Characterization of *Escherichia Coli* HM22 Persister Cells with AFM Imaging

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Statement of Purpose: Approximately 65% of surgery related infections in developed countries are caused by biofilms that exhibit multidrug tolerance (MDT)[1]. One of the major mechanisms of biofilm MDT is the formation of metabolically inactive persister cells that are found in low concentrations within normal cell populations. Persisters are naturally occurring phenotypic variants, rather than genetic mutants. Because they are dormant, persisters do not react to antibiotic treatments. Instead, they survive in the body until such time as they awaken and repopulate the colony. This study attempts to categorize the morphological differences between the regular cells in exponential phase and persister cells of *Escherichia coli* HM22 using atomic force microscopy (AFM). The AFM is used to measure the differences in height, length, width, and aspect ratio between typical specimens of regular and persister cells.

Methods: Imaging was performed using a Multi-Mode AFM-2 with a Nanoscope IIIa controller (Veeco Instruments, Inc). AFM contact mode was used. This mode directly measures specimen height, friction, and deflection to obtain the sought after morphological characteristics. Quantitative measurement of cell morphology was performed directly from AFM images.

E. coli HM22 was first cultured overnight in 25 mL LB medium supplemented with 25 uL DPAat 37°C with shaking at 200 rpm. Then it was subcultured for 3 hours in the same medium before 100 µg/mL ampicillin was added to lyse regular cells. The persister cells were collected by centrifugation and plated onto circular glass cover slips. These cells were then vacuum dehydrated and imaged using AFM.

Results: AFM demonstrated that *E. coli* persister cells exhibit significant morphological differences from normal, exponential cells, as seen in Figure 3. Exponential cells are longer than persister cells ($p < 0.05$). Persister cells are taller than exponential cells ($p < 0.05$). Cell widths of the two cell types were, however, quite similar ($p > 0.05$).

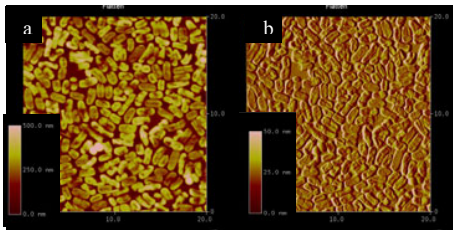

Figure 1. Exponential HM22 cells. Image 1a displays height data. Image 1b displays deflection data.

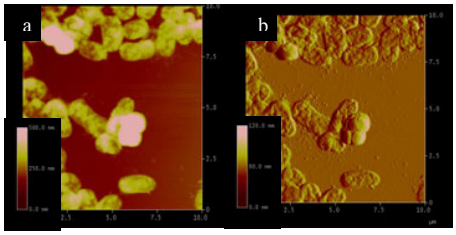

Figure 2. Round, orbital shaped persisters among longer exponentials. Image 2a displays height data. Image 2b displays deflection data.

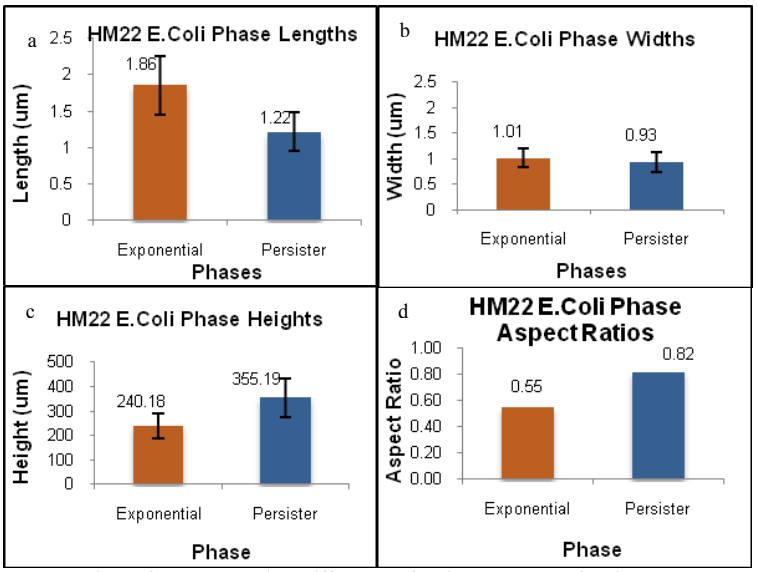

Figure 3. Morphological differences. 3a displays length. 3b displays width. 3c displays height. 3d displays aspect ratio.

HM22 exponential cells appear in Figure 1. Note the elongated shape of the exponential cells, with an average aspect ratio of 0.55. Closer inspection of Figure 1a – the height image – displays three persister cells resting among exponential cells. These cells are strongly differentiable by height. Figure 2 shows four relatively small and round persisters among a population of longer exponential cells.

Discussions and Conclusions: This study demonstrates that AFM imaging is an effective method of qualitatively and quantitatively characterizing bacterial phenotypes. The atomic force microscope is largely underutilized in the field of bacterial studies. That AFM allows for the study of live specimens, possesses superior clarity compared to other similarly powered microscopes and is capable of producing images of surface details and features should prompt its use in biological research.

The aspect ratios of HM22 persister cells are clearly and visibly different than their exponential counterparts. The 0.55 aspect ratio of a typical exponential cell suggests an elongated, pill-like shape. The 0.82 aspect ratio of the typical persister cell suggests a much more rounded shape. Further research is warranted to determine whether the unique shape of persisters reveal insights into their physiological stage. By conserving volume and decreasing surface area, persister cells may be able to conserve energy while minimizing extracellular interactions; ideal for a dormant state. Membrane differences may play a role in persister's MDT.

References: (1) Lewis, K., "Persister cells, dormancy and infectious disease," Nature, vol. 5, pp. 48-56, Jan. 2007. **Acknowledgements:** G. Choudhary, Dr. H. Ehsan, N. Jawrani, S. Sivan, Syracuse Biomaterials Institute REU Program. We also thank Dr. Kim Lewis at Northeastern University for providing the strain of *E. coli* HM22.