we are cellix

# Application Note B100

## Bacteriology: *E. Coli*; Adhesion; Biofilm Formation

**Cellix Ltd.**
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

## 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 colonisation of surfaces are the first steps toward forming biofilms, which are a major concern for implanted medical devices and in many diseases [1]. 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

1. 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 $10^8$ 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.

2. Biochip Coating Procedure

Vena8 Fluoro+ biochip (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.

3. 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/cm$^2$, 100 s per shear stress level. *E. coli* adhesion profiles of single cells were recorded using Venaflux assay software. Cell images were captured from three microscopic fields from each channel and further analysed by ImageJ (http://rsb.info.nih.gob/ij) and Image Pro

**Cellix Ltd.**
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

Premier 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).

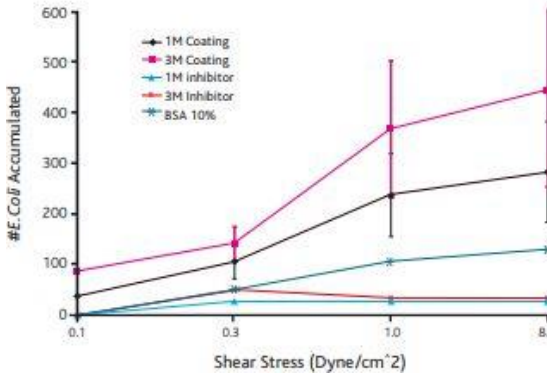


*Figure 1*

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 Fluoro+ biochips 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/cm$^2$.

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/cm$^2$ result in a high percentage of mobility of the *E. coli* (Figure 2). At higher shear stress of 8.0 dyne/cm$^2$ the mobility of the bacteria is low (Figure 2) as the bacteria binding to the surfaces convert into stationary firm adhesion (Figure 3).

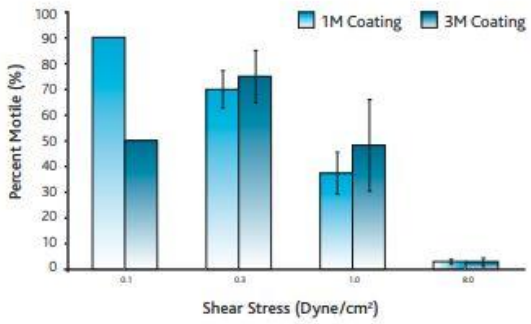


*Figure 2*

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/cm$^2$) the *E. coli* are infused into 1M and 3M coated channels.

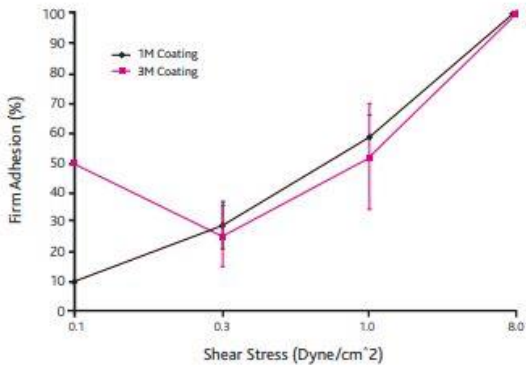


*Figure 3*

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

**Cellix Ltd.**
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

dyne/cm$^2$ (Figure 4). A four-fold increase in bacterial surface coverage is noted over the time course of 3 hrs.

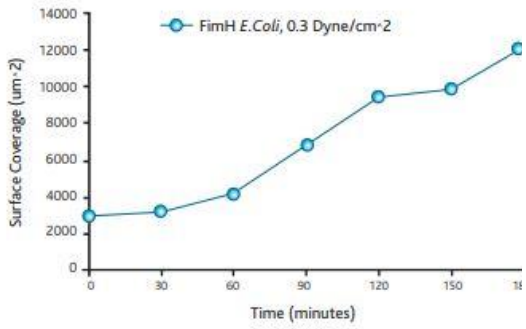


*Figure 4*

Figure 4: Time-dependent formation of biofilm. *E. coli* was subjected to shear stress of 0.3 dyne/cm$^2$ for time course of 3 hrs on 1M coated channels. Surface coverage of bacteria measured as µm$^2$.

## 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 formation [2]. 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 or shear-independent adhesion of *E. coli* in the urinary tract or other bacteria in pathological conditions.

## Acknowledgements

We thank Evgeni V. Sokurenko, Veronika Tchesnokova and Olga Yakovenko, Department of Microbiology University of Washington School of Medicine, Seattle, USA for experimental execution and fruitful collaboration.

## 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.