Adhesion and internalization of functionalized polystyrene latex nanoparticles toward the yeast Saccharomyces cerevisiae

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The toxicity and the internalization, adhesion, and dispersion behavior of manufactured polystyrene latex (PSL) nanoparticles (nominal diameter: 50 nm) with various functional groups toward the yeast Saccharomyces cerevisiae (which was applied as a model eukaryote) were examined using the colony count method, and microscopic observations. The colony count tests suggested that PSL nanoparticles with a negative surface charge showed little or no toxicity toward the yeast. In contrast, PSL nanoparticles with an amine functional group and a positive surface charge (p-Amine) displayed a high toxicity in 5 mM NaCl. However, the yeast cells were mostly unharmed by the p-Amine in 154 mM NaCl, results that were quite different from the toxicological effects observed when Escherichia coli was used as a model prokaryote. Confocal and atomic force microscopes indicated that in 5 mM NaCl, the p-Amine nanoparticles entirely covered the surface of the yeast, and cell death occurred; in contrast, in 154 mM NaCl, the p-Amine nanoparticles were internalized via endocytosis, and cell death did not occur.

1. Introduction

Many products using nanoparticles (NPs) are now commercially available, after the recent rapid progress made in the nanotechnology field. Although commercial products including NPs may yield large profits, the potential for these NPs to adversely affect the environment and human health is of significant concern. It is fascinating to note that, despite the potentially harmful nature of these particles, the approximate ratio of the number of the articles returned in a search using the keywords “nanoparticles”, “nanoparticles and toxic”, and “nanoparticles and ecotoxic” was 1000:10:1 [1]. Thus, the information presently available regarding the ecotoxicity of NPs is clearly lacking. NPs have a number of characteristics that can be varied to produce desirable effects; these characteristics include the particle diameter, electrification, wettability, morphology, surface functional groups, specific surface area, and dispersibility, and these characteristics can be varied even in the same material composition. In contrast, organisms are classified diversely by their cellular structure and evolutionary processes. It is therefore difficult to predict the toxicity of NPs toward organisms, since the toxicity cannot be directly linked to the material properties in a one-to-one relationship.

Miyamoto et al. [2] performed toxicity tests to determine whether polystyrene latex (PSL) NPs affected Escherichia coli, which acted as a model prokaryote, and reported that positively charged, amine-modified NPs covered the surface of the microbial cells under the influence of electrostatic interactions, and cell death subsequently occurred. Yeast is widely used as a unicellular eukaryotic model microorganism. However, few studies have investigated the potential impact of NPs on yeast, and the studies that do exist reported that NPs exhibit low or no toxicity toward yeast, compared with prokaryotes [3–8]. NPs have attracted considerable attention for bio-industry applications such as biosensors and drug delivery. There have been many studies reporting the interactions between NPs such as gold [9,10], silica [11,12], PSL [13], chitosan [14], and quantum dot [15] NPs and mammalian cells, macrophages, and red blood cells; these reports have also considered the uptake of NPs. The uptake of gold NPs by yeast spheroplasts whose cell walls had been almost completely removed was reported [16]; in contrast, the uptake of NPs by yeast with a rigid cell wall was not observed under normal conditions [4].
In this study, the toxicity and the internalization, adhesion, and dispersion behavior of manufactured PSL NPs with various functional groups (nominal diameter: 50 nm) toward the yeast *Saccharomyces cerevisiae* (which acted as a model eukaryote) were examined, and the toxicity of the NPs toward yeast was compared with that of the particles toward prokaryote *E. coli* [2].

2. Materials and methods

2.1. Microorganisms and nanoparticles

Budding yeast *S. cerevisiae* (strain JCM 7255) was used as a unicellular eukaryotic model microorganism. *S. cerevisiae* was grown at 30 °C in yeast extract (YE) medium (5.0 g/L yeast extract and 30 g/L glucose) under agitation, and were harvested using centrifugation. The harvested yeast cells were washed three times with a 5 or 154 mM NaCl aqueous solution, and resuspended in the same aqueous solution. Here, the 154 mM NaCl aqueous solution used was an isotonic solution.

The NPs used in this study were as follows: Non-,-carboxylate-, and amine-modified PSL NPs labeled fluorescently with FITC (nominal diameter: 50 nm, micromere-greenF, micromod Partikeltechnologie GmbH, Rostock, Germany), and amine-modified PSL NPs labeled with blue fluorophore (nominal diameter; 50 nm, Sigma, St. Louis, MO, USA). The PSL NPs were suspended in a 5 or 154 mM NaCl aqueous solution prior to use.

The electrophoretic mobility (EPM) and the median diameter of the PSL NPs and the yeast cells were measured using a zeta potential and particle size analyzer (ELS-Z, Otsuka Electronics, Osaka, Japan). Based on the measured EPM values, the zeta potential of the NPs was estimated using the Smoluchowski equation, and the surface potential of the yeast cells was estimated using a soft particle model proposed by Ohshima [17,18]. The characteristics of the PSL NPs and the *S. cerevisiae* used in this study are listed in Table 1. The characters “n” and “p” denote “negatively charged” and “positively charged”, respectively, n-Plain and n-Amine aggregated when they were dispersed in the 154 mM NaCl aqueous solution.

2.2. Estimation of the toxicity of the PSL NPs

0.5 mL of the PSL NP suspensions (0–160 mg/L) was mixed with 0.5 mL of a yeast cell suspension (1 × 10^8 cells/mL) in a microtube, and the microtube was then placed on a Duck rotor at 60 rpm for 1 h, at room temperature. After exposure, 0.1 mL of the diluted suspension was spread on YE agar plates and incubated for 2 days at 30 °C. The number of living cells was determined by counting the number of colony-forming units (CFUs) on the YE agar plates. The cell viability ratio was evaluated by comparing the number of CFUs on the YE agar plates with the number of CFUs on a control plate; the suspension spread on the control plate did not include NPs.

To determine the location of the NPs and the cell viability, the yeast cells were observed after they were exposed to the NP suspension, using a confocal laser scanning microscope (CLSM) (FV-1000D, Olympus, Tokyo, Japan). A 60 × water-immersion objective lens UPLSAPO 60XW (N.A. = 1.20) was used. Dead cells were stained using membrane-impermeable propidium iodide (PI). All cells were verified using differential interference contrast (DIC) imaging. The CLSM observation conditions used were as follows: PI (excitation/emission wavelengths 543/655–755 nm), Micromod PSL NPs (excitation/emission wavelengths 543/485–515 nm), and Sigma PSL NPs (excitation/emission wavelengths 405/420–450 nm).

The NPs adhered on the yeast cell surface after the NP exposure were observed directly using AFM (MFP-3D-BIO-J, Asylum Research, Santa Barbara, CA, USA), using a silicon cantilever probe (OMCL-AC200TS, Olympus, Tokyo, Japan) in tapping mode under ambient laboratory conditions.

3. Results and discussion

The attractive electrostatic force between *E. coli* and the positively charged PSL NPs in the 5 mM solution was stronger than that in the 154 mM solution. The NPs therefore adhered more easily on the cell surface, and showed relatively higher toxicity, when the *E. coli* was exposed to the NPs in the 5 mM solution [2]. The tests assessing the toxicity of the PSL NPs toward yeast cells was then carried out in environments with different ionic strengths. Fig. 1 shows the viability of the yeast cells after 1 h of exposure to 80 mg/L of PSL NPs. When exposed to n-Plain, n-Carboxyl, and n-Amine PSL NPs, many of the yeast cells remained alive, regardless of the NaCl concentration in the dispersion medium. The trend shown by the cell viability of the yeast was similar to that of *E. coli*. Because the surface of the cell was negatively charged, an electrostatic repulsive force acted between the cell and the negatively charged NPs, and the NPs did not adhere on the cell surface. When they were exposed to p-Amine NPs in 5 mM NaCl, the yeast cells almost all died (cell viability <0.01%), whereas almost all of the yeast cells were alive after they were exposed to 154 mM NaCl. In the case of 154 mM NaCl, the trend shown by the cell viability of the yeast was clearly different from that of *E. coli*. Additionally, no significant difference was observed between the number of

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**Fig. 1.** Cell viability after 1 h of exposure to 80 mg/L surface-modified PSL NPs dispersed in NaCl aqueous solutions. The yeast cell concentration was 5 × 10^8 cells/L. The p-Amine concentration was 80 mg/L. (*) P < 0.05 and (**) P < 0.01 versus controls, mean ± SEM, N = 3.
CFUs present when the yeast cells were exposed to the 5 mM and 154 mM NaCl aqueous solutions without NPs. These results indicated that the hypotonic solution showed little toxicity toward the yeast cells as well as the E. coli. Fig. 2 shows the cell viability as a function of NP concentration after 1 h exposure to the p-Amine particles. In the 5 mM case, the cell viability decreased drastically with increasing NP concentration, and all of the cells were observed to be dead when an NP concentration of 40 mg/L was used. In contrast, almost all of the yeast cells remained alive in the 154 mM solution, regardless of the NP concentration.

Typical CLSM images of the yeast cell suspensions after 1 h of exposure to the 80 mg/L p-Amine PSL NP suspensions in 5 mM and 154 mM NaCl aqueous solutions are shown in Fig. 3. The yeast cells were exposed to the p-Amine particles in (A) 5 mM, and (B) 154 mM solutions, and observations were made of (i) all cells, using DIC imaging, (ii) dead cells, via staining using membrane-impermeable PI, and (iii) PSL NPs labeled with a fluorophore. In the 5 mM case, the surface of the yeast cells was covered with p-Amine particles, and cell death subsequently occurred, whereas the NPs were taken up into the living cells in the 154 mM solution. The NP uptake behavior observed was significantly different for the E. coli [2] and the yeast. It is believed that the NPs were entrapped in the cells because eukaryotic yeast has a function for taking in materials outside of the cell via endocytosis [19,20]. In contrast, the prokaryotic E. coli, which has no endocytic function, was not able to take up the NPs [2].

CLSM can be used to identify the location of NPs using a fluorophore. However, analysis at the nano-level is impossible. Thus, the surface of the yeast cells after NP exposure was observed using AFM. Fig. 4 shows AFM images of the yeast cell surface after exposure to 80 mg/L p-Amine particles in 5 mM NaCl (corresponding to the results shown in Fig. 3A). According to the AFM analysis, the surface of the yeast cell was very smooth prior to NP exposure (Fig. 4A). In contrast, following exposure to NPs in 5 mM NaCl, the yeast cell surface was completely covered with NPs, and a smooth cell surface was no longer visible. In the case of metal oxide NPs such as ZnO and TiO2, reactive oxygen species and dissolved ions damage the cell membrane, DNA, and mitochondria [21,22]. However, it is thought that the high toxicity of high-polymer PSL NPs is not produced by these types of mechanisms. Additionally, the CLSM observations revealed that the negatively charged NPs were not adhered on the cell surface. Positively charged ZnO, SiO2, and gold NPs show higher toxicity toward mammalian cells compared with negatively charged particles, and the electrostatic charge affects the cell viability [23–26]. In light of these findings, it was considered that the damage to the cell membrane resulting from the adhesion of NPs on the surface (owing to attractive electrostatic forces) was one of the main factors responsible for the cell death. It is likely that the reason for the differences observed in the positively charged PSL NPs’ behavior in dispersion media with different ionic strengths was as follows: in the 5 mM solution, strong attractive electrostatic forces acted between the yeast cells and the NPs, and cell death subsequently occurred; in the 154 mM solution the electrostatic forces were weaker, and the yeast cells entrapped the NPs via endocytosis. It is believed that the rate of adhesion of the NPs onto the cell surface by the electrostatic interactions and the rate of the uptake of NPs via endocytosis affect the
colloidal behavior of the NPs (i.e., the adhesion and internalization). Furthermore, similar results were obtained for PSL NPs with a nominal diameter of 100 nm [27]. Thus the effect of NP size on the toxicity and the uptake of NPs was small when the NP diameter was less than 100 nm. In contrast, the effect of the surface charge of NPs on the uptake is different in mammalian cells [13,28,29]. Thus, further investigation is required for the yeast cells.

4. Conclusions

The behavior and toxicity of PSL NPs (nominal diameter: 50 nm) with various functional groups toward the budding yeast S. cerevisiae were investigated. When the yeast cells were exposed to suspensions of negatively charged NPs, it was found that the yeast cells did not die, because the NPs did not adhere to the cell surface. In contrast, the positively charged NPs exhibited a high toxicity in a 5 mM NaCl solution; however, the positive NPs exhibited low toxicity in 154 mM NaCl, and the yeast cells did not die, results that was clearly different from that observed for the toxicity of NPs toward E. coli. According to the direct images obtained using CLSM and AFM after exposure to the positively charged NPs, the yeast cells were covered with NPs and died in 5 mM NaCl, whereas the yeast cells absorbed the NPs and remained alive in 154 mM NaCl. The difference in the NP location observed in dispersion media with different ionic strengths suggested that the strong attractive electrostatic forces acting between the cells and the NPs led to the cell death in the 5 mM NaCl, whereas the NPs were internalized via endocytosis in the 154 mM NaCl because of the decrease in the electrostatic interactions.

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References