A Safer Formulation Concept for Flame-generated Engineered Nanomaterials

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ABSTRACT

Engineering less toxic nanomaterials that maintain valuable functional properties is crucial to the sustainability of the nanotech industry. Herein, a safer formulation concept for flame-generated nanomaterials based on the encapsulation of potentially toxic nanomaterials by a biologically inert nanothin amorphous SiO_2 layer was explored. The core-shell particles maintain specific properties of their core material but exhibit surface properties of their SiO_2 shell. The SiO_2-coating was performed using a previously developed flame spray pyrolysis (FSP) based Versatile Engineered Nanomaterial Generation System (VENGES) in which core ENMs are coated in-flight by the swirl injection of hexamethyldisoloxane (HMDSO). The versatility of the proposed SiO_2-coating process was demonstrated on a number of ENMs (CeO_2, Fe_2O_3, ZnO, Ag) marked by their prevalence in consumer products as well as their range in toxicity. Furthermore, the fundamentals of the SiO_2-coating process were investigated for each ENM. State of the art analytical methods were used to assess the core material structure, composition and morphology (XRD, BET, and TEM). The coating efficiency for each ENM was also assessed by XPS and Chemisorption. Moreover, the effect of the SiO_2-coating on the mobility and aggregation potential of ENMs in water, biological media and air were explored using state of the art analytical methods and instrumentation (DLS, SMPS). Finally, the effect of the SiO_2 coating on the particle-cellular interactions was investigated using a variety of cellular lines (A459, THP1, and human primary monocyte-derived macrophages) and cellular toxicological assays (MTT, LDH and Live/Dead). Results confirmed that the SiO_2 coating of ENMs can significantly reduce the toxicity of the the core material. This method can be scaled up by the nanotechnology industry and used to mediate environmental healh and safety issues related to flame generated ENMs.

Keywords: ENM, nanotoxicology, FSP, core-shell nanoparticles, SiO_2 coating, mitigating toxicity

1 INTRODUCTION

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. Preliminary evidence demonstrates the potential for ENMs to cause adverse biological effects [4]. The potential of nanoparticles and nanofibers to translocate through the air-blood barriers, and thus to reach the pulmonary connective tissues, lymphatic system, or even to reach the circulating blood and thus have access to other critical organs, is of concern. Nano-sized particles (NPs) may enter cells and be more biologically active than their micron-sized counterparts due to their small size and large surface to volume ratio.

While significant research has been directed toward understanding nano-bio interactions as well as fundamental rules of nanotoxicology, research toward devising safer ENM formulation concepts that can readily be adopted by the nanotechnology industry is very sparse. Here, the investigators have developed a "safer formulation concept" for one of the largest ENM families by volume of production [5], 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 [6, 7]. In this study, a recently developed, flame spray pyrolysis ENM synthesis platform (VENGES) at Harvard University [8, 9] was modified to allow for the coating of ENMs with a nano-thin layer of amorphous SiO_2. Amorphous SiO_2 is considered a biologically and environmentally inert material, often used as a negative control material in in-vitro ENM screening assays [10, 11]. Such nanothin SiO_2 is therefore ideal to shield otherwise potentially toxic core-materials from any interactions with environmental and biological media. The

"core-shell" ENMs exhibit the surface properties of their shell while preserving certain important bulk properties (i.e. optical, magnetic properties) of their core materials [12-14].

The primary goal of this study was to demonstrate the versatility of this coating process by applying it to a comprehensive and industry-relevant nanopanel of ENMs (CeO_2, Fe_2O_3, ZnO, Ag) marked by their range in toxicity as well as their prevalence in consumer products. This included investigating (1) the effect of the SiO_2-coating on core material structure, compostion and morphology (XRD, BET, and TEM), as well as on the mobility and aggregation of SiO2-cated and uncoated ENMs in DI-water, biological media (DLS) and air (SMPS), and (2) the coating efficiency (XPS, Chemisorption) of the process for each ENM (XPS and Isopropanol Chemisorption). The secondary goal was to provide in-vitro toxicological evidence for the safety of this novel formulation.

2 RESULTS AND DISCUSSION

2.1 SiO_2 Encapsulation Strategy

Our approach to the synthesis and SiO_2-encapsulation of ENMs is illustrated in Figure 1. Core-ENMs are synthesized by the flame spray pyrolysis (FSP) of precursor solutions containging organometallic compounds dissolved in high enthalpy solvents. The freshly formed core-ENMs are convected to an in line SiO_2 coating reactor, where they are encapsulated with a nanothin amorphous SiO_2 layer by the swirl-injection of HMDSO-laden N_2. The gas phase HMDSO conversion reaction to SiO_2 results from the high temperatures generated by the combustion of the high enthalpy solvents used in the core particle formation. Under optimal supersaturation conditions, defined by the temperature profile in the reactor's coating zone as well as the HMDSO injection mass, the SiO_2 vapor condenses onto the surface of the core ENMs creating the desired nanothin coating layer of SiO_2 (heterogeneous nucleation).

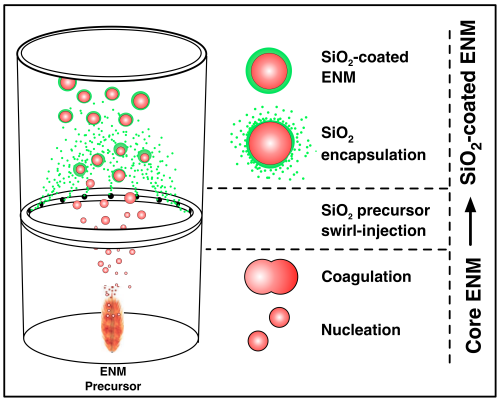

Figure 1: Approach to SiO_2-coated ENM synthesis.

Figure 2 shows transmission microscope images of uncoated and SiO_2-coated CeO_2, Fe_2O_3, Ag and ZnO synthesized by the aforementioned process. Due to agglomeration prior to the HMDSO injection point, the SiO_2 layer (2-5 nm) is shown to encapsulate entire agglomerates rather than individual particles. The characteristic shapes of the core particles (CeO_2: polyhedral, Fe_2O_3: hexagonal/spherical, Ag: spherical, ZnO: rod-like) are preserved by the SiO_2-coating.

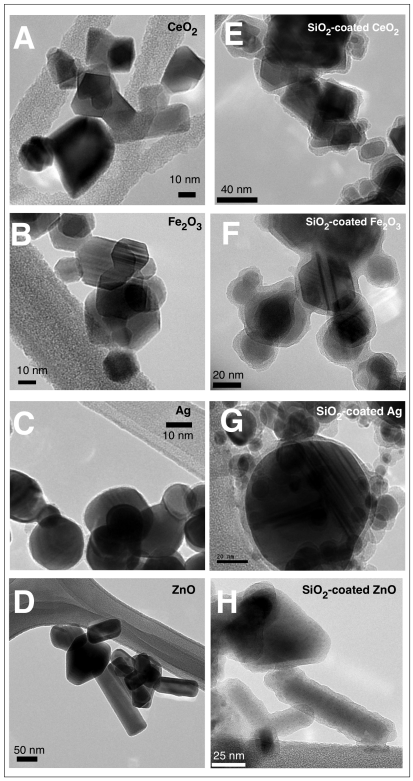

Figure 2: TEM images of uncoated (A-D) and SiO_2-coated (E-H) CeO_2, Fe_2O_3, Ag, and ZnO. SiO_2-coating appears as lighter contrast contour around stronger contrast core particles.

2.2 Assessing SiO_2 Coating Efficiency

XPS provides a highly sensitive and quantitative method of evaluating the coating quality of the ENMs. Figure 3 shows XPS survey spectra for the representative case of CeO_2 with varied SiO_2 wt%. A continuous decrease in Ce electron peaks is observed as the SiO_2 wt% (coating thickness) is increased. At 20 wt% SiO_2, the spectra of the coated CeO_2 matches that of the pure SiO_2, clear indication of a full coating of the CeO_2 particles. Quantitatively this is also confirmed by the atomic concentration ratio, (Ce/(Ce+Si)), which was found to be less than 1%. TEM imaging of these samples further substantiates the XPS spectra analysis (not shown here). While some patchy coatings are visible at 15 wt% SiO_2, the CeO_2 particles are fully encapsulated by SiO_2 at 20 wt%. All other materials in the nanopanel exhibit similar behavior.

2.3 ENM-Cell Interactions

The role of ENM surface coatings on ENM-cell interactions was assessed. Three cell lines of interest were used: human primary monocyte-derived macrophages, PMA-matured

THP-1 macrophages and human alveolar basal epithelial A549 cells. Cells were exposed for 24 hours to a liquid dispersion of coated and uncoated ENMs over a dose range, as described in the method section below. ENM-cellular interactions and cytotoxicity were assessed using a variety of bio-assays such as LDH absorbance, a measure of cytotoxicity, MTT absorbance, a measure of cell viability, and live/dead screening assay. Figure 4 illustrates the cellular toxicity data between ZnO (d_{XRD} = 23.2 nm) and SiO_2-coated ZnO (47 wt% SiO_2) (d_{XRD} = 24.6 nm), as compared to SiO_2 (d_{BET} = 18.6 nm). The uncoated ZnO ENM exhibited a dose response curve with increasing toxicity as doses rise above 50 µg/ml. The SiO_2 coated ZnO ENMs exhibited minimal toxicity similar to that observed for pure SiO_2, a material demonstrated to be non-toxic at these doses in the literature [15]. Cellular toxicity results following exposure to Ag supported on SiO_2 (d_{XRD} = 20.4 nm) (Ag/SiO_2, 50 wt% SiO_2), SiO_2-coated Ag (d_{XRD} = 28 nm) (10 wt% SiO_2), and SiO_2 (d_{BET} = 18.6 nm) are also shown in Figure 5. In THP-1 macrophages (Figure 5 A) toxicity of SiO_2-coated Ag particles was minimal and similar to that of SiO_2, whereas uncoated Ag was toxic at doses greater than 50 ug/ml. In the more sensitive primary monocyte-derived macrophages (Figure 5 B) uncoated Ag was extremely toxic at all doses evaluated. SiO_2-coated Ag was substantially less toxic, although more toxic than SiO_2.

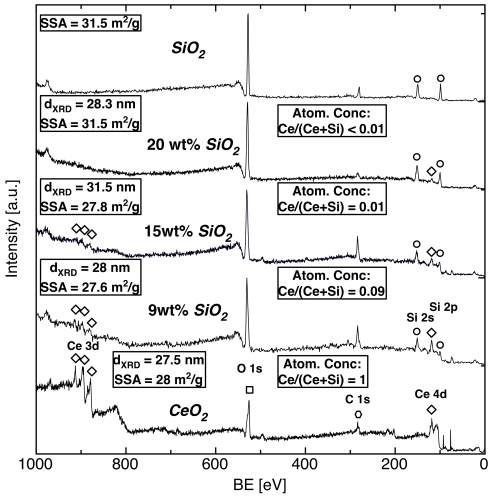

Figure 3: XPS survey scans for CeO_2, SiO_2-coated CeO_2 (9, 15, 20 wt% SiO_2), and SiO_2 with relevant photoelectron peaks (diamonds).

3 CONCLUSIONS

In conclusion, we showcase the effectiveness of a novel safer formulation concept for flame-generated ENMs by applying it to a comprehensive and industry-relevant nanopanel. We present a valuable technique for quantitatively assessing the coating efficiency of the SiO_2-encapsulation process (XPS). Finally, we provide valuable toxicological evidence for the safety of this novel formulation. The described concept bears great promise for large-scale industrial application as a means of effectively inhibiting nanoparticle toxicity.

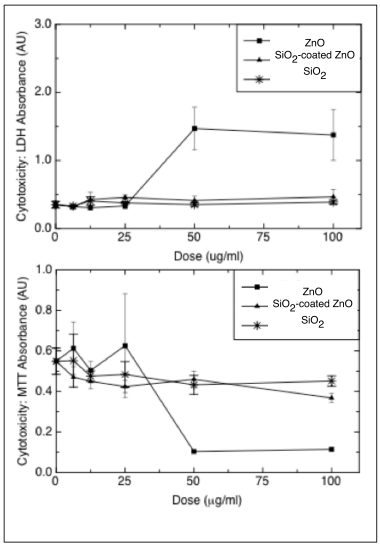

Figure 4: Cytoxicity of ZnO and SiO_2-coated ZnO (A549, MTT/LDH assays).

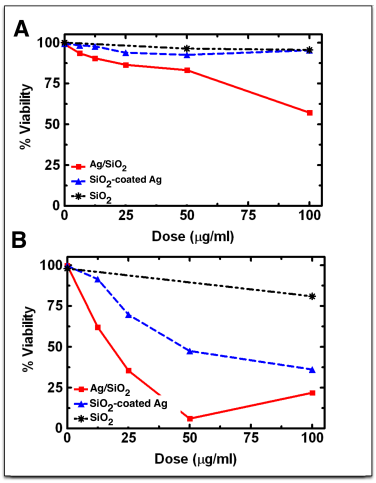

Figure 5: Cytoxicity of Ag/SiO2 and SiO_2-coated Ag (THP-1 (A), monocyte-derived macrophages (B))

4 METHODS

CeO_2, Fe_2O_3, ZnO and Ag core particles were synthesized by the Flame Spray Pyrolysis (FSP) of precursor solutions containing organometallic compounds (Cerium(III) ethylhexanoate, Iron(III) acetylacetonate, Zinc naphthenate, and Silver acetate, 0.5 M, respectively). Optimal synthesis parameters deterimed by previous computational [16] and experimental work [17] were applied to the SiO_2-caotings synthesis.

X-ray Diffraction (XRD) patterns were obtained using a Scintag XDS2000 powder diffractometer (Cu Kα (λ = 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. The Brunauer-

Emmett-Teller (BET) powder-specific surface area (SSA) of all samples was measured by nitrogen adsorption at 77 K (Micromeritics TriStar), after sample degassing for 1 h at 150 °C in nitrogen. ENMs were deposited onto lacey carbon grids for TEM imaging (TEM: Libra 120). Highly surface sensitive X-Ray Photoelectron Spectroscopy (XPS) (ESCA SSX-100, X-ray source: monochromatic Al Kα, 10 kV, 10 mA, detector: hemispherical electron energy spectrometer, spot size: 600 μm) was used to assess SiO_2 coating efficiency [30, 31]. 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.

ENMs were dispersed and delivered to the cells as described in [18]. Human alveolar basal epithelial A549, cultured in F-12/K supplemented with 3% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES, were exposed to 0 – 100 μg/ml ENMs for 24 h. 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. THP-1 monocytes and human primary monocyte-derived macrophages, cultured in RPMI supplemented with 10% heat-inactivated FBS, were also exposed to 0 -100 μg/ml ENMs for 24 h. Cytoxicity was evaluated by dual live/dead staining and fluorescence microscopy.

REFERENCES

[1] "2010 Nanotechnology Research Review," *BBC Research,* p. NAN04713, 2011.

[2] "Nanotechnology: A Realistic Market Assessment," *BBC Research,* p. NAN031D, 2010.

[3] "Nanoscience and Nanotechnologies: Opportunities and Uncertainties," *The Royal Society and the Royal Academy of Engineering,* 2004.

[4] A. Nel, T. Xia, L. Madler, and N. Li, "Toxic potential of materials at the nanolevel," *Science,* vol. 311, pp. 622-627, Feb 2006.

[5] K. Wegner and S. E. Pratsinis, "Scale-up of nanoparticle synthesis in diffusion flame reactors," *Chemical Engineering Science,* vol. 58, pp. 4581-4589, Oct 2003.

[6] S. E. Pratsinis, "Flame aerosol synthesis of ceramic powders," *Progress in Energy and Combustion Science,* vol. 24, pp. 197-219, 1998.

[7] S. E. Pratsinis, "Aerosol-based Technologies in Nanoscale Manufacturing: from Functional Materials to Devices through Core Chemical Engineering," *Aiche Journal,* vol. 56, pp. 3028-3035, Dec 2010.

[8] G. A. Sotiriou, E. Diaz, M. S. Long, J. Godleski, J. Brain, S. E. Pratsinis, and P. Demokritou, "A novel platform for pulmonary and cardiovascular toxicological characterization of inhaled engineered nanomaterials," *Nanotoxicology,* Aug 2 2011.

[9] P. Demokritou, R. Buchel, R. M. Molina, G. M. Deloid, J. D. Brain, and S. E. Pratsinis, "Development and characterization of a Versatile Engineered Nanomaterial Generation System (VENGES) suitable for toxicological studies," *Inhal Toxicol,* vol. 22 Suppl 2, pp. 107-16, Dec 2010.

[10] Y. H. Jin, S. Kannan, M. Wu, and J. X. J. Zhao, "Toxicity of luminescent silica nanoparticles to living cells," *Chemical Research in Toxicology,* vol. 20, pp. 1126-1133, Aug 2007.

[11] D. Napierska, L. C. J. Thomassen, V. Rabolli, D. Lison, L. Gonzalez, M. Kirsch-Volders, J. A. Martens, and P. H. Hoet, "Size-Dependent Cytotoxicity of Monodisperse Silica Nanoparticles in Human Endothelial Cells," *Small,* vol. 5, pp. 846-853, Apr 2009.

[12] B. Buesser and S. E. Pratsinis, "Design of Gas-Phase Synthesis of Core-Shell Particles by Computational Fluid-Aerosol Dynamics," *Aiche Journal,* vol. 57, pp. 3132-3142, Nov 2011.

[13] A. Teleki, M. C. Heine, F. Krumeich, M. K. Akhtar, and S. E. Pratsinis, "In situ coating of flame-made TiO_2 particles with nanothin SiO_2 films," *Langmuir,* vol. 24, pp. 12553-12558, Nov 2008.

[14] A. Teleki, M. Suter, P. R. Kidambi, O. Ergeneman, F. Krumeich, B. J. Nelson, and S. E. Pratsinis, "Hermetically coated superparamagnetic Fe_2O_3 particles with SiO_2 nanofilms," *Chemistry of Materials,* vol. 21, pp. 2094-2100, May 2009.

[15] S. George, S. Pokhrel, T. Xia, B. Gilbert, Z. X. Ji, M. Schowalter, A. Rosenauer, R. Damoiseaux, K. A. Bradley, L. Madler, and A. E. Nel, "Use of a Rapid Cytotoxicity Screening Approach To Engineer a Safer Zinc Oxide Nanoparticle through Iron Doping," *Acs Nano,* vol. 4, pp. 15-29, Jan 2010.

[16] B. Buesser and S. E. Pratsinis, "Design of gas-phase synthesis of core-shell particles by computational fluid–aerosol dynamics," *AIChE Journal,* vol. 57, pp. 3132-3142, 2011.

[17] A. Teleki, B. Buesser, M. C. Heine, F. Krumeich, M. K. Akhtar, and S. E. Pratsinis, "Role of Gas-Aerosol Mixing during in Situ Coating of Flame-Made Titania Particles," *Industrial & Engineering Chemistry Research,* vol. 48, pp. 85-92, Jan 7 2009.

[18] J. Cohen, DeLoid, G., Pyrgiotakis, G., Demokritou, P., "Interactions of Engineered Nanomaterials in Physiological Media and Implications for In Vitro Dosimetry," *Nanotoxicology,* 2012.