Formation and Stimuli-Directed Migration of *D. discoideum* Slugs in Microchips

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Abstract

This paper presents a microfluidic device that geometrically constrains the development of individual *Dictyostelium discoideum* cells into multicellular organisms (slugs). A microchip for the stimuli-directed migration of slugs is also presented. To demonstrate the formation of slugs in a predetermined shape, a microchip is designed to confine the slugs in the vertical direction. In the microchip, sufficient oxygen is supplied to the cells via a membrane, allowing the formation and adaptation of slugs to the shape of the channel. In addition, the manipulation of slug migration direction in a microchip via external stimuli, such as light and temperature gradients, that induce phototaxis and thermotaxis of slugs, respectively, is demonstrated. To direct slug migration with external stimuli, an optical fiber is used for phototaxis and an electrical wire heater is used for thermotaxis. Experimental results show slug formation in a predefined geometry in the microchip, suggesting that this chip is potentially useful for understanding the relationship between the shape and function of cells or tissue. The controlled migration of slugs demonstrated in the microchips can potentially be employed in biologically based microactuators or microrobots.

Keywords: Microfluidic device, Microorganism, *Dictyostelium discoideum*, Phototaxis, Thermotaxis, Bio-microactuator.

1. Introduction

Microfluidic devices have been recognized as great tools for cell research, including studies on cell development, culture, and migration [1,2]. Such devices provide unique advantages such as precisely control buffer and gas flows during cell development [3]. In addition, due to advancements in microfabrication techniques, microdevices can be easily prepared [4], with significantly reduced sample and reagent consumption [5]. As a result, microfluidic devices have been intensively used for the study of cells [6].

Various approaches of using microfluidic devices have been explored for specific applications in cell studies [7]. For instance, a microfluidic bioreactor with an oxygen-supplying component has been developed for liver cell culture [8]. Microchips have been used for the investigation of tissue migration, proliferation, and differentiation [9].

Although the ability to manipulate the shape of cells could be very useful for studying the effect of cell shape on cell function [10], most existing devices do not fully allow the development of cells into controlled shapes. In addition, studies on the controlled migration of microorganisms using microchips could be very useful for bio-actuation applications. For example, small objects can be chemically or physically attached to a slug and carried to specific locations via phototaxis or thermotaxis. This capability could be useful, where transportation may be very difficult using conventional microactuators [11].

The present study investigates the formation of multi-cellular organisms (slugs) of *Dictyostelium discoideum* (*D. discoideum*) using a microchip that can physically confines slugs in a restricted geometry. The directed migration of slugs induced by external stimuli is also demonstrated using microchips in which slug movements are stimulated by light and a temperature gradient via an optical fiber and an electrical wire heater, respectively. *D. discoideum* is employed since its individual cells can develop into slugs under conditions of adequate moisture and oxygen [12]. The migration of *D. discoideum* slugs can be easily induced because a slug can respond and follow very low gradients of external stimuli such as light, temperature, and chemicals [13]. Specifically, a slug detects the light focused on the inner surface of its front tip

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Individual amoeba cells in a slug sense a temperature gradient through the slug skin [14].

Experimental results show that the shapes of multi-cellular organisms of *D. discoideum* can be manipulated by physical confinement in a microchip. Additionally, slug migration can be effectively manipulated using microchips via phototaxis and thermotaxis, demonstrating potential applications in biologically based microactuators.

2. Design of microchip

A schematic of the microchip used for the development of individual cells of *D. discoideum* into slugs in a confined geometry is shown in (Figs. 1(a) and 1(b)). In the chip, a hydrophobic membrane (pore size: 200 nm) is sandwiched between a polydimethylsiloxane (PDMS) microchannel and a glass substrate with an air opening (length: 5 mm, width: 1 mm). Through the inlet, cells in buffer solution are introduced into the channel (length: 15 mm, height: 60 μm, width: 1 mm). The hydrophobic membrane allows air to enter the channel while decreasing the evaporation rate of buffer solution (Figs. 1(a) and 1(b)) [15]. A microchip without an air supply component for a negative control experiment is also developed to examine the effects of air on cell development (Figs. 1(c) and 1(d)).

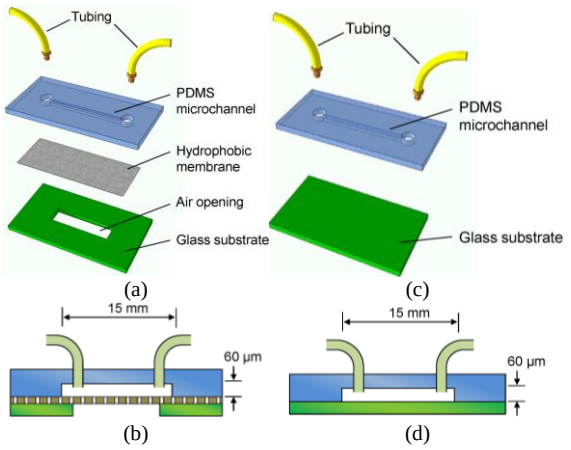


Figure 1. (a) Design schematic and (b) cross-section view of a microchip with an air perfusion component. (c) Design schematic and (d) cross-section view of the chip lacking an air perfusion component.

The agar microchip for the directed slug phototaxis is schematically shown in Fig. 2(a). The microchip is similar to the one reported in our earlier publication [2]. The microchip has a triangle-shaped microchamber converging toward a microchannel (length: 1 cm, width: 500 μm, height: 500 μm). In the chamber, a hydrophobic filter paper (2 mm × 2 mm) is placed for cell inoculation. One end of an optical fiber (diameter: 250 μm) is connected to an external light source while the other end is integrated into the channel to transmit light to the cells in the microchip to induce phototaxis. The agar chip used for the simultaneous phototaxis and thermotaxis of slugs is shown in Fig. 2(b). The agar substrate (5 cm × 5 cm) has a thickness of 3 mm. Three hydrophobic filter papers (1.5 mm × 1.5 mm) for cell inoculation are placed 1 cm apart from each other. A light-transmitting optical fiber is located near the filter paper in the middle. An electrical wire (diameter: 700 μm) that generates heat to induce thermotaxis is placed underneath the agar substrate 1 cm away from the closest filter paper. The wire is connected to a power supply.

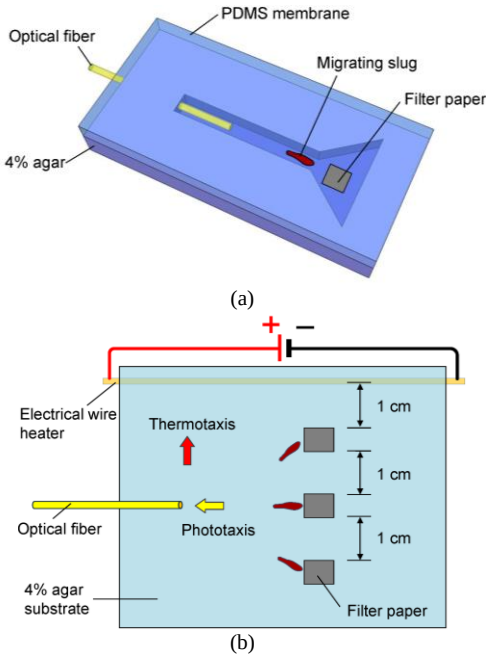


Figure 2. Schematics of agar microchips used for (a) slug phototaxis in a microchannel and (b) simultaneous phototaxis and thermotaxis on an agar substrate.

3. Experimental design

3.1 Materials

Strain HR23 of *D. discoideum* that expresses green fluorescent protein (GFP) when differentiated into prestalk cells was used in the cell development experiments and strain NC4 was used in slug migration experiments [16]. The strain of *D. discoideum* was grown on nutrient agar plates with *Klebsiella aerogenes* in a dark room at 24 ℃ [17]. Sörensen's buffer was used to wash to induce cell starvation. A 4% agar mixture was prepared by adding 1 g of non-nutrient agar (Difco Laboratory, Detroit, MI, USA) into 250 mL of DI water in a glass bottle.

3.2 Fabrication

The cell-development microchip was fabricated from a PDMS microchannel layer bonded on a hydrophobic polycarbonate membrane filter (PCTF25SP, Sterlitech Corp., Kent, WA, USA) placed over a hole in a glass substrate. The SU-8 mold for the PDMS microchannel was prepared using photolithography techniques. Similarly, a 4% agar microchip for slug phototaxis experiments was fabricated via SU-8 replica molding. An agar plate for the experiment of slug phototaxis and thermotaxis was prepared with molten 4% agar solution solidified in a petridish.

3.3 Experimental setup

Throughout the experiments, the microchips were placed in a light enclosure fabricated from 6 sheets of acrylic plastic (7 cm long and 0.45 cm thick) covered with black electrical tape to prevent unintended ambient light entering the chip. On the top and bottom faces of the enclosure, long-pass filters (5 cm × 5 cm, cutoff wavelength: 695 nm, Andover) were attached for observations of the chip in the enclosure. To insert the optical fiber and electrical wires, holes were drilled in the side faces of the enclosure. For cell viability, humidity was maintained by placing wet tissues in the enclosure. The temperature was maintained at 23 ℃ during the experiment. Time-lapse micrograph images of cell development and slug migration were obtained using a fluorescence microscope (LSM 510, Zeiss Corp., Jena, Germany) and a microscope (Nikon Corp., Japan) equipped with a charge-coupled device (CCD) camera (PVC 100C, Pixera Corp., Los Gatos, CA, USA), respectively (Fig. 3).

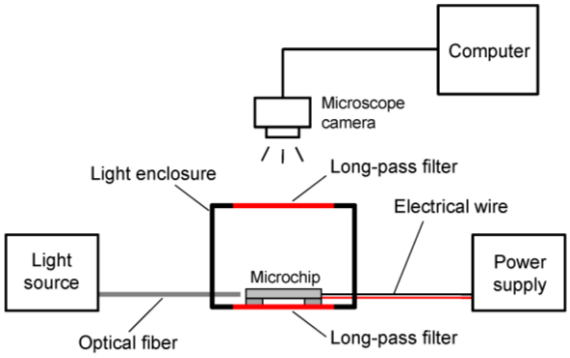


Figure 3. Schematic of experimental setup.

3.4 Experimental procedure

The cell development experiment, solution containing *D. discoideum* cells was injected through the inlet of the microchip using a syringe for both the positive and negative control experiments. Then, the microchips were placed in the light enclosure overnight for cell development. Approximately 20 h later, the microchannels were inspected for slug formation using a fluorescence microscope.

For slug phototaxis experiment, *D. discoideum* cells in a small drop of DI water (50 μL) were inoculated on a filter paper (2 mm × 2 mm, Millipore Corp., Burlington, MA, USA). After excess water was removed, the filter paper was placed in the microchamber of the microchip which was then placed in the light enclosure while an optical fiber was inserted into the microchannel to transmit light. The light-emitting end of the optical fiber was placed 5 mm away from the channel inlet to ensure that migrating slugs did not come into contact with the fiber. Using a CCD camera, images of the slugs were taken at 1-h intervals. Then, the velocities of the migrating slugs were estimated by analyzing the images using ImageJ software (National Institutes of Health).

For the experiment of simultaneous slug phototaxis and thermotaxis, cells were first inoculated on 3 filter papers (1.5 mm × 1.5 mm) on a 4% agar substrate. Then, the substrate was loaded in the enclosure while an electrical wire was placed under the agar substrate. A light-transmitting optical fiber was placed approximately 4 mm away from the filter paper in the middle on the agar substrate. After slug formation within 20 h followed by 3 h of slug phototaxis, the optical fiber was removed and a constant electric current was supplied to the electrical wire to generate a temperature gradient on the surface of the agar substrate to induce slug thermotaxis. The temperature gradient on the surface of the agar substrate was measured using a thermocouple.

To estimate the migration distance of slugs, images of the migrating slugs were taken at 1-h intervals using a CCD camera. The positions of the front tip of each slug were then determined by analyzing the images and the linear displacement distance of the tip during each time interval was measured using Image J. A slug's total migration distance was then calculated by summing the linear displacements measured at each time interval. Slug velocity was defined as the total migration distance per monitoring duration time.

4. Results and discussion

4.1 Slug formation in a confined geometry

In the slug formation experiment, the cells inoculated on a glass substrate initially tended to clump to each other upon inoculation (Fig. 4(a)) [18]. In 18 h, the cells formed multiple cellular aggregates that are typically observed in *D. discoideum* cells suspended in buffer (Fig. 4(b)) [19]. In approximately 30 h, the cells further developed to the culminant stage (Fig. 5c), which is a later stage than the slug stage in *D. discoideum* development. When the glass substrate was kept in the enclosure without a humidity source, the cells died due to desiccation (Fig. 4(d)). These results indicate that individual *D. discoideum* cells can differentiate into slugs and later stages on a glass when ample oxygen and sufficient moisture are provided.

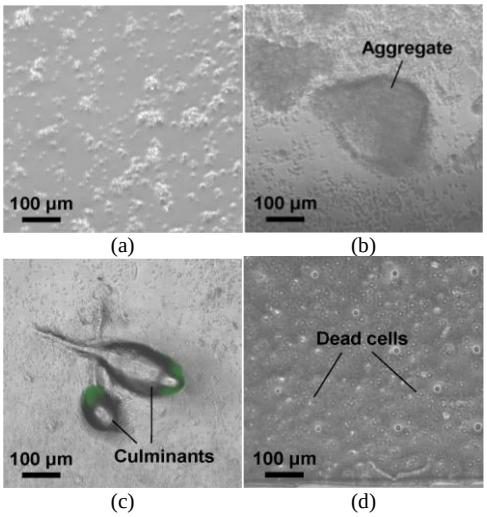
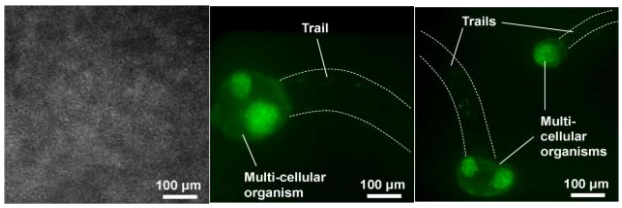


Figure 4. Slug formation on a glass substrate in humid environment. (a) $t = 0$ h, (b) $t = 18$ h, and (c) $t = 30$ h. (d) Negative control showing cell desiccation on a glass substrate in a dry condition.

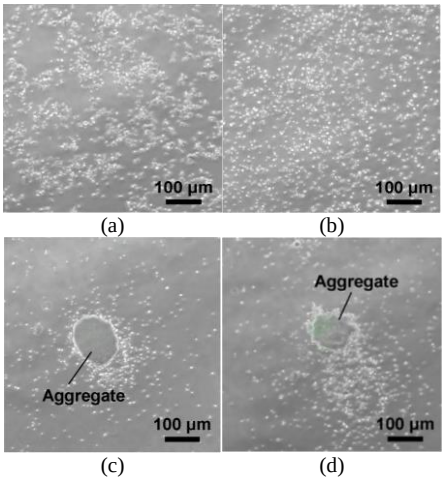


(a) (b) (c)

Figure 5. (a) Bright-field and (b, c) fluorescence micrographs of slugs formed on hydrophobic membrane with a predefined shape in the cell-development microchip at 30 h.

The formation of slugs in a PDMS microfluidic chip with an oxygen perfusion component was investigated. Because of extremely poor contrast, a slug formed on a hydrophobic membrane cannot be visualized using bright-field microscopy (Fig. 5(a)). However, the strong fluorescence intensities expressed in the organisms in the fluorescence micrographs indicate that individual cells differentiated into slugs in the channel after 30 h. The trail left behind the migrating slugs in the channel can also be seen in the images. The slugs formed in the channel appear to be flattened from adapting to the geometry of the channel. The microchannel (height: 60 μm) geometrically constrained the slugs (average thickness: ~100 μm) in the vertical direction (Figs. 5(b) and 5(c)). Results show that the hydrophobic membrane provided sufficient oxygen to the cells while preventing buffer from evaporating from the microchannel.

The development of *D. discoideum* cells in a microchip without an oxygen perfusion component was then investigated. The micrographs show that individual cells were initially clumped to each other (Fig. 6(a)) and then dispersed within 10 h (Fig. 6(b)). After 20 h, a cellular aggregate formed in the channel, with some individual cells also present (Fig. 6(c)). The cellular aggregate did not further differentiate into slugs after 30 h, as evidenced by very weak fluorescence intensity. In addition, a thin outer layer that is uniquely found in a slug did not appear in the cellular aggregate (Fig. 6(d)) [20]. This indicates that the differentiation process was significantly hindered due to the lack of oxygen available to the cells.

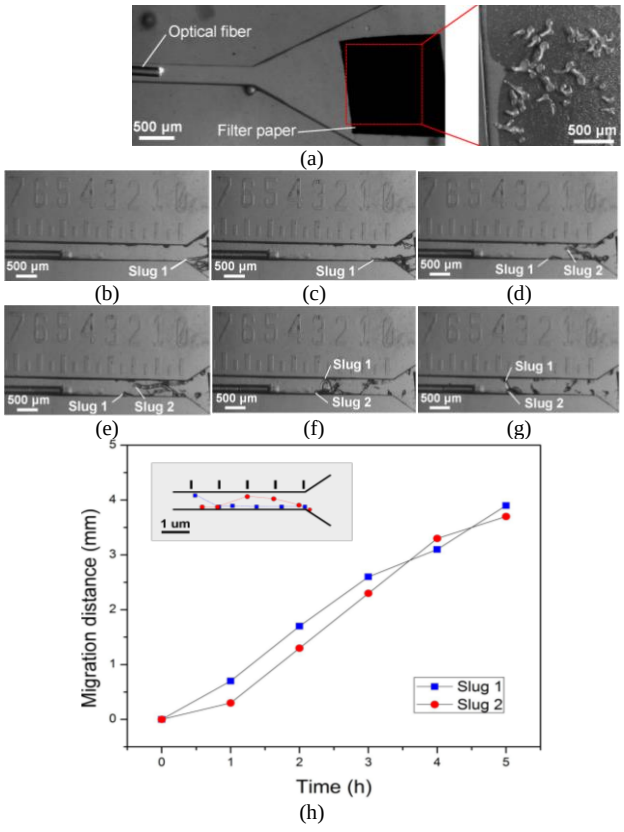


(a) (b) (c) (d)

Figure 6. Overlay images of bright-field and fluorescence micrographs of cell development in the microchip without an oxygen supply over 30 h. (a) t = 0 h, (b) t = 10 h, (c) t = 20 h, and (d) t = 30 h.

4.2 Slug phototaxis through a microchannel

The light-directed migration (i.e., phototaxis) of slugs in the agar microchannel was investigated. Multiple slugs formed on the filter paper and the front ends of slugs pointed in the direction of the optical fiber within 18 h (Fig. 7(a)). Two slugs (i.e., Slugs 1 and 2) were tracked for 5 h of their phototaxis. At t = 21 h, multiple slugs reached an area near the entrance of the channel (Fig. 7(b)). The migration of the slugs on the side wall toward the optical fiber clearly indicates that the lateral movement of slugs was geometrically constrained within the channel (Figs. 7(c)-7(e)). Without the geometrical restriction imposed by the channel, the slugs would migrate in a relatively uncontrolled manner, as exhibited by the slugs migrating in the microchamber. While Slug 2 moved to the lower side wall, Slug 1 migrated in a serpentine pattern, alternating its migration trajectory between upper and lower side walls (Figs. 7(f) and 7(g)). The overall travel distances of Slugs 1 and 2 in the channel measured at their front tips during the 5 h of phototaxis were approximately 3.9 mm and 3.8 mm, respectively (Fig. 7(h)). These results are consistent with those previously reported for a similar experiment [2].



(a) (b) (c) (d) (e) (f) (g) (h)

Figure 7. Time lapse images of slug formation and migration in an agar microchip via phototaxis. (a) t = 18 h, (b) t = 21 h, (c) t = 22 h, (d) t = 23 h, (e) t = 24 h, (f) t = 25 h, and (g) t = 26 h. (h) Migration distances of selected slugs (Slug 1 and Slug 2) in the channel. Inset shows tracings of the front tips of the slugs.

4.3 Slug phototaxis and thermotaxis on an agar substrate

The phototaxis and thermotaxis of slugs were further studied by simultaneously applying light and heat stimuli to the slugs on an agar substrate. A temperature gradient of

approximately 0.5 ℃/cm was generated on an agar substrate with an electrical wire placed underneath to which a constant electric current of 2 A was applied for 30 min. Since a slug is sensitive to an extremely small temperature gradient (i.e., 0.05 ℃/cm [12]), this experimental setup was suitable for slug thermotaxis (Fig. 8a). Slugs formed on 3 different filter papers placed in different locations on an agar substrate migrated via phototaxis toward an optical fiber (Figs. 8b-8d). At t = 22 h, the optical fiber was removed and a constant electrical current was applied the electrical wire heater to induce slug thermotaxis. Within an hour, the slugs that had initially migrated toward the light source changed their migration direction toward a higher temperature (Figs. 8e-8g).

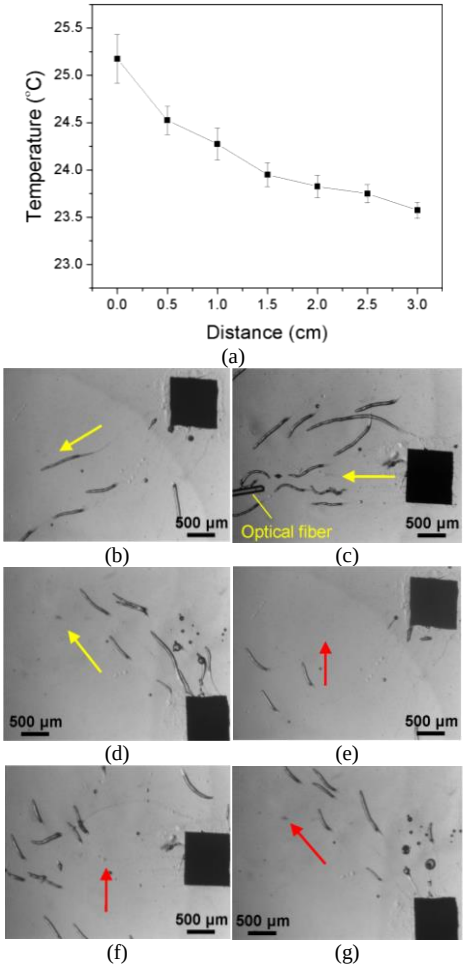


Figure 8. (a) Temperature distribution on the agar substrate along the distance from the electrical wire heater to the filter paper. Time lapse images of multiple slugs migrating on an agar substrate via (b-d) phototaxis at t = 22 h and (e-g) thermotaxis at t = 23 h. Arrows indicate the directions of slug migration.

The slug migration was continuously monitored in the region near the wire heater. Two representative slugs (Slugs 3 and 4) were tracked to investigate their migration behaviors. At t = 27 h, Slugs 3 and 4 approached the monitoring site (Fig. 9a). About an hour later, whole bodies of the slugs became visible in the images (Fig. 9b). While Slug 3 slightly turned to the left, Slug 4 moved forward without any significant change in its migration direction (Figs. 9(c-d)). During the 3 h of tracking, the total traveled distances were 4.75 mm and 5.15 mm for Slugs 3 and 4, respectively. Slugs 3 and 4 migrated at almost constant velocities of 1.6 and 1.7 mm/h, respectively. The migration velocity of the slugs is consistent with the range of slug velocities reported in the literature [21] (Fig. 9e). During thermotaxis, a slug detects the temperature gradient on the surface of its skin. Thus, extra motions such as sweeping are not required for a slug during thermotaxis, resulting in a constant migration velocity.

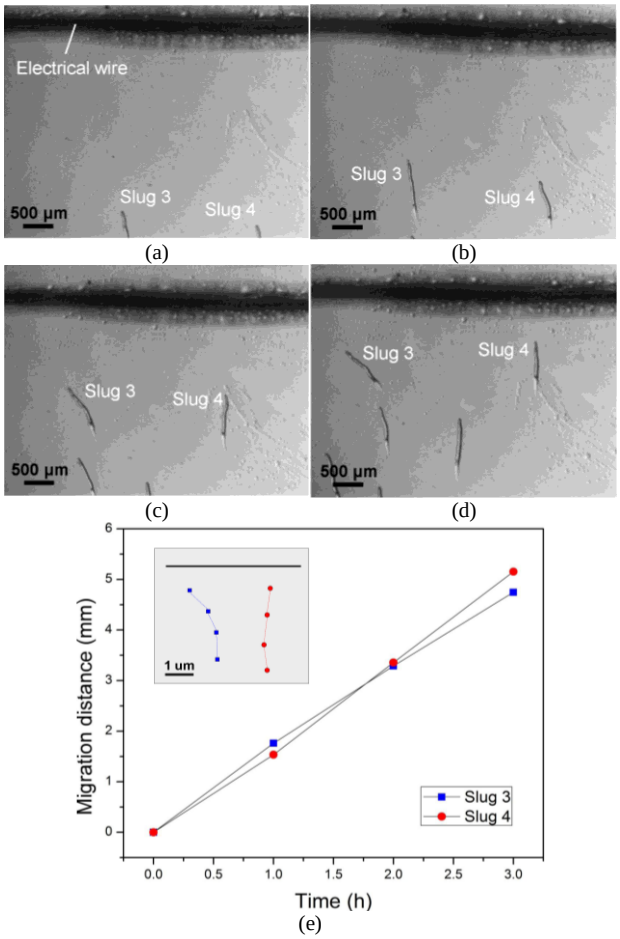


Figure 9. Time lapse images of multiple slugs migrating on an agar substrate via thermotaxis. (a) t = 27 h, (b) t = 28 h, (c) t = 29 h, and (d) t = 30 h. (e) Migration distances of selected slugs (Slug 3 and Slug 4) on the agar substrate. Inset shows tracings of the front tips of the slugs.

5. Conclusion

The formation of slugs with a controlled shape was investigated and the directed migration of slugs in microchips was demonstrated. To study slug formation in a geometrically restricted condition, a microchip that physically confined the slugs was used. Slugs that formed in the microchip had controlled final shapes adapting the microchannel geometry. The migration of slugs could be controlled in a microchip by using light and heat stimuli via an optical fiber and an electrical wire heater, respectively. The results demonstrate that the shape of slugs can be manipulated by the imposed physical confinements in a microchip. This allows the role of cell shape

in cell function to be examined during cell development. Furthermore, the effective manipulation of the migration direction of slugs via light and temperature gradients can be useful for application in biologically based microactuators or microrobots.

Acknowledgments

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