

Biomechanics of Bacteria

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Primary CNF Tools Used: ASML, Oxford 100, AJA sputter deposition, VersaLaser engraver/cutter, MOS clean anneal

Abstract:

The mechanical properties of the bacterial cell envelope influence cell growth, cell division and subcellular localization of membrane proteins. Here we demonstrate the ability to apply mechanical loads to live bacteria, the first step toward determination of mechanical properties of bacterial components *in vivo*. Additionally, we show that devices based on the same concept have the ability to separate bacterial species/strains from one another based on the cell mechanical phenotype.

Summary of Research:

In bacteria, the ability to resist mechanical forces is necessary for survival and growth, allowing cells to withstand osmotic pressures while maintaining cell shape, cell growth and division. Hence, the mechanical properties of bacteria and bacterial structural components influence species competition and resistance to toxins and antibiotics. Our work involves the use of micro/nano fabricated devices as tools for mechanical testing of live bacteria. Within our devices, individual bacteria are flowed into tapered channels travel a distance related to whole cell stiffness: less stiff cells are able to travel further in to the channels (Figure 1).

Key advantages of this microfluidic platform for profiling the biomechanical properties of bacteria include: minimal sample preparation, no chemical immobilization or labeling, and the ability to analyze hundreds of cells at once.

In our first series of experiments we manufactured devices on silica glass wafers using Deep UV photolithography to achieve nano-scale features (250 nm smallest dimension). These glass on glass devices were manufactured using the ASML, Oxford 100, AJA sputter deposition, VersaLaser and MOS clean anneal tools at the Cornell NanoScale Science and Technology Center.

In the first device, design bacteria in liquid culture were submitted to up to 12 different applied pressures to establish the biomechanical profile of two model organisms, *E. coli* and *B. subtilis*.

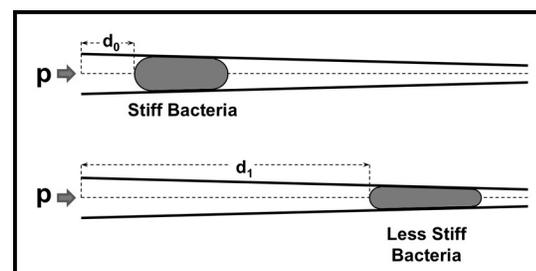


Figure 1: Bacteria under fluid pressure (p) are forced into tapered channels. The distance a cell travels into a tapered channel depends on cell stiffness with more compliant cells traveling further into the channels. The distance traveled by a cell into the tapered channel (d_1) is therefore an indicator of cell stiffness. Viewing the deformation of a cell under two different applied pressures can be used to determine the mechanical properties of the cell envelope.

Our results demonstrated differences in stiffness between *E. coli* and *B. subtilis* (Figure 2) and suggested that a device with a shorter channel length would allow transport of *E. coli*, but not *B. subtilis*, potentially allowing for separation of bacteria based on the biomechanical properties [1]. When combined with theoretical mechanics models it allowed us to determine the stress distribution within individual bacteria and study their response to mechanical stimulation [2].

In our recent work we have explored the effects of mechanical loads on the assembly/disassembly of multicomponent efflux pumps. Multicomponent efflux pumps are three-part channels that cross the inner membrane, periplasm and outer membrane of bacteria and are used to remove toxins (excessive metal ions, antibiotics, etc.). Our data suggests that the assembly and function of multicomponent efflux pumps is sensitive to mechanical stress and strain [4].

References:

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- [3] M.F. Roberts, A. Srivastava, L.M. Wang, C-Y Hui, L.A. Genova, P. Chen, C.J. Hernandez, "A microfluidic system for mechanical characterization and stimulus of individual bacteria," European Society of Biomechanics 2017.
- [4] M.F. Roberts, L.A. Genova, L.M. Wang, P. Chen, C.J. Hernandez, "Non-Uniform Mechanical Stress Promotes Metal Efflux Pump Disassembly," 62nd Annual Meeting, Biophysical Society. San Francisco, CA, USA. 2018.

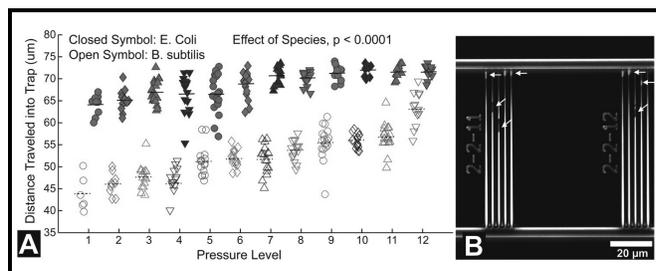


Figure 2: (A) The position of bacteria occupying trap channels at twelve different pressure levels (where level 1 is lowest and level 12 is greatest) in a single experiment are shown. Horizontal lines indicate averages at each pressure level. *E. coli* travel further into the traps than *B. subtilis* overall ($p < 0.0001$, ANCOVA) as well as at each individual pressure level ($p < 0.0001$, *t* tests). (B) Differences in bacterial stiffness between species can be detected in a mixed culture. *E. coli* expressing GFP (green, indicated by the upper horizontal arrows) traveled further into the trap channels than *B. subtilis* (indicated by tilted arrows), demonstrating the possibility of separating bacteria based on mechanical phenotype.