Expert Opinion on Drug Delivery

ISSN: 1742-5247 (Print) 1744-7593 (Online) Journal homepage: https://www.tandfonline.com/loi/iedd20

Overcoming the stability, toxicity, and biodegradation challenges of tumor stimuli-responsive inorganic nanoparticles for delivery of cancer therapeutics

Juan L. Paris, Alejandro Baeza & María Vallet-Regí

To cite this article: Juan L. Paris, Alejandro Baeza & María Vallet-Regí (2019): Overcoming the stability, toxicity, and biodegradation challenges of tumor stimuli-responsive inorganic nanoparticles for delivery of cancer therapeutics, Expert Opinion on Drug Delivery, DOI: 10.1080/17425247.2019.1662786

To link to this article: https://doi.org/10.1080/17425247.2019.1662786

Accepted author version posted online: 30 Aug 2019.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Drug Delivery*

DOI: 10.1080/17425247.2019.1662786

Overcoming the stability, toxicity, and biodegradation challenges of tumor stimuli-responsive inorganic nanoparticles for delivery of cancer therapeutics

Juan L. Paris[1,#], Alejandro Baeza[2,#], María Vallet-Regí[3,*]

1. Department of Life Sciences, Nano4Health Unit, Nanomedicine Group. International Iberian Nanotechnology Laboratory (INL). Av. Mestre José Veiga s/n, 4715-330 Braga – Portugal.

2. Materials and Aeroespatial Production Department, Polymer Materials Research Group, Madrid, 28040, Spain

3. Dpto. Química en Ciencias Farmacéuticas (Unidad Docente de Química Inorgánica y Bioinorgánica), Facultad de Farmacia, UCM, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), 28040-Madrid, Spain. Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

[#] Authors contributed equally to this work

***Correspondence**

María Vallet-Regí

Email: vallet@ucm.es

Abstract

Introduction: Stimuli-responsive nanomaterials for cancer therapy have attracted much interest recently due to their potential for improving the current standard of care. Different types of inorganic nanoparticles are widely employed for the development of these strategies, but in some cases safety concerns hinder their clinical translation. This review aims to provide an overview of the challenges that inorganic nanoparticles face regarding their stability, toxicity and biodegradability, as well as the strategies that have been proposed to overcome them.

Areas covered: The available information about the *in vitro* and *in vivo* biocompatibility, as well as the biodegradability of the following nanoparticles is presented and discussed: superparamagnetic iron oxide nanoparticles, gold nanoparticles, graphene and mesoporous nanoparticles made of silicon or silicon oxide. The toxicology of inorganic nanoparticles is greatly affected by many physicochemical parameters, and their surface modification emerges as the main intervention to improve their biocompatibility and tailor their performance for specific biomedical applications.

Expert opinion: Even though many different studies have been performed regarding the biological behavior of inorganic nanoparticles, long-term *in vivo* data is still scarce, limiting our capacity to evaluate the proposed nanomaterials for clinical use. The role of biodegradability in different therapeutic contexts is also discussed.

Keywords: Inorganic nanoparticles, mesoporous silica nanoparticles. nanomedicine, nano-toxicology, superparamagnetic iron oxide nanoparticles.

Article highlights

- A series of inorganic nanoparticles have become fundamental tools in the development of stimuli-responsive nanoparticles for oncological treatment, making necessary their nano-toxicological evaluation.
- SPION have been employed as drug delivery systems and magnetically triggered heaters. These nanoparticles present potential toxicity by the production of radical oxidative species but this undesired property has been diminished by tuning their size and/or coating their surface with different moieties.
- GNP present unique optical properties which have been exploited for the production of hyperthermia seeds triggered by near-infrared radiation and imaging agents in combination with drug delivery agents. Their biocompatibility is excellent but they are barely degraded in physiological conditions. Therefore, their potential toxicity should be evaluated in long time assays.
- Graphene has been thoroughly studied for drug and gene delivery, biosensing and imaging. Some studies have shown potential toxicity in physiological and pathological conditions, although the chemical modification and surface coating with different polymers can improve its biocompatibility profile.
- Mesoporous particles made of silicon or silica present many advantages as drug delivery systems. Their biodegradability can be controlled by modifying a series of physicochemical parameters, which enables tailoring these materials for specific biomedical applications and preventing their long-term bioaccumulation.

1. Introduction

Nanoparticle use for cancer treatment has attracted significant attention, and several products are already approved for clinical use.[1] The main rationale for the

development of this discipline was the enhanced permeability and retention (EPR) effect.[2] The EPR effect describes the capacity of macromolecules and nanoparticles to extravasate and be retained in solid tumors due to their impaired vascular network. This preferential accumulation in cancerous tissues was expected to drastically improve the therapeutic efficacy while also greatly decreasing the dreaded side effects of traditional chemotherapeutic treatments in oncology. However, the main advantages of the nano-formulations employed in the clinic has been a moderate improvement in their toxicological profile, many times due to the possibility of avoiding toxic excipients that were being used prior to the development of these formulations.[1] Nanomaterials that can respond to changes in their environment materials have been proposed to overcome some of the limitations of the nano-drug delivery systems (nano-DDS) currently employed in the clinic, and some strategies have already entered into clinical evaluation.[3,4] These stimuli-responsive materials can react when exposed to either internal physiopathological factors or externally-induced changes, and their design and production has become an extremely active area of research. Different types of responses can be triggered by the stimulus, from releasing a drug (the most common strategy) to inducing tissue extravasation or cell uptake.[5–9] In the context of developing stimuli-responsive materials, a series of inorganic nanostructures have emerged as fundamental for this task.

We believe that among the materials that can be highlighted, especial attention can be directed upon superparamagnetic iron oxide nanoparticles (SPION), gold nanoparticles, graphene and mesoporous nanoparticles made of silicon or silicon oxide (silica). SPION have become key components in the development of magnetic-responsive nanomaterials due to their capacity of inducing a temperature increase when exposed to alternating magnetic fields.[10] On the other hand, gold nanoparticles can also increase the surrounding temperature when stimulated with light in the appropriate wavelength, and are therefore very used in light-responsive nano-DDS.[11] Graphene shows a large surface area, since all of its atoms are exposed on its surface, which enables binding various molecules for drug and gene delivery. The high near-infrared (NIR) absorbance of nano-graphene also enables its use for photothermal therapy, and for developing light-responsive nanosystems.[12] Finally, the porous structure of

mesoporous silicon and silica nanoparticles (with very large surface areas) can act as a drug reservoir, and when different gatekeepers are grafted on the nanoparticle surface, the release of the loaded drugs can be postponed until the desired stimulus is present.[13] A particular type of this kind of materials, lipid bilayer-coated mesoporous silica nanoparticles (often called protocells) can also be highlighted as particularly promising, and will also be discussed here.[14]

When considered collectively, inorganic nanoparticles present several common characteristics that make them promising therapeutic agents.[15,16] Among them, we can point out their physicochemical stability, which will enable successful protection of loaded drugs throughout their journey in the body, and also enabling a long shelf-life, which might be important for their clinical implementation. They are also generally easy to obtain in a finely-tunable manner, allowing us to optimize their size, shape and surface properties for particular medical conditions, administration routes or to formulate a specific drug. Despite the great promise that all of these nanoparticle types have for the development of therapeutic formulations, a common complaint presented against their use is the lack of certainty about the toxicological behavior of these inorganic structures.[15,16] The main safety concerns can derive from direct cell toxicity induced by the particles, potential nanoparticle aggregation (which can lead to vascular obstruction), long-term bioaccumulation, hemolytic activity and immune recognition and toxicity. Several strategies have been adopted to tackle these issues, for example, by chemically customizing the nanoparticle surface to modify their interaction with other nanoparticles or with the biological environment, decreasing the risk of aggregation, immune recognition of hemolysis. Although it has already been highlighted that inorganic nanoparticles present high physicochemical stability, the nanoparticle matrix can in some cases be modified to enable biodegradation or excretion, to prevent long-term bioaccumulation of the nanotherapeutic agents. The primary objective of this article is to provide an overview of the existing data regarding the stability, toxicity and biodegradability of these inorganic nanostructures, and presenting the strategies developed to improve these parameters as well as presenting our perspective on the current developments and future needs in this area.

2. Superparamagnetic iron oxide nanoparticles (SPION)

Iron oxide nanoparticles have received huge attention for the treatment of solid tumors due to their superparamagnetic properties that produce heat under the application of alternative magnetic fields.[10] The temperature increase achieved by the nanoparticle accumulated in the diseased tissue followed by the exposition to magnetic field cause a significant cellular stress, by itself or in combination with chemotherapy, which provoke the destruction of the tumoral mass. That is the basics of the so-called magnetic hyperthermia therapy.[17] These particles can be easily synthesized following different methods such as co-precipitation, thermal decomposition or solvothermal synthesis, among others.[18] SPION have been recognized as biocompatible materials showing scarce cytotoxicity in *in vitro* cell culture studies at concentrations below 100 $\mu g \cdot mL^{-1}$.[19] However, the toxicity of these particles is strongly dependent of their surface properties and therefore can be modulated by the type of coating or functional groups on their surface. Ankamwar *et al*. reported that Fe_3O_4 nanoparticles coated with tetramethylammonium 11-aminoundecanoate cause significant toxicity in different cell populations at concentrations higher than 10 $\mu g \cdot mL^{-1}$.[20] Therefore, it is necessary to carefully design the biocompatible coatings to allow the safe use of these particles. The cell toxicity caused by SPION is mainly associated to their capacity to generate radical oxidative species (ROS) such as superoxide, hydroxyl radicals or hydrogen peroxide through Fenton reaction ($H_2O_2+Fe^{2+} \rightarrow Fe^{3+}+HO^{-}+HO^{\cdot}$).[21] The generation of ROS can be catalysed on the surface of the nanoparticle or by iron leached to the media. ROS harm cells through different mechanisms as lipid peroxidation which induce membrane malfunction, mitochondrial injury, DNA disruption and the oxidation of key proteins that provoke multiple alterations in the normal cell cycle. ROS generation particularly affects to organs that present high mitochondrial activity that produces O^{2-} and H_2O_2 as heart or liver making these tissues more sensitive to iron toxicity.[22] Sung *et al*. have reported that SPION toxicity is strongly related with their degradation rate within the cell.[23] Thus, when the nanoparticles are exposed to acidic environments, as lysosomal compartments where the pH can reach values up to 5.0, they suffer a rapid degradation

by hydrolysis into free ions ($Fe^{2+/}Fe^{3+}$) increasing ROS formation. Additionally, the released iron can provoke an homeostasis imbalance that induce DNA damage which can trigger carcinogenesis, epigenetic events and inflammatory processes.[24] Hosseinkhani *et al*. studied in detail the toxicities of SPION with different surface chemistries (COOH and NH_2).[25] In this study, the authors found that positively charged amino-functionalized particles induced lower cell viability that acid-coated ones which exhibit a negative surface. The reasons of this fact are double, on one hand the positive surface of the particles induces higher uptake due to the Coulombic attraction with the negatively charged cell membrane. On other hand, the presence of amino groups on the particle surface provokes lysosomal rupture by proton sponge effect releasing the particles into the cytosol together with the lysosomal content enhancing their cytotoxicity. As it has been mentioned before, surface coating plays a critical role in the SPION toxicity. One of the most employed type of coatings are polysaccharides as dextrans which showed no effect in cell viability and functionality in human monocyte-macrophages at concentrations as high as 1 $mg{\cdot}mL^{-1}$ when were employed for coating ultrasmall SPION (Ferumoxtran-10).[26] Pompa *et al*. have reported that the use of a silica shell on the SPION surface significantly reduces the cytotoxicity of the nanoparticles mainly due to a decrease in ROS production.[27] Uncoated SPION are more susceptible to degradation releasing 2-folds iron to the media which catalyze the ROS formation. Additionally, the introduction of amino groups on the silica surface provides a higher protection against the acid environment of lysosomal compartments reducing even more the iron leakage. Silica coated SPION of 30~40nm have been tested in murine models as magnetic resonance imaging (MRI) T_1 contrast agents providing strong positive signal enhancement in different tissues as heart, liver, bladder and kidney without inducing any toxicity in the host.[28] David *et al*. have evaluated the effect in the cytotoxicity of diverse coatings as starch, amino groups and polyethylene glycol (PEG) of different chain length (2k, 5k and 20k Da) employing Chinese Hamster ovaries (CHO-K1 cells).[29] These authors found that the toxicity was strongly related with the particle uptake being the particles decorated with PEG 2kDa the best tolerated at concentrations up to 100 $\mu g{\cdot}mL^{-1}$ showing the lower uptake into the cells. Poly(lactic-co-glycolic acid) (PLGA) a biocompatible polymer widely employed in nanomedicine,

has been employed to coat SPION to enhanced their biocompatibility for MRI,[30] drug delivery[31] and immune stimulation.[32] Mei *et al*. have reported that PLGA coating prevents autophagosome accumulation within the cell which is a typical source of toxicity of metal-based nanoparticles.[33] Proteins have also been used as coatings for SPION. As example, Nosrati *et al*. have employed bovine serum albumin (BSA) as biocompatible coating of Fe_3O_4 nanoparticles for transporting curcumin to breast cancer cells inducing potent therapeutic responses.[34] Once the nanoparticles are exposed to biological fluids, they are immediately covered by the proteins present in the media forming the so-called protein corona. The protein corona is the “readable” part of the nanoparticle for the cells and is the responsible of its behavior in terms of cellular uptake and potential toxicity. Dawson *et al*. have studied the protein corona formation in SPION coated with citric acid, poly(acrylic acid) and a double layer of oleic acid.[35] They found that particles coated with negatively charged moieties (citrate and poly(acrylic acid)) form their corona in the first 2 hours whereas particles coated with oleic acid requires more time due to the non-covalent nature of the double oleic layer. Interestingly, in this last group of SPION, the protein corona is lesser enriched in complement and immunoglobulin proteins than the other ones which make them better for escape to immune clearance. Protein corona formation is a dynamic process which should be studied in detail in order to evaluate the potential fate of the nanoparticle within the host. This process depends on different factors which should be considered in each case, not only to the nanoparticle surface chemistry; even the slight temperature changes provoked by circadian rhythm caused significant variations in the protein composition.[36]

In vitro cytotoxicity evaluation provides valuable information about the safety of these particles, but this information should be contrasted with *in vivo* assays. Employing appropriate animal models, biodistribution and realistic toxicity assessment can be conveniently studied. The degradation products of SPION, Fe^{2+} and Fe^{3+} are generally incorporated into iron-storage proteins as hemoglobin, transferrin or ferritin,[37] but the toxicity of the nanoparticles should be evaluated for each system because it depends on the size, coating and surface chemistry. The biodistribution of SPION once they are administered in the blood stream can be determined and quantified by different

techniques such as MRI, near infrared fluorescence imaging by labelling the nanoparticles with the corresponding fluorophore, positron emission tomography (PET) through the introduction of radionuclides or single photon emission computed tomography (SPECT), among others.[38] Wei *et al*. reported that carboxyl-coated Fe_3O_4 nanoparticles exhibited different organ accumulation depending on their size.[39] The smallest nanoparticles (10 nm) were mainly located in the liver whereas the bigger ones (40 nm) were located mostly in the spleen. In all the cases, the nanoparticles did not induce significant toxicity, but the smallest ones produce an alteration in the genes related with oxidative stress in the cell. Xiao *et al*. have studied the *in vivo* distribution of SPION in the body are mainly with different sizes (10 and 30 nm) and decorated with PEG or polyethylene imine (PEI).[40] They found that SPION coated with PEI were rapidly cleared by macrophages due to the high affinitive of their positively charged surface with plasma proteins and exhibited the lower tumoral uptake. Particles of 10 nm coated with PEG presented the higher tumoral uptake followed by the bigger particles of 30 nm. In all cases, SPION were also accumulated in liver and spleen. PEG-coated SPION remains during more than two weeks without producing toxicity, only a slight increase in alanine-transaminase (ALT) enzyme and inconsequential histopathological alterations in these organs. PEI-coated nanoparticles were removed from these organs faster, but they produce severe toxicity leading to the animal death when the administered dose reached 2.5 $mg \cdot kg^{-1}$. The high toxicity observed in these nanoparticles could be explained by different mechanisms such as cell membrane and mitochondrial membrane disruption which provoke apoptosis, higher hemolytic capacity due to their positive charge and capillary blockage due to the aggregation tendency of positively charged nanoparticles in biological milieu. In a recent study, Wang *et al*. have reported that SPION coated with PEI, which was employed to complex siRNA by electrostatic interactions between the positive charges of the polymer with the negatively charged phosphate backbone of the oligonucleotide strand, did not induce toxicity in rat model.[41] Despite the high accumulation in liver and spleen, these organs presented a normal histopathological analysis and similar ALT enzymatic expression that controls. Moreover, the administration of these particles did not cause renal toxicity in sight of the similar levels of blood urea nitrogen and creatinine with the untreated

animals. Therefore, these nanosystems are suitable for the transportation of therapeutic oligonucleotides. SPION conjugated with human relaxin-2 (RLX) have been employed for the modulation of tumour stroma in the treatment of pancreatic cancer.[42] Pancreatic tumors are characterized by presenting high content of cancer-associated fibroblasts (CAFs) which form a dense extracellular matrix around the tumour hampering the diffusion of chemotherapeutic agents inside the malignancy. In this work, the nanosystems were able to reach the tumoral area and once there, to release RLX which reduces fibrosis and improves the penetration of chemotherapeutic drugs in the tumoral tissue. It is interesting to point out that in the case of antitumoral applications, the capacity of SPION to generate ROS can be employed for enhancing the cytotoxic effects of certain antitumoral drugs. This is the case of platinum drugs as *cis*-platin. This drug activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) which generates $O_2^{\bullet -}$ that are transformed into H_2O_2 by the action of superoxide dismutase (SOD).[43] Lin *et al*. have reported the use of iron oxide nanoparticles coated with polyethylene imine (PEI) and PEG and loaded with cisplatin(IV) prodrugs in liver cancer murine models.[44] These particles induce the generation of H_2O_2 in the cytosolic space by the activation of Pt(IV) drug in the intracellular reductive environment. The produced H_2O_2 was rapidly transformed into highly toxic ROS by the action of the released iron causing the tumoral cell destruction (**Figure 1**). The particles were directed to the tumoral lesion by the action of magnetic fields which reduces their toxicity in other organs.

Finally, as another interesting example, SPION clusters coated with the photosensitizer photoporphyrin IX (PpIX) have been successfully applied for the activatable destruction of tumoral cells by illumination with near infrared radiation (NIR).[45] In this work, the photosensitizer acted as SPION cluster coating enhancing its colloidal stability in aqueous media and as an activatable drug which generates highly cytotoxic singlet oxygen at the same time. The results showed that these nanoparticles in combination with NIR exposition caused 82.60% tumour volume reduction compared with free photoporphyrin.

3. Gold nanoparticles

The application of gold nanoparticles (GNP) in the clinic field has received huge attention in the recent years due to the unique optical properties in combination with their high biocompatibility and lack of toxicity.[46] Colloidal gold has been employed in different materials from ancient times being one of the most impressive example the Lycurgus cup which exhibits different colors depending on the incidence light direction. Gold nanoparticles exhibit high absorption and scattering of light from the visible region (390-650 nm) to near infrared or NIR (650 to 1350 nm). When a metallic nanoparticle, as is the case of GNP, is irradiated with light which has smaller wavelength than its size, it appears a coherent oscillation of the free electrons present on the surface, a phenomenon called surface plasmon resonance (SPR).[47] SPR is highly dependent on the size and shape of GNP. Thus, the change in the optical properties of these particles depending on their size has been employed for the detection of different biomolecules,[48] and even cells,[49] by the colour shift observed as a consequence of the aggregation of GNP in the presence of the analyte in each case. Spherical GNP presents only one SPR band located in the visible region (around 520 nm) due to their symmetry in all axes. In the case of non-symmetrical GNP, as is the case of rod-like GNP, a second SPR band appears because there are two different oscillation modes along the nanoparticle (longitudinal and transversal). This second band is located at higher wavelength and can be tuned by the modification of the length to width relation (aspect ratio). When the aspect ratio of GNP is higher than 2, the second SPR band can be placed in the near infrared region (NIR) which is especially valuable because living tissues are transparent to this wavelength. Thus, NIR radiation produces the excitation of the electrons located on the particle surface that lost the acquired energy by heat transfer increasing the temperature in the surroundings. Therefore, this property has been widely employed for the selective destruction of tumoral cells by the controlled irradiation with NIR in the diseased zone.[50] GNP with diverse sizes and shapes can be precisely synthesized employing different techniques such as photochemical,[51] electrochemical[52] and perhaps the most used one, seed mediated approach.[53] In the last strategy, gold seeds with size around 3-4 nm are produced by reduction of gold salts. After this stem, the seeds are added to a solution of gold salts in the presence of

a weak redactor and a surfactant, usually cetyl trimethylammonium bromide (CTAB), producing the seed growth up to the desired final size and shape depending on the conditions employed. CTAB is highly toxic for the cells due to its capacity to disrupt the cellular membrane and therefore it is usually removed from the surface by more biocompatible molecules as phosphatidylcholine,[54] polyelectrolyte-based coats[55] or polyethylenglycol (PEG) chains.[56] *In vitro* toxicity of GNP depends on their size, shape and surface coating. GNP with size around 1-1.5 nm exhibited the higher cytotoxicity, showing an IC_{50} 30-56 μM, in comparison with bigger particles of 15 nm which were nontoxic at concentrations 60-fold and 100-fold higher.[57] In a later work, the origin of the cytotoxicity of the tinier particles was deeply studied concluding that was due to oxidative stress caused by the generation of ROS on the particle surface.[58] The high surface/volume ratio of the smallest particles produced the larger amount of ROS and therefore, the higher toxicity. Interestingly, the toxicity was avoided by surface passivation with thiol-contained antioxidants which confirms the oxidative mechanism of cell damage. In any case, particle uptake depends on the cell line and should be studied for each situation. GNP of 45 nm were engulfed in higher amount than smaller particles of 13 nm in human dermal fibroblast being the uptake mechanism different for each case; clathrin-mediated endocytosis for the bigger particles and phagocytosis for the smaller ones.[59] The bigger particles exhibited the higher toxicity by cytoskeleton disruption due to their higher tendency to escape from the endosomes and be accumulated in the cytoplasm. Not only the own size of the nanoparticle present strong influence in its cellular uptake and therefore, in the cytotoxicity, but also the aggregation state of them. Chan *et al*. have reported that have reported that particle aggregation reduces the uptake up to 25% in comparison with well dispersed nanoparticles in different cell populations.[60] Nanoparticle shape presents a significant influence in the cellular uptake. Chan *et al*. studied the effect of size (1-100 nm) and shape (1:1 to 1:5 aspect ratio) stabilized with citrate in particle uptake employing HeLa cells.[61] The authors reported an optimal size around 50 nm and aspect-ratio (1:1) which exhibited the higher uptake whereas the uptake was lower in the case of rod-like particles. More exotic shapes as nanotriangles[62] or nanostars[63] have presented significant lower cytotoxicity than the corresponding spheres at the same

concentrations. Surface charge is another parameter which exerts influence in the GNP cytotoxicity. Hussain *et al*. have compared the effect of GNP positively, negatively charged or neutral in the viability of human keratinocyte cell line (HaCaT).[64] Neutral nanoparticles presented the lower toxicity while charged ones (both positive and negative) induced higher amounts of cell alterations as mitochondrial stress, DNA damage and apoptosis induction by expression of caspase-3. Liver is the main organ on charge of blood detoxification and it is well reported that practically all nanoparticles are accumulated there.[65] Monteiro-Riviere *et al*. have studied the cytotoxicity in human hepatocytes which induce GNP covered with polymeric coatings as branched polyethylenimine (BPEI), lipoic acid (LA) polyethylene and glycol (PEG) as a model of positive, negative and neutral coatings.[66] BPEI nanoparticles were found toxic for the cells at concentrations around 50 $\mu g \cdot mL^{-1}$, mainly by the induction of persistent ROS production, whereas the other ones were non-toxic. Interestingly, the toxic effect could be completely avoided by the previous incubation of the nanoparticles with human serum albumin (HSA) which form a protein corona around the particle which reduces the particle uptake in the hepatocyte and the oxidative stress inside the cell. As is was mentioned in the case of SPION, the *in vitro* evaluation of the toxicity provides important information but does not guarantee the safety of a certain treatment based on nanoparticles. Obviously, the toxicity and biodistribution of a nanoparticle in *in vivo* models depends on the administration route and should be considered in each case.[67] For *in vivo* applications, the particle surface must be decorated with biocompatible moieties which maintain the colloidal stability of GNP in physiological media. Glutathione-coated GNP of 1-2 nm were injected subcutaneously at different concentrations showing excellent biocompatibility.[68] The particles were cleared during the first week by the kidneys and after this time, by the liver, without inducing any significative alterations in these organs. GNP coated with a silica shell decorated with PEG chains have also been employed for *in vivo* imaging by Raman spectroscopy without provoking cytotoxicity through intravenous and rectal administration in rat models.[69] The toxic effect and biodistribution of citrate-coated GNP of 12 nm after three repeated intraperitoneal administration during 8 days at different concentrations (40, 200, and 400 $\mu g \cdot kg^{-1} \cdot day^{-1}$) were evaluated.[70] Gold content was measured in

blood and tissues by atomic absorption and inductively coupled plasma-mass spectroscopy showing that gold content in blood did not increase in proportion to the dosage. This fact indicated that gold accumulation was produced mainly in the organs, being liver, kidney and spleen the organs which retained higher gold amounts and brain the lower. Importantly, gold administration in all cases did not lead to any mortality, none of the organs showed any histological alterations and the biochemical and haematological analysis presented normal values. Villaverde *et al*. have encapsulated gold nanorods inside mesoporous silica nanoparticles loaded with doxorubicin and coated with a thermosensitive polymer to prevent the premature drug departure (**Figure 2**).[71] The external surface of these nanocarriers was decorated with a specific peptide (Napamide) in order to enhance their uptake in melanoma cells. In this system, gold nanorods were employed as activatable heaters under NIR radiation. Under light exposition, the temperature in the surroundings exceeded the transition temperature of the polymeric coating which suffered a collapse releasing the cytotoxic drug trapped within the silica network. Thus, this system was able to induce a potent cytotoxic response in melanoma cells exposed to NIR whereas was lesser toxic for fibroblast cells used as control.

Parak *et al*. have studied the fate of the organic surface coating after injection in mice grafting a polymer poly(isobutylene-alt-maleic anhydride)-graft-dodecyl labelled with a radioactive isotope ^{111}In on the gold surface.[72] Inorganic core was synthesized using ^{198}Au in order to trace the fate of the gold nuclei in the host. The results indicated that the inorganic cores were retained in the liver whereas the polymeric shells were excreted through the kidneys. *In vitro* evaluation employing HUVEC and Kupffer cells showed that the nanoparticles were retained in endosomes and lysosomes and the polymer shell was degraded by proteolytic enzymes present in these organs. Rengan *et al*. have encapsulated GNP within liposomes of 100 nm of diameter achieving the complete ablation of the tumoral mass in a fibroblast (HT1080) tumour xenograft model using NIR laser irradiation at 750 nm.[73] As in the previous case, these liposomes were mainly accumulated in liver, spleen and kidneys but they did not induce any sign of acute toxicity in these organs. Only the tumoral region which was exposed to NIR light suffered an extensive necrotic response which leads to complete tumour relapse.

Encapsulation of therapeutic agents into exosomes has revealed as a promising strategy for the selective transportation of cargo to diseased tissues.[74] Exosomes are extracellular vesicles secreted by cells which have been used for the delivery of sensitive payloads as oligonucleotide strands or proteins. This strategy combines the advantage of the use of soft coatings, like liposomes, which are perfectly biocompatible and provides outstanding colloidal stability with the targeted delivery of cargo thanks to the presence of specific receptors located on the surface of these biological moieties. Betzer *et al*. have encapsulated glucose-coated 5 nm GNP for imaging brain damage in a ischemic stroke mouse model.[75] The intranasal administration of these particles lead to their accumulation in the damaged lesion of the brain providing a non-invasive approach for the visualization of brain pathologies. In the case of thermal ablation of tumoral cells which employs GNP as heat source under NIR radiation, one of the main limitations is that only spherical particles which sizes higher than 50 nm presents strong NIR absorption. Larger nanoparticles present the lower penetration in living tissues due to their hampered diffusion. Gao *et al*. have reported an interesting work in which small GNP decorated with PEG chains functionalized with aziridine groups suffered controlled aggregation under laser excitation at 405 nm by covalent cross-linking with the resulting carbene formed by the light exposition.[76] After 15 min of light exposition, the aggregation cause the apparition of a strong NIR absorption band at 700-900 nm. This phenomenon was reproduced in a tumour-bearing murine model yielding to a significant tumour shrinkage and extended life expectancy of the mice exposed to both light treatments, 405 nm for inducing the aggregation and 808 nm irradiation for enhancing the temperature in the tumoral tissue.

4. Graphene

Graphene consists in a hexagonally-arranged two-dimensional network of sp^2-hybridized carbon atoms.[77] Graphene is being thoroughly investigated for several biomedical applications (drug and gene delivery, biosensing and imaging, among others) due to its outstanding properties, such as electronic and thermal conductivity, high surface area and mechanical strength.[77] However, one of the main limitations of

unmodified graphene for biomedical application is the difficulty in obtaining stable aqueous suspensions, which is necessary in the biological context. For this reason, graphene oxide (GO) is commonly employed for biomedical applications, since a stable suspension of GO in aqueous medium can be obtained through hydrogen bonding.[78]

The toxicity of graphene-based materials has been a matter of discussion in recent years, since many parameters appear to deeply affect their biocompatibility.[79–82] In healthy human lung cells, graphene toxicity was seen to depend on concentration and exposure time.[83] Mitochondrial injury in PC12 neuronal cells was seen to be dose and shape-dependent.[84] Also in PC12 cells, reduced GO (rGO) toxicity was mediated by an increase in caspase 3 activation, generation of ROS and release of lactate dehydrogenase (LDH), while it was shown to be less toxic than carbon nanotubes.[85] GO toxicity in A549 cells due to oxidative stress was also dose and size-dependent, despite showing low uptake.[86] Graphene has also been observed to be able of causing DNA damage.[87] Modification of particle surface has been thoroughly employed to improve the biocompatibility of graphene-based materials, with PEGylation as the most widely used strategy.[78] In fact, PEGylation has been shown to improve the *in vitro* biocompatibility of graphene in a wide variety of cell lines.[78,88–91]

Regarding their hemocompatibility profile, while hemolysis was insignificant for both graphene and GO in some studies,[92] other authors appear to have found both hemolytic and thrombotic potential in these materials.[93,94] Some data indicate that both effects might be dependent on morphology as well as chemical structure.[78] For example, GO modification with amino groups [93] or coating with chitosan eliminated its hemolytic effect.[95]

Several studies have also been performed to study the toxicity of graphene-based structures *in vivo.* Different authors have found pulmonary toxicity after intravenous administration of GO and BSA-capped graphene, perhaps linked to the thrombogenic potential mentioned above.[96–99] On the other hand, graphene administration did not affect survival rate of zebrafish embryos.[100] No toxic effects (neither in longevity nor reproductive capacity) were observed after administration in *Caenorhabditis elegans* nematode either.[101] When testing GO and PEGylated Poly-L-Lysine (PEG-PLL)-

modified GO in *C. elegans*, Zhang *et al*. observed that, while the materials were not toxic under normal conditions, severe toxicity was observed under oxidative stress induced by juglone (**Figure 3**).[102]

This study highlights the importance of taking into account not just the material structure and composition, but also the ongoing physio-pathological context, since this can deeply affect its biological behavior. The preparation method of graphene derivatives can also significantly modify their behavior. Green synthesis strategies being proposed to decrease the toxicity of these materials. As a few examples, reduction of GO with ascorbic acid, stabilization of rGO with L-tryptophan and the use of reducing sugars such as glucose and fructose have been proposed.[103–105] While obtaining magnetic graphene-derived materials, Urbas *et al*. observed that the employed chemical functionalization of GO and Fe_3O_4 also enhanced the biocompatibility of the material.[106] Graphene quantum dots were seen to be well tolerated *in vivo*, and easily excreted in urine thanks to their small size.[107] Thickness of functionalized graphene oxide sheets has been shown to play a critical role in tissue accumulation and urinary excretion, with the thinner GO sheets being mainly excreted through urine and with a larger fraction of the thicker GO sheets remaining mainly in the spleen and liver 24 h after intravenous injection.[108]

Yang *et al*. showed that PEGylated graphene did not induce any adverse toxic effects in mice.[109] This indicates that, although graphene is not biodegradable, coating with hydrophilic polymers can enhance its *in vivo* biocompatibility.[110] PEGylated graphene showed no absorption after oral administration with almost complete excretion.[111] Intraocularly administered GO was well tolerated.[112] Upon intraperitoneal injection, PEGylated GO was seen to accumulate in liver and spleen. [78,111] Besides PEGylation, graphene surface modification with other polymers has also been proposed to improve its biocompatibility.[113] Modification of GO with dextran improved its stability in suspension (**Figure 4**), while it also enhanced its biocompatibility with HeLa cells.[114] Modification of GO with Poly(amido amine) (PAMAM) dendrimer enhanced aqueous dispersibility and produced a hybrid material with almost no toxicity towards MDA-MB-231 cells, although the PAMAM dendrimer alone did show some toxicity for

the same cells.[115] Modification of GO with pluronic F127 greatly enhanced dispersibility, although in this case the modified material did show some toxicity in vitro.[116] Polydopamine-modified rGO had ultralow hemolytic potential and exhibited very low toxicity towards HUVEC cells.[117] Xu *et al*. have suggested that graphene functionalization with poly(acrylic acid) (PAA) could be an alternative to PEGylation with better performance in improving *in vitro* and *in vivo* biocompatibility.[118]

5. Mesoporous silicon nanoparticles (MSiNs)

MSiNs possess a semicristalline network and present inherent luminescence with a broad excitation band and narrow emission (tipically in the NIR region).[119,120] This characteristic makes them specially interesting for the development of theranostic nanomaterials, in which real time follow-up by optical methods can be carried out while the particles are carrying out their therapeutic function. Furthermore, their mesoporous structure provides them with large surface areas (200-500 m^2/g) that enable loading considerable amounts of drugs.[120] MSiNs can be prepared by a top-down or a bottom-up approach[119], being the pore structure highly dependent on the fabrication conditions. In order to stabilize the surface of as-prepared MSiNs, different modifications routes are followed, being the main ones oxidation, hydrocarbonization, carbonization, silylation and silanization.[120] Further chemical modifications can be performed to allow stimuli-responsive or other forms of controlled drug release.[121,122]

MSiNs are generally considered to be safe, as the silicon network is known to easily degrade into non-toxic products (mainly related to silicic acid), which can be then safely excreted.[120] In an excellent review article, Croissant *et al*. described the different parameters that have been seen to affect this degradation behavior.[119] A deep understanding of the parameters that can regulate the dissolution kinetics would enable the development of safer and more effective nanotherapeutics. For example, while nanoparticle size is thought to play only a minor role in the degradation kinetics[119], their porosity is a key parameter, with particles with larger pores having faster dissolution kinetics.[119,123–125] Oxidation of the MSiN surface, as well as coating

with a silica layer, are known to slow down the dissolution process.[126] MSiN coating with dextran improved the stability of the nanoparticles against dissolution, enabling longer-term particle tracking.[127] Covalent attachment of PEG on the surface of MSiNs also slowed down particle degradation, with decreasing degradation rate as the polymer molecular weight was increased.[123] The medium in which the MSiNs are dispersed greatly influences dissolution, with accelerated degradation in protein-containing medium [128] and in basic environments [123]. In the biomedical context, the changing environment to which the nanoparticles will be exposed can also be of great importance, not just in the context of pH, since both intra- and extracellular environments contain a complex mixture of different biomolecules. In this context, almost complete biodegradation of MSiNs inside MCF-17 breast cancer cells was seen after 13 days, as evaluated by Raman micro-spectroscopy.[129] Tzur-Balter *et al*. also demonstrated that *MSiN in vitro* and *in vivo* degradation was accelerated with higher concentration of ROS, conditions that can be found in different pathological conditions.[130]

Different studies have been performed regarding MSiN biocompatibility *in vitro*. For example, no toxicity could be seen in endothelial cells *in vitro*, neither before nor after modification of MSiNs with RGD peptides.[131] However, modifications on the material structure and composition can alter their toxicity. The effect of surface properties of MSiNs on their biocompatibility was thoroughly evaluated by Shahbazi *et al*.[132] They found that MSiN toxicity is more dependent on surface charge than on their hydrophobicity/hydrophilicity, being the aminopropyl-functionalized MSiNs the most toxic, and negatively-charged MSiNs the least toxic for the studied cells.[132] Aminopropyl- and undecylenic acid-functionalized MSiNs were also seen to produce the largest amount of hemolysis.[132] When cultured with macrophages, PEGylated MSiNs were also shown not to induce the release of proinflammatory cytokines.[123] *In vivo* evaluation of these same materials correlated well with the toxicity observed *in vitro*, showing no significant changes in biochemical or hematological parameters, although some mild effects were appreciated in kidneys, liver and spleen.[132] Ivanov *et al*. showed that MSiNs presented a good biocompatibility profile *in vivo* after intravenous

injection, with a significantly reduced number of foreign body-type granulomas compared to animals injected with mesoporous silica nanoparticles.[133]

The main strategies employed to improve the *in vivo* performance of MSiNs are based on chemically decorating the particle surface. Hydrophobin-functionalized MSiNs showed improved dispersability in plasma (due to their increased hydrophilicity), and reduced the amount of particles trapped in the lung after IV injection, increasing also the liver-to-spleen ratio of coated particles compared to non-coated ones.[134] On the other hand, similar Hydrophobin-functionalized MSiNs administered orally were observed not to cross the intestinal wall, and to present extended transit time in the gastrointestinal tract, due to their mucoadhesion in the stomach.[135] Poly(methyl vinyl ether-co-maleic acid) (PMVE-MA)-grafted MSiNs showed also improved colloidal and plasma stability through charge repulsion.[136] Surface modification with BSA reduced non-specific cellular uptake *in vitro* and prolonged circulation time *in vivo*.[137] In healthy animals, dextran-modified MSiNs were seen to accumulate mainly in the liver, where they were slowly degraded when compared to non-modified MSiNs.[127]

6. Mesoporous silica nanoparticles (MSNs)

MSNs are constituted by an amorphous silicon oxide network with porosity in the mesopore range (2-50 nm in diameter). Their high surface area (around 100 m^2/g) enables loading large amounts of drugs, and their easy chemical modification by silanol chemistry allows for the development of highly efficient multifunctional stimuli-responsive drug delivery systems.[138–143] Many different types of therapeutic agents can be housed inside the silica matrix and their release can be triggered at demand anchoring different stimuli-responsive gatekeepers on the nanoparticle surface.[144] Thus, MSN have been engineered to release the payload in response to external stimuli as light,[145,146] magnetic fields[147–149] and ultrasounds,[150] or internal stimuli, characteristic of the pathology treated, as pH,[151] presence of enzymes[152] or redox changes,[153] among others. Regarding one of their main limitations, although colloidal stability of unmodified MSNs is challenging, Lin *et al*. showed that PEGylation could provide MSNs with long term stability in different media at physiological temperature,

while also enhancing their biocompatibility and reducing their uptake by macrophages.[154] PEGylation has therefore become a widespread process in the field to improve this parameter.

Silica nanoparticles are generally considered to have a good biocompatibility profile, although some inconsistencies between *in vitro* and *in vivo* data have been found.[155] Many parameters are known to affect the toxicity of silica nanoparticles *in vitro*, such as their size, dose and cell type.[156] Kim *et al*. showed in a microfluidic setup that unmodified MSNs present a flow-dependent toxicity in human endothelial cells, while PEGylated MSNs did not show relevant toxicity, neither under static conditions nor under flow.[157] When evaluating the immune response towards cargo-free MSNs in primary immune cells, Heidegger *et al*. observed only a very low immune response as determined by the release of inflammatory cytokines.[158] Lin *et al*. showed that non-porous silica nanoparticles induced a larger degree of hemolysis than MSNs (**Figure 5**).[159] Moreover, Slowing *et al.* showed that PEGylation of MSNs further reduced their hemolytic activity.[160] Urata *et al.* also proved that the introduction of ethenylene-bridged silsesquioxanes into MSNs could also reduce their hemolytic activity without the need for PEGylation.[161]

Liu *et al*. showed a very good safety profile of Hollow-MSNs (HMSNs) *in vivo*, both after single and repeated injections (up to 4 injections were performed per mouse).[162] Additionally, they observed that all of the injected dose on HMSNs had been excreted 4 weeks after injection.[162] Fu *et al*. evaluated the safety profile of MSNs after administration by different routes.[163] They found that systemic distribution after hypodermic and intramuscular administration was almost negligible, and that the particles were well tolerated and had good tissue biocompatibility even after oral or intravenous administration.[163] Hudson *et al*. also analyzed the *in vivo* biocompatibility of mesoporous silica materials with sizes between 150 nm and 4 μm, and with varying pore sizes after subcutaneous, intraperitoneal and intravenous administration in mice.[164] Materials that had been injected subcutaneously showed good, and the material was progressively degraded over three months. However, severe toxicity was observed after intraperitoneal and intravenous injection.[164] Lung tissue evaluation

showed that the toxicity was likely due to thrombosis. We should take into account that the materials employed here were not PEGylated and lacked any other kind of surface modification that could prevent aggregation *in vivo*, and that the doses employed (30 mg/animal) could be considered as large when compared to the amounts of particles employed in other studies.[165,166] Yu *et al*. also found that particle porosity and surface characteristics altered the toxicological profile *in vivo*.[167] In this study, they found that the maximum tolerated dose was highest for non-porous silica, followed by amino-functionalized MSNs and the worst safety profile was seen for non-functionalized MSNs. The authors noted that the main cause for the adverse reactions observed was the mechanical obstruction of the vasculature due to nanoparticle aggregation in the bloodstream.[167] Lee *et al*. also compared the *in vivo* toxicity of MSNs and non-porous silica nanoparticles and observed a larger dysregulation of spleen function when MSNs were injected.[168] The authors highlighted the lack of consistency with *in vitro* data, which had shown a better safety profile for MSNs. Ivanov *et al*. showed that while small (13 nm in diameter) MSNs were generally well tolerated, some foreign body-type granulomas in liver spleen as well as liver microgranulation could be seen after intravenous injection in mice.[133] Li *et al*. showed that a PEGylated mesoporous silica nanorattle showed very low systemic toxicity in healthy mice.[169] PEGylation or other strategies aimed at decreasing the possibility of particle aggregation appear therefore as critical for the safe use of MSNs with systemic distribution.

One of the main concerns regarding inorganic nanoparticle toxicity is the possibility for bioaccumulation of the employed nanoparticles, especially after repeated administration. To prevent chronic toxicity associated with bioaccumulation, the employed nanoparticles should be biodegraded and/or excreted by some elimination route in a reasonable time frame after they have exerted their function. MSNs decompose in physiological environments, giving rise to soluble silicon species like monosilicic acid, which can be excreted in urine (**Figure 6**).[119,138,170]

Several factor have been seen to regulate MSN solubility *in vitro*. MSN dissolution rate depends on the medium in which they are, with faster dissolution in simulated lung fluid (SLF) than in simulated body fluid (SBF) or phosphate buffered saline (PBS), and with

the slowest release happening in simulated gastric fluid.[171] Braun *et al*. as well as Yamada *et al*. have observed that the dissolution kinetics of MSNs *in vitro* are generally independent of particle size.[171,172] Hao *et al*. saw that the degradation of MSNs in medium with fetal bovine serum (FBS) was dependent on nanoparticle morphology, with slower dissolution kinetics for rod-shaped MSNs than for spherical particles.[173] Townley *et al*. al showed that nanoparticle surface area is directly related to dissolution, with faster kinetics for MSNs with larger surface areas.[174] Particle composition also greatly affects dissolution kinetics, both when functionalized on their external surface (seeing that phenyl-functionalization accelerates dissolution the most)[175], with different dopings (Ca- and Mn-doping accelerate dissolution [176,177], while zirconia-doping slows it down[178]), or with the inclusion of breakable bonds within the silica network (the inclusion of S-S bonds would accelerate particle dissolution in reducing environments[179,180]). Cauda *et al*. showed that PEGylation significantly slowed down the dissolution of MSNs, also as a function of the coverage density and the molecular weight of the polymer[181]. This finding was further confirmed by Hao *et al*. [173], and it has also been seen to be true for the presence of other polymers grafted on MSN surface[150]. The method used to produce the MSNs can also have a critical impact on the dissolution kinetics. Shen *et al*. showed that, by preparing the particles with a biphase stratification approach, they could obtain 3D-dendritic MSNs for which their simulated biodegradation could be tuned to be complete in just 24 h (compared to 2 weeks for more traditional architectures).[182] Möller *et al*. recently described the systematic evaluation of dissolution kinetics of MSNs with different functionalities prepared at acidic, neutral or basic pH following a common recipe.[183] Their findings show that the dissolution at low concentrations is mainly directed by the silica network connectivity and the silica building blocks, with MSNs with interrupted networks prepared under basic conditions degraded the fastest (almost completely within a few hours). Surprisingly, additional disulfide linkers in the pore walls retarded this process, which the authors ascribed to the hydrophobicity of such linkers.[183]

Regarding the biodegradation behavior of MSNs *in vivo*, Zhang *et al* showed that after injection in mice, most of the administered dose of PEGylated and folic-acid targeted 48 nm-MSNs could be safely excreted from the animal body.[184] He *et al*. evaluated the

biodistribution of MSNs of different sizes (80-360 nm) with or without PEGylation, and their results show that PEGylated smaller particles escape more easily from liver and spleen, and are degraded mores slowly as a consequence.[185] No toxicity was observed up to 1 month after injection for any of the MSNs tested.[185] The shape of MSNs has also been shown to affect nanoparticle biodistribution and elimination routes. Short rod-shaped MSNs were found to be trapped more efficiently by the liver, compared to the larger presence of long rod-shaped MSNs, which were found more in the spleen.[186] In that study, MSN clearance was also found to be faster for the short rod-shaped particles, through the two main excretion routes seen (in urine and feces). MSNs here did not cause significant toxicity *in vivo*, although some biliary excretion and glomerular filtration dysfunction could have been induced.[186] Nanoparticle shape has also been shown to affect biodistribution of orally-administered MSNs, with particles with larger aspect ratios presenting decreased biodegradation, systemic absorption and especially, reduced liver distribution and excretion in urine.[187]

6.1 Protocells

As it has mentioned along this section, mesoporous silica is an excellent material for drug delivery due to its extremely high loading capacity and excellent biocompatibility, and the possibility of developing a wide variety of stimuli-responsive nano-DDS. Despite the efficacy showed by these smart nanocarriers, the introduction of complex gatekeepers complicates the translation to the clinic of these systems. A Protocell is a nanosystem composed by a mesoporous silica core coated with a lipid bilayer which avoids the premature drug departure until the system enters into the target cells and provides astonishing colloidal stability to the system in aqueous environments.[188] Additionally, lipophilic drugs can be transported within the lipidic shell enhancing even more the cargo capacity of these nanocarriers.[14] The external surface of protocells has been decorated with targeting moieties in order to provide selectivity against the target cells. As an example, Rosenholm *et al*. have attached folic acid on the protocell surface to deliver zoledronic acid (ZOL) specifically to breast cancer cells in a murine tumour-bearing model of this pathology.[189] ZOL is a nitrogen-containing

bisphosphonate which is widely employed in bone pathologies, but it also induces apoptosis in tumour cells and inhibits their growth. However, this drug is rapidly excreted and therefore, its application in antitumoral therapy requires an efficient transport mechanism. In this work, ZOL was transported to tumoral cells which engulfed the protocells due to the overexpression of folate receptors on their membrane suppressing the tumour growth and angiogenesis and avoiding the accumulation of ZOL in bone tissues. Recently, Mantilla et *al*. have decorated the external surface of protocells with the atoxic subunit B of the cholera toxin which binds to ganglioside GM1 receptor present on the neuron membrane.[190] This system has been able to deliver cargo molecules specifically to motoneurons without affecting the adjacent muscular cells. Not only small drugs can be transported by these protocells but also big macromolecules as siRNA,[191] ricin toxin[192] and even magnetic nanoparticles.[193] The lipid bilayer can be anchored on the MSN surface employing sensitive bonds in order to provide stimuli-responsive behavior in the drug departure process. Thus, Wang *et al*. have decorated the external surface of MSN with dithiol bonds which retain the lipid bilayer by hydrophobic interactions until the system reaches the inner cellular space.[194] Once the particle arrives there, the presence of glutathione induces the rupture of the dithiol bond releasing the lipidic shell and therefore, allowing the drug departure. One of the main limitation of nanoparticles is their poor tissue penetration, which is especially aggravated in the case of tumoral tissues because they are denser than healthy ones. This fact hampers even more the navigation of the nanoparticles within the malignancy reducing their effect to the tumoral periphery. Villegas *et al*. have described a promising strategy to overcome this limitation.[195] In this work, polymeric nanocapsules which contains collagenase, a proteolytic enzyme which digest the collagen present in the extracellular matrix softening the tissue, were anchored on the surface of the protocells. These nanocapsules were engineered to release the enzyme when the pH dropped to mild acidic conditions (pH around 5.5) which is a common condition in tumoral tissues. Therefore, when this nanosystem arrived to tumoral tissue, the presence of the acidic environment triggered the collagenase release and then, these proteolytic enzymes digested the ECM provoking a higher penetration of the protocell into the tumoral tissue.

6. Expert opinion

The use of nanoparticles as drug delivery carriers has been widely explored in the last decades. Nowadays, a large arsenal of nanocarriers is available thanks to the effort carried out by many research groups along the world, from soft nanodevices as liposomes, polymersomes or polymeric micelles to rigid structures as is the case of metallic or ceramic-type nanoparticles. Among this vast collection, inorganic nanocarriers present unique characteristics which have been exploited for the delivery of therapeutic agents housed inside their hard matrix or on the surface. Different strategies have been developed to achieve a controlled drug release in response to certain external or internal stimuli, avoiding the apparition of side effects which are common when these drugs are administered in free form. Inorganic nanocarriers present high chemical and mechanical stability due to the strong nature of the bonds which compose their matrices. This property is especially important in the transportation of sensitive molecules such as proteins, small siRNA or labile molecules which can be housed inside the porous matrix of mesoporous silica or porous silicon. On contrary, the rigidity of these nanoparticles presents also important drawbacks. One of the most important one is their poor penetration in tumoral tissues. As it has been mentioned above, the lymphatic vessel collapse causes the retention of nanoparticles during long periods of time. Moreover, the impaired drainage system also increases the interstitial pressure in the malignant tissue which strongly compromises the diffusion of the nanoparticles within the tissue. This effect is even more severe in the case of rigid nanoparticles which cannot alter their form to navigate throughout the usually dense tumoral microenvironment. This important liability would be solved anchoring proteolytic enzymes on the nanocarrier surface, as was mentioned above,[195,196] or employing ultrasounds for propelling them deep inside the solid tumor.[6] Another interesting alternative to the poor penetration problem consists of the employment of bacteria as nanoparticle carriers.[197] Therefore, due to the self-propelled behavior and sensing

capacities of bacteria, the nanoparticles can be transported to inner zones of the tumor and once there, to release the payload achieving a significant therapeutic enhancement. This last strategy is certainly promising because allow to combine the effect of the released drugs with the immunogenic nature of bacteria which would trigger an efficient immune response capable to destroy the tumoral mass. As it has been widely described along the manuscript, inorganic nanoparticles present low degradation rates, especially in the case of SPION and gold nanoparticles. The biological behavior of these particles can be improved by employing different surface modifications or biocompatible coatings (summarized in **Table 1**) and other strategies mentioned above. However, it is necessary to carry out long term *in vivo* assays which provide information about the toxicity issues related with the exposition to these nanodevices during long periods of time.

Regarding this degradability issue, it has been generally accepted that nanomaterials employed in biomedical applications should be either biodegradable or at least somehow excretable, in order to prevent undesired toxic effects deriving from their bioaccumulation. Therefore, biodegradable inorganic nanomaterials, such as MSiNs and MSNs could, in principle, hold great promise for drug delivery, since their degradation products will be eventually removed from the body in a safe way. The parameters governing this dissolution have been therefore thoroughly studied, and a wide variety of design and synthesis strategies have been developed to tailor material dissolution to the target application and administration route. However, we have also discussed here that in some cases, such as SPION, the degradation of the material can actually lead to increased toxicity (by increasing ROS generation in this case), and different modification or coating strategies are employed to tackle this issue. The recent approval of Hensify® (NBTXR3) for the treatment of soft tissue sarcoma in the European market also challenges the conventional reasoning that non-degradable nanostructures are not suitable for biomedical use.[198] NBTXR3 is composed of crystalline hafnium oxide (HfO_2) nanoparticles that are not degradable and act by amplifying the localized killing effect of radiotherapy. This particles are designed for one single intratumoral injection before radiotherapy, and the particles remain in the area after the treatment is over. The current evidence seems to indicate that the subsequent

presence of these non-degradable particles in the injection site does not induce any evident signs of toxicity. Consequently, it is possible that, for applications in which a single injection could be enough, non-biodegradable nanoparticles could still constitute a helpful tool in therapeutic interventions. However, for applications for which repeated administrations are necessary (as is the case for most drug delivery strategies), biodegradability and safe excretion of the employed nanostructures will still be necessary to ensure that no long-term toxicity arises from excessive bioaccumulation.

Funding

This paper was funded by the European Research Council (Grant VERDI; ERC-2015 AdG no. 694160). JL Paris is supported by a post-doctoral fellowship from the Ramón Areces Foundation.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

[1] Dawidczyk CM, Kim C, Park JH, et al. State-of-the-art in design rules for drug delivery platforms: Lessons learned from FDA-approved nanomedicines. J. Control. Release. 2014;187:133–144.

[2] Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv. Drug Deliv. Rev. 2013;65:71–79.

[3] Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nat. Mater. 2013;12:991–1003.

[4] Lyon PC, Gray MD, Mannaris C, et al. Safety and feasibility of ultrasound-triggered targeted drug delivery of doxorubicin from thermosensitive liposomes in liver tumours (TARDOX): a single-centre, open-label, phase 1 trial. Lancet Oncol. 2018;19:1027–1039.

[5] Ganta S, Devalapally H, Shahiwala A, et al. A review of stimuli-responsive nanocarriers for drug and gene delivery. J. Control. Release. 2008;126:187–204.

[6] Paris JL, Mannaris C, Cabañas MV, et al. Ultrasound-mediated cavitation-enhanced extravasation of mesoporous silica nanoparticles for controlled-release drug delivery. Chem. Eng. J. 2018;340:2–8.

[7] Paris JL, Manzano M, Cabañas MV, et al. Mesoporous silica nanoparticles engineered for ultrasound-induced uptake by cancer cells. Nanoscale. 2018;10:6402–6408.

[8] Wang S, Huang P, Chen X. Hierarchical Targeting Strategy for Enhanced Tumor Tissue Accumulation/Retention and Cellular Internalization. Adv. Mater.

2016;28:7340–7364.

[9] Kwan JJ, Graham S, Myers R, et al. Ultrasound-induced inertial cavitation from gas-stabilizing nanoparticles. Phys. Rev. E. 2015;92:023019.

[10] Mahmoudi M, Sant S, Wang B, et al. Superparamagnetic iron oxide nanoparticles (SPIONs): Development, surface modification and applications in chemotherapy. Adv. Drug Deliv. Rev. 2011;63:24–46.

•• Review which describes the synthesis, characteristics and clinical application of SPION.

[11] Liu J, Detrembleur C, De Pauw-Gillet M-C, et al. Gold Nanorods Coated with Mesoporous Silica Shell as Drug Delivery System for Remote Near Infrared Light-Activated Release and Potential Phototherapy. Small. 2015;11:2323–2332.

[12] Yang K, Feng L, Liu Z. Stimuli responsive drug delivery systems based on nano-graphene for cancer therapy. Adv. Drug Deliv. Rev. 2016;105:228–241.

[13] Argyo C, Weiss V, Bräuchle C, et al. Multifunctional Mesoporous Silica Nanoparticles as a Universal Platform for Drug Delivery. Chem. Mater. 2014;26:435–451.

[14] Butler KS, Durfee PN, Theron C, et al. Protocells: Modular Mesoporous Silica Nanoparticle-Supported Lipid Bilayers for Drug Delivery. Small. 2016;12:2173–2185.

[15] Fadeel B, Garcia-Bennett AE. Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Adv. Drug Deliv. Rev. 2010;62:362–374.

[16] Mukherjee S, Patra CR. Therapeutic application of anti-angiogenic nanomaterials in cancers. Nanoscale. 2016;8:12444–12470.

[17] Pankhurst QA, Connolly J, Jones SK, et al. Applications of magnetic nanoparticles in biomedicine. J. Phys. D. Appl. Phys. 2003;36:R167–R181.

[18] Hu Y, Mignani S, Majoral J-P, et al. Construction of iron oxide nanoparticle-based hybrid platforms for tumor imaging and therapy. Chem. Soc. Rev. 2018;47:1874–1900.

•• Review which describes in detail the synthesis and principal uses in the clinical field of SPION.

[19] Kunzmann A, Andersson B, Vogt C, et al. Efficient internalization of silica-coated iron oxide nanoparticles of different sizes by primary human macrophages and dendritic cells. Toxicol. Appl. Pharmacol. 2011;253:81–93.

[20] Ankamwar B, Lai TC, Huang JH, et al. Biocompatibility of Fe 3 O 4 nanoparticles evaluated by in vitro cytotoxicity assays using normal, glia and breast cancer cells. Nanotechnology. 2010;21:075102.

[21] Shubayev VI, Pisanic TR, Jin S. Magnetic nanoparticles for theragnostics. Adv. Drug Deliv. Rev. 2009;61:467–477.

[22] Eaton JW, Qian M. Molecular bases of cellular iron toxicity. Free Radic. Biol. Med. 2002;32:833–840.

[23] Huang C-C, Liao Z-X, Lu H-M, et al. Cellular Organelle-Dependent Cytotoxicity of Iron Oxide Nanoparticles and Its Implications for Cancer Diagnosis and Treatment: A Mechanistic Investigation. Chem. Mater. 2016;28:9017–9025.

[24] Singh N, Jenkins GJS, Asadi R, et al. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). Nano Rev. 2010;1:5358.

• Review which describes the principal mechanisms of toxicity caused by SPION.

[25] Mahmoudi M, Laurent S, Shokrgozar MA, et al. Toxicity Evaluations of Superparamagnetic Iron Oxide Nanoparticles: Cell “Vision” versus Physicochemical Properties of Nanoparticles. ACS Nano. 2011;5:7263–7276.

[26] Müller K, Skepper JN, Posfai M, et al. Effect of ultrasmall superparamagnetic iron oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro. Biomaterials. 2007;28:1629–1642.

[27] Malvindi MA, De Matteis V, Galeone A, et al. Toxicity Assessment of Silica Coated Iron Oxide Nanoparticles and Biocompatibility Improvement by Surface Engineering. Xu B, editor. PLoS One. 2014;9:e85835.

[28] Iqbal MZ, Ma X, Chen T, et al. Silica-coated super-paramagnetic iron oxide nanoparticles (SPIONPs): a new type contrast agent of T 1 magnetic resonance imaging (MRI). J. Mater. Chem. B. 2015;3:5172–5181.

[29] Hanot C, Choi Y, Anani T, et al. Effects of Iron-Oxide Nanoparticle Surface Chemistry on Uptake Kinetics and Cytotoxicity in CHO-K1 Cells. Int. J. Mol. Sci. 2015;17:54.

[30] Patel D, Moon JY, Chang Y, et al. Poly(d,l-lactide-co-glycolide) coated superparamagnetic iron oxide nanoparticles: Synthesis, characterization and in vivo study as MRI contrast agent. Colloids Surfaces A Physicochem. Eng. Asp. 2008;313–314:91–94.

[31] Carenza E, Jordan O, Martínez-San Segundo P, et al. Encapsulation of VEGF 165 into magnetic PLGA nanocapsules for potential local delivery and bioactivity in human brain endothelial cells. J. Mater. Chem. B. 2015;3:2538–2544.

[32] Saengruengrit C, Ritprajak P, Wanichwecharungruang S, et al. The combined magnetic field and iron oxide-PLGA composite particles: Effective protein antigen delivery and immune stimulation in dendritic cells. J. Colloid Interface Sci. 2018;520:101–111.

[33] Zhang X, Zhang H, Liang X, et al. Iron Oxide Nanoparticles Induce Autophagosome Accumulation through Multiple Mechanisms: Lysosome Impairment, Mitochondrial Damage, and ER Stress. Mol. Pharm. 2016;13:2578–2587.

[34] Nosrati H, Sefidi N, Sharafi A, et al. Bovine Serum Albumin (BSA) coated iron oxide magnetic nanoparticles as biocompatible carriers for curcumin-anticancer drug. Bioorg. Chem. 2018;76:501–509.

[35] Jedlovszky-Hajdú A, Bombelli FB, Monopoli MP, et al. Surface Coatings Shape the Protein Corona of SPIONs with Relevance to Their Application in Vivo. Langmuir. 2012;28:14983–14991.

[36] Mahmoudi M, Shokrgozar MA, Behzadi S. Slight temperature changes affect protein affinity and cellular uptake/toxicity of nanoparticles. Nanoscale. 2013;5:3240.

[37] Bao Y, Wen T, Samia ACS, et al. Magnetic nanoparticles: material engineering and emerging applications in lithography and biomedicine. J. Mater. Sci. 2016;51:513–553.

[38] Patil U, Adireddy S, Jaiswal A, et al. In Vitro/In Vivo Toxicity Evaluation and Quantification of Iron Oxide Nanoparticles. Int. J. Mol. Sci. 2015;16:24417–24450.

[39] Yang L, Kuang H, Zhang W, et al. Size dependent biodistribution and toxicokinetics of iron oxide magnetic nanoparticles in mice. Nanoscale.

2015;7:625–636.

[40] Feng Q, Liu Y, Huang J, et al. Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. Sci. Rep. 2018;8:2082.

[41] Yu Q, Xiong X, Zhao L, et al. Biodistribution and Toxicity Assessment of Superparamagnetic Iron Oxide Nanoparticles In Vitro and In Vivo. Curr. Med. Sci. 2018;38:1096–1102.

[42] Mardhian DF, Storm G, Bansal R, et al. Nano-targeted relaxin impairs fibrosis and tumor growth in pancreatic cancer and improves the efficacy of gemcitabine in vivo. J. Control. Release. 2018;290:1–10.

[43] Kim H-J, Lee J-H, Kim S-J, et al. Roles of NADPH Oxidases in Cisplatin-Induced Reactive Oxygen Species Generation and Ototoxicity. J. Neurosci. 2010;30:3933–3946.

[44] Ma P, Xiao H, Yu C, et al. Enhanced Cisplatin Chemotherapy by Iron Oxide Nanocarrier-Mediated Generation of Highly Toxic Reactive Oxygen Species. Nano Lett. 2017;17:928–937.

[45] Yan L, Amirshaghaghi A, Huang D, et al. Protoporphyrin IX (PpIX)-Coated Superparamagnetic Iron Oxide Nanoparticle (SPION) Nanoclusters for Magnetic Resonance Imaging and Photodynamic Therapy. Adv. Funct. Mater. 2018;28:1707030.

[46] Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem. Soc. Rev. 2009;38:1759.

•• Review which describes in detail the optical properties and clinical applications of GNP.

[47] Umair M, Javed I, Rehman M, et al. Nanotoxicity of Inert Materials: The Case of Gold, Silver and Iron. J. Pharm. Pharm. Sci. 2016;19:161.

[48] Han MS, Lytton-Jean AKR, Oh B-K, et al. Colorimetric Screening of DNA-Binding Molecules with Gold Nanoparticle Probes. Angew. Chemie Int. Ed. 2006;45:1807–1810.

[49] Medley CD, Smith JE, Tang Z, et al. Gold Nanoparticle-Based Colorimetric Assay for the Direct Detection of Cancerous Cells. Anal. Chem. 2008;80:1067–1072.

[50] Guisasola E, Baeza A, Asín L, et al. Heating at the Nanoscale through Drug-

Delivery Devices: Fabrication and Synergic Effects in Cancer Treatment with Nanoparticles. Small Methods. 2018;2:1800007.

• Review which describes the use of GNP in hyperthermia treatment of tumours.

[51] Kim F, Song JH, Yang P. Photochemical Synthesis of Gold Nanorods. J. Am. Chem. Soc. 2002;124:14316–14317.

[52] Yu, Chang S-S, Lee C-L, et al. Gold Nanorods: Electrochemical Synthesis and Optical Properties. J. Phys. Chem. B. 1997;101:6661–6664.

[53] Gole A, Murphy CJ. Seed-Mediated Synthesis of Gold Nanorods: Role of the Size and Nature of the Seed. Chem. Mater. 2004;16:3633–3640.

[54] Takahashi H, Niidome Y, Niidome T, et al. Modification of Gold Nanorods Using Phosphatidylcholine to Reduce Cytotoxicity. Langmuir. 2006;22:2–5.

[55] Hauck TS, Ghazani AA, Chan WCW. Assessing the Effect of Surface Chemistry on Gold Nanorod Uptake, Toxicity, and Gene Expression in Mammalian Cells. Small. 2008;4:153–159.

[56] Rayavarapu RG, Petersen W, Hartsuiker L, et al. In vitro toxicity studies of polymer-coated gold nanorods. Nanotechnology. 2010;21:145101.

[57] Pan Y, Neuss S, Leifert A, et al. Size-Dependent Cytotoxicity of Gold Nanoparticles. Small. 2007;3:1941–1949.

[58] Pan Y, Leifert A, Ruau D, et al. Gold Nanoparticles of Diameter 1.4 nm Trigger Necrosis by Oxidative Stress and Mitochondrial Damage. Small. 2009;5:2067–2076.

[59] Mironava T, Hadjiargyrou M, Simon M, et al. Gold nanoparticles cellular toxicity and recovery: Effect of size, concentration and exposure time. Nanotoxicology. 2010;4:120–137.

[60] Albanese A, Chan WCW. Effect of Gold Nanoparticle Aggregation on Cell Uptake and Toxicity. ACS Nano. 2011;5:5478–5489.

[61] Chithrani BD, Ghazani AA, Chan WCW. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. Nano Lett. 2006;6:662–668.

[62] Singh A, Shukla R, Hassan S, et al. Cytotoxicity and Cellular Internalization Studies of Biogenic Gold Nanotriangles in Animal Cell Lines. Int. J. Green

Nanotechnol. 2011;3:251–263.

[63] Favi PM, Gao M, Johana Sepúlveda Arango L, et al. Shape and surface effects on the cytotoxicity of nanoparticles: Gold nanospheres versus gold nanostars. J. Biomed. Mater. Res. Part A. 2015;103:3449–3462.

[64] Schaeublin NM, Braydich-Stolle LK, Schrand AM, et al. Surface charge of gold nanoparticles mediates mechanism of toxicity. Nanoscale. 2011;3:410.

[65] Bertrand N, Leroux J-C. The journey of a drug-carrier in the body: An anatomo-physiological perspective. J. Control. Release. 2012;161:152–163.

[66] Choi K, Riviere JE, Monteiro-Riviere NA. Protein corona modulation of hepatocyte uptake and molecular mechanisms of gold nanoparticle toxicity. Nanotoxicology. 2017;11:64–75.

[67] Johnston HJ, Hutchison G, Christensen FM, et al. A review of the in vivo and in vitro toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. Crit. Rev. Toxicol. 2010;40:328–346.

[68] Simpson CA, Salleng KJ, Cliffel DE, et al. In vivo toxicity, biodistribution, and clearance of glutathione-coated gold nanoparticles. Nanomedicine Nanotechnology, Biol. Med.. 2013;9:257–263.

[69] Thakor AS, Luong R, Paulmurugan R, et al. The Fate and Toxicity of Raman-Active Silica-Gold Nanoparticles in Mice. Sci. Transl. Med. 2011;3:79ra33-79ra33.

[70] Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, et al. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. Biochem. Biophys. Res. Commun. 2010;393:649–655.

[71] Villaverde G, Gómez-Graña S, Guisasola E, et al. Targeted Chemo-Photothermal Therapy: A Nanomedicine Approximation to Selective Melanoma Treatment. Part. Part. Syst. Charact. 2018;35:1800148.

[72] Kreyling WG, Abdelmonem AM, Ali Z, et al. In vivo integrity of polymer-coated gold nanoparticles. Nat. Nanotechnol. 2015;10:619–623.

[73] Rengan AK, Bukhari AB, Pradhan A, et al. In Vivo Analysis of Biodegradable Liposome Gold Nanoparticles as Efficient Agents for Photothermal Therapy of Cancer. Nano Lett. 2015;15:842–848.

[74] Bunggulawa EJ, Wang W, Yin T, et al. Recent advancements in the use of exosomes as drug delivery systems. J. Nanobiotechnology. 2018;16:81.

[75] Betzer O, Perets N, Angel A, et al. In Vivo Neuroimaging of Exosomes Using Gold Nanoparticles. ACS Nano. 2017;11:10883–10893.

[76] Cheng X, Sun R, Yin L, et al. Light-Triggered Assembly of Gold Nanoparticles for Photothermal Therapy and Photoacoustic Imaging of Tumors In Vivo. Adv. Mater. 2017;29:1604894.

[77] Shen H, Zhang L, Liu M, et al. Biomedical Applications of Graphene. Theranostics. 2012;2:283–294.

•• Review on the biomedical applications of graphene.

[78] Seabra AB, Paula AJ, de Lima R, et al. Nanotoxicity of Graphene and Graphene Oxide. Chem. Res. Toxicol. 2014;27:159–168.

[79] Ou L, Song B, Liang H, et al. Toxicity of graphene-family nanoparticles: a general review of the origins and mechanisms. Part. Fibre Toxicol. 2016;13:57.

• Review which describes the toxicity of graphene and related nanostructures.

[80] Jastrzębska AM, Kurtycz P, Olszyna AR. Recent advances in graphene family materials toxicity investigations. J. Nanoparticle Res. 2012;14:1320.

[81] Yang K, Li Y, Tan X, et al. Behavior and Toxicity of Graphene and Its Functionalized Derivatives in Biological Systems. Small. 2013;9:1492–1503.

[82] Gurunathan S, Han JW, Eppakayala V, et al. Green synthesis of graphene and its cytotoxic effects in human breast cancercells. Int. J. Nanomedicine. 2013;8:1015.

[83] Vallabani NVS, Mittal S, Shukla R, et al. Toxicity of Graphene in Normal Human Lung Cells (BEAS-2B). J. Biomed. Nanotechnol. 2011;7:106–107.

[84] Zhang L, Xia J, Zhao Q, et al. Functional Graphene Oxide as a Nanocarrier for Controlled Loading and Targeted Delivery of Mixed Anticancer Drugs. Small. 2010;6:537–544.

[85] Zhang Y, Ali SF, Dervishi E, et al. Cytotoxicity Effects of Graphene and Single-Wall Carbon Nanotubes in Neural Phaeochromocytoma-Derived PC12 Cells. ACS Nano. 2010;4:3181–3186.

[86] Chang Y, Yang S-T, Liu J-H, et al. In vitro toxicity evaluation of graphene oxide on

A549 cells. Toxicol. Lett. 2011;200:201–210.

[87] Qiao Y, An J, Ma L. Single Cell Array Based Assay for in Vitro Genotoxicity Study of Nanomaterials. Anal. Chem. 2013;85:4107–4112.

[88] Robinson JT, Tabakman SM, Liang Y, et al. Ultrasmall Reduced Graphene Oxide with High Near-Infrared Absorbance for Photothermal Therapy. J. Am. Chem. Soc. 2011;133:6825–6831.

[89] Feng L, Liu Z. Graphene in biomedicine: opportunities and challenges. Nanomedicine. 2011;6:317–324.

[90] Liu Z, Robinson JT, Sun X, et al. PEGylated Nanographene Oxide for Delivery of Water-Insoluble Cancer Drugs. J. Am. Chem. Soc. 2008;130:10876–10877.

[91] Sun X, Liu Z, Welsher K, et al. Nano-graphene oxide for cellular imaging and drug delivery. Nano Res. 2008;1:203–212.

[92] Sasidharan A, Panchakarla LS, Sadanandan AR, et al. Hemocompatibility and Macrophage Response of Pristine and Functionalized Graphene. Small. 2012;8:1251–1263.

[93] Singh SK, Singh MK, Kulkarni PP, et al. Amine-Modified Graphene: Thrombo-Protective Safer Alternative to Graphene Oxide for Biomedical Applications. ACS Nano. 2012;6:2731–2740.

[94] Zhang X, Yin J, Peng C, et al. Distribution and biocompatibility studies of graphene oxide in mice after intravenous administration. Carbon N. Y. 2011;49:986–995.

[95] Liao K, Lin Y, Macosko CW, et al. Cytotoxicity of Graphene Oxide and Graphene in Human Erythrocytes and Skin Fibroblasts. ACS Appl. Mater. Interfaces. 2011;3:2607–2615.

[96] Wen K-P, Chen Y-C, Chuang C-H, et al. Accumulation and toxicity of intravenously-injected functionalized graphene oxide in mice. J. Appl. Toxicol. 2015;35:1211–1218.

[97] Schinwald A, Murphy FA, Jones A, et al. Graphene-Based Nanoplatelets: A New Risk to the Respiratory System as a Consequence of Their Unusual Aerodynamic Properties. ACS Nano. 2012;6:736–746.

[98] Zhang S, Yang K, Feng L, et al. In vitro and in vivo behaviors of dextran

functionalized graphene. Carbon N. Y. 2011;49:4040–4049.

[99] Wang K, Ruan J, Song H, et al. Biocompatibility of Graphene Oxide. Nanoscale Res. Lett. 2011;6:8.

[100] Gollavelli G, Ling Y-C. Multi-functional graphene as an in vitro and in vivo imaging probe. Biomaterials. 2012;33:2532–2545.

[101] Zanni E, De Bellis G, Bracciale MP, et al. Graphite Nanoplatelets and *Caenorhabditis elegans*: Insights from an *in Vivo* Model. Nano Lett. 2012;12:2740–2744.

[102] Zhang W, Wang C, Li Z, et al. Unraveling Stress-Induced Toxicity Properties of Graphene Oxide and the Underlying Mechanism. Adv. Mater. 2012;24:5391–5397.

[103] Syama S, Mohanan PV. Safety and biocompatibility of graphene: A new generation nanomaterial for biomedical application. Int. J. Biol. Macromol. 2016;86:546–555.

[104] Zhu C, Guo S, Fang Y, et al. Reducing Sugar: New Functional Molecules for the Green Synthesis of Graphene Nanosheets. ACS Nano. 2010;4:2429–2437.

[105] Gao J, Liu F, Liu Y, et al. Environment-Friendly Method To Produce Graphene That Employs Vitamin C and Amino Acid. Chem. Mater. 2010;22:2213–2218.

[106] Urbas K, Aleksandrzak M, Jedrzejczak M, et al. Chemical and magnetic functionalization of graphene oxide as a route to enhance its biocompatibility. Nanoscale Res. Lett. 2014;9:656.

[107] Chong Y, Ma Y, Shen H, et al. The in vitro and in vivo toxicity of graphene quantum dots. Biomaterials. 2014;35:5041–5048.

[108] Jasim DA, Boutin H, Fairclough M, et al. Thickness of functionalized graphene oxide sheets plays critical role in tissue accumulation and urinary excretion: A pilot PET/CT study. Appl. Mater. Today. 2016;4:24–30.

[109] Yang K, Zhang S, Zhang G, et al. Graphene in Mice: Ultrahigh In Vivo Tumor Uptake and Efficient Photothermal Therapy. Nano Lett. 2010;10:3318–3323.

[110] Miao W, Shim G, Lee S, et al. Safety and tumor tissue accumulation of pegylated graphene oxide nanosheets for co-delivery of anticancer drug and photosensitizer. Biomaterials. 2013;34:3402–3410.

[111] Yang K, Gong H, Shi X, et al. In vivo biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. Biomaterials. 2013;34:2787–2795.

[112] Yan L, Wang Y, Xu X, et al. Can Graphene Oxide Cause Damage to Eyesight? Chem. Res. Toxicol. 2012;25:1265–1270.

[113] Kiew SF, Kiew LV, Lee HB, et al. Assessing biocompatibility of graphene oxide-based nanocarriers: A review. J. Control. Release. 2016;226:217–228.

[114] Young-Kwan Kim, Mi-Hee Kim, Dal-Hee Min. Biocompatible reduced graphene oxide prepared by using dextran as a multifunctional reducing agent. Chem. Commun. 2011;47:3195–3197.

[115] Wate PS, Banerjee SS, Jalota-Badhwar A, et al. Cellular imaging using biocompatible dendrimer-functionalized graphene oxide-based fluorescent probe anchored with magnetic nanoparticles. Nanotechnology. 2012;23:415101.

[116] Hong BJ, Compton OC, An Z, et al. Successful Stabilization of Graphene Oxide in Electrolyte Solutions: Enhancement of Biofunctionalization and Cellular Uptake. ACS Nano. 2012;6:63–73.

[117] Cheng C, Nie S, Li S, et al. Biopolymer functionalized reduced graphene oxide with enhanced biocompatibility via mussel inspired coatings/anchors. J. Mater. Chem. B. 2013;1:265–275.

[118] Xu M, Zhu J, Wang F, et al. Improved In Vitro and In Vivo Biocompatibility of Graphene Oxide through Surface Modification: Poly(Acrylic Acid)-Functionalization is Superior to PEGylation. ACS Nano. 2016;10:3267-3281

[119] Croissant JG, Fatieiev Y, Khashab NM. Degradability and Clearance of Silicon, Organosilica, Silsesquioxane, Silica Mixed Oxide, and Mesoporous Silica Nanoparticles. Adv. Mater. 2017;29:1604634.

•• Review which describes the degradability and clearance of silicon and silica nanoparticles.

[120] Santos HA, Mäkilä E, Airaksinen AJ, et al. Porous silicon nanoparticles for nanomedicine: preparation and biomedical applications. Nanomedicine. 2014;9:535–554.

•• Review on the preparation and biomedical applications of MSiNs.

[121] Xu W, Rytkönen J, Rönkkö S, et al. A nanostopper approach to selectively engineer the surfaces of mesoporous silicon. Chem. Mater. 2014;26:6734–6742.
[122] Kafshgari MH, Delalat B, Tong WY, et al. Oligonucleotide delivery by chitosan-functionalized porous silicon nanoparticles. Nano Res. 2015;8:2033–2046.
[123] Godin B, Gu J, Serda RE, et al. Tailoring the degradation kinetics of mesoporous silicon structures through PEGylation. J. Biomed. Mater. Res. - Part A. 2010;94:1236–1243.
[124] Hou H, Nieto A, Ma F, et al. Tunable sustained intravitreal drug delivery system for daunorubicin using oxidized porous silicon. J. Control. Release. 2014;178:46–54.
[125] Martinez JO, Chiappini C, Ziemys A, et al. Engineering multi-stage nanovectors for controlled degradation and tunable release kinetics. Biomaterials. 2013;34:8469–8477.
[126] Hon NK, Shaposhnik Z, Diebold ED, et al. Tailoring the biodegradability of porous silicon nanoparticles. J. Biomed. Mater. Res. Part A. 2012;100A:3416–3421.
[127] Park J-H, Gu L, von Maltzahn G, et al. Biodegradable luminescent porous silicon nanoparticles for in vivo applications. Nat. Mater. 2009;8:331–336.
[128] Martinez JO, Evangelopoulos M, Chiappini C, et al. Degradation and biocompatibility of multistage nanovectors in physiological systems. J. Biomed. Mater. Res. Part A. 2014;102:3540–3549.
[129] Tolstik E, Osminkina LA, Matthäus C, et al. Studies of silicon nanoparticles uptake and biodegradation in cancer cells by Raman spectroscopy. Nanomedicine Nanotechnology, Biol. Med. 2016;12:1931–1940.
[130] Tzur-Balter A, Shatsberg Z, Beckerman M, et al. Mechanism of erosion of nanostructured porous silicon drug carriers in neoplastic tissues. Nat. Commun. 2015;6:6208.
[131] Wang C-F, Mäkilä EM, Kaasalainen MH, et al. Copper-free azide–alkyne cycloaddition of targeting peptides to porous silicon nanoparticles for intracellular drug uptake. Biomaterials. 2014;35:1257–1266.
[132] Shahbazi MA, Hamidi M, Mäkilä EM, et al. The mechanisms of surface chemistry effects of mesoporous silicon nanoparticles on immunotoxicity and

biocompatibility. Biomaterials. 2013;34:7776–7789.

[133] Ivanov S, Zhuravsky S, Yukina G, et al. In vivo toxicity of intravenously administered silica and silicon nanoparticles. Materials. 2012;5:1873–1889.

[134] Sarparanta M, Bimbo LM, Rytkoänen J, et al. Intravenous delivery of hydrophobin-functionalized porous silicon nanoparticles: Stability, plasma protein adsorption and biodistribution. Mol. Pharm. 2012;9:654–663.

[135] Sarparanta MP, Bimbo LM, Mäkilä EM, et al. The mucoadhesive and gastroretentive properties of hydrophobin-coated porous silicon nanoparticle oral drug delivery systems. Biomaterials. 2012;33:3353–3362.

[136] Shahbazi M, Almeida P V, Mäkilä E, et al. Functionalized Porous Silicon Nanoparticles for Enhanced Stability and Cellular Internalization. Macromol. Rapid Commun. 2014;35:624–629.

[137] Xia B, Zhang W, Shi J, et al. Engineered stealth porous silicon nanoparticles via surface encapsulation of bovine serum albumin for prolonging blood circulation in vivo. ACS Appl. Mater. Interfaces. 2013;5:11718–11724.

[138] Paris JL, Colilla M, Izquierdo-Barba I, et al. Tuning mesoporous silica dissolution in physiological environments: a review. J. Mater. Sci. 2017;52:8761–8771.

[139] Vallet-Regí M, Rámila A, del Real RP, et al. A New Property of MCM-41: Drug Delivery System. Chem. Mater. 2001;13:308–311.

[140] Vallet-Regí M, Ruiz-Hernández E. Bioceramics: from bone regeneration to cancer nanomedicine. Adv. Mater. 2011;23:5177–5218.

[141] Villaverde G, Alfranca A, Gonzalez-Murillo Á, et al. Molecular Scaffolds as Double-Targeting Agents For the Diagnosis and Treatment of Neuroblastoma. Angew. Chemie. 2019;131:3099–3104.

[142] Mora-Raimundo P, Lozano D, Manzano M, et al. Nanoparticles to Knockdown Osteoporosis-Related Gene and Promote Osteogenic Marker Expression for Osteoporosis Treatment. ACS Nano. 2019;13:5451–5464.

[143] Baeza A, Colilla M, Vallet-Regí M. Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery. Expert Opin. Drug Deliv. 2015;12:319–337.

•• Review on stimuli-responsive MSNs for drug delivery.

[144] Baeza A, Ruiz-Molina D, Vallet-Regí M. Recent advances in porous nanoparticles for drug delivery in antitumoral applications: inorganic nanoparticles and nanoscale metal-organic frameworks. Expert Opin. Drug Deliv. 2017;14:783–796.
[145] Martínez-Carmona M, Lozano D, Baeza A, et al. A novel visible light responsive nanosystem for cancer treatment. Nanoscale. 2017;9:15967–15973.
[146] Martínez-Carmona M, Baeza A, Rodriguez-Milla MA, et al. Mesoporous silica nanoparticles grafted with a light-responsive protein shell for highly cytotoxic antitumoral therapy. J. Mater. Chem. B. 2015;3:5746–5752.
[147] Guisasola E, Asín L, Beola L, et al. Beyond Traditional Hyperthermia: In Vivo Cancer Treatment with Magnetic-Responsive Mesoporous Silica Nanocarriers. ACS Appl. Mater. Interfaces. 2018;10:12518–12525.
[148] Baeza A, Guisasola E, Ruiz-Hernández E, et al. Magnetically Triggered Multidrug Release by Hybrid Mesoporous Silica Nanoparticles. Chem. Mater. 2012;24:517–524.
[149] Guisasola E, Baeza A, Talelli M, et al. Magnetic-Responsive Release Controlled by Hot Spot Effect. Langmuir. 2015;31:12777–12782.
[150] Paris JL, Cabañas MV, Manzano M, et al. Polymer-Grafted Mesoporous Silica Nanoparticles as Ultrasound-Responsive Drug Carriers. ACS Nano. 2015;9:11023–11033.
[151] Martínez-Carmona M, Lozano D, Colilla M, et al. Selective topotecan delivery to cancer cells by targeted pH-sensitive mesoporous silica nanoparticles. RSC Adv. 2016;6:50923–50932.
[152] Hu C, Huang P, Zheng Z, et al. A Facile Strategy to Prepare an Enzyme-Responsive Mussel Mimetic Coating for Drug Delivery Based on Mesoporous Silica Nanoparticles. Langmuir. 2017;33:5511–5518.
[153] Wu M, Meng Q, Chen Y, et al. Large Pore-Sized Hollow Mesoporous Organosilica for Redox-Responsive Gene Delivery and Synergistic Cancer Chemotherapy. Adv. Mater.. 2016;28:1963–1969.
[154] Lin YS, Abadeer N, Haynes CL. Stability of small mesoporous silica nanoparticles in biological media. Chem. Commun. 2011;47:532–534.
[155] Murugadoss S, Lison D, Godderis L, et al. Toxicology of silica nanoparticles: an

update. Arch. Toxicol. 2017;91:2967–3010.

[156] Kim I-Y, Joachim E, Choi H, et al. Toxicity of silica nanoparticles depends on size, dose, and cell type. Nanomedicine Nanotechnology, Biol. Med. 2015;11:1407–1416.

[157] Kim D, Lin YS, Haynes CL. On-chip evaluation of shear stress effect on cytotoxicity of mesoporous silica nanoparticles. Anal. Chem. 2011;83:8377–8382.

[158] Heidegger S, Gößl D, Schmidt A, et al. Immune response to functionalized mesoporous silica nanoparticles for targeted drug delivery. Nanoscale. 2016;8:938–948.

[159] Lin Y-S, Haynes CL. Impacts of Mesoporous Silica Nanoparticle Size, Pore Ordering, and Pore Integrity on Hemolytic Activity. J. Am. Chem. Soc. 2010;132:4834–4842.

[160] Slowing II, Wu CW, Vivero-Escoto JL, et al. Mesoporous silica nanoparticles for reducing hemolytic activity towards mammalian red blood cells. Small. 2009;5:57–62.

[161] Urata C, Yamada H, Wakabayashi R, et al. Aqueous colloidal mesoporous nanoparticles with ethenylene-bridged silsesquioxane frameworks. J. Am. Chem. Soc. 2011;133:8102–8105.

[162] Liu T, Li L, Teng X, et al. Single and repeated dose toxicity of mesoporous hollow silica nanoparticles in intravenously exposed mice. Biomaterials. 2011;32:1657–1668.

[163] Fu C, Liu T, Li L, et al. The absorption, distribution, excretion and toxicity of mesoporous silica nanoparticles in mice following different exposure routes. Biomaterials. 2013;34:2565–2575.

[164] Hudson SP, Padera RF, Langer R, et al. The biocompatibility of mesoporous silicates. Biomaterials. 2008;29:4045–4055.

[165] Villaverde G, Baeza A, Melen GJ, et al. A new targeting agent for the selective drug delivery of nanocarriers for treating neuroblastoma. J. Mater. Chem. B. 2015;3:4831–4842.

[166] Dogra P, Adolphi NL, Wang Z, et al. Establishing the effects of mesoporous silica nanoparticle properties on in vivo disposition using imaging-based

pharmacokinetics. Nat. Commun. 2018;9:4551.

[167] Yu T, Greish K, McGill LD, et al. Influence of geometry, porosity, and surface characteristics of silica nanoparticles on acute toxicity: Their vasculature effect and tolerance threshold. ACS Nano. 2012;6:2289–2301.

[168] Lee S, Kim MS, Lee D, et al. The comparative immunotoxicity of mesoporous silica nanoparticles and colloidal silica nanoparticles in mice. Int. J. Nanomedicine. 2013;8:147–158.

[169] Li L, Tang F, Liu H, et al. In Vivo Delivery of Silica Nanorattle Encapsulated Docetaxel for Liver Cancer Therapy with Low Toxicity and High Efficacy. ACS Nano. 2011;5:679–679.

[170] He Q, Shi J, Zhu M, et al. The three-stage in vitro degradation behavior of mesoporous silica in simulated body fluid. Microporous Mesoporous Mater. 2010;131:314–320.

[171] Braun K, Pochert A, Beck M, et al. Dissolution kinetics of mesoporous silica nanoparticles in different simulated body fluids. J. Sol-Gel Sci. Technol. 2016;79:319–327.

[172] Yamada H, Urata C, Aoyama Y, et al. Preparation of Colloidal Mesoporous Silica Nanoparticles with Different Diameters and Their Unique Degradation Behavior in Static Aqueous Systems. Chem. Mater. 2012;24:1462–1471.

[173] Hao N, Liu H, Li L, et al. In Vitro Degradation Behavior of Silica Nanoparticles Under Physiological Conditions. J. Nanosci. Nanotechnol. 2012;12:6346–6354.

[174] Huang X, Young NP, Townley HE. Characterization and Comparison of Mesoporous Silica Particles for Optimized Drug Delivery. Nanomater. Nanotechnol. 2014;4:2.

[175] Cauda V, Schlossbauer A, Bein T. Bio-degradation study of colloidal mesoporous silica nanoparticles: Effect of surface functionalization with organo-silanes and poly(ethylene glycol). Microporous Mesoporous Mater. 2010;132:60–71.

[176] Li X, Zhang L, Dong X, et al. Preparation of mesoporous calcium doped silica spheres with narrow size dispersion and their drug loading and degradation behavior. Microporous Mesoporous Mater. 2007;102:151–158.

[177] Yu L, Chen Y, Wu M, et al. “Manganese Extraction” Strategy Enables Tumor-

Sensitive Biodegradability and Theranostics of Nanoparticles. J. Am. Chem. Soc. 2016;138:9881–9894.

[178] Fontecave T, Sanchez C, Azaïs T, et al. Chemical Modification As a Versatile Tool for Tuning Stability of Silica Based Mesoporous Carriers in Biologically Relevant Conditions. Chem. Mater. 2012;24:4326–4336.

[179] Maggini L, Cabrera I, Ruiz-Carretero A, et al. Breakable mesoporous silica nanoparticles for targeted drug delivery. Nanoscale. 2016;8:7240–7247.

[180] Chen Y, Meng Q, Wu M, et al. Hollow Mesoporous Organosilica Nanoparticles: A Generic Intelligent Framework-Hybridization Approach for Biomedicine. J. Am. Chem. Soc. 2014;136:16326–16334.

[181] Cauda V, Argyo C, Bein T. Impact of different PEGylation patterns on the long-term bio-stability of colloidal mesoporous silica nanoparticles. J. Mater. Chem. 2010;20:8693–8699.

[182] Shen D, Yang J, Li X, et al. Biphase stratification approach to three-dimensional dendritic biodegradable mesoporous silica nanospheres. Nano Lett. 2014;14:923–932.

[183] Möller K, Bein T. Degradable Drug Carriers: Vanishing Mesoporous Silica Nanoparticles. Chem. Mater. 2019;31:4364-4378.

[184] Zhang Q, Wang X, Li PZ, et al. Biocompatible, uniform, and redispersible mesoporous silica nanoparticles for cancer-targeted drug delivery in vivo. Adv. Funct. Mater. 2014;24:2450–2461.

[185] He Q, Zhang Z, Gao F, et al. In vivo Biodistribution and Urinary Excretion of Mesoporous Silica Nanoparticles: Effects of Particle Size and PEGylation. Small. 2011;7:271–280.

[186] Huang X, Li L, Liu T, et al. The Shape Effect of Mesoporous Silica Nanoparticles on Biodistribution, Clearance, and Biocompatibility in Vivo. ACS Nano. 2011;5:5390–5399.

[187] Li L, Liu T, Fu C, et al. Biodistribution, excretion, and toxicity of mesoporous silica nanoparticles after oral administration depend on their shape. Nanomedicine Nanotechnology, Biol. Med. 2015;11:1915–1924.

[188] Tarn D, Ashley CE, Xue M, et al. Mesoporous Silica Nanoparticle Nanocarriers:

Biofunctionality and Biocompatibility. Acc. Chem. Res. 2013;46:792–801.

[189] Desai D, Zhang J, Sandholm J, et al. Lipid Bilayer-Gated Mesoporous Silica Nanocarriers for Tumor-Targeted Delivery of Zoledronic Acid in Vivo. Mol. Pharm. 2017;14:3218–3227.

[190] Gonzalez Porras MA, Durfee PN, Gregory AM, et al. A novel approach for targeted delivery to motoneurons using cholera toxin-B modified protocells. J. Neurosci. Methods. 2016;273:160–174.

[191] Ashley CE, Carnes EC, Epler KE, et al. Delivery of Small Interfering RNA by Peptide-Targeted Mesoporous Silica Nanoparticle-Supported Lipid Bilayers. ACS Nano. 2012;6:2174–2188.

[192] Epler K, Padilla D, Phillips G, et al. Delivery of Ricin Toxin A-Chain by Peptide-Targeted Mesoporous Silica Nanoparticle-Supported Lipid Bilayers. Adv. Healthc. Mater. 2012;1:348–353.

[193] Sharifabad ME, Mercer T, Sen T. Drug-loaded liposome-capped mesoporous core–shell magnetic nanoparticles for cellular toxicity study. Nanomedicine. 2016;11:2753–2755.

[194] Han N, Zhao Q, Wan L, et al. Hybrid Lipid-Capped Mesoporous Silica for Stimuli-Responsive Drug Release and Overcoming Multidrug Resistance. ACS Appl. Mater. Interfaces. 2015;7:3342–3351.

[195] Villegas MR, Baeza A, Noureddine A, et al. Multifunctional Protocells for Enhanced Penetration in 3D Extracellular Tumoral Matrices. Chem. Mater. 2018;30:112–120.

[196] Villegas MR, Baeza A, Vallet-Regí M. Hybrid Collagenase Nanocapsules for Enhanced Nanocarrier Penetration in Tumoral Tissues. ACS Appl. Mater. Interfaces. 2015;7:24075–24081.

[197] Akin D, Sturgis J, Ragheb K, et al. Bacteria-mediated delivery of nanoparticles and cargo into cells. Nat. Nanotechnol. 2007;2:441–449.

[198] Bonvalot S, Le Pechoux C, De Baere T, et al. First-in-human study testing a new radioenhancer using nanoparticles (NBTXR3) activated by radiation therapy in patients with locally advanced soft tissue sarcomas. Clin. Cancer Res. 2017;23:908–917.

Figures and captions:

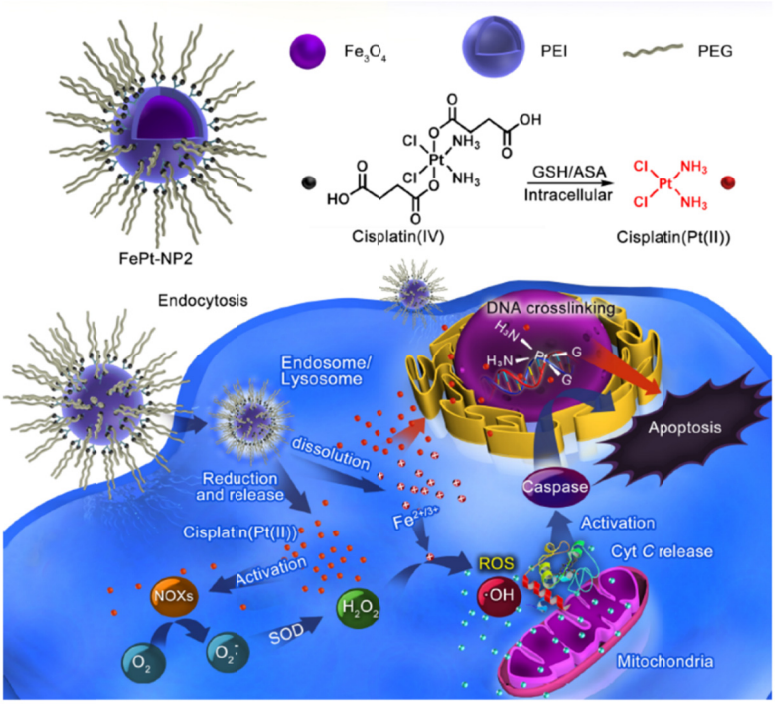

Figure 1. *SPION coated with PEI and PEG which enhance the cytotoxicity of cisplatin(IV) prodrugs. This image is used without modifications from reference 38. Copyright © 2017, American Chemical Society.*

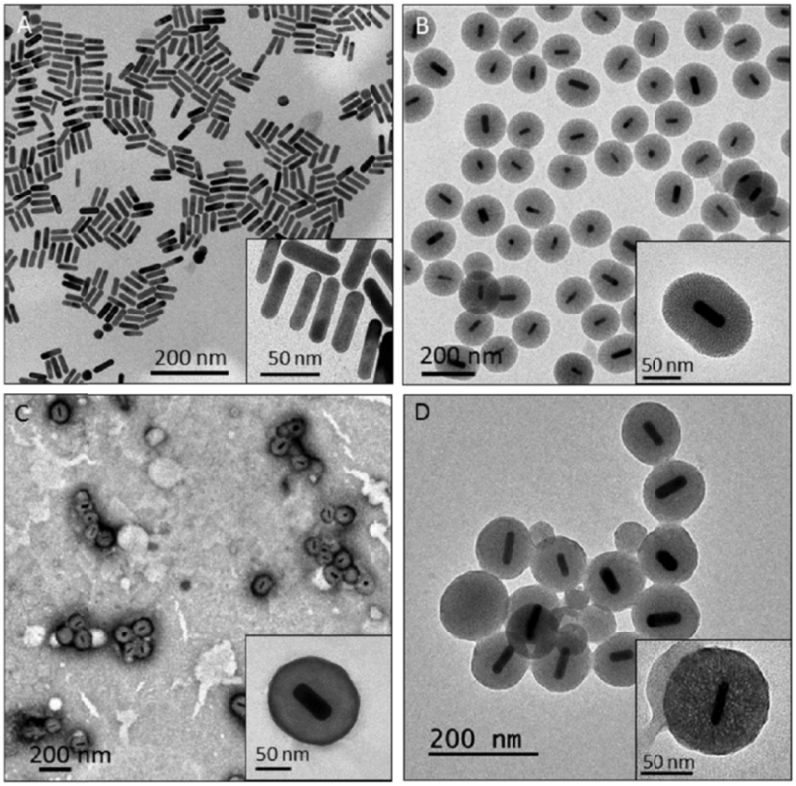

Figure 2. *A) Gold nanorods (GNR) B) GNR embedded within silica matrix (GNR-SiO_2) C) GNR-SiO_2 coated with thermosensitive polymer (GNR-SiO_2-Pol) and D) GNR-SiO_2-Pol decorated with napamide as melanoma targeting agent. This image is used without modifications from reference 65. Copyright © 2018, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

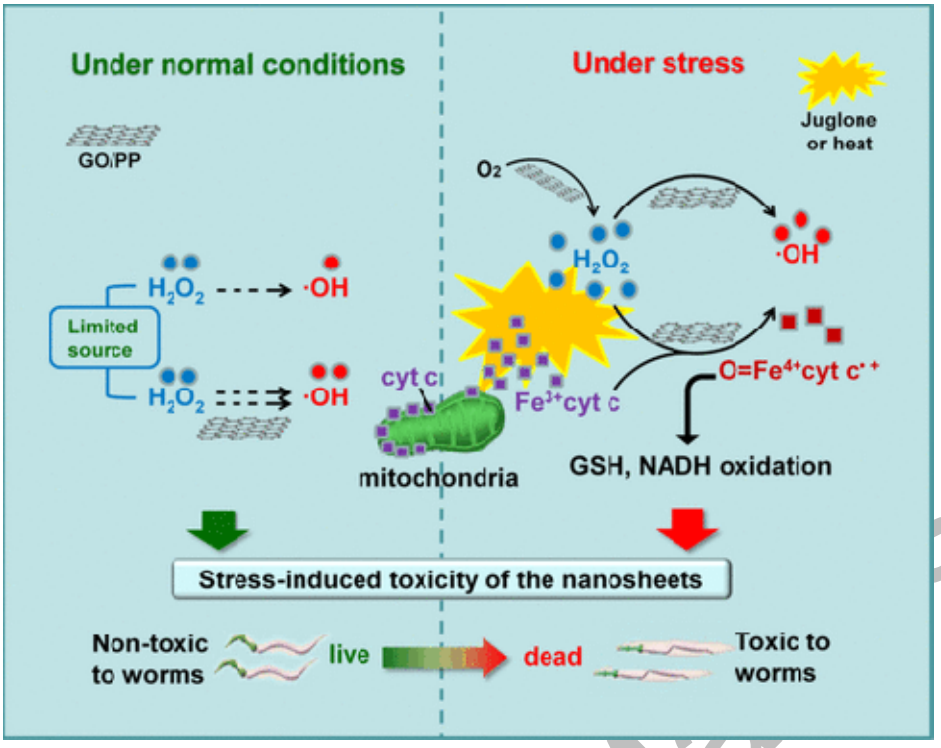

***Figure 3**. Schematic representation of stress-induced toxicity of graphene oxide on Caenorhabditis elegans. This image is used without modifications from reference 96. Copyright © 2012, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

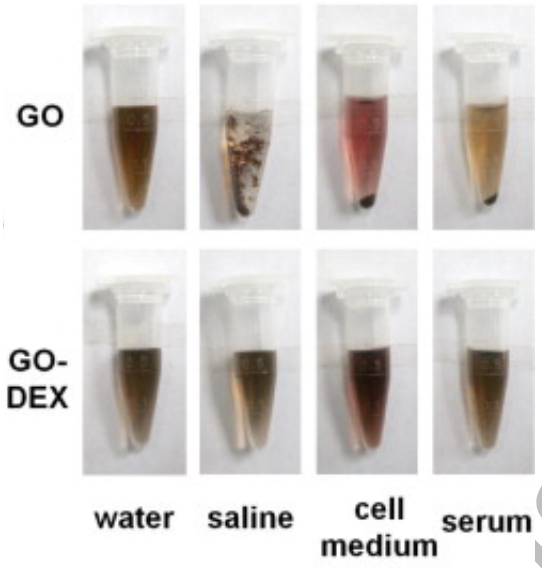

***Figure 4**. Suspension stability of GO and dextran-coated GO in different media. This image is used without modifications from reference 98. Copyright © 2011, Elsevier.*

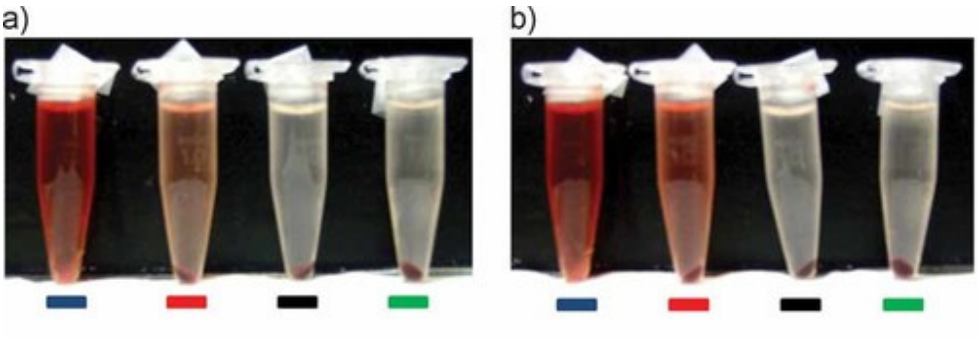

***Figure 5**. Hemolysis assay for non-porous silica nanoparticles (red line) and MSN (green line), water as positive control (blue line) and PBS as negative control (black line). The materials were suspended at 60 (a) and 100 μg mL^{-1} (b). Samples were centrifuged to detect the presence of hemoglobin (red color) in the supernatant. This image is used without modifications from reference 160. Copyright © 2009, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

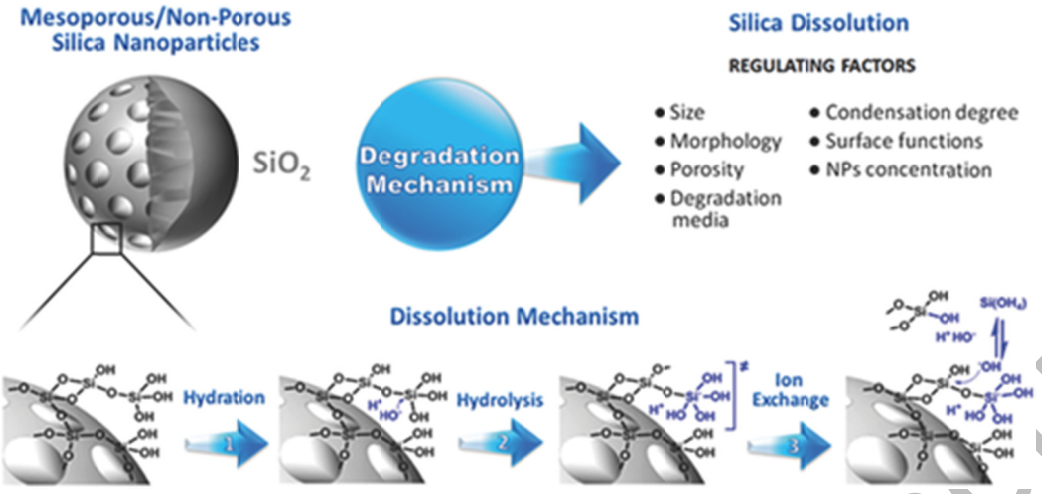

Figure 6. *Schematic representation of the degradation/dissolution process of silica nanoparticles along with its main regulating factors. This image is used without modifications from reference 113. Copyright © 2017, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

Table 1. Summary of the strategies based on nanoparticle surface modification presented in this review,

Nanoparticle	Surface modification	Effect	Refs
SPION	Cationic groups	Increase cytotoxicity by higher cellular uptake and lysosomal rupture	20, 25
	Polysaccharides	Scarce toxicity even at concentrations up to 1 $mg \cdot mL^{-1}$	26
	Silica	Increased biocompatibility by lower production of ROS within the cell. Improved resistance to degradation.	27, 28
	PEG	Reduced toxicity by lower cellular uptake	29
	PLGA	Prevent autophagosome accumulation	33
	PEI	Rapid clearance from the blood stream by macrophages and high toxicity caused by hemolysis and mithocondria/membrane cell disruption.	40
GNP	Thiol-contained antioxidants or gluthatione	Reduced toxicity by the elimination of ROS	58, 68
	Branched PEI	High toxicity at concentrations of 50 $\mu g \cdot mL^{-1}$	66
	Negative and neutral coatings: Lipoic acid, citrate or PEG	Low toxicity *in vitro* and *in vivo*	66, 70
	Silica	Low toxicity *in vivo*	69
	Silica coated with PolyNIPAM	Low toxicity *in vitro*	71
	Liposomes and exosomes	Low toxicity *in vivo*	73, 74
Graphene	Amino groups	Reduction in hemolytic and thrombotic potential	93
	BSA	Reduction in hemolytic and thrombotic potential	96-99
	PEG	Improved colloidal stability and biocompatibility	78, 88-91, 102, 109-112
	Dextrans	Improved colloidal stability and biocompatibility	114
	PAMAM dendrimers	Low toxicity *in vitro*	115
	Pluronic F127	Significant toxicity *in vitro*	116
	Polydopamine	Ultralow hemolytic potential and low toxicity	117
	Polyacrylic acid	Low *in vitro* and *in vivo* toxicity	118
Mesoporous Silicon	Cationic groups	High toxicity and hemolytic potential	132
	Silica	Increase degradation time, low toxicity	126
	Dextran	Increase degradation time, low toxicity	127
	PEG	Increase degradation time, low toxicity	123, 132
	Hydrophobin	Improved dispersibility in plasma reducing lung accumulation	134, 135
	PMVE-MA	Improved colloidal and plasma stability	136
	BSA	Reduced cell uptake/improved circulation lime	137
Mesoporous Silica	PEG	Increase colloidal stability and circulation time. Low toxicity and hemolytic potential. Enhanced degradation time.	154, 157-159, 169, 181, 184, 185
	Ethenylene-bridged silsesquioxanes in network	Reduced hemolytic potential	161
	Calcium, manganese or breakable groups as doping agents	Accelerated degradability	176, 177, 179, 180
	Zirconia doping	Delayed degradability	178
	Lipid bilayers (Protocells)	Increased colloidal stability and biocompatibility	188-195

and the effect associated with each modification.