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## Toxicity test: Fluorescent silicon nanoparticles

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**Abstract.** Semiconductor nanoparticles ('quantum dots', QDs) are useful fluorescent materials because of their high fluorescent stability compared with existing organic fluorescent dyes. QDs were tested in many biochemical experiments, and the reported results suggested their advantages. However, when we consider their application at the clinical level, their large-scale use may be problematic because of their influence on the environment and the living body as a result of cadmium contained in existing mainstream QDs. Here we report on the characteristics of silicon particles (synthesised using the gas phase method and liquid phase method, currently in the development stage) as a substitute material, focusing on cell-level safety and the potential mechanisms of toxicity.

### 1. Introduction

Semiconductor quantum dots (QDs) exhibit brighter and longer fluorescence than organic dyes. Taking advantage of these characteristics, QDs are used as fluorescent labels in many biomedical studies [1-3]. Currently, a CdSe core with a ZnS shell is one of the most popular QDs used in

biological experiments. However, for clinical technologies, the potential toxicological problems because of the CdSe core should be considered [4]. As a substitute for cadmium-based QDs, we selected silicon as the semiconductor material and have developed photoluminescent stable silicon dots [5-9].

Silicon nanocrystals have been synthesised using aerosol, colloids, laser pyrolysis, plasma deposition and electrochemical etching. Recently, we reported two methods; silicon particles produced in a gas phase [5,6] and in a liquid phase [7-9]. In the former method, silicon chips/SiO<sub>2</sub> targets were sputtered in the argon phase and then the SiO<sub>2</sub> layer surrounding silicon nanocrystals was chemically etched. Finally, the surfaces of the silicon particles were oxidised. The latter method involved synthesising in inverse micelles and stabilising with ‘double-bond molecules’ capping. Therefore, the particles synthesised using latter method has a high modification property. Both types of particles have blue or blue green fluorescence in culture medium or phosphate buffered saline (PBS) when excited with UV light. In co-cultures of the particles and cells, fluorescence was observed in the cells [5,7,9].

## 2. Silicon particle properties

Table 1 shows the characteristics of silicon particles synthesised using the two methods. Of the particles synthesised using the gas phase method, the size of the primary particle was 2.5–6.5 nm. The dynamic light scattering (DLS) measurement in liquid was 338 ± 22 nm. We believe that these measurements represent the sizes of secondary and tertiary particles. We reported that the particle surface is oxidised, and IR measurements suggest presence of some hydroxyl groups [6]. Hence, agglomeration and sedimentation may occur in several hours probably due to a small number of hydroxyl groups. As such, in the future, dispersal enhancement through surface modification will be needed.

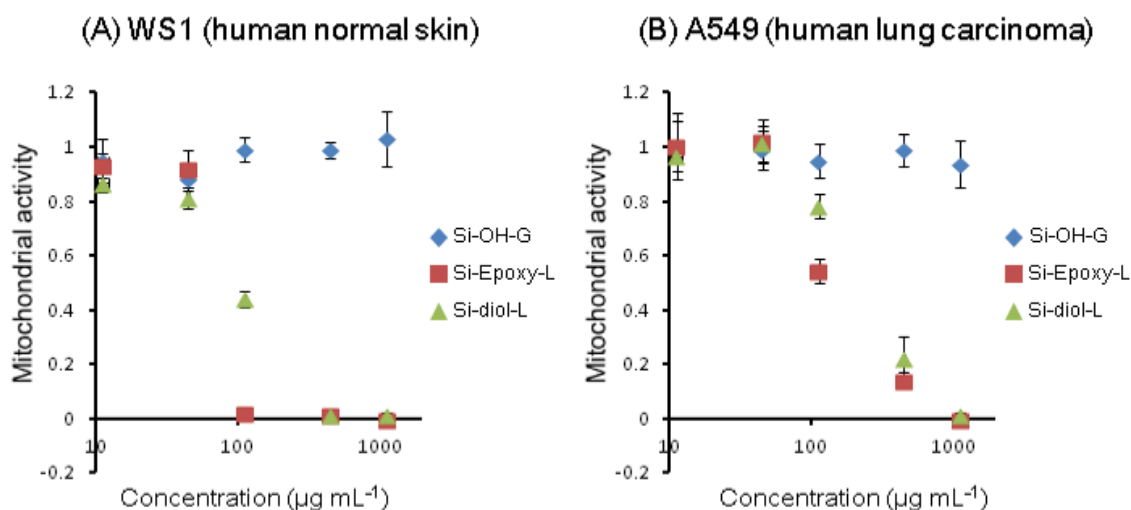
On the other hand, the size of the particles synthesised using the liquid phase method was 1–2 nm and the DLS measurement was 144 ± 11 nm. Note that on the particle surface, the molecules with double bonds can be modified using a platinum catalyst.

**Table 1.** Characteristics of silicon particles synthesised using the gas phase method or the liquid phase method

	Gas Phase Method	Liquid Phase Method
<b>Diameters of primary particles (TEM)</b>	2.5–6.5 nm	1–2 nm
<b>Diameters of agglomeration of NPs (Z-average; DLS)</b>	338 ± 22 nm	144 ± 11 nm
<b>Surface modification</b>	Oxide-passivated	Tuneable (Si-C bond)
<b>Water dispersion</b>	△: Precipitates within a few hours	○: Depends on the surface chemicals
<b>Radial species generation</b>	Yes	Not detected

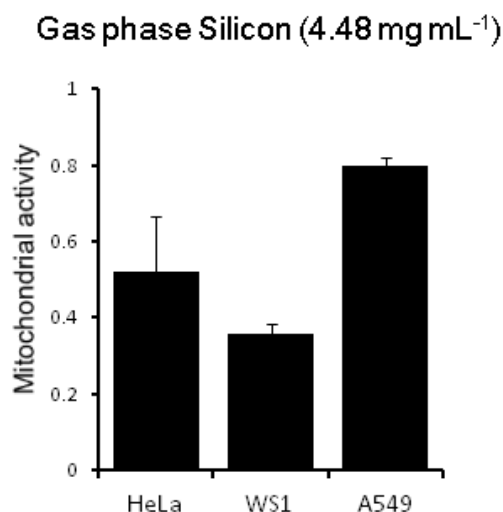
## 3. Silicon particle toxicity in skin and lung cell lines

Figure 1 shows the results of toxicity tests using human skin and lung cell lines, since the skin and lungs are the body tissues contact with the silicon particles firstly. In our previous study, we reported the results of mitochondria activity test for the particles synthesised using the liquid phase method [7]. For comparison, in this article, we added present test results for particles synthesised using the gas phase method.



**Figure 1.** Toxicity test of silicon particles in human cell lines. A: WS1 normal skin cell line, B: A549 lung carcinoma cell line. Si-OH-G: Gas phase silicon (non-coating); Si-Epoxy-L: Liquid phase silicon (Epoxy coating) [7]; Si-diol-L: Liquid phase silicon (diol coating) [7]

For both skin and lung cell lines, the toxicity was the lowest with the silicon particles synthesised using the gas phase method (Figure 1). The low toxicity may depend on the unmodified surface state. Even in the particles of this type, the influence of toxicity can be observed in both WS1 and A549 cells, but more greatly in WS1 cells, at the high concentration of  $4.48 \text{ mg mL}^{-1}$  (Figure 2).

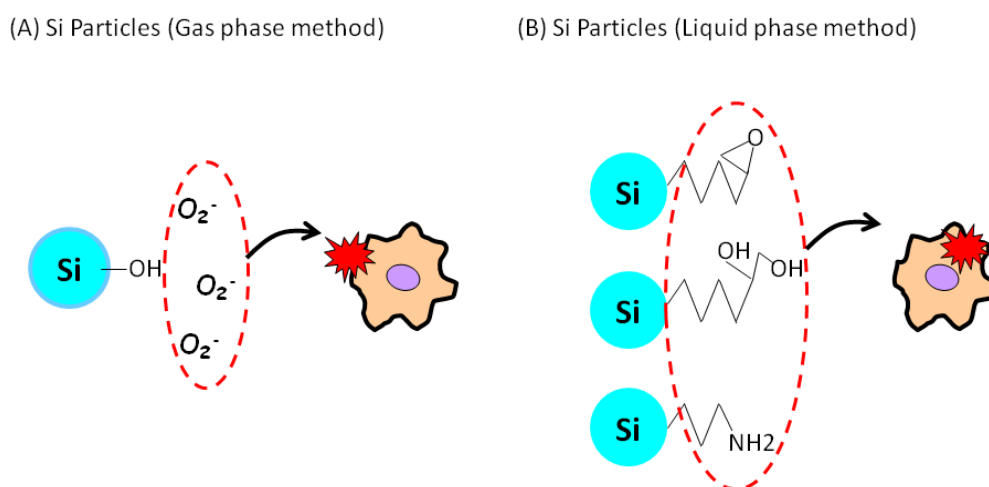


**Figure 2.** Mitochondrial activity at high concentrations of silicon particles synthesised using the gas phase method. HeLa: Human cervical carcinoma; WS1: Human normal skin; A549: Human lung carcinoma.

On the other hand, in case of particles synthesised using the liquid phase method, the influence of toxicity was more evident in the WS1 skin cell line than in the A549 lung cell line. A large difference was noted in the mitochondria activity at concentrations exceeding  $112 \mu\text{g mL}^{-1}$ . We believe that the cell type-dependent sensitivity is due to the metabolic capability of each cell line. Thus, while using these particles, we must consider the influence of the particles on body tissues and accordingly select the particle concentration.

#### 4. Potential toxicity mechanisms of silicon particles

We report the toxic mechanism of silicon particles that we have clarified. As we mentioned earlier, the toxicity of silicon particles synthesized in gas phase method is lower compared with that of mercaptoacetic acid coated QDs in MTT assay method [5]. However, toxicity of silicon particles can be observed at higher concentrations. We confirmed that from the silicon particles using the gas phase method, radicals were produced in small quantities and the cell membrane was oxidised [5]. We suggest that the cell membrane is damaged by the radical at higher concentrations, leading to cell death (Figure 3(A)). In addition, by using superoxide dismutase and *N*-acetylcysteine, we succeeded in suppressing radicals that would have been generated from silicon particles synthesised using the gas phase method [10]. Thus, we may reduce toxicity by adding materials with radical inhibitory activity in the surface modification step or in use of silicon particles.



**Figure 3.** Scheme of potential toxicity mechanisms by silicon particles synthesised using gas phase method (A) or liquid phase method (B).

On the other hand, we suggest that particles synthesised using the liquid phase method have a different toxic mechanism (Figure 3(B)). We did not observe any radical generation from the liquid-phase synthesized particles (data not shown). Limited objective data show that toxicity is higher in epoxy in which chemical activity is high than in diol, suggesting that surface molecules affect toxicity [7].

#### 5. Conclusion

In this report, we compared the characteristics of silicon particles synthesised using the gas phase method and the liquid phase method, focusing on the toxicity test using cells and the toxic mechanism. Radicals and surface molecules were identified as potential causes of toxicity. At present, despite the limitation of lower luminance relative to existing QDs, silicon particles have good potential as fluorescent materials considering the relatively low toxicity and the abundance of silicon in the earth. Given their small influence on the environment and the living body, they may have a bright future if we develop particles with increased fluorescence.

#### 6. Materials and Methods

##### 6.1. Assays for Cellular toxicity

We have reported the detail method in previous papers [5,7]. Briefly, the cell lines were cultured in each well of 96-well plates for 48 hr at 37 °C, and then, the silicon nanoparticles were co-cultured in indicated concentration for 48 hr. Finally, for the measurement of mitochondrial activity, Cell

Counting Kit-8 (Dojindo, Japan) was added and the 450-nm absorption of formazans were measured with plate reader.

## 6.2. Measurement of Dynamic Light Scattering

The diameters of particles in liquid were measured with dynamic light scattering using Zetasizer nano (Malvern instruments, UK).

## Acknowledgement

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## References

- [1] Manabe N, Hoshino A, Liang Y, Goto T, Kato N and Yamamoto K 2006 *IEEE Trans. Nanobiosci.* **5** 263-7
- [2] Wu X, Liu H, Liu J, Haley K N, Treadway J A, Larson J P, Ge N, Peale F and Bruchez M P 2003 *Nat. Biotechnol.* **21** 41-6
- [3] Yamamoto S, Manabe N, Fujioka K, Hoshino A and Yamamoto K 2007 *IEEE Trans. Nanobiosci.* **6** 94-8
- [4] Derfus A, M., Chan W C W and Bhatia S N 2004 *Nano Lett.* **4** 11-8
- [5] Fujioka K, Hiruoka M, Sato K, Manabe N, Miyasaka R, Hanada S, Hoshino A, Tilley R D, Manome Y, Hirakuri K and Yamamoto K 2008 *Nanotechnology* **19** 415102
- [6] Shinoda K, Yanagisawa S, Sato K and Hirakuri K 2006 *J. Cryst. Growth.* **288** 84-6
- [7] Shiohara A, Hanada S, Prabakar S, Fujioka K, Lim T H, Yamamoto K, Northcote P T and Tilley R D 2010 *J. Am. Chem. Soc.* **132** 248-53
- [8] Tilley R D, Warner J H, Yamamoto K, Matsui I and Fujimori H 2005 *Chem. Commun.* **2005** 1833-35
- [9] Warner J H, Hoshino A, Yamamoto K and Tilley R D 2005 *Angew. Chem. Int. Ed.* **44** 2-6
- [10] Hanada S, Fujioka K, Hoshino A, Manabe N, Hirakuri K and Yamamoto K 2009 *Proc. SPIE, Colloidal Quantum Dots for Biomedical Applications IV*, vol 7189 (The International Society for Optical Engineering) 71891A