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***In Vivo* Biodistribution and Pharmacokinetics of Silica Nanoparticles as a Function of Geometry, Porosity and Surface Characteristics**

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Abstract

The *in vivo* biodistribution and pharmacokinetics of silica nanoparticles (SiO₂) with systematically varied geometries, porosities, and surface characteristics were investigated in immune-competent CD-1 mice *via* the intravenous injection. The nanoparticles were taken up extensively by the liver and spleen. Mesoporous SiO₂ exhibited higher accumulation in the lung than nonporous SiO₂ of similar size. This accumulation was reduced by primary amine modification of the nanoparticles. High aspect ratio, amine-modified mesoporous nanorods showed enhanced lung accumulation compared to amine-modified mesoporous nanospheres. Accumulation of the nanoparticles was mainly caused by passive entrapment in the discontinuous openings in the endothelium of the liver and spleen or in the pulmonary capillaries, and was highly dependent on nanoparticle hydrodynamic size in circulation. The SiO₂ were likely internalized by the reticulo-endothelial system (RES) following physical sequestration in the liver and spleen. The nanoparticles that were transiently associated with the lung were re-distributed out of this organ without significant internalization. Pharmacokinetic analysis showed that all SiO₂ were rapidly cleared from systemic circulation. Amine-modified or nonporous nanoparticles possessed a higher volume of distribution at steady state than their pristine counterparts or mesoporous SiO₂. In all, surface characteristics and porosity played important roles in influencing SiO₂ biodistribution and pharmacokinetics. Increasing the aspect ratio of amine-modified mesoporous SiO₂ from 1 to 8 resulted in increased accumulation in the lung.

Keywords

silica nanoparticles; nanotoxicity; biodistribution; geometry; porosity

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1. Introduction

Silica nanoparticles (SiO_2) have utility in a wide range of applications such as biologic delivery platforms [1–3], imaging and diagnostic agents [4–7], and targeted therapeutic carriers [8–10]. Recent improvements in regulating the geometry, porosity, and surface characteristics of SiO_2 have further facilitated their biomedical applications [9, 11–15]. Previous studies have been conducted to show the effect of size, pegylation, and surface charge on biodistribution and *in vivo* toxicity of SiO_2 [16–18]. It has been reported that mesoporous SiO_2 of smaller size with surface pegylation had lower capture by the RES and were more slowly degraded [16]. Organically modified silica nanoparticles (ORMOSIL) with diameters of 20 – 25 nm exhibited effective clearance *via* the hepatobiliary route without any sign of organ toxicity [17]. Cationic mesoporous SiO_2 were excreted rapidly by the hepatobiliary route probably due to charge-dependent serum protein adsorption [18]. Limited information however is available about the impact of geometry of SiO_2 on biodistribution and toxicity.

Recent studies have demonstrated that geometry of nanocarriers can influence their circulation half-life and other pharmacokinetic parameters [19–22]. For example, pegylated polymeric micelles of long, filamentous shape persisted in the circulation up to one week after intravenous injection, approximately 10 times longer than their spherical counterparts [19]. It was suggested that the spherical micelles were taken up by the cells more readily than the long filaments under fluid flow conditions since the cellular entry of the latter was opposed by flow [19]. Cyclic polymers composed of α -cholo- ϵ -caprolactone and ϵ -caprolactone, which had molecular weights greater than the renal threshold, showed longer blood circulation time in mice than linear polymers of similar composition and comparable molecular weight [20]. This effect was attributed to the fact that linear polymers traverse nanopores in glomeruli by end-on motion of one chain end, while cyclic polymers transit through by entering the pores with two chain segments since they lack chain ends [20]. While the studies above relate to more flexible polymeric systems, the influence of geometry on biological system has also been studied for more rigid nanoparticles. For example, it was shown that the anti-intercellular adhesion molecule 1 elliptical polystyrene disks ($0.1 \times 1 \times 3 \mu\text{m}$) had higher endothelial targeting specificity in the lung than spheres of different sizes (0.1, 1, 5 μm) [21]. We previously demonstrated that pegylated gold nanorods ($10 \times 45 \text{ nm}$, 1.13 mV) exhibited longer blood circulation half-life and higher tumor accumulation than pegylated gold nanospheres (50 nm, –27.1 mV) in ovarian tumor bearing mice [22]. These studies suggest that geometry and carrier architecture can influence the *in vivo* behavior of nanoscale platforms. However much needs to be examined in this area since factors such as porosity and surface characteristics can further influence biodistribution and pharmacokinetics.

Our previous studies on lung cancerous epithelial cells and macrophages showed that *in vitro* toxicity of spherical or rod-shaped SiO_2 was mainly determined by porosity and surface characteristics irrespective of geometric features [23]. Further *in vivo* studies demonstrated that the systemic toxicity of these nanoparticles was also mainly influenced by nanoparticle porosity and surface characteristics. Mesoporous SiO_2 tended to be less tolerated than nonporous SiO_2 while amine modification on mesoporous nanoparticles improved the tolerated dose threshold [24]. Geometry did not make a significant difference in the mechanism or extent of the systemic toxicity. The next logical step is to investigate the distribution of these SiO_2 in animals to shed light on the causes for *in vivo* toxicity observed in mice beyond maximum tolerated doses (MTDs) and to relate the *in vitro* toxicity and cellular uptake with *in vivo* toxicity and biodistribution. Herein, we report the biodistribution and pharmacokinetics of SiO_2 in mice as a function of geometry, porosity, and surface characteristics.

2. Materials and methods

2.1. Materials

Nonporous silica nanospheres (Stöber) or mesoporous SiO₂ with distinct geometrical features (nanospheres, Meso S; aspect ratio 8 nanorods, AR8), and their amine-modified counterparts (SA, MA, 8A) were prepared as reported previously [23]. Briefly, nonporous SiO₂ were synthesized by Stöber method and mesoporous SiO₂ were synthesized through a one-step condensation under dilute silica source and low surfactant concentration conditions with ammonium hydroxide as the base catalyst [23]. Monoiodinated Bolton-Hunter Reagent, 1 mCi/37 MBq in benzene, was purchased from American Radiolabeled Chemicals (St. Louis, MO). CD-1 mouse serum was a customized bio-specimen order from Charles River Laboratories, from which the CD-1 mice were ordered for this study. All other chemicals were of reagent grade from Sigma-Aldrich.

2.2. Pre-modification of SiO₂ for radiolabeling experiments

Cationic, amine-modified SiO₂ were produced by reacting the nanoparticles with 3-(aminopropyl)triethoxysilane (APTES) at a weight ratio of 1:1 in anhydrous ethanol for 20 hours at room temperature as described previously [23]. To obtain anionic, slightly amine-modified SiO₂, the same procedure was used except that APTES reacted with SiO₂ at the weight ratio of 1:50 to make sure there were available primary amine groups on the surface to conjugate with monoiodinated Bolton-Hunter Reagent while the surface charge of SiO₂ remained negative. The nanoparticles were stored in ethanol and thoroughly washed in water and borate buffer immediately before radiolabeling experiments.

2.3. SiO₂ radiolabeling experiments

The radiolabeling protocol was adapted from an established Bolton-Hunter method, whereby the primary amine groups available on nanoparticle surface formed an amide bond with *N*-hydroxysuccinimide group from monoiodinated Bolton-Hunter Reagent [25]. To react with nanoparticles, 20 μ L monoiodinated Bolton-Hunter Reagent was transferred to a glass vial and the solvent was allowed to dry in the air for extended time (1 hour). 10 mg of SiO₂ prepared in above section (10 mg/mL) in 0.05 M borate buffer (pH 8.5) was quickly added to the glass vial and stirred on ice for 45 minutes. Then the mixture was transferred to a dialysis cellulose ester membrane with a cutoff size of 3.5 – 5 kD (Float-A-Lyzer G2, Spectra/Por, Specutrum Laboratories, Inc) and dialyzed against 4 L water at room temperature for 20 days with water changing on a daily basis. The unreacted Bolton-Hunter Reagent was readily hydrolyzed in the aqueous medium and was removed by dialysis. The hydrolyzed product is referred to as ¹²⁵I-BHR in this article. Thin layer chromatography (TLC) silica gel was used to check the presence of unbound ¹²⁵I-BHR in radiolabeled SiO₂ (¹²⁵I-SiO₂) using methanol water (4:1 volume ratio) solvent as the mobile phase. The radioactivity on silica gel was measured by the Packard Cobra auto-gamma counter (GMI, Ramsey, Minnesota). For SA and slightly amine-modified Stöber, dialysis did not completely remove ¹²⁵I-BHR from nanoparticles and thus an alternative centrifugation method described below was used. The mixture from radiolabeling reaction was collected into a 2.0 mL microtube and spun at 15,000 $\times$ g for 30 minutes in an Eppendorf centrifuge 5415D (Eppendorf, Hamburg, Germany). Nanoparticles were extensively washed in water and methanol and finally in water. Then TLC method was applied to check the presence of ¹²⁵I-BHR in nanoparticles from each washing cycle until unbound ¹²⁵I-BHR was confirmed to be absent in ¹²⁵I-SiO₂.

2.4. Serum stability of ^{125}I -SiO₂

The stability of radiolabeling on SiO₂ was tested in mouse serum before the biodistribution study. 0.5 mg ^{125}I -SiO₂ was added to 1 ml 50% CD-1 mouse serum in saline and incubated at 37 °C for 72 hours. The experiment was done in triplicate with ^{125}I -BHR in 50% mouse serum as the positive control. At the end of 72 hours, an aliquot of mixture was withdrawn by a glass capillary and analyzed by TLC. The stability of ^{125}I -SiO₂ was expressed as percentage of radioactivity in the original spotting site out of the total radioactivity on the plate.

2.5. Biodistribution and pharmacokinetic analysis

Animal studies were conducted under an approved protocol of the University of Utah Institutional Animal Care and Use Committee (IACUC). Female CD-1 mice, 6 – 8 weeks old, were purchased from Charles River Laboratories and housed in standard cages with five animals per cage. All animals were acclimated to the animal facility for at least one week prior to experimental procedures. CD-1 mice were injected *via* the lateral tail vein with 20 mg/kg SiO₂ suspension in 200 μL sterile saline. The SiO₂ suspension was a mixture of ^{125}I -SiO₂ and SiO₂ of the same type to make a radioactivity dose of 60,000 cpm per animal for pristine SiO₂ (Stöber, Meso S, AR8) or a radioactivity dose of 120,000 cpm per animal for amine-modified SiO₂ (SA, MA, 8A). The weight content of ^{125}I -SiO₂ which contributed to the dose of SiO₂ in injection formulation was considered to be negligible. The mice were sacrificed at 5 minutes, 30 minutes, 2 hours, 24 hours and 72 hours post intravenous injection. At each time point, animals were terminated by CO₂ asphyxiation and blood samples were collected via inferior vena cava by a heparin coated syringe immediately post euthanasia. The animals were flushed with 20 mL sterile saline to remove blood that remained in the organs in order to obtain accurate tissue accumulation counts based on nanoparticle tissue association or uptake rather than blood content. During necropsy, organs of interest (heart, liver, spleen, lung, kidneys, brain, stomach, small intestine, large intestine, tail, thyroid) and the rest of carcass (bones, muscle and skin) were dissected and weighed followed by tissue radioactivity measurement by a gamma counter. Radioactivity obtained from different organs was calculated as the percentage of the injected dose per gram tissue. Compartmental analysis of the pharmacokinetic data was performed using WinNonlin Professional, version 5.3 (Pharsight Corporation, CA). A two-compartmental pharmacokinetic model was utilized with first order elimination.

2.6. Urinary and hepatobiliary excretion studies

To measure the excretion of SiO₂ into urine and feces, five animals received the intravenous injection of SiO₂ of each type at 20 mg/kg and were individually housed in special single-mouse metabolic cages. Urine and feces were collected into separate tubes at 2 hours, 24 hours, 48 hours and 72 hours. The samples were immediately weighed and their radioactivity was measured by a gamma counter. In order to identify the radioactive species in urine, the urine samples, positive controls (950 μL normal urine + 50 μL 2.5 mg/mL SiO₂ + 5 μL 15,000 cpm ^{125}I -BHR), and negative controls (950 μL normal urine + 50 μL 15,000 cpm 2.5 mg/mL SiO₂ injection formulation) were centrifuged at $15,000 \times g$ for 20 minutes to obtain the supernatant. The supernatant was removed and measured by a gamma counter. The percentage of radioactivity in the supernatant out of the overall urine sample is indicative of percentage of unbound radioactivity or degraded product in the urine.

2.7. Statistical analysis

Experiments were performed in triplicate with results present as average value or mean $\pm$ standard deviation. For *in vivo* studies, five animals were used per group and differences in *in vivo* data were analyzed using one-way ANOVA by GraphPad Prism (GraphPad

Software, CA). Where detected, Tukey's test was used to evaluate pairwise differences between the groups.

3. Results

3.1. ^{125}I - SiO_2 characterization and serum stability

Nonporous nanospheres (Stöber), mesoporous nanospheres (Meso S), or mesoporous nanorods (high aspect ratio 8, AR8) were previously synthesized and stored in ethanol [23]. The pristine SiO_2 were further modified with APTES to obtain their highly cationic counterparts (SA, MA, 8A). These amine-modified SiO_2 were directly used in radiolabeling experiments for SA, MA, and 8A. To track the distribution of pristine SiO_2 *in vivo*, the SiO_2 were slightly modified with APTES to generate available primary amine groups for radioisotope conjugation while the anionic surface charge was maintained for comparison with highly cationic, amine-modified SiO_2 (Fig. 1). The content of unbound ^{125}I -BHR in the ^{125}I - SiO_2 product post purification was analyzed by TLC. There was minimum presence of unbound radioisotope molecules associated with ^{125}I - SiO_2 product (Supplemental Fig. 1, Supplemental Table 1). Serum stability study on a typical ^{125}I - SiO_2 , ^{125}I -MA, demonstrated that the amide bond formed during radiolabeling reaction was stable in 50% mouse serum at 37 °C for 72 hours (Supplemental Fig. 2).

3.2. *In vivo* biodistribution of SiO_2 in healthy mice

Biodistribution of a series of SiO_2 with varied shapes, porosities, and surface characteristics was evaluated in immune-competent CD-1 mice *via* bolus tail vein injection by tracing the radioactivity distribution as depicted in Fig. 1. Results show that SiO_2 of various physiochemical properties mainly accumulated in the liver and spleen with differential distribution into the lung (Fig. 2, Supplemental Table 2). To evaluate the effect of geometry on biodistribution, spherical mesoporous SiO_2 (Meso S, MA) were compared with rod-shaped mesoporous SiO_2 with aspect ratio of 8 (AR8, 8A). Both Meso S and AR8 exhibited extensive lung accumulation. This accumulation was almost eliminated with amine-modified nanospheres MA but not with amine-modified nanorods 8A. All the accumulation in the lung showed a rapid elimination from this organ within 24 hours post injection. To examine the influence of porosity on nanoparticle biodistribution, mesoporous nanospheres (Meso S) were compared with nonporous nanospheres (Stöber). Results show that Meso S was primarily accumulated in the lung while Stöber had negligible accumulation in this organ. Stöber also exhibited increased percentage of liver accumulation out of total recovered dose compared with Meso S. To analyze the surface modification effect, amine-modified SiO_2 were compared with their pristine counterparts. It was revealed that amine modification could efficiently reduce nanoparticle lung accumulation (Fig. 2B, D, F).

3.3. Pharmacokinetic analysis

The blood profiles of SiO_2 were fitted to a two-compartmental pharmacokinetic model. All nanoparticles studied were rapidly cleared from blood circulation within 2 hours of injection followed by a slow elimination phase which indicated the slow re-distribution between the blood and organs/tissues (Fig. 3). There was no significant difference in the terminal clearance rates of various types of SiO_2 ($p > 0.05$) (Fig. 4A). Stöber showed a significantly higher volume of distribution at steady state (V_{ss}) than Meso S ($p < 0.05$), while amine-modified, spherical SiO_2 (MA, SA) exhibited a significant increase in V_{ss} compared to their pristine counterparts (Meso S, $p < 0.01$; Stöber, $p < 0.001$) (Fig. 4B).

3.4. Tissue and blood partitioning of SiO_2

The tissue affinity indices, calculated as the ratio of area under the curve from a specific organ over area under the curve of blood, reflect the affinity and capacity of nanoparticle

association with the specific organ of interest [26]. Various SiO₂ showed high affinity for the spleen and low affinity for the kidneys across the board (Table 1). High aspect ratio 8A showed on average higher lung affinity than MA. Stöber and SA had on average higher liver uptake than mesoporous nanoparticles with or without amine modification. Mesoporous SiO₂ showed on average higher affinity to the lung than Stöber. The lung exposure was drastically reduced by amine modification as indicated by decreased tissue affinity indices from amine-modified nanoparticles (SA, MA, 8A) compared with their pristine counterparts (Stöber, Meso S, AR8). The same trend was also observed for kidney exposure of various SiO₂.

The tissue/blood concentration ratio of various nanoparticles in major organs, liver, spleen, lung, and kidneys, was used as an indicator of changes in organ penetrability and retention over time [27]. The SiO₂ across the board generally showed an increase in partitioning in the liver and spleen over time, but the partitioning remained constant in the lung and kidneys over 72 hours (Fig. 5). In the lung, mesoporous nanoparticles (Meso S) demonstrated a significantly higher lung/blood concentration ratio than Stöber ($p < 0.001$) or MA ($p < 0.001$) over 3 days.

3.5. Urinary and hepatobiliary excretion

Excretion of the radioactive material through urinary or hepatobiliary routes followed a similar pattern where radioactivity was excreted through urine more than feces at all time-points (2 hours, 24 hours, 48 hours, and 72 hours) and the excretion peaked at 24 hours post injection for both routes (Supplemental Table 3). The overall excreted radioactivity reached 15% - 38% of injected dose by end of study. To investigate the radioactive species in the excrement, we centrifuged down the urine samples as well as various controls mentioned in the method section to identify radioactive material in the supernatant (Supplemental Fig. 3). Results from control groups show that ¹²⁵I-BHR did not have physical adsorption with SiO₂ and 100% was recovered in the supernatant while 4% of ¹²⁵I-AR8, a typical ¹²⁵I-SiO₂, was recovered in the supernatant. This indicates that the 40% recovered radioactivity in supernatant of urine samples from AR8 treatment in mice was most likely to be unbound radioisotopes or small ¹²⁵I-SiO₂ degraded product. Similar results were found for urine samples from other nanoparticle treatment groups. We also collected the feces from nanoparticle treated animals, suspended in saline followed by centrifugation. Results showed that at least 36% radioactivity in feces was from unbound radioisotopes or small degraded product. The rest of radioactivity recovered in the pellet of urine or feces samples post centrifugation could be ¹²⁵I-SiO₂ or their relatively large degraded product.

4. Discussion

In this study the effect of geometry, porosity and surface characteristics of SiO₂ on biodistribution and pharmacokinetics upon intravenous injection into healthy mice was evaluated. The overall effect of physicochemical parameters of nanoparticles on the studied biological systems is summarized in Table 2 to enable the relation of biodistribution patterns observed here with cellular uptake and toxicity profiles of similar nanoparticles observed previously [23, 24].

The biodistribution results show that the majority of SiO₂ accumulated in the liver and spleen post injection (Fig. 2). This is due to the fact that the discontinuous gaps in the endothelium which lines the sinusoidal walls of liver and spleen allow the passive entrapment of foreign particulates [28, 29]. The continuously increased organ/blood concentration ratios for liver and spleen indicate that nanoparticles were internalized post physical sequestration due to the prevalent presence of macrophages in these organs (Fig. 5A–B). The difference in accumulation of SiO₂ in various organs corroborates with our

previous *in vitro* studies which showed that the cellular response to nanoparticle exposure was cell type dependent; macrophages had extensively higher association with the nanoparticles than epithelial cells [23]. Thus, the liver and spleen, where most macrophages reside, showed the most extensive SiO₂ accumulation in the biodistribution study. Nonporous SiO₂ (Stöber) and their amine-modified counterparts (SA) exhibited highest liver affinity among all types of nanoparticles (Table 1). These results agree with previous *in vitro* studies which showed that porosity played a predominant role in determining nanoparticle cellular association; nonporous nanoparticles with or without amine modification had the highest cellular association among all types of nanoparticles [23]. The high affinity of Stöber for liver could be responsible for the significant increase in liver enzyme levels in plasma beyond MTDs compared with controls as shown in previous *in vivo* toxicity studies [24]. It suggests that porosity plays an important role in influencing nanoparticle biodistribution pattern.

The SiO₂ of various types exhibited differential accumulation in the lung post injection (Fig. 2). The constant lung/blood concentration ratio over time indicated that the accumulation of SiO₂ in the lung was because of transient association with capillary rather than internalization (Fig. 5C). The association was balanced between SiO₂ organ concentration and SiO₂ blood concentration by SiO₂ translocation and redistribution into other organs. Thus, the accumulation in the lung was mostly in capillaries rather than in pulmonary cells. Mesoporous SiO₂ exhibited a higher lung affinity than nonporous SiO₂, and amine modification reduced lung affinity compared with the pristine SiO₂ (Fig. 5C). This pattern could be related to the changes in nanoparticle hydrodynamic size in the presence of serum (Table 2). Though produced with similar size as confirmed by transmission electron microscopy (TEM), mesoporous SiO₂ had significantly higher hydrodynamic size than nonporous SiO₂ in serum [24]. This is probably because individual nonporous nanoparticle was stabilized by protein adsorption as the hydrodynamic size decreased in serum compared to that in saline. Whereas the presence of protein molecules could not dissociate the slightly aggregated mesoporous SiO₂ in aqueous suspension possibly due to the enhanced inter-nanoparticle interaction of these high surface area mesoporous SiO₂. Thus, the addition of protein layer on the surface may have contributed to the increase in hydrodynamic size of mesoporous SiO₂ in serum compared to that in saline. Mesoporous nanoparticles with relatively large hydrodynamic size in serum are more likely to cause obstruction in vessels and can partially explain the increased lung accumulation compared with nonporous SiO₂ (Fig. 2A, E). The amine-modified SiO₂ showed smaller hydrodynamic sizes in serum probably due to steric stabilization from adsorbed protein molecules than their pristine counterparts [24], which causes lower pulmonary accumulation (Fig. 2B, D, F) and decreased tissue affinity indices (Table 1). However, amine-modified mesoporous nanorods (8A) showed higher lung affinity than amine-modified mesoporous nanospheres (MA) (Table 1), demonstrating that geometry of these nanoparticles influences biodistribution to a certain extent. In all, lung accumulation of nanoparticles was mostly influenced by porosity and surface characteristics, however elongated geometrical shape (rods *versus* spheres) increased accumulation in this organ for amine-modified SiO₂. Detailed investigation should be made to confirm the mechanism of nanoparticle-induced obstruction in the environment mimicking the *in vivo* circulation system with whole blood as the medium for future studies.

Our previous *in vivo* toxicity studies showed that the onset of adverse reactions was mainly due to the mechanical obstruction of nanoparticles in the vasculature that led to congestion in organs and subsequent functional failure [24]. It appears that it is the “vasculature impact” rather than cellular toxicity that limits silica nanoparticle safety *in vivo*. In *in vitro* studies, nanoparticle toxicity was mainly influenced by surface characteristics; primary amine modification significantly reduced cellular toxicity as shown by the increased 50% cell inhibitory concentration (IC₅₀) values compared with pristine nanoparticles probably due to

the differential subcellular localization, whereas porosity and geometry did not seem to affect the IC₅₀ [23]. In *in vivo* studies, porosity and surface characteristics influenced hydrodynamic sizes of SiO₂ in circulation, which had an important implication in their vasculature impact and resultant tolerance threshold [24]. Lung and kidneys were shown to be most susceptible to nanoparticle obstruction in vasculature above MTDs probably due to their abundant blood supply and special anatomic structures [24]. Mesoporous SiO₂, which potentially had the largest hydrodynamic size in circulation as evidenced by hydrodynamic size analysis in serum, were most prone to cause vasculature obstruction and subsequent renal failure, resulting in the lowest MTDs at 30–45 mg/kg irrespective of geometrical features [24]. Amine modification on mesoporous SiO₂ reduced the hydrodynamic size in serum and raised the MTDs to 100–150 mg/kg [24]. Nonporous SiO₂ had the smallest hydrodynamic size in serum and thus reached the highest MTDs at 450 mg/kg as observed previously [24]. These previous observations show that porosity and surface characteristics are major factors that influence *in vitro* or *in vivo* toxicity of SiO₂. Our current studies evaluating the biodistribution of these nanoparticles also ascertain the predominant effects of porosity and surface characteristics on organ accumulation.

Pharmacokinetic analysis demonstrates that majority of SiO₂ of all types were rapidly cleared from circulation at a similar rate (Fig. 4A). Pristine SiO₂ had lower V_{ss} than the amine-modified counterparts while nonporous SiO₂ showed higher V_{ss} than mesoporous SiO₂ (Fig. 4B). This agrees with previous *in vitro* studies on both macrophages and epithelial cells that mesoporous SiO₂ had lower cellular association than their amine-modified counterparts while nonporous SiO₂ showed higher association than mesoporous SiO₂ [23]. These *in vivo* observations suggest that amine-modified SiO₂ or nonporous SiO₂ tended to associate and be taken up by RES in liver or spleen, leading into increased V_{ss}.

Further excretion experiments showed that radioactivity originally from ¹²⁵I-SiO₂ dosed intravenously was found in urine and feces (Supplemental Table 3), indicating possible excretion of SiO₂ or their degraded product. Nanoparticle accumulation in the kidneys was low (Fig. 2) and there was limited affinity of SiO₂ to this organ (Fig. 5D). Due to the very dilute radioactivity concentration in urine and low loading capacity of TLC assays, we could not quantitatively identify each radioactive species in the urine by TLC. Based on the centrifugation method, it was shown that there was possible presence of ¹²⁵I-SiO₂ or their degraded product in urine even though a certain extent of possible unbound radioisotopes from bond breakage from ¹²⁵I-SiO₂ *in vivo* was detected in supernatant of urine (Supplemental Fig. 3). It is possible that nanoparticles were degraded into orthosilicic acid species smaller than the reported renal excretion threshold of 7 nm, and were cleared through the renal route [8]. Previous studies by Tang's group have suggested that intact SiO₂ larger than 100 nm in size can be excreted in urine post intravenous injection as evidenced by TEM imaging [30]. The mechanism of large nanoparticle excretion into urine is not fully understood and warrants further studies. The examination into radioactivity in feces indicated a similar fact that SiO₂ was likely excreted through hepatobiliary route into feces as the dense silicate form present in the pellet of feces from centrifugation method, which agrees with previous studies by Lo's group that reported the hepatobiliary excretion of SiO₂ by fluorescence imaging [18]. Our results from excretion experiments suggest that SiO₂ could be biodegraded and excreted out of body.

Previous studies have reported the biodistribution of nanoparticulate systems of similar size range with the SiO₂ system evaluated in our study (100 nm and up) [31–35]. Results suggest that the biodistribution pattern varies distinctively between inorganic nanoparticles and organic nanoparticles of similar dimensions. For example, colloidal gold nanoparticles (100 nm, 200 nm) showed high degree of uptake by the liver, spleen and lung with limited presence in blood [31], which is similar to the biodistribution of SiO₂ used in our study.

Whereas, polymeric systems, such as chitosan nanoparticles [32], poly(lactic-co-glycolic acid) (PLGA) nanoparticles [33], or liposomes [34], exhibited sustained presence in blood circulation in addition to RES accumulation. It has been demonstrated that nanoparticles of ultra-low size (< 100 nm in diameter) or with surface hydrophilicity evade the RES and have long circulation [35]. Hence in addition to the core composition, size, surface functionalization and other physicochemical properties play crucial roles.

5. Conclusions

Of the materials tested in our study, it was demonstrated that SiO₂ biodistribution was influenced more by nanoparticle porosity, surface characteristics, and less by geometry. The nanoparticles across the board showed extensive distribution into liver and spleen with different concentrations in the lung. Mesoporous SiO₂ accumulated in the lung to a higher extent than nonporous SiO₂ of similar size. Such accumulation was reduced by primary amine modification. However, high aspect ratio amine-modified nanorods showed higher lung accumulation than the amine-modified nanospheres. Results from tissue affinity indices and tissue/blood concentration ratio kinetic analyses suggest that tissue affinity was mainly porosity and surface characteristics dependent. Nonporous SiO₂ exhibited high affinity to the liver, and mesoporous SiO₂ had higher affinity to the lung. Amine modification reduced the affinity of SiO₂ to the lung and kidneys. Two-compartmental pharmacokinetic analysis showed that amine-modified SiO₂ tended to have higher V_{ss} than the pristine counterparts and that nonporous SiO₂ exhibited a higher V_{ss} than mesoporous SiO₂. SiO₂ could be degraded and excreted out of the body by both urinary and hepatobiliary routes. This study enables the systematic understanding of how physicochemical factors affect the living system and facilitates the rational design of SiO₂ for their intended applications in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Schematic illustration of nanoparticle selection, radiolabeling, and animal administration for biodistribution and pharmacokinetics studies. The insert table [23] shows the overall physicochemical characterization of SiO₂.

Fig. 2. Biodistribution of SiO_2 with varied geometry, porosity, and surface characteristics: A) Meso S, B) MA, C) AR8, D) 8A, E) Stöber, and F) SA in healthy mice post bolus tail vein injection at a dose of 20 mg/kg. Organ accumulation is expressed as percent of injected dose per gram of tissue post euthanasia at 5 minutes, 30 minutes, 2 hours, 24 hours, and 72 hours. Data are presented as mean $\pm$ standard deviation ($n = 5$).

Fig. 3.

Two-compartmental pharmacokinetic analysis of SiO₂ biodistribution: A) Meso S, B) MA, C) AR8, D) 8A, E) Stöber, F) SA in healthy mice. Activity in the blood is converted to nanoparticle concentration using percent injected dose per gram blood and assumes a blood density of 1.0 g/mL.

Fig. 4. Pharmacokinetic parameters (A) clearance and B) V_{ss} based on the two-compartmental analysis for the nanoparticles. There was no significant difference in clearance among all nanoparticles in blood ($p > 0.05$). Amine-modified SiO₂, MA or SA, exhibited significantly higher V_{ss} than their pristine counterparts, Meso S (** $p < 0.01$) or Stöber (*** $p < 0.001$). Stöber showed a significantly higher V_{ss} than Meso S (* $p < 0.05$).

Fig. 5. Tissue/blood concentration ratio of various SiO₂ in A) liver, B) spleen, C) lung, and D) kidneys. Meso S showed a significantly higher lung/blood concentration ratio than Stöber (***) ($p < 0.001$) or MA (***) ($p < 0.001$) within 3 days. Data are presented as mean $\pm$ standard deviation (n = 5).

Table 1

Tissue affinity indices of SiO₂ of various geometries, porosities, and surface characteristics in major organs of CD-1 mice.

Treatment	Tissue affinity index			
	Liver	Spleen	Lung	Kidneys
Meso S	46.9	93.5	138.9	3.2
MA	82.9	172.3	1.4	0.8
AR8	80.7	193.9	41.8	3.4
8A	49.4	180.5	17.2	2.0
Stöber	186.0	148.1	6.0	3.6
SA	249.5	113.9	4.5	1.3

Summary of engineered SiO₂ with various physicochemical properties and their *in vitro* and *in vivo* evaluation results

Table 2

Nanoparticle type	Physicochemical properties ^a				In vitro ^d			In vivo				Pharmaco-kinetics		
	Geometry by TEM (nm)	Porosity	Surface charge in water	DLS size in serum (nm)	IC ₅₀ (µg/mL)		Cellular association (µg Si/100 µg protein)		Safety ^b		Biodistribution			
					M	E	M	E	MTD	Impaired organ (s) above MTD	Geometry		Porosity	Surface characteristics
Stöber	115	Nonporous	----	121.6	73	/	21.2	1.5	450	Heart, lung, spleen	/	Liver	Lung, kidneys	0.20
Meso S	120	Mesoporous	---	268.9	89	/	0.7	0	30	Kidneys	/	Lung	Lung, kidneys	0.09
AR8	136 × 1028	Mesoporous	---	N/A	74	/	0.4	0	65	Kidneys	/	/	Lung, kidneys	0.07
SA	115	Nonporous	++	N/A	254	/	14.8	1.0	450	Lung, kidneys	/	Liver	/	0.37
MA	120	Mesoporous	+++	150.3	182	/	3.3	0.4	150	Lung, kidneys	/	/	/	0.23
8A	136 × 1028	Mesoporous	+++	N/A	225	/	4.1	0.8	100	Lung, kidneys	Lung	/	/	0.09

^a content adapted from reference [23], surface charge is ranked as ---- highly negative -60 – -40 mV, --- highly negative -40 – -30 mV, ++ moderately positive 10 – 20 mV, +++ highly positive 20 – 40 mV. N/A means not available. M or E refers to macrophages RAW 264.7 or cancerous epithelial cells A549, / means not observed in the study design.

^b content adapted from reference [24], for column under biodistribution, it means that the physicochemical property (indicated in the corresponding column) of nanoparticles of a specific type (indicated in the corresponding row) led to higher affinity in specific organs (indicated in the crossed cell) than nanoparticles of correspondingly different property (nonporous *versus* mesoporous, pristine *versus* amine-modified, or nanospheres *versus* nanorods). / means that this effect is not observed in the study design.