A Microfluidic Device for Measuring Aerotaxis

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1 Abstract

Bacteria rely heavily on sensing environmental gradients to survive and reproduce. While significant effort has been made to understand responses to chemical gradients, or chemotactic responses, less literature exists characterizing responses to gas gradients, or aerotactic responses. This study employed a three-channel microfluidic device fabricated from PDMS to characterize aerotactic bacterial motion. In the presence of a gradient of nitrogen and oxygen, movement of the organism *B. subtilis* tended toward oxygen and away from nitrogen, demonstrating an aerotactic response.

2 Introduction

The movement of bacteria in response to a gas gradient, aerotaxis, is a field that has emerged within the past 15 years [1]. As such, it has not been studied to the extent of the similar process chemotaxis. Chemotaxis is the movement of bacteria in response to a chemical gradient, where the chemical can be anything from food to toxic molecules. Models for chemotaxis in bacteria have been characterized, both for populations and single bacterial cells [2] [3].

Aerotaxis was studied in a microfluidic device using a custom optical microscope. The microfluidic device allowed for development of a gas gradient along its length; the bacterias response to the gradient was visualized with the microscope. This setup can be utilized for studying different bacteria species and potentially other cell types.

Aerotaxis has a broad range of applications; the signaling proteins utilized in for aerotactic responses in bacteria can be potentially engineered for turning on gene expression in the presence of a certain oxygen concentration, or lack thereof. Since aerotaxis occurs on the order of minutes, a response can be expressed fairly quickly. Additionally, modeling aerotaxis is key to understanding how applications such as the aforementioned can be utilized.

3 Experimental apparatus

The aerotaxis experiments are run in a microfluidic device that contains the bacteria and the gases, nitrogen and oxygen, in parallel channels separated by thin layers of PDMS that are permeable to the gases. The device is located on the stage of a custom-built fluorescence microscope. A photo of the overall setup is shown in Figure **??** on page ??

fig:SetupPhoto Full experimental setup. The microfluidic device is on the stage of the microscope, which was custom-built for these experiments. The gas regulators allow for controlled flow of O2 and N2 gas into the device via syringe tips inserted into the device inlets.

3.1 The microscope

The microscope used for the project is a standard 4f microscope with 200mm Nikon objectives. The objective is mounted inverted to facilitate access to the sample. 10X and 20X objectives were used to collect micrographs during the course of the experiments. Brightfield illumination is provided by a red LED and collimated by a small lens. A barrier filter filters out other illumination from the CCD. These components are shown in Figure **??** on page ??.

A fluorescence pathway was also built to aid in cell tracking. The fluorescence illumination is provided by a 532 nm green laser source, which is expanded and then focused onto the surface of the objective in order to produce a collimated beam on the sample. A dichroic mirror reflects laser light onto the sample while allowing light emitted by fluorescence to pass through to the CCD.

Calibration was done optically using an Air Force Microscope Target and an image capture program to verify the magnification. By measuring the pixels in a photograph of a known distance, and knowing the dimensions of the imager in the CCD, the magnification can be calculated.

fig:MicroscopeDiagram The fluorescence path (green), brightfield light source, and imaging path (red) of the microscope used to capture data from the microfluidic device with a CCD camera.

3.2 The microfluidic device

The microfluidic device is composed of the elastomeric material Polydimethylsiloxane (PDMS). The mask, a silicon wafer obtained from the MIT Environmental Microfluidics Group, was used in conjunction with UV-curing photopolymers to create a master mold for the PDMS devices. PDMS and its curing agent were mixed in a 10:1 ratio, respectively, and poured over the mold in a dish. The dish was degassed in a vacuum chamber and then cured for approximately 12 hours. After the PDMS had cured, the excess around the device was cut away, yielding the rectangular shape shown in

The device has three parallel channels formed by indentations in the PDMS; the glass slide creates the fourth wall of each channel, closing them. Each channel is 600 microns wide and 50 microns deep, with 200 microns of separation between each channel. Bacteria was injected into the center channel and allowed to settle before data collection began. Oxygen and nitrogen flows were directed down the outside channels. The devices were cleaned with isopropanol, which does not degrade the PDMS or the plasma bond, and reused for multiple experiments.

fig:DevicePhoto The microfluidic device. It is composed of PDMS, a silicone-like polymer, and features 3 channels parallel to each other. The center is wider and holds the bacteria, while the other two are thinner and flow gas through them. PDMS is gas-permeable, so the gas will diffuse into the center channel where the bacteria are located.

3.3 Gas flow

The essence of aerotaxis is the gas gradient to which bacteria respond. The gas gradient was achieved by routing oxygen and nitrogen through the outside microfluidic channels parallel to the bacteria chamber. Both gases originate in storage cylinders attached to primary regulators. These regulators drop the gas pressure from about 2000 psi to 25 psi. After exiting the primary regulator, each gas flows through 1/8" ID Tygon E-3603 tubing to a secondary regulator that drops the pressure further, providing 2 psi to the lines connected to the device.

These lines have two different configurations during the experiment, providing either N2 to one microfluidic gas channel and O2 to the other, or providing N2 to both microfluidic gas channels. These configurations are shown in

fig:GasFlow The gas flow path, from the cylinders to the microfluidic device, which ultimately exhausts to air. The gas lines switch, providing N2 to one microfluidic gas channel and either N2 or O2 to the other gas channel, depending on the experiment phase.

3.4 Bacteria culturing

4 Results

5 Discussion

References

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