Surface Amination Enhances the Toxicity of Silica-Coated Silver Nanoparticles

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ABSTRACT

Surface charge can greatly influence the toxicity and bioavailability of engineered nanoparticles (NPs). Positively charged NPs generally elicit greater toxicity than comparable negative or neutrally charged particles. The effect of amination on NP toxicity was investigated in embryonic zebrafish, a model vertebrate. Embryos were exposed to 0.5-100ppm suspensions of 80nm silica (Si) or 70nm silver with 20nm Si-coating (AgSi), or aminated NPs of like size and composition. Surface amination significantly increased the toxicity of the NPs. Si NPs did not induce mortality or morbidity at the tested concentrations, whereas aminated Si induced 58% mortality at 100ppm. Both AgSi NPs were significantly more toxic to embryos than SI NPs. AgSi NPs significantly delayed development at 24 hours post fertilization in 25 (50%), 50 (64%) and 100 (75%) ppm treatments, and caused significant mortality beginning at 10ppm. In comparison, aminated AgSi NPs delayed development significantly as low as 1 ppm and induced significant mortality at 5ppm, with 100% mortality above 50ppm. Both AgSi NPs induced significant sublethal effects, including craniofacial and fin malformations, edemas, and body curvatures. When similar silica coated Ag NPs with varied surface amination levels (0.5x, 1x, and 2x) were tested at the same concentrations, the AgSi-1x was significantly more toxic than the 0.5 or 2x AgSi NPs, inducing mortality at 100 (95%) ppm. In contrast, AgSi-2x only induced 38% mortality at 100 ppm, and AgSi-0.5x NPs did not cause toxicity at any concentration tested. Increased amination was observed to alter the stability of the aminated NP dispersions in the exposure media, with the 2x NPs being the least stable in suspension. Our results suggest that increasing surface amination only leads to increased toxicity when bioavailability is held constant, highlighting the importance of understanding NP stability and bioavailability during exposure.

BACKGROUND

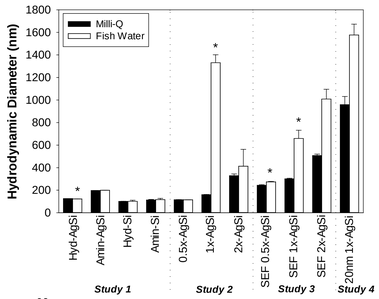
- Surface charge plays a key role in cellular uptake and biological actions of nanomaterials [1].
- Cationic groups on NP surfaces are more likely to interact with the negatively charged cell membrane surface: enhancing **binding** and **internalization** [1-3].
- Amination has been shown to increase nanoparticle uptake in multiple cell culture systems [4-6].

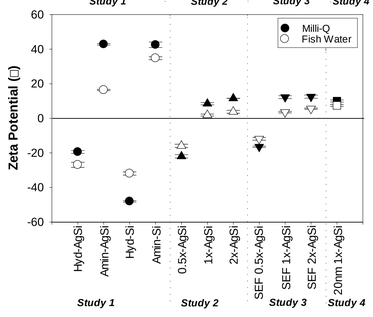
❖ *The overall objective is to investigate the role of core, shell, and surface amination on uptake and toxicity of NPs.*

HYPOTHESES

1. Surface amination is a greater indicator of toxicity than core in like-sized NPs.
2. Increased amination will lead to increased NP toxicity.
3. Increased amination will lead to increased NP uptake.
4. Where surface amination is equivalent, size will be the dominant factor in driving toxicity.

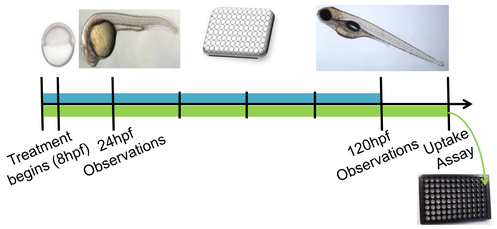
NP Characterization





- Hydrodynamic diameter and zeta potential were measured for 50ppm suspensions of the different NPs (* indicate sig. different)
- Terminal amines increased the hydrodynamic diameter of the NP, and shifted the zeta potential from negative to positive
- Fish water significantly decreased the absolute value of zeta for most of the NPs tested, and increased polydispersity (not shown)

EXPERIMENTAL DESIGN



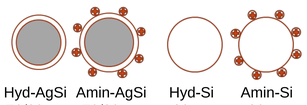
Embryonic Zebrafish Assay

- Dechorionated embryos are individually exposed in 96-well plates at 8 hours post fertilization (hpf) to nominal NP concentrations (0.1-100 ppm)
- 24hpf observations: mortality, developmental progression, notochord anomalies, and spontaneous movement
- 120hpf observations: mortality, axis, brain, circulation, eye, caudal/pectoral fins, jaw, otic, pigment, heart, yolk sac edema, snout, swim bladder, somites, trunk, and 'touch response'

Uptake Assay

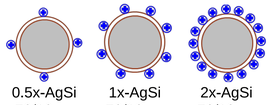
- Dechorionated embryos are individually exposed at 8 hpf to 50 ppm of 0.5x, 1x, or 2x AgSi-SEF NPs (FITC labeled)
- Embryos were rinsed to remove loosely associated NPs (approximately. 20ml/8 embryos) prior to homogenization
- Homogenate was scanned on a spectrofluorometer (excitation = 490nm, emission = 525nm), and quantified using a calibration curve made from the NP + embryo homogenate

Study 1



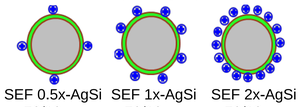
Hydroxyl- or Amine-terminated silca-coated silver (AgSi) or silica (Si) NPs

Study 2



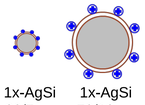
AgSi NPs with relative variation in surface amination

Study 3



Varied-amine AgSi NPs with FITC embedded in the silica shell

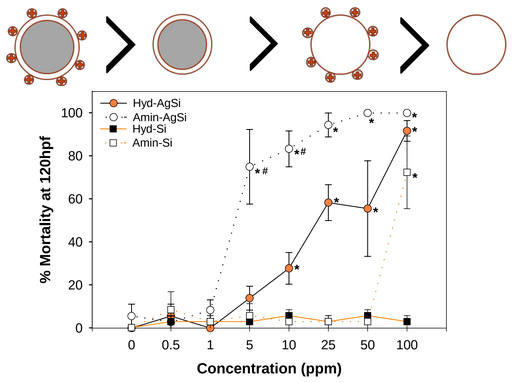
Study 4



1x-AgSi NPs with two different sized cores

RESULTS

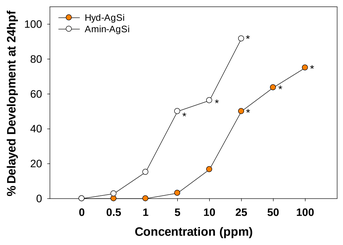
Study 1



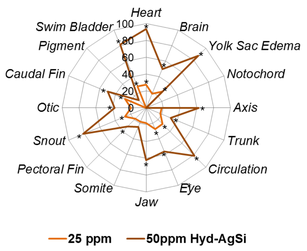
- Amine-terminated NP were more toxic than the hydroxyl-terminated forms for both the AgSi and the Si NPs
- Both AgSi NPs were more toxic than the Si NPs, indicating that silver played a role the toxicities of those NPs

(*significantly different from control, #significantly different from other NP)

Developmental Delay



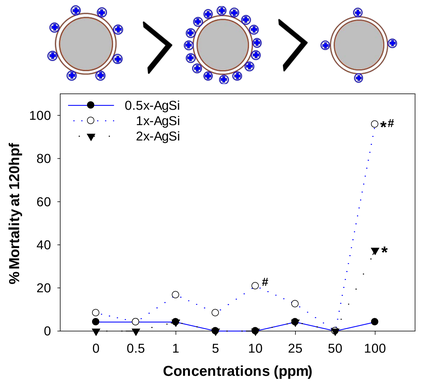
Malformations



- Both hydroxyl- and amine-terminated AgSi NPs delayed development at 24 hpf
- Significant malformations were observed in zebrafish exposed to 25 and 50ppm hydroxyl-AgSi NPs

(*significantly different from control)

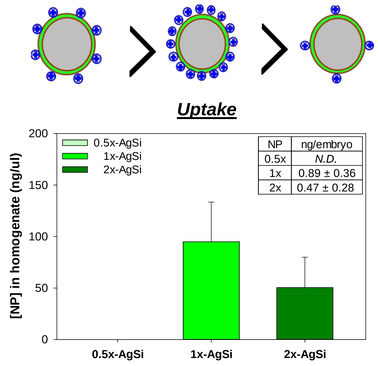
Study 2



- 1x-AgSi NPs were the most toxic of the varied-amination NPs
- 2x-AgSi NPs exhibited moderate toxicity, while 0.5x-AgSi NPs did no induce toxicity at the concentrations tested

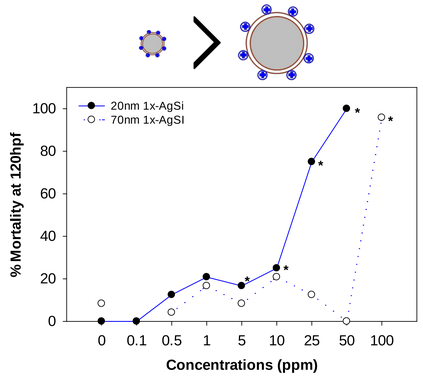
(*significantly different from control, #significantly different from other NPs)

Study 3

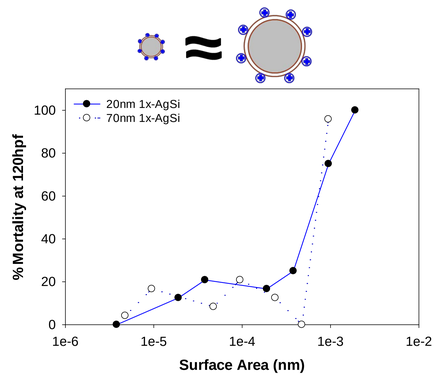


- 1x-AgSi NPs were taken up almost twice as much as the 2x-AgSi NPs, while uptake of the 0.5x-AgSi NPs was below detection

Study 4



- 20nm 1x-AgSi NPs were more toxic than 70nm 1x-AgSi NPs when compared with mass-based doses



- However, when mass doses were converted to surface area of the NPs, the dose responses were not different

SUMMARY

- Surface amination increased the toxicity of the NP, but the composition of the NP also contributed to toxicity. (Study 1)
- Increased toxicity was not directly related to increased amination, indicating other factors played a role in NP uptake and toxicity. (Study 2 & 3)
- When surface area was applied as the dose metric, the differences in toxicity attributed to NP size appeared to be a product of mass-based concentrations. (Study 4)

CONCLUSIONS

- Surface amination only increases toxicity when bioavailability is held constant, thus future studies should look at the impact of amination on NP bioavailability and stability within a suspension.
- A surface area-based dose metric may more accurately depict the dose response of NP toxicity, when comparing across sizes of NPs of similar composition and surface chemistries.

Acknowledgements

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