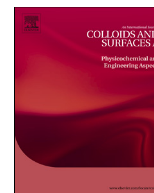
Colloids and Surfaces A 555 (2018) 653–659

Contents lists available at ScienceDirect

Colloids and Surfaces A

journal homepage: www.elsevier.com/locate/colsurfa

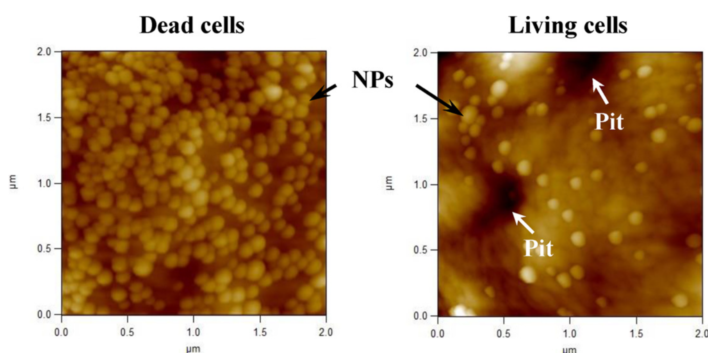


Direct measurements of colloidal behavior of polystyrene nanoparticles into budding yeast cells using atomic force microscopy and confocal microscopy

Toshiyuki Nomura*, Yuta Kuriyama, Shunsuke Toyoda, Yasuhiro Konishi

Department of Chemical Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cytotoxicity
Polystyrene nanoparticles
Uptake
Adhesion
Saccharomyces cerevisiae

ABSTRACT

The colloidal behavior of positively charged polystyrene latex (PSL) nanoparticles (NPs) toward yeast cells (*Saccharomyces cerevisiae*) was investigated using atomic force microscopy (AFM) and confocal laser scanning microscopy (CLSM). AFM imaging revealed that the conically-shaped pits connected with endocytosis were observed in 154 mM NaCl, and the surface was completely covered with NPs within 60 s in 5 mM NaCl. AFM force measurements revealed that the adhesion rate of NPs in 5 mM NaCl was faster than that in 154 mM NaCl. This strongly suggested that in 5 mM NaCl, the NPs accumulated on the cell surface over a short time followed by cell death. Furthermore, the accumulation of NPs on the cell surface in 5 mM NaCl could be suppressed by adding polyethylene glycol to the medium, resulting in an increase in the number of living cells. This suggests that the adhesion rate of NPs primarily depended on the interaction forces between the surfaces, and the viscosity of the medium. Thus, the colloidal behavior of the positively charged PSL NPs toward yeast cells was controlled by the balance between the adhesion rate of NPs on the cells and the uptake rate of NPs into the cells. Additionally, the uptake of the PSL NPs and an endocytosis marker into the cells was inhibited by Latrunculin B and NaN_3. However, their locations without inhibitor treatment were obviously different, indicating that NPs were not transported to the vacuole and accumulated in the vesicles.

1. Introduction

Understanding the interactions between engineered nanoparticles (NPs) and biological cells has received considerable attention because of the potential use of NPs as drug and gene delivery systems among other applications [1–6] and their adverse impacts on human health

* Corresponding author.
E-mail address: nomura@chemeng.osakafu-u.ac.jp (T. Nomura).

https://doi.org/10.1016/j.colsurfa.2018.07.057
Received 8 June 2018; Received in revised form 31 July 2018; Accepted 31 July 2018
Available online 31 July 2018
0927-7757/ © 2018 Elsevier B.V. All rights reserved.

and the environment [7,8]. Yeast has many similarities with animal cells and plant cells. Therefore, it is widely used as a eukaryotic model cell. However, reports on the risk assessment of NPs such as metal oxides, silver, fullerenes, and polymers using yeast cells are limited. This is because various types of NPs have been reported to show little or no cytotoxicity toward yeast [9–13]. In addition, the delivery of NPs to mammalian cells has been extensively studied in relation to drug and gene delivery systems [1–6]. However, uptake of NPs into eukaryotic cells using yeast cells has not been studied. This is because yeast cells are surrounded by robust cell walls [9]. Here, it has been reported that yeast spheroplasts with cell walls almost completely removed take up positively charged nanogold [14]. We demonstrated that in physiological saline (154 mM NaCl solution) positively charged amine modified polystyrene (PS) NPs with particle size less than 100 nm were taken up by living yeast cells [15–17]. In contrast, in 5 mM NaCl solution, the surface of yeast cells was completely covered with the NPs and caused cell death.

Confocal laser scanning microscopy (CLSM) is one technique used to directly observe dynamic NP behavior at the micro-level, but it is not suitable for distinguishing the location of the NPs at the nano-level. Instead, atomic force microscopy (AFM) is a powerful tool for nanoscale imaging of biological surface topography. In this study, we used AFM to visualize yeast cell topography at the nano-level after exposure to PS NPs in the presence of different concentrations of NaCl, as a way to better understand the colloidal behavior of the NPs toward the yeast cells, along with the direct measurement of interaction forces between the NP and the cell. Additionally, an attempt was made to control the NP behavior by adding a thickener to the dispersion medium, and the mechanism of NP uptake was investigated using an inhibitor and an endocytosis marker. The location of PSL NPs and the cell viability after NP exposure were observed directly using CLSM at the macro-level. *Saccharomyces cerevisiae* and positively charged amine-modified PSL NPs were used as a model eukaryotic microorganism and model NPs, respectively.

2. Materials and methods

2.1. Yeast strain and growth condition

The budding yeast *Saccharomyces cerevisiae* JCM 7255™ was purchased from the Japan Collection of Microorganisms. *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 harvested in the late exponential growth phase by centrifugation at 8400 × *g* and 4 °C for 10 min. To remove the leftover medium components, the harvested cells were washed three times with a sterilized NaCl solution used as a dispersion medium in the following experiments. The washed cells were re-suspended in the sterilized dispersion medium. The concentration of the yeast cells in the suspension was adjusted using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) and a Petroff-Hausser counting chamber.

2.2. Polystyrene nanoparticles

Positively charged amine-modified PSL NP with a fluorophore (nominal diameter: 100 nm) was purchased from Sigma–Aldrich (L9904, St. Louis, MO, U.S.A.) and was named PS-NH_2. The PSL NPs were suspended in the sterilized dispersion medium with a vortex for 10 s prior to use. The hydrodynamic diameter and the surface potential of PS-NH_2 and yeast cells dispersed in 5 mM and 154 mM NaCl solution are shown in Table S1 [15].

2.3. Preparation of yeast cells immobilized on the substrate

Slide glass (S7224, Matsunami Glass, Kishiwada, Japan) was rinsed in deionized water and then ethanol for 15 min each. After drying with nitrogen gas, 100 µl of 4 mg/ml dopamine hydrochloride (Sigma–Aldrich) in 10 mM TRIS − HCl buffer (pH 8.5) solution was placed on the cleaned glass surface, and was allowed to stand for 1 h at room temperature to create a coating consisting of a thin adherent polymer film derived from dopamine polymerization [18,19]. The polydopamine coated glass was then washed with deionized water, and dried under vacuum for 10 min A total of 100 µl of the yeast cell suspension (1×10^6 cells/ml) was placed on the prepared polydopamine coated glass (PDA glass), and the sample was allowed to stand for 1 h to allow the cells to immobilize on the PDA glass. The glass was then rinsed with dispersion medium to remove any non-immobilized yeast cells in the suspension.

2.4. Atomic force microscopy imaging

A total of 100 µl of 40 µg/ml PS-NH_2 suspension was placed on the yeast cells immobilized on the PDA glass. After the desired NP exposure time, the NP suspension was removed with the running 2.5% glutaraldehyde solution and was allowed to stand for 1 h. Images of the yeast cell surface were captured using an AFM (MFP-3D-BIO-J, Oxford Instruments Asylum Research, Santa Barbara, CA, USA) in tapping mode in an air-conditioned laboratory (24 ± 2 °C). Rectangular cantilevers with a silicon tip (OMCL-AC200TS, Olympus, Tokyo, Japan, nominal spring constant = 9 N/m) were used at a scan speed of 0.2 Hz and 1024 pixels per line scan. The concentrations of the NaCl solution used as the dispersion medium were 5 mM and 154 mM.

2.5. Preparation of the nanoparticle probe

The NP probe was prepared by the following simple method. A V-shape cantilever (OMCL-TR400PSA, Olympus, nominal spring constant = 0.08 N/m) with a silicon nitride pyramidal tip was sequentially rinsed with chloroform, ethanol, and deionized water for 15 min. The cantilever was then cleaned in plasma cleaner (PDC-32 G, Harrick Plasma, Ithaca, NY, USA) operating at the middle level under reduced air pressure for 5 min. The cleaned cantilever was immersed in 5 mM NaOH solution for 15 min, then rinsed with deionized water. Last, the cantilever was immersed in 40 µg/ml PS-NH_2 suspension dispersed in ethanol, and dried under vacuum for 10 min.

2.6. Force measurements

Force measurements were carried out between the NP surface of the NP probe and the soft surface of a living yeast cell in 5 mM or 154 mM NaCl solution in an air-conditioned laboratory (24 ± 2 °C) using the AFM integrated with an inverted optical microscope (Eclipse TE2000, Nikon, Tokyo, Japan) equipped for biological research. The NP probe was engaged onto the yeast cell surface using the inverted optical microscope. Multiple force curves were recorded at various spots using a maximum applied force of 100 pN, a contact time of 100 ms, and contact approach and retraction rates of 100 nm/s. The spring constant of each individual cantilever was calibrated by employing the thermal-noise method [20] embedded in the AFM software. The cantilevers' spring constants were 0.094 ± 0.001 N/m. For each condition, three yeast cells from independent cultures were probed. The maximum downward force exerted on the NP probe is referred to as the adhesion force, which is measured relative to the baseline [21].

2.7. Confocal microscopy imaging

A confocal laser scanning microscope (FV-1000D, Olympus, Tokyo, Japan) with oil-immersion objective lenses of N.A. = 1.40 (UPLSAPO 100XO, Olympus) was used to determine the location of the PSL NPs in conjunction with a fluorophore and the viability of the yeast cells.

Download English Version:

https://daneshyari.com/en/article/6977237

Download Persian Version:

https://daneshyari.com/article/6977237

Daneshyari.com