Immunotoxicity of Silica Nanoparticles as a Function of Physicochemical Properties

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Objective

To synthesize and characterize four silica nanoparticles with distinct physicochemical properties and evaluate the effect of size and porosity on cytotoxicity, cell uptake, saturation and immune function in RAW 264.7 macrophages.

Introduction

Silica-based nanoparticles have shown significant potential in biomedical applications such as controlled drug delivery, theranostics, and imaging due to their tunable physicochemical properties. The safety profile of silica nanoparticles (SNPs) has not been fully established, which is crucial for clinical translation and advancement. A detailed examination of the interaction of silica nanoparticles with our immune system and the downstream effects is warranted. There is a knowledge gap on how the physiochemical properties of SNPs such as size, charge, geometry and porosity influence their immunotoxicity. A major concern is the saturation of mononuclear phagocytic system. Our objective in this work is to find the saturation doses of SNPs in murine macrophages and investigate their immunotoxicity at these doses and as a function of silica nanoparticle physiochemical properties.

Methods

Nonporous spherical SNPs with diameters of 68.4 ± 19.3 nm and 111.0 ± 27.9 nm (SNP50 and SNP100, respectively) were fabricated using the modified Stöber process. Mesoporous and hollow mesoporous SNPs with diameters of 134.4 ± 447.2 nm and 131.6 ± 41.3 nm (MSNP100 and HMSNP100, respectively) were fabricated using the surfactant hexadecyltrimethylammonium bromide (CTAB). CTAB was removed by acidic ethanol wash and was confirmed via FTIR analysis. The size and morphology of all particles were determined using DLS and TEM. RAW 264.7 murine macrophages were cultured according to standard protocol and used for all following experiments. Cytotoxicity and maximum tolerated dose (MTD) of SNPs were determined in a concentration range of 800-6.25 µg/mL and analyzed using CCK-8. The rate of uptake and saturation doses was analyzed at MTD concentrations of SNPs and particle internalization was determined through quantification of silicon via ICP-MS. Cells were also imaged via TEM to visualize nanoparticle uptake and storage. Cell death at saturation doses was evaluated by Annexin/PI assay. Lastly, immune response gene expression analysis was conducted at SNP saturation doses via real-time PCR.

Conclusion

We conclude that SNP saturation of murine macrophages is dependent on nanoparticle physicochemical properties. Macrophages exhibit minimal toxicity at SNP saturation doses. This gives us the opportunity to further investigate the correlation between the physicochemical properties of SNPs, as a carrier platform, and the mechanisms of immune response alteration *in vitro* and *in vivo*.

Acknowledgements

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Results

Silica Nanoparticle Characterization

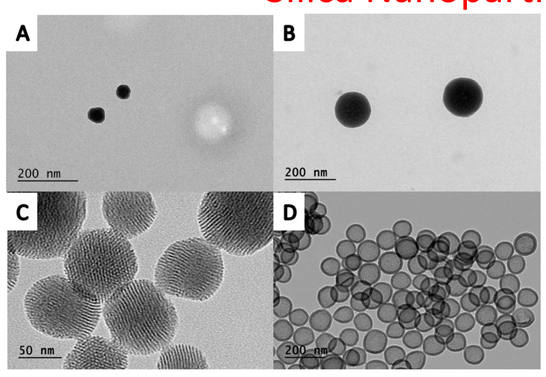

Figure 1. Transmission electron microscopy (TEM) images of (A) SNP50; (B) SNP100; (C) MSNP100; and (D) HMSNP100

Nanoparticle	Hydrodynamic Diameter	PDI	Zeta Potential
SNP50	68.4 ± 19.3	0.137	-9.9 ± 3.9
SNP100	111.0 ± 27.9	0.039	-48.3 ± 7.1
MSNP100	134.4 ± 47.2	0.121	-6.8 ± 3.6
HMSNP100	131.6 ± 41.3	0.087	-16.1 ± 4.3

Table 1. Size and zeta potential of silica nanoparticles

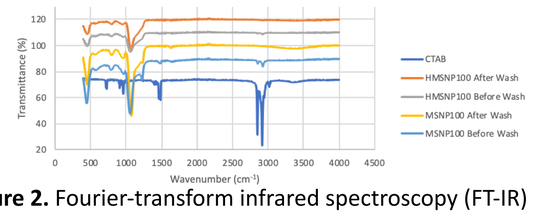

Figure 2. Fourier-transform infrared spectroscopy (FT-IR) spectra of porous silica nanoparticles before and after CTAB extraction

Cytotoxicity and Maximum Tolerated Dose

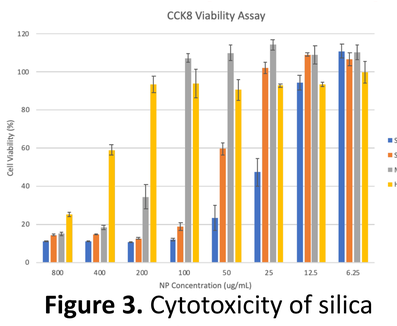

Figure 3. Cytotoxicity of silica nanoparticles in RAW 267.4 macrophages after 24 h

Nanoparticle	Maximum Tolerated Dose mg/mL (24 h exposure)
SNP50	50
SNP100	100
MSNP100	200
HMSNP100	200

Table 2. MTD of silica nanoparticles in RAW 267.4 macrophages

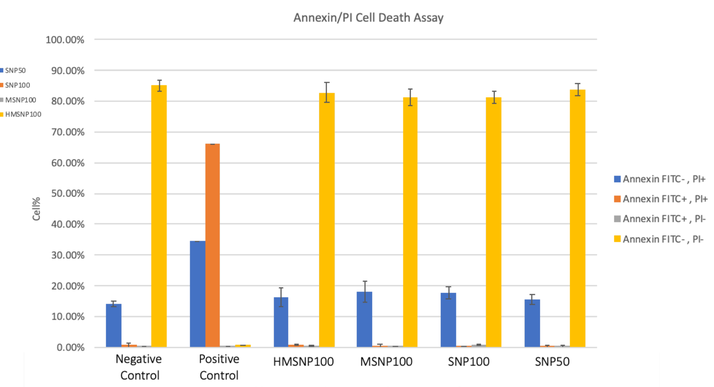

Figure 4. Cell death analysis of RAW 267.4 macrophages at silica nanoparticle MTDs

Silica Nanoparticle Uptake

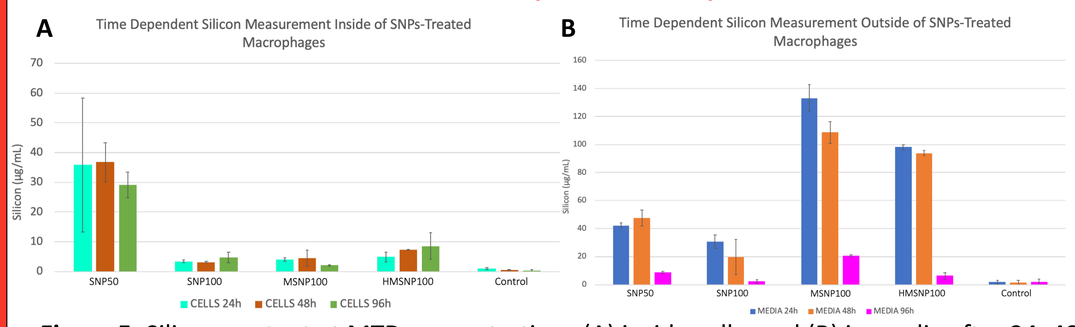

Figure 5. Silicon content at MTD concentrations (A) inside cells; and (B) in media after 24, 48, and 96 h incubation

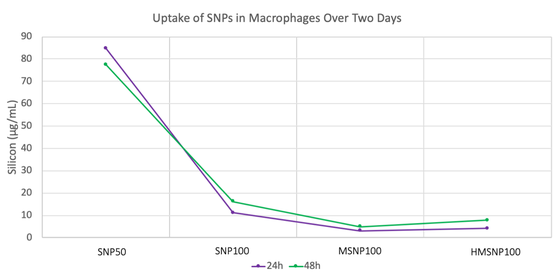

Figure 6. Intracellular silicon content at MTD concentrations showing saturation occurring at 24 h incubation

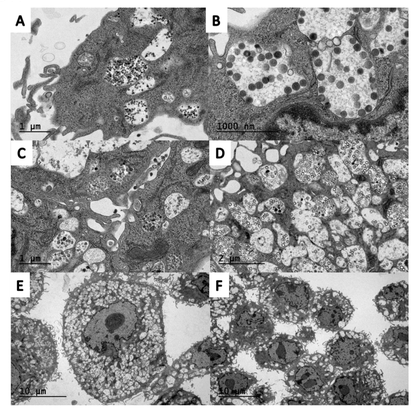

Figure 7. TEM images of SNP-saturated macrophages. (A) SNP50; (B) SNP100; (C) MSNP100; (D) HMSNP100; (E) Giant Macrophage; (F) Control

Gene Expression Analysis

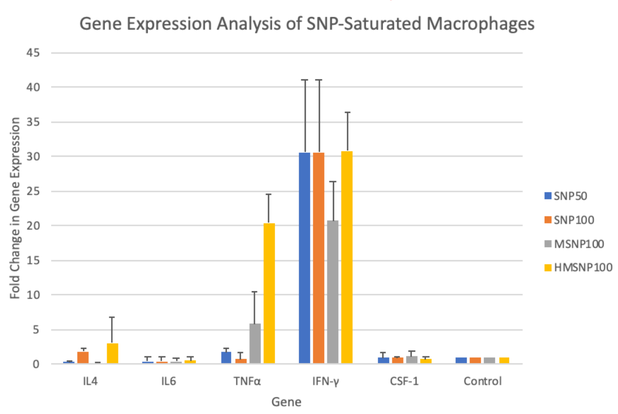

Figure 8. Real-time PCR analysis of immune response mRNA expression of SNP-saturated RAW 267.4 macrophages at 24 h incubation