Organically Modified Silica Nanoparticles Interaction with Macrophage Cells: Assessment of Cell Viability on the Basis of Physicochemical Properties

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ABSTRACT: Silica nanoparticles have drawn a lot of attention for nanomedicine application, and this is attributed to their biocompatibility and ease of surface functionalization. However, successful utilization of these inorganic systems for biomedical application depends on their physicochemical properties. This study, therefore, discusses *in vitro* toxicity of organically modified silica nanoparticles on the basis of size, shape, and surface properties of silica nanoparticles. Spherical- and oval-shaped nanoparticles having hydroxyl and amine groups were synthesized in Tween 80 micelles using different organosilanes. Nanoparticles of similar size and morphology were considered for comparative assessment. "As-prepared" nanoparticles were characterized in terms of size, shape, and surface properties using ZetaSizer, transmission electron microscopy, and Fourier transform infrared to establish the above parameters. *In vitro* analysis in terms of nanoparticle-based toxicity was performed on J-774 (macrophage) cell line using propidium iodide-4′,6-diamidino-2-phenylindol and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays. Fluorescent dye-entrapped nanoparticles were used to visualize the uptake of the nanoparticles by macrophage cells. Results from cell studies suggested low levels of toxicity for different nanoparticle formulations studied, therefore are suitable for nanocarrier application for poorly soluble molecules. On the contrary, the nanoparticles of similar size and shape, having amine groups and low net negative charge, do not exhibit any *in vitro* cytotoxicity. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3943–3951, 2015

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INTRODUCTION

Nanomaterials have drawn great interest for biomedical applications, which include high availability of drugs at the target site with minimal side effects. However, an area of concern in the application of these engineered nanoparticles is potentially the adverse environmental and health effects because of their unique chemical and physical properties at the nanoscale.[1] Considerable variation in cell behavior has been reported with the changes in size, shape, and surface properties of synthesized nanomaterials.[2] In the context of biomedical application, the particle size, morphology, and surface properties such as charge, functional groups, and ligand have a very critical role to play.[3] These properties need to be modulated to increase the stability of the nanoparticles in the systemic circulation and/or cell culture media, and also to enhance cell and nanoparticle interaction.[4] Among different nanoparticle systems, silica-based nanomaterials have drawn a lot of attention for biomedical application.[5] This is because of their biocompatibility, biodegradability, and ease with which these nanoformulations can be synthesized at large scale and chemically modified for varying surface functionalization.[6,7]

All these advantages have resulted in utilization of silica as organically modified silica (ORMOSIL) (shell) coating[8] on different metal/metal oxide nanoparticles such as gold,[9] silver,[10] and ferrous (iron)[11,12] for properties such as antibacterial[10] (Gram-negative *E. coli* and *P. aeruginosa*, and Gram-positive *S. aureus* and *B. subtilis*) and cancer cell targeting along with imaging when labeled with transferrin and quantum dots.[13,14] This suggests that encapsulation of metallic core in polymeric structure not only makes formulation biocompatible, but also resulted in stable multifunctional advanced system.[11] Apart from being utilized as coating, "ORMOSIL" nanoparticles have also been synthesized with diverse surface properties[15] for different biomedical applications such as the delivery of DNA,[16,17] biosensing for glucose or ethanol,[18] biomedical imaging,[19] gene delivery,[20] and live cell imaging,[21] as drug delivery systems[22,23] and in photodynamic therapy.[24] When functionalized with Near-Infrared fluorophore and radiolabeled iodine-124 act as probe for safe *in vivo* bio-imaging,[25] and tagged with antibodies such as antimesothelin and anticlaudin-4 can target pancreatic cancer cells.[26] *In vitro* detection of cells, microbes, and biomolecules[27] has been carried out using fluorescent dye-tagged ORMOSIL nanoparticles where required.[28]

Organically modified silica formulation proves to be potential vector for biomedical application as treatment with

Abbreviations used: ORMOSIL, organically modified silica; DLS, dynamic light scattering; TEM, transmission electron microscopy; FT-IR, Fourier transform infrared; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI-DAPI, propidium iodide-4′,6-diamidino-2-phenylindole; OTES, octyltriethoxysilane; VTES, vinyltriethoxysilane; APTES, 3-aminopropyl triethoxy silane.

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silica facilitates a more stable suspension; presence of ligand groups allows conjugation with biomolecules, peptides, and drug molecules;[11]; in addition, the core of nanoparticles allows encapsulation of poorly soluble or hydrophobic drug.[28] Also, the addition of silane during synthesis leads to controlled size and size distribution of metal/metal oxide nanostructure.[12,29] ORMOSIL nanoparticles can be synthesized with different functional groups by utilizing the relevant organosilane[17,26]; thus, particles with different physicochemical characteristics can be generated.[30] Besides, different shapes and sizes of the nanoparticles can be formed, particularly by using micellar systems for synthesis.[6] All these advantages make the silica-based systems ideal for biomedical application; yet, there is a lack of studies related to the safety of these nanostructures. Variability in nanoparticle physicochemical properties can arise when more than one type of organosilane is utilized. For the nanoparticles with ORMOSIL shell, the safety profile of the carrier depends largely on its chemical characteristics. With the development of nanoscale silica-based materials, it has become necessary to fully understand how they interact with the biological environment to ensure safety. In literature, studies suggest that a high concentration of silica nanoparticles of any size range shows toxicity. Also, the geometry and surface functionality of the particles relates to cell uptake and cell apoptosis.[6,31] Several reports suggest that there is no cyto- or geno-toxicity associated with silica nanoparticles.[32,33] These variations in observation can possibly be because of differences in nanoparticle systems used in terms of their physicochemical properties and/or cell lines used to assess cytotoxicity. To validate the existing knowledge as well as that of future investigations, studies to correlate physicochemical properties and biological phenomenon is imperative.

In the present work, we have selectively studied the unmodified and amine-modified silica nanoparticles of two specific shapes and two size ranges. Amine functionality was considered as important as it can participate easily in bond formation with several types of molecules. The two size ranges selected were 1–10 and 20–100 nm that are relevant for a vast majority of *in vivo* applications. We synthesized ORMOSIL nanoparticles of variable size, morphology, and surface functionality by altering the silane precursors used in the process, and also by the use of Tween 80 micellar systems. Synthesized nanoparticles were then characterized to determine their size, shape, and surface charge, followed by systematic *in vitro* investigation based on the viability of mouse macrophage cells following their interaction with these nanoparticles.

MATERIALS AND METHODS

Materials

Butanol, Tween 80, pyrene, dimethyl formamide, octyltriethoxysilane (OTES), vinyltriethoxysilane (VTES), 3-amino propyl triethoxy silane (APTES), and liquor ammonia (analytical grade) were purchased from Sigma–Aldrich, St. Louis, USA. Disodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride were procured from Merck, Mumbai (Maharashtra) India. J-774 mouse macrophage cell lines were bought from ATCC. Culture media, fetal bovine serum (FBS), antibiotics, and culture plates were purchased from Gibco. 4,6-Diamidino-2-phenylindole, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and propidium iodide were purchased from Roche Diagnostics (Mumbai, India).

Table 1. Size by DLS with Poly Dispersity Index (PDI) Values and Zeta Potential Data for Samples A–G with Respective Amount (μL) of OTES/VTES and APTES

	Sample	DLS (PDI; nm)	Zeta (mV)	Shape
A	OTES6	6 (0.535)	−6.20	Spherical
B	OTES25	33 (0.356)	−6.22	Spherical
C	VTES25	50 (0.392)	−4.56	Spherical
D	VTES100	75 (0.530)	−4.82	Oval
E	OTES25APTES10	37 (0.335)	−2.90	Spherical
F	VTES25APTES10	60 (0.228)	−2.53	Spherical
G	VTES100APTES10	90 (0.714)	−3.85	Oval

Also, shape as observed by TEM.

Methods

Nanoparticle Synthesis

Nanoparticles were synthesized by microemulsion-based sol-gel approach in which Tween 80 micelles worked as a nanoreactor site for silane hydrolysis and polymerization as reported earlier.[34] In brief, calculated amounts of butanol (400 μL) and Tween 80 (0.22 g) were stirred for 5–6 h in 10 mL of DI water in a 100-mL round-bottom flask. Following stirring, varying amounts of OTES (0–25 μL) or VTES (25–100 μL) were added to the micellar system, to obtain nanoparticles of variable sizes with hydroxyl surface functionality. The solution was stirred overnight, following which 10 μL of liquid ammonia were added to ensure hydrolysis and polycondensation. For -NH_2 (amine) functionalization, APTES was incorporated in the system in the given volume size as in Table 1. In order to synthesize dye-entrapped nanoparticles, the hydrophobic dye pyrene was dissolved in the micellar core formed during nanoparticle synthesis. From the particles synthesized, seven samples were selected and designated as A, B, C, D (from OTES/VTES) and E, F, G (with APTES). The schematic representation of nanoparticle synthesis protocol is given in Figure 1. The prepared nanoparticles were dialyzed three times in 1000 mL DI water using 14 kD dialysis membrane to ensure the removal of any unreacted silane and/or surfactant Tween 80 before characterization and cell studies.

Characterization of the nanoparticles

The hydrodynamic diameter and zeta (ζ) potential of nanoparticles were determined using Malvern Nano ZetaSizer ZS system (Malvern Instruments, Malvern, UK). The shape and size of all the nanoparticles of the high aspect ratio were determined by transmission electron microscopy (TEM). To confirm the surface functionality of different nanoformulations, Fourier transform infrared (FT-IR) spectroscopy was used.

Dynamic Light Scattering and Zeta

The size (hydrodynamic diameter), size distribution, and zeta potential of prepared nanoparticles was determined by photon correlation spectroscopy using Malvern Nano ZetaSizer ZS system (Malvern Instruments, Malvern, UK). The measurements were performed at standard condition of 25°C with a detection angle at 173°C. For determining surface charge, measurements were performed at 25°C using a disposable U-shaped cuvette