Modeling of chemotactic steering of bacteria-based microrobot using a population-scale approach

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The bacteria-based microrobot (Bacteriobot) is one of the most effective vehicles for drug delivery systems. The bacteriobot consists of a microbead containing therapeutic drugs and bacteria as a sensor and an actuator that can target and guide the bacteriobot to its destination. Many researchers are developing bacteria-based microrobots and establishing the model. In spite of these efforts, a motility model for bacteriobots steered by chemotaxis remains elusive. Because bacterial movement is random and should be described using a stochastic model, bacterial response to the chemo-attractant is difficult to anticipate. In this research, we used a population-scale approach to overcome the main obstacle to the stochastic motion of single bacterium. Also known as Keller-Segel's equation in chemotaxis research, the population-scale approach is not new. It is a well-designed model derived from transport theory and adaptable to any chemotaxis experiment. In addition, we have considered the self-propelled Brownian motion of the bacteriobot in order to represent its stochastic properties. From this perspective, we have proposed a new numerical modelling method combining chemotaxis and Brownian motion to create a bacteriobot model steered by chemotaxis. To obtain modeling parameters, we executed motility analyses of microbeads and bacteriobots without chemotactic steering as well as chemotactic steering analysis of the bacteriobots. The resulting proposed model shows sound agreement with experimental data with a confidence level <0.01 . © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4932304>]

I. INTRODUCTION

The bacteria-based microrobot (bacteriobot) is a hybrid concept microrobot, which has propelling bacteria and a drug-loaded microstructure. The bacteriobot represents a potential alternative vehicle for targeted drug delivery systems (DDS). Researchers are particularly interested in developing the bacteriobot for tumor therapy, as such a development could reduce the side effects of tumor treatment.^{1–4} Studies have investigated various bacteria strains, biocompatible materials, and microstructure shapes in order to enhance bacteriobot function. Researchers have also modeled movement of various bacteria and bacteriobots.

The bacteriobots generally show directionless random motilities in isotropic environments, as bacteria do.^{5–7} However, for elevated directionality, researchers have employed the intrinsic characteristics of bacteria, including chemotaxis,^{4,8,9} magnetotaxis,^{2,10–12} galvanotaxis,¹³ and phototaxis.¹⁴ These taxes of bacteria also correspond to external stimuli, such as chemical attractants, electromagnetic actuation, electrophoresis systems, and ultraviolet light, respectively. Therefore, the bacteriobot can be considered to be the phoretic microrobot mentioned by Golestanian because of its bacterial phoresis properties.¹⁵ However, because bacteria are

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bio-organisms with different paths, there are many uncertainties, making it difficult to predict the individual paths of bacteriobots.

Most of bacteria such as *Escherichia coli*, *Salmonella typhimurium*, and *Serratia marcescens*, which are used for bacteriobot fabrication, have two phases in their motion: (1) the run phase moving straight forward and (2) the tumbling phase changing direction. The transition between these phases is essentially random and usually is described by using the two-state continuous Markov chain model.^{9,13} One can use an ensemble of bacteria with this model to explain the movement of a bacteriobot; however, this individual bacteria approach creates complex problems in terms of formulating directional forces and direct attachments between bacteria and microstructure. To solve this problem, researchers estimated the sum of overall propulsive direction of the attached bacteria based on information about the bacteriobot's movement and introduced a bacterial flagella flexibility constant to consider the flow stream.^{13,16,17} However, these individual approaches still have considerable issues in bacteriobot modeling. For example, using these individual phoretic microrobot approaches, we should consider the change in transition time between the run phase and tumbling phase, the variation of the bacterium tumbling angles, and the frequencies with respect to external stimulations. One report refers to bacterial tumbling probability in chemical gradients to analyze bacteriobots' properties.⁹ However, no report uses analytic results from the bacterial tumbling angle. Although the tumbling angle of some kind of bacteria shows the average of the value of zero degree, that of some others does not. It is known that the mean values of the tumbling angles are about 62° and 50° in the case of *E. coli* and *S. marcescens*, respectively.¹⁸ In addition, their tumbling angular distributions do not show a Gaussian distribution.^{19,20}

However, the motility of bacteriobots without external stimulation in isotropic environments can be considered through the Brownian motion of active particles. This approach handles the bacteriobot not as a combined object of bacteria and microstructure but as a single self-propelled particle using the perspective of Brownian motion. The advantage of this method is that it can be used without measuring precise parameters about the bacterial tumbling and the bacterial directional attachment to the microstructure. Therefore, it has been used for bacteriobot motility analysis with or without a single-cell based approach.^{16,21,22} However, this method cannot create a bacteriobot chemotactic steering model in suspended bacterial media; an additional term is required to express external steering.

In this paper, to model the bacteriobot's motion in chemotaxis, we adopted a population-scale approach instead of a single-cell based approach to describe bacterial motion. The population-scale approach, which is compatible with the single-cell based approach, has the advantage of allowing effective observation of bacteria at the macro scale.²³ We propose a new modeling method through the combination of this approach and the Brownian motion of active particles. Therefore, this research can be considered the first to use a chemotaxis equation for bacteriobot modeling. We conclude by verifying the proposed model through chemotaxis experiments with bacteriobots using a customized agarose three-channel chemical gradient microfluidic device.

II. MODELING OF BACTERIOBOT

The ideal conditions for bacteriobots are as follows: the bacteria exist isotropically in media with no external force; in such conditions, they can only be propelled by the attached bacteria without bias toward any direction. We assume that the microstructure is a sphere, namely, a microbead. Then, the time variations of the bacteriobot's mean squared displacement (MSD) can be described as follows:¹⁶

$$\Delta L^2_{\text{without steering}} = 6Dt + \frac{V^2}{2D_R^2} (2D_R t + e^{-2D_R t} - 1), \quad (1)$$

where L is the displacement of the bacteriobot after t seconds, D is the translational diffusion coefficient, V is the propulsion speed in x-y plane, and D_R is the rotational diffusion coefficient.

The equation assumes that the probability of bacterial motility along z-axis (gravitational direction) is the same with x- and y-axis. In Eq. (1), the stochastic motion of the bacteriobot can be described as the coupling of the translational diffusion and the rotational diffusion. This diffusion can be affected by the following three factors: pure Brownian motion of the microbead in media, the collision force of any unattached bacteria around the bacteriobot, and the propulsion force of the attached bacteria. First, pure Brownian motion comes from molecular level collisions, such as water and media molecules. Second, the collision force of unattached bacteria around the bacteriobot directly depends on the bacterial density in the media. Finally, bacterial propulsion by attached bacteria is a key component in the actuation of the bacteriobot, which depends on the number of attached bacteria and the transition frequency between the tumbling and run of the attached bacteria. A microbead of $10\ \mu\text{m}$ diameter in pure water has a molecular level diffusion coefficient D of about $0.02\ \mu\text{m}^2/\text{s}$; however, the diffusion coefficient of the microbead can be increased to over $100\ \mu\text{m}^2/\text{s}$ by increasing bacterial density in media.²⁴

In the presence of an external steering control, bacteriobots undergo additional directional forces, obtaining better directionality and motility, as shown in Fig. 1. Based on Einstein's relation, under a specific condition, we assumed that the time average of drift velocity owing to a small external force is proportional to the time average of the diffusion coefficient with no external force.^{25–27} If the assumption between the time averaged diffusion constant and the small external force was true, the proportional relation can be used in here. A diffusion coefficient requiring a calculation of drift velocity can be obtained approximately from Eq. (1). If the Brownian relaxation time, τ ($\tau = 1/D_R$, the inverse of D_R), is small compared to t , then Eq. (1) becomes $\Delta L^2 = 6Dt + V^2\tau t$ approximately. If it is large compared to t , then Eq. (1) becomes $\Delta L^2 = 6Dt + V^2t^2$. From these relationships, we can define a parameter, the effective diffusion coefficient of a bacteriobot (D_{eff}), which satisfies $\Delta L^2 = 6D_{\text{eff}}t$, as follows:

$$D_{\text{eff}} = \begin{cases} D + \frac{1}{6}V^2\tau & (\tau \ll t) \\ D + \frac{1}{6}V^2t & (\tau \gg t). \end{cases} \quad (2)$$

Because pure Brownian motion is relatively small compared to other motions, we can ignore the pure Brownian motion in our case. Therefore, the calculation of the bacteriobot's total displacement using both mean drift velocity of the bacteriobot and environmental bacterial collision by means of a steering force can be written as follows:

$$\Delta L_{\text{with steering force}} = k_l t \langle D_{\text{eff}} \rangle + k_c L_C, \quad (3)$$

where k_l is a coefficient that reflects how much steering force affects the diffusion behavior of the bacteriobot itself. It depends on the proportionality factor of Einstein relation and changes in the attached bacterial thrust force when inducing chemo-attractants. $\langle D_{\text{eff}} \rangle$ indicates time average of D_{eff} , L_C represents the mean displacement of environmental bacteria caused by the

FIG. 1. A schematic drawing of bacterial forces on the bacteriobot. With the condition of no external stimulation, the attached bacteria generate thrust forces in random directions, and environmental bacteria also generate a random motion for the microbead with occasional collision (a). With a chemo-attractant, the directions of the attached bacterial thrust force and of the environmental bacterial collision are shifted toward the attractant (b). Note that the relative length of the arrows does not represent the real magnitude ratio of forces.

steering force, and k_c is the efficiency coefficient—how much force is transferred to the microbead from the environmental bacterial collision, moreover, how frequently collision is occurred by environmental bacteria, where the unit of k_l is $1/\mu\text{m}$, and k_c is a non-dimensional variable. In Eq. (3), the first term represents the motility caused by the attached bacteria, and the second term represents the motility caused by the environmental bacteria. If there is no steering control, bacterial thrust and collision with the microbeads from every direction create a random walk motion for the bacteriobot. However, if a chemotactic stimulation (chemical gradient) is applied for steering control, bacteriobots can move toward the chemo-attractant region.^{8,9,28}

To estimate the bacteriobot's motility by chemotaxis, the change of L_C by a chemical gradient should be considered. Using a basic definition of flux, L_C is derived from bacterial flux J ,

$$L_C = r_s \iiint J \cdot dA dt, \quad (4)$$

where r_s is a mean distance among the bacteria, which can be calculated through an approximation of mean inter-particle distance and can be approximated as a Wigner-Seitz radius $[3/(4\pi n)]^{1/3}$. A is a microbead cross-section. When a chemical gradient is generated along the x -axis, chemotactic steering will be applied along the x -direction. Therefore, the mean displacement (L_C) of bacterial flux along the x -axis is defined as Eq. (4), which indicates the multiplication of the mean distance among the bacteria and the number of bacteria that can cross the microbead's cross-section A on the y - z plane during a given time.

Many researchers have already studied bacterial flux J in the presence of a chemical gradient.^{21,29–31} Keller-Segel's equation is widely used for bacterial chemotaxis.³² During a short time that can ignore the birth and death of bacteria, they derived a bacterial flux from transport theory as follows:

$$J = n\chi(C)\nabla C - \mu\nabla n, \quad (5)$$

where χ is the chemotactic coefficient, C is the concentration of chemo-attractant, μ is the bacterial random motility coefficient, and n is the number of bacteria per unit volume. The chemotactic coefficient is dependent on the chemical concentration, speed of bacteria, and dissociation constant between the attractant and the receptor. Among many equations for chemotactic coefficients, the following equation, derived by Chen *et al.*³³ and noted by Tindall *et al.*,²³ is frequently used:

$$\chi(C) = \frac{8v}{3} \tan\left(\frac{\chi_0}{8v} \frac{K_d}{(K_d + C)^2} \nabla C\right), \quad (6)$$

where v is the bacterial swimming speed in two dimensions, χ_0 is chemotaxis sensitivity coefficient, and K_d is the chemotaxis receptor constant. μ is also known as the bacterial diffusion coefficient, which depends on the chemical gradient. However, to simplify the model, we adopted the value from a previous paper.³⁴ In Eq. (5), the first term on the right-hand side indicates chemophoretic force, which reflects the bacterial movement induced by the chemical gradient. The second term describes the bacterial random motion, which reflects a diffusion of bacteria using Fick's first law. With the boundary conditions and initial conditions as in experiment, we can calculate J as described in Section III B. Finally, we can obtain the bacterial displacement and bacteriobot's displacement through Eqs. (1) and (3).

III. MODEL DEMONSTRATION AND TESTING

Fig. 2 illustrates the procedure used to evaluate the displacement of the bacteriobot in bacterial media with chemical steering. As described in Section II, the displacement of bacteriobot (ΔL) is a function of the bacteriobot's effective diffusion coefficient (D_{eff}), bacterial steering

FIG. 2. Procedures to evaluate the displacement of bacteriobots in the bacterial media with chemical steering. The values D , V , τ can be obtained from experiments; n , C can be obtained from simulation, and k_l , k_c can be estimated through the combination of the experiment and simulation.

(L_C) to the chemo-attractant, and the coefficients k_l and k_c . To evaluate the effective diffusion coefficient (D_{eff}), we execute an experimental estimation for the diffusion coefficient of microbeads (D) with non-attached bacteria and without chemo-attractant in the media. The bacteriobot's motility parameters, such as propulsion speed (V) and Brownian relaxation time (τ), are also estimated through experiments because D_{eff} depends on D , V , and τ . For the evaluation of bacterial displacement in the presence of chemo-attractant (L_C), we simulated the changes of the chemical concentrations (C) and bacterial densities (n) in the microchannel. Finally, we estimated k_l and k_c values through a fitting of experimental results.

A. Experimental setup for motility analysis

For motility analysis of bacteriobots, we adopted a customized three-channel microfluidic device, which is used in experiments and simulations. We made the 3-channel microfluidic device using conventional photolithography and soft lithography with agarose. In short, through conventional photography, a master mold of the three-channel microfluidic device first was fabricated with SU-8 photo-resistor, an emulsion mask, and an aligner. Second, we poured and gelled a 3% high temperature agarose solution in a petri dish and pushed the master mold into the agarose gel. Through this method, we obtained the final three-channel microfluidic device. The width of the left and right channels was $3000\ \mu\text{m}$ in contrast to the central channel, which was $400\ \mu\text{m}$, to allow the channels to maintain their role as chemical sources for a sufficient amount of time. Fig. 3 shows a conceptual schematic of the 3-channel microfluidic device with a chemical gradient, where the loaded bacteriobots exist in the central channel, and the chemo-attractant and phosphate buffered saline (PBS) exist in the left and right channels, respectively.

The microbeads for the experiment were fabricated by spraying 1% 4-armed thiolated polyethylene glycol (PEG-SH) into 1% 4-arm maleimide modified PEG (PEG-MAL). After selecting microbeads of $5\text{--}10\ \mu\text{m}$ in diameter using the $5\ \mu\text{m}$ and $10\ \mu\text{m}$ filters, we soaked the selected microbeads for about 1 h in 0.001% Poly-L-lysine (PLL); the PLL coating enhances bacterial attachment for bacteriobot fabrication. Bacteria, which are used for the bacteriobot fabrication, usually adhere to the hydrophobic surface of polystyrene microbeads but do not adhere to the hydrophilic surface of PEG microbeads. Therefore, the PLL coating on the PEG microbeads makes their surfaces hydrophobic and the bacteria can easily adhere to the PLL coated PEG microbeads.³⁵ After 3 or 4 repeated washings of the microbeads with distilled

FIG. 3. The geometry of the microchannel used for testing bacteria and bacteriobot motility. Aspartic acid and PBS were loaded in the left- and right-side channels to make a chemical gradient after bacteriobot loading into the central channel.

water, we mixed them with bacteria for over 30 min, allowing us to obtain the final bacteriobots. In every experiment, we used the *S. typhimurium* bacterial strain, which is known as an effective bacterial strain for tumor therapy.³⁶

We executed the experiments for microbeads' and bacteriobots' motility analysis in chemotaxis using the three-channel microfluidic device according to the following routine. First, we injected the microbeads or bacteriobots into the central channel of the agarose microfluidic device. Second, in the chemo-attractant test, we loaded 0.1 mM aspartic acid as a chemo-attractant and a PBS as a control medium in the agarose microfluidic device's left and right side channels, respectively. In a control test, we loaded a PBS both in the left- and right-side channels. After loading the chemo-attractant/PBS solutions in the left- and right-side channels, we recorded the images in the central channel at 15 frames per second for over 90 s. Our MATLAB[®] tracking program,³⁷ which contains Darnton and Jaffe's particle tracking code,³⁸ analyzed the recorded videos, as the program can recognize the centroids of the microbeads and bacteriobots with pre-defined thresholds, and it can calculate their distances and velocities. When the recognition times of the microbeads and bacteriobots were less than 30 s, or when they were out of focus or made contact with the boundary of the channel, their motions were not included in the motility analysis.

To validate our model according to the condition changes of the experiments, the initial concentration of aspartic acid and the initial density of the environmental bacteria were regulated. Specifically, for the initial concentration change of the chemo-attractant, 0.025 mM, 0.05 mM, and 0.075 mM of aspartic acid were used with the environmental bacteria optical density of 1.0 (8×10^8 bacteria/ml³). In addition, for the initial density change of the environmental bacteria, the optical densities of 0.25, 0.5, and 0.75 were used with the 0.1 mM aspartic acid. To regulate the initial density of the environmental bacteria, we diluted the bacteria-microbead mixture after the mixing step of the bacteriobot fabrication.

B. Simulation model for motility analysis in chemotaxis

To simulate the chemotaxis of bacteria and bacteriobots, we evaluated bacterial density $n(x,t)$ and chemical concentration $C(x,t)$ using COMSOL Multiphysics[®]. It is necessary to evaluate n and C for obtaining the bacterial flux. First, for the bacterial population (n), one can introduce a continuity equation to Keller and Segel's equation as follows:

$$\frac{\partial n}{\partial t} = -\nabla \cdot (n\chi \nabla C - \mu \nabla n). \quad (7)$$

Second, we can derive a chemical concentration (C) with time dependence through Fick's second law while introducing D_c (a diffusion coefficient of chemo-attractant)

$$\frac{\partial C}{\partial t} = D_c \nabla^2 C. \quad (8)$$

In the experiment, the distance (l) between left and right channels was $1000\text{ }\mu\text{m}$. And, we set the center coordinate of the central agarose channel as a zero, while the chemo-attractant was at a region of $x \leq -l/2$ in our simulation. Through COMSOL Multiphysics[®] software tool, we computed the number of bacteria in unit volume ($n(x,t)$) and the concentration of chemo-attractant ($C(x,t)$), where the initial conditions and boundary conditions of $n(x,t)$ and $C(x,t)$ are as follows:

$$C(x, 0) = \begin{cases} C_0 & (x = -l/2) \\ 0 & (-l/2 < x \leq l/2), \end{cases} \quad (9)$$

$$C(0, t) = C_0, \quad C(l/2, t) = 0, \quad \frac{\partial C}{\partial t}(x, 0) = 0, \quad (10)$$

$$n(x, 0) = \begin{cases} n_0 & (-l/5 < x < l/5) \\ 0 & (x \leq -l/5 \text{ or } x \geq l/5), \end{cases} \quad (11)$$

$$n(x \leq -l/5 \text{ or } x \geq l/5, t) = 0, \quad \frac{\partial n}{\partial t}(x, 0) = 0, \quad (12)$$

where C_0 is the initial concentration of chemo-attractant and n_0 is the initial density of bacteria. As the equation shows, our simulation assumed chemo-attractant and PBS to be infinite sources. In addition, because agarose has a similar diffusion coefficient as water, we did not discriminate the diffusion coefficient of agarose.³⁹

The three-dimensional simulation model with $1000\text{ }\mu\text{m}$ height and $400\text{ }\mu\text{m}$ depth was meshed as 39380 elements, and the simulation time interval was 0.1 s. After reaching the numerical solutions of n and C through the simulation, we computed the bacterial flux $J(x,t)$ and the displacement of environmental bacteria $L_C(x,t)$ using Eqs. (4) and (5). The parameters, which were used in the simulation, are listed in Table I. Because our bacteria are very similar to *E. coli*, we adopt some characteristic values of *E. coli*, which were already well analyzed.

IV. RESULTS AND DISCUSSION

A. Aspects of bacteriobot's motility with and without chemotaxis

Fig. 4 shows the motility experimental results of bacteriobot with and without a chemo-attractant. When there was no chemotaxis, the bacteriobots showed random directionalities, namely, no bias; meanwhile, their average velocity in the horizontal direction was approximately $0.02\text{ }\mu\text{m/s}$. However, bacteriobots show biased movements to the left direction in the presence of the chemo-attractant on the left side; the average velocity in the horizontal direction was about $-0.28\text{ }\mu\text{m/s}$, and it was relatively larger than without chemotaxis steering. As

TABLE I. Parameters for numerical simulation of the chemotaxis of the bacteriobot.

Symbol	Meaning	Value	Reference
χ_0	Chemotactic sensitivity coefficient	$5.7 \times 10^{-4}\text{ cm}^2/\text{s}$	^a
K_d	Chemotaxis receptor constant	1.0 mM	^b
μ	Random motility coefficient	$8.9 \times 10^{-7}\text{ cm}^2/\text{s}$	^c
A	Cross-section of microbeads	$25\pi\text{ }\mu\text{m}^2$	...
v	Swimming speed of bacteria	$20\text{ }\mu\text{m/s}$	...
n_0	Initial density of bacteria	$8.0 \times 10^8\text{ cells/ml}$	...
C_0	Initial concentration of aspartic acid	0.1 mM	...

^aReference 45.

^bReference 46.

^cReference 34.

expected, the bacteriobots with chemotactic steering showed enhanced directionality compared to the bacteriobots without chemotactic steering. Most bacteriobots went to the left side in the end, but a few bacteriobots were driven to the right direction with small displacements from the starting point. These experimental results provide strong evidence that bacteriobots can be steered to the chemo-attractant.

B. Estimation of simulation parameters using experimental data

To calculate the diffusion coefficient of bacteriobots (D_{eff}), we executed a motility experiment for the microbeads and the bacteriobots without chemotaxis. Because microbeads in media do not self-propel or move by Brownian motion, the propulsion speed (V) is nearly zero, and MSD is $\Delta L^2 = 6Dt$. Based on the MSD measurement of the microbeads in a non-chemotaxis condition with the bacterial optical density of 1.0 (data are not shown), we calculated the D to be approximately $2.81 \mu\text{m}^2/\text{s}$. Because this value is greater than that of normal Brownian motion and less than that of the bacterial carpet in other group's experiment,⁴⁰ we found the calculated value is reasonable.

We executed the motility experiment for bacteriobots under the same conditions and applied the value D in Eq. (1). Consequently, we estimated the V and τ to be about $0.69 \mu\text{m}/\text{s}$ and 677 s , respectively. Fig. 5 shows the comparison between the experimental MSD data and the fitted MSD curve using the obtained values.

C. Modeling of bacteriobots with chemotactic steering

Through COMSOL Multiphysics® simulation, we estimated the bacterial density and flux, as shown in Fig. 6. In their initial state, the environmental bacteria were uniformly distributed

FIG. 4. The bacteriobot's motility without a chemo-attractant (a)-(b) and with a chemo-attractant (c)-(d). These plots show merged pathways of bacteriobots (a), (c) and relative pathways by shifting the starting point to zero (b), (d). The bacteriobots without and with a chemo-attractant showed the average velocity about $0.02 \mu\text{m}/\text{s}$ and $-0.28 \mu\text{m}/\text{s}$, respectively. The results show that the bacteriobots' movement is biased to the left direction where the chemo-attractant is located.

FIG. 5. Mean squared displacement of bacteriobots without a chemo-attractant. Propulsion speed and Brownian relaxation time can be evaluated in analyzing the bacteriobots' motion as a Brownian motion of active particles. Equation (1) was fitted to 32 bacteriobots' data by using two parameters fitting, where R-square is 0.9926.

with a density of 8.0×10^8 cells per milliliter, and they started to move toward the left direction with the chemo-attract diffusion. Then, the bacterial density difference between the left and right region increased. After some time, if the gradient of bacterial density reached equilibrium with the chemotactic force, the bacteria migration also reduced, and the bacterial flux reached nearly zero.

Using this simulation result, we obtained the numerical solutions of bacterial density $n(x,t)$ and bacterial flux $J(x,t)$; we computed consequentially $L_C(x,t)$ using Eq. (6). To compare between the modeling results and the experimental results, we used only the $L_C(0,t)$ value. The value of D_{eff} was also approximately calculated using $D_{\text{eff}} = D + V^2t/6$, because the experimental time (t) was so small compared to the calculated Brownian relaxation time (τ). This approximation comes from a Taylor series expansion of the exponential term of Eq. (1); the ignored series showed only a 1% difference in D_{eff} value, with an experiment time of 60 s in this study. In addition, we also estimated k_l and k_c in Eq. (3) as 0.036 and 0.028, respectively. Based on these results, we could estimate that the pushing efficiency of the environmental bacteria to the bacteriobots was about 2.8%. The calculation parameters using experimental data are shown in Table II.

FIG. 6. The results of the bacterial chemotaxis simulation. The difference in bacterial density between the left and right region in the agarose channel increases (a) and the magnitude of bacterial flux decreases (b) as time proceeds.

TABLE II. Calculation parameters using experimental data.

Symbol	Meaning	Value
D	Diffusion coefficient of the microbead in the condition of bacterial media	$2.81 \mu\text{m}^2/\text{s}$
V	Propulsion speed of the bacteriobot in two dimensions	$0.69 \mu\text{m}/\text{s}$
τ	Brownian relaxation time of the bacteriobot	677 s
$\langle D_{\text{eff}} \rangle$	Time average of the bacteriobot's effective diffusion coefficient for 60 s	$5.20 \mu\text{m}^2/\text{s}$
k_l	Steering coefficient of the bacteriobot	0.036
k_c	Collision efficiency of the environmental bacteria to the bacteriobot	0.028

D. Validation of bacteriobot model with chemotactic steering

Fig. 7 shows the comparison between the numerical solution of the proposed modeling and the experimental data. In the modeling and experiment, the bacteriobots' motility slightly decreased with the passage of time because the chemical gradient degraded. The bacterial movement speed to the chemo-attractant (bacterial flux) depends on the gradient of the chemical concentration according to the chemotaxis model. Therefore, the bacteria or bacteriobots near the left channel with the chemo-attractant initially moved quickly toward the left channel because that region has the maximum chemical gradient just after chemo-attractant loading. After that, the chemo-attractant spontaneously diffused in the channel, the chemical gradient reduced as time passed, and the velocity of the bacteria/bacteriobots decreased. If the chemo-attractant and PBS were not refreshed infinitely, the chemical gradient would become zero along with the velocity of the bacteria/bacteriobots.

As shown in Fig. 7, the expected results based on the modeling coincided with the experimental results within the standard error range. To validate the proposed model, we evaluated p-value using Pearson's chi-square test. The p-value was about 0.0022, where it means the model fits statistically with the experimental results. The standard error increases in this graph can be interpreted as evidence that the bacteriobots undergo a stochastic process.

FIG. 7. Experimental results and theoretical results of the bacteriobots' motility steered by chemotaxis. This theoretical curve from Eq. (3) has a nonlinear shape due to the integral calculus of Eq. (4) and the partial differential equation of Eq. (5). As a result, the nonlinear curve shows a positive second derivative around 10 s and a negative second derivative after about 20 s. Comparing results of the time variations in bacteriobot displacement shows that the proposed model corresponds with experimental data within the experimental standard error range.

FIG. 8. Modeling and experimental results, and variations of modeling coefficients according to the initial condition changes. Modeling and experimental results, and variations of modeling coefficients according to the initial chemo-attractant (C_0) changes (a). Modeling and experimental results, and variations of modeling coefficients according to the initial number of the environmental bacteria (n_0) changes (b).

E. Further analysis by changing initial condition of the modeling and experiment

For the validation of our model, we executed two additional experiments. First, we investigated the change of bacteriobot's motility according to initial concentration of chemo-attractant (C_0). Second, we examined the change of bacteriobot's motility according to initial density of environmental bacteria (n_0). Fig. 8 shows the experimental results and the simulation results at the same time. From the experimental results, it was confirmed that the bacteriobot's motility can be affected by the initial chemo-attractant. And, the effect of the environmental bacteria on the bacteriobot, which was always neglected in other bacteriobot studies, came out. In addition, we found that there is a strong positive correlation between the collision efficiency (k_c) and the initial density (n_0) of the environmental bacteria. The corresponding results of the experiments and the simulations are shown in the supplementary material (Fig. S1-S4).⁴¹

V. CONCLUSION

In summary, we proposed a motility model of bacteriobots with chemotaxis steering. This model involves both the population-scale view of the bacterial chemotaxis in biology and the self-propulsive Brownian particle view in physics. We also considered the movement of the environmental bacteria in a real experimental condition. We analyzed the motilities of bacteriobots without a chemo-attractant in order to calculate modeling parameters; thus, the diffusion coefficient and the Brownian relaxation time of bacteriobots could be evaluated. In addition, by comparing the simulation results using these parameters and the real chemotaxis experimental data, we also validated the proposed bacteriobot motility model. Through the additional experiments for the validation of our model, the effect of the initial density of the environmental bacteria on the bacteriobot is analyzed quantitatively. The three-channel microfluidic device generated a linear gradient of chemo-attractant chemical in the central channel, diffusing it through the hydrogel channel wall. These microfluidic channel devices have been fabricated for a linear chemical gradient using various materials, such as agarose, nitrocellulose, PEG, and other variations.^{39,42–44} We customized this channel for our purposes and used it to evaluate the chemotaxis of the bacteriobots. In contrast to other studies about bacteriobot motility, we separately considered the effects of environmental bacteria and attached bacteria. The environmental bacteria are the remaining bacteria (unattached bacteria) after mixing bacteria and microbeads (the bacteria attachment process in the fabrication of bacteriobots). They could increase their population radically on account of their fast doubling speed, increasing the collision with

bacteriobots. Because of this phenomenon, we also paid attention to environmental bacteria in this study.

Among our results, the diffusion coefficient of bacteriobots (about $2.81 \mu\text{m}^2/\text{s}$) corresponds to a diffusion coefficient of a normal 100 nm–200 nm diameter nanoparticle. In other words, stochastic modeling of bacteriobots is necessary for the motility analysis of nanoparticles and nanorobotics. Therefore, to describe the motilities of the particle, one should choose the single particle perspective or the population-scale perspective. In this respect, this paper represents the first study using a population-scale approach to phoretic-micro/nanorobotic motility modeling.

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