Supplementary Information

Supplementary Materials and Methods

Microfluidic biofilm assays Microfluidic devices were created out of poly(Dimethyl-siloxane) (PDMS) and glass by photolithography and replica molding as previously described (2). Master molds were fabricated by spinning KMPR 1025 negative photoresist (MicroChem) onto clean silicon 4 inch wafers at 2500 rpm for 30 sec, resulting in channels with an average height of 30 μm. PDMS and crosslinker (Dow Corning; Sylgard 184) were mixed to a 10:1 m/m ratio, poured over the molds, and degassed in vacuum for 1 hour, followed by curing at 60^{o}C for 6 hours. PDMS negatives were bonded to glass slides or 1.5 type coverslips (Fisher) by activating both with O_2 plasma using a Techniques MicroRIE plasma etcher. Plasma was generate at a pressure of 500 mTorr and RF power of 75 W for 10 seconds.

The device utilized for this study operates in two modes: (i) seeding of bacteria in a pattern to areas of known shear, and (ii) subsequent exposure of isolated populations of sessile bacteria to shear. All flow is achieved using a syringe pump (Harvard apparatus) and plastic syringes (Becton Dickenson), connected to devices using PEEK tubing (Upchurch Scientific) and luer lock stubs (Fisher).

Prior to seeding, channels were converted to a hydrophobic state utilizing a slight modification of our previously published method of *in situ* silane chemistry (1), using an octyl(tri-ethoxy)silane (OTES). After bonding PDMS and glass slides, devices were placed under vacuum for 1 hour to remove gas from the PDMS bulk.

During degassing, a 1%v/v OTES in 95:5 EtOH:H_2O is made, and after addition of 10% v/v Acetic Acid, the reaction is allowed to occur under vigorous shaking for 15 min, protected from light. After the devices are brought out of the vacuum, a droplet of the OTES solution is added all inlets and outlets. The solution is drawn into the channels as air fills the PDMS, then OTES is injected into inlets (a), (b), and (c) (Figure 1A and Figure S2A) at a flow rate of 10 μl/min for 35 min. The solution is allowed to react for a 10 more min without flow in the channels, followed by a wash with 600 μl pure ethanol at 20 μl/min into (a), (b), and (c), using a syringe pump to inject the fluid.

Bacteria were seeded in a specific pattern within the device by using a modification of the functionalization setup previously described (1). Devices were pre-equilibrated with phosphate buffered saline (PBS) pH 7.4, and inlets (a) and (c) (Figure 1A and Figure S2A) were injected at 10 μl/min for 2 min with inlet (b) unplugged, followed by lowering the flow rate of inlets (a) and (c) to 500 nl/min, with inlet (b) unplugged, for at least 2 min. Next, while inlets (a) and (c) are flowing, inlet (b) was plugged in with the bacteria solution, consisting of overnight culture at OD_{600} = 0.6, washed 3 times in PBS and diluted 1:10 in PBS. Subsequently, inlets (a) and (c) were flowed at 500 nl/min, and inlet (b) was flowed at 125 nl/min for 1 hour to seed bacteria. After seeding, inlet 2 was stopped, and inlets 1 and 3 were flowed for 5 min at 500 nl/min, and finally inlet (b) was unplugged. After seeding, conversion to perfusion mode was achieved by changing inlet (c) to an outlet, and blocking inlet (b) and outlet (d) (Figure 1 C, Figure S2 B). Channel blocking was achieved using a Syringe leur stub (Fisher) filled with cured PDMS and crosslinker.

Perfusion was carried out in a 37°C incubator, flowing TSB with 2.5% glucose at 18 μl/min for high flow rate devices, and 4.5 μl/min for low flow rates.

After perfusion for 6, 12, or 24 hours, biofilms in the microfluidic assay were first washed at 4°C with PBS at 18 μl/min or 4.5 μl/min for 20 min and fixed by flowing through 400 μl of 4% paraformaldehyde at the respective flow rate used for culture. The devices were incubated at 4°C for 12 hours, and then washed with 400 μl of PBS at 10 μl/min.

References

1. **Weaver W. M., S. Dharmaraja, V. Milisavljevic, and D. Di Carlo**. 2011. The effects of shear stress on isolated receptor-ligand interactions of Staphylococcus epidermidis and human plasma fibrinogen using molecularly patterned microfluidics. Lab Chip **11**:883–889.
2. **Xia Y., and G. M. Whitesides**. 1998. Soft Lithography. Annual Review of Materials Science **28**:153–184.

Supplementary Figures

Supplementary Figure 1: Determining the operational shear stresses present in catheter lumens. (A) Cross sectional surface plots of the velocity fields in single and double lumen catheters created in COMSOL Multiphysics. Dimensions were taken from measurement of Argyle and BD catheters. (B) Extraction of the cross-sectional velocity profile from A, illustrating how the wall shear stresses were calculated in each scenario. (C) Plots of the wall shear stresses in both single and double lumen catheters as a function of radial wall position. X-axis markers correspond to the markers on the double lumen catheter in A. Maximum shear occurs on the rounded face, where as shear stress minimum occurs in the corners. (D) A comprehensive plot illustrating the calculated operational ranges of various catheter sizes and types.

Supplementary Figure 2: Operational and initial conditions in the microfluidic biofilm assay. (A) DAPI stained cells just after seeding, prior to exposure to fluid shear. (B) The sizes of the patterns differ between chambers, chamber 1 having the largest pattern and chamber 4 having the smallest. (C) Despite differences in size, there is no significant difference in the surface density of the cells within the patterns between each chamber. (D) Using low volume percentages of 1 μm fluorescent beads (~0.00005 %), streak images of beads in flow were used to measure the average fluid velocity within the chambers. Dot plots show the velocity for each bead measured, and the black bars represent average velocities. Red Bars show the theoretical average fluid velocities calculated from fluidic resistances of

555 the channels and injected flow rates, assuming ideal fluidic resistance theory. Black
556 numbers give the average velocity calculated for each chamber. (E) Average
557 velocities measured in the resistor channels between the four chambers, where ($R_{i-(i+1)}$)
558 indicates flow between the ith and ith +1 chambers. Average velocities are
559 written above each dot plot in black.
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561 **Supplementary Figure 3:** (A-B) Microtiter plate assays verified that the strains
562 used in this study represent the three main biofilm phenotypes. (A) Strains 35984
563 and A-26 form biofilms under normal culture conditions, where as 12228 and A-10
564 form no biofilms under any culture conditions. (B) Strains A-5, W-166, and Z-173
565 are induced to form biofilms upon supplementation of media with increasing
566 concentrations of ethanol. These results agree with those obtained by Milisavljevic
567 et al. Measurements are presented as fold increase relative to control wells with no
568 seeded bacteria. (D-E) Both W-166 and Z-173 are induced to produce biofilms
569 when exposed to fluid flow. Both strains form biofilms after 12 hours and show no
570 significant increase in biofilm after 24 hours compared to 12 hours, indicating
571 biofilm formation sooner than A-5 (shown in Figure 3B). (§ : $P < 0.025$, ** : $P < 0.01$,
572 all measured against 1)
573
574 **Supplementary Figure 4:** A-5 clones collected from the microfluidic biofilm assay
575 revert to the original phenotype in microtiter plate assay. (A) A flow chart
576 illustrating the process of excising the biofilms from the microfluidic chip. The
577 Phase image indicates that there is biofilm in the chamber before excision. (B)

578 Streaks of clones from Chambers 3 and 4 from the 18 μl/min device (HQ1) and also
579 a control device where no bacteria were seeded (HQ3 ,-Bact) to show that colonies
580 picked from HQ1 and HQ2 were in fact from the channels, and not contaminants.
581 (C) Representative images of microtiter plate assays for all six clones assayed from
582 HQ1 and HQ2, as well as the A-5 input. (D) Quantitation of the microtiter plate
583 assays using fold increase in absorbance at 570 nm measured against a well in
584 which no bacteria were seeded.