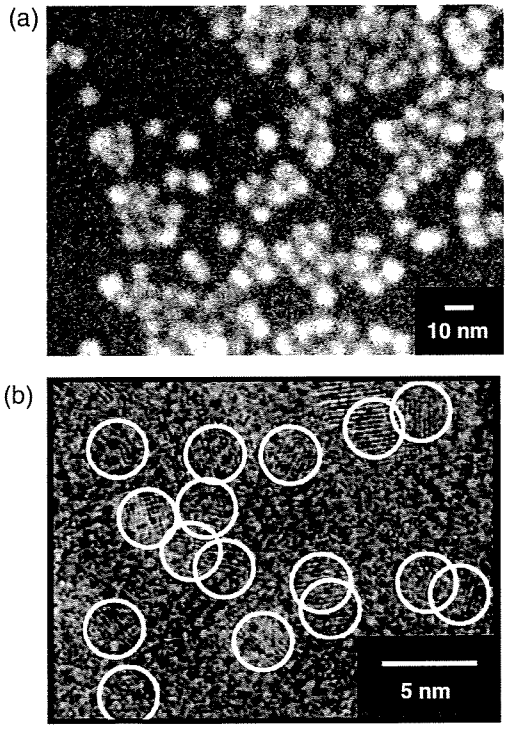

Figure 1. (a) SEM image and (b) HRTEM image of silicon quantum dots (Si-QDs).

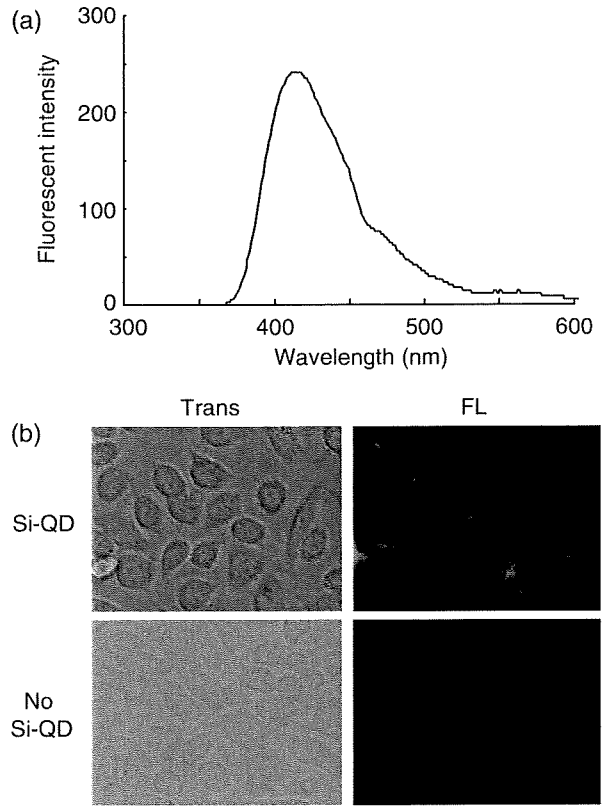

Figure 2. (a) Photoluminescent spectrum of Si-QDs excited at 300 nm. (b) Transmitted (trans) and fluorescent (FL) microscopy images of HeLa cells labelled with Si-QDs for 48 h incubation (upper), and without Si-QDs (lower).

(This figure is in colour only in the electronic version)

450 nm absorption of formazans produced by the enzyme was measured with an E max (Molecular Devices, USA) microplate reader ($n = 2$–6). The activities were calculated as the ratio of the absorbance value against those of the control. For the measurement of LDH releases from cells, we used an LDH Cytotoxicity Detection Kit (Takara Bio, Japan) as an indicator for plasma membrane leakage ($n = 5$). The 490 nm absorption of formazans as an indicator of LDH releases were measured with the E max microplate reader. The cytotoxicity was calculated as the ratio of the LDH release from the sample against that of the positive control for cell-death, which was co-cultured with 200 μM $CdCl_2$ (Wako Pure Chemicals, Japan), and the negative control, which was cultured in MEM-α. For statistical analysis, the data were analysed using the Tukey–Kramer's test for multi-group comparison. Differences were considered significant at $P < 0.05$.

2.5. *Radical assays*

Radical species was measured with a LumiMax Superoxide Anion Detection Kit (Stratagene, USA). Si-QDs in the concentration of 1120 μg ml^{-1} were mixed with the reagents of the kit 10 min before the measurements. The chemiluminescence as an indicator of oxiradicals was measured with a microplate fluorometer, Fluoroskan Ascent FL (Thermo scientific, USA), with 5 min intervals ($n = 2$). Lipid peroxidation was assayed with a BioxTech MDA-586 assay kit (OXIS International, USA). The cells were cultured with Si-QDs at the concentration of 1120 μg ml^{-1} for 48 h, the same as for the cytotoxicity assay ($n = 2$). As an indicator of peroxy radicals, we assayed malondialdehyde, which can react with *N*-methyl-2-phenylindole and yield a stable chromophore with maximal absorbance at 586 nm, using an Ultrospec 2100 pro (GE Healthcare, UK) absorbance spectrometer.

3. Results and discussion

3.1. *Characterization and bioimaging studies*

Figure 1(a) shows the SEM image of a number of water-soluble Si-QDs on a carbon grid. The mean diameter of Si-QDs was 6.5 ± 1.5 nm. The TEM image shows the core structure of Si-QDs (figure 1(b)). The average structure size was 2.5 nm.

The photoluminescence spectrum in the phosphate buffered saline (PBS) excited at 300 nm shows a peak at 414 nm (figure 2(a)). The suitability of Si-QDs as a chromophore for biological imaging is demonstrated in figure 2(b). The Si-QDs were incubated with HeLa cells for 48 h and observed with a fluorescence microscope. Compared to the control, which was cultured in no-particle media, green fluorescence was observed brightly in the HeLa cells cultured with the Si-QDs. From this result, the green fluorescence has arisen from the emission of Si-QDs. Furthermore, the fluorescence was observed mainly on the cell surface.

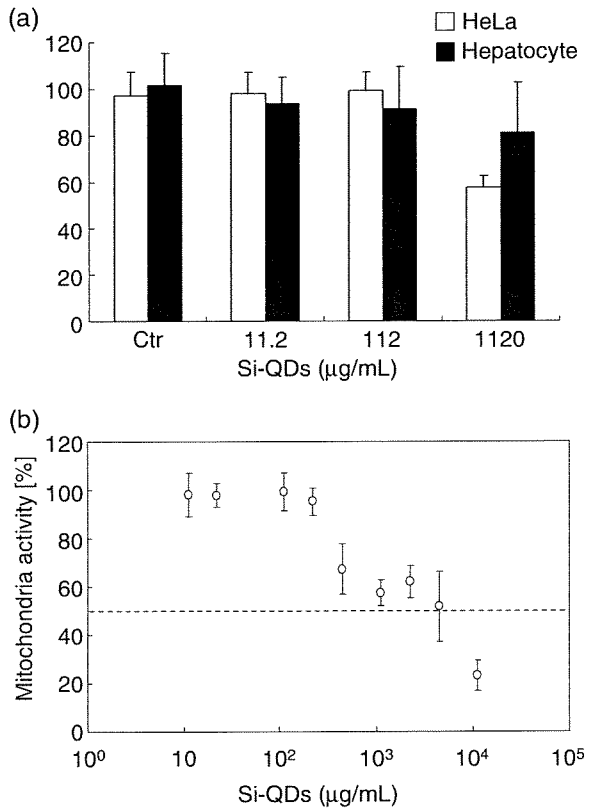

Figure 3. Effects of QDs on human cells. (a) Comparison of Si-QD toxicity against HeLa cells and hepatocytes. (b) Mitochondrial activity of HeLa cells cultured with Si-QDs.

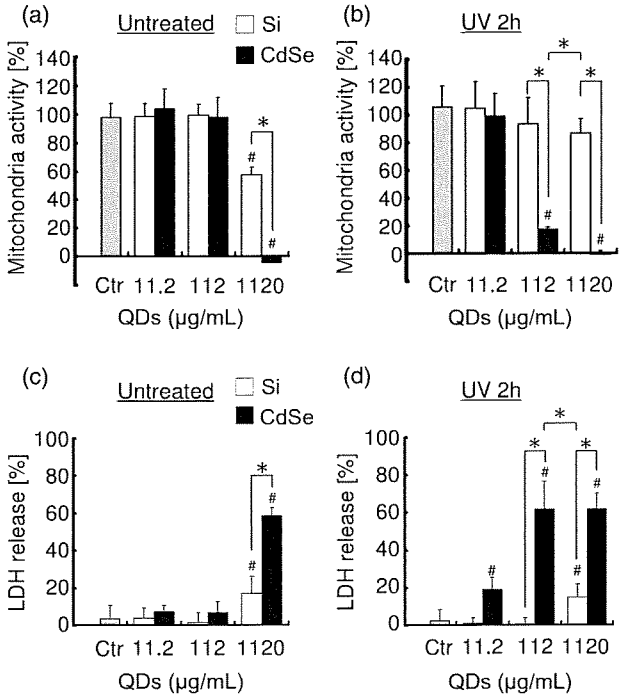

Figure 4. Comparison of QD toxicity on the mitochondrial activity ((a), (b)) and the LDH release ((c), (d)) after 48 h incubation. HeLa cells were incubated with untreated QDs ((a), (c)) and UV-exposed (2 h) QDs ((b), (d)). Si: Si-QDs, CdSe: CdSe-QDs. Values are mean ± standard deviations. # represents the value being significantly different from the control ($P < 0.05$). * represents the difference being significant ($P < 0.05$).

These findings indicated the possibility of the Si-QDs as chromophores in bioimaging.

3.2. *In vitro cytotoxicity and TC50*

In order to use Si-QDs widely in bioimaging, we have to know the limit concentration at which the Si-QDs do not affect cell activity.

First, we measured the mitochondrial activities in 0–1120 μg ml^{-1} of Si-QDs with HeLa cells as a carcinoma cell line, and also primary human hepatocytes for the simulation of *in vivo* toxicity. Mitochondrial activity assays indicate cell viability. As shown in figure 3(a), the Si-QDs at 1120 μg ml^{-1} were less toxic against the hepatocytes than HeLa cells. Since our previous study also showed that HeLa cells were more sensitive to the toxicity test than hepatocytes [18], we used HeLa cells in further toxicity tests. In figure 3(b), we show the mitochondrial activities of HeLa cells in 11.2–11 200 μg ml^{-1} of Si-QDs. From the result, we calculated the 50%-inhibitory toxicity concentration (TC50) with HeLa cells as 4761 μg ml^{-1}.

Second, in comparison with CdSe-QDs, we performed two types of cytotoxicity assay: a mitochondrial activity assay, and a lactate dehydrogenase (LDH) release assay, which indicates cellular membrane damages. The cells were cultured with the QDs in the medium for 48 h and tested. Additionally, in order to investigate the effects of degradation, we also used the QDs exposed to UV (254 nm) for 2 h before the co-culture.

Figure 4(a) shows the results of the mitochondrial activity assays with untreated Si-QDs. At a concentration of 1120 μg ml^{-1}, the mitochondrial activity with the Si-QDs decreased to 57 ± 5.3% of control ($P < 0.01$), which was significantly higher than that of CdSe-QDs ($P < 0.01$) (figure 4(a)). Additionally, with the UV-exposed Si-QDs at 1120 μg ml^{-1}, the activity decreased to 87 ± 10% (n.s.) and it was significantly higher than that of CdSe-QDs at 112 μg ml^{-1} ($P < 0.01$) (figure 4(b)).

In the LDH assays, at a concentration of 1120 μg ml^{-1}, the LDH release with Si-QDs increased to 17 ± 9.0% ($P < 0.01$), which was significantly lower than that of CdSe-QDs ($P < 0.01$) (figure 4(c)). Using the UV-exposed Si-QDs at 1120 μg ml^{-1}, the release increased 15 ± 6.8% ($P < 0.05$) (figure 4(d)). In concentrations of more than 11.2 μg ml^{-1}, the releases with Si-QDs are significantly lower than those with CdSe-QDs at the same concentrations ($P < 0.01$) (figure 4(d)).

These results from both cytotoxicity assays suggested that we should use Si-QDs at concentrations less than 112 μg ml^{-1}. However, Si-QDs were less toxic than CdSe-QDs against the HeLa cells at 1120 μg ml^{-1}. In particular, after being exposed to UV, the Si-QDs were at least ten times less toxic than the CdSe-QDs in concentrations more than 112 μg ml^{-1}.

The cytotoxicities of several QDs have been reported [17, 19, 20, 42, 43]. Derfus *et al* studied the cytotoxicity of UV-exposed CdSe-QDs against hepatocytes and indicated that the cell damages were caused by cadmium ions released from CdSe-QDs [17]. We also identified that cadmium ions were released from our CdSe-QDs in similar conditions, such as UV or H_2O_2 exposure (UV exposure for 2 h: 48 ppm; H_2O_2 exposure for 48 h: 3.6 ppm). This result suggested that cadmium ions are released from CdSe-QDs in highly oxidative conditions and that they then degrade, and then the ions induce cytotoxicity.

On the other hand, the cytotoxicity of Si-QDs and its mechanism has not been reported. Compared to CdSe-QDs, Si-QDs do not consist of inherently toxic elements. There are some reports that several nanocrystals, such as fullerenes and CdTe-QDs, produced reactive oxygen species and that the radicals induced cell damage [19, 20, 43, 44]. Since Si-QDs at high concentration caused LDH release, an indicator of cellular membrane damage, we suspected that Si-QDs produced oxygen radicals and that the radicals affect the cell membrane.

3.3. Toxicity mechanism of silicon quantum dots

To test the hypothesis that cell toxicity caused by Si-QDs is due to radicals, we investigated the ability of Si-QDs to provide oxygen radicals and detected the peroxy radicals in co-cultured cells. In the presence of oxygen radical species, peroxy radicals are formed on the alkene termini of the cellular lipid bilayer and they can associate, forming a hole in cytoplasmic membrane [45].

First, we confirmed the ability of Si-QDs to provide oxygen radicals using a luminol reaction. Figure 5(a) shows that Si-QDs in aqueous solutions can produce oxygen radicals. The maximum yield of oxygen radicals from Si-QDs was seven times higher and their generations were longer in duration than those of the CdSe-QDs (figure 5(a)). When Si-QDs were exposed to UV, the maximum yield was lower than for untreated Si-QDs (figure 5(a)). These results suggested that Si-QDs produce oxygen radicals more readily than CdSe-QDs and that the yields are reduced by UV exposure.

Second, we confirmed the formation of peroxy radicals in the co-cultured cells with malondialdehyde (MDA) as the indicator of peroxy radicals. We show in figure 5(b) that the concentration of peroxy radicals increased with 1120 μg ml^{-1} of Si-QDs with and without UV exposure. The mean concentration of the peroxy radicals in untreated Si-QDs was higher than that of UV-exposed Si-QDs.

Taken together, these radical results suggested that Si-QDs produce oxygen radicals and then the radicals affect the cytoplasmic membrane. Since the yields of oxygen radicals were reduced by UV exposure, the mechanism may be associated with the oxidative states of Si-QDs. Our previous study showed that the surface condition of Si-QDs was changed by oxidation [38, 39]. In addition, their surface potentials were reduced by UV exposure (data not shown).

Nanocrystals, such as fullerenes and CdTe-QDs, can also produce radical species, and the toxicities were reduced by surface modification [20, 44]. Sayes *et al* showed that the cytotoxicity of fullerenes was changed by their surface functional group [44]. Choi *et al* showed that the modification with *N*-acetylcysteine (NAC), which has anti-oxidant property [46], reduced the lipid peroxidation from CdTe-QDs [20]. In this study, we showed the reduction of radicals with UV exposure (figure 5(a)). Although the Si-QDs showed less toxicity than the CdSe-QDs at high concentration, in order to be a much safer chromophore *in vivo* and to protect the environment, Si-QDs also need a suitable surface modification, such as NAC, and oxidative-state control with UV exposure. This study may therefore provide the basis for future improvements on the cytotoxicity amelioration of Si-QDs and safety precautions required.

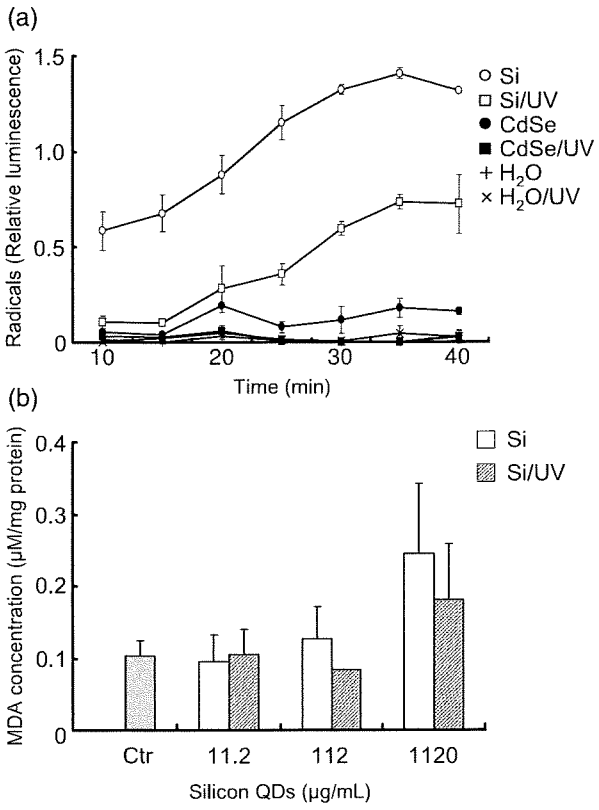

Figure 5. (a) Oxygen radical detection in QD solutions. QDs with and without UV exposure were incubated with luminol, and the chemiluminescence was measured at 5 min intervals after 10 min pre-incubation. Si: Si-QDs; Si/UV: UV-exposed Si-QDs; CdSe: CdSe-QDs; CdSe/UV: UV-exposed CdSe-QDs; H_2O: H_2O without UV exposure; H_2O /UV: H_2O with UV exposure. (b) Peroxy radical detection from the HeLa cells cultured with Si-QDs with and without UV exposure. Malondialdehyde (MDA) was used as the peroxy radical indicator. Si: Si-QDs, Si/UV: UV-exposed (2 h) Si-QDs. Values are mean ± standard deviations.

4. Conclusion

In conclusion, passive-oxidized Si-QDs can be used as bioimaging probes. The mitochondrial activity assays and LDH release assays revealed that the Si-QDs were safer than CdSe-QDs at high concentration, especially under UV

exposure conditions. However, the Si-QDs may become slightly toxic to cultured human cells at high concentration. As one of the toxicity mechanisms, we suggest that oxygen radicals from Si-QDs affect the plasma membrane of cells. These results are useful information to future applications of Si-QDs in biological and medical areas.

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