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REVIEW PAPER

# Silica NPs–Cytotoxicity Cross-Talk: Physicochemical Principles and Cell Biology Responses

**Houra Nekounam[1] · Javad Malakootikhah[2] · Donya Shaterabadi[3] · Babak Negahdari[4] · Mohammad Reza Kandi[3] · Michael R. Hamblin[5]**

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**Abstract**
Many advances have been made in the preparation, optimization, and applications of silica nanoparticles (NPs) in biomedical nanotechnology. Considering this, the broader human, environmental, and industrial contacts with these NPs are inevitable. Improved knowledge of the physicochemical properties of silica NPs and their interactions with biological systems at the cellular level is essential for the rational design of silica NPs. This can involve the deliberately enhanced or decreased cellular responses and toxicity. Therefore, controlling the risk of toxicity can better guide the design of silica NPs for drug delivery and bioimaging systems. Various reports have discussed the toxicology of silica NPs. However, the mechanisms underlying the cellular responses to these NPs remain unclear. Here, we discuss the physicochemical-biological interactions governing the cellular responses and toxicity of silica NPs.

**Keywords** Silica nanoparticles · Physicochemical properties · Cellular responses · Toxicology

## 1 Introduction

According to the 'Nanotechnology Consumer Products Inventory,' silica NPs are the third most frequently investigated products after carbon and silver NPs. Silica NPs have unique advantages, such as large surface area, biocompatibility [1], biodegradability [2], high capacity for cargo loading, and tunable pore size [3]. Silica NPs are already employed as a food additive to control the viscosity and acidity of edible products [4]. They are also used as a cosmetic ingredient to increase the efficacy of these products for topical application [5]. Silica-based NPs have been proposed as drug nanocarriers, biomodulators, and tissue engineering agents [6, 7]. There are some available commercial brands of silica NPs, such as Aerosil® or C-Spec®, which are presently employed in pharmaceutical science [8, 9].

The use of silica in various industries has increased to such an extent that the daily ingestion of silicate particles by adults in the United Kingdom, is estimated at 35 mg in the form of food additives and excipients [10]. Therefore, discussion about the wider safety and toxicology of this material is necessary [11].

There is some literature discussing the toxicity of silica NPs. Nevertheless, there are gaps in our knowledge about the association between the physicochemical properties and cellular physiology relevant to the toxicity of silica NPs. There are discrepancies in the literature about the effects of similar NPs, or how the physicochemical properties of different NPs affects their toxicity, which may be due to variation in cellular physiology or biomolecular interactions [12]. For example, cells may employ different intracellular signaling pathways to modify their response after exposure to NPs

✉ Babak Negahdari
b-negahdari@sina.tums.ac.ir

✉ Mohammad Reza Kandi
kandi.nanomedicine@gmail.com

✉ Michael R. Hamblin
Hamblin.lab@gmail.com

[1] Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran

[3] Department of Medical Nanotechnology, Faculty of Advanced Medical Science, Tabriz University of Medical Science, Tabriz, Iran

[4] Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

[5] Laser Research Centre, University of Johannesburg, Doornfontein, South Africa

[13]. Therefore, the effects of cellular physiology and the physicochemical properties of silica NPs, and how these two parameters can interact to affect cell responses, especially cytotoxicity, remain poorly understood.

Moreover, better understanding of this cross-talk between physicochemical properties and cellular physiology, will help us to take full advantage of these particles. For example, the physicochemical properties of the NPs can be tailored to affect particular cellular pathways and to maximize cellular uptake [14].

Here, we review the effects of the physicochemical properties of silica NPs (size, shape, surface chemistry, concentration, and crystallinity) on cellular processes, such as cell proliferation, endocytosis, exocytosis, cell death mechanism, cell signaling, and the effect of these responses on the modulation, exacerbation or inhibition of silica NP cytotoxicity in vitro studies.

## 2 Silica NPs Cell Death Mechanism

### 2.1 overview

Whether silica NPs can cause apoptotic death is a controversial issue. Here, we present the three basic types of cell death and describe reports linking silica NPs to distinct types of cell death. Apoptosis is the well-known form of programmed cell death with two distinct mechanisms: extrinsic (based on cell membrane receptors pathway) and intrinsic (based on mitochondria signaling pathway) [15]. A decrease in the mitochondrial membrane potential indicates that the mitochondria have participated in the induction of silica NP toxicity [16]. On the other hand, changes in the expression of mitochondrial pathway markers with NP sizes below 50 nm, such as the up-regulation of proapoptotic factors and the down-regulation of anti-apoptotic factors [17], and increased caspase activity have been reported [18, 19].

Necrosis is a nonspecific cell injury and consider as the accidental cell death in several pathological or injury conditions[20, 21]. Lee et al. reported high levels of apoptosis rather than necrosis in Human umbilical vein endothelial cells (HUVEC) treated with 20 nm silica NPs. In contrast, the activation of either of these two death pathways was negligible with other sizes (30 nm, 40 nm, or 50 nm) [22]. Another mode of cell death, autophagy, is identified by engulfment a wide range of cytoplasmic materials in the double-membrane vesicles, called autophagosomes [23]. In the nutshell, different modes of death mechanism may be triggered singly or simultaneously based cell response to certain stimuli. Moreover, Endoplasmic reticulum (ER) stress, including perturbation of calcium homeostasis can induce apoptosis death [24]. The stimulation of calcium influx by silica NPs was independent of whether the NPs were internalized, but was closely correlated with the size and surface chemistry of these NPs [20]. Unlike the 200 nm diameter, the 50 nm silica NPs induced a high and persistent intracellular calcium influx accompanied by apoptotic death in a Mouse Hypothalamic GnRH Neuronal Cells (GT1-7) [21]. It seems that silica NPs stimulated calcium influx through triggering transient receptor potential vanilloid (TRPV), especially TRPV4, which then led to activation of voltage-dependent calcium channels and induced even more calcium influx [20]. Although, Endothelial NO synthase (eNOS)—activated phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway plays a major function in endothelial cell protection against apoptosis [25]. Lee et al. reported two independently size-dependent death pathways in HUVEC cells treated with silica NPs. The induction of apoptotic associated with ER stress, and the induction of necrotic death related to autophagy were demonstrated in the treated with 20 nm silica NPs. The ER stress was related to the generation of reactive oxygen species (ROS). At the same time, autophagy-related necrotic death was dependent on PI3K/AKT/eNOS but was independent of ROS or the AMP-activated protein kinase (AMPK) signaling pathway [22]. Ultrasmall silica NPs (6 nm) induced mitochondrial-independent apoptosis or cell death receptor-mediated extrinsic apoptosis involving caspase 8 in the A549 cells [26]. These reports may indicate that silica NPs can induce autophagy-apoptosis cross-talk [27].

Oxidative stress is proposed in the toxicity of silica NPs [28]. The glutathione (GSH) and its redox potential in mouse keratinocytes was significantly decreased after treatment with 30 nm silica NPs, but larger than 30 nm NPs did not produce any changes in GSH levels [11]. Silica NPs caused intracellular pH changes in a size-dependent manner. There was a direct relationship between the reduced size, reduced intracellular pH, and cytotoxicity. Nevertheless, silica NPs larger than 135 nm did not show any pH change or cytotoxicity [29].

To better understand the effect of crystallinity on cytotoxicity, it is relevant to consider crystallopathies, because crystals can induce various medical disorders [30]. The best known crystallopathy caused by crystalline silica is called silicosis. The main difference between crystalline and amorphous silica is caused by the arrangement of their atoms (Fig. 1) [31]. The genotoxic behavior of crystalline silica NPs was described as a so-called "threshold carcinogen" [32]. Exposure to low doses (5 μg/cm$^2$) of these NPs (Min-U-Sil 5) caused DNA damage accumulation. Crystalline silica NPs induced a DNA damage response (DDR) via NLR Family Pyrin Domain Containing 3 (NLRP3) in a mitochondrial depolarization-dependent manner, which was not related to increased levels of ROS [33]. While, the cytotoxicity of amorphous silica NPs is due to the high content of strained 3-membered siloxane rings which can react with water and produce more hydroxyl radicals [34].

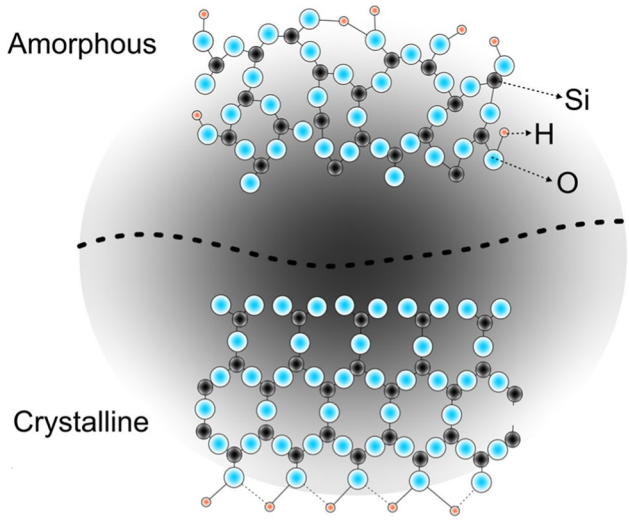


**Fig. 1** Both amorphous and crystalline silica NPs contain siloxane bonds, but with different arrangement of silica and oxygen atoms

### 2.2 Synthesis Method and Controversial Cytotoxicity Report

The formation mechanism of silica NPs largely based on the Stöber protocol (known as sol–gel method) generally includes hydrolysis and condensation of silanes [35].

Siloxane (Si–O-Si) and silanol (Si–OH) are the groups primarily present on the surface of silica NPs prepared by the flame pyrolysis (fumed silica) and sol–gel (colloidal silica) synthetic methods, respectively. Different interactions between fumed silica aggregates with the outer layer of the plasma membrane led to the activation of the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (Nalp3) inflammasome, and subsequent cytokine secretion by the Tamm-Horsfall protein 1 (THP-1) macrophage cells. Inflammasome activation is associated with a higher generation of hydroxyl radicals in the fumed rather than colloidal silica NPs. It is also worth noting that different radicals can be formed by the amorphous silica NP [12]. Altogether, one of the main reasons for the conflicting data about the toxicity of silica NPs may be their different synthetic methods [36].

## 3 Silica NPs-Cell Membrane Interactions

### 3.1 Size Effects

The size-dependent toxicity is based on the fact that NPs with a high ratio of surface area to mass have higher surface reactivity than bulk particles [37]. For example, cell viability was reduced twofold in cells treated with 20 nm silica NPs compared to 30, 40, or 50 nm NPs at a concentration of 25 µg/ml in HUVEC cells [22].

A cell free assay (such as liposomal models) showed complete dye release when interacting with 50 nm silica NPs due to lipid bilayer disruption [38] and creating pore in the vesicles [39]. Silica NP-treated cells showed particle size-dependent Lactate dehydrogenase (LDH) (plasma membrane integrity marker) release. The smallest size (30 nm) silica NPs showed the highest release of LDH [11]. The available information on silica NP toxicity indicates that a diameter smaller than 20 nm is critical for the toxicological effects [22].

### 3.2 Surface Chemistry Effects

Surface chemistry was found to affect the aggregation and toxicity of NPs [40]. The modification of silica NPs dictates their interaction with cells [41]. For example, the interaction between tetra-alkyl ammonium groups on the membrane of Red blood cells (RBC) and silanol groups on the silica NPs could explain the RBC hemolytic mechanism of silica NPs [42].

In contrast to native-silica NPs, most modifications (amination, carboxylation, or protein coating) strongly reduced or prevented dye leakage from liposomal models. The common assumption that cationic modifications induce membrane-activity for NPs, does not hold true for silica NPs [38]. Nevertheless, cationic surface charges above a certain threshold (> 30 mV) in amine-modified particles increased the hemolysis of RBCs [43]. Positively charged silica NPs are taken up more rapidly compared to neutral/negatively charged (hydroxyl/carboxyl modified) silica NPs with identical diameters. The positively charged silica NPs can electrostatically interact with the negatively charged surface of the plasma membrane, and thereby trigger endocytosis [44, 45]. Quaternary ammonium groups have been reported to be biocompatible, and can enhance the cellular uptake of silica NPs [46]. Nevertheless, amine-modified silica NPs, due to the interparticle hydrogen bonding between amine and silanol groups, show increased particle aggregation and reduced cellular uptake. A significant decrease in aggregation and a considerable increase in the dispersion of amine-modified silica NPs, was achieved by modifying the residual surface silanol groups by reacting them with trihydroxysilylpropyl methyl phosphonate (THMP) [47].

Among different surface modifications (carboxylic acid (-COOH), thiol (-SH), amine ( -NH2)) that have been tested in mesoporous silica NPs (MSNs), carboxylic modification showed better RBC membrane coating efficiency [48]. In addition, reductions in LDH release, ROS production, and DNA damage have all been observed in cells treated with carboxylic modified silica NPs compared to non-modified NPs [60]. Moreover, carboxylic modified-silica NPs showed a lower agglomeration rate compared to unmodified or amine modified-silica NPs [49].

### 3.3 Concentration Effects

Cellular responses are caused by the number of cell-associated NPs and not by the number of NPs in the cellular microenvironment [9]. In this regard, Horie et al. investigated silica NP agglomerate formation, and reported that the concentrations of the silica NPs did not correctly reflect the cell-NP interactions [50]. The diffusion and dosimetry (ISDD) model showed that the 100 nm silica NPs contacted the cell surface more efficiently than 20 nm silica NPs. Therefore, silica NPs with larger diameters have a higher effective exposure concentration than smaller diameters [51]. It seems that the increased concentration and subsequent increase in agglomeration are directly related to the toxicity of silica NPs. Nevertheless, most studies have shown that silica NPs of any kind or size induce cytotoxic effects only at relatively high concentrations (> 25 ug/mL) [28].

## 4 Silica NPs-Cellular Uptake

### 4.1 Size Effects

A water-soluble tetrazolium salt (WST-1) cytotoxicity assay was showed that 15 nm NPs induced more cytotoxicity, while the 60 nm NPs led to less cytotoxicity. In contrast, the 200 nm NPs did not display any cytotoxicity. The size-dependent cytotoxicity for these silica NPs was strongly correlated with the uptake mechanism. Uptake in serum-free conditions showed that clathrin-mediated was the dominant mechanism for all sizes. Smaller diameter (15 nm) also used other pathways such as clathrin-independent and caveolin-independent. Caveolin-mediated had a minor role in the uptake mechanism, and this pathway was only active for 60 nm and 200 nm silica NPs (Fig. 2) [52]. In a nutshell, the higher cell uptake seen for 50 nm silica NPs suggests this would be a suitable diameter to be used as a carrier in biomedical science [53].

### 4.2 Surface Chemistry Effects

The functional groups on the NPs surface determines the biological fate of them. The dominant internalization pathways in bare silica NPs were clathrin-mediated and macropinocytosis. Decoration of silica NPs with folic acid limited the uptake pathway to clathrin-mediated, but guanidino-functionalization did not show any evidence of clathrin, caveolin, or macropinocytosis uptake [42]. Among the various endocytosis mechanisms, caveolin-mediated appears to more prominent for organically modified silica NPs (ORMOSIL) [14].

Non-modified, amine-modified, and thiol-modified silica NPs at high concentrations all formed agglomerates.

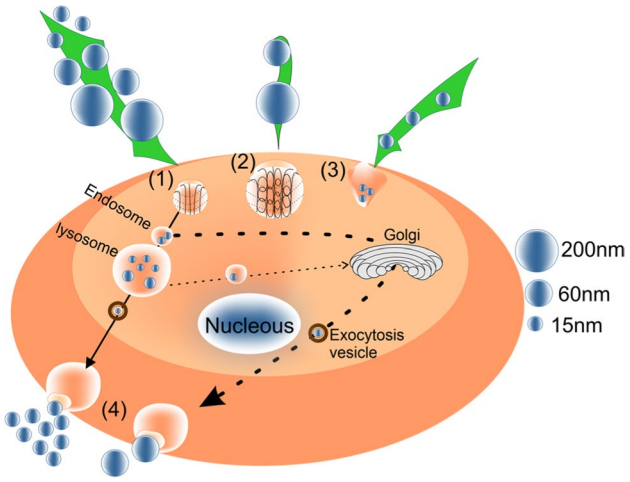


**Fig. 2** Cell uptake under serum-free conditions showed that clathrin-mediated endocytosis is the dominant uptake pathway for all sizes. Smaller diameter NPs (15 nm) also used other pathways such as clathrin-independent and caveolin-independent endocytosis. The caveolin-mediated endocytosis plays a minor role in the uptake mechanism. The possible pathway for the silica NP exocytosis is the localization in lysosomes and excretion by the Golgi apparatus or lysosome mediated exocytosis. The effect of size on silica NPs exocytosis shows that silica NPs with smaller sizes are more easily excreted

In contrast, Polyvinylpyrrolidone (PVP)-coated silica NPs did not show significant agglomeration or toxic effects at high concentrations [44]. The viability of cells treated with PVP-modified silica NPs was similar to untreated cells [44]. PVP significantly reduce most silica-biomolecule interactions [54]. Analysis demonstrated that a single serum protein (albumin) was adsorbed on the surface of PVP-silica NPs. Therefore, this coating can decrease the endocytotic uptake and increase cell viability [44].

Polyethylene glycol (PEG) is used to reduce the cytotoxicity of NPs by improving their solubility [55]. C-Spec® (pegylated silica NPs) has been tested as a cancer-selective probe [9]. PEGylated MSNs showed significantly reduced protein absorption and cellular uptake [56]. Silica NPs with a lower-density polymer coating showed more cytotoxicity compared to NPs with a higher-polymer density, because the silica core was less protected [57] (Fig. 3).

The effects of surface modification on cellular uptake may be different for variant cell types. The internalization of bare MSNs in both Human mesenchymal stem/stromal cells (hMSCs) and mouse embryonic fibroblast cells (3T3-L1) was mediated by clathrin and actin-dependent. Endocytosis inhibitors blocked the internalization of native silica NPs in these two cells. However, with positively charged silica NPs, inhibitory effects only occurred in 3T3-L1 cells, but not in hMSC cells [46]. As a result, by modulating the surface charge, the uptake of silica NPs by a specific cell type could be tailored [58].

### 4.3 Concentration Effects

The relationship between the concentration of silica NPs and cellular behavior can be explained by the agglomeration of the particles, and saturation of the uptake. The 45 nm C-Spec® induced no adverse effects at a concentration of 50 µg/mL. This high safe concentration allowed saturatable uptake and cell fluorescence tracking for an extended period of time [9].

### 4.4 Shape and Aspect Ratio (AR) Effects

It has been proposed that MSNs have a high affinity for clathrin-coated vesicles due to their hexagonal exterior and internal hexagonal mesopores [13]. Spherical MSNs and longer rod MSNs (AR = 4) were internalized via the clathrin-mediated and caveolin-mediated respectively [45]. "Wrapping time" refers to the rate of NP trapping by cell membranes based on the surface area of the particles. Indeed, spherical NPs show higher uptake compared to rod NPs owing to the shorter wrapping time [42].

The predominant internalization mechanism of rod MSNs in HeLa (human cervical cancer) and A549 (human lung carcinoma) cells was macropinocytosis. Among the various ARs investigated, including short (1–1.7), medium (2.1–2.5), and long (4–4.5), the medium AR showed the most evidence for the micropinocytosis. The cellular integrin adhesome network is able to respond to mechanical stimuli and changes in extracellular matrix (ECM) topography. This network acts as a mechanosensor to detect changes in the contact site of the different ARs by particle-cell membrane interactions, and leads to subsequent uptake by a small GTPase-dependent macropinocytosis [59].The effects of silica NPs on cellular functions (uptake, proliferation and cell death) are in the order; sphere > short rod > long rod [42].

## 5 Silica NPs – Subcellular Interactions

### 5.1 Size Effects

Zhu et al. reported that silica NPs with a diameter ≤ 160 nm could only cross the cell membrane but remained in the cytoplasm, while silica NPs with a diameter ≤ 50 nm could easily enter the nucleus [58]. Nevertheless, Hsiao et al. reported that none of the silica NPs tested (15, 60, or 200 nm diameter) were able to enter the nucleus in several

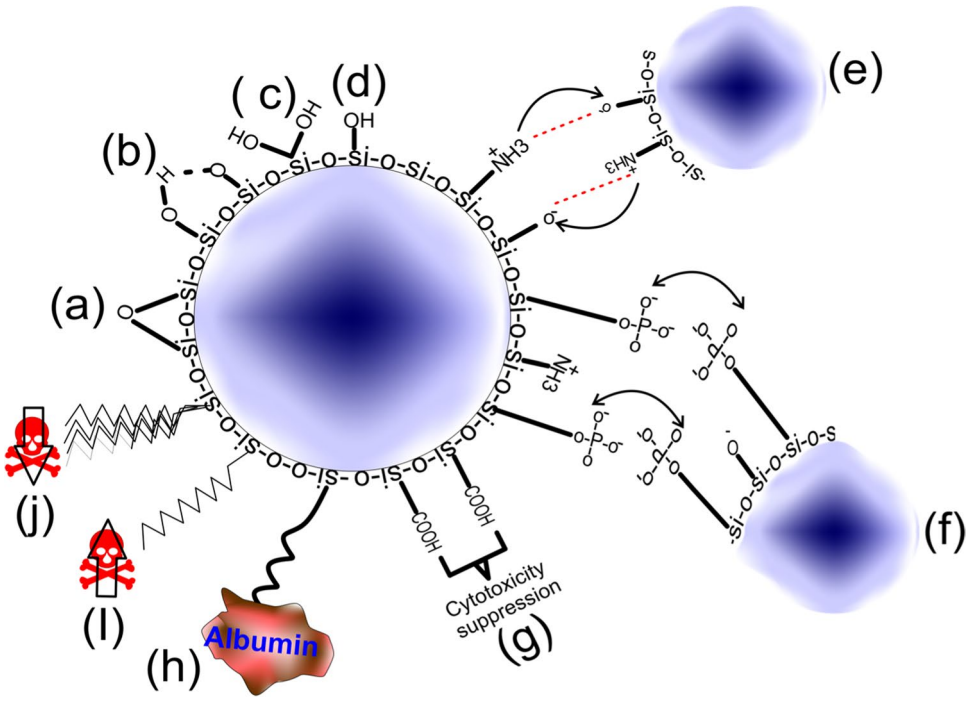


**Fig. 3** Surface chemistry effects. Siloxane (A) and silanol (B, C, D) are primarily present on the surface of silica NPs prepared by the flame pyrolysis and sol–gel synthesis method respectively. Amine-modified silica NPs due to interparticle hydrogen bonding between amine and silanol groups, show increased particle aggregation (E). Considerable increase in dispersion of amine-modified silica NPs was achieved by using THMP (F). Carboxylic modified-silica NPs showed a lower agglomeration rate and less cytotoxicity (G). Albumin is the dominant protein adsorbed on the surface of PVP-silica NPs, and this coating prevents the interparticle agglomeration (H). Silica NPs with lower-density polymer show more cytotoxicity compared to NPs with a higher-polymer density (I, H)

different cells (THP-1, A549, HaCaT (human keratinocyte), and NRK-52E (rat renal proximal tubular)) [52]. Soenen et al. observed that silica NPs of small hydrodynamic diameter were localized mostly in the perinuclear region [9]. It is known that the rigid size of the nuclear pores present a barrier for the passage diameter up to 9 nm [60].

### 5.2 Surface Chemistry Effects

Differently decorated NPs could have varying intracellular localizations. A report by Sun et al. found that there were no silica NPs with a diameter of ~ 100 nm and different surface modifications (hydroxyl (OH), COOH and NH2) detected in the nucleus [45].

A comparative study demonstrated that unmodified silica NPs, unlike amine and carboxylate modified with the same diameter (~ 70 nm), penetrated the nucleus, therefore genotoxicity could be associated with unmodified silica NPs [41]. Negatively charged silica NPs were widely distributed on the cell membrane, in the cytoplasm, and in lysosomes [61]. Internalized ORMOSIL NPs were translocated from primary endosomes to lysosomes, and accumulated in the lysosomes. The accumulation of ORMOSIL NPs in the lysosomes was independent of autophagosomal/lysosomal fusion. Among the various endocytosis mechanisms, caveolin-mediated appears to more prominent [14]. A549 cells treated with lysine coated 10 nm silica NPs in the presence or absence of 10% serum, did not show any genotoxic effects up to 5 mg/L concentration [62].

Lipid bilayer-coated silica NPs, namely protocells (biocompatible NPs) in the nanomedicine, are developed by the fusion of liposomes to the surface of silica NPs [35]. Lipid coating shows 1,2-Dioleoyl-sn-glycerophosphoethanolamine (DOPE)-coated MSNs display earlier lysis times than Dipalmitoylphosphatidylcholine (DOPC)-coated MSNs. DOPE owing to fusogenic properties show better cell membrane fusion rather than DOPC [63]. When carboxylic (-COOH) groups were added to the surface of silica NPs, calcium flux and cytotoxicity were suppressed in Balb/3T3 cells [64].

### 5.3 Crystallinity Effects

Reports have found higher accumulation of amorphous silica NPs in lysosomes, while crystalline silica NPs accumulated in the cytoplasm [65]. In terms of toxicity, crystalline silica NPs are more toxic than the amorphous type, because of more pronounced rupture of organelle membranes and higher production of ROS. Crystalline silica NPs did not show significant cytotoxicity in H1299 (human non-small cell lung carcinoma) cells or NE083 (human esophageal epithelial) cells at low doses (< 2.5 mg/mL) [65].

### 5.4 Silica NPs-Exocytosis

Possible pathways for the exocytosis of silica NPs are the excretion by the Golgi apparatus or lysosome-mediated exocytosis (Fig. 2) [66]. The effect of size on silica NP exocytosis shows that smaller sizes are more easily excreted, and the effect of NP size in HepG2 (Hepatocellular carcinoma) cells is 180 nm > 60 nm > 370 nm > 600 nm [61].

None-modified silica NPs are exocytosis through the Golgi or lysosomal or theme cooperation. Modified phosphonate-MSNs secretion by lysosome. Exocytosis rates of modified MSNs are in the order of Phosphonate (P)-MSN > Folate-MSN > Polyethyleneimine (PEI)-MSN in the A549 cells [66]. It is also necessary to mention that the PEI is a cationic polymer in gene delivery [67].

Interestingly, difference in the intracellular biodistribution of amorphous and crystalline silica NPs correlated with their cellular excretion, because membrane encapsulated NPs underwent exocytosis more readily than cytoplasmic NPs [68].

## 6 Silica NPs- Biological Environment Interactions

The mechanobiology of the cell microenvironment will change the uptake of silica NPs [69, 70]. The cytotoxicity of silica NPs in endothelial cells under stressful conditions was not significantly different compared to static conditions, but the uptake of these NPs by cells that were stretched was lower due to the reduction of endocytosis [71]. Increasing ionic strength of protein-free fluids rapidly induced agglomeration of silica NPs, and changed the properties of theme [44]. Surprisingly, silica NPs were agglomerated in LHC-9 ( Lechner's serum-free bronchial epithelial growth) medium, but showed nearly no agglomeration in DMEM:F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) medium [72]. Some properties related to the toxicity of silica NPs could vary in different culture media. For example, changing the hydrodynamic diameter of some silica NPs in the culture medium is a reason for reducing the genotoxicity of theme [73].

It is well known that biomolecules, especially serum proteins, can form a coating around NPs, called a "protein corona" [74]. The protein corona formed around the NPs reduces their nonspecific interactions with the cell surface [75]. Protein corona coated cationic silica NPs (-NH2) and anionic silica NPs (-COOH) bound to albumin receptors and scavenger receptors on the cell surface respectively [45].

The longitudinal axis of rod-MSNs, because these particles are two-dimensional rather than sphere-MSNs which are zero-dimensional, increases protein adsorption. In contrast to the type of proteins which are adsorbed on the surface

of both shapes, the total amount of proteins does not differ significantly. Although the most abundant plasma protein is albumin, it constitutes only a small amount of the protein corona, 12% on rod shaped, and 2% on spherical MSNs. Instead, the most abundant absorbed proteins were immunoglobulins (57% on spherical and 42% on rod-shaped). In particular, the immunoglobulin gamma-2 chain C protein was 38% on rod-shaped and 30% on spherical MSNs [76].

Protein corona-coated silica NPs are generally less toxic than bare silica NPs. This could be due to reducing the surface energy of silica NPs due to the coverage of the silanol groups, leading to less binding to the cell surface. This reduced adsorption, in turn, reduces the interaction of these particles with cellular surface and receptors, followed by a decrease in uptake [34]. On the other hand, the presence of protein serum in the medium promotes the exocytosis [77].

## 7 Silica NPs—Cell Type Interactions

The use of in vitro cellular assays as a model for body tissues, may not accurately replicate the cell behavior within their natural niche. These considerations may affect the predicted cytotoxicity of NPs. This difference was significant between A549 and 16HBE as two types of lung cells. A549 are adenocarcinoma cells while 16HBE are normal human bronchial epithelial cells. Silica NPs at 128 µg/mL concentration induced toxicity in 16HBE cells without any effect on A549 cells. In addition, the internalization rate of silica NPs was higher in 16HBE cells compared to A549 cells.

The presence of dipalmitoylphosphatidylcholine (DPPC) as a surfactant component inhibited the silica NPs induced-cytotoxicity in 16HBE cells. The presence of DPPC reduced the internalization of silica NPs in both cells types [78].

### 7.1 Cell-Type Dependent Toxicity

According to the cell type, cells can carry out various detoxification strategies. This particular cellular behavior was described as a rudimentary type of "cellular vision" [79]. The slightest difference, such as the zeta potential of the cell membrane or the presence of a specific receptor on the cell surface, can affect the behavior of NPs [42]. For example, the zeta potentials of HeLa cells and erythrocytes are – 19.4 mV and – 31.8 mV respectively [80]. The lower uptake of silica NPs could be caused the limited surface area of PC12 (rat pheochromocytoma) cells compared to C17.2 (mouse-derived neural stem cells) or HUVEC cells [9]. Different toxicity of silica NPs in various cell lines could be correlated with varying rates of uptake. For example, the higher uptake of silica NPs in THP-1 and A549 cells compared to HaCaT and NRK-52E (Normal Rat Kidney-52E Epithelial) cells could be a reason for the higher toxicity found in THP-1 and A549 cells [52]. Silica NPs generally induce less toxicity in tumor cells (A549, MKN-28 (human gastric cancer), HT-29) compared to fibroblast cells [81]. Moreover, epithelial cells as well as tumor cells have more tolerance to silica NPs compared to phagocytic or fibroblast cells [52].

Cell-type dependent toxicity could sometimes arise from the indirect toxic effects produced by other cells. For example, the supernatant from silica-treated Kupffer cells induced the release of Tumor Necrosis Factor Alpha (TNF-α), nitric oxide, and ROS in BRL (rat liver-derived fibroblast-like cell) cells. Treatment with this supernatant even led to the inhibition of mitochondrial electron transport chain complex I activity in the BRL cells [17].

### 7.2 Cell Sensitivity

Another factor that determines cellular response to NPs is NP-induced stress. For instance, cytotoxicity with higher concentrations of silica NPs in PC12 cells may be due to more sensitivity of them to stress [9]. The greater cell death in HUVEC cells, and the higher survival of HeLa cells suggested that HUVEC was more sensitive to silica NPs [82].

The lower sensitivity of T cells compared to B cells or monocytes is due to the lower intracellular uptake of silica NPs. It seems that the high sensitivity of dendritic cells and monocytes is due to the presence of other endocytosis pathways in addition to phagocytosis [10]. The sensitivity of immune cells to silica NPs is in the following order: T-cells ≈ monocytes > macrophages ≈ B-cells [83]. Phagocytes are first responders in the presence of nanomaterials. A study by Herd et al. showed that the macrophage response, such as uptake and toxicity after exposure to silica NPs was phenotype dependent. High and low cellular uptake was reported for macrophage cells with the M1 and M2 phenotypes, respectively. This may be because M1 macrophages are more likely to phagocytose invading pathogens [84]. M1/M2 refers the two significant different activities of macrophage populations. M1 and M2 causes tissue damage and tissue repair respectively [35].

### 7.3 Cell Specific Toxicity

Brain (A-172) and liver (Huh-7) cells showed more adverse effects on DNA contents compared to stomach (MKN-1) or lung (A549) cells after exposure to amorphous silica NPs [85]. Silica NPs induced micronuclei formation in Balb/3T3 cells, while A549 cells did not show any significant micronuclei formation. This finding illustrates cell type-dependent toxicity at the genomic level [86]. The dopaminergic neuron-like cell line (PC12) after treatment with 25 nm silica NPs at 25–200 mg/L concentrations, showed inhibition of the ubiquitin–proteasome system (UPS), and subsequent α-synuclein

aggregation. Amyloid formation of α-synuclein is a marker of Parkinson's disease (PD) [87].

## 7.4 Internalization Mechanisms

The internalization mechanisms of non-modified, amine-modified, and thiol-modified silica NPs were investigated in neural stem cells and astrocytes. It was found that neurons do not internalize any of these particles. The microglial cells took up all the above-mentioned types of NPs, but not PVP-coated silica NPs. The explanation of these differences is related to the physiological behavior of these cells. The neuronal cell bodies and axons of mature neuron cells possess a rigid membrane structure. On the other hand, neurons do not have any phagocytotic activity. Also, according to the very high rate of uptake by activated microglial cells (macrophage cells), they are more susceptible to particle uptake and NP-related death than other neural cell types [44].

The order of cell uptake after two hours of incubation was HaCat > THP-1 > A549 > NRK-52E, while the order changed to A549 > THP-1 > NRK-52E > HaCat after 24 h, showing the uptake was both cell type and incubation time dependent [52].

The biological behavior of NPs may be different in an original cell type compared to variant cells derived from the parent. For instance, the F-actin-dependent pathway was involved in the uptake of amino-modified silica NPs in the human breast adenocarcinoma cell (MCF-7). However, in MCF-7-derived breast cancer stem cells (BCSCs), the scavenger receptor mediated a different internalization route for silica NPs. The effect of the amino-modified silica NPs in BCSCs was to up-regulate the expression of the scavenger receptor compared to MCF-7 parental cells [45].

### 7.4.1 Endocytosis factors

The major route employed by cells to uptake NPs is endocytosis. Endocytosis includes the introversion of cell membrane and organization of endocytic pit and formation endocytic vesicles. The optimization of cell targeting and cell uptake of NPs are imperative results that are obtained from understanding of endocytosis mechanism. Nevertheless, there are different bounds to access to a public understanding of the recent findings owing to the diversity mechanisms based on cell types and NPs [88]. Different endocytosis factors (NP load per endosome, endosome size, lysis rate, etc.) can affect the results of cell uptake. Silica NPs with a diameter of around 200 nm and 300 nm did not show any cytotoxicity, because the 3T3 cells were not able to internalize these massive particles [22]. The lower uptake in renal carcinoma cells (Renca cells) compared to 3T3 cells (fibroblast cells) was associated with larger endosomes and a lower load of silica NPs per endosome in Renca cells. The long-term expression of higher levels of Ras-related protein in brain 5 (Rab5) in cancer cell lines, such as Renca, Hela, etc. could be a reason for the smaller size of their endosomes because Rab5 controls endosomal fusion and size distribution [63] (Fig. 4).

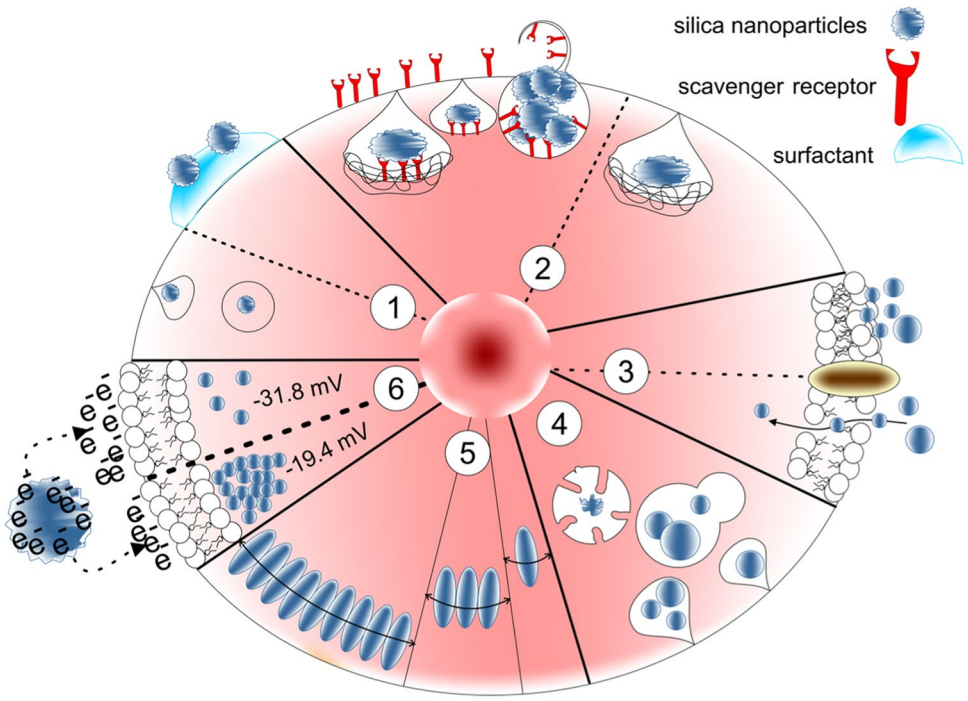


**Fig. 4** The presence of surfactant reduces the cellular internalization of silica NPs (1). The scavenger receptor mediates a different route internalization of silica NPs in BCSCs (2). The rigid cell membrane structure alters silica NPs internalization (3). Different cytoplasmic properties such as endosome characters can affect the outcome of cellular uptake (4). Cell surface area can limit or improve the cellular uptake of silica NPs (5). Dual influences of zeta potential of silica NPs and cell membrane in the outcome of internalization (6)

### 7.5 Cell Exocytosis

The exocytosis of particles is an integral part of cellular physiology, and this is also dependent on cell type. The exocytosis profile of phosphonate-modified MSNs in different cells was in the order, A549 (81%) > MDA-MB231(human breast cancer) (81%) > PANC-1(human pancreatic cancer cell) (75%) > MCF-7 (breast cancer cell) (61%) > MDA-MB 435 (metastatic human breast cancer) (36%) > H9 (human embryonic stem cell) (4%). This information is useful in designing biomedical applications. For example, the lower exocytosis and higher persistence of phosphonate modified MSNs in the H9 human embryonic stem cell suggested that these particles might be a good candidate for embryonic stem cell tracking in cellular transplantation procedures [66].

## 8 Conclusion

The physicochemical properties of NPs and the particular cellular function can dramatically affect each other. In order to design nontoxic NPs for specific biomedical applications, it is necessary to consider the nano-bio interactions. The mechanistic elucidation of the intracellular pathways could provide improved formulations of silica NPs. Different internalization pathway in various cells could be employed by tailored silica NPs as an optimized platform for targeted therapy. Caveolin-mediated endocytosis, which has a longer retention time of the silica NPs inside the cells, could increase drug delivery efficacy. Since the applied concentrations of NPs incubated with the cells did not correctly reflect the concentration at the cell-NP interface, this parameter can be affected by cell function. This insight is important for developing the next generation of nano-formulations that could allow a predetermined behavior and interaction with the microenvironment and/or the cells. Tailored selectivity for targeted cells and intracellular compartments could reduce the overall toxicity, and improve the utility of silica NPs for medicine applications.

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### Declarations

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