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A Safer Formulation Concept for Flame-Generated Engineered Nanomaterials

Samuel Gass^{1,2}, Joel M. Cohen¹, Georgios Pyrgiotakis¹, Georgios A. Sotiriou^{1,2}, Sotiris E. Pratsinis², and Philip Demokritou^{1,*}

¹Center for Nanotechnology and Nanotoxicology at Harvard School of Public Health, Harvard University, 665 Huntington Avenue, 02115 Boston, MA U.S.A ²Particle Technology Laboratory, Institute of Process Engineering, Department of Mechanical and Process Engineering, Swiss Federal Institute of Technology Zurich (ETH Zurich), Sonneggstrasse 3, CH-8092, Zurich, Switzerland

Abstract

The likely success or failure of the nanotechnology industry depends on the environmental health and safety of engineered nanomaterials (ENMs). While efforts toward engineering safer ENMs are sparse, such efforts are considered crucial to the sustainability of the nanotech industry. A promising approach in this regard is to coat potentially toxic nanomaterials with a biologically inert layer of amorphous SiO₂. Core-shell particles exhibit the surface properties of their amorphous SiO₂ shell while maintaining specific functional properties of their core material. A major challenge in the development of functional core-shell particles is the design of scalable high-yield processes that can meet large-scale industrial demand. Here, we present a safer formulation concept for flame-generated ENMs based on a one-step, in flight SiO₂ encapsulation process, which was recently introduced by the authors as a means for a scalable manufacturing of SiO₂ coated ENMs. Firstly, the versatility of the SiO₂-coating process is demonstrated by applying it to four ENMs (CeO₂, ZnO, Fe₂O₃, Ag) marked by their prevalence in consumer products as well as their range in toxicity. The ENM-dependent coating fundamentals are assessed and process parameters are optimized for each ENM investigated. The effects of the SiO₂-coating on core material structure, composition and morphology, as well as the coating efficiency on each nanostructured material, are evaluated using state-of-the-art analytical methods (XRD, N₂ adsorption, TEM, XPS, isopropanol chemisorption). Finally, the biological interactions of SiO₂-coated vs. uncoated ENMs are evaluated using cellular bioassays, providing valuable evidence for reduced toxicity for the SiO₂-coated ENMs. Results indicate that the proposed 'safer by design' concept bears great promise for scaled-up application in industry in order to reduce the toxicological profile of ENMs for certain applications.

Keywords

safer nano-formulations; flame generated nanomaterials; nanotechnology; nanotoxicology; core-shell particles

*Corresponding author: Philip Demokritou, PhD, pdemokri@hsph.harvard.edu.

Supplementary Materials

Supplemental Figures 1–4 showing the optical absorbance of the ZnO nanoparticles, the VENGES used for the in-vivo inhalation studies, the XRD patterns of the ZnO nanoparticles, and the in vivo toxicity data for CeO₂ and SiO₂ coated CeO₂ ENMs, and Supplemental Table 1 with all precursor information are available free of charge via the Internet at <http://pubs.acs.org>.

The global nanotechnology industry reached over 1.5 trillion USD last year and has become a major economic force in the 21st century.¹ Engineered nanomaterials (ENMs) are by far the largest segment of the nanotechnology market, accounting for 80% of all revenues.² Global ENM production rates are expected to increase by more than 20 times within the next 15 years.³ Meanwhile, the number of consumer products containing ENMs is growing at a similarly exponential pace.⁴ ENMs are found in products ranging from sunscreens and cosmetics to food and beverages, building materials and toner formulations, making human and environmental exposures inevitable.^{5, 6} Preliminary evidence demonstrates the potential for ENMs to cause adverse biological effects.⁷ Nano-sized particles (NPs) may enter cells and exhibit greater toxic potential than their micron-sized counterparts due to their small size and corresponding large surface to volume ratio.⁷ The potential of nanoparticles and nanofibers to translocate across air-blood barriers, accessing critical extrapulmonary organs is also of concern.^{8–11}

While significant research has been directed toward understanding nano-bio interactions, research toward devising safer ENM formulation concepts that can readily be adopted by the nanotechnology industry is very sparse.^{12, 13} A promising approach in this regard is coating potentially toxic nanomaterials with a nanothin layer of amorphous SiO₂. Amorphous SiO₂ is considered a biologically and environmentally inert material, often used as a negative control material in *in-vitro* ENM screening assays.¹⁴ Such nanothin SiO₂ is therefore ideal to shield otherwise potentially toxic core-materials from any interactions with environmental and biological systems. The “core-shell” ENMs exhibit the surface properties of their shell while preserving certain important intrinsic functional properties (i.e. optical,¹⁵ magnetic properties,^{16, 17} plasmonic,¹⁸ phosphorescence¹⁹) of their core materials.

Here, a “safer formulation concept” based on this SiO₂ coating process is presented. By design, this concept is tailored to a family of ENMs with the highest volume of production,²⁰ namely, flame-generated nanomaterials. Gas phase (flame aerosol) processes are the preferred routes for scalable ENM synthesis as they do not create liquid by-products, offer easier particle collection from gases than liquids, usually include fewer process steps, and result in high purity materials with unique morphologies including the synthesis of metastable phases.^{21, 22} The applied SiO₂-coating process in flames has major advantages over wet-methods, such as sol-gel^{23, 24} and reverse micro-emulsion²⁵ which are frequently applied to the synthesis of SiO₂-coated nanoparticles by industry. These low yield, multi-step, wet synthesis methods often produce porous SiO₂ coatings,²⁶ which cannot sufficiently protect surrounding media from any potential toxic implications of the core materials.

In this study, a flame spray pyrolysis ENM synthesis platform (VENGES)^{27, 28} recently developed by the authors was modified to allow for in-flight coating of ENMs with a nanothin layer of amorphous SiO₂.¹⁵ This flame-based SiO₂-coating process has recently been explored by the authors as a means of high yield scalable nanomanufacturing of SiO₂-coated ENMs such as TiO₂, Fe₂O₃, and Ag. However, its versatility as well as the implication of the SiO₂-coating on the toxicological profiles of these particles was never investigated. Herein, we showcase the versatility of the SiO₂ coating process by applying it to a comprehensive and industry-relevant nanopanel (CeO₂, Fe₂O₃, ZnO, Ag). ENMs were chosen based on their range in toxicity²⁹ and prevalence in consumer products.⁴ Moreover, we characterize the effect of the SiO₂-coating on core material structure, composition and morphology, as well as the coating efficiency of the process using state-of-the-art analytical methods. Finally, the influence of surface coating on ENM-bio interactions is evaluated using several *in-vitro* bio-assays and cellular toxicity is assessed.

RESULTS AND DISCUSSION

SiO₂-encapsulation Strategy- Coating reactor

Figures 1 (a) and (b) illustrate the proposed approach and underlying theory to the one-step nanoparticle synthesis, and in-flight SiO₂-encapsulation, respectively. As presented in detail in the methods section, core nanoparticles are formed in the gas phase by flame spray pyrolysis of organometallic compounds dissolved in high enthalpy solvents.³⁰ The freshly formed core nanoparticles pass through the SiO₂ coating region, where they are encapsulated with a nanothin (1–5 nm) amorphous SiO₂ layer by the swirl-injection of hexamethyldisiloxane (HMDSO) vapor-laden N₂.

The key to successful hermetic coatings is to reduce the temperature in the coating region as to inhibit further core particle growth, maintaining however, sufficient temperatures for the conversion of HMDSO to SiO₂-coatings (heterogenous nucleation). The quality of SiO₂-coating on the core nanoparticles is influenced mainly by the coating reactor temperature profile, the mixing profile of the SiO₂ precursor vapor with the core nanoparticles, and the HMDSO injection molar flow rate. This has previously been described by the authors for the case of SiO₂-coated TiO₂.¹⁵

Figures 1c–f representatively show particle morphology of SiO₂-coated ZnO synthesized for different process parameters and illustrates the effect of various process parameters on the coating efficiency (Table S1). For example, in the case of ZnO, when using xylene as the high enthalpy solvent in the core particle synthesis step, the temperature profile in the SiO₂-coating zone is very high (> 1500 K). This leads to a reaction between the core ZnO nanoparticles and the SiO₂ coating, thereby forming sub-stoichiometric zinc silicates (Zn_{1.7}SiO₄, XRD, Figure S3) on the surface of the ZnO core nanoparticles (dark spots in Figure 1d). However, by carefully selecting a solvent with lower specific combustion enthalpy density (here ethanol), the zinc silicate formation can be inhibited and a homogeneous smooth nanothin SiO₂-coating is obtained (Figure 1c).

Varying molar flow rate ratio of the amount of HMDSO vapor that is injected to the one of the core material ($\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$) enables precise control over the SiO₂-coating thickness.^{15, 16, 18} However, if this ratio or the absolute HMDSO concentration in the reactor is increased over a certain threshold, the HMDSO vapor can nucleate homogeneously, thereby forming segregated SiO₂. This results in some core ZnO nanoparticles that are fully coated with SiO₂, but also segregated free SiO₂ nanoparticles (Figures 1e,f). These separate SiO₂ nanoparticles may further sinter with the SiO₂-coated ZnO ENMs forming rather rough SiO₂ surface coatings (Figure 1e). The particular temperature profile is also likely responsible for various combinations of these possibilities.

From the aforementioned examples, it is evident that the process is highly core ENM material-dependent and therefore needs to be optimized for each case individually. For the purpose of this study, ENM-dependent process parameters related to synthesis of all ENMs were optimized in order to enable complete encapsulation of the core ENMs with a smooth SiO₂ nanothin layer of a controlled thickness, avoiding the generation of free standing SiO₂ and rough SiO₂ surfaces, as it will be presented in the section below.

Effect of SiO₂-coating on core material structure and composition

The effect of the SiO₂ coating on the core material was investigated with Transmission Electron Microscopy (TEM) for the overall structural properties. X-Ray diffraction (XRD) was used to assess the crystal structure of the materials, and nitrogen absorption via the Brunauer-Emmett-Teller (BET) method for the specific surface area.

Figure 2 shows transmission electron microscope (TEM) images of the resulting bare and SiO₂-coated ENMs for each of the ENMs in the study (CeO₂, Fe₂O₃, Ag and ZnO). The amorphous,^{16–18, 31, 32} smooth and relatively homogeneous SiO₂ layer (2–5 nm) around each material is indicative of optimal synthesis parameters (Table S1). As shown in Figure 2, the core nanoparticles form agglomerated and/or aggregated chain-like structures, which is typical with gas phase synthesis of nanostructured materials. Therefore, the SiO₂-coating also encapsulates these agglomerates rather than individual core nanoparticles.¹⁸ The characteristic shape of the primary particles (CeO₂: polyhedral, Fe₂O₃: hexagonal, Ag: spherical, ZnO: rodlike), however, is unaltered by the smooth SiO₂-coating (Figure 2). The absence of segregated free SiO₂ domains or co-oxidized SiO₂-core material particles is indicative of the evenly distributed heterogeneous nucleation onto the entire core ENM particle surface.

Figure 3 illustrates the effect of increased HMDSO injection volume ($\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$), which corresponds to a specific theoretical coating thickness (TCT, upper axis in Figures 3a–d), on core particle crystal structure and size, as measured by X-ray Diffraction (XRD), as well as composite particle specific surface area (SSA), measured by N₂ adsorption (BET). It is worth noting that the TCT is estimated based on $\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$ flow ratio, assuming full HMDSO conversion to an anhydrous and perfectly dense SiO₂-coating, as well as spherical primary particles of a known diameter (d_{XRD}) and is just an indicator of how much HMDSO injection volume was used. Based on these parameters and assumptions, the TCT can be calculated as:

$$TCT = \left[\left(\frac{2MW_{\text{SiO}_2} \dot{n}_{\text{HMDSO}} \rho_{\text{core}}}{MW_{\text{core}} \dot{n}_{\text{core}} \rho_{\text{SiO}_2}} + 1 \right)^3 - 1 \right] \frac{d_{\text{XRD}}}{2} \quad (1)$$

is the HMDSO injection rate [mol/s],

$\dot{n}_{\text{core}}$ is the core material synthesis rate [mol/s],

MW_{SiO_2} is the molecular weight of SiO₂ [g/mol]

MW_{core} is the molecular weight of core material [g/mol]

ρ_{SiO_2} is the density of SiO₂ [g/cm³],

ρ_{core} is the density of Core Material [g/cm³],

and d_{XRD} is the XRD determined crystal size.

For increasing HMDSO injection molar flow rate (increased TCT), the average crystal sizes of CeO₂ (a), Fe₂O₃ (b) and ZnO (d) remained constant indicating that core-particle growth was minimal at the point of HMDSO injection. This is in agreement with previous studies for the case of SiO₂-coated TiO₂¹⁵ and Fe₂O₃.¹⁶ Therefore, for the materials used in this study (CeO₂, Fe₂O₃, ZnO), the process enabled independent control over primary particle size and SiO₂-coating thickness. In the case of Ag, crystal size decreased progressively with increased HMDSO injection volume (Figure 3c). This indicates that the growth of Ag is not terminated at the HMDSO injection point, likely attributable to the low melting point of silver. However, with an increasing $\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$ flow ratio, the core nanosilver size decreases, exhibiting further the core size tuneability as shown in previous studies.¹⁸

Assessing segregated SiO₂ formation during coating process—The SSA of SiO₂-coated CeO₂ and SiO₂-coated Fe₂O₃ remained constant when increasing the HMDSO injection volume, indicating minimal separate SiO₂ nanoparticle formation and surface roughness (Figures 3a,b). Free SiO₂ formation would lead to larger SSA (> 100 m²/g).^{15, 33}

In the cases of both Ag and ZnO (Figures 3c,d), however, an increase in SSA is observed for higher HMDSO injection volumes, which would indicate the formation of separate SiO₂ nanoparticles or rough surface coatings.³⁴ However, TEM images of SiO₂-coated Ag (Figure 2g) reveal minimal free SiO₂ nanoparticle formation. Therefore, such increase in SSA for SiO₂-coated nanosilver might also stem from (i) the large density difference between Ag and SiO₂, (ii) the decrease in Ag primary particle sizes and (iii) an increase in SiO₂ surface roughness with increased HMDSO injection molar flow rate. In the case of ZnO (Figure 3d), the formation of segregated (free) SiO₂ was also observed beyond a certain HMDSO injection volume ($\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}} > 0.2$). This is also evident from the high SSA obtained for $\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}} > 0.2$ (105 m²/g, Figure 3d) as well as TEM images (Figure 1c) of this sample. Higher HMDSO concentrations can lead to the spontaneous (homogenous) nucleation of SiO₂ due to oversaturation as well as insufficient core material surface for heterogeneous nucleation and needs to be avoided, as part of the optimization process for each ENM.³¹

In conclusion, for all ENMs investigated, SiO₂-encapsulation showed no significant effect on the crystal structure and composition of the core-material. The locations of X-ray diffraction peaks of the SiO₂-coated materials coincide with those of the bare materials (Figure 3e). Some minor differences in diffraction patterns for the case of Fe₂O₃ might be attributed to the formation of α -Fe₂O₃ ([104] plane peak which appears suppressed in the presence of SiO₂). In the case of Ag, as explained above, the SiO₂-coating leads to smaller Ag crystal sizes, broadening the characteristic diffraction peaks of metallic silver (Figure 3e). It should also be noted that the absence of crystalline SiO₂ peaks in the XRD patterns is a clear indication that the SiO₂ formed under these conditions is indeed amorphous. This is very important for the toxicological profile of SiO₂, as it is known in the literature that crystalline SiO₂ is highly toxic.³⁵

Assessing coating quality—TEM imaging was utilized to evaluate coating uniformity, coating roughness, and the formation of free silica. To confirm the absence of the free silica, the specific surface area was also measured. As described in the previous section, SiO₂ has a very high surface area (~200 m²/g) as compared to the core particles (30–50 m²/g), and therefore the presence of free silica will immediately increase the specific surface area of the coated particles compared to that of the uncoated ones. We also employed the isopropanol chemisorption as an additional qualitative method to monitor reactions that are catalyzed only by the core material, ensuring that the particles are completely coated.

X-ray Photoelectron Spectroscopy (XPS) was used to assess the coating quality quantitatively. XPS has very short penetration depth, ~5 nm, and therefore was scanning only the upper most atomic layers of the nanoparticles.^{36–38} In the case that the coating is sufficient in thickness, the only XPS peaks detected will be SiO₂ peaks. Typically, however, electrons with lower binding energy have higher kinetic energy, increasing therefore their inelastic mean free path (IMFP), giving strong signal even for coatings thicker than 5 nm.³⁹ Additionally, the peak intensity depends on the relative surface area of silica to the core material. It is therefore likely that the presence of free SiO₂ can give false results by increasing the silica signal, even for partially coated core particles. Therefore XPS was always assessed in conjunction with SSA data and the TEM imaging, in order to rule out the free SiO₂ scenario.

As previously discussed, the TEM and SSA measurements confirmed both the uniform smooth coating of the ENMs (TEM, Figure 2e–h) and the lack of surface area increase (BET, Figure 3a–d), clear indication of minimum free SiO₂ formation. Moreover, Figures 4a–d show the XPS survey spectra of the materials investigated with increasing SiO₂-coating thickness as defined by the Theoretical Coating Thickness (TCT, explained in detail

in the methods section). For each spectrum shown, core metal electron transitions as well as Si and O electron transitions are highlighted with diamond, circles and squares, respectively. With increasing TCT values the XPS survey spectra (Figures 4a–d) of the ENMs here showed a rapid decrease in the core metal electron peaks (for the peaks with the higher binding energy) and a corresponding increase in the Si electron transitions. In the case of CeO₂ and Fe₂O₃, the spectrum of SiO₂-coated CeO₂ (TCT = 3.0 nm) and SiO₂-coated Fe₂O₃ (TCT = 2.7 nm) matches that of the pure SiO₂, clearly indicating a hermetic encapsulation of the ENMs. For Ag and ZnO, the characteristic core metal electron peaks remain visible, even for the highest TCT, but the TEM shows a smooth uniform coating for both cases. This is attributed to low binding energy electrons coming from the bulk material, which still make it through the SiO₂ layer.⁴⁰ Similarly the low binding energy peaks of Ce in Figure 4a are still present even when the higher binding energy peaks have disappeared. As a result, a portion of the core electron peaks observed for small TCTs (1–2 nm) does, in fact, stem from core electrons below the SiO₂ coating layer, and not because of the lack of hermetic coating.

The XPS spectra can also be used to quantify the hermetic coating nature of ENMs.^{37, 38} Here the following elemental ratio X_{el} metric was proposed and defined as the ratio of the core material elemental concentration to the total elemental concentration (Equation 2):

$$X_{el} = \frac{Me_{core}}{Me_{core} + Si} \quad (2)$$

where Me_{core} and Si are the core-material and Silicon elemental concentration, respectively. For elements having more than one binding energy peak, the highest one is regarded. Figure 5 shows corresponding X_{el} derived from the XPS survey spectra as a function of TCT for all 4 different materials (a–d). As expected, the X_{el} decreases rapidly as a function of TCT for all materials investigated.¹⁵ It must be noted, however, that the XPS probing depth can be a few nanometers deep. For cases in which the TCT is between 0 nm and 2 nm, a fraction of electrons from the core material may still be ejected. However, XPS cannot distinguish if these electrons originate from core electrons penetrating through the SiO₂ coating or uncoated areas on the particle surface. Therefore, we expect the proposed metric to underestimate the coating efficiency of certain materials when the TCT is inferior to the core electron IMFP. However, in our case the optimum SiO₂ coating thickness is greater than 2 nm, therefore the XPS data can be used to demonstrate quantitatively the hermetic nature of SiO₂ coating for all particles.

In order to further eliminate the possibility of patchy coatings, and to cross validate the XPS coating efficiency data, isopropanol chemisorption¹⁶ on SiO₂-coated and bare CeO₂ particles was performed. Since the chemisorption of isopropanol on SiO₂ is minimal, no sharp drop in thermal conductivity (TC) is visible for the SiO₂ curve (Figure 6a). In contrast, two pronounced peaks (shaded regions) were seen for bare CeO₂ (165 °C, 283 °C). The first may be attributed to the release of adsorbed isopropanol or surface reaction products, as it corresponds fairly well to the surface isopropoxide decomposition temperature of 190 °C.⁴¹ The second peak may be attributed to other, more tightly adsorbed, isopropanol decomposition products.⁴² For an increasing SiO₂ TCT up to 3 nm, the peaks almost disappear and only very small bands are visible. Even at TCT of 1.3 nm, ENM samples show no significant reduction in TC at the expected desorption temperatures, indicating minimal bare CeO₂ surface.

Furthermore, the coating efficiency on the core ZnO nanoparticles was evaluated by monitoring methylene blue degradation in aqueous solution in the presence of ZnO nanoparticles and UV light (254 nm). Uncoated ZnO nanoparticles are photocatalytic

nanoparticles that can degrade organic dyes, such as methylene blue.⁴³ Such methylene blue degradation can be seen in Figure 6b, where the normalized methylene blue concentration (C/C_0) is plotted as a function of time in the presence of uncoated (circles) and SiO₂-coated ZnO nanoparticles (squares) and UV light (254 nm). In the case of the uncoated ZnO nanoparticles, the methylene blue concentration decreases over time, indicating its degradation because of the photocatalytic ZnO surface. However, in the presence of the SiO₂-coated ZnO nanoparticles the methylene blue concentration in solution remains rather constant over time, indicating the minimal photocatalytic activity of those nanoparticles. This further verifies that the ZnO core nanoparticles are fully coated. Both the isopropanol chemisorption and the photocatalytic activity data results are in agreement with the XPS data, a clear indication that the proposed use of XPS spectra as a means to quantify the coating quality is valid.

In conclusion, the aforementioned results demonstrate the ability of this scalable process to hermetically coat a wide range of ENMs. Moreover, the particular combination of characterization methods applied to evaluating the coatings (BET, TEM, XPS and isopropanol chemisorption) enables an accurate qualitative and quantitative indication of the coating efficiency.

While this study focuses on the environmental health and safety implications of the SiO₂-coated ENMs, the resulting coated particles have great potential for many other applications. It is worth mentioning that SiO₂ surface can be easily further functionalized to facilitate dispersibility in liquids and other possible applications in nanomedicine.

Effect on SiO₂ coatings on core material functional properties—It has been shown in other recently published studies by the authors that a nanothin layer of SiO₂ coating can maintain other specific optical,¹⁵ plasmonic,¹⁸ phosphorescent,³³ and magnetic¹⁶ properties of the core ENM. This is essential for the whole safer formulation concept, as the target is to maintain the desirable inherent core ENM specific functional properties and simultaneously exploit the surface properties of SiO₂ as well as inhibit any toxicological outcomes. However, this approach cannot be considered a “swiss army knife” for all ENMs and all applications. For example, coating the surface with amorphous SiO₂ will likely eliminate the desirable catalytic properties of CeO₂, or bacteriacidal properties of Ag associated with their specific chemistries. On the contrary, coating Ag surface with SiO₂ used for plasmonic applications while reducing ion leaching and toxicological outcomes may be beneficial.

In this study, we selected to showcase the minimal effect of SiO₂ coating on optical properties of ZnO. Both the coated and uncoated ZnO ENMs demonstrated a similar ultraviolet A (UVA) absorbance peak around 370nm (supplement, Figure S-1), clear indication of no change on the optical properties. Therefore, the SiO₂ coated ZnO prepared with this high yield scalable nanomanufacturing process makes it ideal for use in cosmetics and sunscreens. It is worth making the point here, that this proposed approach for a safer formulation of ZnO is preferable to other proposed safer formulation approaches such as Fe doping ZnO ENMs to reduce Zn solubility, as such doping ultimately alters the optical properties of ZnO.¹³ Thus, such hermetically SiO₂-coated ZnO nanoparticles could be used in specific applications as UV-filters (e.g. in sunscreens) without compromising their desired performance while reducing their toxicity, as it will be demonstrated in sections below.

Effect of SiO₂ coating on ENM-bio Interactions

Characterization of ENM behaviour when suspended in physiological media: The hydrodynamic diameter and zeta potential of all uncoated and SiO₂-coated ENMs under study dispersed in both de-ionized (DI) H₂O, and in physiological media commonly used for

cellular bio-assays, are summarized in Table 1 (see method section for dispersion protocol details). The zeta-potential measured for all four SiO₂ coated ENMs dispersed in DI H₂O were strongly negative, and comparable to that of bare SiO₂.⁴⁴ These values were distinct from the strongly positive values, measured for their uncoated counterparts with the exception of Ag (Table 1, supplemental Figure S1).⁴⁴ Furthermore, All SiO₂-coated ENMs exhibit similar agglomeration in deionized (DI) water and match that of pure SiO₂ particles of same primary particle size, and were stable over the course of 24 hours. This is another confirmation for the successful encapsulation of ENMs by SiO₂.

In addition, in physiologic media used in cellular bio-assays, protein adsorption to the nanoparticle surface strongly influenced agglomeration state and is reflected in the measured hydrodynamic diameter and zeta-potential (Table 1, Figure S1). This is in agreement with previously reported studies.⁴⁴ The high hydrodynamic diameter of the Fe₂O₃ nanoparticles in biological medium are likely attributable to the interparticle magnetic attractive forces.^{45–47}

ENM-cellular interactions: The biologically inert nature of amorphous SiO₂ ENMs has been reported in a number of recently published *in vitro* studies.^{48, 49} However conflicting evidence has also been reported, identifying silanol groups on the surface of amorphous SiO₂ ENMs as key mediators that can induce hemolysis⁵⁰ as well as alveolar epithelial cell toxicity at high doses.⁵¹ *In vivo*, the anti-inflammatory nature of amorphous SiO₂ is documented in a number of studies,^{52, 53} providing supportive evidence for our “safer-formulation” approach. Sayes et al. reported short-term acute aerosol exposures produced no significant pulmonary inflammatory, genotoxic, or adverse lung histopathological effects in rats, even at very high doses up to 86 mg/m³.⁵³

It is worth mentioning the differential pulmonary response associated with crystalline, colloidal or amorphous silica dusts.³⁵ It was shown that amorphous SiO₂ induced a low and transient pulmonary inflammatory response in rats, whereas crystalline silica induced large and persistent pulmonary inflammatory responses.³⁵ Synthetic amorphous silica, including colloidal, precipitated, and as in our case, fumed silica, is not involved in progressive fibrosis of the lung.^{54, 55} However, high doses of amorphous silica may result in acute pulmonary inflammatory responses in exposed animals.⁵⁶ Surface impurities on the surface of amorphous SiO₂ particles may lead to the formation of highly toxic silanes with implications for cellular viability. However, there are no impurities or silanes on the surface of the flame generated SiO₂-coated particles as shown by our XPS data, which is also in agreement with thermogravimetric analyses of the surface chemistry of fumed silica particles previously reported in the literature.⁵⁷ While more research is needed to clearly identify and characterize the health risks associated with respiratory exposure to amorphous fumed SiO₂, current evidence suggest those risks are minimal.⁵²

The role of the SiO₂ surface coatings on ENM-cell interactions, and its effect on biological properties, was assessed in this study using human alveolar basal epithelial A549 cells and two different commonly used bio-assays (LDH absorbance, a measure of cytotoxicity, and MTT absorbance, a measure of cell viability, see method section for details). Figures 7a and 7b report cell viability for A549 cells exposed to varied doses of a liquid ENM dispersions (0 – 50 µg/ml) after 24 hours exposure, for both bare and SiO₂-coated ENMs.

As shown in Figure 7, at the doses investigated, exposure to pure SiO₂ resulted in no significant decrease in cell viability as measured by our LDH and MTT assays (Figure 7a, b). Additionally, SiO₂-coated ZnO nanoparticles show reduced toxicity than their bare counterparts, further indication for the hermetic nature of the coating (Figure 7a, b).

Recently published studies also report that amorphous SiO₂ is relatively insoluble in both water and in various cell culture media,⁴⁹ suggesting the ENMs under study remain encapsulated in a layer of relatively inert SiO₂.

ENM-cellular interactions: Reduction of toxicity for the case of ZnO: As shown in Figure 6, exposure to uncoated ZnO nanoparticles resulted in significant decreases in cell viability as measured by our LDH and MTT assays (Figure 7a, b). Our results are consistent with previously reported studies demonstrating that nano sized ZnO particles exert toxicity in mammalian cells, as measured by lysosomal damage, mitochondrial perturbation, reactive oxygen species generation, induction of pro-inflammatory cytokine release, and cell death.^{58, 59} In addition, recent studies investigating the effects of nano-sized ZnO *in vivo* report substantial lung inflammatory responses including LDH release and recruitment of polymorphonuclear monocytes (PMNs) in rats following intratracheal instillation.^{12, 60} As shown in Figures 7a and b, the SiO₂ encapsulation effectively increased cell viability (p value < 0.05), especially at higher administered doses. This protective effect is likely due to inhibition of Zn ion leaching into the cellular environment,¹² thereby minimizing cytotoxicity. Therefore, the dissolution of ZnO is inhibited by the presence of the hermetic SiO₂ coating, and the nanoparticle toxicity is reduced.

ENM-cellular interactions: Reduction of toxicity for the case of CeO₂: No toxicity was observed *in vitro* for either bare CeO₂, or the SiO₂-coated CeO₂ with the A549 cell line (Figure 7a, b). Therefore, the reduction of toxicity *in vitro* could not be made for the case of SiO₂ coated CeO₂ given the fact that the uncoated CeO₂ ENMs were found with minimal toxicity. The authors recognize that additional *in vitro* assays, testing various end points across different cell lines, may improve sensitivity for detecting toxicity *in vitro* beyond the LDH and MTT assays reported here for A549 cells. However, several studies investigating the *in vitro* toxicity of CeO₂ across a broad range of cell lines from various tissues, and examining a variety of biological endpoints have already been reported in the literature, and found CeO₂ to be relatively non-toxic across the board.^{49, 58, 59} Kroll et al. reported CeO₂ ENMs do not induce cytotoxicity measured by LDH and MTT assays, and do not increase oxidative stress or generation of reactive oxygen species as measured by the DCF assay. These results were consistent across multiple cell lines including A549 cells, heterogeneous human epithelial colorectal adenocarcinoma cells (Caco-2), adenocarcinoma derived human lung epithelial cells (Calu-3), canine kidney epithelial cells (MDCK, MDCK II), mouse derived fibroblast cells (NIH-3T3), rat kidney cells (NRK52E), rat alveolar macrophages (RAW 264.7), and rat lung epithelial-T-antigen negative cells (RLE-6TN).⁵⁸ Similarly, Zhang et al. reported no toxicity and minimal oxidative stress for multiple cell lines exposed to CeO₂ ENMs (human bronchial epithelial cells -BEAS-2B, and rat alveolar macrophages - RAW 264.7).⁴⁹ Xia et al. investigated inflammatory cytokine release in response to CeO₂ exposure *in vitro*, and reported no increase in TNF- α production after the ENMs are internalized by rat alveolar macrophages (RAW 264.7), and no IL-8 production after they are internalized by non-cancerous human bronchial epithelial cells (BEAS-2B).⁵⁹

Despite the compelling *in-vitro* evidence presented above for the minimal toxicological properties of uncoated CeO₂, *in vitro* assays only provide limited information for realistic exposure risk assessment and are not necessarily in agreement with *in vivo* animal studies. Whole-animal *in vivo* inhalation studies are the “gold standard” for pulmonary toxicology due to their physiologically relevant exposure mechanisms, and discrepancies with reported toxicity results *in vitro* highlight major concerns about the predictive capacity for cellular nanotoxicology studies.

In the case of CeO₂, several recent studies report large discrepancies between *in vitro* models and more realistic *in vivo* models for CeO₂ ENMs. Recent instillation studies

measuring pulmonary toxicity in rats have reported significant pulmonary toxicity for CeO₂ ENMs.^{61, 62}

In another recently published study performed by our group, Sprague Dawley rats were exposed via inhalation to either CeO₂ or SiO₂ encapsulated CeO₂ ENMs in order to assess the toxicological impact of SiO₂ encapsulation *in vivo*.⁶³ Animals exposed to bare CeO₂ exhibited eight times greater inflammation, as measured by influx of polymorphonuclear leukocytes in the bronchoalveolar lavage fluid (BALF), than those exposed to the SiO₂-coated ENMs. Additionally, reported LDH release was significantly elevated in the BALF of rats exposed to bare CeO₂ compared with those exposed to SiO₂-coated CeO₂. For both outcomes measured, the SiO₂-encapsulated CeO₂ ENMs induced minimal pulmonary toxicity and was comparable to the particle-free control, thereby supporting the proposed safer formulations concept (Supplemental Figure S-4).

ENM-cellular interactions: Fe₂O₃ and Ag: Similar to the bare CeO₂, SiO₂, and SiO₂ coated ENMs, no toxicity was observed *in vitro* for either the bare Fe₂O₃ or the Ag with the A549 cell line (Figures 7a, b). Therefore, making any conclusion based solely on *in vitro* evidence for the SiO₂ coated materials is not possible. These findings for minimal *in vitro* toxicity in mammalian epithelial cells are consistent with results reported in the literature for pure Fe₂O₃, and Ag ENMs at the employed nanoparticle sizes and measured concentrations across various cell lines and measured biological endpoints.^{49, 58, 59, 64}

It is again important to note that *in vitro* assays only provide limited information for realistic exposure risk assessment, and several recent studies report large discrepancies between *in vitro* models and more realistic *in vivo* models.^{13, 17–19, 65} For example, while Fe₂O₃ exhibited minimal toxicity on A549 cells evaluated in this study, a recent *in-vivo* study by the authors, suggests that moderate and acute exposure to Fe₂O₃ nanoparticles can cause lung injury and inflammation in Sprague Dawley rats.²⁸ With regards to Ag, limited data is available for *in vivo* pulmonary toxicity. One group reported inhalation exposure to 18nm silver particles resulted in decreased lung function (including tidal volume, minute volume and peak inspiration flow), as well as inflammatory lesions in the lung morphology and increased inflammatory markers during a 90 day rat study.⁶⁶ Considering the relatively mild effects reported for SiO₂ *in vivo*, we expect the SiO₂ coated Fe₂O₃ and Ag to demonstrate decreased toxicity *in vivo* as well, similar to the protective effects observed for ZnO and CeO₂. Again, conflicting evidence between *in vitro* and *in vivo* studies highlight limitations in current *in vitro* protocols at predicting toxicity, and emphasize the need for biologically complex and physiologically relevant exposure systems *in vitro*.^{61–63}

The authors recognize the need to further study *in vivo* the effect of SiO₂ coating on these ENMs, and plans are underway to generate *in vivo* data on the effect of SiO₂ coating on their toxicity. However, our compelling *in vitro* and *in vivo* evidence presented here for the case of SiO₂ coated CeO₂ and ZnO as well as the data on the biological inert nature of amorphous SiO₂ reported in the literature suggests hermetic encapsulation with amorphous SiO₂ is an appropriate safer formulation approach.

CONCLUSIONS

In summary, we showcase the effectiveness of a novel safer formulation concept for flame-generated ENMs by applying it to a comprehensive and industry-relevant nanopanel. The versatile and scalable process described here enables the one step, inflight, hermetic encapsulation of potentially toxic ENMs by a nanothin SiO₂ layer. An effective technique for quantitatively assessing the coating efficiency of the SiO₂-encapsulation process based on XPS was also presented and validated. Finally, we provide valuable *in-vitro* and *in-vivo*

toxicological evidence for the ability of the proposed concept to reduce the toxicological profile of the ENMs, while maintaining the functional properties of the core materials. The optimum theoretical SiO₂ coating thickness for each nanoparticle type sufficient to render core materials hermetically coated and therefore non-toxic is greater than 2 nm. The TEM imaging confirms an approximate 3nm coating thickness, but it is semi-quantitative. The described concept bears great promise for large-scale industrial application as a means of effectively inhibiting nanoparticle toxicity.

MATERIALS AND METHODS

ENM Synthesis

The synthesis of the bare and SiO₂-coated nanoparticles was performed using the Flame Spray Pyrolysis (FSP) based Harvard Versatile Engineered Nanomaterial Generation system (VENGES^{27, 28}). In brief, Fe₂O₃, Ag, ZnO and CeO₂ and core particles were synthesized by combustion of organometallic precursors listed in Table 2. Precursor solutions were fed to the FSP nozzle through a stainless steel capillary at 5 ml/min, dispersed by 5 l/min O₂ (Air Gas, purity >99%, pressure drop at nozzle tip: $p_{\text{drop}} = 2$ bar) and combusted to form the desired nanomaterials. A premixed stoichiometric methane-oxygen (1.5 l/min, 3.2 l/min) supporting flame was used along with 40 l/min O₂ (Air Gas, purity >99%) sheath gas.

The FSP burner was enclosed with a 200 mm quartz glass tube (i.d. 45 mm). On top of this tube, HMDSO (Sigma Aldrich) vapor was swirl-injected through a torus ring with 16 equidistant and of equal size ($d_{\text{inner}} = 0.6$ mm) openings. A total gas flow of 16 l/min, consisting of N₂ carrying HMDSO vapor ($V_{\text{N}_2, \text{coat}}$) and pure N₂ ($V_{\text{N}_2, \text{swirl}}$) for mixing, was injected through the torus ring jets. The torus ring jet injection angles were 20° in the downstream direction, in order to avoid stagnation flow, and 10° away from the centerline, in order to induce the necessary mixing swirl.⁶⁷ The reactor was terminated by a 200–400 mm quartz tube. HMDSO vapor was obtained by bubbling N₂ ($V_{\text{N}_2, \text{coat}}$) gas through liquid HMDSO (500 ml), maintained at a controlled temperature using a temperature-controlled water bath. At saturated conditions, the HMDSO content within the $\text{N}_{2, \text{coat}}$ flow is determined by the HMDSO partial vapor pressure, which is a function of the bubbler temperature and can be estimated using the Antoine equation.³¹ We define the molar ratio of injected HMDSO to synthesized core particles as $\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$. A theoretical coating thickness (TCT) for the individual particles was estimated based on $\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$, assuming full HMDSO conversion to SiO₂ coatings (no separate SiO₂ formation)³¹ as well as monodisperse spherical primary particles of a known diameter (d_{XRD}).

The bare nanoparticles were synthesized at identical conditions, in the absence, however, of the HMDSO vapor. For the purpose of evaluating ENM-bio interactions, bare Ag was cooxidized with SiO₂, in order to provide sufficient control over Ag primary particle size.¹⁸ After synthesis, particles were collected for physico-chemical characterization and *in-vitro* toxicity assessments on a water-cooled glass fiber filter (Whatman) located 800 mm above the reactor.^{27, 28} A schematic of the coating reactor used is provided in Figure 1.

PHYSICOCHEMICAL CHARACTERIZATION

STRUCTURE, COMPOSITION, MORPHOLOGY: X-ray Diffraction (XRD) patterns for ZnO and CeO₂ were obtained using a Scintag XDS2000 powder diffractometer (Cu K α ($\lambda = 0.154$ nm), –40 kV, 40 mA, stepsize = 0.02°). The crystal size was determined by applying the Sherrer Shape Equation to the Gaussian fit of the major diffraction peak. Fe₂O₃ and Ag XRD patterns were obtained using a Bruker D8 diffractometer (Cu K α ($\lambda = 0.154$ nm), –40 kV, 40 mA, stepsize = 0.02°). The crystal size was determined by Rietveld analysis and the crystal size of the major diffraction peak is reported. The Brunauer-

Emmett-Teller (BET) powder-specific surface area (SSA) of all samples was measured by N₂ adsorption at 77 K (Micromeritics TriStar), after sample degassing for 1 h at 150 °C in nitrogen. ENMs were also deposited onto carbon grids for TEM imaging (TEM: Libra 120).

COATING EFFICIENCY CHARACTERIZATION: Highly surface sensitive X-Ray Photoelectron Spectroscopy (XPS) (ESCA SSX-100, X-ray source: monochromatic Al K α , 10 kV, 10 mA, detector: hemispherical electron energy spectrometer, spot size: 600 μ m) was used to assess SiO₂ coating efficiency. Survey scans (binding energy range: 0 – 1100 eV, pass energy: 100 eV, step size: 0.65 eV), were used for surface elemental quantification. All XPS spectra were calibrated using the C1s hydrocarbon contamination peak (BE: 284.6 eV). Atomic concentrations were determined using CASA XPS software and respective sensitivity factors for relevant elements.

XPS results were supported by isopropanol chemisorption on SiO₂ coated and bare CeO₂ particles following methods described previously.^{19, 32} In brief, 50mg of sample were heated at 10°C/min to 400°C and kept there for 30 minutes in a 20%O₂ atmosphere (50ml/min) to remove surface water and carbonaceous species. Samples were then cooled to 110°C and flushed with He for 10 minutes. 2-Propanol (2000ppm; Messer) in N₂ (50ml/min) was then introduced at 110°C for 30 minutes to minimize physisorption and optimize chemisorption of the 2-propanol. The gas atmosphere was again changed to He, and after 10 minutes the sample was heated at 10°C/min to 500°C. During this final heating stage the thermal conductivity of off-gases was monitored by an Autochem II 2920 thermal conductivity detector (Micromeritics, Georgia).

Additional photocatalytic experiments were conducted for ZnO and SiO₂-coated ZnO nanoparticles. In brief, 18 mg of the ZnO or SiO₂-coated ZnO nanoparticles were added in 100 mL of a 10 ppm methylene blue aqueous solution. The suspension was sonicated for 20 s with a Branson Sonifier S-450A (Branson Ultrasonics, Danbury, CT) fitted with a 3 inch cup horn (maximum power output of 400 W at 60 Hz, continuous mode, output level 3), and left in the dark for 20 min. The suspension then was irradiated with an 8 W 254 nm UV lamp for one hour, and 1ml samples were collected after 10, 20, 45, and 60 mins. The samples were placed in 2 ml centrifuge tubes and were spun at 20,000 rpm. The supernatant was removed and the adsorption spectrum was measured with the Synergy 2 Multi-Mode Microplate Reader (Bio-tek Instruments, Winooski, VT) from 550 nm to 700 nm. The intensity of three wavelengths (615, 630 and 660 nm) was measured with reference at 700 nm. The data were plotted normalized to the initial concentration (C_i/C_0) where C_0 is the concentration at $t=0$ and C_i is the concentration at a given time. The data were fit with an exponential decay $C_i/C_0 = \text{Exp}(-t/\tau_0)$.

ENM DISPERSAL AND CHARACTERIZATION IN LIQUID—ENM dispersion was performed using a protocol previously described,⁴⁴ and included the calibration of sonication equipment and standardized reporting of sonication energy. We identified for each ENM the material-specific critical sonication energy required to achieve monodisperse solutions at the lowest agglomeration state as measured by dynamic light scattering (DLS). Cup horn sonication was performed in deionized water (DI H₂O) to minimize reactive oxygen species generation via sonolysis, to minimize ionic strength and specific conductance, and hence particle interactions, during sonication, and to avoid denaturation of proteins in the final cell delivery media. Stock solutions in DI H₂O were then diluted to desired concentrations (0, 6.25, 12.5, 25, 50, or 100 μ g/ml) in F-12K cell culture media supplemented with 3% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 10 mM HEPES (F-12K/3%FBS) and vortexed for 30 seconds. ENM suspensions in F-12K/3% FBS were then characterized for hydrodynamic diameter (dH), polydispersity index (PDI), zeta potential (ζ), and specific conductance (σ) by dynamic

light scattering (DLS) using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). pH was also measured using a VWR sympHony pH meter (VWR International, Radnor, PA, USA). Following comprehensive characterization, dispersions were applied to cells for toxicological evaluation.

The critical delivered sonication energy (DSE_{cr}) was determined for each ENM as follows. ENMs were dispersed at 1 mg/ml in 10 ml of solute in 50 ml, 24 mm diameter conical polyethylene tubes, by sonication with a Branson Sonifier S-450A (Branson Ultrasonics, Danbury, CT) fitted with a 3 inch cup horn (maximum power output of 400 W at 60 Hz, continuous mode, output level 3). The tube was immersed so that the sample liquid aligned with the level of water in the cup. The system was calibrated by the calorimetric calibration method previously described,^{44, 68} whereby the power delivered to the sample was determined to be 1.75 W. Dispersions were analyzed by DLS, and hydrodynamic diameter as a function of DSE exhibiting asymptotic de-agglomeration trends were derived for each ENM. From each curve, an ENM-specific DSE_{cr} was estimated by determining the DSE value at which the dispersed ENMs were within 10% of their observed minimum dH value (theoretical dH at $DSE = +\infty$).

Absorbance of ZnO and SiO₂-coated ZnO particles dispersed in DI H₂O was measured at 5nm intervals across a spectrum of wavelengths ranging from 315–800nm using a Spectramax Multi-Mode Microplate Reader (Molecular Devices, USA).

IN-VITRO TOXICITY

Cell lines: Human alveolar basal epithelial A549 cells were cultured in F-12/K supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 10 mM HEPES. All incubations were performed at 37°C/5% CO₂ unless otherwise specified. Assays were repeated three times for each ENM. F-12/K cell culture media, Penicillin/Streptomycin, HEPES and BSA were obtained from Sigma Aldrich (St. Louis, MO), and FBS and heat-inactivated FBS were obtained from Atlanta Biologicals (Atlanta, GA).

Bio-assays: Cellular metabolic activity and cytotoxicity were measured via the 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay and lactate dehydrogenase (LDH) assay respectively according to the standard protocols. In short, cells were seeded in 96-well microtiter plates (Corning Inc., New York, NY) at a concentration of 1×10^5 cells per well. The next day, culture media was replaced with 100 μ l of ENM dispersions in either F-12K/3%FBS at various concentrations, and incubated for 24 hours. Following 24 hours of exposure, 50 μ l of supernatant was collected and transferred to fresh 96-well plates and 100 μ l of LDH-reagent was applied. These plates were then left for 30 minutes in the dark at room temperature. During this incubation period, the presence of LDH released by dead or dying cells into the supernatant due to plasma membrane damage was identified by the reduction of the reagent tetrazolium salt, measureable by absorbance. Plates were read immediately following incubation at 490nm with a Synergy 2 Multi-Mode Microplate Reader (Bio-tek Instruments, Winooski, VT).

Exposure media was removed from the plates still containing cells, replaced with fresh medium containing MTT reagent, and incubated for an additional 4 hours. Vital cells integrated the dye and transformed the yellow tetrazolium salt (MTT) into purple formazan crystals, an indicator of active metabolism. Following the 4-hour incubation period, 100 μ l of MTT solubilization solution (10% Triton X-100 with 0.1 N HCl in anhydrous isopropanol) were added to the wells to stop the reaction. Plates were placed on an orbital plate shaker for 30 minutes to dissolve the formazan crystals, and then centrifuged at 250 rpm for 10 minutes to ensure that particles or dying cells were removed from the solution.

100 µl of the supernatant was then removed and placed in a fresh 96 well plate to be read at 570nm with a Synergy 2 Multi-Mode Microplate Reader (Bio-tek Instruments, Winooski, VT). MTT Cell Proliferation kits and LDH Cytotoxicity Detection kits were obtained from Roche Applied Science (Indianapolis, IN). All experiments were conducted in triplicate for each ENM.

CALCULATIONS AND STATISTICS: Absorbance values measured for LDH and MTT *in-vitro* assays were normalized with negative control (particle free media) and positive control (Triton X) absorbance values, to a metric of cell viability (%) using the following equation:

$$\text{cell viability}(\%) = \frac{\text{Sample Absorbance} - \text{Negative Control Absorbance}}{\text{Positive Control Absorbance} - \text{Negative Control Absorbance}}$$

Statistics: Variance of toxicity results for each ENM was tested by post hoc one-way ANOVA with significance set at p 0.05. One-tailed unpaired student T test was used for significance testing, with significance set at p 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

ENM synthesis and SiO₂ coating fundamentals. (a) A schematic of the coating reactor highlighting the most important elements. Core ENMs are formed through the combustion of organometallic compounds dissolved in high enthalpy solvents. The HMDSO vapor is injected into the particulate flow in an in-line SiO₂-coating reactor. (b) The mechanism of the coating and the possible results. The high temperatures obtained in the core particle synthesis provide the necessary energy for the HMDSO conversion to SiO₂ vapor. Under optimal SiO₂ vapor supersaturation conditions, the SiO₂ vapor nucleates heterogeneously onto the core ENM surface, thereby forming a desired SiO₂ coating layer of a controlled thickness (Case I). The ENM specific optimization of the coating reactor temperature and mixing profiles is required in order to prevent: (1) surface reactions between the core material and the SiO₂ vapor (alloying) (Case II) (2) homogenous SiO₂ nucleation which may result in segregated SiO₂ domains and/or rough surface coatings (Case III); and (3) inhomogeneous SiO₂ coatings (patchy coatings) or free silica (case IV). Above-discussed cases are representatively shown for the coating of ZnO: smooth coating (c), alloying (forming of zinc silicates, zinc oxide and silica) (d), rough coating (e), and free (segregated) silica formation (f).

Figure 2. TEM images of bare (a – d) and SiO₂-coated (e – h) CeO₂, Fe₂O₃, Ag, and ZnO. SiO₂-coating layer (2 – 5 nm) appears as lighter contrast contour around stronger contrast core particles.

Figure 3. SSA [m²/g] (right axis) and Crystal Size [nm] (left axis) of SiO₂-coated CeO₂ (a), Fe₂O₃ (b), Ag (c), and ZnO (d) as a function of core particle to HMDSO molar ratio ($\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{Core}}$) (lower axis) and theoretical coating thickness (TCT) (upper axis). XRD patterns of bare (black) and SiO₂-coated (red) ENMs (e).

Figure 4. Coating Efficiency: XPS survey spectra of bare and SiO₂-coated CeO₂ (a), Fe₂O₃ (b), Ag (c), and ZnO (d). Spectra shown for increasing TCTs. Pure SiO₂ (upper most spectrum) included for reference purposes.

Figure 5. XPS surface atomic concentration ratio $[Me_{core}/(Me_{core} + Si)]$ for bare and SiO₂-coated ENMs as a function of TCT [nm].

Figure 6.

Off-gas thermal conductivity (TC) as a function of temperature during isopropanol desorption from pure SiO₂, SiO₂-coated CeO₂ and bare CeO₂ (a). Concentration of methylene blue in aqueous solutions measured by optical absorption spectra in the presence of ZnO and SiO₂-coated ZnO nanoparticles under UV irradiation (254 nm) for varying time (b).

Figure 7.

Cytotoxicity as measured by LDH (a) and MTT (b) absorbance after 24 h exposures to 0 – 50 (left to right) in A549 cells. # mean ug/ml bare and SiO₂-coated Ag, CeO₂, Fe₂O₃, ZnO and pure SiO₂ values for each dose are not all equal (ANOVA) for $p < 0.05$; *significantly different from immediate right neighbor for $p < 0.05$

Table 1

Summary of ENMs (bare and SiO₂-coated) used to evaluate ENM-cellular interactions.

Material	Theoretical SiO ₂ [wt%]	TCT [nm]	d _{XRD,core}	SSA [m ² /g]	d _H (nm)	ζ (mV)
SiO ₂	100	N/A	19 (d _{BET})	147	DI H ₂ O	-28.4±6.90
					F12K/3% FBS	-9.73±2.84
CeO ₂	0	0	28	32.8	DI H ₂ O	37.8±1.85
					F12K/3% FBS	-12.5±0.289
SiO ₂ -coated CeO ₂	20	3	31	37	DI H ₂ O	-38.4±1.86
					F12K/3% FBS	-11.6±0.451
Fe ₂ O ₃	0	N/A	19.6	42	DI H ₂ O	40.7±17.6
					F12K/3% FBS	-10.1±1.45
SiO ₂ -coated Fe ₂ O ₃	33	2.7	21.3	49	DI H ₂ O	-53.6±19.9
					F12K/3% FBS	-11.4±1.54
Ag	50	N/A	20.4	57	DI H ₂ O	-19.5±1.31
					F12K/3% FBS	-11±0.551
SiO ₂ -coated Ag	10	1.8	28	29	DI H ₂ O	-18.5±1.5
					F12K/3% FBS	-11.6±0.153
ZnO	0	N/A	28	36	DI H ₂ O	23.0±0.462
					F12K/3% FBS	-9.89±2.05
SiO ₂ -coated ZnO	23	2.5	27.0	66	DI H ₂ O	-15.9±1.27
					F12K/3% FBS	-11.1±0.814

Note: The high hydrodynamic diameter of the Fe₂O₃ nanoparticles in biological medium are likely attributable to the interparticle magnetic attractive forces.⁴⁵⁻⁴⁷

Table 2

Relevant synthesis parameters for particles presented in Figure 1c–f. Parameters which are not listed were not changed and are as described in the method section.

TEM Figures	Precursor	Solvent	Precursor Molarity	$\bar{n}_{\text{HMDSO}}/\bar{n}_{\text{Core}}$
Figure 1c	Zinc naphthenate, 65% mineral spirits (10% Zn) (STREM)	ethanol (95%, Sigma Aldrich)	0.5	0.2
Figure 1d	Zinc naphthenate, 65% mineral spirits (10% Zn) (STREM)	xylene (>99%, Sigma Aldrich)	0.5	0.2
Figure 1e,f	Zinc naphthenate, 65% mineral spirits (10% Zn) (STREM)	ethanol (95%, Sigma Aldrich)	0.5	0.41