

Secondary Flow Mixing Due to Biofilm Growth in Capillaries of Varying Dimensions

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ABSTRACT: Using a magnetic resonance microscopy (MRM) technique, velocity perturbations due to biofouling in capillaries were detected in 3D velocity maps. The velocity images in each of the three square capillary sizes (2, 0.9, and 0.5 mm i.d.) tested indicate secondary flow in both the *x*- and *y*-directions for the biofouled capillaries. Similar flow maps generated in a clean square capillary show only an axial component. Investigation of these secondary flows and their geometric and dynamic similarity is the focus of this study. The results showed significant secondary flows present in the 0.9 mm i.d. capillary, on the scale of 20% of the bulk fluid flow. Since this is the “standard 1 mm” size capillary used in confocal microscopy laboratory bioreactors to investigate biofilm properties, it is important to understand how these enhanced flows impact bioreactor transport.

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Introduction

Control of mixing and transport in capillary bioreactors and biofouled capillary membranes is an important engineering

The presence of spatially varying secondary flows generated by the heterogeneous biofilm plays a role in the spatial distributing of biological function through varying speciation and chemical communication. The data presented show reactor size impacts studies of spatially distributed biological activity and the idea that scaling of transport models in biofilm impacted devices is possible.

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challenge in industrial and biomedical systems. *Staphylococcus epidermidis* is a Gram-positive, coagulase negative member of the bacterial genus *Staphylococcus*. Commonly found on human skin, *S. epidermidis* is the most common species used in laboratory tests. *S. epidermidis* is a main cause of infection in patients with medical implants (Costerton et al., 2005). Unfortunately for these patients, *S. epidermidis* biofilms are resistant to most common antibiotics (Brock et al., 1994). These troublesome microcolonies primarily consist of bacterial cells and extracellular polymeric substance (EPS). Early studies indicated that the EPS attaches to a surface by the way of adhesive polysaccharides excreted by the cells (Costerton, 1999), but more recently EPS is known to be a conglomeration of many biopolymers (Sutherland, 2001). EPS may account for 50–90% of the total organic carbon of biofilms and can be considered the primary matrix material (Xavier et al., 2005). The EPS matrix hydrogel is important when studying biofilm transport properties in square capillaries due to its viscoelastic behavior (Rupp et al., 2005; Shaw et al., 2004; Stoodley et al., 1994; Towler et al., 2007).

This study presents magnetic resonance microscopy (MRM) data for testing and development of bioreactor system transport models. Biofilms were imaged in their natural state allowing for accurate study of their structure and impact on advective transport. This non-invasive technique allows images to be taken of biofilms in an environment similar to their native environment (Gjersing et al., 2005; Seymour et al., 2004a). Biofilms can be grown in capillaries of various sizes using similar techniques. Using standard protocols for growing capillary biofilms to be imaged using a confocal microscope (Rani et al., 2005), the resulting biofilm thickness is 10–20% of capillary cross-section where the growth period varies according to capillary size. MRM is a unique tool for measuring

quantitative flow maps and transport parameters, and allows determination of similitude conditions (Bird et al., 2002) in different-sized systems where similar flow characteristics exist.

Classical approaches to interfacial transport using an empirical mass transfer coefficient proportional to power law scaling of the Reynolds and Schmidt numbers (Bird et al., 2002) do not capture the role of secondary flows. The spatial variation of advective transport generates a spatially heterogeneous rate of mass flux in the capillary system. In the case of biomedical separations, for example, blood filtration, which uses capillary membranes, these variations can result in spatially varying driving forces and contaminant concentration. The square capillaries used in this work (Gjersing et al., 2005) are used extensively in confocal microscopy studies (Rani et al., 2005; Stewart et al., 2007; Takenaka et al., 2008) of fluorescent biomolecular binding. Previous studies on spatial variation of genetic and metabolic biofilm behavior (Kirisits et al., 2007; Majors et al., 2005a,b; McLean et al., 2008a,b) are impacted by the flow behaviors elucidated here. Of broader engineering interest is the impact of soft interfaces on mixing and transport in a range of systems (de Gennes, 1994; Debeer et al., 1994; Pathak et al., 2004; Qi and Hou, 2006; Ziebis, 1996), and the results of this work are discussed in that context.

MRM is a non-invasive tool able to access several observable quantities in biofilms such as chemical composition (Majors et al., 2005a; Vogt et al., 2000), diffusion (Hornemann et al., 2008), and macroscale structure and transport (Gjersing et al., 2005; Manz et al., 2005; Seymour et al., 2004a,b). This research uses MRM to characterize velocity profiles for flow in capillary bioreactors. It was found that the presence of biofilms in capillary reactors generates significant secondary flows in the non-axial directions and that the orthogonal components of these secondary flows tend to be out of phase (Gjersing et al., 2005). Additionally, MRM has been shown to characterize a biofilm's internal structure by revealing the contrast in density, via T_2 relaxation (Hoskins et al., 1999; Lewandowski et al., 1992; Manz et al., 2003) throughout the biofilm (Gjersing et al., 2005; Seymour et al., 2004a). T_2 is a nuclear spin relaxation time, which varies depending on molecular rotational freedom and is typically shorter for more viscous fluids or more solid-like materials such as gels, for example, biofilms. These same studies indicated that the generation of significant secondary flows by biofilms can require modification of current mass transport models (Beyenal and Lewandowski, 2002; Chambless et al., 2006; Eberl et al., 2000; Horn and Hempel, 1997; Lewandowski and Beyenal, 2003; Picioreanu et al., 2000a,b) for transport from the bulk fluid to the biofilm (Gjersing et al., 2005).

Theory

Many unique biofilm features have not been sufficiently characterized by experimental data. An area of particular

interest to biomedical researchers is bioreactor fluid dynamics because of its applications to certain biomedical devices, clinical procedures, and research on biomolecular microbiology of biofilms. More insight into biofilm properties and their impact on fluid flow could prove beneficial to the design of implanted biomedical devices that commonly become infected with biofilm-forming bacteria and in bioseparation devices. MRM has proven to be an informative bioreactor experimental technique. Its ability to produce accurate images of changes in the biofilm structure and reactor transport non-invasively provides unique experimental data (Gjersing et al., 2005; Hoskins et al., 1999; Lewandowski et al., 1992; Manz et al., 2003; Seymour et al., 2004a).

Transport Impacted by Biofilm Growth

Confocal microscopy is a prominent experimental tool to analyze microbial biofilm spatial structure (Nivens et al., 2001; Stoodley et al., 1994) and spatially distributed biological activity (Debeer et al., 1994; Heydorn et al., 2002). A typical biofilm confocal microscopy study uses a 1.0 mm o.d. with a 0.9 mm i.d. square cross-section capillary bioreactor (Kirisits et al., 2007; Rupp et al., 2005; Stewart et al., 2007). The transport of biomolecules, composing nutrients, metabolites, RNA, DNA proteins, and enzymes, depends on the interaction between the free stream velocity field and the biofilm biomass. The relative amplitude of advective and diffusive transport mechanisms varies as a function of spatial location. The presence of spatially varying secondary flows generated by the heterogeneous biofilm potentially plays a role in the spatial distribution of biological function through spatial distribution of microbe genetic variation and chemical communication (Heydorn et al., 2002). In classical mass transfer approaches, mass transfer coefficients dependent on Reynolds number correlations based on the bulk axial velocity v_z are used (Bird et al., 2002; Gjersing et al., 2005; Lewandowski and Beyenal 2007; Lewandowski et al., 1995). In non-turbulent ($Re < 2100$) systems where temporally or spatially irregular boundary conditions generate secondary flows with non-zero, non-axial v_x and v_y velocity components, the classical theory fails to account for spatially dependent advective mixing and transport (Gjersing et al., 2005). In turbulent ($Re > 2100$) flow transport theory, models incorporate additional turbulent fluxes resulting from fluctuations in point velocity about the mean (Bird et al., 2002). Such approaches also do not capture the physics associated with the transport processes in capillary bioreactors. The data presented here indicate that due to the presence of significant secondary flows, full solution of the velocity field is needed to quantifiably model mass transport (Picioreanu et al., 2000b). Significant work along these lines is being undertaken (Kapelos et al., 2006; Picioreanu et al., 2000a).

Biofilms represent a viscoelastic surface perturbation, which is heterogeneous both in biopolymer spatial

distribution and hence material response. An interesting question in scale up or down of bioreactor systems is the role of such a fixed thickness perturbation as the reactor system dimension varies. The concept of dynamic similitude in fluid dynamics implies that for the same Reynolds number in similar geometries the dynamics are reproducible (Bird et al., 2002). A classic example is the Taylor–Couette hydrodynamic instability for fluid flow in the gap of two concentric cylinders with the inner cylinder rotating. Above a critical rotation rate, secondary flow in the radial v_r and axial v_z directions is generated as a perturbation on the primary angular v_θ flow (Chandrasekhar, 1981). The wavelength of the secondary axial flow velocity scales with cylinder gap at similar Reynolds number indicating dynamic similitude.

Materials and Methods

Growing Biofilms

The stages of growing a *S. epidermidis* biofilm include setting up the bioreactor system, growth of suspended bacterial cells, inoculation of the bioreactor system, and a monitored growth period. An initial liquid culture was prepared using 10 mL of 30 g L⁻¹ tryptic soy broth (TSB; Difco Becton–Dickinson, Sparks, MD) and *S. epidermidis* cells from frozen stock (-70°C) ATCC# 35984. Both were mixed in a micro centrifuge tube, and the suspended bacteria cells were shaken at 37°C overnight (12 h). Once the suspended bacterial cells reach a desired concentration as determined by the optical density measured with a spectrophotometer at 600 nm, the solution was used to inoculate the bioreactor system.

The bioreactor is gravity fed with a nutrient solution of 30 g TSB per 10 L of water and Magnevist (20 mL of pure Magnevist per 500 mL of DI H₂O). Magnevist (Berlex Laboratories, Mfd., Wayne, NJ) reduces the necessary MRM experiment duration by decreasing T_1 relaxation and allowing more rapid signal acquisition, and due to chelating does not penetrate cell membranes. At the concentrations used, Magnevist has been shown not to affect biofilm growth (Lewandowski et al., 1992). The system components consist of a square capillary (Friedrich and Dimmock, Millville, NJ) bioreactor in a protective glass casing, two 10 L carboys (one for feed and one for waste), an incubator set at 37°C which provides optimal conditions for biofilm growth, a glass flow break upstream of the capillary bioreactor that maintains steady-state flow, and an inoculation chamber downstream of the capillary. The area upstream and downstream of the square capillary was clamped to prevent flow for a 4 h period after inoculation. This time allows the bacteria cells to “settle” onto the capillary walls whilst it is in a horizontal position. In order to maximize biofilm properties for MRM experiments, the biofilm was fed nutrient at a fixed flow rate of 16.7 mL s⁻¹ using a peristaltic pump ($Re = 8–33$) for 48–96 h depending on capillary size. Shear during growth is

important in determining the resultant mechanical properties of a biofilm (Stoodley et al., 1994). Therefore, flow rates and Re were kept as fixed as possible during biofilm growth. During MR experiments, the constant flow rate was controlled with a gravitational head to eliminate peristaltic pump vibrations and to maintain velocities within the desired MR measurement window. Flow rates during MR experiments varied depending on capillary size: $Re = 110 \pm 3.8$ for the 2 mm capillary, $Re = 430 \pm 30$ for the 0.9 mm capillary, and $Re = 562 \pm 172$ for the 0.5 mm capillary. This study focuses on six biofilm experiments; there were two for each of following capillary cross-section lengths: 2, 0.9, and 0.5 mm. At the end of the growth period, the bioreactor system was transferred into the MRM instrument.

MRM Biofilm Experiments

MRM measurements are made using a Bruker DRX spectrometer ($B_0 = 5.9$ T), a 5-mm saddle radio frequency coil, and magnetic field gradients up to 1.7 T m⁻¹. For each capillary size, velocity maps were taken using a velocity phase encoding pulse sequence (Callaghan, 1991) shown in Figure 1 and a specific set of experimental parameters.

The sequence combines a standard spin warp magnetic resonance imaging (MRI) sequence with the basic pulsed gradient spin echo (PGSE) experiment and is used to measure translational spin displacement over a specific time interval Δ with a magnetic gradient pair applied for a time δ (Callaghan, 1991; Fukushima, 1999). This ordering of rf pulses, magnetic field gradients, and data acquisition is used to examine position exchange (Blumich, 2000). The first gradient encodes for position, r , waiting a time Δ for the spins to move, and using a second gradient to encode for the new position, r' . The signal is then encoded for displacements ($r' - r$). Knowing the spin displacement over a fixed time interval Δ allows for velocity calculations from the net phase shift of the magnetization (Blumich and Kuhn, 1992; Callaghan, 1991). Two images are taken, the first image without motion encoding PGSE gradients ($g = 0$ mT m⁻¹),

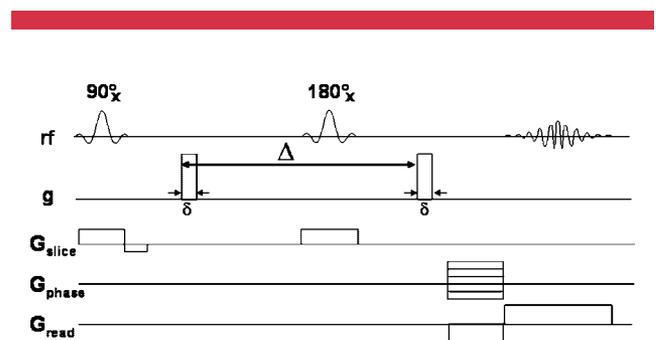


Figure 1. Pulse sequence used to make displacement measurements and create velocity maps.

and the second image with PGSE gradient values ranging from 150 to 800 mT m⁻¹. Depending on the capillary size, various gradients were required due to the range of velocities present. The gradients for each of the sizes were 800 mT m⁻¹ for the 2 mm, 300 mT m⁻¹ for the 0.9 mm, and 150 mT m⁻¹ for the 0.5 mm capillary. The phase difference, Φ , for each pixel between the images is dependent on the velocity v in that pixel via $\Phi = 2\pi/\gamma\delta\Delta v$, where δ is the time the gradient is applied and Δ is the delay time between gradients. For our experiments $\delta = 1$ ms, $\Delta = 10$ ms, the repetition time was 500 ms, the echo time was 17.9 ms, and four averages were used. The velocity images were averaged over a 0.3-mm slice thickness. All three velocity components v_x , v_y , and v_z were measured with a total experimental time varying from 51 min for the 0.5 mm up to 90 min for the 2 mm capillary. This timescale is short relative to biological growth timescales, and any sloughing events would manifest themselves as image artifacts and were not detected. The experiments used a spatial orientation: the read gradient in z -direction, the phase gradient in x -direction, and the slice gradient in the y -direction. The magnetic field gradients applied give spatial resolution of the (x,z) plane over the 0.3 mm slice in y -direction, where the z -axis is the long axis of the capillary. The velocity profiles measured are bulk flow $v_z(x,z)$, the cross-stream x - and y -directions, $v_x(x,z)$ and $v_y(x,z)$. The field of view (FOV) decreased with capillary size: 20 mm \times 3 mm for the 2 mm capillary, 20 mm \times 2.5 mm for the 0.9 mm capillary, and 20 mm \times 1.5 mm for the 0.5 mm capillary. Using 128 \times 64 pixels, the resulting spatial resolution was 156 \times 47 $\mu\text{m pixel}^{-1}$ for the 2 mm capillary, 156 \times 39 $\mu\text{m pixel}^{-1}$ for the 0.9 mm capillary, and 156 \times 23 $\mu\text{m pixel}^{-1}$ for the 0.5 mm capillary.

All of the data presented in this article address the known variations in biological samples by performing multiple experiments, averaging the results, and reporting the trends.

Results and Discussion

The velocity profile for clean capillaries (no biofilm) shows fastest flow at the capillary mid-section and no flow at the sides (Seymour et al., 2004a), as is typical for laminar flow of a Newtonian fluid through a conduit. Clean capillaries also have no secondary flows $v_x, v_y = 0$. Velocity maps for each capillary size in this study after biofouling are shown in Figures 2–4. Since previous data (Gjersing et al., 2005; Seymour et al., 2004a) indicate significant secondary flow, one of the objectives of this research is to determine if conditions of hydrodynamic similitude, geometric, and dynamic similarity exist in biofouled capillaries. Of particular interest is whether the secondary flow structures scale with capillary length scale despite the fact that the biofilms have an average thickness of the order of 100 μm and occupy a different proportion of the capillary (varies 5–20%) as the capillary cross-sectional length scale is varied.

The velocity profiles in the biofouled capillaries clearly indicate significant non-axial flow components, which exhibit oscillatory flow behavior throughout the capillary

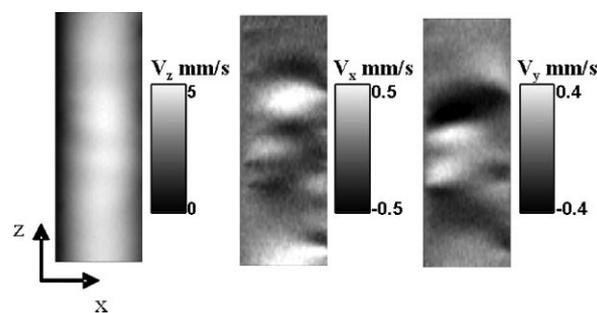


Figure 2. Biofouled 2 mm capillary velocity maps with biofouling on the left side of the capillary. The v_x and v_y components exhibit significant non-axial flow components inside the capillary bioreactor. The slice thickness is 0.3 mm with spatial resolution of 156 \times 47 $\mu\text{m pixel}^{-1}$. FOV shown (2 mm \times 11.72 mm) is a cropped section from the entire image and is magnified in the x -direction.

bioreactor (Figs. 2–4) as previously demonstrated for a 0.9 mm capillary (Gjersing et al., 2005; Seymour et al., 2004a). The spatial flow frequencies present were quantified by performing a Fourier transformation (FT) of the data points, after appropriate baseline corrections of a line drawn through the capillary center along the longitudinal axis as indicated in Figure 5. The Fourier transform statistically characterizes the periodicity of the non-axial streamlines. Data in Figure 6 show distinguishable peaks in the FT graphs. By observing the dominant frequency for each size capillary, it is evident that the peak is proportional to the capillary size. For example, in the 2 mm graph, the dominant frequency is 40 mm⁻¹ ($\lambda = 2.5$ mm). Scaling down to a 0.9 mm cross-section gives a frequency that is close to

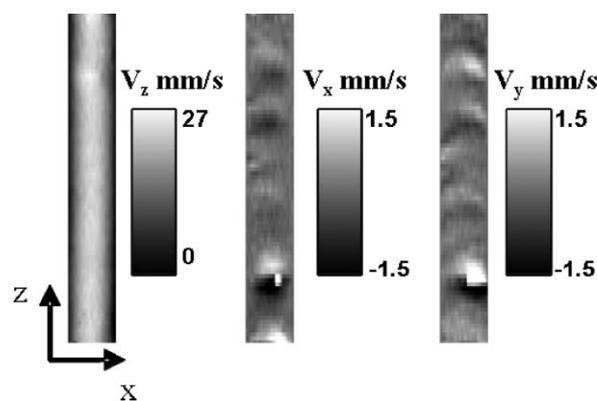


Figure 3. Biofouled 0.9 mm capillary velocity maps with biofouling on the left side of the capillary. There are obvious non-axial flow components inside the capillary bioreactor. The slice thickness is 0.5 mm with a spatial resolution of 156 \times 39 $\mu\text{m pixel}^{-1}$. FOV shown (0.9 mm \times 11.72 mm) is a cropped section from the entire image and is magnified in the x -direction. *Note:* A large biofilm cluster is causing some faster velocities and velocity wrapping in a few pixels towards the bottom of the images.

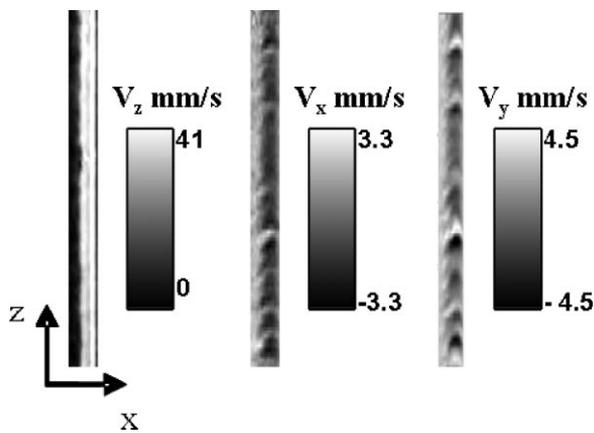


Figure 4. Biofouled 0.5 mm capillary velocity maps with biofouling on the left side of the capillary. Non-axial flow components are apparent in the capillary bioreactor. The slice thickness is 0.3 mm with a spatial resolution of $156 \times 23 \mu\text{m pixel}^{-1}$. FOV shown ($0.5 \text{ mm} \times 11.72 \text{ mm}$) is a cropped section from the entire image and is magnified in the x -direction. Note that this narrow capillary was slightly misaligned with the gradient axis and hence v_x and v_y show a small baseline component due to the large axial flow that was removed before FT analysis.

80 mm^{-1} ($\lambda = 1.25 \text{ mm}$). Likewise, for the 0.5 mm capillary, the dominant frequency is found at 160 mm^{-1} ($\lambda = 0.625 \text{ mm}$). The ratio of the wavelength to channel width is 1.25 ± 0.14 for all three capillary sizes.

Averaging the maximum non-axial velocity observed at the capillary centerline for multiple biofilms in the same capillary size allows comparison of the relative amplitude of the secondary flows between the different size capillaries. The secondary flows are most significant at the centerline in the 0.9 mm capillary at approximately 20% of the maximum

z -direction flow. This appears to be due to the capillary size in combination with the size of the biofilm, a resonant-like effect. That is, in the 2 mm capillary the secondary flows are damped at the centerline by viscosity due to the larger cross-section. While in the 0.5 mm capillary, the upper wall is close enough to the biofilm to cause damping. Table I summarizes an average value for the experimentally observed secondary flow amplitudes at the capillary centerline.

Another comparison of the 2 mm capillary flow data to the values from the 0.9 mm capillaries is shown through analysis of the secondary flows as a function of the distance from the biofilm. The bottom surface inside the capillary is referenced as 0 mm. The top capillary surface is the full inner width. To determine the impact of the biofilm surface on the flow field, several velocity profiles were analyzed at locations above the biofilm surface. T_2 images and the zero flow regions in the velocity maps were used to ensure an average biofilm height of $100 \mu\text{m}$ for every sample used in this study. A combination of established protocols (Gjersing et al., 2005) were used to estimate the necessary growth time to get the desired ($100 \mu\text{m}$) thickness, but also checked visually and verified with MR T_2 and velocity maps before using a sample for this study. These locations are shown in Figure 7 for clarification via a schematic. At positions closer to the biofilm–fluid interface in the 2 mm capillary, secondary flows as a percentage of the bulk flow increase to the same order of magnitudes observed in the 0.9 mm capillary. For example, the 0.9 mm centerline is $350 \mu\text{m}$ from the average biofilm surface where the secondary x -direction velocity ratio is approximately 20% for $v_{x,\text{max}}/v_{z,\text{max}}$ and in the 2 mm at similar distance above the average biofilm surface ($300 \mu\text{m}$), $v_{x,\text{max}}/v_{z,\text{max}}$ is 22.65%. The velocity values were not so closely related in the y -direction when comparing the same positions ($300\text{--}350 \mu\text{m}$) where the 0.9 mm retained the 20% for $v_{y,\text{max}}/v_{z,\text{max}}$, but only 14% in the 2 mm

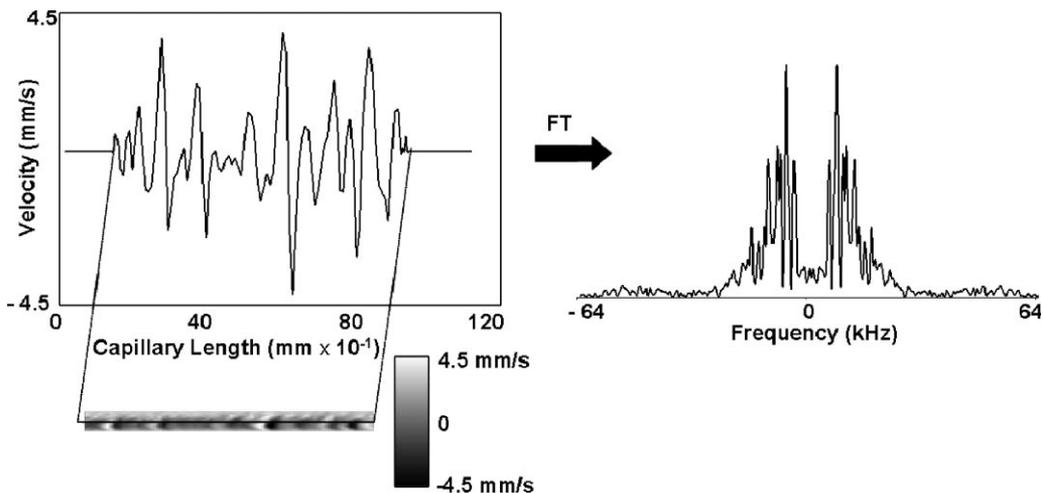


Figure 5. A Fourier transformation (right) of a line through the 0.5 mm velocity map shows the spatial frequencies (mm^{-1}) of the flow through the capillary bioreactor.

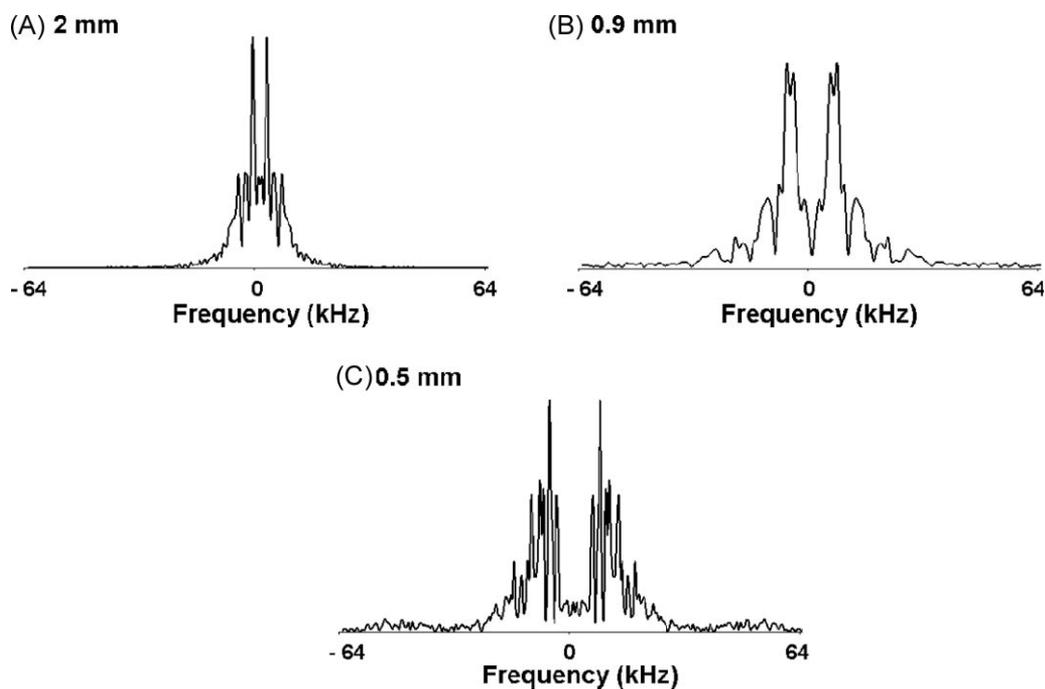


Figure 6. Fourier transformations of velocity data for (A) 2 mm, (B) 0.9 mm, and (C) 0.5 mm capillaries. The strongest peaks at a frequency are inversely related to the capillary cross-section.

indicating asymmetry in the larger capillary. The data clearly indicate that the proportion of the channel width the biofilm occupies impacts the variation in mixing by secondary flow. This leads to the idea that confocal microscopy data on spatial variation of biological function (Heydorn et al., 2002; Kirisits et al., 2007; Nivens et al., 2001) based on data from studies on single bioreactor sizes is prone to reactor size dependency. Table II shows the secondary velocities as a percentage of the axial velocity as data analysis lines move from the centerline towards the biofilm.

Conclusions

This article presents analysis of the dependence of secondary flows generated by biofilms of approximately 100 μm thickness on capillary bioreactor cross-section size. The

Table I. The x - and y -direction average maximum velocity as a percentage of the maximum z -direction velocity for the three different capillary cross-sections.

	0.5 mm	0.9 mm	2 mm
$v_{x,\text{max}}/v_{z,\text{max}} \times 100$	4.9 ± 0.1	20 ± 0.3	12 ± 2.8
$v_{y,\text{max}}/v_{z,\text{max}} \times 100$	5.6 ± 0.9	21 ± 4.3	8.7 ± 2.2

Data are taken from the centerline of the capillary. The biofilm surface has a different impact on the flow depending on capillary size. The biggest impact is in the 0.9 mm capillary where the biofilm occupies approximately 11% of the capillary inner cross-section.

wavelength of the secondary flows is shown to scale with the capillary bioreactor cross-section in analogy with the scaling of wavelength with Couette gap size in hydrodynamic instabilities like Taylor vortices. However, the amplitude of the secondary flows, represented as a percentage of the bulk axial flow, depends on the distance from the biofilm. The capillary bioreactor cross-section size thus interacts with the biofilm and generates stronger secondary flows at the bioreactor centerline as shown for the 0.9 mm capillary or weaker flows due to solid boundary effects, as in the 0.5 mm capillary or viscous damping as in the 2 mm capillary. The presence of spatially varying secondary flows generated by the heterogeneous biofilm plays a role in the spatial distribution of biological function through varying speciation and chemical communication. The data presented here clearly support the conclusion that reactor size impacts studies of spatially distributed biological activity, and the idea that, scaling of transport models in biofilm impacted devices is possible but requires more study. 2D numerical simulations with either random or periodic solid surfaces did not introduce secondary flows of as large an amplitude as observed in the biofilm samples. Whether the viscoelastic nature of the biofilm or the spatial growth pattern of the biofilm causes the enhanced oscillatory motion observed in the biofouled capillaries is the subject of ongoing experiments on model soft interfacial surfaces. It should be noted that the numerical simulation of fluid dynamics with soft viscoelastic interfacial boundary conditions is an open topic of research. The biological impact of these secondary flows

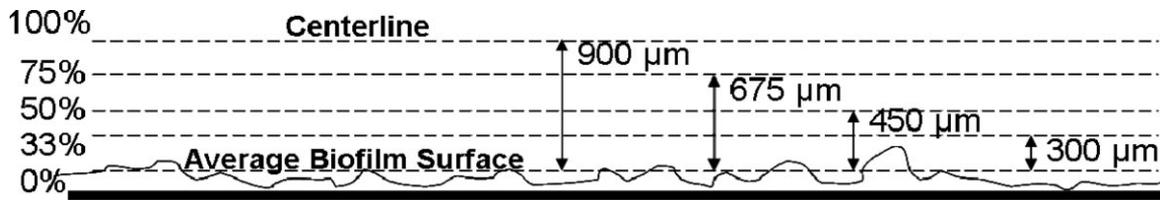


Figure 7. Schematic explaining the data analysis process of choosing velocity profiles at various distances from the average biofilm surface–liquid interface in 2 mm capillary.

Table II. The percentage maximum x - and y -direction flow of the maximum z -direction flow to relate distance from fluid–biofilm interface to secondary flow significance.

Vertical capillary position	$v_{x,max}/v_{z,max} \times 100$	$v_{y,max}/v_{z,max} \times 100$
0.9 mm centerline (350 μm to biofilm surface)	20	21
2 mm (300 μm to biofilm surface)	23	14
2 mm (450 μm to biofilm surface)	18	13
2 mm (675 μm to biofilm surface)	16	11
2 mm centerline	12	8.7

The average biofilm–fluid interface is designated as 100 μm with distance increasing towards the 1.0 mm centerline to a maximum of 900 μm from the biofilm. Both non-axial flow ratios are significant and, in particular, the x component in both the 0.9 mm and 2 mm capillaries shows the same magnitude in the 300–400 μm range from the biofilm surface independent of capillary dimension. Since the biofilm grows asymmetrically perpendicular to the x -direction, the y -component may be more of a function of capillary size.

is also an open question as they induce spatially variable advection of nutrients and the biofilm structure may assist in optimizing this transport.

Nomenclature

B_0	external magnetic field (T)
g_i	magnetic field gradient for motion sensitivity (T m^{-1})
G_i	magnetic field gradient in the i direction (T m^{-1})
r	spatial coordinate vector (m)
Re	Reynolds number ($Re = v_{z,max}l/\nu$)
T_2	transverse, spin–spin relaxation time (s)
$v_i, i = x, y, z, r, \theta$	components of velocity vector (m s^{-1})
$v_{i,max}$	maximum velocity component (m s^{-1})
x	Cartesian spatial coordinate
y	Cartesian spatial coordinate
z	Cartesian spatial coordinate, axis of capillary

Greek Letters

δ	duration of velocity encoding gradient pulses (s)
Δ	observation time for encoding displacement (s)
γ	gyromagnetic ratio, for protons $\gamma = 2.675\text{E}8$ ($\text{rad s}^{-1} \text{T}^{-1}$)
ν	kinematic viscosity ($\text{m}^2 \text{s}^{-1}$)
Φ	phase difference (rad)

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