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Motility steering of bacteriobots using chemical gradient microchannel

Shaohui Zheng, Jiwon Han, Sunghoon Cho, Van Du Nguyen, Seong Young Ko, Jong-Oh Park*, and Sukho Park*, *IEEE Member*

Abstract— **In the recent years, several groups focused on the development of bacteria based microrobots (bacteriobots) using microbeads and flagellar bacteria. The bacteriobots will be a promising cancer therapeutic method in the future with drug encapsulation inside microbeads. However, it remains elusive that how to steer the motion of bacteriobots. In this study, we attempted to steer the motion of bacteriobots with the intrinsic bacterial chemotaxis to particular chemicals. Therefore, a new microfluidic channel was designed and fabricated through micro-molding method of hydrogel patterns, which a sustained chemical gradient was investigated using rhodamine B at various determined time intervals. Thereafter, the bacteriobots solution was injected into the central channel with chemoattractant gradient, then the chemotactic motion of bacteriobots was investigated through a microscope and analyzed with MATLAB program. Moreover, some other chemoattractant chemicals, secreted from tumor cells could also stimulate the tumor targeting ability possible with bacteriobots. Overall, the motion of bacteriobots can be steered through bacterial chemotaxis, and we expect drug embedded bacteriobots to be a new targeted therapy in cancer treatment.**

Index Terms— **Bacteriobots, Motility steering, Chemical gradient microchannel, Chemotaxis, Aspartic acid**

I. Introduction

In the recent years, as the development of micro-nanotechnology, many groups focused on the fabrication of micro/nano robotics for therapeutic and diagnostic applications through minimally invasive interventions [1-8]. However, in spite the efforts in the development of these devices, an intractable problem still remains to be addressed that how to actuate the micro-robots before large-scale biomedical application. Normally, external magnetic fields, ultrasound and on board biological motors such as bacteria and immune cells were applied to propel micro-devices according to recent reports [5-18]. However, a huge external actuating system is essential to generate driving force with magnetic fields or ultrasound. Biological motors are of great advantages in actuation of microsystems, such as high motility, micro-size, self-replication, high efficiency and taxis. Particularly, flagellar bacteria have been proposed as a promising motor in assembly of microrobots among all types of biological motors. Generally, bacteria could be attached to the surface of microstructures (body of the micro-devices) through specific physical or chemical binding strategies in the assembly process of bacteriobots [5, 10-12].

Several groups have reported the development of various bacteriobots with various microstructures and bacteria strains recently. Behkam and Sitti group propelled PS (polystyrene) microbeads using motion of *S. marcescens* by bacterial chemotaxis [10]. While, Kojima *et al* successfully developed liposome based microsystems by attaching bacteria to the surface of liposome through a raft domain binding method [17]. Moreover, we also developed the several bacteriobots with biocompatible PEG-DA (poly ethylene glycol-diacrylate), alginate, hyaluronic acid (HA) and *S. typhimurium*, which we also called the bacteria based microrobots as bacteriobots [8-10]. In these studies, the bacteriobots showed excellent motility owing to flagellar movement, and a tumor therapeutic effect was also investigated with the HA based bacteriobots [11-13]. However, a steering strategy is still indispensable to realize the motion control of bacteriobots. Martel's group proposed magnetotactic bacteria microrobots and remotely steered these microrobots with external magnetic field for drug delivery and MRI imaging. However, a complicated magnetic system is still mandatory for external actuation [18].

Flagellar bacteria possess an excellent motility because the flagella enable forward and rotatory movement at the low Reynolds microenvironment [19]. Generally, several factors can affect the motion form of bacteria, such as temperatures, pH, chemical materials and magnetic field [20]. *Salmonella* was known as a high chemotactic response strain to various chemicals, especially some specific chemicals secreted by tumor cells, thus, possess an active tumor targeting ability. Herein, *Salmonella enteritidis* were used to actuate the microbeads and attempted to stir their movement in a

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Shaohui Zheng, Jiwon Han, Sunghoon Cho, Van Du Nguyen, Seong Young Ko are with School of Mechanical Systems Engineering, Chonnam National University, Gwangju 500-757, Korea. (Email: shaohui19910@gmail.com, judyvet@jnu.ac.kr, cho82@jnu.ac.kr , nvdu81@gmail.com , sko@jnu.ac.kr).

Jong-Oh Park and Sukho Park are with the Robot Research Initiative, School of Mechanical Systems Engineering, Chonnam National University, Gwangju 500-757, Korea. (Corresponding author phone: 82-62-530-1687; fax: 82-62-530-0267; email: jop@jnu.ac.kr and spark@jnu.ac.kr).

chemoattractant gradient microfluidic channel with agarose gel. *Salmonella* strain has high motility and can be affected by chemical materials such as certain kind of amino acid [21]. Thus, in this research, aspartic acid was employed as the chemoattractant chemical to steer the motion of bacteriobot in a chemical gradient microfluidic channel [16].

In this paper, firstly, according to previous researches, a new type of chemical gradient channel was designed and fabricated with agarose hydrogel and the chemical gradient distribution was observed with red fluorescent material rhodamine B to substitute aspartic acid as a marking chemical [22, 25]. Then bacteria based microrobots were developed by attaching *S. enteritidis* to poly-l-lysine (PLL) coated HA microbeads. Then observation of chemotactic migration of bacteriobots was performed in previous agarose gradient channel and analyzed through MATLAB program [11]. According to the chemotactic migration movement of bacteriobots, we demonstrated the possibility to control the motion of bacteriobots.

II. Materials and Methods

A. Design and analysis of chemotaxis channel

Based on conventional researches of microfluidic channels and chemotaxis chamber, an agarose hydrogel based microfluidic chemotaxis channel was designed as Fig.1 to facilitate the micro-movement towards a chemical attractant [22-25]. The channel was fabricated with micro-molding method to realize required pattern on hydrogels.

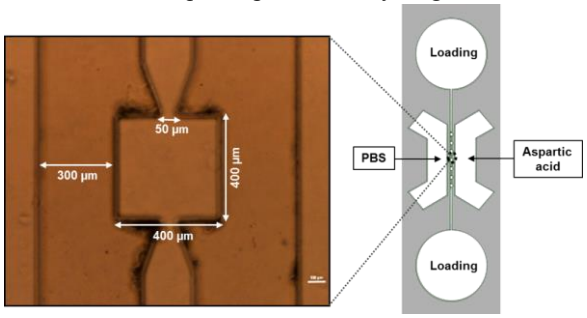

Fig. 1 The design scheme of chemotaxis microfluidic channel

Firstly, the pattern of the channel was designed with CAD program and the channel mask was fabricated in POSCO, Korea. Then, through the photo- and soft-lithography procedures, the SU-8 2150 photoresist was coated on a Si wafer, covered with the mask and exposed to UV rays, then the wafer was soaked in SU-8 developer to eliminate unexposed SU-8 2150 to complete the channel mold with a thickness of 600μm [11, 24]. Thereafter, heated agarose solution was poured onside the mold, the microchannel can be obtained by separating the mold and agarose hydrogel after cooled to room temperature. The gradient formation was examined based on the diffusion of red fluorescent rhodamine B which was loaded in the right (aspartic acid) side chamber due to the similar permeability between rhodamine B and aspartic acid in agarose gel. And the time dependent gradient formation was observed by optical microscope at various time intervals to investigate the chemical gradient generation, and quantitatively evaluated with ImageJ program.

B. Development of bacteriobots

a. Fabrication of HA microbeads

HA microbeads were prepared using micro-droplet method through the crosslinking reaction between HA and divinyl sulfone (DVS) (Fig. 2) [26-28]. The HA was prepared in a 0.5% (w/v) aqueous solution by mixing 0.01g HA with 2 ml NaOH solution at a pH of 10~13. While, the crosslinker solution was prepared by adding 80μl DVS in 20 ml isobutanol solution at a concentration of 0.4% (vol%). HA micro-droplets were generated through aeration gun by air pump felled to DVS/isobutannol solution to be solidified by DVS to form HA microbeads. After reaction, to remove the residual DVS and isobutanol reagent, the microbeads were soaked into ethanol solution and then centrifuged in centrifuge (HM-150IV, Hanil Science, S. Korea) for 10 minutes at 8000rpm. Then the supernatant was removed, the microbeads were washed again using ethanol and DI water each for twice sequentially, and finally dried in air.

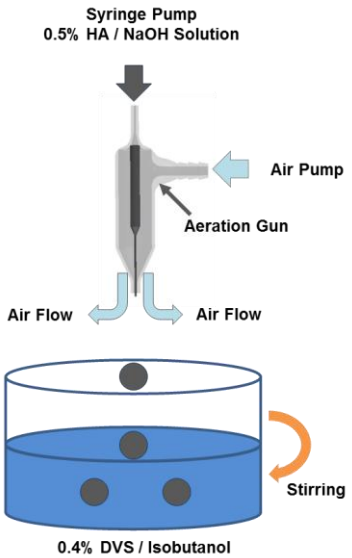

Fig. 2. Schematic diagram of HA microbeads fabrication progress with a aeration method.

b. Bacteria culture

Salmonella. enteritidis (ATCC® 13076™) were purchased from American Type Culture Collection (Manassas, VA). The bacteria were cultured through the following process. First, the bacteria were cultured for 12 hours on Luria-Bertani (LB) agar plate (1% Bacto-Tryptone, 0.5% Bacto-Yeast extract, 1% NaCl, 1.5% Agar), containing Kanamycin (Duchefa Biochemie) with a concentration of 25μg/ml. Then a bacterial colony was cultured in LB broth (without agar) for

4 hours again until the bacteria density was as OD 600 value 0.8~1.2 for following experiments, measured by spectrophotometer (UV mini-1240, Shimadzu, Japan).

c. Bacteria attachment to microbeads

In order to fabricate the bacteriobots, surface of the HA microbeads was modified with positively charged PLL to facilitate the attachment of negative charged bacteria on the HA microbeads. Firstly, HA microbeads were coated with 0.01% PLL solution in the 30×10 mm petri dish for 1 h, then the residual PLL was washed with DI water on 0.45μm filter (Nylon membrane, R1JA00667, Millipore, Ireland) to harvest the PLL coated HA microbeads. After that, HA microbeads solution (10^5/ml) was mixed with bacteria solution (OD 600 value= 0.8~1.2) at equivalent volume, then the result solution was incubated for 20min at 30℃ incubator to allow bacterial attachment. Then, bacteria were attached onside PLL coated HA microbeads and finally bacteriobots were fabricated. Thereafter, the morphology of bacteriobots was investigated with scanning electron microscope (SEM, SS-550, Shimadzu, Japan) after a dehydration procedure for bacteriobot sample preparation to maintain the morphology of bacteria.

C. Analysis of motion of bacteriobots with chemotaxis

The chemotaxis characteristic of bacteriobots was investigated with aspartic acid. First, the chemical gradient channel was fabricated with agarose hydrogel as previous reports. Then 0.1mM aspartic acid and PBS solutions were loaded at the right and left side chamber of the channel, respectively. After 10 min gradient generating process, the channel was fixed under the optical microscope and 0.5ml of bacteriobot solution was inoculated inside the up and down loading chambers, gently. When the flow became static, the movement of bacteriobots was videoed with microscope for 1 min and repeated for 10 times at the same experiment condition. Then the videos were analyzed with the Tracking program, based on Darnton and Jaffe's particle tracking code, developed with MATLAB and calculated the movement of bacteriobots, the moving distances and velocity of bacteriobots , thus the chemotactic movement could be analyzed with the tracking data in other programs based on MATLAB.

III. RESULTS

A. Analysis of chemical gradient in microfluidic channel

For the channel of chemotaxis analysis, it is essential to generate a stable gradient during evaluation period. The red fluorescent dye rhodamine B was employed to visualize the diffusion-derived chemical gradient inside the microchannel for different time intervals until 30 min (Fig.3.a). Results showed that a stable and high concentration gradient can be generated and maintained for a quiet long time more than 30 min. Moreover, high intensity of rhodamine B along the chemical channel was evaluated as shown in Fig.3. Consequently, the gradient concentration and the duration of chemical were enough for the observation of bacterial migration micro-movement towards chemotactic chemical.

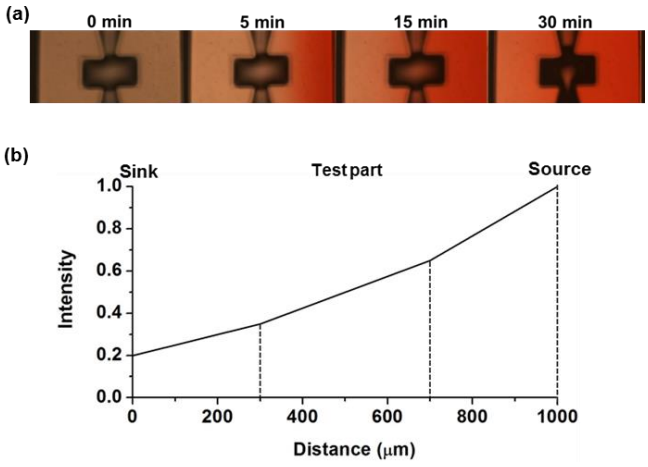

Fig. 3. Chemical gradient analysis using rhodamine B dye. (a) Fluorescent image of three parts at various time intervals, (b) Evolution of rhodamine B concentration profiles at experimental chamber after 30 minutes' diffusion. Rhodamine B was loaded right chamber of microfluidic channel.

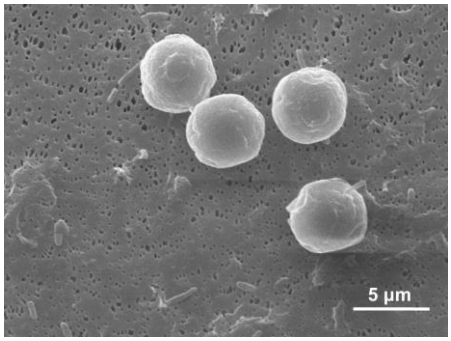

Fig. 4. SEM image of bacteriobots (bacteria and microbeads)

B. Bacteriobots

The crosslinking of HA microdroplets with DVS led to uniform spherical microbeads with an average diameter around 5μm (Fig. 4). In addition, size of microbeads can also be adjusted by aeration setting. After surface modification to microbeads with PLL, bacteria were attached at the surface of microbeads and imaged with scanning electron microscope (SEM). As shown in Fig. 4, bacteria were attached on the surface of PLL coated HA microbeads. However, bacteria shrunk seriously owing to the dehydration during SEM sample preparation procedure, which showed smaller morphology than that in aqueous medium environment. Moreover, some tumor therapeutic drugs could also be embedded inside the microbeads with physical encapsulation methods, therefore, these kind bacteriobots would be capable to be applied in biomedical fields, regarding as another kind of active drug delivery system based on biological motors.

C. Motion investigation and analysis

In micro-environment, bacteriobots showed disordered motion form owing to the random motion of bacteria, which could not be distinguished by naked eyes. However, video tracking of bacteriobots inside the microfluidic channel revealed the directional migration of these bacteriobots towards chemoattractant chemical side in the presence of aspartic acid gradient, which the terminal points of motion were labelled with numbers always located on the right side of start points, showed in Fig. 5(a).

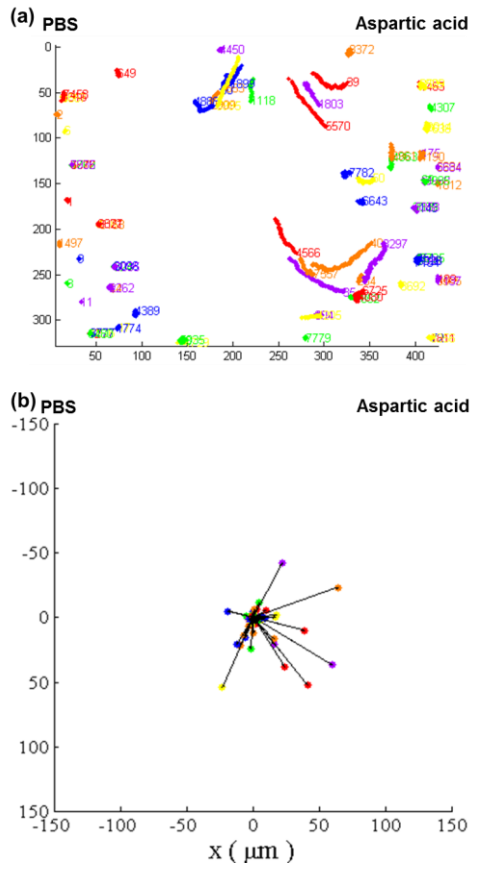

Fig. 5. The motility of bacteriobots exposed to chemo-attractant. (a) Movement trajectories of bactriobots, (b) The distribution of bactariobots' movement end points when gathering the start points into the same time (0, 0).

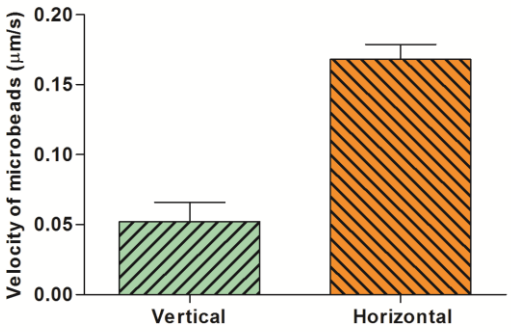

Fig. 6. The velocity of bacteriobots in chemical gradient channel in vertical and horizontal directions.

Combining all of the motion tracking images into one image with MATLAB and aggregating all the trajectory start points of all bacteriobots (0, 0) led to distribution image of terminal points of movements showed in Fig. 5. b. These results demonstrated that a great majority of bacteriobots migrated towards the side with high aspartic acid concentration. Moreover, the migration velocity of bacteriobots to chemoattractant was calculated in MATLAB as 0.17 μm/s (horizontal direction), however, velocity in vertical direction was just 0.05 μm/s (Fig. 6), which furtherly demonstrated that bacteriobots tend to migrate towards the chemoattractant direction. These experimental results provided strong evidence that motion of bacteriobots could be steered with some kind of chemoattractant chemicals.

IV. Discussion

In this study, we aimed at the steering of the movement of bacteriobots with bacterial chemotaxis characteristic. 5 μm microbeads were fabricated with hyaluronic acid and *S. enteritidis* were employed to attach on the surface of microbeads to develop the bacteriobots (Fig.4). An agarose hydrogel based chemical gradient microfluidic device was fabricated to evaluate the chemotactic movements of bacteria based microrobots (Fig.1). In addition, the stable gradient formation of agarose microchannel was examined with red fluorescent rhodamine B. The gradient distribution was observed and imaged with optical microscope (Fig.3.a) and quantitatively analyzed with Image J program (Fig.3.b). These results demonstrated that the microfluidic channel was capable to be applied to create a micro-environment for bacteriobots chemotactic motility investigation.

The chemotactic movement of bacteriobots was investigated at the microfluidic channel and a large majority bacteriobots moved towards chemoattractant direction (horizontal direction), which the velocity was 0.17 μm/s (Fig.5.b, Fig. 6). However, there was no remarkable movement discrepancy in non-chemoattractant direction (vertical direction). Therefore, the bacteriobots could be guided by the chemoattractant chemicals, generally. Thus, we demonstrated that it was possible to steer the motion through chemoattractant chemicals.

We aimed at the treatment of cancer using bacteriobots. Actually, the bacteria have tumor targeting and therapeutic effect which were reported in various previous studies. And if we encapsulated anticancer drug inside microbeads, the bacteriobots would have dual anticancer effect. In this study, the chemotactic property of bacteriobots was investigated in the gradient channel with aspartic acid, moreover, tumor cells can also secrete similar chemoattractant chemicals, in other words, bacteriobots could be capable to recognize and target to tumor cell which we will investigate the tumor targeting ability of bacteriobots through animal experiments in future work. However, some problem, such as biocompatibility of bacteria in human body and targeting

efficiency of bacteriobots to tumor should be further addressed. Moreover, more effective drug delivery vesicles should be developed to enhance the therapeutic effect of nanomedicine. Thereafter, these tumor targeted bacteriobots proposed a new promising theranostic methodology in cancer treatment, which deserves special attentions and further researches.

V. CONCLUSION

In this study, bacteriobots were developed by attaching *S. enteritidis* to PLL coated HA microbeads. Motility steering of bacteriobots was carried out in a newly designed chemical gradient microfluidic channel owing to bacterial chemotaxis, tracked and analyzed with MATLAB program. Owing to these results, the movement of bacteriobots can be steered with chemoattractant chemicals at a certain extent. In a word, the chemotactic characteristic of bacteriobots will be an essential motion steering factor and a promising tumor targeting strategy if we apply bacteriobots as a cancer therapeutic method in the future.

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