



Bacteriology:

E. Coli - Adhesion - Biofilm Formation

OBJECTIVES

To elucidate the importance of physiological **shear stress** environment required for *E. coli* **adhesion, colonisation** and **biofilm formation** using the **Cellix VenaFlux™ Platform**.

INTRODUCTION

Escherichia coli typically colonises the gastrointestinal tract of human infants within a few hours after birth. Usually, *E. coli* and its human host coexist in good health and with mutual benefit for decades. However, there are several highly adapted *E. coli* clones that have acquired specific virulence attributes, which confirm an increased ability to adapt to new niches and allow them to cause a broad spectrum of disease. Three general clinical syndromes can result from *E. coli* infection: enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis (Application Note I100).

Bacterial adhesion to and subsequent colonization of surfaces are the first steps toward forming biofilms, which are a major concern for implanted medical devices and in many diseases¹. Biofilms are resistant to innate host defences, mechanical removal and antibiotic treatments. It is therefore important to understand the physiological environment and mechanisms that lead to the spread of bacteria.

Type 1 fimbriae are the most common type of adhesive organelles in *E. coli* and mediate mannose-specific adhesion via the fimbrial tip-associated lectin-like subunit FimH. FimH mediates 'catch-bonds' with mannose that are strengthened by tensile mechanical force. The environmental shear stress is of great importance in gaining an insight into the mechanism by which bacteria thrive, becoming resistant to therapies.

METHODS

i) Bacteria Strains/Culture:

The *E. coli* K-12 strain expressing FimH-wt was supplied by Prof. Evgeni Sokurenko for accumulation and mobility/firm adhesion assays along with FimH-j96 (A188D) for biofilm formation studies. Bacteria were grown overnight in Super-broth with appropriate antibiotics, washed twice in PBS, and brought to 10⁸ colony-forming units/ml in PBS containing 0.2% BSA (PBS-BSA). For *E. coli* accumulation assays, the K12 bacteria were premixed with 1% inhibitor, 50 nM in HBS-EP.

ii) Biochip Coating Procedure:

Each microchannel (400 μm wide, 100 μm deep) was coated in humid conditions at 37°C for 45 min with 10 μl of either 200 μg/ml mannose-BSA (monomannase), 20 μg/ml RnaseB (trimannose); or 10% BSA. Prior to flow experiments each channel was washed three times with PBS-0.2% BSA and quenched with PBS-0.2% BSA for 15 min to decrease non-specific binding.

iii) Adhesion Profiles/Image Analysis:

Bacteria was infused into the 1M, 3M and BSA coated channels using pre-defined shear steps from 0.1, 0.3, 1.0 and 8.0 dyne/cm², 100s per shear stress level. *E. coli* adhesion profiles of single cells were recorded using MetaMorph software. Cell images were captured from three microscopic fields from each channel and further analysed by ImageJ (<http://rsb.info.nih.gov/ij/>) and Cellix's DucoCell software. Data was exported into Excel to allow further analysis.

RESULTS

Shear Stress Enhances Accumulation of *E. coli* on Surfaces Coated with a 1M and 3M Ligand The bacteria adhered readily on the 1M and 3M surfaces at the lowest shears tested, and accumulation increased with increased shear (Figure 1). Inhibitors for 1M and 3M binding were used as controls to inhibit accumulation of the bacteria (Figure 1).

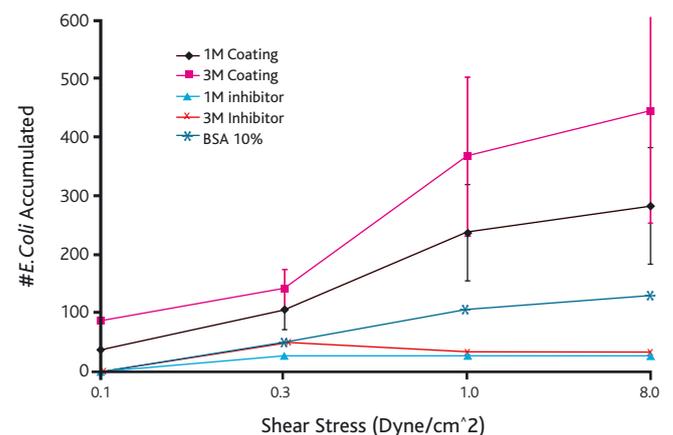


Figure 1: Accumulation Profile Of *E. Coli* Subjected To Shear Stresses On 1M And 3M Surfaces. *E. coli* treated or untreated with inhibitors were infused into Vena8™ channels coated with either 1M or 3M ligands and subjected to an increasing shear stress of 0.1, 0.3, 1.0 and 8.0 dyne/cm².



Increased Shear Stress Results in a Transition from Rolling to Stationary *E. coli* Adhesion Following the FimH-expressing *E. coli* accumulation to 1M and 3M ligands, the bacteria exhibit two adhesion modes: weak rolling adhesion (Figure 2) and firm stationary binding (Figure 3). The rolling mode dominates at lower shears. Shear stresses of 0.1, 0.3 and 1.0 dyne/cm² result in a high percentage of motility of the *E. coli* (Figure 2). At higher shear stress of 8.0 dyne/cm² the mobility of the bacteria is low (Figure 2) as the bacteria binding to the surfaces convert into stationary firm adhesion (Figure 3).

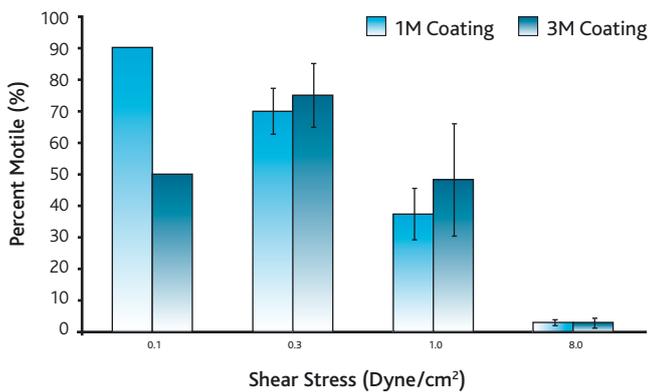


Figure 2: Motility Of *E. Coli* Under Shear Stress. Under a range of increasing shear stresses (0.1, 0.3, 1.0 and 8.0 dyne/cm²) the *E. coli* are infused into 1M and 3M coated channels.

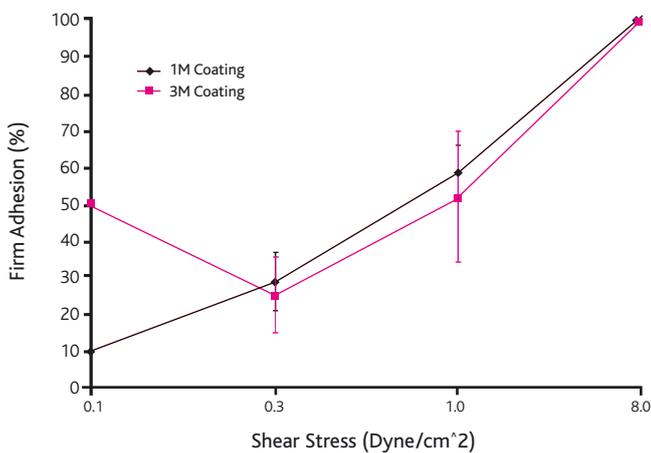


Figure 3: Firm Adhesion Profile Of *E. Coli* Under Shear Stress. The percentage of *E. coli* firmly adhering to 1M or 3M coated surfaces examined at increasing shear stresses.

Low Shear Stress On Accumulated FimH *E. coli* Results In Time Dependent Biofilm Formation Rapid spreading of the FimH *E. coli* results via weak rolling adhesion on 1M coated.

surface at low shear stress of 0.3 dyne/cm² (Figure 4). A four-fold increase in bacterial surface coverage is noted over the time course of 3 hrs.

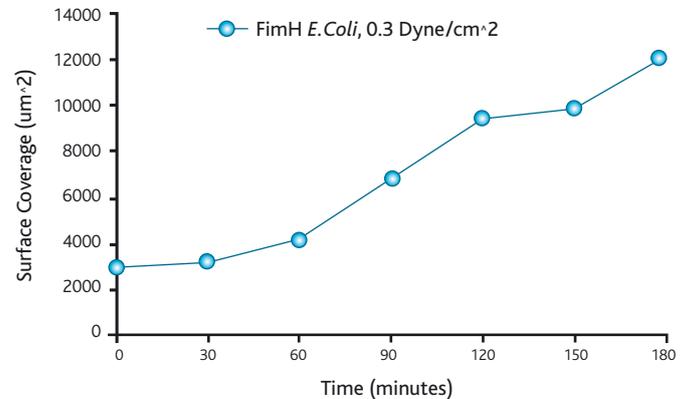


Figure 4: Time-Dependent Formation Of Biofilm. *E. coli* was subjected to shear stress of 0.3 dyne/cm² for time course of 3hrs on 1M coated channels. Surface coverage of bacteria measured as µm².

DISCUSSION

To reiterate the importance of shear stress in *E. coli* adhesion, colonisation and biofilm formation, we subjected the bacteria to a range of shear stress that determined the fate of bacterial adhesion. Previously published data supports our finding that bacteria weakly adhere at low shear stress and in a time-dependent manner results in biofilm formation². This is due to the degree of mobility of the bacteria at low shear stress. Also, at higher shear stresses the bacteria accumulate on the 1M and 3M ligands indicated by the firm adhesion and the low mobility of the *E. coli* bacteria. These studies gain insights into the mechanism by which bacterial infections spread. Cellix's VenaFlux™ platform provides researchers the opportunity to further investigate shear-dependent/-independent adhesion of *E. coli* in the urinary tract or other bacteria in pathological conditions.

REFERENCES

- ¹-Nilsson L, Thomas W, Trintchina E, Vogel V and Sokurenko E. Catch Bond-mediated Adhesion without a Shear Threshold. *Journal of Biological Chemistry*; 281; 24, 16656-16663, 2006.
- ²-Anderson B et al. Weak Rolling Adhesion Enhances Bacterial Surface Colonization. *Journal of Bacteriology*; 189; 5, 1794-1802, 2007.

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