3×10^{-3}	[47]
Acetylcholinesterase/choline oxidase in gold nanoparticles and MWCNTs by silica sol gel process	Amperometric	0.005–0.4 mM	3.395	15	-	[48]

MWCNT: Multi-walled carbon nanotubes; HRP: Horseradish peroxidase; APDMOS: (3-aoryloxypropyl) dimethoxymethylsilane

Table 2: Encapsulation of cells into silica matrices.

Cell and/or factors	Silica source	Application	Comments	Reference
3T3 mouse fibroblasts and CRL-2595 epithelial cells	TEOS	Wound healing	Survive of cells after encapsulation; cell death due to the lack of cell attachment	[52]
Myoblasts and VEGF	TMOS	Diaphragm repair	Control release of VEGF; nontoxicity; response inflammatory by high VEGF concentration.	[53]
Rat (DRG) neurons	TMOS/Polyurea	Neural repair	Attachment, confinement and growth of DRG Neurons on the surface.	[54]
SaOs-2 osteosarcoma	GPTMS/γ-PGA	Bone defect	Hybrid with good mechanical properties and nanoscale level; Support and growth of cell line with not toxicity.	[55]
hMSCs	TEOS-PEG-RGD	Neural, muscular and bone repair	Nanocomposite thixotropic gel for 3D cell culture. High expression and differentiation of cells. Differences in cell morphology with stiffnesses changes.	[56]

VEGF: vascular endothelium growth factor; DRG: dorsal root ganglia; L1: laminin; GPTMS: glycidoxypropyl trimethoxysilane; γ-PGA: Poly(γ-glutamic acid); MSCs: mesenchymal stem cells

Table 3: Drugs incorporated in SiNP.

Incorporated drugs	Properties	Reference
Camptothecin	Suppression of tumor growth in mice with low toxicity effects	[100]
Camptothecin	Surface functionalization of SiNP with THSC demonstrated high colloidal stability in aqueous medium. The particles were internalized by cancer cells leading to the suppression of tumor growth in mice	[101]
Rifampin/ Isoniazid	MSNs coated with PEI or cyclodextrin for rifampin and isoniazid delivery respectively showed high internalization efficacy in *M. tuberculosis*-infected macrophages	[102]
Paclitaxel	MSNs functionalized-PEI for siRNA attachment with enhancing paclitaxel release to pancreatic cancer cells with reduced cytotoxicity	[103]
Doxorubicin	Delivering of siRNA simultaneously with doxorubicin into multidrug-resistance human ovarian cancer cells suppress the nonpump resistance and improve the anticancer action of doxorubicin	[104]
Ibuprofen	MSNs-coated chitosan polymer for pH-responsive delivery of ibuprofen. Under low pH, the amino groups on chitosan are protonated and, thus, ipuprofen can be released. In contrast, at physiological conditions, a chitosan insoluble gel is formed, avoiding the release of ibuprofen	[105]
Insulin	Insulin release was dependent on porous size. The large porous led to faster release of peptide	[99]

SiRNA: small interfering RNA; PEI: polyethlyenimine

Figure 1: Chemical reaction of Stober method.