Bacterial Mechanics and Mechanobiology

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Primary CNF Tools Used: AJA sputter deposition, ASML stepper, PT 770, Oxford 100, MOS clean anneal

Abstract:

Bacteria naturally experience mechanical forces in the environment. Bacteria experience mechanical forces as they grow and divide, swim in fluids, attach to surfaces, and grow in biofilms. Although it has been well established that mechanical forces are key signals for eukaryotic cell development and physiology, much less is known about the importance of mechanical forces for bacterial cells. This is in part because applying controlled mechanical stimuli to bacterial cells is technically challenging due to the small scale of bacteria (~ 1 μm wide). We developed a microfluidic platform to apply mechanical stimuli to individual, live bacteria cells. We use this microfluidic platform to apply mechanical loads to *E. coli* and *V. cholerae* cells in order to understand how mechanisms of antibiotic resistance respond to mechanical stimuli. Additionally, we use this microfluidic platform in conjunction with finite element modelling to quantify the mechanical properties of the bacterial cell envelope.

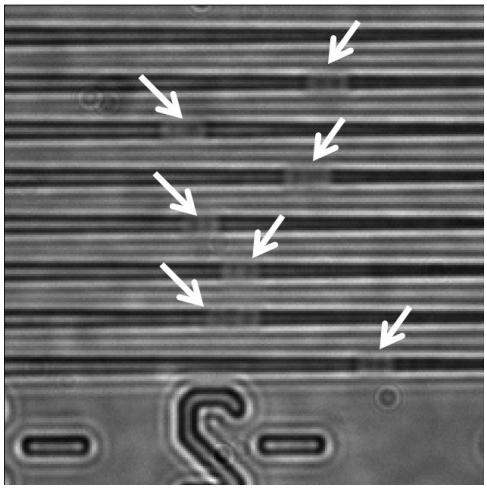

Figure 1: E. coli *cells trapped within the tapered channels of the microfluidic device. Fluid pressure is used to flow the bacteria into the tapered channels.*

Summary of Research:

Our work involves the use of microfluidic devices to apply a mechanical stimuli to individual bacteria. Within our devices, fluid pressure pushes individual bacteria into narrow tapered channels (Figure 1). The bacteria experience mechanical loading from the hydrostatic fluid pressure as well as contact with the tapered channels walls (Figure 2). The amount of mechanical loading a cell experiences depends on the fluid pressure, which is varied strategically within the device. During a single experiment, different cells experiencing different magnitudes of mechanical loading can be observed simultaneously. The bacteria remain alive while trapped in the tapered channels and continue to elongate and divide. Cells remain viable for up to 12 hours in the devices.

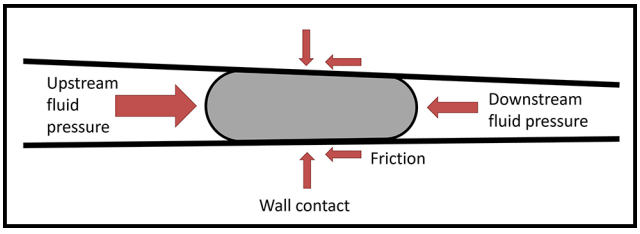

Figure 2: The bacteria cells experience mechanical loading in the tapered channels due to the fluid pressure, which varies from the upstream end to the downstream end.

Key advantages of this microfluidic platform include minimal sample preparation, no chemical immobilization or labeling, and the ability to analyze hundreds of cells at once [1].

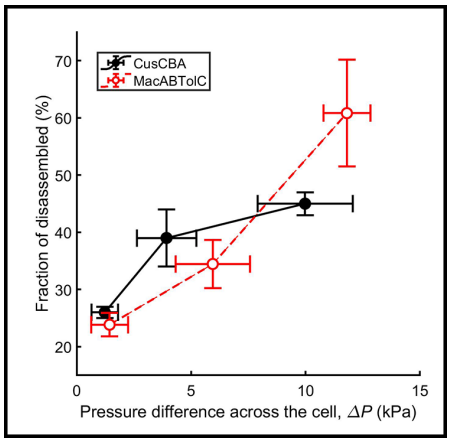

Figure 3: Increased mechanical loading (pressure difference across the cell), was shown to increase disassembly of the multicomponent efflux complexes CusCBA and MacABTolC in E. coli.

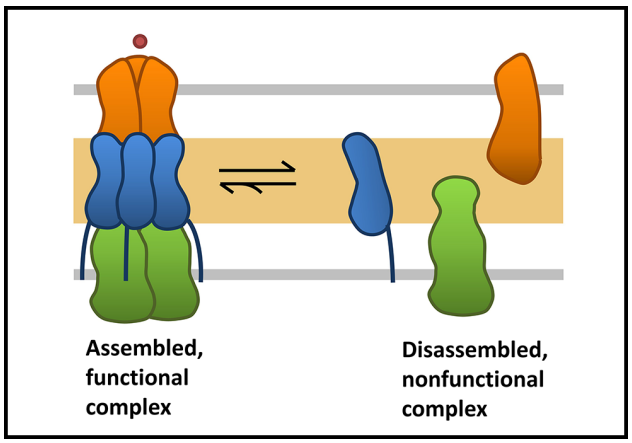

Figure 4: Multicomponent efflux complexes can be in an assembled and functional form or a disassembled and nonfunctional form. Disassembled complexes are unable to efflux toxins and antibiotics.

We manufacture our microfluidic devices on fused silica glass wafers using deep UV photolithography to achieve nano-scale features (250 nm smallest dimension). These glass-on-glass devices are manufactured using the AJA sputter deposition, ASML, PT770, Oxford 100, VersaLaser, and MOS clean anneal tools.

We are investigating the effects of mechanical stress and strain on two systems of antibiotic resistance found in bacteria: multicomponent efflux complexes and two component signal transduction systems. Multicomponent efflux complexes create channels that cross the cell envelope of bacteria and are used to pump toxins and antibiotics out of the cell. Our data suggests that the assembly and function of the multicomponent efflux complex CusCBA in *E. coli*, which effluxes the toxin copper, is impaired by increased mechanical loading (Figure 3) [2].

Preliminary evidence shows that other trans-envelope multicomponent complexes are also sensitive to the mechanical stress experienced by the cell. Disassembly of the multicomponent efflux complex MacABTolC, which effluxes macrolide antibiotics, also increases with the magnitude of mechanical loading (Figure 3) [3]. Disassembled CusCBA and MacABTolC complexes are nonfunctional and incapable exporting copper toxins and antibiotics, suggesting toxin and antibiotic resistance of mechanically stressed cells is reduced (Figure 4).

Two-component signal transduction systems are a key mechanism bacteria use to sense external stimuli and respond by altering gene expression. We are currently investigating a two-component system in *V. cholerae* that controls cell wall homeostasis and is essential for resistance to antibiotics that damage the cell wall. Preliminary work shows that this signaling pathway is activated by mechanical loading in our microfluidic device, providing exciting evidence that mechanical stimuli can affect gene expression in bacteria.

We are also working to better understand bacterial mechanical properties by combining experimental data from the microfluidic devices with finite element modelling to calculate numerical estimates for the Young's Modulus of the bacterial cell envelope. Establishing a reliable method of measuring the mechanical properties of the bacterial cell envelope will help us identify subcellular components that contribute to bacterial mechanics as well as how different environmental factors such as antibiotic treatment can change bacterial mechanical properties.

Conclusions and Future Steps:

So far our research has shown that mechanical stress and strain impairs the proper assembly and function of the cellular machinery needed for toxin and antibiotic efflux in *E. coli*. We also have preliminary evidence that a two-component system in *V. cholerae* regulates gene expression in response to mechanical stimuli.

In the future we will focus on using our microfluidic device to quantify the mechanical properties of different components of the bacterial cell envelope.

References:

[1] X. Sun, W.H. Weinlandt. H. Patel, M. Wu, C.J. Hernandez. (2014) "A Microfluidic Platform for Profiling Biomechanical Properties of Bacteria." Lab on a Chip. 14 (14), 2491-2498. NIHMS600175.

[2] L.A. Genova, M.F. Roberts, Y.C. Wong, C.E. Harper, A.G. Santiago, B. Fu, A. Srivastava, W. Jung, L.M Wang, Krzemiski, X. Mao, X. Sun, C.Y. Hui, P. Chen, C.J. Hernandez. (2019) "Mechanical stress compromises multicomponent efflux complexes in bacteria". Proc. of the National Academy of Sciences U. S. A. 116 (51) 25462-25467.

[3] C.E. Harper, W. Zhang, P. Chen, C.J. Hernandez. (2020) "Mechanical stress promotes disassembly of the antibiotic efflux complex MacAB-TolC". Biophysical Society AM. San Diego, CA, USA.