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Cytotoxicity and behavior of polystyrene latex nanoparticles to budding yeast

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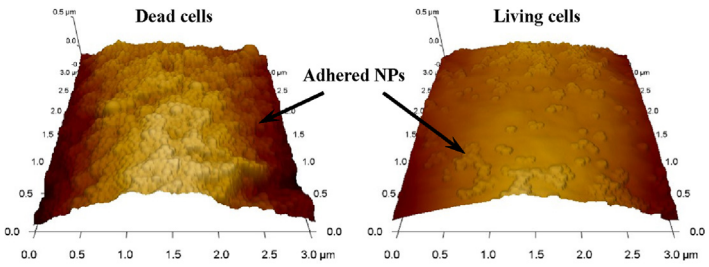
HIGHLIGHTS

- NPs adhered on the yeast cell surface showed high toxicity in low ionic strength.
- NPs entrapped in the yeast cells showed little toxicity in high ionic strength.
- The electrostatic attractive force between yeast and NP affected cell viability.
- The osmotic pressure inhibited the uptake of NPs.

GRAPHICAL ABSTRACT



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ABSTRACT

The cytotoxicity and behavior of positively charged amine-modified polystyrene latex nanoparticles (NPs) toward budding yeast cells were examined in different environments. The yeast cells, dispersed in either 27.8 mM glucose or low-ionic strength aqueous electrolyte solution, were covered with NPs regardless of the ionic species or diameter of the NPs. The NPs that adhered to the cell surface showed high toxicity. The cell viability following NP exposure increased with the NaCl concentration independently of the glucose concentration in the dispersion medium. Therefore, the strong electrostatic attraction between the negatively charged yeast cells and positively charged NPs resulted in NP adhesion to the cell surface and subsequent cell death. However, the amount of endocytosis-like NPs inside the cell, dispersed in the hypertonic solution, was lower than that in the isotonic solution. These results indicate that the osmotic pressure affects the uptake of NPs rather than the cell viability. In contrast, NPs dispersed in a high-ionic strength electrolyte solution with a diameter of ~100 nm or less were absorbed by the yeast cells. NPs incorporated in the living yeast cells showed little toxicity.

1. Introduction

Recently, nanotoxicology has gained considerable attention owing to the increasing presence of nanoparticles (NPs) in commercially available products [1]. However, there is some concern that the unique physicochemical characteristics of NPs, though advantageous to us, may adversely affect human health and the environment. NPs are available in many forms according to their physical characteristics such as particle diameter, electrification, wettability, morphology, surface functional groups, specific surface area, and aggregability. Thus, further knowledge and evaluation of the potential adverse environmental and biological effects of NPs are important.

Yeast, because of its many similarities with animal and plant cells (specifically its cellular structure and functional organization),

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is widely used as a model unicellular eukaryotic microorganism. However, evidence for the cytotoxicity of NPs toward yeast is still limited [2]. A few studies have investigated the potential impact of NPs, composed of materials like metal oxides, silver, and fullerene, on yeast and other unicellular microorganisms such as bacteria and algae [2–6], but these studies found that NPs exhibit little or no toxicity toward yeast. The uptake of NPs by yeast cells has not been studied, although uptake by mammalian cells has been demonstrated for various cell types [7–11]. In yeast, many proteins involved in endocytic internalization have been reported [12,13], however it has been thought that the uptake of NPs is difficult because yeast has a rigid cell wall [3]. Prescianotto-Baschong and Riezman mentioned that positively charged nanogold could be entrapped in yeast spheroplasts whose cell walls had been almost completely removed [14]. Interestingly, the authors [15,16] demonstrated that the uptake of endocytosis-like NPs by live budding yeast cells could be directly observed using confocal laser scanning microscopy (CLSM) in time-lapse mode when exposed to positively charged amine-modified polystyrene latex (PSL) NPs (diameter ≤100 nm) dispersed in physiological aqueous saline (154 mM NaCl). Conversely, in lower ionic strength aqueous NaCl solution, the NPs covered the budding yeast cell surface, consequently leading to cell death.

In this study, the cytotoxicity and behavior of positively charged PSL NPs toward budding yeast cells were examined using a colony count method and microscopy techniques. *Saccharomyces cerevisiae* and commercial amine-modified PSL NPs with varying diameters (50, 100, 500 nm) were used. Electrolyte (NaCl, KCl, $CaCl_2$, $MgCl_2$), glucose, and corresponding mixed aqueous solutions were used as the dispersion media to expose yeast cells to NP suspensions. The behavior of the PSL NPs, such as uptake, adhesion, and dispersion, was observed using CLSM, atomic force microscopy (AFM), and transmission electron microscopy (TEM).

2. Materials and methods

2.1. Yeast strain

Budding yeast *S. cerevisiae* JCM 7255^T (JCM, Japan Collection of Microorganisms, Wako, Japan) was grown at 30 °C in yeast extract (YE) medium (5.0 g/L yeast extract and 30 g/L glucose). Yeast cells were collected in the late exponential growth phase by centrifugation at 8400 × *g* for 10 min at 4 °C, washed three times with the sterilized electrolyte or glucose aqueous solution used in the cytotoxicity test as dispersion medium, and re-suspended in the same aqueous solution. The concentration of yeast cells in the suspension was estimated using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) and a Petroff-Hausser counting chamber.

2.2. Polystyrene NPs

Amine-modified PSL NPs of different diameters (50, 100, 500 nm) labeled with a fluorophore were purchased from Sigma–Aldrich (L0780; L9904; L9779, St. Louis, MO, USA). The NPs were labeled pA50, pA100, and pA500, respectively, where "p" and "A" stand for positively charged and amine-modified, respectively. The PSL NPs were suspended in the desired concentration of sterilized electrolyte or glucose aqueous solution with a vortex for 10 s prior to use.

2.3. Characterization of NP and yeast cell properties

The electrophoretic mobility (EPM) and median diameter of the PSL NPs and yeast cells as a function of ionic strength were measured using a zeta potential and particle size analyzer (ELS-Z, Otsuka Electronics, Hirakata, Japan). The zeta potential of the NPs was calculated using the Smoluchowski equation for a hard particle: $u = (\varepsilon_r \varepsilon_0 \zeta)/\eta$, where u is the EPM, ε_r is the relative permittivity of the medium, ε_0 is the permittivity of the vacuum, ζ is the zeta potential, andη is the viscosity of the medium. Previous studies have reported that the zeta potential of a soft particle, such as microbial cells, does not follow the Smoluchowski equation because charged polymers at the cell surface affect the EPM [17–20]. Thus, the zeta potential of microbial cells was estimated using the Ohshima equation which is applicable to soft particles and considers the effect of charged polymers [21,22].

2.4. Cytotoxicity of PSL NPs toward yeast cells

Cytotoxicity tests of the amine-modified PSL NPs toward the yeast cells were carried out using the colony count method according to the reported procedure [15]. First, 0.5 mL of yeast cell suspension (1×10^6 cells/mL) and 0.5 mL of PSL NP suspension were vortexed together in a microtube for 10 s. The microtube was then placed on a tube rotator (TR-350, AS ONE, Osaka, Japan) at 60 rpm at room temperature. After 1 h of exposure to the NPs, 0.1 mL of diluted suspension was spread on YE agar plates, prepared by adding 2.0% (w/v) agar to the YE medium, and incubated for 2 days at 30 °C. The number of viable cells in the suspension was estimated by counting the number of colony-forming units (CFUs) on the YE agar plates. The cytotoxicity was evaluated by comparing the number of CFUs on the YE agar plates with that on the control plate; the suspension spread over the control plate did not include NPs. The electrolyte (NaCl, KCl, $CaCl_2$, and $MgCl_2$) and glucose aqueous solutions were used as the dispersion medium, and the initial ionic strength of the aqueous electrolyte solution varied from 5 to 154 mM. The initial concentrations of the glucose aqueous solutions were 27.8 and 83.3 mM. An isotonic solution (a mixture of NaCl and glucose) was also prepared. The initial concentration of the PSL NP suspensions was varied between 0 and 160 mg/L to investigate the effects of NP concentration on NP toxicity toward yeast cells.

2.5. Confocal microscopy

The cell suspension was stained with membrane-impermeable propidium iodide (PI), which only infiltrates cells with disrupted membranes. The suspension was then placed on a coverslip. The location of the PSL NPs and cell viability were evaluated by CLSM (FV-1000D, Olympus, Tokyo, Japan) with a water-immersion objective lenses of N.A. = 1.20 (UPLSAPO 60XW, Olympus, Tokyo, Japan). All cells were identified using differential interference contrast (DIC) imaging.

2.6. Atomic force microscopy

The NP locations (at the nano-scale level) on the yeast cells after NP exposure was determined by AFM (MFP-3D-BIO-J, Oxford Instruments, Santa Barbara, CA, USA) in tapping mode at room temperature in an air-conditioned laboratory (24 ± 2 °C). AFM imaging was carried out using a silicon cantilever probe (OMCL-AC200TS, Olympus, Tokyo, Japan) with a nominal spring constant of 9 N/m at a scan speed of 0.2 Hz and 1024 pixels per line scan. The sample was prepared as follows: the cell suspension was placed on a gelatin coated glass slide, which was prepared according to the reported procedure [23]. The sample was allowed to stand for 10 min to allow the cells to become immobilized on the substrate. The substrate was then rinsed with deionized water to removeany weakly