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Oscillational motion properties of bacteria and polystyrene particles on a positively polarized substrate surface

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ABSTRACT

The oscillational motion of bacteria and non-biological particles on a positively polarized substrate surface were investigated in this study using several bacterial species (*Staphylococcus epidermidis* ATCC12228 and *Pseudomonas aeruginosa* PA14) and polystyrene particles (modified with sulfate or carboxylate) that have different cell/particle size, surface potential, surface ionizable functional group, and surface appendage with respect to the mean square displacement (MSD) and motion trajectory. The attractive/repulsive interactions between the bacteria/particle and a positively polarized substrate surface are further discussed with the results of the motion analysis based on the extended Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. As our major findings, all the bacterial species and particles showed oscillational motion, a kind of sub-diffusive motion that is more limited than the Brownian motion of the suspended bacteria/particles, on a positively polarized substrate surface. However, the motion properties among the bacteria/particles were found to differ in motion radius and MSD. As the size and negative surface potential of the bacteria/particle got smaller, the oscillational motion became more active, which may result from a decrease in attractive interactions such as van der Waals interaction and electrostatic attractive interaction. In the case in which some surface functional group (e.g., sulfate group) contributed to the formation of a strong Lewis acid–base interaction, the oscillational motion was significantly reduced regardless of the surface potential of the particle. The bacterial surface appendages were found to have no influence in explaining motion differences between the bacteria and non-biological particle.

1. Introduction

A method of bacterial adhesion control using electrostatic interactions between adhered bacteria, and a polarized substrate surface has received attention because it does not require a chemical agent [1–5]. The electrical method is classified into two approaches. One applies a negative electric current (potential) to improve the electrostatic repulsion between the negatively polarized substrate surface and the adhered bacteria generally negatively charged [2,3]. The other uses a positive electric current that induces an oscillational motion of the adhered bacteria and bacterial inactivation. If some external force such as shear stress is applied to the oscillating and inactivated bacteria, the adhered bacteria can be effectively removed from the substrate surface [1,6].

In the method applying a positive current, electrostatic attraction is generally derived from the overlapped electric double layer between the negatively charged bacteria and positively polarized substrate surface [3]. Despite the electrostatic attraction, an electro-hydrodynamic flow referring to a convective flow generated under positive current prevents bacteria from stably adhering to the positively polarized substrate surface, resulting in the bacteria irregularly moving at the boundary of a substrate surface [7,8]. The bacteria's oscillational motion is also observed under non-polarized condition, but this motion occurs in a nano-scale range, differentiating from the oscillation on a positively polarized surface that occurs in a micro-scale range [9]. As the oscillational motion on a positively polarized surface facilitates bacterial detachment [1], a clear understanding of the bacteria's oscillational motion reflecting the interactions with a positively polarized substrate surface becomes important as an electrical approach for bacterial adhesion control [7].

As results of our prior study investigating the effects of electric field conditions (i.e., current intensity and ionic strength) on the oscillational motion of adhered bacteria and the development of

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Table 1
Surface characteristics of the model bacteria and particles.

	Division	Shape	Size (cross-sectional area)	Surface potential (zeta potential, mV)
P. aeruginosa PA14 (wild type)	Gram-negative bacteria	Rod	Avg. 1.5 μm^2 (0.5–0.8 μm of diameter × 1.5–3.0 μm of length)	−26.7 ± 0.9
P. aeruginosa PA14 flgK$^-$	Gram-negative bacteria	Rod	Same with wild type	–
P. aeruginosa PA14 pilA$^-$	Gram-negative bacteria	Rod	Same with wild type	–
S. epidermidis ATCC12228	Gram-positive bacteria	Sphere	Avg. 0.8 μm^2 (0.5–1.5 μm of dia.)	−20.6 ± 0.5
PS-sulfate	Non-biological particle	Sphere	0.8 μm^2 (1.0 μm of dia.)	−22.9 ± 0.1
PS-carboxylate	Non-biological particle	Sphere	0.8 μm^2 (1.0 μm of dia.)	−52.8 ± 4.1

biofilm structures [7], the bacteria's oscillational motion is more active in the condition of higher positive current and lower ionic strength. Moreover, the increase of current intensity promotes the consumption of ions at the counter electrodes, resulting in an electro-hydrodynamic flow that is a convective flow leading bacterial oscillation to be more active. A lower ionic strength is found to induce a weaker attraction between the adhered bacteria and substrate surface, as the double layer thickness of cell surface is relatively expanded. These findings demonstrate that the bacteria's oscillational motion depends on the physical interactions with a positively polarized substrate surface. Accordingly, a comparative study of motion properties among bacteria and a non-biological particle would help a better understanding of the bacteria's oscillational motion because the particle's physical interactions with a substrate surface have been relatively more researched.

The aim of this study is to investigate the differences in oscillational motion properties among various bacteria and non-biological particles with application of positive current to the substrate surface, and to discuss the physical interactions influencing their attachment to or detachment from a positively polarized substrate surface. A particle tracking method was used to analyze the oscllational motions of bacterial cells and polystyrene (PS) particles having partially different size, surface potential, surface functional group and surface appendage. Firstly, three motion properties of two bacterial species (i.e., *Staphylococcus epidermidis* ATCC12228 and *Pseudomonas aeruginosa* PA14 having a different cell size but similar surface potential) and a non-biological particle (i.e., PS particles modified with sulfate (PS-sulfate) having similar size and surface potential with *S. epidermidis*) were comparatively examined in terms of motion trajectory, MSD curve and the averaged root MSD. Secondly, the oscillational motions of the PS-sulfate and PS particles modified with carboxylate (PS-carboxylate) were compared in order to investigate the effects of the PS particle's surface functional group and surface potential on the oscillational motions. Thirdly, the motions of *P. aeruginosa* mutants lacking either flagella (flgK$^-$) or pili (pilA$^-$) were compared with the motion of *P. aeruginosa* wild type in order to investigate the effects of the surface appendage as well as the surface functional group on the oscillational motions. Lastly, the attractive (return force) and repulsive (driving force) interactions between the adhered bacteria/particle and the positively polarized substrate surface are discussed based on the extended DLVO (XDLVO) theory which has been widely used in interpreting bacterial adhesion [10].

2. Materials and methods

2.1. Model bacterial strain and non-biological particles

S. epidermidis ATCC12228 and *P.* aeruginosa PA14 included in Gram-positive and Gram-negative species, respectively, were chosen as model bacteria to investigate the effect of bacterial cell size on their motion properties (Table 1). Both bacterial species are well known to contribute to biofilm formation on various solid surfaces [11]. *S. epidermidis* is a sphere shaped-bacteria with a diameter ranging from 0.5 to 1.5 μm (average cross-sectional area of cell when assuming as circle shape: 0.8 μm^2), and *P. aeruginosa* is a rod-shaped bacteria with about a 0.5–0.8 μm diameter and about 1.5–3.0 μm in length (average cross-sectional area of cell when assuming as rectangular shape: 1.5 μm^2) [12,13]. The zeta potential of *S. epidermidis* and *P. aeruginosa* representing the net surface potential was measured, respectively, as −20.6 ± 0.5 mV and −26.7 ± 0.9 mV using the ELS-8000 Electrophoretic Light Scattering (Photal Otsuka Electronics, Osaka, Japan) system at 160 Hz and 80 V and not much different between the two species.

S. epidermidis and *P. aeruginosa* were incubated at 37 °C for 24 h after streaking on tryptic soy agar (Difco, USA) plates. Each colony was inoculated into tryptic soy broth (Difco, USA) and cultured at 110 rpm and 37 °C overnight. The cultured cells were collected by centrifuge and washed twice with 20 mM of potassium phosphate buffer (KH_2PO_4, pH 7.1). Subsequently, the bacterial pellets were resuspended in potassium phosphate buffer (20 mM KH_2PO_4, pH 7.1) at 1×10^8 CFU/ml of the initial concentration. In order to investigate the effect of surface appendages on bacterial oscillation, flgK$^-$ and pilA$^-$ were used in the motion analysis under the same experimental conditions as the wild type *P. aeruginosa*. Their culturing methods and preparation processes of bacterial solutions were the same as the wild type *P. aeruginosa*.

Two kinds of PS particles, PS-sulfate and PS-carboxylate (Molecular Probes, USA), were used as model non-biological particles in this study. Both PS particles have a sphere shape with a diameter of about 1 μm (cross-sectional area of 0.8 μm^2), similar to the average diameter of *S. epidermidis*. PS-sulfate has a zeta potential of −22.9 ± 0.1 mV, also comparable with those of the bacterial species. On the other hand, PS-carboxylate has a zeta potential of −52.8 ± 4.1 mV, which is about two times higher (Table 1) implying a stronger negative surface potential [14]. PS-sulfate, which has a similar size and zeta potential value with *S. epidermidis*, was mainly used to compare the motion properties with bacteria, and the oscillational motion of PS-carboxylate was additionally analyzed to investigate the effects of functional groups and surface potentials on oscillational motion compared to PS-sulfate.

Both PS particle solutions (1 vol%) were prepared by diluting each particle species in 20 mM of potassium phosphate buffer (KH_2PO_4, pH 7.1). The concentration of PS particle solutions could not be accurately determined due to the absence of concentration information on the particle stocks.

2.2. Flow chamber and ITO electrodes

An experimental reactor which is composed with a flow chamber (FC 81 flow cell, Biosurface Technologies, USA), and two ITO coated glass electrodes was prepared as previously described [7].