Electronic Supplementary Material (ESI) for Analytical Methods.

Microfluidic flow confinement to avoid chemotaxis-based upstream growth in a biofilm flow cell reactor

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Electronic supporting information:

In brief, a typical experiment included 3 different growth phases, as controlled by the microfluidic system. They include an inoculation phase ($phase_i$), a downstream growth phase ($phase_d$) and an upstream growth phase ($phase_u$). Table 1 (main paper) includes the details of the solution conditions applied to the inlets during each growth phase. During $phase_i$ and $phase_d$, inoculant solution and a nutrient solution, respectively, were added to I_1, while a confinement solution was applied to I_2. As the confinement solution (I_2) did not contain any nutrient source, bacteria were prevented from colonizing the portions of the channel that were upstream of I_1. In this approach bacteria were confined to the downstream portions of the microchannel between I_1 and O. Increased velocity and shear forces in the neck, could have also prevented random swimming of planktonic bacteria into the confinement stream. However, since such bacteria could not grow due to the lack of nutrients, even if some did swim upstream high resolution microscopy would have been required to detect their presence. Instead, during $phase_u$, we applied a nutrient solution to I_2 and stopped the flow into I_1 after a visible biofilm was observed in measurement area 2. The nutrient solution throughout the microbioreactor provided both an incentive for planktonic bacteria to try to move to uncolonized upstream portions, and provided the necessary

nutrients to enable subsequent biofilm growth in the measurement channel. Using low-resolution microscope imaging throughout the channel biofilm growth could be observed as an indicator of the success of the hydrodynamic confinement from the neck. We ran 4 separate experiments with different volumetric flow rates applied to I_1 and I_2 (Q_1 and Q_2, respectively) as summarized in Table 1 (main paper). The volumetric flow rate Q_2 used during $phase_u$ is highlighted (bold box) as the critical parameter for achieving the effect of hydrodynamic isolation between the measurement area and head area via the neck constriction feature. This is the value that is reported as Q_2 in the main paper, section "Measurement of optical density for biofilm growth".

Table S1. Volumetric flow rates for experiments 1-4 in Figure 6.

Experiment number	**Flow rate**	**$Phase_i$**	**$Phase_d$**	**$Phase_u$**
(a)	Q_1	0.3	0.3	0
	Q_2	0.3	0.6	0.3
(b)	Q_1	0.3	0.3	0
	Q_2	0.3	0.6	1
(c)	Q_1	0.3	0.3	0
	Q_2	0.3	0.6	3
(d)	Q_1	0.3	0.3	0
	Q_2	0.3	0.6	4

The corresponding velocity and shear force during the $phase_u$ are the critical parameters in determining the ability to achieve the desired hydrodynamic confinement. Using relevant flow

rates in Table S1 along with equations 1 and 2, we show in Tables S2 and S3 the calculated $v_{1,2,3}$ and $\tau_{1,2,3}$ (respectively) as defined in Figure 3 (main paper).

Table S2. Calculated velocities during upstream growth phase, $phase_u$.

$Phase_u$, Q_1 (mL/h)	**v_h (mm/s)**	**v_n (mm/s)**	**v_m (mm/s)**
0.3	0.83	6.6	0.83
1	2.7	22.0	2.7
3	8.3	66.0	8.3
4	11.0	88.0	11.0

Table S3. Calculated shear force during upstream growth phase.

$Phase_u$ Q_1 (mL/h)	**τ_h (Pa)**	**τ_n (Pa)**	**τ_m (Pa)**
0.3	0.099	0.799	0.099
1	2.65	0.33	2.65
3	0.99	7.99	0.99
4	1.33	10.65	1.33

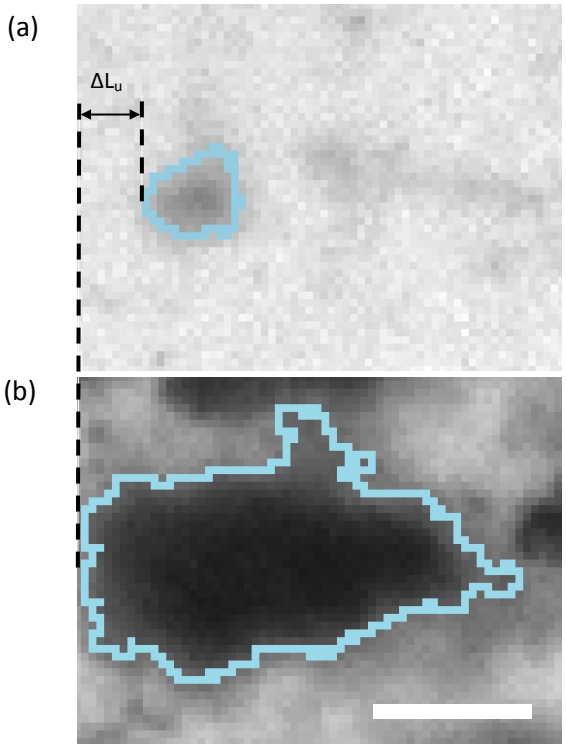

Figure S1 expansion of a biofilm colony in a microchannel in a downstream portion of the channel after 10 hours (a) and 25 hours (b). Blue boarders accentuate the outer edge of the biofilm colony. The upstream change in length (ΔL_u) is shown as the displacement of the trailing edge in the upstream direction. Flow was from left to right. Scale bar is 100 µm.