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Original Research Paper

Control of colloidal behavior of polystyrene latex nanoparticles and their cytotoxicity toward yeast cells using water-soluble polymers

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ABSTRACT

Positively charged polystyrene latex (PSL) nanoparticles (NPs) dispersed in physiological saline (154 mM NaCl solution) are taken up by yeast cells. However, in low ionic strength solutions, the yeast cells are covered with the NPs, leading to cell death. The environmental conditions under which NPs are taken up are therefore limited. In this study, we attempted to control the uptake of positively charged PSL NPs by *Saccharomyces cerevisiae* in 5 mM NaCl solution using a water-soluble polymer. Addition of a small amount of anionic sodium carboxymethylcellulose (CMC), which has a carboxyl group, to 5 mM NaCl solution allowed the uptake of PSL NPs by living yeast cells. In contrast, non-ionic methylcellulose did not affect the NP behavior. This is because the negatively charged CMC adhered to the positively charged PSL NP surfaces and the surface charge changed from positive to negative. Atomic force microscopy using a single-NP probe consisting of one NP immobilized on the flattened end of the silicon nitride tip showed that CMC significantly reduced the interaction force between a negatively charged living yeast cell and a positively charged PSL NP.

1. Introduction

The interaction forces between engineered nanoparticles (NPs) and biological cells have received considerable attention due to the potential use of NPs in biological applications such as drug- and gene-delivery systems [1–6], and their possible adverse impacts on human health and the environment [7,8]. Yeast is widely used as a model unicellular eukaryotic microorganism due to many similarities to animal and plant cells. However, only a few studies have investigated the potential impact of NPs (metal oxides, silver, and fullerene) on yeast cells [9–13]. These studies reported that NPs show little or no toxicity toward yeast. The uptake of positively charged gold NPs by yeast spheroplasts whose cell walls had been almost completely removed was demonstrated [14]. However, although NP uptake by animal cells has been reported, there have been few studies of NP uptake by yeast. This is probably because yeast cells are protected by a robust cell wall [9]. In our previous studies, confocal observation revealed that live yeast cells take up positively charged amine-modified polystyrene latex (PSL) NPs dispersed in physiological saline (154 mM NaCl solution). However, the yeast cells were completely covered with the NPs in a low ionic strength (5 mM NaCl solution), leading to cell death [15–17]. The environmental conditions for NP uptake are therefore limited.

It is important to know the physicochemical properties of biological cells and NPs that affect colloidal behavior (diffusion, adhesion, uptake). However, theoretical estimation of the biological interactions between cells and NPs are difficult because of their heterogeneous surfaces mediated by various complex surface macromolecules, including proteins, polysaccharides, and lipids. Atomic force microscopy (AFM) can directly measure the interaction forces between surfaces and is a powerful tool for measuring biological surface forces [18,19]. Various methods based on AFM have been used to measure the interaction forces between a solid surface and a biological cell, e.g., colloidal probe [20–22], cell probe [19,23,24], and NP probe methods [25]. However, only a few studies have been performed using a single-NP probe, i.e., only one NP immobilized on the end of the AFM cantilever tip [26,27]. To the best of our knowledge, there are few reports of the biological interaction between a single NP and a biological cell.

In this study, we attempted to control the uptake of positively charged PSL NPs by the budding yeast *Saccharomyces cerevisiae* cells in the low ionic strength dispersion medium (5 mM NaCl solution) using a water-soluble polymer, namely anionic sodium carboxymethylcellulose (CMC) or non-ionic methylcellulose (MC). A single-NP probe consisting of one NP immobilized on the flattened end of the silicon nitride tip of the AFM cantilever was

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prepared by a simple method, without surface modification of the tip, and used direct measurement of the force between a living yeast cell and a PSL NP.

2. Materials and methods

2.1. Yeast strain and growth conditions

The budding yeast *S. cerevisiae* JCM 7255 ^{T}, which was used as a model unicellular eukaryotic organism, was purchased from the Japan Collection of Microorganisms (Tsukuba, Japan). *S. cerevisiae* was grown in YE medium (5 g/L yeast extract and 30 g/L glucose) at 30 °C with agitation at 120 rpm. The yeast cells were collected by centrifugation and were washed three times with sterilized 5 mM NaCl solution to remove remaining medium components. A yeast cell suspension was prepared by resuspending the washed cells in the sterilized dispersion medium, and the cell concentration was adjusted using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan).

2.2. Polystyrene latex nanoparticles

Positively charged amine-modified PSL NPs with a fluorophore (nominal diameter: 100 nm) were purchased from Sigma-Aldrich (L9904, St. Louis, MO). A PSL NP suspension was prepared by suspending the NPs in sterilized dispersion medium.

2.3. Dispersion medium

CMC was purchased from Sigma-Aldrich (419273, average molecular weight: ~90 000) and 0.5% (w/v) sterilized MC 400 solution was purchased from Wako Pure Chemical Industries, Ltd. (133-14255, Osaka, Japan). The dispersion medium was prepared by dissolving a water-soluble polymer in 5 mM NaCl solution. The final concentration of the polymer varied from 0 ppm (control) to 4 ppm.

2.4. Cytotoxicity test of polystyrene latex nanoparticles toward yeast cells

The yeast cell suspension (500 μL) and PSL NP suspension (500 μL) were mixed in a microtube. The microtube was then placed on a tube rotator at 60 rpm for 60 min at room temperature. After exposure, the diluted suspension (100 μL) was spread on YE agar plates and incubated for 2 d at 30 °C. The number of colony-forming units (CFUs) on the plates was counted. Cell viability was estimated by comparing the number of CFUs on the plates with that on a control plate. The suspension spread on the control plate did not include NPs. The location of the PSL NPs was observed using a confocal laser scanning microscopy (CLSM; FV-1000D, Olympus, Tokyo, Japan) with oil-immersion objective lenses of numerical aperture 1.40 (UPLSAPO 100XO, Olympus). After CLSM imaging, the dead cells were stained with trypan blue, which only infiltrates cells with disrupted membranes. The final concentrations of the PSL NPs and yeast cells were 40 μg/mL and 5×10^5 cells/mL, respectively.

2.5. Immobilization of living yeast cells on glass substrate

A non-coated glass slide (NC-glass) (S7224, Matsunami Glass, Kishiwada, Japan) was rinsed with deionized water and then ethanol for 15 min each. After drying with nitrogen gas, 4 mg/mL of dopamine hydrochloride (Sigma-Aldrich) in 10 mM Tris–HCl buffer (pH 8.5) solution (100 μL) was placed on the cleaned NC-glass surface and left for 1 h at room temperature to create a coating consisting of a thin polymer film produced by dopamine polymerization [28,29]. The polydopamine-coated glass slide (PDA-glass) was washed with deionized water and dried under vacuum for 10 min. The yeast cell suspension (100 μL, 1×10^6 cells/mL) was placed on the prepared PDA-glass, and the sample was left for 1 h to allow cell immobilization on the PDA-glass. The glass was then rinsed with dispersion medium to remove non-immobilized yeast cells in the suspension. The immobilized cells were stained using a LIVE/DEAD Yeast Viability Kit (Molecular Probes, Eugene, OR) and the cell viability was determined using CLSM.

2.6. Preparation of single nanoparticle probe

An NP probe was simply prepared as follows. A V-shaped cantilever (OMCL-TR400PSA, Olympus, nominal spring constant = 0.08 N/m) with a silicon nitride pyramidal tip was used to prepare an NP probe. To prepare a flattened tip end for single NP immobilization, the cleaned NC-glass imaging was performed in an air-conditioned laboratory (24 ± 2 °C) using AFM (MFP-3D-BIO-J, Oxford Instruments Asylum Research, Santa Barbara, CA) in contact mode (scan range: 20 μm × 20 μm, scan rate: 0.5 Hz, set point: 2.0 V, Z voltage: 130 V, scan time: 90 s). After imaging, the cantilever was sequentially rinsed with chloroform, ethanol, and deionized water for 15 min each. The cantilever was cleaned using a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY) operated at the middle level under reduced air pressure for 15 min. Finally, the flattened tip of the cantilever was immersed in a 40 μg/mL PSL NP suspension dispersed in deionized water and dried under vacuum for 10 min.

The prepared single-NP probe was coated with a thin layer of osmium using an osmium coater (Neoc-ST, Meiwafosis, Osaka, Japan) and was observed using a field-emission scanning electron microscopy (SEM) (JSM-6700F, JEOL, Tokyo, Japan).

2.7. Direct measurement of interaction force between a nanoparticle and a cell

The force between the single NP surface of the NP probe and soft surface of a living yeast cell in dispersion medium was measured using AFM integrated with an inverted optical microscope (Eclipse TE2000, Nikon, Tokyo, Japan). The NP probe was engaged with the yeast cell surface using the inverted optical microscope. Multiple force curves were recorded at various spots (1 μm × 1 μm, 10 pixels × 10 pixels) using a maximum applied force of 100 pN and contact approach and retraction rates of 200 nm/s. The spring constant of each individual cantilever was calibrated using the thermal-noise method [30] embedded in the AFM software. The cantilever spring constants were 0.095 ± 0.002 N/m. Three yeast cells from independent cultures were probed using independent single-NP probes under each condition. The maximum downward force exerted on the NP probe is referred to as the adhesion force, and was measured relative to a baseline [31].

2.8. Hydrodynamic diameter and electrophoretic mobility measurements

The hydrodynamic diameters and electrophoretic mobilities (EPMs) of yeast cells and PSL NPs dispersed in 5 mM NaCl aqueous solutions containing a water-soluble polymer were measured using a zeta potential and particle size analyzer (ELS-Z, Otsuka Electronics, Hirakata, Japan).

2.9. Contact angle measurements and surface energy estimation

The contact angle between the sample and a probe liquid was measured with a contact angle analyzer (FTA125, First Ten