

Using Microfluidic Devices to Understand the Growth and Migration of *S. Aureus* within Submicron Channels

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Primary CNF Tools Used: AJA Sputter Deposition Tool, Gamma Automatic Coat-Develop Tool, ASML Deep UV stepper, Gamma Automatic Coat-Develop Tool, Oxford 82 Tool, Plasma-Therm 770 ICP tool, Oxford 100 Tool, Versalaser, AFM, Profilometer, Zeiss Ultra 55 SEM microscope

Abstract:

Microfluidic devices were created to understand the growth and migration of the human pathogenic bacterium, *Staphylococcus aureus* through nanoscale channels. This work was inspired by human clinical studies, which have found that *S. aureus* is able to colonize channels in the bone tissue matrix that are 2-3 times smaller than cell diameter during infection. It is unknown how *S. aureus* enters channels given that the bacterium is normally non-motile. It has been proposed that cells may enter via growth – such that daughter cells form within channels and then proliferate down the channel length. Using microfluidic devices, we tested the idea that *S. aureus* could enter channels smaller than cell width via growth and division. We found that proliferation could facilitate migration, but only to cell deformations of 600 nm (65% cell width). Below 600 nm, mechanical confinement significantly slowed or completely inhibited division. Thus, *S. aureus* is unlikely to traverse nanoscale channels in bone via division. In contrast, we found that physiologically relevant fluid pressures generated around bone in vivo could force cells into channels smaller than cell width.

Summary of Research:

The Hernandez Research Group has developed a microfluidic device, which can be used to deform single bacterial cells to submicron widths¹. The device is manufactured from etched silica wafers, and consists of a series of tapered channels, that narrow in width from ~1.5 to 0.3mm (Figure 1). When a suspension of bacteria is flowed into the device, individual bacteria are trapped within tapered channels by a pressure differential (ΔP) generated between the channel inlet and outlet. There are 4 distinct loading pressures generated within one

device due to the parallel, ladder-like arrangement of channel sets. Greater pressure differences cause cells to travel further into the channel and experience greater deformation. Our prior works have shown that cell deformation within channels activates mechanosensitive stress response systems and can be used to infer information on cell stiffness^{2,3}.

Recently, we used devices to understand how *S. aureus* colonizes the bone tissue matrix during infection⁴. Specifically, microscopic studies have found *S. aureus* within channels in bone, known as canaliculi⁵. Canaliculi range in width from 300 to 900nm, requiring *S. aureus* to deform to only 1/3 its original size to navigate. The ability of *S. aureus* to colonize canaliculi is likely a major contributor to treatment failure and chronic infection, as bacteria become protected from immune cell clearance and antibiotic therapy within nanochannels. It is generally unknown how *S. aureus* colonizes canaliculi, but it has been proposed that cells may enter through division^{5,6}. Therefore, to test this idea, we determined if proliferation could lead to *S. aureus* migration within our microfluidic device.

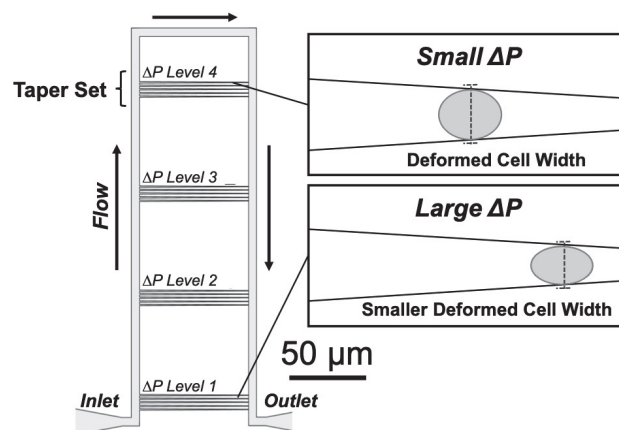


Figure 1: Schematic illustration of the microfluidic device.

We used fluidic pressure to deform 322 individual cells to varying widths within microfluidic channels. We observed that deformation slowed *S. aureus* division, as cells that were more deformed took longer to divide (Figure 2A). When cells were deformed more than 606nm, no divisions were detected. We speculated that mechanical confinement may inhibit proliferation if the division plane is positioned parallel to the channel walls, restricting cell growth across the width of the channel. However, fluorescent labeling of proteins localized to the division plane showed that most cells had planes aligned perpendicular to the channel walls in the orientation least likely to impair division (Figure 2B). Thus, mechanical deformation inhibits *S. aureus* growth through an unidentified stress response, making it unlikely that bacteria enter canaliculi in bone through division.

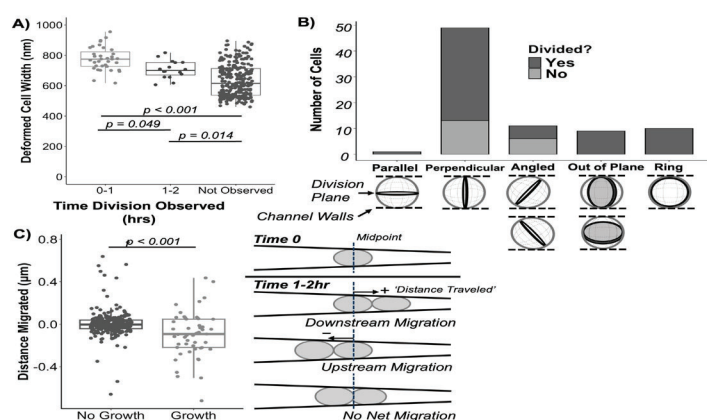


Figure 2: (A) The deformed width of cells that did or did not divide within the device is shown ($n = 322$). (B) Orientation of the division plane is shown relative to channel walls for cells within the device ($n = 80$). (C) The distance migrated for cells that divided within channels ($n = 51$) and for cells that did not divide ($n = 271$) is shown with an illustration of the analysis method.

To further verify that *S. aureus* colonization of canaliculi via growth was unlikely, we determined whether cell division within channels resulted in cell migration. We hypothesized that division would result in the net migration of cells towards the narrower end of the channel if cells could preferentially sense and divide into canaliculi as proposed^{5,6}. We found, however, that growth and division had a marginal effect on cell positioning. On average, dividing cells migrated only 97 ± 215 nm and showed a slight bias (56% of cells studied) to migrate upstream towards the wider end of the channel (Figure 2C).

Conclusions and Future Steps:

Given our observation of impaired growth in deformed cells, coupled with our findings that proliferation does not lead to significant migration, we conclude that *S. aureus* is unlikely to enter canaliculi in bone through growth and division. As *S. aureus* is non-motile, external forces would be required to transport cells into canaliculi. Therefore, we speculate that *S. aureus* transport in bone may be facilitated by fluid pressures. Based on our studies investigating *S. aureus* deformation as a function of applied pressure, we estimate that only 9kPa would be needed to force cells into channels measuring 300nm. Fluid pressures generated in bone during normal physical activity have been reported to be as high as 8 to 20kPa⁷. Thus, the ability of bacteria to colonize canaliculi may be more closely related to physical loads on the bone and the mechanical properties of the bacteria rather than growth. Future work will determine if differences in cell stiffness between clinical isolates of *S. aureus* effect canalicular colonization and subsequent infection severity.

References:

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