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# Development of Bacteria-actuated Microrobots Using the Surface Modification of Microstructures

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***Abstract*— Microrobots are useful for application in various fields. However, they have limitations with respect to their actuators and motilities. In this study, we fabricated the two types of flagellated bacteria-actuated microrobots using the flagellated bacteria *Serratia marcescens* and *Salmonella typhimurium* that could be utilized as microactuators. First, we fabricated the *Serratia marcescens*-actuated microrobot, where we adopted the selective bacteria adhering on the surface of SU-8 microcubes through the selective coating with bovine serum albumin. Many number of *Serratia marcescens* attached on bovine serum albumin-uncoated side of SU-8 microcubes. The average velocity of the selective *Serratia marcescens*-attached SU-8 microcubes was increased more than two times from SU-8 microcubes with a nominal *Serratia marcescens* attachments. Second, we fabricated the *Salmonella typhimurium*-actuated microrobots that have a selective bacteria patterning on the surface of polystyrene microbeads using bovine serum albumin. Similarly, the average velocity of the selective *Salmonella typhimurium* patterned polystyrene microbeads was 5 folds faster than that of the polystyrene microbeads where the bacteria were attached on the whole surface of the microbeads. Consequently, the experimental results mean that the flagellated bacteria could be utilized as microactuators for microrobots and the selective patterning of the bacteria could enhance the velocity of the bacteria-actuated microrobots.**

## I. Introduction

Recently, many researchers have concentrated on the development of micro- and nano-sizing robots using many technologies, such as micro technology, nano technology, and bio technology [1-3] because of their many benefits in various fields, such as the medical, environment monitoring, and military fields [1, 4]. However, the development of microrobots has encountered many technical problems because micro-size, reliable, and high efficiency microactuators are needed [5].

To develop the effective microactuators, many research groups proposed the several types of microactuators. Firstly, some researchers have proposed a bacterial microorganism with flagella motors bioactuators, *Escherichia coli* (*E. coli*), *Serratia marcescens* (*S. marcescens*), and *Salmonella typhimurium* (*S. typhimurium*) [6-9]. The bacteria have the many unique merits as a microactuator because it could obtain the energy source from surrounding environment, excellent swimming ability on soft agar surfaces, high-density adhesion to hydrophobic surfaces and strong force [7, 9].

Bacteria can be regulate their motility by several sensory mechanisms, such as phototaxis, magnetotaxis, and chemotaxis [6, 10]. First, phototaxis is an important role in motility of some cyanobacterium strains, *Cylindrospermum alatosporum*, *Anabaena variabilis*, and *Synechocystis* species [1, 2]. Cylindrospermum alatosporum can be appear the positive phototactic response on wavelength peaks at 450 nm and 640 nm. These similar phenomenon been reported for Anabaena variabilis that two additional wavelength peaks were observed at 550 nm and 730 nm [11]. While phototactic bacteria, such as cyanobacteria and purple photosynthetic bacteria, showed negative phototaxis through the long-wavelength UV irradiation at 360 nm [12]. Second, some magnetotactic bacteria (MTB) strains, (MC-1 and MWB-1), can be motile on magnetic field at a velocity about 200 to 300 μm/sec [13, 14]. B. Nelson's group reported on the artificial bacterial flagella with a magnetic metal head in the shape of a thin square plate. Three orthogonal electromagnetic coil pairs control the artificial bacterial flagella [15]. Moreover, some other groups proposed a neodymium-iron-boron microrobot, which is composed of neodymium–iron–boron with dimensions 250 μm × 130 μm × 10 μm, is actuated and stirred by a system of 6 macro-scale electromagnets, and observed micro-robot translation speeds can exceed 10 mm/sec [16]. Third, *S. typhimurium* and *E. coli* can be recognize the some bacterial chemotactic chemicals, chemo-attractant (L-aspartic acid) and chemo-repellent ($NiSO_4$), by their chemoreceptors and show positive or negative motility against a chemotactic chemicals by flagella motor [17].

In the development of bacteria-actuated microrobots, microstructure fabrication, surface modification of microstructures, and selective bacterial attachment and patterning skills are the key technologies. Many researchers have focused on the bacteria attachment to a selective region using various methods, such as self-assembled monolayers, metal templates, stamped proteins and peptides, bio- and comb polymers, microfluidic channels, and membranes [18-20]. Recently, many research groups focused on surface

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modification of microstructure through the coating with some proteins [21]. Especially, fluorescence labeled proteins isothiocyanate-bovine serum albumin (FITC-BSA) is used on the patterning methods of the microstructures because of their bacterial adherent inhibitory effects [22].

Alternatively, a reactive ion etching plasma-based patterning method was proposed for surface modification of microstructures. Especially, B. Behkam's group suggested that the *S. marcescens* had been utilized as microactuator for polystyrene (PS) microbeads using $O_2$ plasma-based bacteria patterning technique [3]. When RIE plasma is exposed to the microstructure, $O_2$ plasma-irradiated microstructure's surface represents hydrophilic conditions, which are difficult for bacterial attachment onto the microstructure's surface. The bacteria could be attached on $O_2$ plasma-unexposed the other side of the microstructure's surface [3]. However, this technique could make the passive bacterial attachment to only restrict evenly half the microstructure's surface areas, because the straightness.

In this study, we propose several bacterial patterning methodology to microstructures using BSA. And, we investigate the relationships between selective bacterial attachment to microstructures through the coating with BSA and motility of bacteria-actuated microrobot.

## II. Materials and Methods

### A. Bacteria Culture

*Serratia marcescens* (*S. marcescens*) ATCC 13880 were obtained from the American Type Culture Collection. And, the *Salmonella typhimurium* (*S. typhimurium*) strain was provided and genetically modified by Professor J. Min (Chonnam National University Medical School, Korea), which was genetically engineered through the defection of guanosine 5'-diphosphate-3'-diphosphate (ppGpp) to attenuate the pathogenicity and the green fluorescent protein (*gfp*) gene, to generate fluorescence signals. *S. marcescens* were grown in 3.0 g Difco Bacto beef extract, 5.0 g Difco Bacto peptone, 15 g agar, and 1 L distilled water, pH 7.0 containing nutrient agar plate. Then, Bacteria were cultivated in 3.0 g Difco Bactor beef extract and 5.0 g Difco Bactor peptone containing liquid media in a 30°C incubator for 24 hr. *S. marcescens* were inoculated on 20% glucose solutions containing 0.5% agar plate. The inoculation site generally turned pink shortly after the swarming motion developed. The swarms expanded across the plate in waves that appeared as concentric rings with the most active bacteria located along the outermost edge of the swarms. In order to observe the *S. marcescens*, bacteria stained with Fluorescent dye, LIVE/DEAD *Bac*Light™ Bacterial staining Kits (Molecular Probes, Eugene, OR). *S. typhimurium* should be cultured for 8 hr on a mixture of Luria–Bertani (LB) agar plate (0.5 g yeast extract, 1.0 g tryptone, 1.0 g NaCl, 1.0 g agar, 100 mL distilled water), 50 μg/mL Ampicillin, and 50 μg/mL Kanamycin (Duchefa Biochemie, B.V., Haarlem, the Netherlands). 2 mL LB media was added into the culturing agar plate.

### B. Microfabrication of SU-8 Microcubes

The photolithography was performed on a 4-inch silicon wafer with the designed mask pattern using a contact aligner. Firstly, the negative polymeric photoresist SU-8 2035 (Microchem, Newton, MA) was spin-coated over the silicon wafer. The thickness of the photoresist SU-8 was approximately 30 μm and the wafer was soft-baked on a hotplate at 110°C for 25 min and cooled down at the room temperature. The soft bake process enabled the photoresist layer to be solidified. The photoresister SU-8 on the wafer was patterned by UV light exposure for 80 sec (365 nm center wavelength). Then the patterned photoresister on the wafer was baked at 110°C for 15 min, and it was subsequently developed for 5 min. After the development, the substrate was rinsed with isopropanol, and then rinsed with de-ionized water to remove any toxins that may remain on the wafer. Fig. 1 shows the micro-sized cube-like SU-8 structures that were fabricated by the previous mentioned procedure.

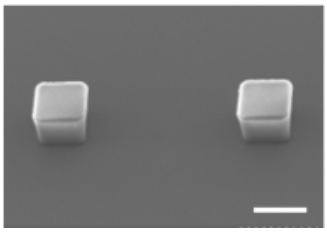

Fig. 1. SEM Photographs of 30 μm SU-8 microcubes on silicon wafer at 15.0 kV and 600 X magnifications after microfabrication (Scale bars = 30 μm).

### C. Scanning Electron Microscopy (SEM)

The SU-8 microcubes on the silicon wafer and submerged PS microbeads on PDMS was observed using a scanning electron microscope (S-4700, Hitachi, Japan).

### D. Protein (BSA) coating on microstructures

For nonspecific bacterial attachment to microstructures, firstly, we treated the blocking protein onto the SU-8 microcubes on the silicon wafer by soaking in 5% BSA (Sigma-Aldrich Chemical Co. St. Louis, MO) for 24 hr. The exposed surfaces of the SU-8 microcubes were coated with 5% BSA, except for the non-exposed a silicon wafers-attached bottom side of SU-8 microcubes. After the incubation, the substrate was rinsed with a phosphate-buffered saline (PBS) buffer, and the SU-8 microcubes could be detached by using a knife. And then, we incubated *S. marcescens* with detached SU-8 microcubes in the motility buffer. Secondly, for BSA coating on restricted regions of 6 μm PS microbeads (Polyscience, Warrington, PA), we used the submerged properties of PS microbeads on hydrogels. 1% agarose solution was poured into a petri dish. After the gelation of the 1% agarose solution, PS microbeads was added onto the surface of the 1% agarose gel. And then, a PDMS (Sylgard 184 A and Sylgard 184 B with a volume ratio of 10:1, Dow Corning, Midland, MI) solution was slowly added onto the top of the 1% agarose gel to submerge the PS microbeads. After curing the PDMS solution in 80°C oven for

24 hr, the submerged PS microbeads-contained PDMS membrane was detached from the agarose surface. The submerged PS microbeads contained PDMS membrane was dipped in 5% BSA solution for 24 hr. The PS microbeads could be extracted from the PDMS membrane using ultra-sonication. Next, detached PS microbeads incubated with *S. typhimurium* in the motility buffer.

### *E. Observation of the bacteria-actuated microrobot movement*

The SU-8 microcubes and PS microdeads were tracked moving distance and calculated average velocity. A set of continuous frame images were captured by inverted phase-contrast microscope (Nikon T*i*-U microscope; Nikon USA, Melville, NY) with 1280 x 960 pixels, then extracted time information and coordinates of the SU-8 microcubes and PS microbeads of each frames using MATLAB. Average velocity per 0.5 sec was calculated and the data were merged with original image and plotted using MATLAB.

## III. RESULTS

### *A. Bacterial attachment by BSA Coating*

Selective bacterial attachment on microstructures plays role in enhancement of the motility of the bacteria-actuated microrobots. In this study, we found that some *S. marcescens* bacteria were attached on the BSA-uncoated surface of the SU-8 microcube because of their hydrophobic properties (Fig. 2A). The other side, when SU-8 microcubes were treated with BSA, *S. marcescens* bacterial adhesion on BSA-uncoated surface of SU-8 microcube increased by approximately 2.0 folds compared with that in all sides of the uncoated groups (Fig. 2B).

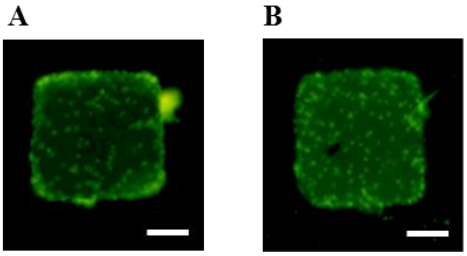


Fig. 2. Fluorescent microscopic images of green fluorescent staining *S. marcescens*-attached SU-8 microcubes. (A) BSA-untreated bottom side of SU-8 microcube and (B) BSA-treated bottom side of SU-8 microcube (Scale bars = 10 μm).

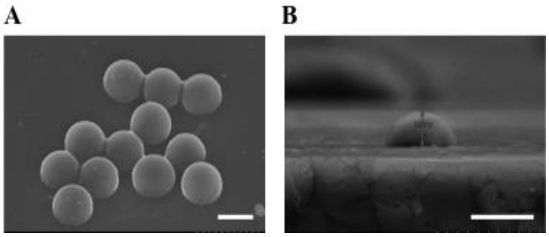


Fig. 3. SEM images of submerged PS microbeads on PDMS surfaces of (A) 3.0 kX magnifications and (B) 5.0 kX magnifications at 15.0 kV (Scale bars = 5 μm).

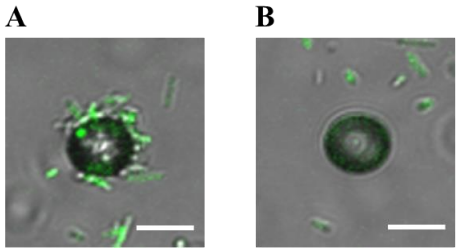


Fig. 4. Fluorescent microscopic images of green fluorescent expressing *S. typhimurium*-attached PS microbeads. (A) BSA-untreated PS microbead and (B) BSA-treated PS microbead (Scale bars = 5 μm).

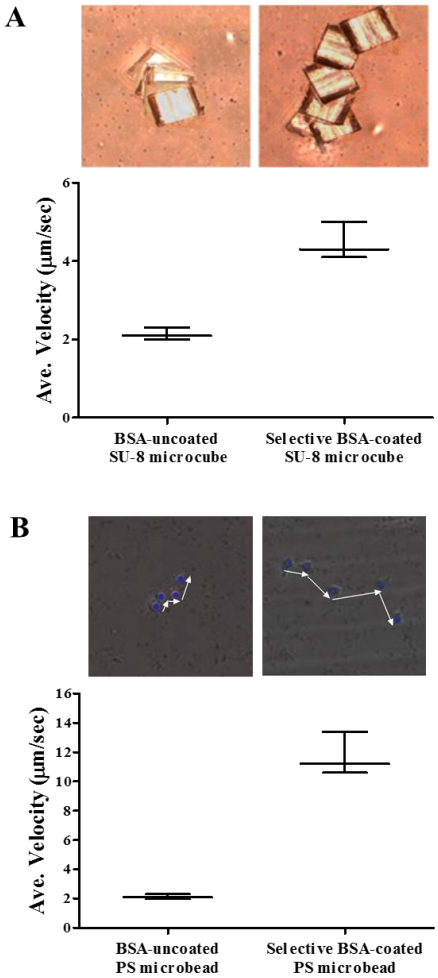


Fig. 5. Average velocities of bacteria-actuated microrobots. (A) Average velocity of BSA-uncoated and -coated SU-8 microcubes and (B) PS microbeads.

Next, we performed that submerged PS microbeads on agarose gel transferred to the PDMS surface for the acquirement of the fine SEM images. We could observe the submerged PS microbeads, which are transferred from the agarose gel to PDMS membrane (Fig. 3A). Moreover, we observed that PS microbeads presented the submerging property about approximately half of the PS microbeads in 1% agarose gels (Fig. 3B). Moreover, we found that several *gfp* expressing *S. typhimurium* bacteria were attached on the BSA-uncoated surface of the PS microbeads because of their

hydrophobic properties (Fig. 4A). But, *gfp*-expressed *S. typhimurium* bacteria not attached on the BSA-coated surface of the PS microbeads (Fig. 4B). These results revealed that selectively bacterial adhesions on restrict regions of microstructures could regulate through their surface modification using BSA, which having an anti-bacterial adhesion property.

*B. Motility of bacteria-actuated microrobots*

The selective bacterial patterning has effect on motility of bacteria-attached microstructures. First, selectively BSA coating SU-8 microcubes has shown actively movement by attaching *S. marcescens* bacteria and moving velocity was increased by 210% than BSA-uncoated groups (Fig. 5A). Second, the selectively BSA-coated PS microbeads has shown bacterial attachment onto restrict region of PS microbeads. As these reason, we observed that the selective BSA-coated PS microbead groups shows longer movement than uncoated PS microbeads. Moreover, it has shown that moving velocity of selective BSA-coated PS microbeads was increased by 560% than uncoated groups through the selective *S. typhimurium* bacterial attachments onto surface of PS microbeads (Fig. 5B). These results reveals that selective patterning of bacteria using BSA could plays an important role in moving velocity of bacteria-actuated microrobots.

## IV. DISCUSSIONS

In the development of microrobots, the most important things are the fabrication of an active microactuator. For this reason, many researchers are focused on bioactuators called as flagella motors that use several flagellated bacterial strains with excellent motility [7-9]. Especially, *S. marcescens* have excellent swarming behavior about 47 μm/sec [7, 9] and magnetotactic bacterial strains, such as MC-1 and MWB-1, which have excellent motility at a velocity exceeding 200 to 300 μm/sec [11, 12]. Moreover, bacterial strains have several advantages in adaptation for development of microrobots, which are controllability on specific conditions using several methodologies, chemotaxis, phototaxis, and magnetotaxis, and possibility of genetically modifications, such as attenuation, therapeutic protein expression, and fluorescence expression [6, 10, 23, 24]. Moreover, bacterial patterning on microstructures are very important role in the motility of bacteria-actuated microrobots. Recently, some research groups have concentrated on the bacterial patterning onto a specific region of microstructures through the several technologies, RIE plasma-based and micro patterning techniques [18-20]. However, the RIE plasma-based patterning methodology has some disadvantages in bacterial patterning, which could not actively regulate the bacteria adherent area to only half surface area of microstructures, because of the straightness of RIE plasma [3].

To overcome the several problems for development of microrobots, we were developed several types of effective bacteria-actuated microrobots using excellent swarming behavior-contained flagellated bacterial strains, *S. marcescens* and *S. typhimurium,* surface modifiable hydrophobic microstructures, SU-8 microcubes and PS microbeads, because bacteria were easily attached on hydrophobic material's surfaces (Fig. 1 and 3). Finally, for the enhancement of directionality and motility of microrobots, we performed the selective coating with BSA, which are contained inhibitory effects of bacterial adhesion, on various types of microstructure. In this study, we proposed that several flagellated bacteria patterning method could modulate the selective bacteria attachment on several types of microstructures using BSA. Many number of bacteria attached on BSA-uncoated bottom sides of BSA-treated SU-8 microcubes and BSA-untreated PS microbeads, while small amount of bacteria attached on BSA-untreated SU-8 microcubes and BSA-treated PS microbeads (Fig. 2 and 4). Moreover, bacteria-based microrobots enhanced the moving distance and velocity by the selective bacteria attachment (Fig. 5). Based on these results, we expect to contribute a development of effective drug delivery systems and active biomedical microrobots.

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