Inducing Bacterial Biofilm Formation by Fluid Forces Using a Microfluidic Shear Array

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ABSTRACT
Staphylococcus epidermidis is a common cause of catheter-related bloodstream infections, resulting in significant morbidity, mortality, and increased hospital costs. The ability to form biofilms greatly increases the ability of *S. epidermidis* to cause these infections, however some clinically isolated strains do not form biofilms *in vitro*, although they contain the necessary genetic components. Significant phenotypic variation of biofilm formation within these strains presents an unanswered question as to the ultimate importance of biofilms in catheter related infections. Using a parallel microfluidic approach to investigate flow as an environmental signal for *S. epidermidis* biofilm formation, we demonstrate that clinically relevant fluid shear induces biofilm formation in certain clinical isolates and influences their biofilm structure. These findings suggest an important role of the catheter fluidic microenvironment in the establishment of *S. epidermidis* infections, and illustrates that characterization of clinical isolates must be conducted in a clinically relevant context.

KEYWORDS
Biofilm, Shear Stress, Microfluidics, *Staphylococcus epidermidis*

INTRODUCTION

Staphyloccocus epidermidis is a significant cause of hospital-acquired infections, specifically blood stream infections (BSI) that result from the use of long-term central catheter lines [1]. The ability to form biofilms, structured assemblies of secreted extracellular biomolecules and cells, has long been considered a key factor in the ability of *S. epidermidis* to cause serious catheter-related BSIs [2]. One caveat, which has long plagued the infectious disease community, lies in the significant variation of biofilm phenotype amongst clinically isolated strains. Even when only considering one type of biofilm matrix, the polysaccharide intracellular adhesin (PIA), there are strains that contain all the genetic components needed to make biofilms, yet form none under normal *in* vitro conditions in a 96-well plate format [3]. Further, some strains have been shown to begin to from biofilms when exposed to low concentrations of Ethanol and Benzyl Alcohol [4], however it is unclear if these conditions persist during infection long enough to effectively induce biofilm *in vivo.*

The catheter lumen, where *S. epidermidis* pathogenesis initiates, is a dynamic microenvironment inherently characterized by fluidic forces, however, little is understood regarding their effects on biofilm formation. Here we use a microfluidic bioreactor shear array coupled to a novel cross-flow seeding mechanism to create isolated populations of bacteria to be cultured under a range of shear stresses in parallel. Using this platform, we show that fluid shear stress, rather than washing biofilm away, actually induces formation of biofilms in clinically isolated strains that would not form biofilms under normal *in vitro* conditions. This platform, along with these initial observations, implicates the catheter environment itself as a factor in bacterial pathogenesis, and warrants consideration of fluidic forces in both the design and operation of catheters.

EXPERIMENTAL

We have employed a robust microfluidic device design (Fig. 1), combining parallel fluidic circuitry and a novel cross-flow seeding channel, to perform a range of shear stress measurements on isolated cell populations in each chamber. This allows for analysis of multiple identical populations without any upstream/downstream effects of metabolic bi-products or soluble signal secretion. Further, there is insignificant flow in the cross channels when in perfusion mode, as the circuit design results in no pressure drop across these channels, negating the need for complex two-layer valve systems. This ensures that each population of cells under a given shear is in fact isolated from its neighbors, experiencing different, but distinct shear stresses.

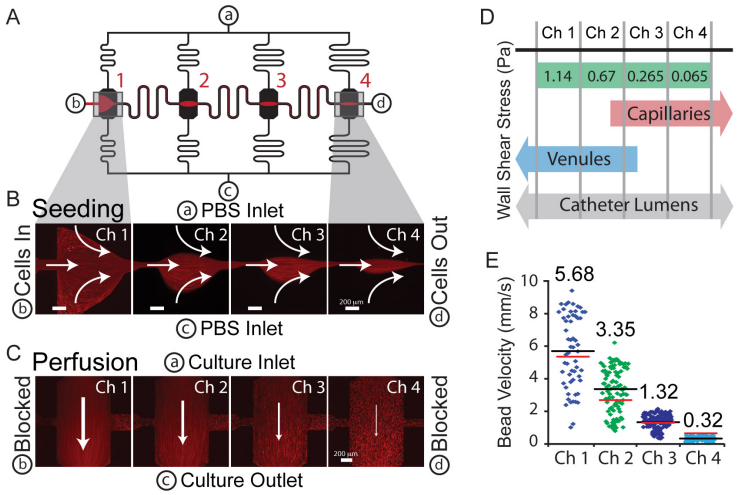

Figure 1: (A) Schematic of the device. (B) Seeding mode, 1 μm fluorescent beads in flow. (C) Perfusion mode used for culture (D) Shear stresses covered in one experiment (E) Fluid velocity in each chamber was measured using streak images of 1μm beads.

In order to characterize the quantitative capabilities of the platform, well-defined (both genotypically and phenotypically) *S. epidermidis* ATCC strains were cultured under flow in the device. Analysis of these strains showed that the microfluidic biofilm assay could differentiate between biofilm$^+$ (strain 35984) and biofilm$^-$ (strain 12228) genotypes with high confidence ($P<0.001$) over a range of shears and culture times. These initial experiments laid the groundwork for assays using clinically isolated strains of *S. epidermidis.*

Clinical isolates of *S. epidermidis* displayed a range of biofilm phenotypes when grown under clinically relevant fluid shear. Strains A-10 and A-26, although both containing biofilm$^+$ genotypes, display significantly different biofilm phenotypes under static conditions and flow. These results are in line with previous observations using conventional 96-well plate assays.

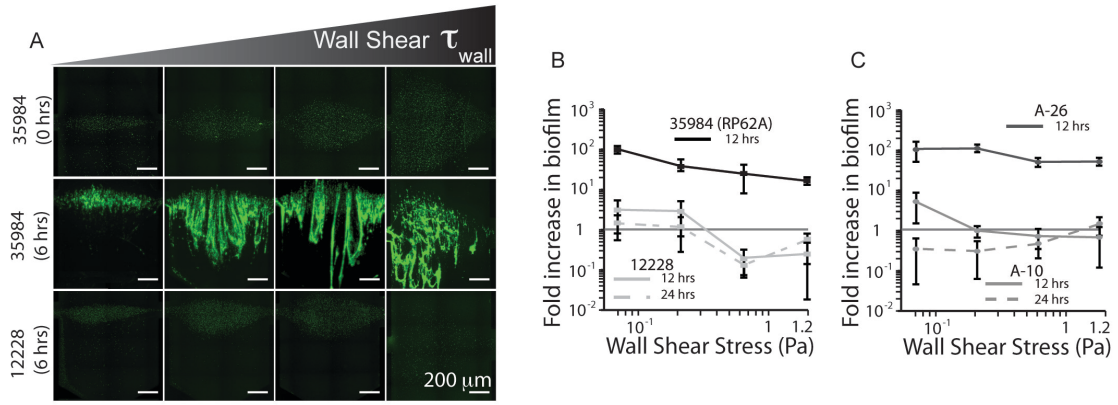

Figure 2: (A) Laboratory ATCC strains of S. epidermidis show distinct biofilm phenotypes under flow. Quantification of biofilm formation in (B) ATCC strains and (C) clinical isolates from blood cultures.

Interestingly, the clinical isolate strain A-5, which is unable to form biofilms when grown in a 96 well-plate assay without flow, was induced to switch to a strong biofilm$^+$ phenotype when grown under flow. Over a range of shear spanning two orders of magnitude, A-5 exhibited a biphasic dependence of biofilm formation on fluid flow (Fig. 3 A, B), increasing biofilm secretion until mechanical forces overcame cell adhesion. Interestingly, this strain has also been shown to be induced to form biofilm when exposed to low concentrations of ethanol [4].

To more rigorously determine if the observed biofilm formation in strain A-5 was due to an induced phenotype, or from selection of a previously existing mutant subpopulation, cells were collected from the biofilms grown under flow, and grown without flow in a 96 well-plate. When cells producing biofilm under flow were extracted and grown in non-inducing 'normal' *in vitro* conditions, they reverted to a wild type biofilm formation phenotype (Fig. 3 C,D).

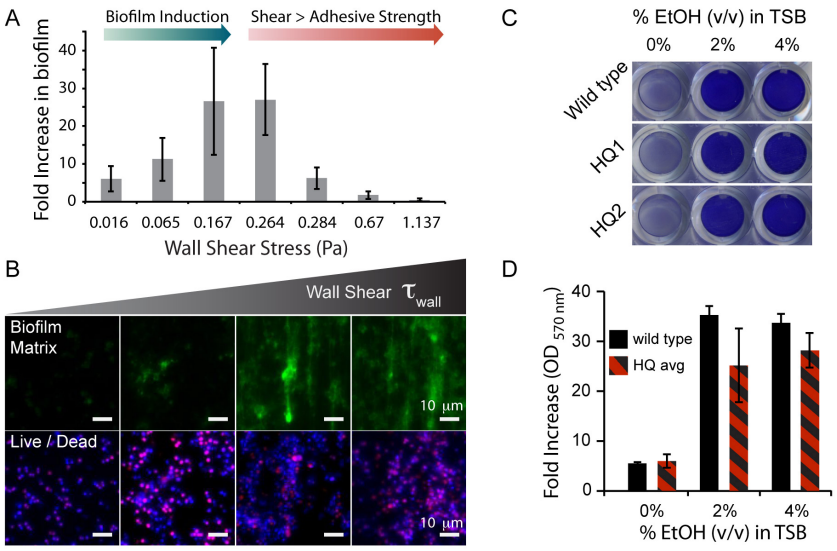

Figure 3: Clinical isolate strain A-5 is induced to form biofilm when exposed to fluid flow. (A) A biphasic dependence on shear. (B) At low shear, no biofilm matrix is present, but live cells are still on the surface (C) Cells collected from flow-induced biofilms revert to a wild type (no biofilm production) phenotype under no flow conditions.

Our results show that the catheter environment may play an important role in formation of biofilms in *S. epidermidis* clinical isolates. Notably, certain strains significantly increase their pathogenic potential when exposed only to fluidic forces present in catheters under clinical operating conditions. This data lays the groundwork for further investigation into the molecular mechanisms involved in shear induced biofilm formation, which may uncover a rare instance of a mechanosensing pathway in what are commonly considered evolutionarily primitive prokaryotic organisms. Most importantly, this warrants consideration of the mechanical forces when designing catheter lumens and operational protocols, offering a potentially simple solution to reduce infection rates.

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