Journal of
Biomaterials and Tissue Engineering
Vol. 3, 108–121, 2013

REVIEW

Controlling the Interaction Between Cells and Silica Nanoparticles

Andrea Mathilde Mebert, Daniela Edhit Camporotondi, María Lucia Foglia, Gisela Solange Alvarez, Pablo Luis Santo Orihuela, Luis Eduardo Diaz, and Martín Federico Desimone*

Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 Piso 3°, (1113) Ciudad Autónoma de Buenos Aires, Argentina

In recent years, the application of silica nanoparticles in the biomedical field experienced a great development, showing a sharp increase in the number of published articles and patents. The driving forces for these and future developments are the possibility to design nanoparticles with homogeneous size and structure and amenable to specific grafting. In this way, it is possible to control the interaction of nanoparticles with cells. Moreover, it is possible to tune the characteristics of the nanoparticles to meet the requirements of each specific cell and desired application. Herein, we present different strategies developed to optimize the size, morphology, surface topography, elemental ratio, hydrophobic/hydrophilic balance, and erosion rate, which contribute to understand the nature of this inherently complicated cell-nanoparticles interactions mechanism, which will determine the resulting function performance.

Keywords: Cells, Nanoparticles, Silica, Toxicity.

CONTENTS

1. INTRODUCTION

The sol–gel process has several well-known advantages such as a choice of high purity precursors (monomers or condensed species), homogeneity of the obtained material with different shapes (i.e., gels, films, particles)[1,2] and especially the possibility of making hybrids and composite materials with new chemical and mechanical properties, conductivity and permeability.[3–5] Moreover, slight changes in experimental parameters such as pH, additives, and concentration can lead to substantial modification of the resulting material.[6,7] In recent years, biomedical applications of sol–gel technology have received extensive coverage.[8–13] Particularly, the application of silica nanoparticles in the biomedical field experienced a great development, showing a sharp increase in the number of published articles and patents.[14,15] The driving forces for these and future developments are the possibility to design nanoparticles with homogeneous size, structure and amenable to specific grafting, which provides structural support and delivery systems for therapeutic purposes.[16–18]

The effect of various silica formulations such as plain, mesoporous and hollow silica nanoparticles are being currently investigated. Nowadays, different silica nanoparticles can be obtained through well known procedures.[19–25] The most popular is undoubtedly the Stöber method.[22] This method allows the synthesis of monodisperse, spherical silica nanoparticles that range from 5 to 2000 nm, through an ammonia-catalyzed reaction of tetraethylorthosilicate (TEOS) with water in low-molecular-weight alcohols.[26] Another method used to obtain solid silica particles involves the preparation of water-in-oil microemulsions, where the droplets of the water polar phase dispersed in a continuous oil phase are used as nanoreactors for the synthesis of silica nanoparticles.[24,27] Secondly, hollow silica

*Author to whom correspondence should be addressed.

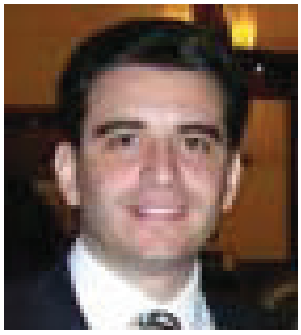
 2157-9083/2013/3/108/014 doi:10.1166/jbt.2013.1069

Andrea Mathilde Mebert was born in Erlangen, Germany, in 1983. As a child she moved to Buenos Aires, Argentina, where she graduated in Pharmacy from the University of Buenos Aires (2010). She currently holds a doctoral fellowship granted by Agencia Nacional de Promoción Científica y Tecnológica and she is working on her Ph.D. She also holds a first level laboratory instructor at the Department of Analytical and Physical Chemistry of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires. Her researches are oriented to biomaterials including nanomaterials.

Daniela Edhit Camporotondi was born in Buenos Aires, Argentina, in 1986. This year, she graduated in Pharmacy at University of Buenos Aires. Since 2009, she has been working as a second level laboratory instructor at the Department of Analytical and Physical Chemistry of the Faculty of Pharmacy and Biochemistry, and she has been taking part of the biocompatible nanomaterials research project as well.

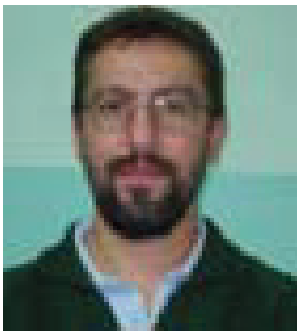
María Lucia Foglia was born in Buenos Aires in 1984. She graduated in Pharmacy from the University of Buenos Aires in 2008 and is working on her Ph.D. since 2009 in the same institution. She currently holds a doctoral fellowship granted by the National Research Council (CONICET) and a teaching assistant position in Instrumental Analysis at the Faculty of Pharmacy and Biochemistry, which belongs to the University of Buenos Aires. Her research interests include the obtention of biomaterials by the sol–gel route and the possibility to incorporate nanoparticles for drug delivery.

Gisela Solange Alvarez was born in Buenos Aires in 1980. She graduated in Biochemistry (2005) and Pharmacy (2008) at Universidad de Buenos Aires and received her Ph.D. degree in the Biotechnology field from the same institution (2010). She currently holds a full teaching assistant position in Instrumental Analysis of the Faculty of Pharmacy and Biochemistry at Universidad de Buenos Aires. Her research interests are focused on biomaterials and nanocarriers for drug delivery and at the moment she is holding a posdoctoral fellowship granted by the National Research Council (CONICET).



Pablo Luis Santo Orihuela was born in Buenos Aires in 1974. He graduated in Biochemistry (2001) at Universidad de Buenos Aires and received his Ph.D. degree in Toxicology from the same institution (2008). He received his M.Sc. in Pest Control and its Environmental Impact from Universidad de General San Martín, Buenos Aires. He currently holds a teaching assistant position in Instrumental Analysis of the Faculty of Pharmacy and Biochemistry at Universidad de Buenos Aires. His research interests are focused on insecticides, nanocarriers, biochemical and molecular bases of insecticides resistance of vector human disease insects.

Luis Eduardo Diaz is full professor of Instrumental Analytical Chemistry and Head of the Department of Analytical, Inorganic, and Physicochemical Chemistry of the Faculty of Pharmacy and Biochemistry at the University of Buenos Aires (Argentina). Today he currently leads a research group working on: (a) biomaterials, especially in bioceramics, drug delivery systems and smart targeted nanosystems for drug delivery, and (b) hybrid bioremediation systems.

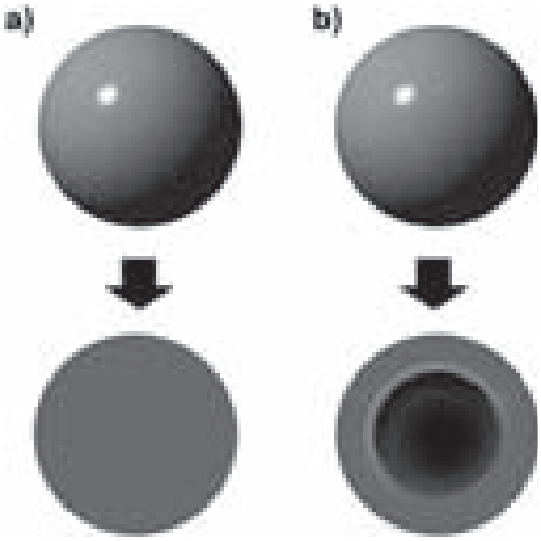


Martún Federico Desimone was born in 1974 in Buenos Aires, Argentina. He graduated from Pharmacy and Biochemistry at University of Buenos Aires and received his Ph.D. degree from the same University. After a postdoctoral period in the University of the Basque Country, Spain, with Professor Dr. J. L. Pedraz, he joined in 2008, the Thibaud Coradin's group in the Laboratoire Chimie de la Matiére Condensée de Paris. Actually, he holds an Adjunct Professor position in the Faculty of Pharmacy and Biochemistry at the University of Buenos Aires. He is a CONICET researcher and the director of the biocompatible nanomaterials project.

nanoparticles (HSNP) are gaining great attention. They consist of hollow spherical particles with typical sizes below 200 nm and a shell of silica. Such structure provides some advantages over solid (plain) ones because of its low density and large surface area (Scheme 1).[20, 21, 28]

In addition, their size will also determine the mechanism and rate of nanoparticles cell uptake and their ability to permeate through tissues. Moreover, particle shape (i.e.,: spherical, tubes) and surface properties (i.e.,: positively or negatively charge) have a great impact on different aspects of cellular functions including cell proliferation, apoptosis, cytoskeleton formation, adhesion and migration. Thus, although silica is generally accepted as having low toxicity, the biocompatibility of silica nanoparticles (SiNPs) as a "new" kind of material should be revisited.[29] In this way, it is possible to control the interaction of nanoparticles with cells. This interaction is sometimes mediated by the type and concentration of the protein layer adsorbed to the nanoparticles that interact with integrins present in the cell membrane. Thus, a careful consideration must be given to control the interaction of nanoparticles with macromolecules and cells present in biological systems. Such interactions play a key role in the stability, aggregation and dissolution of silica nanoparticles and in the uptake by phagocyte mononuclear cells or even other cells types. In addition, the transendothelial permeability in various normal or damaged tissues, the uptake by cells and particle clearance should be taken into account.

Therefore, it is possible to tune the characteristics of the nanoparticles to meet the requirements of each specific cell and desired application.[30] Especially, silica nanoparticles have gained ground in the pharmaceutical field and have been widely studied in the context of drug delivery and drug targeting.[31–36] Indeed, a huge amount of cosmetic products and pharmaceutical formulations contain nanoparticles. In this sense, it was suggested that an ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize.[37, 38] Silica nanoparticles meets nearly all the requirements, thus it is not surprising the great attention that they are receiving from researchers and industries.[39, 40]



Scheme 1. Schematic representation of: (a) plain nanoparticles and (b) hollow nanoparticles.

The great advances in the application of silica nanoparticles also require a careful characterization of the biocompatibility, immune response and toxicological effects that they may cause. The study of the immune response against nanoparticles is actually being studied[41] for multiple reasons such as the elucidation of new targets for drug delivery systems,[42–44] as vaccination adjuvants[45,46] or for toxicological studies.[47–50] It is worth to mention that nanoparticles interact with the immune system in different ways, having the possibility to trigger both the innate and adaptive immune response. Both innate and adaptive immune responses work in a coordinated manner to mount an effective immune response in the body that prevents or protects against foreign material, mostly microorganisms, but also dust and particles, entering and/or affecting the organism.[41] Furthermore, cell cultures can be used in toxicity screening both through estimation of the basal functions of the cell (i.e., those processes common to all types of cells) or through testing on specialized cell functions. Several parameters are used to assess the effect of nanoparticles over cell functions. These parameters include vital staining, proliferation test, cytosolic enzyme release, and cytokine production, among others. Special attention should be given if nanoparticles are intended to treat or prevent infections. In this case, it is highly desirable that the formulation possess a strong activity against microorganisms without affecting host cells.

In the case of cell interaction with nanoparticles, some works have been published which try to elucidate the mechanism either for cell toxicity or for cell growth stimulation in other cases. Herein, we present different strategies developed to optimize the size, morphology, surface topography, elemental ratio, hydrophobic/hydrophilic balance, and erosion rate, which contribute to understand the nature of this inherently complicated cell-nanoparticles interactions mechanism, which will determine the resulting function performance.

2. *IN VITRO* ASSAYS

Biocompatibility is always a matter of study in medical research. In this sense, the effect on human health of a therapeutic, occupational or accidental exposure to nanoparticles should be thoroughly investigated. Consequently, many studies have been performed in this field but still very little is known about the human body's response to nanoparticles.

Research has shown that the response depends not only on nanoparticle composition, but also on many factors like size, shape, mass and the method of synthesis involved, which can influence cell toxicity.[51] Even though there are works whose results seem to contradict those obtained by other researchers, there are some generalizations which are worthwhile of considering when working with nanoparticles.

2.1. Effect of Concentration and Size of SiNPs

From the analysis of the literature available, it has been found that cellular response to SiNPs is concentration dependent,[52–54] even though increasing SiNPs concentration will also increase the effect produced and eventually the probability of cell toxicity.[55]

On the other hand, the size of the nanoparticles is related to the surface which will ultimately be in contact with the cells. In this sense, it would be expected that a higher interaction will be established with smaller sized particles. This higher interaction can be favorable or unfavorable, depending on the characteristics of the material employed. Furthermore, smaller sized nanoparticles are more rapidly taken up by cells, thus speeding the reaction to them. It was reported that as a consequence of the treatment of Langerhans cells with silica particles, with diameters of 70, 300, and 1000 nm, cellular uptake and cytotoxicity increased with the decrease in particle size.[56]

Indeed, when considering size alone, it has been extensively reported that cytotoxicity increases when nanoparticle size is reduced.[50,57] However, this was observed when cells were exposed to silica nanoparticles ranging from 50 to 500 nm and not in those with less than 50 nm.[52,58] Because agglomeration of nanoparticles can take place quite easily, another approach points out the fact that when surface area is taken into account it can be generally accepted that the larger the surface area, the higher the toxicity.[59]

Morishige et al. found that unmodified amorphous microsized silica particles (i.e.,: 1000 nm) induced higher levels of IL-1b production in THP-1 human macrophage like cells whereas smaller particles (30 to 300 nm) did not induce such a significant production.[60] In a related work, Waters et al. suggested that the response of macrophages to SiNPs depends on the nominal surface area basis rather than on particle mass or number. They arrived to this conclusion using unopsonized amorphous silica nanoparticles ranging from 7 to 500 nm diameter and measured the stimulation of inflammatory protein secretion and the induced macrophage cytotoxicity.[59] Oberdörster et al. also reported that particles with greater specific surface areas per mass are more biologically active.[61] Same results were found in mouse keratinocytes (HEL-30) exposed for 24 h to 30, 48, 118, and 535 nm SiNPs. Indeed, silica nanoparticles exhibiting the highest specific surface area showed more toxic effects.[54] When working with endothelial cells (EAHY926 cell line), surface area also seems to be an important parameter in cytoxicity, as it was seen with the smallest SiNPs (14–16 nm diameter), which led to a 50% reduction of viability when used at a lower concentration than 19, 60, 104, and 335 nm monodisperse amorphous spherical silica particles.[62]

2.2. Effect of Crystalline Form and Porosity

Silica can be divided into crystalline or amorphous (noncrystalline), all having the same basic molecular formula

(almost 100% SiO_2).[63] Because inhalation of crystalline silica dust is well known to cause silicosis, it is commonly accepted that this form of silica is more toxic. However, in a recent publication, Constantini et al. compared the uptake and toxicity of amorphous silica to crystalline silica in various cell types, such as mouse alveolar macrophage (MH-S) cells, mouse lung epithelial type II, mouse skin melanoma, monkey kidney fibroblast, human adenocarcinoma cells and cervical epithelial cells among others. They found out that, whereas all cells are able to take up silica nanoparticles, only macrophages showed extreme sensitivity, and in this case crystalline silica and amorphous silica killed the cells with equal strength.[64]

Other works dealing with mesoporous silica nanoparticles try to explain the effect of the porosity on the final response.[65–67] Lee et al. compared the effect *in vitro* and *in vivo* of mesoporous silica nanoparticles (MSNs) with non-porous colloidal silica of the same size and shape. Results showed that MSNs induced less activation of MAPKs, NF-kB and caspase-3 leading to a lower cytotoxicity and inflammatory response when compared to non-porous silica nanoparticles. Moreover, the results of the hazard test for identification of contact hypersensitivity performed *in vivo* were congruent with those obtained *in vitro*.[65]

2.3. Effect of Surface Modification

It seems that covering the anionic surface of silica nanoparticles, often with cationic compounds, results in reduced toxicity when compared to particles without any modification. Tao et al. reported different mesoporosity-dependent and functional group-dependent cytotoxicity and endocytosis of various silica nanomaterials on suspended and adherent cells. They confirmed that the functionalization of mesoporous nanomaterials, namely by amination with positively charged quaternary amines, is able to prevent cellular injury as the rate of cell internalization is significantly diminished.[68] In relation to nanoparticle modification, Chen et al. synthesized silicon nanoparticles chemically modified with sodium chloride or sodium iodide (diameters of 10–100 nm) as novel non-viral vectors for DNA transfer into cells. Results showed a better efficiency of DNA transfection with the advantage of protection of DNA against degradation. Microscopy assays showed no cytotoxicity during adhesion and entry of the nanoparticles into HT1080 cells. *In vivo* experiments with mice revealed accumulation of nanoparticles within the cells of the brain, liver, spleen, lung, kidney, intestine and prostate without pathological cell changes or mortality.[69] There are more works suggesting the potential of modified silica nanoparticles as gene carriers with very low or no cell toxicity observed. As an example, the ability of cationic SiNPs to transfect galactosidase expression plasmid DNA pCMVβ reporter gene was successful in Cos-1 cells *in vitro*[70,71] and in the mouse lung *in vivo*.[71] Furthermore, SiNPs with surface cationic amino groups were efficiently taken up *in vitro* and succeeded to deliver DNA to the nucleus.[72] In addition, Bharali et al. found promising the use of amino-functionalized silica organically modified nanoparticles for future direction and effective therapeutic manipulation of neural stem/progenitor cells and *in vivo* targeted brain therapy.[73] Mumin et al. demonstrated that phosphonate functionalization of mesoporous SiNPs with a resulting foam structure (i.e.,: 65 nm) leads to increased levels of interaction or internalization by dendritic cells.[74]

Surface modification can drastically change cellular response to nanoparticles. For example Gyenge et al. tried core–shell silica nanoparticles with hydroxyl-, aminopropyl- or PEGylated surface modifications (200–300 nm) encapsulating a fluorescent dye to evaluate their uptake in the human head and neck squamous cell carcinoma cell line UMB-SCC 745 and found that the uptake of PEGylated SiNPs was minimal even after 24 h in contrast to hydroxyl- and aminopropyl-modified SiNPs.[75]

In relation to the mechanism involved in the protection given by modification with cationic compounds, which will be further discussed, Dutta et al. proposed that serum proteins which commonly adsorb on the surface of amorphous silica nanoparticles could influence the biological fate of SiNPs and in fact they observed that surfactant coated nanoparticles, which prevented proteins from binding to their surface, inhibited cytotoxicity.[76] Considering the amount of surface modification possibilities this is probably an interesting point for optimization in the design of SiNPs for different proposes.

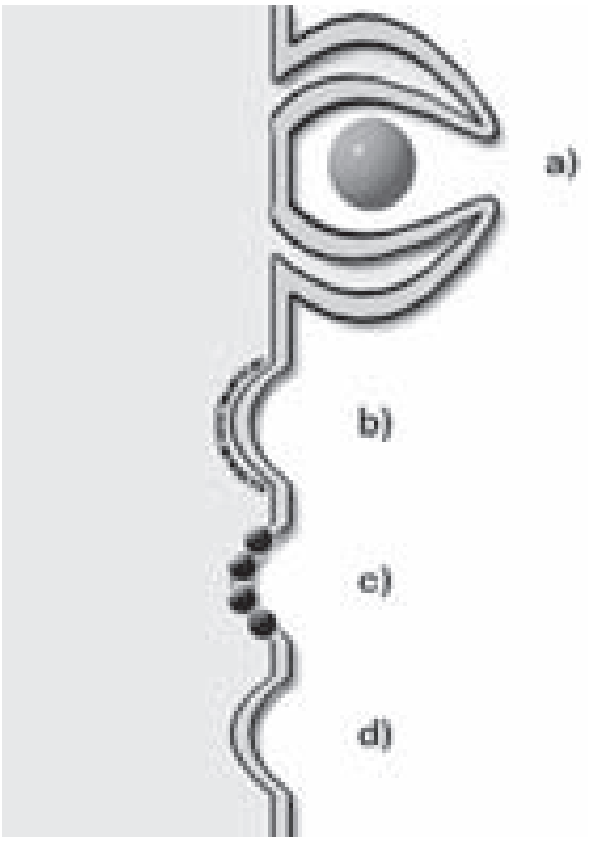
2.4. Cell Type

Another interesting fact is that not all the cells show the same response to silica nanoparticles, which can be related to different interaction mechanisms or to different degrees of interaction. As an example, 3T3 fibroblasts, bronchiolar epithelial cells (hT) and RAW 264.7 macrophages were used to test viability when exposed to 30 nm silica or titanium nanoparticles in different concentrations and three different diameters of multi-wall carbon nanotubes. As expected, it was found that size and composition do affect cellular response but this is cell type dependant in such a way that it is recommended to focus nanoparticle engineering on the potential cell type which may be finally exposed to them. Likewise, Malugin et al. tried modified (–COOH and $–NH_2$) and unmodified silica nanoparticles from 50–500 nm in human prostate carcinoma DU145, colon carcinoma HCT116 and murine macrophages RAW 264.7 cells. They found that phagocytic cells are more susceptible to amorphous silica nanoparticles than epithelial cells.[52]

2.5. Cellular Uptake

In order to design drug loaded nanoparticles as well as to diminish the toxicity of the before mentioned SiNPs,

it is necessary to know more about the different ways the particles obtained will enter the target cell.[77] There are essentially four mechanisms of endocytosis by means of which nanoparticles enter the cells: clathrin-mediated endocytosis, caveolae-mediated endocytosis, phagocytosis and macropinocytosis, though a discussion has grown around this classification and it may need to be updated. Nanoparticles taken up by endocytosis are firstly enclosed within early endosomes, phagosomes and macropinosomes respectively. These vesicles will later become multivesicular bodies, also known as late endosomes, and eventually fuse with lysosomes.[78] In general terms, larger particles are taken up by phagocytosis while smaller ones by endocytosis (Scheme 2). Strictly speaking, phagocytosis is a process occurring only in highly specialized cells, e.g., macrophages, although other cells may use phagocytic-like mechanisms too.[79] In this sense, Zhang et al. identified an optimal NP radius at which the cellular uptake reaches a maximum and they showed that the cellular uptake of NPs is regulated by membrane tension, which can be elaborately controlled by particle size. The optimal NP radius reported for endocytosis was on the order of 25–30 nm.[80] Similarly, SiNPs uptake by HeLa cells is size-dependent and the maximum uptake by cells occurs at a nanoparticle size of 50 nm. These findings suggested that MSNs 50 nm in diameter may be the most suitable candidate to serve as a carrier for further studies in biological applications.[81]



Scheme 2. Mechanisms of endocytosis by means of which nanoparticles enter the cells: (a) phagocytosis (b) endocytosis clathrin dependent (c) endocytosis caveolin dependent (d) endocitosis non-clathrin non-caveolin dependent.

Not only size but also concentration probably influences one of the various uptake mechanisms that have been described as potential cell capture pathways for nanoparticles. In this sense, an interesting observation was made by Choi et al. who described that when working with low SiNPs concentrations, namely 7.28×10^{-4} μg/ml, silica was dispersed throughout the cytoplasm whereas at higher concentrations they appeared to be engulfed in phagocitic vacuoles, and therefore suggested that concentration may affect silica nanoparticles uptake.[82]

When it comes to size, it has been suggested that modified (–COOH, $–NH_2$, $–SO_3H$, –CHO) and unmodified 1000 nm silica particles are taken by THP-1 cells with similar frequencies by actin-dependent phagocytosis.[52,83] On the other hand, other groups working with silica nanoparticles ranging from 50 to 500 nm claimed that within this range they do not enter the cells through the phagocytic pathway but through a different mechanism, presumingly endocytosis.[52] Moreover, recently Quignard et al. demonstrated that positive charged SiNPs (i.e.,: 200 and 40 nm) are easily uptaken by human dermal fibroblast, through a macropinocytosis, without impacting on cell viability. On the other hand, the small SiNPs (i.e.,: 10 nm positively and negatively charged) show high and fast cytotoxicity compared to the largest ones at a similar dose, but only the negatively-charged colloids induced genotoxicity effects.[84] One interesting point that deserves more attention is that SiNPs are prone to dissolve in culture media. The dissolution process was also confirmed by several works that monitors the cell culture medium where it was demonstrated that it contains both colloidal and soluble silica species.[84–87] Apparently, as it was shown by Xing et al. not only size and concentration affect cellular uptake of SiNPs. Their experimental results demonstrated that uptake of silica-coated nanoparticles by HeLa cells was higher at 37 °C than at 4 °C and concluded it was time and energy dependent process.[88] Analysis of the intracellular localization of silica nanoparticles revealed that SiNPs penetrated into the nucleus, whereas SiNPs modified with amine or carboxyl groups showed no nuclear localization. These results suggest that intracellular localization is a critical factor underlying the cytotoxicity of these silica nanoparticles.[89]

2.6. Cell Damage Mechanism

Different cells have different metabolisms and thus it would be expected that the extent of cell interaction and toxicity will vary when considering different cells being exposed to the same silica nanoparticles. Concerning the mechanism of cell toxicity, several studies propose that nanomaterials cause lysosomal membrane destabilization induced by reactive oxygen species (ROS) generation, which would ultimately lead to apoptosis.[31,58,90–94]

Indeed, human embryonic kidney (HEK293) cells[95] and human bronchoalveolar carcinoma-derived cells,[96] for example, showed dose dependent cytotoxicity associated with increased oxidative stress when exposed to SiNPs. In the case of liver cells, Ye et al. reported that exposure to SiNPs of the normal human hepatic cell line (L-02) caused cytotoxicity in size, dose and time dependent manners, with 21 nm SiNPs inducing oxidative stress, apoptosis and increased p53 and Bax expression.[97] On the other hand, Lu et al. tested the biological response of normal human L-02 hepatocytes and HepG2 hepatoma cells to SiNPs and stated that the first ones showed only slight toxicity when compared to the antiproliferation activity seen in the second ones.[98]

The mechanisms of toxicity in human HepG2 hepatoma cells by 7 to 50 nm or 14 nm silica nanoparticles were studied by Lu et al.[98] and Ahmad et al.[99] respectively. Both found that in these cells nanoparticles produce toxicity in a dose dependent manner. The expressions p53 and caspase-3 (proapoptotic) were up-regulated, whereas the expression of bcl-2 (antiapoptotic member of the Bcl-2 family) was down-regulated. Lu et al. found decreased the expression of procaspase-9 and no significant change in expression of Bax (propapoptotic member of the Bcl-2 family), whereas Ahamed et al. found that the proapoptotic gene bax was up-regulated in HepG2 cells treated with silica nanoparticles. Both found an increase in ROS and decrease in glutathione (GSH) (antioxidant) levels. This suggests that in these cells nanoparticles induced apoptosis mediated through ROS via p53, bax/bcl-2 and caspase pathways.[99]

When considering macrophage cells, Morishige et al. suggested that ROS production induced by phagocytosis of unmodified 1000 nm silica particles triggered endosomal rupture followed by the activation of the pro-inflammatory complex NLRP3 inflammasome (NLR family, pyrin domain containing 3) and subsequent interleukin-1b (IL-1b) production.[60] In addition, SiNPs induced an elevated level of reactive oxygen species (ROS), leading to DNA damage.[100] When surface modification with several functional groups (–COOH, $–NH_2$, $–SO_3H$, –CHO) was performed, it dramatically suppressed IL-1b production by reducing ROS production in THP-1 human macrophage-like cells. The inflammatory effect *in vivo* in intraperitoneally injected (1 mg) mice showed the same results when compared to IL-1b production *in vitro*.[52,83] Furthermore, unmodified silica nanoparticles from 50 to 500 nm induced a more severe plasma membrane damage in RAW264.7 macrophages than surface functionalized (–COOH and $–NH_2$).[52] In relation to the immune system, Lucarelli et al. suggested the possibility of inadequate defense against certain infections after exposure to SiNPs (15 nm) due to the inhibition of the expression of toll-like receptor (TLR) and proinflammatory cytokine production in macrophages.[101] Meanwhile, Winter et al. reported that SiNPs (14 nm) activate murine bone marrow-derived dendritic cells and activate inflammasome.[102] In another work, in which human umbilical vein endothelial cells (HUVECs) were exposed to silicon dioxide nanoparticles with diameters of 304 nm and 310 nm, results showed that NP induced exocytosis of Weibel-Palade bodies, associated with the release of von Willebrand factor and necrotic cell death.[83]

However, in some cells, ROS production does not seem to be associated to citotoxicity of silica nanoparticles. In relation to this, Yu et al. did not found significant differences between HEL-30 controls and those treated with different sizes (30, 48, 118, and 535 nm) and different concentrations of amorphous SiNPs and suggested that either direct physical disruption of membranes or another unknown mechanism could be involved in the observed size-dependent toxicity.[54] Moreover, Tao et al. studied the effect of two types of mesoporous silica nanoparticles on mitochondrial oxygen consumption and found out that in their presence glucose supported respiration was delayed without contribution of reactive oxygen species, because cellular GSH remained unchanged.[103]

Finally, Sohaebuddin et al. concluded that, depending on the exposed cell type, the same material can cause different intracellular responses and potential mechanisms of toxicity, as it was shown that nanomaterial-associated lysosomal membrane destabilization is responsible for the nanomaterial-induced toxicity in 3T3 fibroblasts, but not in hT bronchiolar epithelial cells and RAW macrophages and suggest that cytotoxicity of SiNPs in these cell lines may be associated with mitochondrial membrane potential reduction.[51]

Interactions between particles with mitochondria could also generate additional oxidant species. Indeed, increased oxygen consumption was observed in lung tissue cubes of animals exposed to particles.[104] This observation is also supported by an increased NADPH oxidase activity. It is worth to mention that this enzyme play a role as potent superoxide anion producer enzyme which is expressed both in phagocytic and nonphagocytic cells.[105] Moreover, increased mitochondrial oxygen consumption, NO production and phospholipid oxidation in lung homogenates exposed to particles was also observed.

3. *IN VIVO* ASSAYS

Although there are lots of scientific works concerning the potential toxicity of silica nanoparticles over different types of cell culture, little information is provided about the toxicity on living organisms. Furthermore, these *in vivo* results are often contradictory and the question about the possibility to safely administrate silica nanoparticles to living organisms remains open. In this section the results reported on biocompatibility, biodistribution and clearance of silica nanoparticles will be discuss.

3.1. Toxicity of SiNPs

Some authors found low toxicity of silica nanoparticles after their injection in animals. It has been reported

the synthesis of ultrafine organically modified silica (ORMOSIL) nanoparticles with diameters of 20 nm, conjugated with a near-infrared fluorophore for optical bioimaging. ORMOSIL NPs mainly accumulated in the liver and spleen and did not show any toxicity, adverse effect or any other abnormalities in the tissues of injected animals after 15 days studies.[106] In another experiment, the results of single and repeated dose toxicity of mesoporous hollow silica nanoparticles (MHSiNPs) demonstrated low toxicity of these new nanostructures *in vivo* when repeated and single dose toxicity were evaluated for 110 nm (MHSiNPs) after their delivery through the blood stream of mice. The LD50 of MHSNs was greater than 1000 mg/kg, while in repeated administration; 80 mg/kg continuous injection for 14 days did not produce animal death. No toxicity was found in liver, spleen, lung and kidney in MHSiNPs-injected mice at 40 and 160 mg/kg single dose and there were no abnormalities in the spleen, kidney and lung in MHSiNPs-injected mice at 500 and 1280 mg/kg. However, lymphocytic infiltration, microgranulation and degenerative necrosis of hepatocytes were observed in the liver at 500 and 1280 mg/kg single administration. In this study, MHSNs were found to be distributed in the liver and spleen after 24 h intravenous injection. Macrophage resident in liver and spleen was the target cell of MHSiNPs.[107] This is in concordance with other results where liver damage caused by mesoporous hollow silica nanoparticles was also demonstrated after continuous intraperitoneal injection into mice twice a week for 6 weeks. The administration of MHSiNPs at 50 mg/kg increased liver injury markers in serum, such as alanine aminotransferase (ALT), inflammatory cytokines interleukin-1 beta (IL-1b) and tumor necrosis factor-alpha (TNF-α). Histological analysis revealed lymphocytic infiltration and silicotic nodular like lesions in liver where activated kupffer cells played a key role similar to alveolar macrophage in the process of silicosis.[108, 109] Moreover, it was observed that 30 mg of intraperitoneally administered silica particles invariably resulted in death or distress of mice; in contrast it was found that even 100 mg of poly(lactic-co-glycolic) (PLGA) acid microspheres cause no detectable toxicity in mice of the same strain and mass indicating that silica tested would appear 100 times more lethal than PLGA particles.[66]

Apparently, there is a relationship between toxicity and sizes, shapes, surface area or chemical modification on the surface of silica nanoparticles which also condition the grade of dispersion of the particles. An experiment was performed where different sizes (50, 100 and 200 nm) of silica particles were used to evaluate their level of toxicity. The 200 nm particles were taken up faster and more intensively than the other sizes by macrophages of the spleen and liver and disappeared thereafter. In the liver, there was a significant increase in the incidence and severity of inflammation for 100 and 200 nm silica nanoparticles treatment groups at 12 h. The 50 nm particles induced only a slight inflammatory response at the same time.[110] However, all particle sizes remained aggregate in the spleen through macrophage trapping after 4 weeks. The aggregation state of nanoparticles is an important factor for distribution and excretion in the body. A previous study showed quantum dots that remained nano-sized *in vivo* are excreted via the kidney, and quantum dots that aggregated into larger particles remained in liver tissue until 5 days after a single intravenous injection.[111] There are several reports in the literature which indicate that larger particles tend to accumulate more into the liver as compared to the smaller ones.[112, 113]

Other studies have shown that exposures to nanoscale particles produce greater inflammatory and cytotoxic effects when compared to exposures to larger sized particles at equivalent mass concentration. As a particle decreases in size, the surface area increases and a greater proportion of atoms/molecules are found at the surface compared to those inside. Thus, nanoparticles have a much larger surface area per unit mass compared with larger particles. The increase in the surface-to-volume ratio results in the increase of the particle surface energy which may render them more biologically reactive.[114] Therefore it is considered that nanoparticles can be more reactive with biological components and have adverse effects due to large surface area and much particle number. Lu et al. found in their study that all sizes (30, 70 and 300 nm) of silica nanoparticles tested caused injury in the liver and caused lung inflammation without affecting the spleen, heart and kidneys. Furthermore, they proved that particles of 30 nm were the most toxic among the three sizes as determined via biochemical assays and histopathological examinations at 10 mg/kg. However, the other SiNPs sizes could also induce liver injury when the dose was increased. It was reported that, surface area had a greater effect than particle number on the toxicity of silica nanoparticles in the liver.[115] The DNA damage caused by amorphous SiNPs of 15 and 55 nm in liver, lung and blood cells and micronuclei in circulating reticulocytes were measured after three consecutive intravenous injections to rats at 48, 24 and 4 h before sacrifice. Silica particles caused a small but reproducible increase in DNA damage and micronucleated reticulocytes when tested at their maximum tolerated dose (MTD) but no genotoxic effects were observed at lower doses. These effects were probably caused through inflammatory cell-derived oxidants such as inflammatory markers, TNF-α and IL-6, found in rats' plasma. The same experiment was performed using gold nanoparticles and no genotoxic effects were observed.[116]

The toxicity of silica nanoparticles includes many aspects which were evaluated by other authors as well. For example, it has been shown that silica and titanium dioxide nanoparticles with diameters of 70 nm and 35 nm, respectively, can cause pregnancy complications when injected intravenously into pregnant mice. These nanoparticles were found in the placenta, fetal liver and fetal brain, and mice treated with these nanoparticles had smaller uteri and smaller fetuses than untreated controls. However,

it was also found that larger (300 and 1000 nm) silica particles did not induce these complications.[117] In addition, it was reported that systemically administered SiNPs can penetrate the blood-testis barrier and were detected within sertoli cells and spermatocytes, including in the nuclei of spermatocytes.[118, 119]

When SiNPs are not administered to the blood stream but subcutaneously, their toxic effect is also of great importance, particularly for local drug applications. The cytotoxicity and biocompatibility of mesoporous silicates in particle sizes 150, 800 and 4000 nm injected subcutaneously in rats were analyzed. At early time points the authors found large deposits of the material subcutaneously but very small amounts or no particles were observed after 2–3 months and there was an inflammatory reaction at the beginning which decreased substantially with time. There was no significant injury to surrounding tissues like muscle, nerve or blood vessels.[66] Bionanocomposites based on the association between biological polymers and inorganic colloids are an emerging class of materials.[120] Nanocomposite silica-collagen materials derived from concentrated collagen hydrogels and silica nanoparticles were evaluated *in vivo* to establish their potentialities for biological dressings. It was determined through the experiments that silicification significantly improved the mechanical and thermal stability of the collagen network and that nanocomposites favor the metabolic activity of immobilized human dermal fibroblasts while decreasing the hydrogel contraction. When studying *in vivo* implantation of bulk hydrogels in subcutaneous sites of rats after 1 week, it was noticed that these materials were colonized and vascularized without inducing strong inflammatory response.[121]

3.2. Degradation and Excretion

Several reviews suggest that silica nanoparticles are degradable over time in the body. Liu et al. found that about 50% of MHSiNPs was removed from the body over 4 weeks after injection. The particles would be excreted from the body with a clearance time of over 4 weeks and this long circulation time of MHSiNPs provides the possibility of using them as drug carriers for controlled release applications *in vivo*.[107] Excretion to urine and feces showed different patterns depending on particles size. At 12 h, 50 nm SiNPs reached the highest concentration in urine while 100 nm particles had a peak concentration at 24 h and neither three sized silica NPs (50, 100 and 200 nm) were detected in urine 1 week after intravenous injection. Silica nanoparticles eliminated slower via feces than in urine. The 50 nm silica nanoparticles excreted faster than the other two particle sizes and 200 nm silica particles were excreted from urine and feces at lower concentrations than the other two. This fact could be useful as nanoparticles for therapy need to have a long retention time for targeting and therapy. However, a long retention time could also caused *in vivo* toxic effects.[110]

Not only the size and surface area are important to evaluate the toxicity, distribution and excretion of SiNPs but also their surface chemical characteristics. In this sense, highly positive charge, mesoporous silica nanoparticles (MSNs) administered *in vivo* revealed markedly different uptake and elimination behaviors in comparison to less charged moieties. The first ones were quickly excreted from the liver into the gastrointestinal tract, while less charged moieties remained sequestered within the liver. Taken together these findings, the authors suggest that charge-dependent adsorption of serum proteins greatly facilitates the hepatobiliary excretion of silica nanoparticles, and that nanoparticle residence time *in vivo* can be regulated by manipulation of surface charge.[122] Moreover, the biodistribution and urinary excretion of different surface-modified silica nanoparticles (SiNPs) in mice were investigated using an *in vivo* optical imaging system. Three types of surface-modified SiNPs, including OH-SiNPs, COOH-SiNPs, and PEG-SiNPs with a size of 45 nm were prepared. Intravenous injection of these SiNPs followed by fluorescence tracing *in vivo* indicated that OH-SiNPs, COOH-SiNPs, and PEG-SiNPs were all cleared from the systemic blood circulation, but that both the clearance time and subsequent biological organ deposition were dependent on the surface chemical modification. The PEG-SiNPs exhibited relatively longer blood circulation times and lower uptake by the reticuloendothelial system organs than OH-SiNPs and COOH-SiNPs and all three types of SiNPs were partly excreted through the renal excretion route.[123]

One of the main problems in silica nanoparticle administration is their accumulation and rapid clearance by the reticuloendothelial system, which results in short circulation times. This short half-life decreases the exposure of nanoparticles to the blood–brain barrier (BBB) and consequently dissuades the interaction of NPs with the brain vascular endothelial cells and reduces their opportunity to cross the BBB. PEGylation of nanoparticles has then become the most widely used approach to increase the circulation lifetime of nanoparticles. Neutral charge and hydrophilicity are desired surface characteristic of the nanoparticles in order to avoid the rapid uptake by monocytes and consequently by organ-resident phagocytic cells (i.e., Kupffer cells in the liver). Most of the strategies to reach those requirements use hydrophilic polymers (i.e., polyethylene glycol) that, in addition, show steric effects which help to stabilize the nanoparticles preventing agglomeration.[124, 125]

A study was performed where fluorescein-doped magnetic silica nanoparticles (FMSiNPs) and PEGylated PAMAM conjugated fluorescein-doped magnetic silica nanoparticles (PEGylated PFMSNs) were used to demonstrate that PEGylated PFMSNs could penetrate the BBB through transcytosis of vascular endothelial cells, diffusing into cerebral parenchyma and distributing in the neurons. In contrast, non-PEGylated FMSiNPs were not found to cross the BBB. Moreover, the PEGylated PFMSNs may

not induce cumulative intoxication to the brain.[126] It was reported that nanosized silica-overcoated magnetic particles containing Rhodamine B isothiocyanate can also penetrate the blood–brain barrier without any apparent toxicity.[127]

4. CONCLUSION

Silica nanoparticles seem to have low toxicity and to be biocompatible when administered locally but they appear to be quite toxic systemically, leading to lymphocytic infiltration, microgranulation and degenerative necrosis in liver tissue.

Regarding the size, larger silica nanoparticles appear to be taken up faster and more intensively by macrophages than smaller ones, causing liver inflammation. On the other hand, as far as it is possible to assume, the smaller the particle, the greater surface/volume ratio, resulting in surface energy raise and consequently, more biologically reactivity. Additionally, increasing the dose could also induce liver damage, independently of the SiNPs size. Instead, when SiNPs are subcutaneously administered, the inflammatory reaction only appears at the beginning, and then decreases leaving no damage in surrounding tissues.

It was also found that size was related to nanoparticles excretion. Smaller SiNPs are excreted faster than larger one, providing the possibility of getting a long retention time for controlled drug delivery. Modifying the chemical surface could manage the chance to regulate the excretion too. Hepatobiliary excretion is enhanced by giving the nanoparticle's surface a highly positive charge. Alternatively, PEGylation allows obtaining longer blood circulation times, lower uptake by the reticuloendothelial system organs, and also penetration of the blood–brain barrier.

Regarding the possibilities given by surface modification and the results obtained by the different authors, it can be concluded that the properties of SiNPs can be mastered to meet the desired therapeutic requirements. In this sense, when anticancer properties are expected, then naked SiNPs can be useful and higher uptakes rates would cause selective toxicity in the chosen target. On the other side, when a therapeutic action or enhancement is needed, then modifying the surface of the nanoparticles could help reach the final objective.

Acknowledgments: Daniela Edhit Camporotondi is grateful for her doctoral fellowship granted by Universidad de Buenos Aires. María Lucia Foglia and Gisela Solange Alvarez are grateful for their doctoral and posdoctoral fellowship granted by the National Research Council (CONICET). Andrea Mathilde Mebert thanks Agencia Nacional de Promoción Científica y Tecnológica for her doctoral fellowship. The authors would like to acknowledge the support of grants from the University of Buenos Aires UBACYT 20020110100081 (to Martín Federico Desimone) and from Agencia Nacional de Investigaciones Científicas y Técnicas BID 1728/OC-AR PICT 1783.

References and Notes

1. C. Brinker and G. Scherer, Sol–Gel Science, Academic Press, San Diego, USA (**1990**).
2. R. K. Iler, The Chemistry of Silica, J Wiley, New York, USA (**1979**).
3. N. Baccile, F. Babonneau, B. Thomas, and T. Coradin, Introducing ecodesign in silica sol–gel materials. *J. Mater. Chem.* 19, 8537 (**2009**).
4. C. Sanchez, B. Julián, P. Belleville, and M. Popall, Applications of hybrid organic-inorganic nanocomposites. *J. Mater. Chem.* 15, 3559 (**2005**).
5. C. Sanchez, L. Rozes, F. Ribot, C. Laberty-Robert, D. Grosso, C. Sassoye, C. Boissiere, and L. Nicole, "Chimie douce": A land of opportunities for the designed construction of functional inorganic and hybrid organic-inorganic nanomaterials. *Comptes. Rendus. Chimie.* 13, 3 (**2010**).
6. G. J. D. A. A. Soler-Illia, C. Sanchez, B. Lebeau, and J. Patarin, Chemical strategies to design textured materials: From microporous and mesoporous oxides to nanonetworks and hierarchical structures. *Chem. Rev.* 102, 4093 (**2002**).
7. E. Ruiz-Hitzky, P. Aranda, M. Darder, and M. Ogawa, Hybrid and biohybrid silicate based materials: Molecular versus block-assembling bottom-up processes. *Chem. Soc. Rev.* 40, 801 (**2011**).
8. D. Avnir, O. Lev, and J. Livage, Recent bio-applications of sol–gel materials. *J. Mater. Chem.* 16, 1013 (**2006**).
9. T. Coradin, M. Boissiere, and J. Livage, Sol–gel chemistry in medicinal science. *Current Med. Chem.* 13, 99 (**2006**).
10. T. Coradin and J. Livage, Aqueous silicates in biological sol–gel applications: New perspectives for old precursors. *Accounts Chem. Res.* 40, 819 (**2007**).
11. V. Kandimalla, V. S. Tripathi, and H. Ju, Immobilization of biomolecules in sol–gels: Biological and analytical applications. *Critical Rev. Anal. Chem.* 36, 73 (**2006**).
12. S. Y. Gadre and P. I. Gouma, Biodoped ceramics: Synthesis, properties, and applications. *J. Amer. Ceramic Soc.* 89, 2987 (**2006**).
13. M. F. Desimone, G. S. Alvarez, M. L. Foglia, and L. E. Diaz, Development of sol–gel hybrid materials for whole cell immobilization. *Recent Pat. Biotechnol.* 3, 55 (**2009**).
14. M. L. Foglia, G. S. Alvarez, P. N. Catalano, A. M. Mebert, L. E. Diaz, T. Coradin, and M. F. Desimone, Recent patents on the synthesis and application of silica nanoparticles for drug delivery. *Recent Pat. Biotechnol.* 5, 54 (**2011**).
15. Q. He and J. Shi, Mesoporous silica nanoparticle based nano drug delivery systems: Synthesis, controlled drug release and delivery, pharmacokinetics and biocompatibility. *J. Mater. Chem.* 21, 5845 (**2011**).
16. J. Wu, J. Silvent, T. Coradin, and C. Aime, Biochemical investigation of the formation of three-dimensional networks from DNA-grafted large silica particles. *Langmuir* 28, 2156 (**2012**).
17. Y. L. Choi, J. H. Lee, J. Jaworski, and J. H. Jung, Mesoporous silica nanoparticles functionalized with a thymidine derivative for controlled release. *J. Mater. Chem.* 22, 9455 (**2012**).
18. K. C. W. Wu and Y. Yamauchi, Controlling physical features of mesoporous silica nanoparticles (MSNs) for emerging applications. *J. Mater. Chem.* 22, 1251 (**2012**).
19. C. Barbé, J. Bartlett, L. Kong, K. Finnie, H. Q. Lin, M. Larkin, S. Calleja, A. Bush, and G. Calleja, Silica particles: A novel drug-delivery system. *Adv. Mater.* 16, 1959 (**2004**).
20. J.-F. Chen, H.-M. Ding, J.-X. Wang, and L. Shao, Preparation and characterization of porous hollow silica nanoparticles for drug delivery application. *Biomaterials* 25, 723 (**2004**).

21. Z. Z. Li, L. X. Wen, L. Shao, and J. F. Chen, Fabrication of porous hollow silica nanoparticles and their applications in drug release control. *J. Control Release* 98, 245 **(2004)**.

22. W. Stöber, A. Fink, and E. Bohn, Controlled growth of monodisperse silica spheres in the micron size range. *J. Colloids Interf. Sci.* 26, 62 **(1968)**.

23. K. S. Finnie, J. R. Bartlett, C. J. A. Barbé, and L. Kong, Formation of Silica Nanoparticles in Microemulsions. *Langmuir* 23, 3017 **(2007)**.

24. L. M. Gan, K. Zhang, and C. H. Chew, Preparation of silica nanoparticles from sodium orthosilicate in inverse microemulsions. *Colloids Surf. A* 110, 199 **(1996)**.

25. G. H. Bogush, M. A. Tracy, and C. F. Zukoski Iv, Preparation of monodisperse silica particles: Control of size and mass fraction. *J. Non-Crystalline Solids* 104, 95 **(1988)**.

26. K. Nozawa, H. Gailhanou, L. Raison, P. Panizza, H. Ushiki, E. Sellier, J. P. Delville, and M. H. Delville, Smart control of monodisperse stober silica particles: Effect of reactant addition rate on growth process. *Langmuir* 21, 1516 **(2004)**.

27. R. P. Bagwe, C. Yang, L. R. Hilliard, and W. Tan, Optimization of dye-doped silica nanoparticles prepared using a reverse microemulsion method. *Langmuir* 20, 8336 **(2004)**.

28. F. Caruso, R. A. Caruso, and H. Möhwald, Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating. *Science* 282, 1111 **(1998)**.

29. F. Tang, L. Li, and D. Chen, Mesoporous silica nanoparticles: Synthesis, biocompatibility and drug delivery. *Adv. Mater.* 24, 1504 **(2012)**.

30. J. M. Rosenholm, C. Sahlgren, and M. Linden, Towards multifunctional, targeted drug delivery systems using mesoporous silica nanoparticles—Opportunities & challenges. *Nanoscale* 2, 1870 **(2010)**.

31. Y. Liu, C. Lou, H. Yang, M. Shi, and H. Miyoshi, Silica nanoparticles as promising drug/gene delivery carriers and fluorescent nanoprobes: Recent advances. *Current Cancer Drug Targets* 11, 156 **(2010)**.

32. S. C. McBain, H. H. P. Yiu, and J. Dobson, Magnetic nanoparticles for gene and drug delivery. *Int. J. Nanomed.* 3, 169 **(2008)**.

33. C. P. Tsai, C. Y. Chen, Y. Hung, F. H. Chang, and C. Y. Mou, Monoclonal antibody-functionalized mesoporous silica nanoparticles (MSN) for selective targeting breast cancer cells. *J. Mater. Chem.* 19, 5737 **(2009)**.

34. K. Unger, H. Rupprecht, B. Valentin, and W. Kircher, The use of porous and surface modified silicas as drug delivery and stabilizing agents. *Drug Develop. Ind. Pharm.* 9, 69 **(2008)**.

35. L. Pasqua, F. Testa, R. Aiello, S. Cundari, and J. B. Nagy, Preparation of bifunctional hybrid mesoporous silica potentially useful for drug targeting. *Microporous Mesoporous Mater.* 103, 166 **(2007)**.

36. J. M. Rosenholm, A. Meinander, E. Peuhu, R. Niemi, J. E. Eriksson, C. Sahlgren, and M. Linden, Targeting of Porous Hybrid Silica Nanoparticles to Cancer Cells. *ACS Nano* 3, 197 **(2008)**.

37. D. T. Birnbaum and L. Brannon-Peppas, Polymers in controlled release. *Polymer News* 27, 13 **(2002)**.

38. L. Branno-Peppas and L. Brannon-Peppas (ed.), Polymers in Controlled Release, John Wiley & Sons, New York, **(2002)**.

39. M. Vallet-Regí, E. Ruiz-Hernández, B. González, and A. Baeza, Design of Smart Nanomaterials for Drug and Gene Delivery. *J. Biomater. Tissue Eng.* 1, 6 **(2011)**.

40. S.-H. Wu, Y. Hung, and C.-Y. Mou, Mesoporous silica nanoparticles as nanocarriers. *Chem. Comm.* 47, 9972 **(2011)**.

41. S. Hussain, J. A. J. Vanoirbeek, and P. H. M. Hoet, Interactions of nanomaterials with the immune system. *Nanomed. Nanobiotechnol.* 4, 169 **(2012)**.

42. M. Gary-Bobo, O. Hocine, D. Brevet, M. Maynadier, L. Raehm, S. Richeter, V. Charasson, B. Loock, A. Morère, P. Maillard, M. Garcia, and J.-O. Durand, Cancer therapy improvement with mesoporous silica nanoparticles combining targeting, drug delivery and PDT. *Int. J. Pharm.* 423, 509 **(2011)**.

43. E. Verraedt, M. Pendela, E. Adams, J. Hoogmartens, and J. A. Martens, Controlled release of chlorhexidine from amorphous microporous silica. *J. Control Release* 142, 47 **(2010)**.

44. P. Demuth, M. Hurley, C. Wu, S. Galanie, M. R. Zachariah, and P. Deshong, Mesoscale porous silica as drug delivery vehicles: Synthesis, characterization, and pH-sensitive release profiles. *Microporous Mesoporous Mater.* 141, 128 **(2011)**.

45. L. V. Carvalho, R. D. C. Ruiz, K. Scaramuzzi, E. B. Marengo, J. R. Matos, D. V. Tambourgi, M. C. A. Fantini, and O. A. Anna, Immunological parameters related to the adjuvant effect of the ordered mesoporous silica SBA-15. *Vaccine* 28, 7829 **(2010)**.

46. L. P. Mercuri, L. V. Carvalho, F. A. Lima, C. Quayle, M. C. A. Fantini, G. S. Tanaka, W. H. Cabrera, M. F. D. Furtado, D. V. Tambourgi, J. D. R. Matos, M. Jaroniec, and O. A. Sant'Anna, Ordered mesoporous silica SBA-15: A new effective adjuvant to induce antibody response. *Small* 2, 254 **(2006)**.

47. P. Berntsen, C. Y. Park, B. Rothen-Rutishauser, A. Tsuda, T. M. Sager, R. M. Molina, T. C. Donaghey, A. M. Alencar, D. I. Kasahara, T. Ericsson, E. J. Millet, J. Swenson, D. J. Tschumperlin, J. P. Butler, J. D. Brain, J. J. Fredberg, P. Gehr, and E. H. Zhou, Biomechanical effects of environmental and engineered particles on human airway smooth muscle cells. *J. Royal Soc. Inter.* 7, S331 **(2010)**.

48. K. Wittmaack, In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: Particle number, surface area, or what? *Environ. Health Persp.* 115, 187 **(2007)**.

49. B. Fadeel and A. E. Garcia-Bennett, Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. *Adv. Drug. Deliv. Rev.* 62, 362 **(2009)**.

50. A. Nel, T. Xia, L. Mädler, and N. Li, Toxic potential of materials at the nanolevel. *Science* 311, 622 **(2006)**.

51. S. K. Sohaebuddin, P. T. Thevenot, D. Baker, J. W. Eaton, and L. Tang, Nanomaterial cytotoxicity is composition, size, and cell type dependent. *Particle Fibre Toxicol.* 7, 22 **(2010)**.

52. A. Malugin, H. Herd, and H. Ghandehari, Differential toxicity of amorphous silica nanoparticles toward phagocytic and epithelial cells. *J. Nanopart. Res.* 13, 5381 **(2011)**.

53. M. V. D. Z. Park, I. Lynch, S. Ramírez-García, K. A. Dawson, L. De La Fonteyne, E. Gremmer, W. Slob, J. J. Briedé, A. Elsaesser, C. V. Howard, H. Van Loveren, and W. H. De Jong, *In vitro* evaluation of cytotoxic and inflammatory properties of silica nanoparticles of different sizes in murine RAW 264.7 macrophages. *J. Nanopart. Res.* 13, 6775 **(2011)**.

54. K. Yu, C. Grabinski, A. Schrand, R. Murdock, W. Wang, B. Gu, J. Schlager, and S. Hussain, Toxicity of amorphous silica nanoparticles in mouse keratinocytes. *J. Nanopart. Res.* 11, 15 **(2009)**.

55. A. Kunzmann, B. Andersson, C. Vogt, N. Feliu, F. Ye, S. Gabrielsson, M. S. Toprak, T. Buerki-Thurnherr, S. Laurent, M. Vahter, H. Krug, M. Muhammed, A. Scheynius, and B. Fadeel, Efficient internalization of silica-coated iron oxide nanoparticles of different sizes by primary human macrophages and dendritic cells. *Toxicol. Appl. Pharmacol.* 253, 81 **(2011)**.

56. H. Nabeshi, T. Yoshikawa, K. Matsuyama, Y. Nakazato, A. Arimori, M. Isobe, S. Tochigi, S. Kondoh, T. Hirai, T. Akase, T. Yamashita, K. Yamashita, T. Yoshida, K. Nagano, Y. Abe, Y. Yoshioka, H. Kamada, T. Imazawa, N. Itoh, S. I. Tsunoda, and Y. Tsutsumi, Size-dependent cytotoxic effects of amorphous silica nanoparticles on Langerhans cells. *Pharmazie* 65, 199 **(2010)**.

57. T. Hirai, T. Yoshikawa, H. Nabeshi, T. Yoshida, S. Tochigi, M. Uji, K. Ichihashi, T. Akase, T. Yamashita, K. Yamashita, K. Nagano, Y. Abe, H. Kamada, S. Tsunoda, Y. Yoshioka, N. Itoh, and Y. Tsutsumi, Size-dependent immune-modulating effect of amorphous nanosilica particles. *Pharmazie* 66, 727 **(2011)**.

58. E. J. Park, J. Yi, K. H. Chung, D. Y. Ryu, J. Choi, and K. Park, Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. *Toxicol. Lett.* 180, 222 **(2008)**.

59. K. M. Waters, L. M. Masiello, R. C. Zangar, B. J. Tarasevich, N. J. Karin, R. D. Quesenberry, S. Bandyopadhyay, J. G. Teeguarden, J. G. Pounds, and B. D. Thrall, Macrophage responses to silica nanoparticles are highly conserved across particle sizes. *Toxicol. Sci.* 107, 553 **(2009)**.

60. T. Morishige, Y. Yoshioka, H. Inakura, A. Tanabe, X. Yao, S. Narimatsu, Y. Monobe, T. Imazawa, S. I. Tsunoda, Y. Tsutsumi, Y. Mukai, N. Okada, and S. Nakagawa, The effect of surface modification of amorphous silica particles on NLRP3 inflammasome mediated IL-1Î² production, ROS production and endosomal rupture. *Biomaterials* 31, 6833 **(2010)**.

61. G. Oberdörster, E. Oberdörster, and J. Oberdöster, Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Persp.* 113, 823 **(2005)**.

62. D. Napierska, L. C. J. Thomassen, V. Rabolli, D. Lison, L. Gonzalez, M. Kirsch-Volders, J. A. Martens, and P. H. Hoet, Size-dependent cytotoxicity of monodisperse silica nanoparticles in human endothelial cells. *Small* 5, 846 **(2009)**.

63. J. H. E. Arts, H. Muijser, E. Duistermaat, K. Junker, and C. F. Kuper, Five-day inhalation toxicity study of three types of synthetic amorphous silicas in Wistar rats and post-exposure evaluations for up to 3 months. *Food Chem. Toxicol.* 45, 1856 **(2007)**.

64. L. M. Costantini, R. M. Gilberti, and D. A. Knecht, The phagocytosis and toxicity of amorphous silica. *PLoS ONE* 6, e14647 **(2011)**.

65. S. Lee, H. S. Yun, and S. H. Kim, The comparative effects of mesoporous silica nanoparticles and colloidal silica on inflammation and apoptosis. *Biomaterials* 32, 9434 **(2011)**.

66. S. P. Hudson, R. F. Padera, R. Langer, and D. S. Kohane, The biocompatibility of mesoporous silicates. *Biomaterials* 29, 4045 **(2008)**.

67. Y.-S. Lin and C. L. Haynes, Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *J. Amer. Chem. Soc.* 132, 4834 **(2010)**.

68. Z. Tao, B. B. Toms, J. Goodisman, and T. Asefa, Mesoporosity and functional group dependent endocytosis and cytotoxicity of silica nanomaterials. *Chem. Res. Toxicol.* 22, 1869 **(2009)**.

69. Y. Chen, Z. Xue, D. Zheng, K. Xia, Y. Zhao, T. Liu, Z. Long, and J. Xia, Sodium chloride modified silica nanoparticles as a non-viral vector with a high efficiency of DNA transfer into cells. *Current Gene Therapy* 3, 273 **(2003)**.

70. C. Kneuer, M. Sameti, U. Bakowsky, T. Schiestel, H. Schirra, H. Schmidt, and C. M. Lehr, A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells *in vitro*. *Bioconj. Chem.* 11, 926 **(2000)**.

71. M. N. V. Ravi Kumar, M. Sameti, S. S. Mohapatra, X. Kong, R. F. Lockey, U. Bakowsky, G. Lindenblatt, H. Schmidt, and C. M. Lehr, Cationic silica nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency *in vitro* and *In vivo*. *J. Nanosci. Nanotechnol.* 4, 876 **(2004)**.

72. I. Roy, T. Y. Ohulchanskyy, D. J. Bharali, H. E. Pudavar, R. A. Mistretta, N. Kaur, and P. N. Prasad, Optical tracking of organically modified silica nanoparticles as DNA carriers: A nonviral, nanomedicine approach for gene delivery. *PNAS* 102, 279 **(2005)**.

73. D. J. Bharali, I. Klejbor, E. K. Stachowiak, P. Dutta, I. Roy, N. Kaur, E. J. Bergey, P. N. Prasad, and M. K. Stachowiak, Organically modified silica nanoparticles: A nonviral vector for *in vivo* gene delivery and expression in the brain. *PNAS* 102, 11539 **(2005)**.

74. A. M. Mumin, J. W. Barrett, G. A. Dekaban, and J. Zhang, Dendritic cell internalization of foam-structured fluorescent mesoporous silica nanoparticles. *J. Colloids Interf. Sci.* 353, 156 **(2011)**.

75. E. B. Gyenge, X. Darphin, A. Wirth, U. Pieles, H. Walt, M. Bredell, and C. Maake, Uptake and fate of surface modified silica nanoparticles in head and neck squamous cell carcinoma. *J. Nanobiotechnol.* 9, 32 **(2011)**.

76. D. Dutta, S. K. Sundaram, J. G. Teeguarden, B. J. Riley, L. S. Fifield, J. M. Jacobs, S. R. Addleman, G. A. Kaysen, B. M. Moudgil, and T. J. Weber, Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials. *Toxicol. Sci.* 100, 303 **(2007)**.

77. M. Massignani, C. LoPresti, A. Blanazs, J. Madsen, S. P. Armes, A. L. Lewis, and G. Battaglia, Controlling Cellular Uptake by Surface Chemistry, Size, and Surface Topology at the Nanoscale. *Small* 5, 2424 **(2009)**.

78. T.-G. Iversen, T. Skotland, and K. Sandvig, Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. *Nano Today* 6, 176 **(2011)**.

79. N. Daum, C. Tscheka, A. Neumeyer, and M. Schneider, Novel approaches for drug delivery systems in nanomedicine: effects of particle design and shape. *Nanomed. Nanobiotechnol.* 4, 52 **(2012)**.

80. S. Zhang, J. Li, G. Lykotrafitis, G. Bao, and S. Suresh, Size-Dependent Endocytosis of Nanoparticles. *Adv. Mater.* 21, 419 **(2009)**.

81. F. Lu, S.-H. Wu, Y. Hung, and C.-Y. Mou, Size Effect on Cell Uptake in Well-Suspended, Uniform Mesoporous Silica Nanoparticles. *Small* 5, 1408 **(2009)**.

82. J. Choi, Q. Zheng, H. E. Katz, and T. R. Guilarte, Silica-based nanoparticle uptake and cellular response by primary microglia. *Environ. Health Persp.* 118, 589 **(2010)**.

83. A. T. Bauer, E. A. Strozyk, C. Gorzelanny, C. Westerhausen, A. Desch, M. F. Schneider, and S. W. Schneider, Cytotoxicity of silica nanoparticles through exocytosis of von Willebrand factor and necrotic cell death in primary human endothelial cells. *Biomaterials* 32, 8385 **(2011)**.

84. S. Quignard, G. Mosser, M. Boissière, and T. Coradin, Long-term fate of silica nanoparticles interacting with human dermal fibroblasts. *Biomaterials* 33, 4431 **(2012)**.

85. M. F. Desimone, C. Hélary, G. Mosser, M. M. Giraud-Guille, J. Livage, and T. Coradin, Fibroblast encapsulation in hybrid silica-collagen hydrogels. *J. Mater. Chem.* 20, 666 **(2010)**.

86. M. F. Desimone, C. Hélary, I. B. Rietveld, I. Bataille, G. Mosser, M. M. Giraud-Guille, J. Livage, and T. Coradin, Silica-collagen bionanocomposites as three-dimensional scaffolds for fibroblast immobilization. *Acta Biomater.* 6, 3998 **(2010)**.

87. S. Quignard, G. J. Copello, C. Aimé, I. Bataille, C. Helary, M. F. Desimone, and T. Coradin, Influence of silicification on the structural and biological properties of buffer-mediated collagen hydrogels. *Adv. Eng. Mater.* 14, B51 **(2012)**.

88. X. Xing, X. He, J. Peng, K. Wang, and W. Tan, Uptake of silica-coated nanoparticles by HeLa cells. *J. Nanosci. Nanotechnol.* 5, 1688 **(2005)**.

89. H. Nabeshi, T. Yoshikawa, A. Arimori, T. Yoshida, S. Tochigi, T. Hirai, T. Akase, K. Nagano, Y. Abe, H. Kamada, S. Tsunoda, N. Itoh, Y. Yoshioka, and Y. Tsutsumi, Effect of surface properties of silica nanoparticles on their cytotoxicity and cellular distribution in murine macrophages. *Nanoscale Res. Lett.* 6, X1 **(2011)**.

90. T. C. Long, J. Tajuba, P. Sama, N. Saleh, C. Swartz, J. Parker, S. Hester, G. V. Lowry, and B. Veronesi, Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons *in vitro*. *Environ. Health Persp.* 115, 1631 **(2007)**.

91. J. K. Su, M. K. Byeong, J. L. Young, and W. C. Hai, Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. *Environ. Mol. Mutag.* 49, 399 **(2008)**.

92. M. Thibodeau, C. Giardina, and A. K. Hubbard, Silica-induced caspase activation in mouse alveolar macrophages is dependent upon

mitochondrial integrity and aspartic proteolysis. *Toxicol. Sci.* 76, 91 **(2003)**.

93. R. Meneghini, Iron Homeostasis, Oxidative Stress, and DNA Damage. *Free Rad. Biol. Med.* 23, 783 **(1997)**.

94. M. S. Cooke, M. D. Evans, M. Dizdaroglu, and J. Lunec, Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* 17, 1195 **(2003)**.

95. F. Wang, F. Gao, M. Lan, H. Yuan, Y. Huang, and J. Liu, Oxidative stress contributes to silica nanoparticle-induced cytotoxicity in human embryonic kidney cells. *Toxicol. In Vitro* 23, 808 **(2009)**.

96. W. Lin, Y. W. Huang, X. D. Zhou, and Y. Ma, *In vitro* toxicity of silica nanoparticles in human lung cancer cells. *Toxicol. Appl. Pharmacol.* 217, 252 **(2006)**.

97. Y. Ye, J. Liu, J. Xu, L. Sun, M. Chen, and M. Lan, Nano-SiO_2 induces apoptosis via activation of p53 and Bax mediated by oxidative stress in human hepatic cell line. *Toxicol, In Vitro* 24, 751 **(2010)**.

98. Q. J. X. Lu, H. Zhou, Q. Gan, W. Tang, J. Lu, Y. Yuan, and C. Liu, *In vitro* cytotoxicity and induction of apoptosis by silica nanoparticles in human HepG2 hepatoma cells. *Int. J. Nanomed.* 6, 1889 **(2011)**.

99. J. Ahmad, M. Ahamed, M. J. Akhtar, S. A. Alrokayan, M. A. Siddiqui, J. Musarrat, and A. A. Al-Khedhairy, Apoptosis induction by silica nanoparticles mediated through reactive oxygen species in human liver cell line HepG2. *Toxicol. Appl. Pharmacol.* 259, 160 **(2012)**.

100. H. Nabeshi, T. Yoshikawa, K. Matsuyama, Y. Nakazato, S. Tochigi, S. Kondoh, T. Hirai, T. Akase, K. Nagano, Y. Abe, Y. Yoshioka, H. Kamada, N. Itoh, S. I. Tsunoda, and Y. Tsutsumi, Amorphous nanosilica induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. *Particle Fibre Toxicol.* 8, 1 **(2011)**.

101. M. Lucarelli, A. M. Gatti, G. Savarino, P. Quattroni, L. Martinelli, E. Monari, and D. Boraschi, Innate defence functions of macrophages can be biased by nano-sized ceramic and metallic particles. *European Cytokine Network* 15, 339 **(2004)**.

102. M. Winter, H. D. Beer, V. Hornung, U. Kärmer, R. P. F. Schins, and I. Förster, Activation of the inflammasome by amorphous silica and TiO2 nanoparticles in murine dendritic cells. *Nanotoxicol.* 5, 326 **(2011)**.

103. Z. Tao, M. P. Morrow, T. Asefa, K. K. Sharma, C. Duncan, A. Anan, H. S. Penefsky, J. Goodisman, and A. K. Souid, Mesoporous silica nanoparticles inhibit cellular respiration. *Nano Lett.* 8, 1517 **(2008)**.

104. N. D. Magnani, T. Marchini, D. R. Tasat, S. Alvarez, and P. A. Evelson, Lung oxidative metabolism after exposure to ambient particles. *Biochem. Biophys. Res. Commun.* 412, 667 **(2011)**.

105. N. Louhelainen, M. Myllärniemi, I. Rahman, and V. L. Kinnula, Airway biomarkers of the oxidant burden in asthma and chronic obstructive pulmonary disease: Current and future perspectives. *International Journal of COPD* 3, 585 **(2008)**.

106. R. Kumar, I. Roy, T. Y. Ohulchanskky, L. A. Vathy, E. J. Bergey, M. Sajjad, and P. N. Prasad, *In vivo* biodistribution and clearance studies using multimodal organically modified silica nanoparticles. *ACS Nano* 4, 699 **(2010)**.

107. T. Liu, L. Li, X. Teng, X. Huang, H. Liu, D. Chen, J. Ren, J. He, and F. Tang, Single and repeated dose toxicity of mesoporous hollow silica nanoparticles in intravenously exposed mice. *Biomaterials* 32, 1657 **(2011)**.

108. T. Liu, L. Li, C. Fu, H. Liu, D. Chen, and F. Tang, Pathological mechanisms of liver injury caused by continuous intraperitoneal injection of silica nanoparticles. *Biomaterials* 33, 2399 **(2012)**.

109. G. Xie, J. Sun, G. Zhong, L. Shi, and D. Zhang, Biodistribution and toxicity of intravenously administered silica nanoparticles in mice. *Arch. Toxicol.* 84, 183 **(2010)**.

110. M. Cho, W.-S. Cho, M. Choi, S. J. Kim, B. S. Han, S. H. Kim, H. O. Kim, Y. Y. Sheen, and J. Jeong, The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. *Toxicol. Lett.* 189, 177 **(2009)**.

111. Z. Chen, H. Chen, H. Meng, G. Xing, X. Gao, B. Sun, X. Shi, H. Yuan, C. Zhang, R. Liu, F. Zhao, Y. Zhao, and X. Fang, Biodistribution and metabolic paths of silica coated CdSeS quantum dots. *Toxicol. Appl. Pharmacol.* 230, 364 **(2008)**.

112. S. Nagayama, K. Ogawara, Y. Fukuoka, K. Higaki, and T. Kimura, Time-Dependent Changes in Opsonin Amount Associated on Nanoparticles Alter Their Hepatic Uptake Characteristics. *Int. J. Pharm.* 342, 215 **(2007)**.

113. Q. He, Z. Zhang, F. Gao, Y. Li, and J. Shi, *In vivo* Biodistribution and Urinary Excretion of Mesoporous Silica Nanoparticles: Effects of Particle Size and PEGylation. *Small* 7, 271 **(2011)**.

114. G. Oberdörster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, B. Karn, W. Kreyling, D. Lai, S. Olin, N. Monteiro-Riviere, D. Warheit, and H. Yang, Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Particle Fibre Toxicol.* 2, 8 **(2005)**.

115. X. Lu, Y. Tian, Q. Zhao, T. Jin, S. Xiao, and X. Fan, Integrated metabonomics analysis of the size-response relationship of silica nanoparticles-induced toxicity in mice. *Nanotechnol.* 22, 055101 **(2011)**.

116. T. R. Downs, M. E. Crosby, T. Hu, S. Kumar, A. Sullivan, K. Sarlo, B. Reeder, M. Lynch, M. Wagner, T. Mills, and S. Pfuhler, Silica nanoparticles administered at the maximum tolerated dose induce genotoxic effects through an inflammatory reaction while gold nanoparticles do not. *Mutation Research/Genetic Toxicol. Environmental Mutagenesis* 745, 38 **(2012)**.

117. K. Yamashita, Y. Yoshioka, K. Higashisaka, K. Mimura, Y. Morishita, M. Nozaki, T. Yoshida, T. Ogura, H. Nabeshi, K. Nagano, Y. Abe, H. Kamada, Y. Monobe, T. Imazawa, H. Aoshima, K. Shishido, Y. Kawai, T. Mayumi, S. I. Tsunoda, N. Itoh, T. Yoshikawa, I. Yanagihara, S. Saito, and Y. Tsutsumi, Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nature Nanotechnol.* 6, 321 **(2011)**.

118. Y. Morishita, Y. Yoshioka, H. Satoh, N. Nojiri, K. Nagano, Y. Abe, H. Kamada, S. I. Tsunoda, H. Nabeshi, T. Yoshikawa, and Y. Tsutsumi, Distribution and histologic effects of intravenously administered amorphous nanosilica particles in the testes of mice. *Biochem. Biophys. Res. Comm.* 420, 297 **(2012)**.

119. Z. Lan and W. X. Yang, Nanoparticles and spermatogenesis: How do nanoparticles affect spermatogenesis and penetrate the blood-testis barrier. *Nanomed.* 7, 579 **(2012)**.

120. C. Aimé and T. Coradin, Nanocomposites from biopolymer hydrogels: Blueprints for white biotechnology and green materials chemistry. *J. Polymer Sci. Part B: Polymer Phys.* 50, 669 **(2012)**.

121. M. F. Desimone, C. Hélary, S. Quignard, I. B. Rietveld, I. Bataille, G. J. Copello, G. Mosser, M.-M. Giraud-Guille, J. Livage, A. Meddahi-Pellé, and T. Coradin, *In vitro* studies and preliminary *in vivo* evaluation of silicified concentrated collagen hydrogels. *ACS Appl. Mater. Interf.* 3, 3831 **(2011)**.

122. J. S. Souris, C. H. Lee, S. H. Cheng, C. T. Chen, C. S. Yang, J. A. A. Ho, C. Y. Mou, and L. W. Lo, Surface charge-mediated rapid hepatobiliary excretion of mesoporous silica nanoparticles. *Biomaterials* 31, 5564 **(2010)**.

123. X. He, H. Nie, K. Wang, W. Tan, X. Wu, and P. Zhang, *In vivo* study of biodistribution and urinary excretion of surface-modified silica nanoparticles. *Anal. Chem.* 80, 9597 **(2008)**.

124. C. Yagüe, M. Moros, V. Grazu, M. Arruebo, and J. Santamaría, Synthesis and stealthing study of bare and PEGylated silica micro- and nanoparticles as potential drug-delivery vectors. *Chem. Eng. J.* 137, 45 **(2008)**.

REVIEW

125. Q. He, J. Zhang, J. Shi, Z. Zhu, L. Zhang, W. Bu, L. Guo, and Y. Chen, The effect of PEGylation of mesoporous silica nanoparticles on nonspecific binding of serum proteins and cellular responses. *Biomaterials* 31, 1085 **(2009)**.

126. S. Ku, F. Yan, Y. Wang, Y. Sun, N. Yang, and L. Ye, The blood-brain barrier penetration and distribution of PEGylated fluorescein-doped magnetic silica nanoparticles in rat brain. *Biochem. Biophys. Res. Comm.* 394, 871 **(2010)**.

127. J. S. Kim, T.-J. Yoon, K. N. Yu, B. G. Kim, S. J. Park, H. W. Kim, K. H. Lee, S. B. Park, J.-K. Lee, and M. H. Cho, Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicol. Sci.* 89, 338 **(2006)**.

Received: 30 April 2012. Accepted: 9 August 2012.