Video Article

Chemotactic Response of Marine Micro-Organisms to Micro-Scale Nutrient Layers

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Abstract

The degree to which planktonic microbes can exploit microscale resource patches will have considerable implications for oceanic trophodynamics and biogeochemical flux. However, to take advantage of nutrient patches in the ocean, swimming microbes must overcome the influences of physical forces including molecular diffusion and turbulent shear, which will limit the availability of patches and the ability of bacteria to locate them. Until recently, methodological limitations have precluded direct examinations of microbial behaviour within patchy habitats and realistic small-scale flow conditions. Hence, much of our current knowledge regarding microbial behaviour in the ocean has been procured from theoretical predictions. To obtain new information on microbial foraging behaviour in the ocean we have applied soft lithographic fabrication techniques to develop 2 microfluidic devices, which we have used to create (i) microscale nutrient patches with dimensions and diffusive characteristics relevant to oceanic processes and (ii) microscale vortices, with shear rates corresponding to those expected in the ocean. These microfluidic devices have permitted a first direct examination of microbial swimming and chemotactic behaviour within a heterogeneous and dynamic seascape. The combined use of epifluorescence and phase contrast microscopy allow direct examinations of the physical dimensions and diffusive characteristics of nutrient patches, while observing the population-level aggregative response, in addition to the swimming behaviour of individual microbes. These experiments have revealed that some species of phytoplankton, heterotrophic bacteria and phagotrophic protists are adept at locating and exploiting diffusing microscale resource patches within very short time frames. We have also shown that up to moderate shear rates, marine bacteria are able to fight the flow and swim through their environment at their own accord. However, beyond a threshold high shear level, bacteria are aligned in the shear flow and are less capable of swimming without disturbance from the flow. Microfluidics represents a novel and inexpensive approach for studying aquatic microbial ecology, and due to its suitability for accurately creating realistic flow fields and substrate gradients at the microscale, is ideally applicable to examinations of microbial behaviour at the smallest scales of interaction. We therefore suggest that microfluidics represents a valuable tool for obtaining a better understanding of the ecology of microorganisms in the ocean.

Protocol

Preparation

1. Create a Mask

Using a CAD software, design the channel for high-resolution printing on a transparency. This will be the "mask".

In the clean room:

2. Clean and bake the wafer

First, squirt the wafer with Acetone, then quickly with Methanol, then with Isopropanol. Finally, dry the wafer using Nitrogen.

Bake the wafer in the oven (130°C) for 5 min.

3. Coating the wafer

Place the wafer at the center of the spin-coating machine. Pour photoresist (SU-8) from the bottle onto the wafer. Let the SU-8 flow and relax for ~ 10 s. Turn on the spin-coater and ramp its speed up from 0 to 500 rpm over 5 s; keep at 500 rpm for 10 s; ramp up to final speed over 10 s and maintain at final speed for 30 s. The final speed depends on the targeted coating thickness and the SU-8 used. The details can be found at http://www.microchem.com/

4. Soft-bake

After coating the wafer, bake it first at 65°C and then at 95°C. The baking time varies with targeted thickness and type of photoresist used. Then, let the wafer sit at room temperature for at least 5 min.

5. Exposure

Place the mask on top of the wafer and expose the wafer to UV light for the time recommended in the SU-8 manual.

6. Post-exposure bake

Bake the wafer at 65°C and then 95°C following the SU-8 manual's instructions.

7. Developing the wafer to obtain the "master" (mold)

Prepare a beaker filled with the developer (PMMA). Immerse the wafer into the beaker while very gently oscillating the beaker until the unexposed part of the photoresist is washed away.

In our lab:

8. Prepare PDMS and pour it onto the wafer

Mix the PDMS with its curing agent at 10:1 ratio into a cup. Stir and mix it homogenously: this will generate lots of bubbles and make the mixture look opaque. Pour the mixture on the "master".

9. De-bubble in vacuum chamber

To remove the bubbles, placed the master and PDMS mixture that is covering it into a vacuum chamber until all bubbles are gone.

10. Baking in oven

Bake for at least 12 hours in an oven at 65°C to harden the PDMS.

11. Punch holes

Peel off the PDMS from the master and punch holes for inlets and outlets of the channels.

In cleanroom (not shown)

12. Plasma bonding

Channels are bonded to a glass slide after treating both the PDMS layer and the glass slide with oxygen plasma for 1 min.

Experiments:

Exp #1: Investigating the chemotactic response of marine microbes to micro-scale nutrient layers

1) Setting up the experiment

1. Add organisms and substrates to glass syringes
2. Place microfluidic channel onto microscope stage and attach tubing to appropriate inlets and outlets
3. Connect tubing to waste reservoir. Make sure tubing is entirely submerged in the fluid in waste reservoir to avoid pressure oscillations
4. Place syringes onto syringe pump and connect to valves and tubing
5. Set up microscope: light conditions, magnification, etc.
6. Focus on appropriate position in the channel
7. De-bubble channel using larger syringe filled with artificial seawater
8. Set appropriate flow rate on syringe pump. In this case 2 ml/min, which corresponds to a mean flow velocity of 220 μm s^{-1} in the channel

2) Running the experiment

1. Start syringe pump to establish a nutrient gradient in the channel
2. Once flow has stabilized and a band of nutrients has developed, stop the flow on the syringe pump and begin recording time from this point
3. Nutrient band begins to diffuse laterally
4. At regular time intervals, use image analysis software to record sequences of frames to create 'movies'
5. Discriminate swimming organisms by taking time-difference images between two subsequent frames, so that only moving objects are now visualized, allowing us to differentiate motile cells from non-moving particles and background noise
6. Take movies to determine positions of cells in the channel with reference to the position of the nutrient patch
7. Record movies at regular intervals for 10-20 min to analyze the positions and swimming patterns of organisms
8. By using the image analysis software to superimpose the positions of organisms in different frames (assigning to each pixel the maximum light intensity recorded in that pixel over the duration of the movie), we can obtain trajectory information for swimming cells

Exp #2: Investigating the effects of shear on marine bacteria swimming in a vortexZ

1. Using different channel geometry, we can observe the behavior of bacteria swimming in a vortex at different shear rates

Discussion

An understanding of how marine microbes interact with their local chemical and physical environment is imperative for a more complete and precise perception of the role of planktonic microorganisms in the oceans nutrient and carbon cycles (Azam and Malfatti 2007). However, due to the small scales (< mm) over which many important microbial interactions take place, technical limitations have prevented detailed examinations of microbial behaviour within the heterogeneous bio-physico-chemical landscape predicted to be experienced by swimming microbes in the ocean. Recent advances in microfluidics (Whitesides et al. 2001) have enabled detailed analyses of microbial ecology within complex microhabitats (Mao et al. 2003, Park et al. 2003, Keymer et al. 2006, Marcos and Stocker 2006). The microfluidic devices described here allowed us to examine both the chemotactic response of marine microbes to a diffusing nutrient patch (Blackburn et al. 1997, 1998) and the swimming behaviour of microbes within turbulent shear, at a single cell level.

The soft lithographic fabrication process involved in making the microfluidic channels allows for intricate details to be created within the channel architecture, permitting the precise control of flows and gradients within the channels. Flexibility afforded by the fabrication technique allows for

channels of various dimensions to be created for comparative studies. The image analysis system applied here permits the visualization of individual cells and nutrient gradients, providing a platform for the detailed quantitative analysis of microbial swimming and chemotactic behaviour at both a single-cell and population level.

We have applied this microfluidic channel as a sensitive chemotaxis assay for a variety of swimming marine microbes and have found that many species are capable of rapidly responding to a diffusing patch of nutrients, forming dense aggregations of cells within the high nutrient concentrations inside the patch. During the chemotactic accumulation of cells within the nutrient patch, some species have also exhibited marked behavioural shifts, including changes in swimming speed and turning frequency. Our observations provide experimental support for the hypothesis that marine microbes can utilise short-lived nutrient patches in the ocean as important growth habitats.

Using different channel geometry, we are able to generate stable microvortices on scales relevant to microbial dynamics in the aquatic environment. This setup allows us to observe the behaviour of bacteria swimming in response to different shear rates. In contrast to the random swimming behaviour under quiescent flow condition, under the influence of a strong shear, bacteria both follow streamlines of the flow field and are aligned with them. This setup offers valuable insight on the fundamental interaction between microorganisms and their fluid dynamical environment.

In each of these experiments, microfluidics has proven to be an effective tool for studying microbial behaviour within dynamic microhabitats. With an increasing recognition of the importance of microbial dynamics within natural habitats, and novel applications of microfluidic technology, we suggest that the further coupling of microfluidics with microbial ecology will yield important new insights.

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