Video Article

Window on a Microworld: Simple Microfluidic Systems for Studying Microbial Transport in Porous Media

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Abstract

Microbial growth and transport in porous media have important implications for the quality of groundwater and surface water, the recycling of nutrients in the environment, as well as directly for the transmission of pathogens to drinking water supplies. Natural porous media is composed of an intricate physical topology, varied surface chemistries, dynamic gradients of nutrients and electron acceptors, and a patchy distribution of microbes. These features vary substantially over a length scale of microns, making the results of macro-scale investigations of microbial transport difficult to interpret, and the validation of mechanistic models challenging. Here we demonstrate how simple microfluidic devices can be used to visualize microbial interactions with micro-structured habitats, to identify key processes influencing the observed phenomena, and to systematically validate predictive models. Simple, easy-to-use flow cells were constructed out of the transparent, biocompatible and oxygen-permeable material poly(dimethyl siloxane). Standard methods of photolithography were used to make micro-structured masters, and replica molding was used to cast micro-structured flow cells from the masters. The physical design of the flow cell chamber is adaptable to the experimental requirements: microchannels can vary from simple linear connections to complex topologies with feature sizes as small as 2 μm. Our modular EcoChip flow cell array features dozens of identical chambers and flow control by a gravity-driven flow module. We demonstrate that through use of EcoChip devices, physical structures and pressure heads can be held constant or varied systematically while the influence of surface chemistry, fluid properties, or the characteristics of the microbial population is investigated. Through transport experiments using a non-pathogenic, green fluorescent protein-expressing *Vibrio* bacterial strain, we illustrate the importance of habitat structure, flow conditions, and inoculums size on fundamental transport phenomena, and with real-time particle-scale observations, demonstrate that microfluidics offer a compelling view of a hidden world.

Video Link

The video component of this article can be found at https://www.jove.com/video/1741/

Protocol

I. Microfluidic Device Fabrication

1. The first step in creating a microfluidic device is to draw a two dimensional layout of the device in a computer assisted drawing (CAD) program. We have used AutoCAD, but other drawing programs are also available, such as CleWin, or CorelDraw.
2. The next step is to fabricate a photolithographic mask. Depending on the device dimensions, required resolution and budget, these masks could be fabricated in chrome (highest resolution, high cost), created on photographic film, or even printed directly on an overhead transparency using a high resolution printer. Here, we have used Cr masks manufactured by Advance Reproductions Corp., North Andover, MA.
3. The next step is to transfer the pattern from the mask onto a photosensitive epoxy to create a raised-relief mold.
    1. First, a layer of negative SU8 photoresist is spun onto a silicone wafer to the desired thickness, and baked to volatilize excess solvent. Photoresist formulation and spin speeds control the thickness of the deposited layer.
    2. Then, the pattern is exposed to a pre-determined dose of UV light through the mask. A post exposure bake cross-links the light-exposed regions of the photoresist coating, rendering them insoluble.
    3. Next is the development step, where unexposed resist is removed with a chemical developer, leaving a positive raised-relief structure called a master.
    4. A third baking step called a hard bake step is optional to further fix the structures.
4. PDMS molding

1. We use the silicone elastomer poly(dimethyl siloxane) for replica molding of microfluidic devices [1]. First, the base material is mixed in a 10:1 weight ratio with the curing agent, poured over the mold in a shallow container such as a petri dish, and degassed under vacuum until all the visible bubbles are gone.
2. The master with the uncured PDMS is then placed in a carefully leveled oven for at least 4h at 65°C for cross-linking of the polymer to occur.
3. After PDMS is solidified, the molded section is cut out of the petri dish. Holes are punched through the top of the device to access the microfluidic spaces in the finished device.

5. Attachment to glass
    1. Clean PDMS is irreversibly bonded to clean glass by exposure to oxygen plasma. First, the molded and punched PDMS and a clean glass slide are placed bonding surface up into a plasma cleaner. We used a PDC-32G Harrick plasma cleaner.
    2. After the chamber is closed, the operator turns on a vacuum pump and then the radio frequency or rf source. Plasma will self ignite in the chamber, evidenced by a slightly purple glow in the chamber.
    3. After exposure to plasma for 30 seconds, the rf source and then the vacuum pump are turned off.
    4. Quickly, the glass slide and PDMS device are removed from the chamber and brought into direct contact (molded PDMS surface side down). This will form an irreversible bond, and will also make the surface properties hydrophilic.
    5. If desired, different surface chemistries can be achieved with PDMS through immobilization of proteins on the surface by adsorption or covalent bonding.
    6. The device can then be filled with fluid such as deionized water, growth media, or artificial groundwater by capillary forces or use of gentle pressure with a syringe.

II. Flow Quantification of Microfluidic Device by Gravimetric Analysis

1. To calibrate pressure-driven flow in the device, micro-structured flow cells were first preloaded with DI water.
2. A liquid containing reservoir, such as a plastic syringe or the flow module (shown in Figure 1) is connected to the upstream inlet well, and the height of fluid in the reservoir is maintained above the height at the downstream well.
3. Samples are collected at the waste well at regular intervals and weighed on an analytical balance.
4. The slope of the curve plotting total volume vs. total time gives the average volumetric flow rate (Figure 2). We have found reproducible, linear average velocities for a wide range of pressure heads and for different pressure-maintaining systems.

III. Flow Visualization, Velocity Mapping, and Hydrophilic / Hydrophobic Interaction

1. For model calibration, 3 µm fluorescent latex beads in both Carboxy YG and Plain YG surfaces were acquired from Polysciences, Inc. and were diluted in DI water to the concentration of 0.01 % solids.
2. Beads flowing through the habitats were visualized on a Zeiss Axiovert 25 fluorescence microscope equipped with a Mightex BCE-B013-U monochrome camera.
3. Individual frames were captured in rapid succession and assembled into movies with the ImageJ software package (Figure 3).

IV. Microfluidic Device (EcoChip) for Modeling Bacterial Growth and Transport

1. *Vibrio* sp. GFP Kan, a non-pathogenic green fluorescent protein-expressing organism was grown for 14 hours in Luria-Bertani medium to a concentration of approximately 10^9 cells/ml.
2. Bacteria in the growth medium were introduced into EcoChip devices and allowed to colonize the device and establish flocs and films overnight for 19 hours.
3. Then the growth media was removed from the wells and all of the habitats were flushed with artificial seawater (please see Wang et al. [2] for ASW recipe) so that different densities of bacteria were remaining in different habitats.
4. One habitat was kept sealed with no flow, while slow flow conditions were maintained in the 3 other habitats.
5. Fluorescent and white light pictures of the bacterial growth were taken every 15-20 hours for a period of several days (Figure 4).

V. Representative Results

The flow module provides simple means for regulating and distributing flows through multiple habitats. Gravimetric analysis proved to be an easy and straightforward way to determine the flow rates through the habitat structures, which depend on the hydraulic resistance of the habitats and the pressure differences between input and the output wells. For the bead flow experiments we have observed much larger bead accumulation at the device surfaces for the uncarboxylated beads (Figure 2). Additionally, larger diameter beads, 6 and 10 mm, were much more likely to become entrained in the smaller pore openings and begin to accumulate in the device. Faster flow rates reduced particle retention and entrainment.

During the bacteria growth experiments, the influence of flow conditions is clearly evident. Continuous shearing forces cause bacteria to aggregate together and form flocs, and not to be found as individual cells. Transport of large bacterial flocs is an important environmental process which is extremely difficult to study in a macroscopic system.

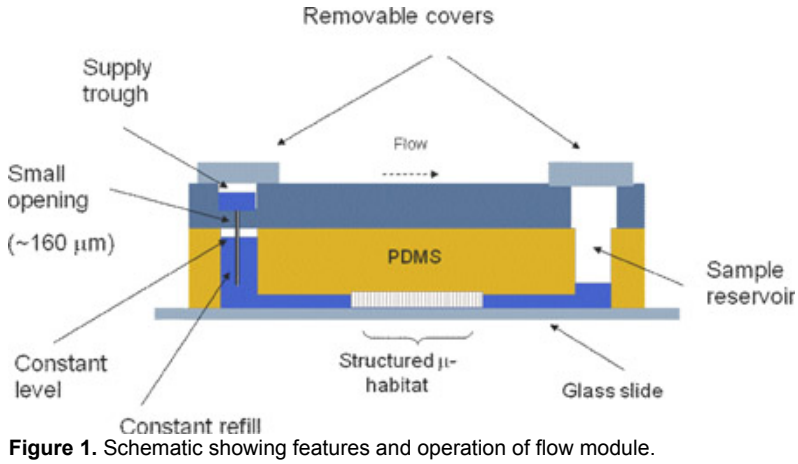

Figure 1. Schematic showing features and operation of flow module.

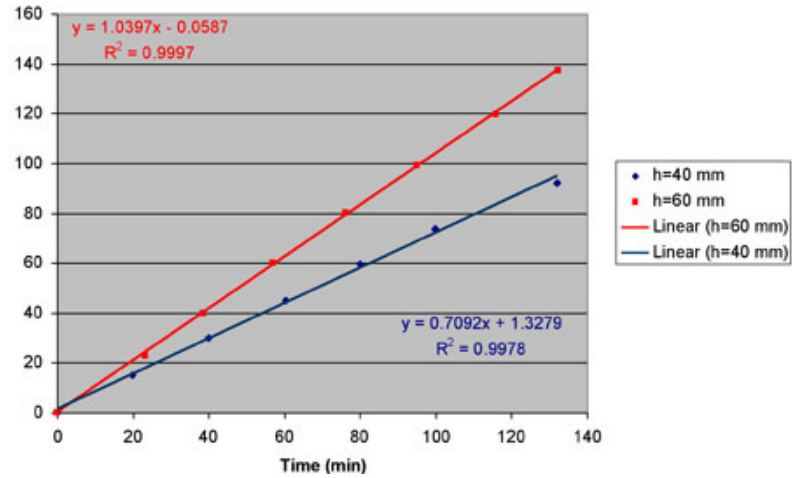

Figure 2. Flow rate through the structured habitat as determined by gravimetric analysis for different column heights. Column height ratio: 60 / 40 = 1.5, determined flow rate ratio: 1.04 / 0.71 = 1.46. Resulted flow rates for H = 40 mm is V = 0.71 μL/min and for H = 60 mm is V = 1.04 μ L/min. Estimated average linear velocities are 3.1 mm/s and 4.6 mm/s respectively.

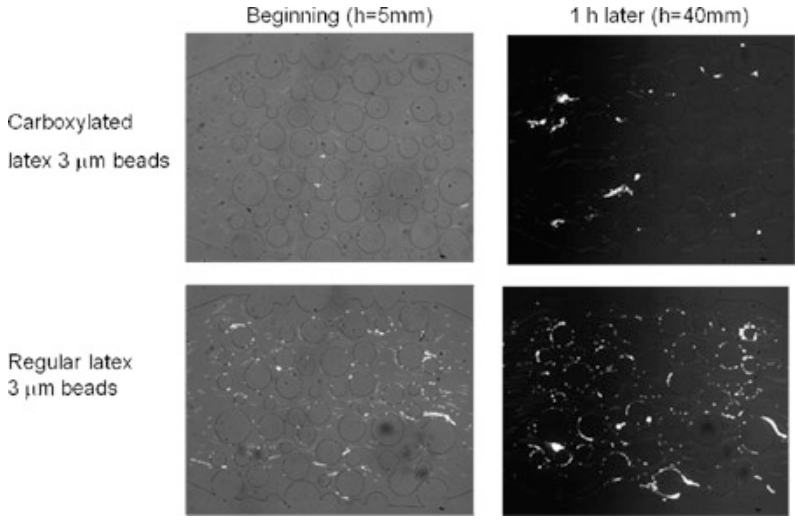

Figure 3. Transport of 3um latex beads with and without carboxylation flowing through the structured habitats.

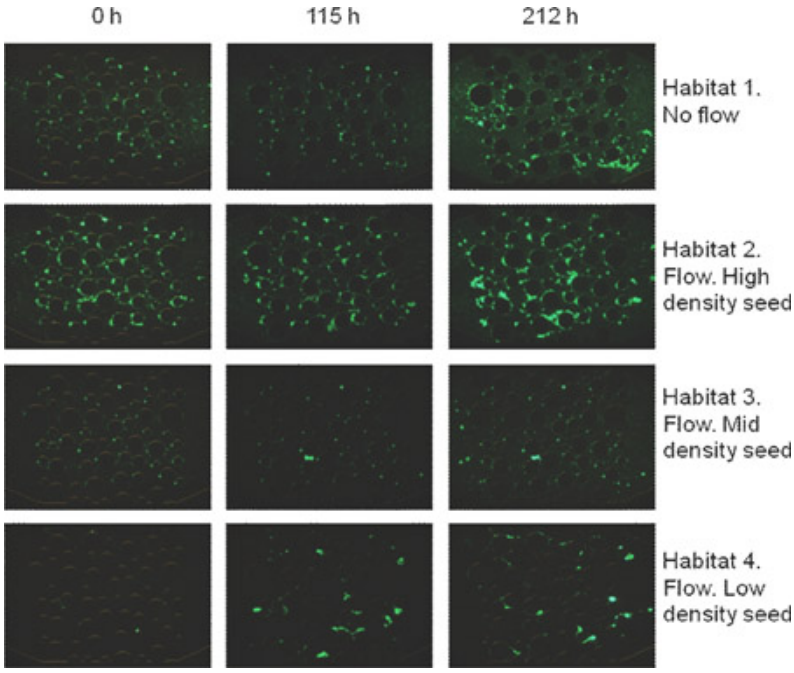

Figure 4. Change in bacterial growth and biofilm formation as a function of seed density in presence of a slow flow.

Discussion

The EcoChip system is adaptable to the needs of an individual experiment. New masters can be created relatively easily, and once a master is fabricated, additional exactly replicated devices can be cast as needed. The flow module is simple to use, requires no special equipment or complex connections, and can be modeled as a simple falling head pressure-driven flow system. Additional extensions to this work are ongoing, and include creating humic acid coated channels, and systematically varying the aqueous chemistry of the flowing fluid. Using this approach, the micro-scale interactions of bacteria with surfaces and growth and transport phenomena in porous media can be observed directly and systematically investigated.

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