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Bacterial Chemotaxis Enabled Autonomous Sorting of Micro-particles

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***Abstract*— Autonomous manipulation and assembly at micro/nanoscale continues to be one of the main challenges of micro/nanorobotics. Biomotors are increasingly being considered as robust, versatile and cost-effective choices for a variety of micro/nanorobotic tasks. Here we propose utilization of motility and chemotaxis in flagellated bacteria for autonomous sorting of $6\ \mu m$ and $10\ \mu m$ micro-particles within a microfluidic platform. Difference in surface chemistry of the $6\ \mu m$ and $10\ \mu m$ particles are exploited to selectively assemble bacteria onto $6\ \mu m$ particles and separate them from $10\ \mu m$ particles via chemotaxis motility of the attached bacteria. It has been shown that within 1 hour, an increasingly larger number of $6\ \mu m$ particles accumulate within a $600\ \mu m$ radius, near the chemo-attractant source.**

I. INTRODUCTION

State-of-the-art robotic manipulation and assembly of micro/nanoscale objects require sophisticated hardware, computationally expensive control strategies and highly skilled users. Existing sorting methods [1] such as optical, magnetic and electrical separation often require set-ups that are costly and do not always perform well when the objects that need to be sorted are of similar size.

Therefore, new approaches are needed in order to increase micro-manipulation and assembly throughput rate and decrease the associated costs. In recent years, a paradigm shifting approach which utilizes biomotors has been proposed by a few researchers [2], [3]. Here, we introduce a bio-hybrid microrobotic approach in which the motility and chemotaxis in flagellated bacteria is harvested for autonomous sorting of micro-particles. It has been shown in the past that bacteria can be effectively used for the controlled actuation of micro/nanoscale objects [4], [5], [6], [7], [8] . Martel *et al*. [9] showed that microscale bricks can be manipulated and assembled into a pyramid using magnetotactic bacteria and a computer controlled external magnetic field. We have previously shown that bacteria-propelled micro-particles are capable of chemotaxis in presence of a chemo-attractant gradient [10].

In this work, we utilize bacteria-based propulsion combined with the concept of chemotaxis (i.e. bacteria response to chemical gradients) to sort micro-particles that have similar sizes density with different surface chemistry. The use of chemical energy as power source, bacteria flagellar motor as actuators, and chemotaxis as controller makes this autonomous micro-particle sorting platform a cost-effective, compact, and fully self-contained system. This platform offers significant flexibility compared with other microfluidic based approaches such as dielectrophoresis or optical tweezers, in which particle dielectric properties or required forces can limit the applications. In contrast with probe-based systems such as atomic force microscope (AFM), the proposed platform can easily achieve parallel operation and higher throughput autonomous manipulation can be more easily achieved.

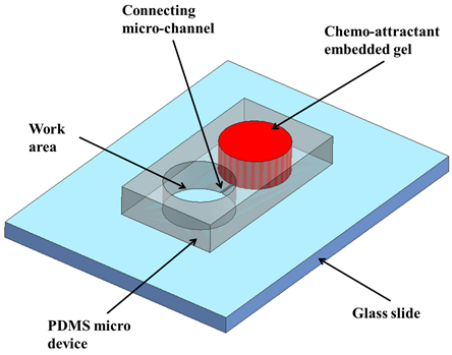

Fig. 1. A schematic of the microfluidic platform used for autonomous micro-particle sorting.

In this paper, we have designed, modeled, and constructed a microfluidic workspace with a $4\ mm$ diameter work area in which $6\ \mu m$ and $10\ \mu m$ particles of same density but different surface chemistry are autonomously sorted using flagellated bacteria with chemotactic capability. The findings from this work will be used towards determining the requisite mechanical, physicochemical, and biological properties of such bio-hybrid manipulation and assembly workspaces.

II. METHODOLOGY

A. Design of Experiment

The proposed microfluidic workspace is composed of a chemo-attractant chamber, a work area and a connecting channel as shown in Fig. 1. The bulk of the device is made from polydimethylsiloxane (PDMS), a biocompatible translucent polymer routinely used in microfluidics and soft lithography. The $4\ mm$ diameter chemo-attractant chamber is filled with polyethylene glycol diacrylate (PEGDA) hydrogel infused with the chemo-attractant casamino acid. PEGDA enables controlled and slow release of chemo-attractant such that the chemical gradient can be maintained for up to a few hours and bacterial chemotaxis can be effectively utilized for sorting during this time period. The work area, $4\ mm$ in diameter, contains $6\ \mu m$, and $10\ \mu m$ diameter polystyrene particles and hyper-flagellated *Serratia*

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marcescens (S. marcescens) bacteria. The two wells are connected by a microfluidic channel which is 100 μm wide and 1 mm long. The 6 μm, and 10 μm diameter polystyrene particles have different surface properties such that bacteria will only attach to the 6 μm particles. In presence of an appropriately designed chemo-attractant gradient, it is expected that the 6 μm particles will be autonomously transported towards the connecting micro-channel while the 10 μm particles will be left behind in the center of the work area due to lack of mobility.

B. Device Fabrication

1) Fabrication of SU-8 mold and micro-device: An SU-8 master template of the microfluidic device was fabricated using standard microfabrication techniques. Briefly, photoresist SU-8 was spun on a silicon wafer and soft baked at 65 $^{\circ}C$ for 3 minutes. Using UV lithography, the micro-device pattern was transferred in SU-8 and developed. After hard bake at 95 $^{\circ}C$ for 6 minutes, the device was coated with C_4F_8. PDMS solution was prepared according to the manufacturer's instruction, poured in the SU-8 mold, degased, and cured at room temperature. After 24 hours, the PDMS layer was peeled off to obtain the micro-device. The PDMS micro-device and a pre-cleaned glass slide were plasma treated using PDC-32G plasma cleaner on high power setting for 30 seconds and were subsequently bonded together to obtain the microfluidic device.

2) Fabrication of PEGDA gels: The liquid hydrogel solution, containing the PEGDA base polymer and Irgacure 2959 photoinitiator was injected in one of the PDMS micro-device wells and then photopolymerized using a UV light source (365 nm, 0.78 mJ/cm^2) for 5 minutes. The obtained gel is then soaked in 0.4 M casamino acid solution (a common bacterial chemo-attractant) for a duration of 30 minutes.

C. Diffusion Coefficient Measurement

The rate at which the chemical attractant diffuses out of the gel affects the chemical concentration field in the work area, bacteria response, and working time. Therefore, for the design of the experiment, it is required to characterize the mass transport properties of the PEGDA gel used in the chemo-attractant chamber. The diffusion coefficient of the fabricated gel for casamino acid solution was determined using a Franz diffusion cell as shown in Fig. 2. At known time periods, a small amount of solution is extracted from the buffer compartment and its absorbance is measured using a DU-800 Beckman Coulter spectrophotometer at 238 nm wavelength. The absorbance value is representative of the amount of chemicals that has diffused through the gel membrane. The permeation curve of the PEGDA gel, shown in Fig. 3, is used to determine the diffusion coefficient value of the gel. Using the mean first passage time measurements method as discussed in [11], the diffusion coefficient of the gel was determined to be $5.19 \times 10^{-6}\ cm^2/s$.

D. COMSOL Simulations

A mass transport simulation of casamino acid solution using finite element analysis software package COMSOL for the determined diffusion coefficient was performed. In this analysis, the chemo-attractant contained in the PEGDA gel will diffuse out through the connecting channel to the well that contains the bacteria and the beads (work area). An example of the chemical concentration profile is shown in Fig. 4.

III. Experimental Work

A. Materials and Methods

A 2 μl aliquot of wild-type *S. marcenscens* (ATCC 274) culture grown in Luria-broth (1% tryptone and 0.5% sodium chloride, 0.5% yeast extract) was transferred to culture plates containing Luria-broth with 0.65% Bacto agar and 5 *g/l* glucose. A swarm of bacteria was grown overnight at 30 $^{\circ}C$. The 6 μm and 10 μm polystyrene particles used in the experiment, were prepared and suspended in motility medium (0.01 M of potassium phosphate, 0.0067 M of sodium chloride, 10^{-4} M of EDTA, 0.01 M of glucose, and 0.002% of Tween-20, pH=7.0). A 20 μl aliquot of the mixture of the two kinds of micro-beads was pipetted behind the edge of the bacteria swarm on the plate and left at room temperature for about 4 minutes. During this time period, bacteria on the swarm plate randomly interacted with the particles and adhered to the ones that had favorable surface property. After the interaction time of 4 minutes, the bacteria and beads suspension (approximately 15 μl of total volume) was aspirated from the plate and pipetted into 1 ml solution of motility medium.

Work area of the device was examined using a Zeiss AxioObserver Z1 inverted microscope equipped with an AxioCam HSm camera. Optical microscopy images were acquired at 20 frames per second. The videos were analyzed using a two-dimensional particle tracking algorithm developed in MATLAB (The MathWorks, Natick, MA).

B. Particle Surface Chemistry and Bacterial Adhesion

Two distinct sizes of polystyrene particles were chosen for these experiments. 6 μm and 10 μm pure polystyrene beads were obtained from Sigma-Aldrich. We intended to actively separate the 6 μm diameter particles from a suspension of the 6 μm and 10 μm. Therefore, we chemically modified the surface properties of the 6 μm particles such that bacteria

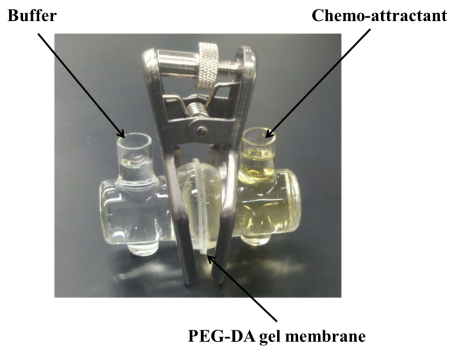

Fig. 2. Franz diffusion cell was used to measure the diffusion coefficient of casamino acids in PEGDA gel.

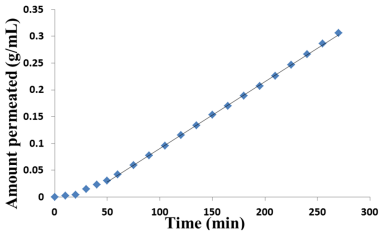

Fig. 3. Permeation curve of casamino acids through a 1.3 mm thick PEGDA gel membrane. The diffusion coefficient of the chemical in the gel is determined using the linear section of the plot.

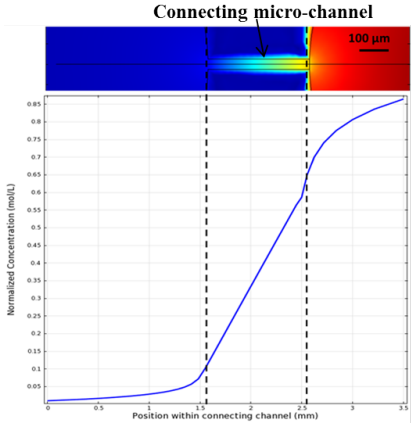

Fig. 4. COMSOL simulation result shows the chemical concentration distribution within the micro-device at time $t = 1000\ s$.

will self-assemble onto them at large numbers. This way, the 6 μm will be transported towards the source of chemo-attractant while the 10 μm particles will be left behind at the center of the work area due to lack of mobility. To achieve this, 10 μm particles were coated with an alkyl sulfonate surfactant which hinders bacteria attachment. The 6 μm polystyrene particles were washed by centrifugation at 1700 rcf and resuspension in 1:1 DI water/IPA solution. This procedure was repeated three times to remove all surfactants from the surface of the beads and promote maximum self-assembly of bacteria to the 6 μm beads.

C. Control Experiment: Micro-bead Propulsion

The propulsion of the micro-beads was studied in the absence of chemical attractant and in a quiescent environment to assay the efficacy of the surface chemistry based selective attachment of bacteria, as described earlier. 6 μm diameter particles which have favorable surface for bacterial attachment and 10 μm diameter particles with unfavorable surface properties were introduced on the bacteria swarm plate. Motion of both particles was tracked at 20 frames per second for 1 minute. The 6 μm diameter particles were highly motile while the 10 μm diameter particles did not move at all. It should be noted that the experiments were also performed for the reverse case in which the 10 μm diameter particles had a more favorable surface property and in that case significantly higher displacement and speed values were observed for the 10 μm particles when compared with the 6 μm diameter particles which had unfavorable surface properties. We elected to conduct the sorting experiments with the first group of particle–6 μm particles with favorable surface for bacterial attachment and 10 μm particles with unfavorable surface for bacterial attachment.

IV. Transport of 6 μm Diameter Particles

Favorable surface chemistry (surface programming) of the 6 μm particles to promote bacteria adhesion can be tailored toward the autonomous guidance of these particles to a specific location using chemotaxis (i.e. the ability in bacteria to move up a chemical attractant gradient [12]). The chemical gradient needed to move the 6 μm diameter particles to a specific location is established within the microfluidic device as demonstrated in section II. The motion of both 6 μm and 10 μm particles was observed over a period of 1 hour. We observed during the course of the experiment, that the motile 6 μm particles propelled by chemotactic bacteria progressively collect near the entrance of the connecting micro-channel, where the chemical concentration is highest. Many of the accumulating particles continue moving up the gradient and into the connecting micro-channel. A succession of the optical microscopy images shows the progression of the accumulation of 6 μm particles near the entrance of the connecting channel of the micro-device (Fig. 5).

The number of 6 μm particles within a 600 μm radius from the entrance of the connecting channel was measured as a function of time. Fig. 6 illustrates the increase in the number of 6 μm particles near the entrance of the connecting channel as time increases. We saw negligible displacement of the 10 μm particles during the 1 hour of experiment. We also observed immobility of the only 10 μm bead within the 600 μm radius from the entrance of the connecting channel.

The strength of the method presented in this paper lies in its ability to sort micro-particles with similar or even identical sizes and densities as long as their surface properties are different. The use of bacteria as actuators allow for selective attachment on micro-particles having different surface properties. Moreover, different bacterial strains having specific affinity to certain chemo-attractants can be used to sort more than two types of micro-particles in terms of surface chemistries. Also, multiple sources of chemo-attractant activated in a pre-designed time controlled manner can be used to establish a spatiotemporal varying chemical gradient and achieve multi-dimensional particle manipulation.

The limitation of the proposed method includes the overall size of the work area which cannot exceed 600 μm for the single chemo-attractant design proposed here. If larger work areas are desired, additional chemo-attractant sources should be implemented to maintain a desired gradient that will continuously guide the particle to the final destination.

V. Conclusions and Future Works

A bio-hybrid microfluidic platform for autonomous sorting of micro-particles is introduced. Variation in surface chemistry properties and its effect on bacterial adhesion along with bacteria chemotaxis were used to autonomously guide 6 μm

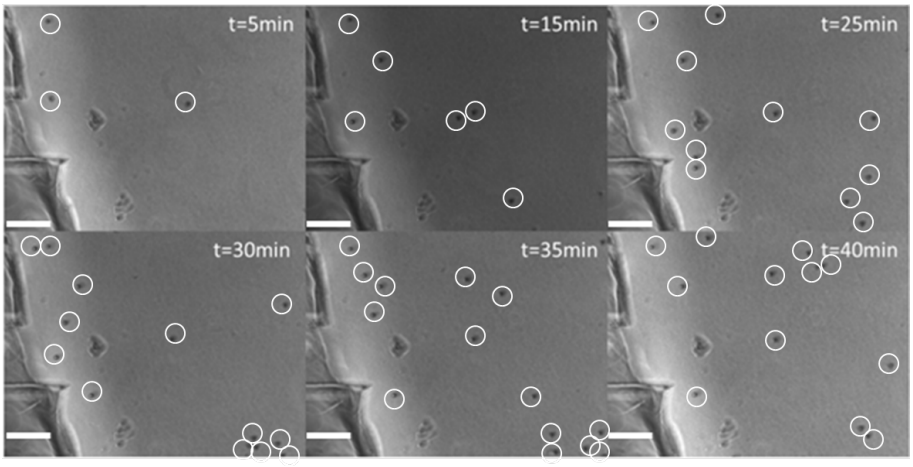

Fig. 5. Optical microscopy images show progression of accumulation of 6 μm particles near the source of the chemo-attractant, at the entrance of the connecting micro-channel. The white circles identify micro-particles

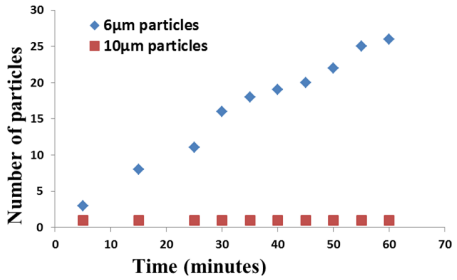

Fig. 6. Accumulation rate of the 6 μm and 10 μm particles near the entrance of connecting micro-channel.

polystyrene particles away from the center of the work area where a mixture of 6 μm and 10 μm polystyrene particles with different surface properties were initially introduced. The number of particles accumulating at a corner of work area, near the source of chemo-attractant, steadily increased during the hour long experiment. The work presented here will serve as a stepping stone towards developing cost-effective, autonomous, and robust manipulation platforms which can in long-term reduce the complexity and costs associated with performance of these tasks at reduced length-scales. The particularly attractive feature of the proposed system is that the biological manipulators and the microfluidic platforms used in this work can be produced cost-effectively and rapidly and are highly scalable in nature. The bio-hybrid manipulation platform as described here is not only very low cost, but it also does not require any electrical or magnetic source of power. It relies on chemical energy source for actuation and chemical signaling for steering. In future, we aspire to develop a bio-hybrid autonomous factory for transport and delivery, sorting, or bottom-up programmed self-assembly of micron-sized objects. Successful development of such assembly and manipulation workspace could revolutionize current practices and enable high throughput and high precision bottom-up assembly strategies. In long-term, this will lead to the development of novel devices and applications in areas such as nano-electronics and advanced materials.

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