ABSTRACT
This paper presents light-directed migration of multi-cellular microorganisms (slugs) of Dictyostelium discoideum in a microchip. The microchip consists of a microchamber and intersecting microchannels fabricated from agar to provide a moist environment necessary for slug survival. Slugs are driven to migrate in the geometric confinements of the microchannels under the stimulation of light, with the direction of migration determined by the position of an optical fiber that introduces light into the microchip. Experimental results demonstrate that this approach effectively allows controlled migration of slugs and has potential applications to biologically based microactuators.

KEYWORDS
Dictyostelium discoideum, microactuators, microchannels, phototaxis, slug

INTRODUCTION
Recently, manipulating movements of microorganisms in microfluidic systems has received increasing attention. In particular, utilizing microorganisms as bio-microactuators to generate useful work in a microchip has been a subject of active research [1]. Bio-microactuators offer distinct advantages over conventional microactuators. When they are living cells, bio-microactuators are powered directly by biochemical reactions, eliminating external energy sources such as batteries. Microorganisms can be self-healing when sustained with physical damages, resulting in higher efficiencies and reliabilities for microsystems utilizing bio-microactuators. In addition, sensory mechanisms to respond to environmental changes are naturally presented in microorganisms, thus migration response can be simply induced by stimuli such as light, temperature, and chemicals.

Studies have shown the possibility of using various microorganisms as biologically based microactuators, or bio-microactuators. For example, flagellated bacteria were able to generate linear and rotational flow patterns and produce sufficient force to move microscale objects. Similarly, a phototaxically driven unicellular alga carried and released a chemically attached microbead in a microchannel [1]. Microfabricated microscale gears unidirectionally rotated under forces exerted by bacteria [2]. Although these works have demonstrated microorganisms as actuation components in microsystems, they do not allow efficient control of the movements of the microorganisms. Efforts to control migration of microorganisms in microscale environments under external stimuli are still rather limited.

We investigate stimuli-responsive migration of multi-cellular microorganisms in microchannels. Specifically, we explore the light-guided migration (phototaxis) of multi-cellular organisms (slugs) of the social amoeba Dictyostelium discoideum (D. discoideum). Since D. discoideum slugs are phototactic, their movements can be driven and controlled by light-based stimulation. We use a microchip that consists of a microchamber connected to a microchannel, which joins two other microchannels to form a T-junction. An optical fiber, connected to an optical source, is coupled into each of the channels. To ensure a moist and sufficiently stiff surface to the slugs, agar is used as channel material. D. discoideum slugs differentiate in the chamber and migrate through the channels under light stimulation from the optical fibers. The channels offer geometrical confinements to guide the slug migration. Moreover, following the change in the light stimulation, the slugs change their migration direction at the channel intersection. Experimental results show that migration of slugs can be effectively controlled by manipulating position of optical fibers in microchannels, demonstrating the potential to exploit stimuli responsiveness of microorganisms to enable innovative bio-microactuators.

DESIGN
D. discoideum cells are soil living amoebae that feed on bacteria. When deprived of food supply, individual D. discoideum cells stream into aggregates by signaling each other using cyclic adenosine monophosphate. The aggregates further differentiate into multi-cellular organisms that are known as slugs with a length of approximately 1 mm and cross-sectional dimensions of about 100 μm. Slugs can respond to very low intensities of light signals, moving up the light gradients [3]. Thus, by defining a gradient of light intensity in a desired direction, the movement of D. discoideum slugs can be
phototaxically controlled. Further control can be obtained by placing the slugs inside microchannels, the geometry of which will constrain their lateral movement.

The phototaxically controlled migration of slugs in conﬁnements of a microchip is schematically shown in Figure 1. The microchip consists of three microchannels (the main, right, and left channels) and a microchamber fabricated from 4% agar, which provides a moist environment to ensure cell viability. The microchamber has an equilateral triangle shape (length of each side: 1 cm) converging toward the main microchannel. The main microchannel is 3 cm in length, and the right and left microchannels are both 0.6 cm in length. All of the channels have the same height (500 μm) and width (500 μm). An optical ﬁber (diameter: 250 μm) is inserted into each microchannel to transmit light from an external source to the cells. Multiple D. discoideum amoeba cells are placed in a microchannel and allowed to differentiate into slugs. The slugs are then directed to move through the microchannels in the direction of light from the optical fibers. A thin air-permeable poly(dimethylsiloxane) membrane is placed on the top of the channel network to vertically confine the slugs while providing additional oxygen necessary for cell survival.

EXPERIMENTAL

Fabrication of 4% Agar Microchannels. The fabrication process of the 4% agar microchip is shown in Figure 2. SU-8 photoresist was spin-coated on a silicon wafer, baked on a hotplate, and exposed to UV light through a photomask. Following a post-exposure bake on a hotplate, the unexposed photoresist was removed in a developer, creating a mold for the microchannel network. One gram of non-nutrient agar (Difco Laboratories) was dissolved in 250 mL of purified water at 95 °C, with heating from a hot plate. Then the molten agar solution was evenly spread on to the SU-8 mold in a petridish and placed on a flat surface at room temperature for 10–15 minutes until the solution was cured. The solidiﬁed 4% agar microchip was ﬁnally peeled off from the mold.

The phototaxically controlled migration of D. discoideum slugs were carried out as follows. A small drop of concentrated cells (~50 μL) was placed on a filter paper (2 mm × 2 mm, Millipore Corporation) to remove excess buffer from the cells. The filter paper was then placed in the microchamber of the microchip while the optical ﬁbers were inserted into the microchannels. A thin PDMS membrane was placed on the top of the microchannel network, and the microchip was kept in a dark room over night.

After 20 hours of incubation at room temperature (~24°C), slugs would start to migrate toward the optical fiber in the main channel. The distance between the light emitting end of the optical fibers and the slug was maintained at 2 to 3 mm by manually adjusting the position of the optical fiber. This ensured that the slugs did not come into contact with a fiber, which would affect their migration. When the slugs reached the end of the main channel, the optical ﬁber in that channel was removed, while another optical ﬁber was inserted into the left or right channel to direct the slugs to make an appropriate turn. During the experiment, the migrating slugs were imaged using a CCD camera, at one-hour intervals in the main channel and at ten-minute intervals in the T-junction. The slug position was then determined by analyzing the images using ImageJ software (National Institutes of Health freeware).

RESULTS AND DISCUSSION

Migration of Slugs through Main Channel. We ﬁrst investigated the migration of slugs in response to light stimulation through the main microchannel. Images of two slugs (Slugs 1 and 2) at different times are shown in Figure 3. At time t = 0 hr, the slugs started to migrate from the microchannel toward the main channel (Figure 3a). In about an hour, Slug 1 had reached near the inlet of the main channel while Slug 2 continued migrating in the microchannel (Figure 3b). At this time, the optical ﬁber was moved backward approximately by 2 mm to provide more space to the migrating slugs. At time t = 2 hr, more than half of Slug 1 had entered the microchannel. Despite the backward movement of the optical ﬁber in the main channel, Slug 2 could still migrate toward the inlet of the channel, most likely by sensing the light reﬂected from the side walls of the microchannel (Figure 3c). At time t = 3 hr, Slug 2 had migrated to the inlet of the main channel, with its anterior part gliding on the channel’s side wall (Figure 3d). At time t = 4 hr, Slug 2 entered the channel and shrank in length due to the resistance from the side wall. Slug 1 migrated through the channel in a serpentine pattern, reﬂecting that the slug was sweeping its anterior to sense light during phototaxis [4] (Figure 3e). (This phenomenon was observed for migrating slugs, including

Cell Preparation. The D. discoideum strain NC4 was grown on slime mold (SM) agar plates containing an organic medium on which Klebsiella aerogenes had inoculated. The SM agar plates were stored in a dark room at 24°C for 72 hours. In order to induce starvation, the cells were collected from the plates and washed in Sörensen’s buffer by centrifugation at 1000 rpm for 5 minutes. Supernatant buffer was discarded and the cells were resuspended in deionized water (concentration: 10^8 cells/mL).
those presented below.) At time $t = 5\, \text{hr}$, Slug 2 appeared elongated because of detachment from the side wall while continuing to migrate, and Slug 1 became shorter as its anterior glided on the side wall (Figure 3f).

Figure 3: Micrograph images of Slugs 1 and 2 at different times as the slugs migrated from a microchamber into the main microchannel. (Scale bar: 500 \(\mu\)m.)

The travel distances of the front tips of Slugs 1 and 2 during their migration from the microchamber to the main channel were obtained by analyzing consecutive images. As shown in Figure 4, Slug 1 migrated approximately 3.94 mm at a nearly constant speed of ~0.81 mm/hr for 5 hours. On the other hand, the total migration distance of Slug 2 was approximately 3.42 mm. The speed of Slug 2 decreased from ~0.72 mm/hr to ~0.53 mm/hr between $t = 3$ and 4 hrs, likely because of the resistance from the side wall that was in contact with the slug’s anterior. Beyond this time, Slug 2 again migrated at its normal speed as it detached from the channel’s side wall.

Figure 4: Migration distances of Slugs 1 and 2 in the main microchannel.

Migration of Slugs into the Left Channel. We also demonstrated the use of light stimulation to guide slugs in a particular direction in the T-junction. We first investigated the migration of slugs into the left channel. For this purpose, when slugs reached the end of the main channel, the optical fiber in that channel was removed, while another optical fiber was inserted in the left channel. Figure 5 shows images of two slugs (Slugs 3 and 4) as they were guided to turn left. At $t = 0\, \text{min}$, the anterior of Slug 3 reached the end of the main channel, while Slug 4 was still migrating inside that channel (Figure 5a). After ten minutes, the optical fiber in the left channel was pulled back by about 3 mm. At $t = 60\, \text{min}$, Slug 3 had exited the main channel completely, turned left, and started to enter the left channel. On the other hand, Slug 4 had reoriented toward the left channel with an angle of about 45º (Figure 5b). At $t = 120\, \text{min}$, Slug 3 had completed entering the left channel, while the anterior of Slug 4 had just reached the entrance of the left channel (Figure 5c). At $t = 180\, \text{min}$, Slug 3 was migrating in a serpentine pattern through the left channel and Slug 4 had completely entered the left channel (Figure 5d).

Figure 5: Micrograph images of Slugs 3 and 4 at different times as the slugs turned from the main channel into the left channel. (Scale bar: 500 \(\mu\)m.)

Figure 6: Centroidal trajectories of Slugs 3 and 4 as the slugs turned from the main channel into the left channel.

Figure 6 shows the trajectories of the centroids of Slugs 3 and 4 determined from consecutive images. It can be seen that Slug 3 was away from the main channel’s side walls and later migrated close to the left channel’s lower side wall, while Slug 4 first approached and then moved away from the main channel’s right side wall. These observations indicate the active geometric...
confinement of the channels on the migration of the slugs. The average migration speeds of Slugs 3 and 4 along these trajectories were 0.48 and 0.51 mm/hr, respectively. The radii of curvature of the trajectories of Slugs 3 and 4 are approximately 0.25 mm and 0.92 mm, respectively. That is, Slug 3 made a sharper turn than Slug 4 in the T-junction; the more gradual turn made by Slug 4 reflects that the slug had not yet reached the end of the main channel when the optical fiber was moved backward.

Migration of Slugs into the Right Channel. We investigated whether phototactic migration direction changes of *D. discoideum* in microchannels are biased, as it is known that individual constituent cells in a slug make clockwise rotations when viewed from behind. To examine if slugs are biased to make turns in a certain direction, we also steered slugs into the right channel, using an optical fiber inserted in that channel. Figure 7 shows resulting images of a slug (Slug 5). At t = 0 min, Slug 5 reached the end of the main channel (Figure 7a). When the optical fiber was pulled back approximately 3 mm along the right channel, the slug continued migrating along the direction of the main channel. At t = 60 min, Slug 5 came in contact with the upper side wall of the T-junction and started to turn to the right (Figure 7b). At t = 90 min, the anterior of Slug 5 had completed the right turn and was gliding on the upper side wall (Figure 7c). The slug had also become thicker and shorter due to the resistance from the wall. At t = 120 min, the slug had completed the right turn, and its whole body was almost in contact with the upper side wall (Figure 7d).

![Figure 7: Micrograph images of Slug 5 at different times as the slug turned from the main channel into the right channel. (Scale bar: 500 μm.)](image)

The trajectory of the centroid of Slug 5 is shown in Figure 8. This trajectory is seen to be in close proximity of the main channel’s left side wall, again reflecting active geometric confinement of the channel on the slug migration. The radius of curvature of Slug 5’s trajectory was approximately 0.43 mm, and the slug’s average migration speed along the trajectory was about 0.77 mm/hr. These are respectively comparable to the afore-mentioned radii of curvature and average migration speeds of the left-turning Slugs 3 and 4, and the differences are within the range of slug-to-slug variations in migration behavior. Thus, it appears that *D. discoideum* slugs do not exhibit a bias in changes in migration directions.

![Figure 8: Trajectory of the centroid of Slugs 5 as the slugs turned from the main channel into the right channel.](image)

**CONCLUSION**

Migration of *D. discoideum* slugs has been driven and controlled by manipulating the position of light introduced into a microchip. In the microchip, a microchamber is connected to a microchannel, which joins two other microchannels to form a T-junction. The microchip is fabricated from 4% agar, which provides a moist and sufficiently stiff environment for the slugs. The geometrical confinement imposed by microchannels and manipulation of light stimulation using optical fibers in the microchip enable effective control of the slug migration. Experimental results have demonstrated that slugs were effectively guided through the microchannels, and exhibited no bias in changing their migration direction at the T-junction. Thus, light-directed migrating *D. discoideum* slugs can be potentially used as biologically based microactuators.

**REFERENCES**


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