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A novel planar flow cell for studies of biofilm heterogeneity and flow-biofilm interactions

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Abstract

Biofilms are microbial communities growing on surfaces, and are ubiquitous in nature, in bioreactors, and in human infection. Coupling between physical, chemical, and biological processes is known to regulate the development of biofilms; however, current experimental systems do not provide sufficient control of environmental conditions to enable detailed investigations of these complex interactions. We developed a novel planar flow cell that supports biofilm growth under complex two-dimensional fluid flow conditions. This device provides precise control of flow conditions and can be used to create well-defined physical and chemical gradients that significantly affect biofilm heterogeneity. Moreover, the top and bottom of the flow chamber are transparent, so biofilm growth and flow conditions are fully observable using non-invasive confocal microscopy and high-resolution video imaging. To demonstrate the capability of the device, we observed the growth of *Pseudomonas aeruginosa* biofilms under imposed flow gradients. We found a positive relationship between patterns of fluid velocity and biofilm biomass because of faster microbial growth under conditions of greater local nutrient influx, but this relationship eventually reversed because high hydrodynamic shear leads to the detachment of cells from the surface. These results reveal that flow gradients play a critical role in the development of biofilm communities. By providing new capability for observing biofilm growth, solute and particle transport, and net chemical transformations under user-specified environmental gradients, this new planar flow cell system has broad utility for studies of environmental biotechnology and basic biofilm microbiology, as well as applications in bioreactor design, environmental engineering, biogeochemistry, geomicrobiology, and biomedical research.

INTRODUCTION

It is now recognized that microbial communities on interfaces, generally termed biofilms, are extremely important in a wide variety of environmental, engineered, and biomedical systems. Biofilms are widely manipulated to consume nutrients (Pynaert et al. 2003), degrade hazardous organic compounds (Nicolella et al. 2005; Paulsen et al. 1999; Vayenas et al. 2002), and immobilize metals (Finlay et al. 1999). Also, biofilms are responsible for more than half of microbial infections of humans, and these infections are highly

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problematic because cells in biofilms are typically more than 500 times more resistant to antimicrobial therapy than planktonic cells (Costerton et al. 1995; Costerton et al. 1999; Kolter and Greenberg 2006), and as a result, biofilm-based infections tend to be persistent, particularly on implanted medical devices (Stewart and Costerton 2001; Wagner and Iglewski 2008). In addition, biofilms play a significant role in global biogeochemical cycling, energy flow, nutrient cycling, particle and solute transport, and weathering (Battin et al. 2003; Larsen and Greenway 2004; Ragusa et al. 2004; Sawicki and Brown 1998; Stott and Tanner 2005).

Biofilms interact with their environment in complex ways. Surrounding environmental conditions are key factors to the growth of biofilms in biotechnological systems, in the human body, and in nature. Prior studies have shown that the microbial growth is strongly influenced by the nutrient availability, physical transport, and biofilm structure (Boessmann et al. 2004; Kuehn et al. 2001; Venugopalan et al. 2005). For example, biofilms have been observed to develop different morphologies under different flow conditions (Besemer et al. 2007; Chang et al. 2003; Purevdorj et al. 2002; Teodosio et al. 2011), hydrodynamic shear forces significantly affect the formation of biofilms in wastewater treatment plants (Liu and Tay 2002), and internal transport limitations are among many factors that cause biofilms to be highly resistant to chemical stresses (Stewart 2002; Stewart and Franklin 2008). In general, distributions of flow, hydrodynamic shear on surfaces, and important chemical constituents are generally heterogeneous in both natural and engineered systems (De la Rosa and Yu 2005; Singer et al. 2010). All of these factors define habitat conditions for microbial growth in biofilms, and can potentially be modified by cellular metabolism and the development of biofilms (Battin et al. 2001; Kreft et al. 2001; Laspidou and Rittmann 2004; Rittmann 1982). Therefore it is important to understand interactions between the biofilm growth and spatial patterns of key environmental conditions.

A variety of bioreactors have been designed and used to study interactions between biofilms and their environment. Multi-well plates or petri dishes are used to grow biofilms under stagnant conditions (Blair et al. 2008; Ramage et al. 2001). Drip-flow reactors are designed to simulate flow conditions at the air-liquid interface (Goeres et al. 2009). Rotating disk reactors are used to grow biofilms under conditions of low hydrodynamic shear (Zelver et al. 1999). The rotating reactors developed by Donlan *et al.* are now one of the standard approaches to grow biofilms under high shear conditions (Donlan et al. 2004). While these types of reactors produce shear stress on growing biofilms and provide a reasonable degree of reproducibility because they allow users to establish repeatable experimental conditions, they do not allow flow heterogeneity to be imposed and therefore cannot be used to observe growth of biofilms relative to flow gradients or to explore feedbacks between biofilm growth, flow patterns, and chemical gradients. Further, the internal flow structure in these reactors is not understood in detail, making the distribution of environmental conditions over the growing biofilm ill-defined. This means that local interactions between biofilms and their surrounding environment will generally vary throughout the device, and that observations in different regions of the flow cell are not comparable. Flow-through systems provide better control of flow conditions because uniform flow can be achieved after a short inlet region (Bakker et al. 2003; Teodosio et al. 2011). In a flow-through configuration, biofilm growth has been observed under continuous unidirectional laminar and turbulent flow in both glass capillaries and various flow cells (Bakker et al. 2003; Davies et al. 1993; Davies et al. 1998; Singh et al. 2000; Sternberg and Tolker-Nielsen 2005; Teodosio et al. 2011). Round capillaries offer the advantage of a very well defined flow condition and ability to observe biofilm growth around the circumference of the capillary, but offer low surface area and are difficult to image. Square capillaries and rectangular flow cell channels offer improved imaging capability but at the expense of less well defined flow conditions. Large flow cell systems grow biofilm samples on coupons, which cannot be observed

directly *in situ* and must instead be removed from the system for analysis. Further, all of these flow-through systems are restricted to unidirectional flow, which can only represent a limited range of systems of interest – though this does include some extremely important applications such as catheters and the vascular system. Improved experimental capability is needed to assess more general interactions between biofilms and their surrounding environment. More specifically, it is desirable to have improved capability to observe the growth of biofilms under imposed environmental gradients, which are very common in nature, as well as to support biofilm growth on more extensive surfaces, where strong interactions between the growing biofilm and surrounding environmental conditions can produce heterogeneity over a wide range of scales.

Here we present a novel planar flow cell that supports biofilm growth under highly controlled two-dimensional (2D) patterns of environmental conditions while enabling macroscopic and microscopic optical measurements. This reactor allows observation of biofilm growth under user-specified environmental gradients, which are required for the investigation of important physical-chemical-biological interactions and the development of emergent properties such as spatial patterns of biofilm growth and net metabolic rates. We also provide a set of experimental results to illustrate how the system can be used to obtain insights into biofilm-environment interactions. Specifically, we show that patterns of *Pseudomonas aeruginosa* biofilm growth are strongly influenced by imposed gradients of nutrient influx and hydrodynamic shear.

MATERIALS AND METHODS

Flow cell fabrication

A schematic and photograph of the flow cell are presented in Figure 1. Two pieces of transparent acrylic with thickness of 2.0 mm were cut with a CO₂ laser (X2-660, Universal Laser Systems Inc.) to 100 mm × 100 mm in size. A square window of 35 mm × 35 mm was cut at the center of the top sheet. Two sheets of 0.8 mm-thick silicone rubber were laser-cut to the same geometry as the top acrylic piece. One sheet is used to form the frame of the flow chamber; while a second is used as a buffer to protect the coverslip. Silicone was selected because it is non-toxic and inert, and soft enough to be penetrated by needles to allow insertion of inflow and outflow ports at any desired locations. In the flow configuration used here, sixteen segments of stainless steel tubing (G25, I.D. = 0.24 mm, and O.D. = 0.50 mm) were then inserted into one of the rubber sheets to provide inlet and outlet ports, spaced evenly around four sides of the rubber sheet. A 45 mm × 50 mm glass microscope coverslip (Fisher Scientific) was sandwiched between the two rubber sheets and between the two pieces of acrylic to form the 2D flow chamber with final dimension of 35 mm × 35 mm × 0.6 mm. Twelve screws were positioned around the perimeter of the flow cell to seal the system by compressing the acrylic sheets, rubber gaskets, and glass coverslip. The coverslip allows direct observation of the biofilm growth by microscopy, while the transparent acrylic bottom allows other optical observations to be conducted, e.g., flow visualization by video microscopy.

UV system fabrication and tests

An in-line UV disinfection system is placed just upstream of the flow cell system to prevent upstream propagation of bacteria from flow cell to sterile growth medium, which typically limits the duration of conventional flow-cell experiments to just a few days. A schematic illustration of the UV disinfection chamber is provided in Figure 2A. The flow circuit for the entire experimental system, including flow cell and in-line UV disinfection, is shown in Figure 3. The UV light source is a Pen-Ray Lamp (11SC-1, UVP LLC) located in the center of the chamber. Influent growth medium is transmitted through the unit via a series of 12

glass capillary tubes (I.D. = 1.0 mm) that traverse the UV chamber. The inner surfaces of the chamber are covered with laser-cut mirrored acrylic to increase the disinfection efficiency by reflecting UV light within the chamber. An exhaust fan is incorporated to eliminate heat generated by the UV lamp. Multiple passes through the UV disinfection unit are used during flow cell operation to improve disinfection efficiency. The single-pass disinfection efficiency of the device was tested by pumping a suspension of planktonic *P. aeruginosa* PAO1 through the system. Flow rates were 0.40 ml/min, and initial *P. aeruginosa* concentrations were 10^3 , 10^4 , 10^5 and 10^6 CFU/ml in Tests 1–4, respectively.

Characterization of fluid flow and solute transport in the flow cell

Transport through the planar flow cell was both observed and simulated. The flow field was characterized by particle tracking velocimetry (PTV) (Devasenathipathy et al. 2003; Nokes 2007). Fluorescent polystyrene latex beads ($d = 5 \mu\text{m}$) were injected into the flow chamber and their paths were observed using a high resolution video camera (PCO 1600, Cooke Corporation) mounted on an epifluorescent microscope (Zeiss Axiophot, Carl Zeiss SMT Inc.). Time series images were captured at a magnification of $40\times$ under control of Camware (Cooke Corporation). PTV calculations were performed on ~ 100 particles in each region using the FluidStream software package v7.01 (Nokes 2007). The flow field was also visualized by observing the migration of dye (FD&C Blue, Sensient Technologies Corporation) through the flow chamber using the same camera system.

Patterns of fluid flow and solute transport in a flow cell were also simulated using the lattice Boltzmann Model (LBM) in order to characterize the distribution of hydrodynamic shear and nutrient influx (Chen and Doolen 1998; Succi 2001). The 2D flow cell domain ($35 \text{ mm} \times 35 \text{ mm}$) was divided into 280×280 lattice nodes, yielding a lattice spacing of 0.125 mm. This resolution was selected to adequately resolve each individual inlet, which has a diameter of 0.24 mm. A D2Q9 lattice model (9 velocity vectors in 2D) was used, with the LBM code taken as a 2D implementation of the 3D code of Chen *et al.* (Chen et al. 2008). These simulations were performed using a steady flow field with the Reynolds number selected to match the flow condition imposed at each influent port. The boundary shear stress was calculated from the velocity field as:

$$\tau_w = \frac{6\mu U}{h} \quad (\text{Eq. 1})$$

where μ is the viscosity of the fluid, $1.005 \text{ N}\cdot\text{s}/\text{m}^2$, U is the average local velocity, and h is the thickness of the chamber (Potter et al. 1997; Usami et al. 1993).

The performance of the LBM was confirmed by comparing against the PTV results. In the experiments performed here, biofilm growth did not significantly alter the flow field in the flow cell. Cases with significant feedbacks between biofilm growth and flow could also readily be observed in the flow cell, in which case the flow model presented here would need to be extended to include these feedback processes. This would likely require three-dimensional simulation of flow, which could readily be implemented using the LB method, e.g. Chen *et al.* (Chen et al. 2008), but such 3D models are computationally expensive and cannot be directly applied at the scale of the entire flow cell. Therefore, new strategies would have to be developed to up-scale local 3D flow-biofilm interactions to assess biofilm growth and detachment relative to the flow field throughout the flow cell.

Solute transport was also simulated to illustrate the propagation of dissolved materials through the flow cell. In these simulations, we used a representative diffusion coefficient ($1 \times 10^{-9} \text{ m}^2/\text{s}$) for the influent medium. The transport of specific solutes, such as nutrients, oxygen, or antibiotics can also be simulated by selection of the appropriate diffusion

coefficient. However, spatiotemporal patterns of substrates, nutrients, and other reactive constituents depend on uptake and other transformation processes as well as transport, so simulation of these constituents would require specific parameterization of microbial metabolism and other processes within the biofilm, as well as *in situ* or whole-flowcell observations to verify the fidelity of the simulations. These are important topics for future research that are uniquely suited to the planar flow cell device.

Preparation and inoculation of the test strain

Flow cell experiments were performed using *P. aeruginosa* PAO1-*gfp*, which has a chromosomally expressed Green Fluorescent Protein (GFP). A single colony was transferred into 5 ml of autoclaved Luria-Bertani broth (LB) which contained 10 g/L tryptone, 5 g/L yeast extract and 5 g/L sodium chloride, and grown overnight at 35°C. The resulting culture was diluted with 1% LB medium to OD₆₀₀ = 0.1, injected into the inverted flow cell, and allowed to sit quiescently for 1 hour to facilitate attachment of cells to the coverslip. Flow of 1% LB medium was then initiated and maintained at a constant rate of 0.16 ml/min (0.04 ml/min per inflow port) or 0.80 ml/min (0.20 ml/min per inflow port) using a Gilson Minipuls-3 Pump (Gilson, Inc.) under the right-angle flow condition shown in Figure 1C. Bacterial abundance was monitored in the flow cell effluent. Viable bacterial cell counts were measured repeatedly in each effluent line. For each sample, a 10-fold dilution series was plated on agar plates in triplicate. Plates were incubated at 35°C for 24 hours before colonies were counted.

Biofilm imaging and analysis

Biofilm growth was observed in the nine regions R1–R9 shown in Figure 4, forming a 10 mm × 10 mm observation window at the center of the flow cell. Three image stacks in each region were collected at 1–2 μm vertical resolution using a Leica Confocal Microscope (DM RXE-7) with 40× oil objective (HCX PL APO 1.25-.75NA CS). The planar images were collected using Leica Confocal Software (LCS), and three-dimensional images were generated from the image stacks using the 3D imaging software VOLOCITY (Improvision Inc.). Initial attachment of cells to the surface was confirmed by confocal microscopy performed after 30 minutes of flow. The biofilm growth was quantitatively analyzed using the COMSTAT image analysis software (Heydorn et al. 2000a; Heydorn et al. 2000b).

RESULTS

Flow and chemical gradients in the planar flow cell

Hydrodynamic and chemical gradients can be imposed on the flow cell by controlling the distribution of inflow and outflow through the 16 ports spaced around the four sides of the flow chamber. The flow configuration within the planar flow cell can be customized by changing the locations of the influent and effluent connections, and chemical gradients can be imposed independently, if desired, by using multiple sources of influent (e.g., growth medium). Examples with imposed heterogeneous flow patterns and uniform flow but imposed chemical gradients are illustrated in Figure 1. The right-angle flow shown in Figure 1C imposes a strong gradient in velocity between the bottom-right corner and the top-left corner of the flow cell. The 360°-turn flow shown in Figure 1D causes high velocities and high rates of solute flux near the inflow/outflow region, but very low velocities and solute fluxes occur on the opposite side of the growth chamber. In Figure 1E, we show a case where the flow is uniform across the flow cell, but a concentration gradient is produced perpendicular to the flow direction by introducing a different concentration of dye to each inflow. Flow patterns shown in Figure 1C and 1E were obtained under a flow rate of 0.8 ml/min, and similar flow patterns can be obtained at inflow rates ranging from 0.16 ml/min to 1.6 ml/min. The flow patterns shown in Figure 1D were obtained under a flow rate of 0.4

ml/min, and similar flow patterns can be achieved at inflow rates ranging from 0.08 ml/min to 0.4 ml/min.

We adopted the right-angle flow pattern shown in Figure 1C for further experimentation and simulation. LBM simulations of the right-angle flow configuration for an inflow rate of 0.80 ml/min are presented in Figure 4. The LBM simulations agreed very well with the PTV observations at the nine observation regions shown in Figure 4 (p-value < 0.001). The relative standard error was $15.4 \pm 3.0\%$ and all model simulations were within the standard error ranges of the PTV observations, as shown in Supplementary Figure 1. The streamlines, indicated as black lines in Figure 4A, generally show a smooth transition from the inlets to the outlets. Flow focusing around the inlet and outlet ports is restricted to a relatively thin region near the right and bottom sides of the flow cell, respectively. There are also recirculations with stagnant regions in the upper- and lower-right corners of the flow cell, while very slow flow occurs along the long flow path that proceeds along the upper and left sides of the flow cell. The desired smooth flow gradient occurs at the center of the flow cell. The flow distribution in the centermost 10 mm $\times$ 10 mm region is illustrated with an expanded velocity scale in Figure 4B. Dye transport through the flow cell for this flow configuration is shown in Supplementary Movie 1.

Patterns of fluid velocity, hydrodynamic shear, and solute transport for the right-angle flow case are illustrated in Figure 5A. Shear stress increases from the top-left to the bottom-right of the flow cell. Mass transfer, which controls solute distribution in the flow chamber, occurs via advection and diffusion processes. Advective transport follows the streamlines shown in Figure 4A, while diffusion is the mechanism of transport across streamlines. Further, because velocities are higher along the shorter flow paths, solute transport (oxygen, nutrients, *etc.*) decreases from the bottom-right to the top-left of the flow cell. Supplementary Movie 1 shows solute transport through the flow cell. There, it can be seen that advection is greater than diffusion throughout most of the flow chamber under a flow rate 0.80 ml/min, but diffusion dominates in the slow flow conditions at the corners and along the left side of the flow cell. These trends were verified by LBM simulations, as illustrated in Figure 5B. That image shows the transport through the bottom-right and central region of the flow cell is dominated by advection, and solute delivery to the left of the flow cell is much slower. The full LBM simulation of solute transport through the flow cell is provided in Supplementary Movie 2.

Efficacy of in-line UV disinfection

Observations of the single-pass performance of the UV disinfection system are presented in Figure 2B. Note that this challenge case is much more severe than the conditions under which we used the UV disinfection system in the flow cell setup (Fig. 3), because these tests involved downstream propagation of planktonic bacteria. We delivered growth medium to the flow cell in four influent lines. Thus each influent stream was subject to three passes through the UV system (via the internal capillary tubes) before discharge to the flow cell. A 2-log removal of bacteria (99% killing efficiency) was obtained during a single pass through the UV disinfection unit, indicating that up to 6-log removal of bacteria would occur with three passes per influent line.

In the configuration used in our flow cell setup, no upstream growth of *P. aeruginosa* was observed to occur through the disinfection unit in any of the continuous flow experiments. In the flow cell setup without the UV disinfection unit, $8.6 \pm 3.7 \times 10^8$ CFU/ml bacteria were observed to grow upstream in the inlet lines, ultimately migrating into the bubble traps and the reservoir of growth medium after five days.

Demonstration of biofilm growth under a flow gradient

We conducted experiments with *P. aeruginosa* biofilms under a simple flow gradient to demonstrate the application of this flow cell system in investigations of flow-biofilm interactions. We selected *P. aeruginosa* for this evaluation because it is ubiquitous in aquatic environments, an opportunistic pathogen that is one of the major causes of nosocomial infections, and also a model organism commonly used to study the growth, consequences, and eradication of bacterial biofilms (Emori and Gaynes 1993; Lyczak et al. 2000).

Single cells attached evenly on the cover glass in all of the nine observations regions, which indicated that initial attachment of cells was homogeneous (results not shown). Bacterial abundance in the flow cell effluent fluctuated between 1.5×10^4 CFU/ml and 4.3×10^6 CFU/ml for the first few days under the slow flow condition (0.16 ml/min), but stabilized at $2.9 \pm 0.3 \times 10^8$ CFU/ml after five days. Under the fast flow condition (0.80 ml/min), bacteria abundance in the effluent also fluctuated between 6.7×10^5 CFU/ml and 2.3×10^7 CFU/ml for the first three days and then stabilized at $1.2 \pm 0.2 \times 10^8$ CFU/ml.

Representative images of biofilm structure relative to the flow gradient after seven days under each of the two imposed flow rates are shown in Figure 6. The biofilm developed into dramatic patterns under both flow conditions, but patterns of biofilm growth were completely opposite relative to the velocity gradient under the slow- vs. fast-flow conditions. Under the slow flow condition, substantial growth occurred only in regions of relatively high fluid influx, corresponding to high inputs of oxygen, carbon, and nutrients. The biofilm developed a columnar morphology in regions of the flow cell with velocities over 0.58 mm/s (R6, R8 and R9 in Fig. 6A), while little growth occurred in regions with velocities below 0.49 mm/s (R1, R2 and R4 in Fig. 6A). Under the 5× higher inflow condition, providing 5× higher influx of oxygen and growth medium, abundant biofilm growth occurred in regions of the cell with relatively low velocities (R1–R5, R7 in Fig. 6B). However, there was little accumulation of biofilm biomass at local velocities above 1.6 mm/s (R6, R8 and R9 in Fig. 6B). Under these high velocities, only isolated, sporadic cells and discontinuous thin biofilms were found. No large cell clusters or mushroom structures were found under this condition.

The planar flow cell is useful not only to impose the flow gradients that led to the dramatic patterns of biofilm growth shown in Figure 6, but also to provide highly detailed observations of local transport conditions. We also evaluated the biofilm growth and biomass accumulation relative to the distribution of fluid flow. The relationship between biomass accumulation and local velocity is shown in Figure 7A. Faster local velocities cause greater delivery of carbon, nutrients, and oxygen to cells residing in the biofilm. Influx appeared to limit biofilm growth under slow inflow conditions, leading to substantial biofilm growth only in regions with the highest velocities. Local bulk concentrations of carbon, nutrients, and oxygen depend on depletion during transport from the flow cell influent ports to each region of the flow cell. To evaluate this effect, solute (growth medium) travel times from the inlets to each interior region of the flow cell were calculated based on the velocity field obtained by LBM simulations. The relationship between biomass accumulation and travel time is shown in Figure 7B. Considerably more biomass and thicker biofilms grew in regions with shorter travel time. Regions R6, R8, and R9 had high local velocities, low travel times, and substantial biofilm growth. In these regions, biomass accumulation increased with decreasing travel time, i.e., with increasing access to fresh growth medium. Conversely, when travel time exceeded 300 seconds, little biofilm developed because of depletion of carbon, nutrients or oxygen. These results agree with Duddu *et al.*'s model that growth preferentially occurs upstream under transport limitations (Duddu et al. 2009).

Completely different patterns of biomass accumulation occurred under the fast inflow condition. Under this condition, local velocities were greater than 0.9 mm/s in all

observation regions, and travel times were lower than 200 s in all regions. Therefore, based on the observations presented previously, transport conditions did not limit biofilm growth. Nonetheless, little biomass accumulation was observed in some regions, but these were the regions of high local velocity – completely opposite the trend observed in the slow inflow condition. Under these conditions, high hydrodynamic shear stress on the biofilm led to biomass loss due to detachment. Relationships between biomass, biofilm roughness, and boundary shear are presented in Figure 7C–D. The results in Figure 7C show that substantial biofilm detachment occurred at local shear greater than ~ 0.018 Pa, and that almost all cells were removed from the flow cell surface at >0.019 Pa. For the Poiseuille flow that occurs at the center of the flow cell, detachment is also directly proportional to the local mean velocity measured by PTV and simulated using the LBM model. Further, the observations of spatial patterns of biofilm roughness presented in Figure 7D indicate that detachment also strongly influenced the biofilm structure. Under moderately high local velocity and shear, detachment led to the formation of smooth biofilms (low roughness). Under local velocity and shear of $U > 1.6$ mm/s and $\tau_0 > 0.018$ Pa, the detachment rate became sufficiently high to remove most of the biofilm from the surface, leaving only small amounts of biomass in the form of isolated clusters with high roughness (compare quantitative results presented in Figure 7A and 7D with biofilm images R6, R8, and R9 in Figure 6).

DISCUSSION

We designed a planar flow cell that enables detailed observation of biofilm growth under well controlled physical and chemical gradients.

The new flow cell offers several advantages over prior designs. First, it allows imposition of complex flow patterns over the surface where biofilm growth occurs. Many other biofilm culturing systems present unusual flow conditions not normally found in nature, such as the rotating flows generated in stirred and rotating reactors. Conventional flow cells provide only unidirectional flow, whereas 2D flow patterns are easily obtained in the planar flow cell. It is also highly advantageous that the flow field in the planar flow cell is very well defined. Here, the velocity distribution between the parallel plates follows Poiseuille's law, and the 2D velocity field across the flow cell can be readily analyzed as Hele-Shaw flow (Potter et al. 1997; Todd 1980; Usami et al. 1993). The Hele-Shaw model has been widely used to study 2D potential flow fields (Todd 1980). Theoretically this requires Stokes flow conditions, with Reynolds number (Re) < 1 , but it has been shown to be valid in practice for $Re \approx 10$. Here, we kept $Re = 4.68$ under slow flow conditions and $Re = 23.4$ under fast flow conditions which is within the range of applicability for potential flow theory. Similar laminar flows are commonly found in blood vessels, catheters, small pipes in engineered water systems, shallow overland flow, groundwater aquifers and a variety of other porous media (Sutera and Skalak 1993). This high degree of confidence in the determination of the flow distribution not only facilitates interpretation and generalization of the results, but also provides a high degree of confidence when configuring the flow cell to achieve desired flow patterns. In conventional reactors, the user can only control the general magnitude of the flow, and this greatly restricts the range of factors affecting the growth of biofilms that can be investigated. In addition, inlet effects of the planar flow cell are localized, leading to smooth gradients across the center of the flow cell. These gradients allow simultaneous observation of biofilm growth under a wide range of local conditions (Figure 1). These broad lateral gradients also occur at a substantially larger scale than the lateral velocity profile in traditional unidirectional flow cells. Transverse velocity distributions also cannot be controlled in conventional flow cell designs. Moreover, in systems with one inflow, many individual experiments must be performed to observe biofilm growth under different flow rates, shear stresses, or chemical concentrations (Busscher and van der Mei 2006), but the

planar flow cell provides a spectrum of local environmental conditions in a single experiment.

The flow cell was also carefully designed so that optical measurements can be made through both sides of the device. Biofilms in rotating and stirred reactors are grown on coupons that need to be removed before measurements can be made, and conventional unidirectional flow cells can be observed only from one side. In the planar flow cell, multiple, repeated *in situ* optical observations can be performed without disturbing either the biofilm structure or the fluid flow. As an example, here an upright confocal laser scanning microscope was used to directly analyze biofilm structure (Fig. 6). A high-resolution video camera mounted on an optical microscope was used to observe the transport of injected dissolved tracers and micro-particles in order to characterize the flow field at different scales: through the entire flow cell (macro-scale) and around biofilm cell clusters (micro-scale). As a result, the device can be used for observing interactions of macro-scale environmental gradients and micro-scale biological processes. The same methods can also be used to directly visualize the transport and interactions of many important solutes in biofilms, such as redox sensitive dyes and antibiotics functionalized with fluorophores (Duplessis et al. 2004; Haggerty et al. 2009; Lin et al. 2008).

In addition, we designed an in-line UV disinfection system that effectively prevents the back-growth of bacteria in the inflow tubing, enabling long-term experiments on interactions between biofilm growth and environmental heterogeneity. Compared with other methods for preventing growth of bacteria out of flow cells, such as flow breakers and filters, the UV system provides more precise flow control because it is non-invasive and maintains a fully pressurized system.

An exemplary set of results was presented to illustrate how fluid flow conditions affect biofilm growth both by delivering nutrients, carbon, and oxygen to growing cells, and by imposing physical stresses on the biofilm structure. The development of a *P. aeruginosa* biofilm was observed under a flow condition that provides a consistent environmental gradient in growth medium influx and hydrodynamic shear. A positive relationship was observed between velocity and biomass accumulation because higher nutrient influx supported faster microbial growth, but this relationship eventually reversed because high hydrodynamic shear led to detachment of cells from the surface. This not only shows the utility of the device for culturing biofilms under physical and chemical gradients, but also dramatic heterogeneity in biofilm development under laminar flow gradients. Transport-associated nutrient limitations restricted growth under slow-flow conditions, but the nutritional environment no longer limited biofilm development under high-flow conditions, and instead the mechanics of the flow-biofilm interaction, the adhesive properties of cells, and the structural properties of the biofilm controlled the overall pattern of biofilm development under high-flow conditions. For *P. aeruginosa* biofilms grown under 1% LB medium, we found that cell detachment substantially hindered biofilm development at a boundary shear of ~ 0.018 Pa and essentially completely removed cell clusters from the surface at boundary shear > 0.019 Pa. However, observed biofilm thicknesses were less than 5% of the gap spacing of the flow chamber, indicating that there were not strong feedbacks between biofilm growth and the flow field for the conditions we tested. Feedbacks with biofilm growth should be considered at the scale of the biofilm roughness, where biofilm structures generate 3D secondary flows. The device can be used to observe flow-biofilm interactions in 3D, if a suitable method is implemented to observe the 3D flow field, e.g. using fast 3D confocal laser scanning microscopy (Lima et al. 2006), holographic 3D video imaging (Katz and Sheng 2010), or magnetic resonant microscopy (Hornemann et al. 2009). In addition, further advances in biofilm models are needed to simulate flow-biofilm interactions in 3D (von der Schulenburg et al. 2009). The planar flowcell device presented

here provides an excellent experimental platform for generating results on local-scale flow-biofilm interactions and feedbacks on the redistribution of flow at the centimeter-scale.

The flexible construction of the planar flow cell allows it to be used in a wide variety of configurations. The ability to position inflow and outflow ports as desired allows many different flow patterns to be obtained. Several example flow configurations can be seen in Figure 1, and more complicated 2D flow patterns can be achieved by careful selection of inflow and outflow conditions. Chemical gradients can be controlled by introducing solutions with different chemical concentrations in each inflow, as illustrated in Figure 1E. The ability to combine imposed chemical gradients with flow gradients allows establishment of a much wider array of conditions than conventional unidirectional flows cells, wherein concentration gradients develop passively due to solute consumption or accumulation. The capability of customizability of this device makes it in particular appropriate for evaluating new techniques for controlling biofilm growth in biomedical or industrial applications. The device can be used with a variety of cover slips, making it suitable for evaluating the efficacy of anti-microbial/anti-fouling coatings. In addition, the bottom acrylic sheet can be replaced with another coverslip to support detailed 3D observations of biofilm growth on both the top and bottom surfaces. Further, the base of the flow cell can also readily be constructed of various materials, such as biomaterials, plastic, or various metals to observe biofouling of these materials or evaluate their anti-biofouling properties. This capability is particularly important for materials designed for biomedical use in implanted or inserted devices, e.g., long-term orthopedic, neurological, cardiac or vascular implants, and short- or long-term urinary or vascular catheters (Campoccia et al. 2006; Etienne et al. 2005; Jones et al. 2005). Finally, turbulent flow fields could be created by increasing the thickness of the flow chamber. This would expand the range of application, but would require more advanced 3D simulations of the turbulent flow field and flow-biofilms interactions.

The novel planar flow cell can be used to investigate a wide range of processes involving the interaction of microbial growth and environmental conditions. It can be used to study the growth of individual cells (e.g., conditions that influence cellular metabolism and chemotaxis of cells) and the emergent properties of the entire biofilm community (e.g., morphology associated with both growth and detachment of many individual cells). Moreover, this flow cell system is uniquely capable of supporting investigations of biofilm growth under environmental gradients, such as gradients in the nutritional environment involving the mixing of influents with different chemical composition, patterns of killing associated with delivery of biocides, and the role of cellular dispersal (detachment/transmission/deposition) in the ecology of complex microbial communities. Particle transport can also be readily observed in the flow cell, as illustrated through the use of particle-tracking velocimetry. Particle deposition and re-suspension in the biofilm are highly relevant to waterborne disease transmission, carbon dynamics, and many other environmental processes. Finally, the device can be used to explore the manipulation of microbial growth for engineering purposes, e.g. in bioenergy production and biochemical reactors, as well as to the prevention of undesirable microbial growth in engineered water systems, e.g., in hospitals and other facilities. Many of these applications place a premium on understanding interactions between microbial growth and local environmental conditions. The innovative flow cell and combined experimental and modeling work described here provide great promise for exploring interactions between material properties, cellular adhesion and growth, biofilm heterogeneity, and the surrounding aqueous environment.

CONCLUSIONS

We developed a planar flow cell that support biofilms growth under well defined, user-selected 2D flow conditions. The significance of the present device is the development and

construction of a 2D flow chamber, in which planar flow gradients can be created, controlled, and directly observed. A demonstration of *P. aeruginosa* growth under imposed flow gradients revealed the important effects of environmental heterogeneity on biofilm development. This novel system provides unique capability for studying the influence of a wide range of flow conditions on biofilms in one experiment, and allows investigation of large-scale interactions between heterogeneities of biofilms and their external conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Bakker DP, van der Mats A, Verkerke GJ, Busscher HJ, van der Mei HC. Comparison of velocity profiles for different flow chamber designs used in studies of microbial adhesion to surfaces. *Applied and Environmental Microbiology*. 2003; 69(10):6280–6287. [PubMed: 14532092]
- Battin TJ, Kaplan LA, Newbold JD, Hansen CME. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*. 2003; 426(6965):439–442. [PubMed: 14647381]
- Battin TJ, Wille A, Sattler B, Psenner R. Phylogenetic and functional heterogeneity of sediment biofilms along environmental gradients in a glacial stream. *Applied and Environmental Microbiology*. 2001; 67(2):799–807. [PubMed: 11157246]
- Besemer K, Singer G, Limberger R, Chlup AK, Hochedlinger G, Hodl I, Baranyi C, Battin TJ. Biophysical controls on community succession in stream biofilms. *Applied and Environmental Microbiology*. 2007; 73(15):4966–4974. [PubMed: 17557861]
- Blair KM, Turner L, Winkelman JT, Berg HC, Kearns DB. A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. *Science*. 2008; 320(5883):1636–1638. [PubMed: 18566286]
- Boessmann M, Neu TR, Horn H, Hempel DC. Growth, structure and oxygen penetration in particle supported autotrophic biofilms. *Water Science and Technology*. 2004; 49(11–12):371–377. [PubMed: 15303764]
- Busscher HJ, van der Mei HC. Microbial adhesion in flow displacement systems. *Clinical Microbiology Reviews*. 2006; 19(1):127–+. [PubMed: 16418527]
- Campoccia D, Montanaro L, Arciola CR. The significance of infection related to orthopedic devices and issues of antibiotic resistance. *Biomaterials*. 2006; 27(11):2331–2339. [PubMed: 16364434]
- Chang I, Gilbert ES, Eliashberg N, Keasling JD. A three-dimensional, stochastic simulation of biofilm growth and transport-related factors that affect structure. *Microbiology-Sgm*. 2003; 149:2859–2871.
- Chen C, Packman AI, Gaillard JF. Pore-scale analysis of permeability reduction resulting from colloid deposition. *Geophysical Research Letters*. 2008; 35(7)
- Chen S, Doolen GD. Lattice Boltzmann method for fluid flows. *Annual Review of Fluid Mechanics*. 1998; 30:329–364.
- Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappinscott HM. Microbial Biofilms. *Annual Review of Microbiology*. 1995; 49:711–745.
- Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. *Science*. 1999; 284(5418):1318–1322. [PubMed: 10334980]
- Davies DG, Chakrabarty AM, Geesey GG. Exopolysaccharide Production in Biofilms - Substratum Activation of Alginate Gene-Expression by *Pseudomonas-Aeruginosa*. *Applied and Environmental Microbiology*. 1993; 59(4):1181–1186. [PubMed: 8476292]

- Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*. 1998; 280(5361):295–298. [PubMed: 9535661]
- De la Rosa C, Yu T. Three-dimensional mapping of oxygen distribution in wastewater biofilms using an automation system and microelectrodes. *Environmental Science & Technology*. 2005; 39(14): 5196–5202. [PubMed: 16082947]
- Devasenathipathy S, Santiago JG, Wereley ST, Meinhart CD, Takehara K. Particle imaging techniques for microfabricated fluidic systems. *Experiments in Fluids*. 2003; 34(4):504–514.
- Donlan RM, Piede JA, Heyes CD, Sanii L, Murga R, Edmonds P, El-Sayed I, El-Sayed MA. Model system for growing and quantifying *Streptococcus pneumoniae* biofilms in situ and in real time. *Applied and Environmental Microbiology*. 2004; 70(8):4980–4988. [PubMed: 15294838]
- Duddu R, Chopp DL, Moran B. A Two-Dimensional Continuum Model of Biofilm Growth Incorporating Fluid Flow and Shear Stress Based Detachment. *Biotechnology and Bioengineering*. 2009; 103(1):92–104. [PubMed: 19213021]
- Duplessis MR, Ziebis W, Gros O, Caro A, Robidart J, Felbeck H. Respiration strategies utilized by the gill endosymbiont from the host lucinid *Codakia orbicularis* (Bivalvia : Lucinidae). *Applied and Environmental Microbiology*. 2004; 70(7):4144–4150. [PubMed: 15240294]
- Emori TG, Gaynes RP. An Overview of Nosocomial Infections, Including the Role of the Microbiology Laboratory. *Clinical Microbiology Reviews*. 1993; 6(4):428–442. [PubMed: 8269394]
- Etienne O, Gasnier C, Taddei C, Voegel JC, Aunis D, Schaaf P, Metz-Boutigue MH, Bolcato-Bellemin AL, Egles C. Antifungal coating by biofunctionalized polyelectrolyte multilayered films. *Biomaterials*. 2005; 26(33):6704–6712. [PubMed: 15992921]
- Finlay JA, Allan VJM, Conner A, Callow ME, Basnakova G, Macaskie LE. Phosphate release and heavy metal accumulation by biofilm-immobilized and chemically-coupled cells of a *Citrobacter* sp. pre-grown in continuous culture. *Biotechnology and Bioengineering*. 1999; 63(1):87–97. [PubMed: 10099584]
- Goeres DM, Hamilton MA, Beck NA, Buckingham-Meyer K, Hilyard JD, Loetterle LR, Lorenz LA, Walker DK, Stewart PS. A method for growing a biofilm under low shear at the air-liquid interface using the drip flow biofilm reactor. *Nature Protocols*. 2009; 4(5):783–788.
- Haggerty R, Marti E, Argerich A, von Schiller D, Grimm NB. Resazurin as a “smart” tracer for quantifying metabolically active transient storage in stream ecosystems. *Journal of Geophysical Research-Biogeosciences*. 2009; 114
- Heydorn A, Ersboll BK, Hentzer M, Parsek MR, Givskov M, Molin S. Experimental reproducibility in flow-chamber biofilms. *Microbiology-Uk*. 2000a; 146:2409–2415.
- Heydorn A, Nielsen AT, Hentzer M, Sternberg C, Givskov M, Ersboll BK, Molin S. Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology-Uk*. 2000b; 146:2395–2407.
- Hornemann JA, Codd SL, Fell RJ, Stewart PS, Seymour JD. Secondary Flow Mixing Due to Biofilm Growth in Capillaries of Varying Dimensions. *Biotechnology and Bioengineering*. 2009; 103(2): 353–360. [PubMed: 19191352]
- Jones DS, Djokic J, Gorman SP. The resistance of polyvinylpyrrolidone-Iodine-poly(epsilon-caprolactone) blends to adherence of *Escherichia coli*. *Biomaterials*. 2005; 26(14):2013–2020. [PubMed: 15576175]
- Katz J, Sheng J. Applications of Holography in Fluid Mechanics and Particle Dynamics. *Annual Review of Fluid Mechanics*. 2010; 42:531–555.
- Kolter R, Greenberg EP. Microbial sciences - The superficial life of microbes. *Nature*. 2006; 441(7091):300–302. [PubMed: 16710410]
- Kreft JU, Picioreanu C, Wimpenny JWT, van Loosdrecht MCM. Individual-based modelling of biofilms. *Microbiology-Sgm*. 2001; 147:2897–2912.
- Kuehn M, Mehl M, Hausner M, Bungartz HJ, Wuertz S. Time-resolved study of biofilm architecture and transport processes using experimental and simulation techniques: the role of EPS. *Water Science and Technology*. 2001; 43(6):143–150. [PubMed: 11381960]

- Larsen E, Greenway M. Quantification of biofilms in a sub-surface flow wetland and their role in nutrient removal. *Water Science and Technology*. 2004; 49(11–12):115–122. [PubMed: 15303731]
- Laspidou CS, Rittmann BE. Evaluating trends in biofilm density using the UMCCA model. *Water Research*. 2004; 38(14–15):3362–3372. [PubMed: 15276753]
- Lima R, Wada S, Tsubota K, Yamaguchi T. Confocal micro-PIV measurements of three-dimensional profiles of cell suspension flow in a square microchannel. *Measurement Science & Technology*. 2006; 17(4):797–808.
- Lin YF, Wang YC, Chang SY. Capillary electrophoresis of aminoglycosides with argon-ion laser-induced fluorescence detection. *Journal of Chromatography A*. 2008; 1188(2):331–333. [PubMed: 18343388]
- Liu Y, Tay JH. The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. *Water Research*. 2002; 36(7):1653–1665. [PubMed: 12044065]
- Lyczak JB, Cannon CL, Pier GB. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes and Infection*. 2000; 2(9):1051–1060. [PubMed: 10967285]
- Nicolella C, Zoezzi M, Rabino M, Furfaro M, Rovatti M. Development of particle-based biofilms for degradation of xenobiotic organic compounds. *Water Research*. 2005; 39(12):2495–2504. [PubMed: 15978651]
- Nokes, R. FluidStream 7.0 – System Theory and Design: Department of Civil Engineering. University of Canterbury; 2007.
- Paulsen JE, Ekrann S, Oppen E. Visualisation of bacterial degradation and mobilisation of oil in a porous medium. *Environmental Geology*. 1999; 38(3):204–208.
- Potter, MC.; Wiggert, DC.; Hondzo, M. *Mechanics of fluids*. Prentice Hall; Upper Saddle River, NJ: 1997. p. xvp. 752
- Purevdorj B, Costerton JW, Stoodley P. Influence of hydrodynamics and cell signaling on the structure and behavior of *Pseudomonas aeruginosa* biofilms. *Applied and Environmental Microbiology*. 2002; 68(9):4457–4464. [PubMed: 12200300]
- Pynaert K, Smets BF, Wyffels S, Beheydt D, Siciliano SD, Verstraete W. Characterization of an autotrophic nitrogen-removing biofilm from a highly loaded lab-scale rotating biological contactor. *Applied and Environmental Microbiology*. 2003; 69(6):3626–3635. [PubMed: 12788771]
- Ragusa SR, McNevin D, Qasem S, Mitchell C. Indicators of biofilm development and activity in constructed wetlands microcosms. *Water Research*. 2004; 38(12):2865–2873. [PubMed: 15223281]
- Ramage G, vande Walle K, Wickes BL, Lopez-Ribot JL. Standardized method for in vitro antifungal susceptibility testing of *Candida albicans* biofilms. *Antimicrobial Agents and Chemotherapy*. 2001; 45(9):2475–2479. [PubMed: 11502517]
- Rittmann BE. The Effect of Shear-Stress on Biofilm Loss Rate. *Biotechnology and Bioengineering*. 1982; 24(2):501–506. [PubMed: 18546318]
- Sawicki JA, Brown DA. Investigation of microbial-mineral interactions by Mossbauer spectroscopy. *Hyperfine Interactions*. 1998; 117(1–4):371–382.
- Singer G, Besemer K, Schmitt-Kopplin P, Hodl I, Battin TJ. Physical Heterogeneity Increases Biofilm Resource Use and Its Molecular Diversity in Stream Mesocosms. *Plos One*. 2010; 5(3)
- Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature*. 2000; 407(6805):762–764. [PubMed: 11048725]
- Sternberg C, Tolker-Nielsen T. Growing and Analyzing Biofilms in Flow Cells. *Current Protocols in Microbiology*. 2005; 1B(2):2.1–2.15.
- Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. *International Journal of Medical Microbiology*. 2002; 292(2):107–113. [PubMed: 12195733]
- Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet*. 2001; 358(9276):135–138. [PubMed: 11463434]
- Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. *Nature Reviews Microbiology*. 2008; 6(3):199–210.

- Stott R, Tanner CC. Influence of biofilm on removal of surrogate faecal microbes in a constructed wetland and maturation pond. *Water Science and Technology*. 2005; 51(9):315–322. [PubMed: 16042273]
- Succi, S. The lattice Boltzmann equation for fluid dynamics and beyond: Oxford science publications. 2001.
- Sutera SP, Skalak R. The History of Poiseuille Law. *Annual Review of Fluid Mechanics*. 1993; 25:1–19.
- Teodosio JS, Simoes M, Melo LF, Mergulhao FJ. Flow cell hydrodynamics and their effects on E-coli biofilm formation under different nutrient conditions and turbulent flow. *Biofouling*. 2011; 27(1): 1–11. [PubMed: 21082456]
- Todd, DK. Groundwater hydrology. Wiley; New York: 1980. p. xiiip. 535
- Usami S, Chen HH, Zhao YH, Chien S, Skalak R. Design and Construction of a Linear Shear-Stress Flow Chamber. *Annals of Biomedical Engineering*. 1993; 21(1):77–83. [PubMed: 8434823]
- Vayenas DV, Michalopoulou E, Constantinides GN, Pavlou S, Payatakes AC. Visualization experiments of biodegradation in porous media and calculation of the biodegradation rate. *Advances in Water Resources*. 2002; 25(2):203–219.
- Venugopalan VP, Kuehn A, Hausner M, Springael D, Wilderer PA, Wuerz S. Architecture of a nascent *Sphingomonas* sp biofilm under varied hydrodynamic conditions. *Applied and Environmental Microbiology*. 2005; 71(5):2677–2686. [PubMed: 15870359]
- von der Schulenburg DAG, Pintelon TRR, Picioreanu C, Van Loosdrecht MCM, Johns ML. Three-Dimensional Simulations of Biofilm Growth in Porous Media. *Aiche Journal*. 2009; 55(2):494–504.
- Wagner VE, Iglewski BH. *P. aeruginosa* Biofilms in CF Infection. *Clinical Reviews in Allergy & Immunology*. 2008; 35(3):124–134. [PubMed: 18509765]
- Zelver N, Hamilton M, Pitts B, Goeres D, Walker D, Sturman P, Heersink J. Measuring antimicrobial effects on biofilm bacteria: From laboratory to field. *Biofilms*. 1999; 310:608–628.

Figure 1.

(A) Construction of the planar flow cell. (a) A 100 mm × 100 mm transparent acrylic piece with a thickness of 2 mm is used as the base support. (b) A silicone rubber sheet with a square hole (35 mm × 35 mm) is used to form the chamber of flow cell. The thickness of this sheet can be varied to control the thickness of the flow chamber. (c) Stainless steel tubing with I.D. = 0.24 mm and O.D. = 0.50 mm is inserted into the silicone rubber sheet to provide connections for flow inlets and outlets. The locations of the inlets and outlets can be varied as desired to achieve different flow patterns. (d) A 45 mm × 50 mm glass microscope coverslip is placed over the chamber to enable observation by microscopy. (e) A rubber mask is placed on top of the coverslip to protect the glass, e.g., during compression when the flow cell is assembled. (f) Another acrylic piece with the same dimensions as the base support, but with the area for the flow chamber cut out, is placed on top in order to provide structural strength during assembly (compression). (g) Twelve screws around the perimeter of the flow cell are used to seal the system (which must be water-tight to prevent leaks and maintain sterility during long-term operation). (B) Side-view photograph of the assembled flow cell. (C, D, E) Typical 2D flow patterns demonstrated by dye injection: (C) a right-angle (90°-turn) flow under an inflow rate of 0.8 ml/min, (D) a 360°-turn flow under an inflow rate of 0.4 ml/min, and (E) concentration gradients formed with straight flow under an inflow rate of 0.8 ml/min. These dye injections were performed in flow cells without biofilms, and so only illustrate the types of flow conditions that can be obtained in the flow cell. There was no sorption or other long-term retention of dye in the flow cell.

Figure 2.

Ultraviolet disinfection system for non-invasive in-line disinfection: (A) Schematic illustration showing the orientation of capillary tubes (blue lines) relative to the UV source. Influent tubing can be directly connected to each capillary tube, or the flow can be redirected through multiple capillaries before being discharged to the flow cell. (B) Observed disinfection efficiency of the in-line UV system for four initial concentrations of *P. aeruginosa* PAO1 under a through-flow rate of 0.4 ml/min. Red columns were controls without UV disinfection and blue columns were the bacterial abundance after single passage of the UV chamber. The killing efficiency is also presented (□). Error bars indicate the standard deviation for triplicate experiments.

Figure 3. Schematic of the flow cell system. Fresh medium or chemicals are pumped through bubble traps, the UV disinfection system and the flow cell, finally go into a waste bottle. Dashed connections can be customized by users to form different flow patterns according to their requirements.

Figure 4.

LBM simulation of a right-angle flow under an inflow rate of 0.80 ml/min. (A) Flow field and streamlines at the scale of the entire flow chamber. (B) Flow field in the inner region where biofilm growth was characterized. The nine inset squares R1–R9 indicate locations where confocal micrographs were obtained. Coordinates are lattice locations normalized by lattice spacing δx , U is a lattice dimensionless parameter as fluid velocity increased from blue to red.

Figure 5.

Trends of hydrodynamic parameters in the planar flow cell under right-angle flow pattern. (A) Black arrow indicates shear stress increasing and red arrow indicates solute influx decreasing. (B) Snap shot of solutes transport from LBM simulation (Supplementary Movie 2): solutes have propagated through the center of the flow cell by advection, but diffusion transport to the left of the flow cell is much slower. In legend, solutes concentration is normalized by the inlet concentration C_0 as C^* and increases from blue to red.

Figure 6.

Growth of *P. aeruginosa* biofilm in the flow cell under two different flow conditions observed by confocal microscopy. The size of each image is 0.375 mm × 0.375 mm, and the positions of the nine regions were shown in Figure 4 with local flow velocities. (A) Under the slower inflow condition (0.16 ml/min), biofilm growth occurred primarily in the region of highest fluid flow (i.e., greatest influx of growth medium), and very little growth was observed in regions with slow flow (low influx of growth medium). (B) Under the faster flow condition (0.80 ml/min), biofilm growth occurred primarily in the region of lowest fluid flow (i.e. influx did not limit growth), while little biofilm growth was observed in the region of highest fluid flow because high hydrodynamic shear detached cells from the surface in these regions. The average local velocity in each region (R1–R9) is reported parenthetically above each confocal micrograph.

Figure 7.

P. aeruginosa biofilm development and biomass detachment relative to local fluid velocity and fluid travel time from an inlet under the slow inflow condition (0.16 ml/min) (◆), and the fast inflow condition (0.80 ml/min) (□). (A) Dependence of biomass accumulation on local velocity. (B) Dependence of biomass accumulation on travel time. (C) Dependence of biomass accumulation on local boundary shear stress. (D) Dependence of biofilm roughness on local boundary shear stress.