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Light-directed migration of *D. discoideum* slugs in microfabricated confinements

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ABSTRACT

This paper investigates the light-driven migration of the multi-cellular microorganism *Dictyostelium discoideum* as a potential bio-actuation mechanism in microsystems. As a platform for slug migration we use microscale confinements, which consist of intersecting microchannels fabricated from solidified agar–water solution. The agar surface provides necessary moisture to the slugs during the experiment while remaining sufficiently stiff to allow effective slug migration. The movements of the slugs in the microchannels are driven and guided by phototaxis via controlling light transmitted through optical fibers. The microchannels impose geometrical confinements on the migrating slugs, improving the spatial precision of the migration. We demonstrate that slugs that form in a microchamber can be driven to migrate through the microchannels, as well as steered to a particular direction at microchannel intersections. Our experimental results indicate that slug movements can be more effectively controlled in microchannels, and potentially useful for bio-actuation applications.

1. Introduction

Controlled movement of microorganisms in microfluidic systems is a subject of active research [1]. In particular, the use of microorganisms as bio-microactuators to generate useful work in a microchip has been receiving increasing attention [2]. Bio-microactuators offer distinct advantages over conventional microactuators. When they are living cells, bio-microactuators are powered directly by biochemical reactions [3], eliminating external energy sources such as batteries. Microorganisms can be self-healing, resulting in higher efficiencies and reliability for microsystems utilizing bio-microactuators that have sustained physical damages [4,5]. In addition, sensory mechanisms that respond to environmental changes are naturally presented in microorganisms [6], whose responses can hence 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. Microorganisms have been used as bio-microactuators to move fluids [6], transport microscale objects [7,8], drive microrotors [4], and deliver drugs [9]. For example, flagellated bacteria were able to generate linear and rotational flow patterns, producing sufficient force to move microscale objects [6,10]. Similarly, a unicellular alga with chemically attached microbeads was driven by phototaxis to carry and release beads in a microchannel [11]. Forces exerted by bacteria were also used to unidirectionally rotate microfabricated gears [12]. Although these works have demonstrated the potential use of microorganisms as actuation components in microsystems, there has been a lack of studies that exploit stimuli responsiveness to precisely control the movements of the microorganisms.

In this paper, we investigate stimuli-responsive migration of multi-cellular microorganisms in microfabricated confinements. 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, light stimulation can be used to induce phototaxis, and effect and control their migration in microchannels. To demonstrate this principle, 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 for 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 coupled into the chip. The channels offer geometrical confinements to guide the slug migration. Moreover, the channel intersections provide

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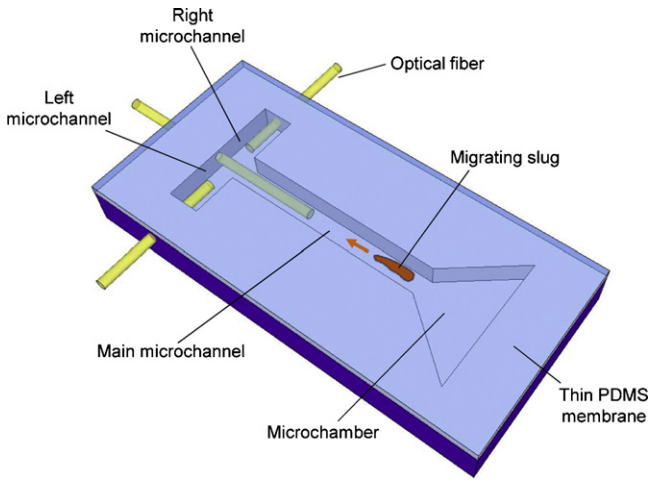

Fig. 1. A schematic representation of phototaxis of a *D. discoideum* slug in a microchip. Migration of the slug is driven and directed by light transmitted through the optical fibers.

geometrical bifurcations that enable the migrating slugs to change directions under light stimulation. Experimental results show that migration of slugs can be effectively controlled by manipulating the position of optical fibers in microchannels, demonstrating the potential to exploit stimuli responsiveness of microorganisms to enable innovative bio-microactuators.

2. Design

D. discoideum is a species of amoeba that normally lives in soil and feeds on bacteria. When starved (i.e., 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. *D. discoideum* slugs are responsive to very low intensities of light signals, moving up the light gradients [13,14]. Thus, by defining a gradient of light intensity in a desired direction, the movement of 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 directed migration of slugs in microfabricated confinements in a microchip is schematically shown in Fig. 1. The microchip consists of a triangular microchamber connected to a microchannel (called the main channel), which in turn joins two other microchannels (respectively called left and right channels) to form a T-shaped junction. The microchamber has an equilateral triangle shape (length of each side: 10 mm) converging toward the main channel. The main channel is 30 mm in length, and the right and left channels are both 6 mm in length. All of the channels were 500 μm wide, and had the same height as the chambers, which was also 500 μm. An optical fiber (diameter: 250 μm) is inserted into each channel to transmit light from an external source to the cells. We use optical fibers because they are inexpensively available and compatible with our microchip, although other illumination methods with appropriate wavelengths (below 650 nm for slug phototaxis [15]) could be used.

The microchip is fabricated from 4% non-nutrient agar to ensure a moist and nutrient-deprived environment as required for cell viability and slug formation [16]. Also the agar provides a sufficiently stiff support surface on which slugs could migrate without slipping [17]. The microchip is sealed from the top with a sheet of polydimethylsiloxane (PDMS) to vertically confine migrating slugs while providing additional oxygen necessary for cell survival by preventing oxygen depletion due to cell respiration [18].

Multiple *D. discoideum* amoeba cells are placed in a microchamber 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. Under stimulation of light from the optical fiber coupled to the main channel at the distal end, the slug migrates into and along the main channel toward the light. As the slug reaches the T-junction, the light source for the optical fiber in the main channel is turned off, while the light source for the optical fiber in the left or right channel is turned on. Following the change in the light stimulation, the slug turns left or right into either of the side channels accordingly.

3. Experimental

3.1. Materials

The following materials were used in the experiments. Cells of *D. discoideum* were prepared as follows. The *D. discoideum* strain NC4 were grown on slime mold (SM) agar plates containing an organic medium on which *Klebsiella aerogenes* had inoculated [19]. The SM agar plates were stored in a dark room at 24 °C for 72 h. To induce starvation, the cells were collected from the plates and washed in Sörensen's buffer by centrifugation at 1000 rpm for 5 min. Supernatant buffer was discarded and the cells were resuspended in deionized water (concentration: 10^8 cells/mL).

Four percent non-nutrient water agar (Difco Laboratories) was prepared by adding 1 g of agar to 250 mL of purified water filtered from a Milli-Q Plus water purification system (Millipore). The mixture was thoroughly mixed in a glass bottle and then was warmed on a hot-plate until the agar powder was completely dissolved [20]. Bubbles formed in the agar–water mixture were removed by applying vacuum to the bottle.

3.2. Microchip fabrication

The fabrication process of the 4% agar microchip is shown in Fig. 2. The microchip was fabricated from 4% non-nutrient agar via replica molding. To fabricate the mold, SU-8 2150 photoresist (MicroChem) was spin-coated on a silicon wafer, baked on a hotplate at 95 °C for 2 h, and exposed to UV light through a photomask (Fig. 2a). Following the post-exposure bake at 95 °C for 30 min on a hotplate, the unexposed photoresist was removed in a developer, creating a mold for the microchannels (Fig. 2b). Molten 4% non-nutrient agar solution was evenly spread onto the SU-8 mold held in a petridish and placed on a flat surface at room temperature for 10–15 min until the agar solution fully solidified (Fig. 2c). The solidified agar was removed from the mold to create the microchannel structure (Fig. 2d). The resulting 4% agar microchip with a PDMS cover was approximately 5 cm (along the main channel) × 3 cm (along the side channels) in size. In parallel to agar molding, a sheet of PDMS (thickness: 500 μm) was fabricated by spin-coating of a PDMS prepolymer solution of *Sylgard* 184 (Dow Corning) that was mixed with the manufacturer-supplied curing agent at a volume ratio of 10:1 on a glass substrate. Then the prepolymer was degassed by vacuum for 30 min and cured on a hot plate at 75 °C for 1 h. The PDMS sheet then was bonded onto the agar chip by spontaneous adhesion. An optical fiber 250 μm in diameter (The Fiber Optic Store.com) was inserted into each of the microchannels through access holes fabricated within the agar chip.

3.3. Experimental setup

Phototaxis experiments were performed with the microchip placed in an acrylic plastic enclosure fabricated as follows (Fig. 3).