Functional-dependent and size-dependent uptake of nanoparticles in PC12

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Abstract. It is suggested that the uptake of nanoparticles is changed by the particle size or the surface modification. In this study, we quantified the uptake of nanoparticles in PC12 cells exposed Quantum Dots with different surface modification or fluorescent polystyrene particles with different particle size. The PC12 cells were exposed three types of the Quantum Dots (carboxyl base-functionalized, amino base-functionalized or non-base-functionalized) or three types of the fluorescent particles (22 nm, 100 nm or 1000 nm) for 3 hours. The uptake of the nanoparticles was quantified with a spectrofluorophotometer. The carboxyl base-functionalized Quantum Dots were considerably taken up by the cells than the non-base-functionalized Quantum Dots. Conversely, the amino base-functionalized Quantum Dots were taken up by the cells less frequently than the non-base-functionalized Quantum Dots. The particle number of the 22 nm-nanoparticles taken up by the cells was about 53 times higher than the 100 nm-particles. However, the particle weight of the 100 nm-particles taken up by the cells was higher than that of the 22 nm-nanoparticles. The 1000 nm-particles were adhered to the cell membrane, but they were little taken up by the cells. We concluded that nanoparticles can be taken up nerve cells in functional-dependent and size-dependent manners.

1. Introduction

Previous studies suggested that nanoparticles were taken up by cells and invaded tissues [1,2]. Oberdorster et al. (2004) showed that Fischer-344 rats were exposed 13C nanoparticles (36 nm) by inhalation and consequently the 13C nanoparticles reached to the brain [3]. Although the exposure pathway was not observed, it was suggested that the nanoparticles would be translocated to the brain via the olfactory nerves. Furthermore, de Lorenzo (1970) visualized the silver-coated colloidal gold (50 nm) taken up by the olfactory nerves [4]. Therefore, the uptake of the nanoparticles in the olfactory nerves may be occurred when we inhale the nanoparticles in the air. Thus, it should be quantified to consider the biological risk to the brain.

Many studies showed that the uptake of the nanoparticles was affected by the particle size and surface modification. Rejman et al. (2004) showed that non-phagocytic mouse melanoma B16 cells
took up particles up to a size of 500 nm at 37°C, but no uptake was seen of particles 1 μm in size [5]. Also, Sur et al. (2010) showed that the uptake of the nanoparticles was changed by the surface modification [6]. However, these tendencies may be not applicable to the nerve cells because the uptake of the nanoparticles can be different by the species. Although the uptake of the nanoparticles in the nerve cells was visualized [7], it is still unknown how the particle size and the surface modification affect the uptake of the nanoparticles.

In this study, we exposed three types of fluorescent particles with different sizes or three types of Quantum Dots with different surface modifications to rat pheochromocytoma (PC12) cells, as a model of nerve cells. The particle number and the particle weight of these nanoparticles which were taken up by the cells or adhered to the cell membrane were quantified with a spectrophotometer. Thus, we studied how the uptake of the nanoparticles in the nerve cells was affected by the particle size and the surface modification.

2. Materials and Methods

2.1. The preparation of nanoparticles for exposure
Three types of the fluorescent polystyrene particle suspensions (MORITEX Ltd., Tokyo, Japan) and three types of the Quantum Dot suspensions (Invitrogen Ltd., Tokyo, Japan) were purchased. The particle diameter of the fluorescent particles was 22 nm, 100 nm or 1000 nm, respectively. Also, two types of the Quantum Dots were functionalized by carboxyl base or amino base and another type of the Quantum Dots were not functionalized. The characterization of these particles is shown in table 1.

Each particle suspension was mixed with RPMI 1640 medium (GIBCO Ltd., Tokyo, Japan). The concentration of the suspensions in the medium was adjusted 0.5% (v/v). The particle diameter and number distribution of these particles were analyzed with a dynamic light-scattering particle-size analyzer (ELSZ-0, Otsuka Electronics Company, Limited, Osaka, Japan) 5 minutes and 3 hours after the mixture with the medium.

2.2. Cell culture
The PC12 cell line, derived from rat pheochromocytoma (CRL-1721.1™), was obtained from American Type Tissue Culture. The PC12 cells were cultured on poly-D-lysine cellware dishes (φ 100 mm, Becton Dickinson Ltd., Tokyo, Japan) and grown in the RPMI 1640 medium supplemented with 5% fetal bovine serum, 10% horse serum (both heat inactivated) in a humidified atmosphere with 5% CO2 at 37°C. The PC12 cells were repeatedly-subcultured when the cells reached 80% confluent and seeded in the range of 1×106 cells/dish to 4×106 cells/dish.

Table 1. Characterization of fluorescent particles and Quantum Dots.

| Characteristic                        | Fluorescent particles | Quantum Dots |
|--------------------------------------|-----------------------|--------------|
|                                      | 22 nm | 100 nm | 1000 nm | Carboxyl base | Amino base | Non-base |
| Primary diameter (nm)                | 22    | 100    | 1000    | 10–20        | 10–20      | 10–20    |
| Composition                          | Polystyrene | Polystyrene | Polystyrene | CdSe        | CdSe       | CdSe     |
| Shape                                | Spherical | Spherical | Spherical | Spherical    | Spherical  | Spherical |
| Surface modification                  | —      | —      | —       | Carboxyl base | Amino base | —        |
| Density (g/cm³)                      | 1.06   | 1.06   | 1.06    | —            | —          | —        |
| Number concentration (particles/mL)  | 1.7×10¹⁵ | 1.8×10¹⁵ | 1.8×10¹⁰ | 4.8×10¹⁵ * | 4.8×10¹⁵ * | 1.2×10¹⁵ * |
| Maximum excitation wavelength (nm)   | 542    | 542    | 542     | 350          | 350        | 350      |
| Maximum fluorescent wavelength (nm)  | 612    | 612    | 612     | 565          | 565        | 565      |

*The number concentration was calculated as one particle includes 1000 CdSe molecules.
2.3. Uptake test

The PC12 cells which reached 80% confluent were exposed three types of the fluorescent particles (22 nm, 100 nm or 1000 nm) or three types of the Quantum Dots (carboxyl base-functionalized particles, amino base-functionalized particles or non-base-functionalized particles). Each particle suspension was mixed with RPMI 1640 medium at 0.5% (v/v) just before the exposure. The medium in the dishes was aspirated and 2 mL of the mixed suspension was added into three dishes. As a negative control group, three dishes were added 2 mL of the RPMI 1640 medium. All the dishes were incubated in a humidified atmosphere with 5% CO\(_2\) at 37°C for 3 hours. After the exposure, the exposure medium was aspirated and the cells collected into a centrifugal tube. The cells were washed with PBS and centrifuged at 1000 rpm for 10 minutes. The wash was repeated three times and the number of the collected cells was measured after the wash. Then, 1 mL of 1% triton-X100 containing PBS was added into the tube and homogenized to break the cell membrane. The tube was centrifuged at 1000 rpm for 10 minutes and the supernatant was separated into a micro tube. The pellet was washed gently by 1 mL of PBS three times and then suspended in 1 mL of PBS. The fluorescent intensity in the supernatant and the pellet was measured with a spectrofluorophotometer (RF-5300PC, Shimadzu Ltd., Kyoto, Japan). The standard curves of the fluorescent intensity in each particle were made by the dilution series of the particle suspensions mixed with PBS. The fluorescent intensity of the diluted particle suspensions was measured with the spectrofluorophotometer and the particle number in the supernatant and the pellet was calculated by the standard curves.

2.4. Cell viability test

The PC12 cells were seeded in a poly-D-lysine cellware 96 well Plate (Becton Dickinson Ltd., Tokyo, Japan) at 1×10^4 cells/well and incubated in a humidified atmosphere with 5% CO\(_2\) at 37°C for 3 days. The PC12 cells were exposed 0.2 mL of the 0.01%, 0.1% or 1% (v/v) fluorescent particles containing RPMI 1640 medium or the 0.1%, 1% or 10% (v/v) Quantum Dots containing RPMI 1640 medium and incubated in a humidified atmosphere with 5% CO\(_2\) at 37°C for 24 hours. As a negative control group, three wells were added 2 mL of the RPMI 1640 medium. After the exposure, 0.01 mL of Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) was added into each well and incubated in a humidified atmosphere with 5% CO\(_2\) at 37°C for 3 hours. The absorbance at 450 nm in each well was measured with Microplate Reader (Bio-Rad Laboratories Inc., Tokyo, Japan).

3. Results

3.1. Particle characterization

The number distribution of the fluorescent particles or the Quantum Dots in the RPMI 1640 medium was measured with a dynamic light-scattering particle-size analyzer. Figure 1 shows the number distribution of three types of the fluorescent particles (22 nm, 100 nm or 1000 nm) 5 minutes or 3 hours after mixing with the RPMI 1640 medium. The number distribution of the 22 nm-particles 5 minutes after the mixture appeared two peaks. The mean diameter of the first peak and the second peak was 25.6 nm and 88.2 nm, respectively. The 100 nm-particles and 1000 nm-particles showed monodisperse. The mean particle diameter of the peak was 158.1 nm in the 100 nm-particles and 1089.8 nm in the 1000 nm-particles. The size distribution of these suspensions was in the range of 22.1 nm to 225.9 nm in the 22 nm-particles, 126.4 nm to 594.6 nm in the 100 nm-particles and 875.7 nm to 2795.5 nm in the 1000 nm-particles. After 3 hours, the second peak in the 22 nm-particles was disappeared and showed monodisperse. Similarly, The 100 nm-particles showed monodisperse and the spectrum was almost same as that measured 5 minutes after the mixture. However, the particle diameter became larger in the 1000 nm-particles than that measured 5 minutes after the mixture, though the spectrum was still monodisperse. The mean particle diameter of the peak was 27.3 nm in the 22 nm-particles, 155.5 nm in the 100 nm-particles and 1326.3 nm in the 1000 nm-particles. Also, the size distribution of these suspensions was in the range of 22.1 nm to 104.1 nm in the
22 nm-particles, 126.4 nm to 403.7 nm in the 100 nm-particles and 1062.7 nm to 3395.0 nm in the 1000 nm-particles.

**Figure 1.** The number distribution of three types of the fluorescent particles 5 minutes after mixing with the RPMI 1640 medium (open rhomboid) and 3 hours after mixing with the medium (solid triangle). (a); 22 nm-particles, (b); 100 nm-particles, (c); 1000 nm-particles.

**Figure 2.** The number distribution of three types of the Quantum Dots 5 minutes after mixing with the RPMI 1640 medium (open rhomboid) and 3 hours after mixing with the medium (solid triangle). (a); carboxyl base-functionalized particles, (b); amino base-functionalized particles, (c); non-base-functionalized particles.

Figure 2 shows the number distribution of three types of the Quantum Dots (carboxyl base-functionalized particles, amino base-functionalized particles and non-base-functionalized particles) 5 minutes or 3 hours after mixing with the RPMI 1640 medium. The number distribution of these suspensions 5 minutes after the mixture showed monodisperse. The mean particle diameter of the peak was 10.0 nm in the carboxyl base-functionalized particles, 10.4 nm in the amino base-functionalized particles and 8.7 nm in the non-base-functionalized particles. The size distribution of these suspensions was in the range of 8.4 nm to 39.6 nm in the carboxyl base-functionalized particles, 8.4 nm to 39.6 nm in the amino base-functionalized particles and 6.9 nm to 70.7 nm in the non-base-functionalized particles. After 3 hours, the particle diameter in these suspensions became larger than that measured 5 minutes after the mixture, though the spectrum was still monodisperse. The mean particle diameter of the peak was 13.7 nm in the carboxyl base-functionalized particles, 16.6 nm in the amino base-functionalized particles and 13.4 nm in the non-base-functionalized particles. Also, the size distribution of the suspensions became broad in the range of 12.3 nm to 153.4 nm in the carboxyl base-functionalized particles, 15.0 nm to 225.9 nm in the amino base-functionalized particles and...
12.4 nm to 186.1 nm in the non-base-functionalized particles. The summery of the particle diameter and the size distribution of these suspensions is shown in Table 2.

**Table 2.** The particle diameter and the size distribution after mixing with the RPMI 1640 medium.

| Characteristic       | Fluorescent particles | Quantum Dots |
|----------------------|-----------------------|--------------|
|                      | 22 nm 100 nm 1000 nm | Carboxyl base Amino base Non-base |
| Primary diameter (nm)| 22 100 1000         | 10—20 10—20 10—20 |

5 min after mixing with RPMI 1640:

|                      | 25.6 158.1 1089.8 | 10 10.4 8.7 |
| Size distribution (nm)| 22.1—225.9 126.4—594.6 875.7—2795.5 | 8.4—39.6 8.4—39.6 6.9—70.7 |

3 h after mixing with RPMI 1640:

|                      | 27.3 155.5 1326.3 | 13.7 16.6 13.4 |
| Size distribution (nm)| 22.1—104.1 126.4—403.7 1062.7—3395.0 | 12.4—153.4 15.0—225.9 12.4—186.1 |

3.2. Uptake test

The particle number of three types of the fluorescent particles and the Quantum Dots taken up by the PC12 cells or adhered to the cell membrane was quantified with a spectrofluorophotometer. We confirmed that there was no fluorescence in the PBS used for the third washing after the exposure. Therefore, the fluorescence in the supernatant and the pellet collected after the washing was probably derived of the particles taken up by the cells and adhered to the cell membrane, respectively.

![Figure 3](image.png)

*Figure 3.** The particle number of three types of the fluorescent particles (22 nm, 100 nm, 1000 nm) in the supernatant including the particles taken up by the PC12 cells (a) or in the pellet including the particles adhered to the cell membrane (b). The PC12 cells were exposed 0.5% (v/v) fluorescent particles containing RPMI 1640 medium and incubated in a humidified atmosphere with 5% CO₂ at 37°C for 3 hours.

* p < 0.05 versus 1000 nm-particle-exposed cells.
Figure 3 shows the particle number in the supernatant and the pellet in the fluorescent particle-exposed PC12 cells. The particle number in the supernatant was considerably higher in the 22 nm-particle-exposed cells (20654 particles/cell) than that in the 100 nm-particle-exposed cells (390 particles/cell) and in the 1000 nm-particle-exposed cells (0.02 particles/cell). The total surface area of the fluorescent particles in the supernatant was also higher in the 22 nm-particle-exposed cells (31.2 μm$^2$/cell) than that in the 100 nm-particle-exposed cells (12.3 μm$^2$/cell) and in the 1000 nm-particle-exposed cells (0.1 μm$^2$/cell). Similarly, the particle number in the pellet was obviously higher in the 22 nm-particle-exposed cells (16468 particles/cell) than that in the 100 nm-particle-exposed cells (139 particles/cell) and in the 1000 nm-particle-exposed cells (0.23 particles/cell). The total surface area of the fluorescent particles in the pellet was calculated 25.0 μm$^2$/cell in the 22 nm-particle-exposed cells, 4.4 μm$^2$/cell in the 100 nm-particle-exposed cells and 0.7 μm$^2$/cell in the 1000 nm-particle-exposed cells.

However, the particle weight in the supernatant was higher in the 100 nm-particle-exposed cells (0.41 pg/cell) than that in the 22 nm-particle-exposed cells (0.23 pg/cell) and in the 1000 nm-particle-exposed cells (0.025 pg/cell), as shown in figure 4. It was calculated that the percent of the dose in the supernatant was 0.12 percent/10$^6$ cells in the 22 nm-particle-exposed cells, 0.22 percent/10$^6$ cells in the 100 nm-particle-exposed cells and 0.01 percent/10$^6$ cells in the 1000 nm-particle-exposed cells. On the other hand, the particle weight in the pellet was higher in the 1000 nm-particle-exposed cells (0.072 pg/cell) than that in the 22 nm-particle-exposed cells (0.056 pg/cell) and in the 100 nm-particle-exposed cells (0.044 pg/cell). The percent of the dose in the pellet was calculated 0.10 percent/10$^6$ cells in the 22 nm-particle-exposed cells, 0.08 percent/10$^6$ cells in the 100 nm-particle-exposed cells and 0.13 percent/10$^6$ cells in the 1000 nm-particle-exposed cells.

**Figure 4.** The particle weight of three types of the fluorescent particles (22 nm, 100 nm, 1000 nm) in the supernatant including the particles taken up by the PC12 cells (a) or in the pellet including the particles adhered to the cell membrane (b). The PC12 cells were exposed 0.5% (v/v) fluorescent particles containing RPMI 1640 medium in a humidified atmosphere with 5% CO$_2$ at 37°C for 3 hours.

* $p < 0.05$ versus 1000 nm-particle-exposed cells.

Figure 5 shows the particle number in the supernatant and the pellet in the Quantum Dot-exposed PC12 cells. The particle number in the supernatant was considerably higher in the carboxyl base-functionalized particle-exposed cells (26488 particles/cell) than that in the amino base-functionalized
particle-exposed cells (4321 particles/cell) and in the non-base-functionalized particle-exposed cells (1769 particles/cell). Also, the percent of the dose and the total surface area of the Quantum Dots in the supernatant were higher in the carboxyl base-functionalized particle-exposed cells (0.055 percent/10^6 cells and 74.9 μm^2/cell, respectively) than those in the non-base-functionalized Quantum Dots (0.015 percent/10^6 cells and 12.2 μm^2/cell, respectively). Conversely, they were lower in the amino base-functionalized Quantum Dots than the non-base-functionalized Quantum Dots; 0.009 percent/10^6 cells and 5.0 μm^2/cell, respectively.

The particle number in the pellet was also higher in the carboxyl base-functionalized particle-exposed cells (8590 particles/cell) than that in the amino base-functionalized particle-exposed cells (2701 particles/cell) and in the non-base-functionalized particle-exposed cells (1859 particles/cell). The total surface area and the percent of the dose of these Quantum Dots in the pellet were calculated 24.3 μm^2/cell and 0.018 percent/10^6 cells in the carboxyl base-functionalized Quantum Dots, 7.6 μm^2/cell and 0.006 percent/10^6 cells in the amino base-functionalized Quantum Dots and 5.3 μm^2/cell and 0.015 percent/10^6 cells in the non-base-functionalized Quantum Dots.

3.3. Cell viability test

Figure 6 shows the cell viability of the PC12 cells exposed the Quantum Dots or the fluorescent particles for 24 hours. The cell viability was little changed between three types of the Quantum Dots exposed cells up to 1% particle concentration in the medium, compared to the negative control group. Similarly, the cell viability exposed three types of the fluorescent particles was almost same as the negative control until 1% particle concentration in the medium. However, the cell viability was decreased at the 10% particle concentration and it was different by the particle size. Although the 22 nm-particle-exposed cells were completely died, the 100 nm-particle-exposed cells and the 1000 nm-particle-exposed cells were still alive to some extent. The cell viability in the 100 nm-particle-exposed cells and the 1000 nm-particle exposed cells was 73% and 54%, respectively.
4. Discussion
The particle diameter of the fluorescent particles was little changed in the 22 nm-particles and the 100 nm-particles. Conversely, the 1000 nm-particles were aggregated 3 hours after the mixture with RPMI 1640 medium. It is suggested that the 1000 nm-particles were settled out in the medium and aggregated due to the high particle concentration on the bottom. According to Stokes' theorem, the sedimentation distance of these particles 3 hours after the mixture with the medium (viscosity: 0.71 cP) is calculated as $2.4\times10^{-4}$ mm in the 22 nm-particles, $5.0\times10^{-3}$ mm in the 100 nm-particles and $0.50$ mm in the 100 nm-particles. Therefore, the 1000 nm-particles were accumulated on the bottom of the dish faster than the 22 nm-particles and the 100 nm-particles, and consequently they might be aggregated.

The particle diameter of the Quantum Dots was increased 3 hours after the mixture with RPMI 1640 medium. It is suggested that nanoparticles are likely to be aggregated in medium because of the large amount of electrolyte ions and the protein adsorption [6, 8]. Sur et al. (2010) showed that the particles diameter in the medium was increased in the range of 26 to 37 nm due to the protein adsorption [6]. In this study, the mean particle diameter of the Quantum Dots was also increased in the range of 3.7 nm to 6.2 nm. Therefore, the Quantum Dots were possibly adsorbed by the proteins in the medium and consequently the particle size became larger than the original size.

*Figure 6.* The viability of the PC12 cells exposed three types of the Quantum Dots or three types of the fluorescent particles. (a); The cell viability of the PC12 cells exposed the carboxyl base-functionalized particles (open rhomboid), the amino base-functionalized particles (open circle) or the non-base-functionalized particles (open triangle) mixed with the RPMI 1640 medium (particle concentration; 0.01%, 0.1% or 1%) for 24 hours. (b); The cell viability of the PC12 cells exposed the 22 nm-particles (open rhomboid), the 100 nm-particles (open circle) or the 1000 nm-particles (open triangle) mixed with the RPMI 1640 medium (particle concentration; 0.1%, 1% or 10%) for 24 hours. * $p < 0.05$ versus untreated controls.

The particle number of the fluorescent particles taken up by the PC12 cells was increased in a size-dependent manner (figure 3). The 22 nm-particles were taken up by the cells over 20000 particles in a cell and the particle number was about 53 times higher than that of the 100 nm-particles. Furthermore, the 1000 nm-particles were rarely taken up by the cells, which was a same result as Rejman’s study [4]. Similarly, the particle number of the fluorescent particles adhered to the cell membrane was increased in a size-dependent manner. Therefore, we suggest that the particle number of particles taken up by nerve cells may be exponentially increased as the particle size becomes small. On the
other hand, the particle weight/the percent of the dose was about 1.8 times higher in the 100 nm-particles than that in the 22 nm-particles (figure 4). It means that the 100 nm-particles were taken up by the cells more effectively than the 22 nm-particles. Rejman et al. (2004) showed that the effects of the uptake of the particles by caveolae-mediated endocytosis and clathrin-mediated endocytosis were differently by the particle size [4]. Therefore, the 100 nm-particles might be taken up effectively by such uptake mechanisms. In addition, the particle weight of the 1000 nm-particles in the pellet was the highest among the particle types and also higher than that in the supernatant. Therefore, it was suggested that the 1000 nm-particles were interacted with the cells and adhered to the cell membrane, but it was too large to be taken up by the cells.

The cell viability test of the fluorescent particles showed that the 22 nm-particles completely killed the cells but the 100 nm-particle-exposed cells and the 1000 nm-particle-exposed cells were still alive (figure 6). It might be due to the high surface area in the 22 nm-particles (31.2 μm²/cell) rather than that in the 100 nm-particles (12.3 μm²/cell) and in the 1000 nm-particles (0.1 μm²/cell). Oberdorster et al. (2000) showed that the cytotoxicity of TiO₂ particles (20 nm and 250 nm) was related to the surface area [9]. However, despite the surface area of the 1000 nm-particles was lower than that of the 100 nm-particles, the cell viability in the 1000 nm-particle-exposed cells was decreased to 54% more than the 100 nm-particle-exposed cells (73%). It might be caused by the sedimentation of the 1000 nm-particles during the exposure. As shown in figure 1, the 1000 nm-particles were aggregated and these aggregates probably settled out in the dish. Consequently, unexpected higher amount of the 1000 nm-particles might be interacted with the adherent PC12 cells. Further studies are needed to determine the accurate relationship between the particle size and the cell viability.

The particle number of the Quantum Dots taken up by the PC12 cells was different by the surface modification (figure 5). The particle number and the percent of the dose of these particles in the supernatant were higher in the carboxyl base-functionalized particles but lower in the amino base-functionalized particles than those of the non-base-functionalized particles. The positively charged particles were likely to be taken up by the cells effectively because the cell membrane was charged negatively. Geys et al. (2008) showed that the isoelectric point of the carboxyl-functionalized Quantum Dots and the amine-functionalized Quantum Dots was pH 1.71 and 3.19, respectively, and both particles were charged negatively in the medium [10]. Therefore, the amino base-functionalized particles might be less interacted with the cells. Nevertheless, the carboxyl base-functionalized particles were taken up more frequently than the non-base-functionalized particles. Although the uptake mechanism of these particles was still unknown, it was clearly that the particles were selectively taken up by the cells according to the surface modification.

The cell viability test of the Quantum Dots showed almost no difference compared to the negative control cells (figure 6). It may be changed between the particle types because the surface area of the Quantum Dots taken up by the cells was considerably higher in the carboxyl base-functionalized Quantum Dots (74.9 μm²/cell) than that in the amino base-functionalized Quantum Dots (5.0 μm²/cell) and in the non-base-functionalized Quantum Dots (12.2 μm²/cell). Similarly, Geys et al. (2008) showed that Quantum Dots caused pulmonary vascular thrombosis, with carboxyl-functionalized Quantum Dots being more potent in inducing this effect than amine-functionalized Quantum Dots [10]. Therefore, it should be concerned more in further studies how the surface modification of the particles affects to the cells.

5. Conclusion
In this study, the uptake of the nanoparticles in the PC12 cells was quantified and the effects of the particle size and the surface modification were tested. The large number of the 22 nm-fluorescent particles was taken up by the PC12 cells than the 100 nm-particles. However, the 100 nm-particles were taken up more frequently than the 22 nm-particles. Conversely, the 1000 nm-particles were little taken up by the cells. It is suggested that nanoparticles are likely to be taken up by the cells than micro-sized particles. Furthermore, the uptake of the Quantum Dots was changed by the surface modification. Although the carboxyl base-functionalized Quantum Dots were taken up by the cells
more frequently than the non-base-functionalized particles, the amino base-functionalized Quantum Dots were less taken up by the cells. These findings suggest that the nanoparticles are taken up by cells in size-dependent and functional-dependent manners. Although the uptake mechanism of the nanoparticles is still too complicated to clarify, our present study is valuable to consider the biological risk of the nanoparticles to nerve tissues.

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