

Electronic Supplementary Information (ESI)

S1†: *Pseudomonas Aeruginosa (PA14)* Culture and Experimental protocol

S2†: PDMS device washing procedures

S3†: Non-dimensionalizing biofilm area with Buckingham Pi theorem

S4†: Diffusion of growth limited substrates in biofilms

S5†: Shear stress at the boundary of biofilms

ESI S1†: *Pseudomonas Aeruginosa* (PA14) Culture and Experimental protocol

All procedures for PA14 culture and seeding to PDMS devices were carried out according to the guidelines of Environmental Bio Technology Laboratory, Korea University. PA14 constitutively expressing green fluorescent protein (GFP) were used as the model bacterium. All of experimental procedures were conducted at darkroom to protecting GFP. As medium, Luria-Bertani broth (L3022, Sigma, USA) was used. The microfluidic devices was fabricated using silicon wafers with SU-8 patterns as a mold. After *Polydimethylsiloxane* (PDMS) was curing on the silicon wafer at 80°C, the PDMS layer was bonded with a bare PDMS layer by oxygen plasma treatment (**Fig. S1a**). The fabricated PDMS devices were sterilized by autoclave before seeding bacterial cells. Bacterial cells were cultured in a shaking incubator at 37°C and 200 rpm for 24 hours. When we secured sufficient cell density, PA14 were suspended in LB medium as constant density ($OD_{600}=0.1$). To allow cells adhere to the surface of PDMS devices, the suspension were filled micro channels and inoculated for two hours at temperature and humidity controlled incubator. Fresh LB medium was then fed into the channel for 48 hours with desired flow rate by a peristaltic pump (74-128-00000, Thermo Fisher Scientific, USA) (**Fig. S1b**). With the pump, microbore tubing system was used that have 0.19 mm, 0.25 mm and 0.89 mm of inner diameter. After experiment, microfluidic channel was refreshed by PBS solution several times only using hydrostatic pressure to remove suspended bacterial cells and unattached biofilms. Fluorescence microscopy (JuLi, NanoEnTek, Korea) was used to monitor and quantify the growth of biofilms through the change of biofilm coverage in the microfluidic channel.

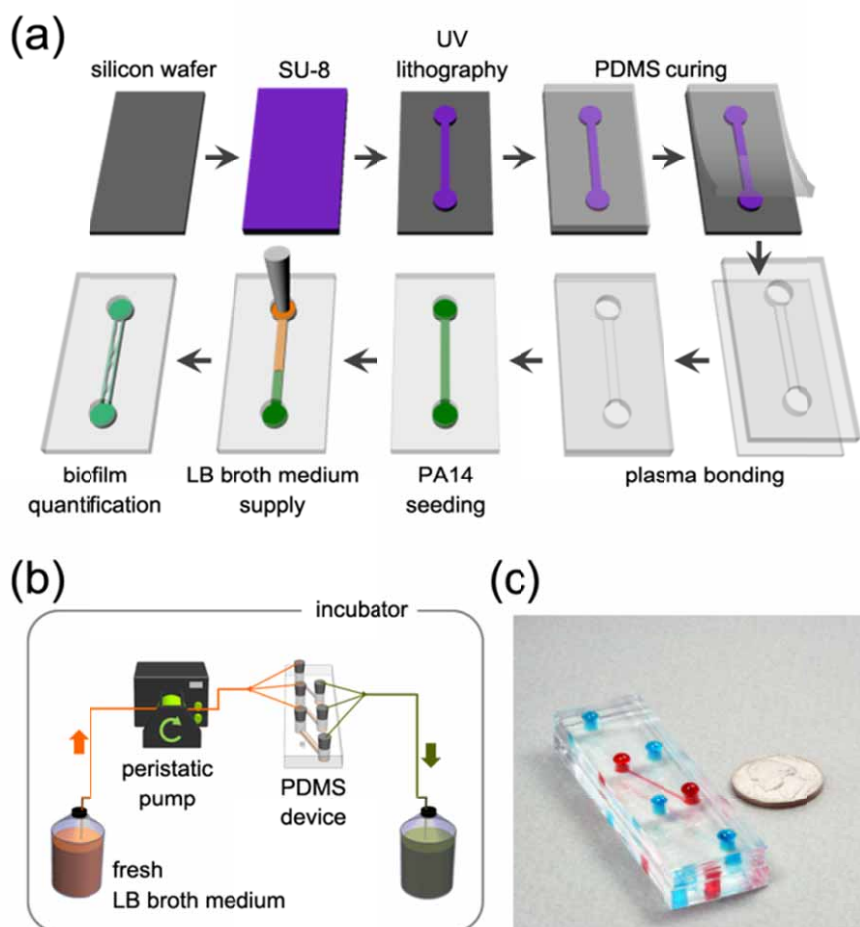


Figure S1. Schematic diagram showing the preparation of microfluidic devices and the experimental protocol. (a) Soft lithography and the experimental procedures. A silicon wafer was used as a mold. PDMS devices were fabricated and bonded to another PDMS layer. PA14 cells were seeded in each microfluidic channel for 2 hours. Fresh LB medium was then fed into the channel for 48 hours. Fluorescence microscopy was used to monitor and quantify the growth of biofilms. (b) A schematic diagram of the fluid-supplying system. A peristaltic pump was used to feed the LB broth medium to the microfluidic channels continuously over two days in a temperature-controlled incubator at 37°C. (c) A photograph of a microfluidic device. The device includes three channels, each with a different width: 500, 1000, and 1500 μm . All channels had the same height and length: 120 and 17,200 μm , respectively.

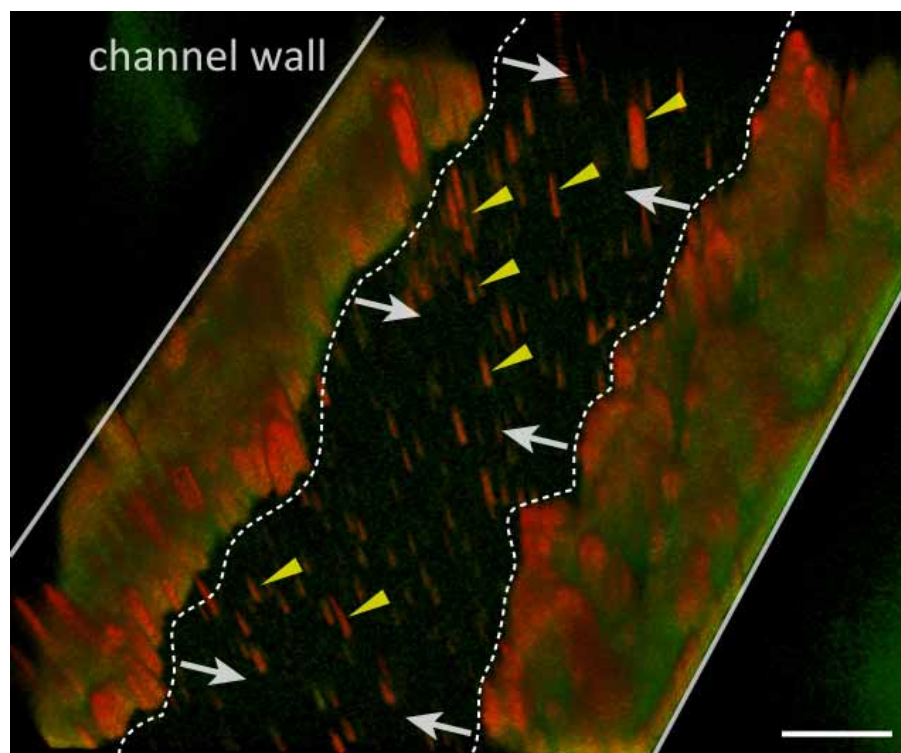


Figure S2. A 3D image of PA14 (green) and the biofilm matrix (red). This image is three dimensional view of figure 1(a). The scale bar indicates 200 μm . Microfluidic devices used in the work had very high aspect ratio ($r=w/h$), and induced development of mat-like biofilms at the sides of channel. The rasping growth of biofilms in vertical dimension indicated that bacterial biofilms filled the microchannel from bottom to top in their coverage area. The thick mat-like biofilm structure can be noticed (white dotted line) and apparently differentiated from the corners (white arrows). Small biofilm colonies developed on the channel surface (yellow arrowheads). The mat-like biofilms have low variation in their coverage area in vertical direction, precisely analyzed by two-dimensional model. The coverage area was monitored and quantified by fluorescence microscopy.

ESI S2†: PDMS device washing procedures

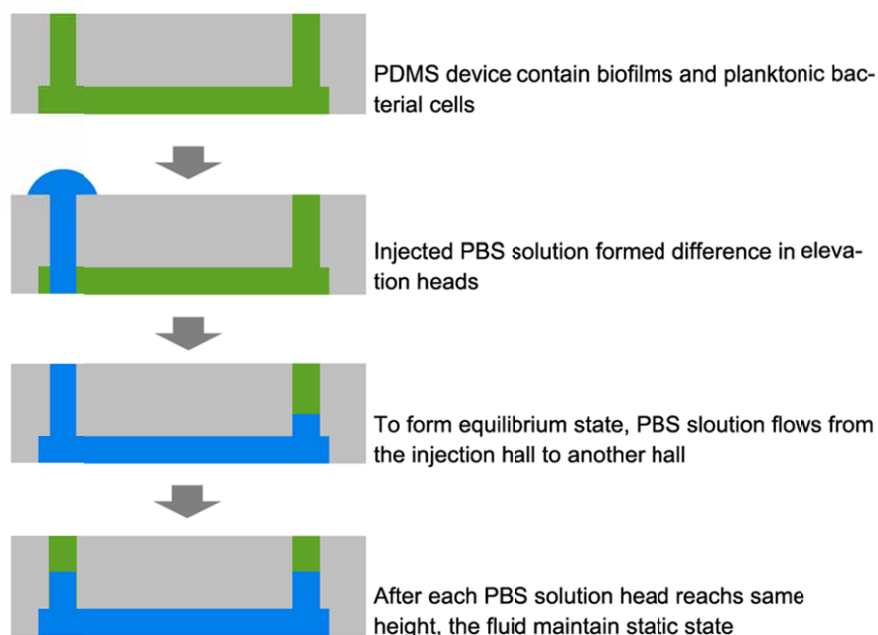


Figure S3. Schematic diagram of refreshment procedures. After two day of culture, the PDMS device contained mat-like biofilms, biofilms, and planktonic bacterial cells. To remove the planktonic bacterial cells, we refreshed microfluidic channel using PBS solution. In these procedures, only head difference was used as flow source to minimize shear flow into developed biofilms in the channel. Through repeating the refreshments several times, we could wash out planktonic cells remaining biofilms.

ESI S3†: Non-dimensionalizing biofilm area with Buckingham Pi theorem

Non-dimensionalized Biofilm area was expressed as a function of non-dimensional numbers through Buckingham PI theorem. It makes it possible that the mathematical model could be used in both micro and macro model of biofilm development.

Step 1; List variables that are involved in the biofilm formation. When bacterial cells form biofilms, diverse factors influence to biofilm development. In this work, we choose six variables focusing on hydrodynamic effects: biofilm area (A_b), shear stress (τ), channel width (w), channel height (h), flow velocity (v), density of the medium (ρ).

$$A_b = fn(\tau, w, h, v, \rho)$$

Step 2; Express each of the variables in terms of basic dimensions. Each five variables was expressed with three basic dimensions, time (T), length (L), and mass (M);

$$A_b = [L^2], \tau = [ML^{-1}T^{-2}], w = [L], h = [L], v = [LT^{-1}], \rho = [ML^{-3}]$$

Step 3; Determine the required number of pi terms. According to Buckingham pi theorem, the number of pi terms equal to the number of variables (Step 1) minus the number of reference dimensions required to describe these variables (Step 2). In this case, we needed three pi terms (6-3=3).

Step 4; Form pi term by multiplying one of the nonrepeating variables by the product of the repeating variables. With nonrepeating variables, biofilm area (A_b), density of the medium (ρ), and channel width (w) or channel height (h), we formed two cases of pi terms;

$$(i) \Pi_1 = A_b h^{-2}, \Pi_2 = \rho v^2 \tau^{-1}, \text{ and } \Pi_3 = w h^{-1}$$

$$(ii) \Pi_1 = A_b w^{-2}, \Pi_2 = \rho v^2 \tau^{-1}, \text{ and } \Pi_3 = h w^{-1}$$

Step 5; Express the final form as a relationship among the pi terms. According to the nonrepeating variables, non-dimensionalized biofilm area was expressed as two type of function. At the first case, non-dimensionalized biofilm area was expressed as a function of the Reynolds number (Re) and the channel aspect ratio (r).

$$A_b/h^2 = fn\left(\frac{v^2 \rho}{\tau_{cs}/2}, \frac{w_{cs}}{h}\right) = fn(Re, r)$$

At the second case, non-dimensionalized biofilm area was expressed as a function of the Reynolds number (Re) and reciprocal number of the channel aspect ratio ($1/r$).

$$A_b/w_{cs}^2 = fn\left(\frac{v^2 \rho}{\tau_{cs}/2}, \frac{h}{w_{cs}}\right) = fn(Re, 1/r)$$

ESI S4†: Diffusion of growth limited substrates in biofilms

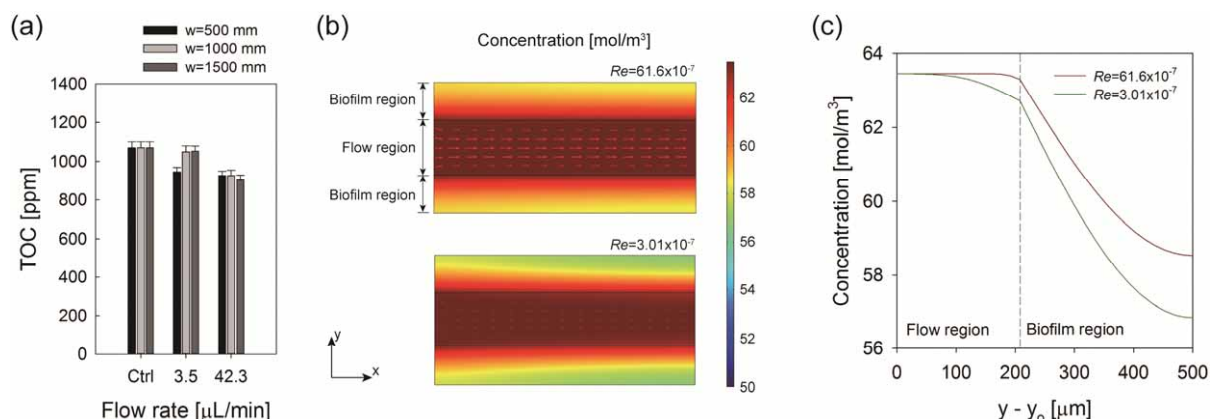


Figure S4. (a) Total Organic Carbon (TOC) concentration in the Luira Bertani (LB) broth medium according to the flow rates. TOC level was measured after LB medium pass through micro-channel which cultured biofilms for two days. ($n=3$, $\pm$ SEM) (b) Gradient of tryptone concentration in biofilm and flow regions with different hydrodynamic conditions, after eight minutes beginning diffusion. (c) Concentration profile of tryptone in biofilm matrix with different Re after eight minutes. The $y-y_0$ indicates distance from the center of the micro-channel to the y -coordinate.

Availability of nutrients is one of the key factors for biofilm development, because it regulates metabolism of bacterial cells in biofilms²⁵. In the processes of supplying of growth-limited substrates in biofilms, diffusion is a predominant process, since diffusion distance is increased in the biofilms and composition of biofilms (e.g. locally high cell density and EPS) arrest the fluid flow²⁶. Therefore, simulation for studying the effects of shear flow on the diffusion of growth-limited substrates could explain the phenomena that higher flow rates promoted biofilm developments in low Re condition. First of all, to confirm that supplied fluid flow has sufficient nutrients, TOC level of LB broth medium which passed through micro-channel containing biofilms was measured. Figure S4 (a) showed that fluid flow in the micro-channel maintained constant nutrients level, regardless of flow rates and channel dimension. Since the LB broth medium is one of the very nutrient-rich medium, we could regard the concentration of nutrients in the flow as a constant at the computer simulation for diffusion of growth-limited substrates. Tryptone (major component of LB broth medium) was used for the simulation as a growth-limited substrate. We compared convective diffusion of tryptone according to the different Re , 61.6×10^{-7} and 3.01×10^{-7} . The diffusion coefficient of tryptone is $10 \times 10^{-6} \text{ cm}^2/\text{s}$ in the water and $2.5 \times 10^{-6} \text{ cm}^2/\text{s}$ in the biofilms²⁶. Figure S4(b) showed concentration gradient of tryptone with different Re in biofilm region. The diffusion of tryptone into the biofilm regions was promoted by higher Re of medium (**Fig.**

S4c). This is because shear flow at the interface between biofilms and micro-flow facilitated convective diffusion into biofilm regions, and it reduced diffusion time of tryptone. These simulated results proposed the reason why faster flow of medium encourages biofilm formation in micro environments. The faster shear flow aided convective diffusion of growth-limited substrates into the biofilms, and it enabled to generate more biofilms in the micro channels.

ESI S5†: Shear stress at the boundary of biofilms

r	$Re [x10^{-7}]$	$w_{flow} [\mu m]$	$\tau_{ESS} [Pa]$	Hydrodynamic condition
12.5	2.08	743.06	0.09	Low Re regime
	25.52	573.94	1.65	
	61.48	371.82	5.65	High Re regime
	100.91	772.69	5.18	
8.33	3.01	591.75	0.13	Low Re regime
	37.11	433.25	2.36	
	88.79	354.50	7.07	High Re regime
	146.05	596.5	6.84	
4.17	5.34	429.25	0.21	Low Re regime
	68.82	197.63	6.59	
	161.11	240.00	11.78	High Re regime
	261.16	407.25	11.78	

Table S1. Wall shear stress according to the Reynolds number of micro-flow

Table S1 showed that each value of shear stress at the boundary of biofilms according to the Reynolds number (Re) and width of flow (r). Compare to the shear stress levels at the low Re regime, shear stress in the high Re regime had very large value. It explained that under high Re condition, viscous effects of shear flow was a main reason for limitation of biofilm growth. In this condition, biofilms had constant value of endurable shear stress (τ_{ESS}) according to the channel aspect ratio (r). As biofilms developed more and more, the width of flow was narrower and narrower, and then it caused increase of wall shear stress at the boundary of biofilms. With faster shear flow, the growth of biofilms was limited to the smaller value, because the faster shear flow was enough to generate the endurable shear stress. On the other hand, with relatively slow shear flow, biofilms could be

developed more until the wall shear stress reach to the endurable shear stress (τ_{ESS}). According to the channel geometry, each case of biofilm had its own range of endurable shear stress (τ_{ESS}). It could be explained by difference on mechanical properties of biofilms. Since biofilms had been developed under different hydrodynamic conditions according to the channel aspect ratio (r), they could have distinct mechanical properties by the geometry of micro channel.