

## The Effect of Pathophysiologic Glucose Concentration on Biofilm Growth In Vitro

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**Statement of Purpose:** It has long been recognized that the growth rate of bacteria is dependent on the concentration of nutrients in the media. Until recently, it was not appreciated that some of those nutrients may play a role as signaling molecules, affecting the behavior of the bacteria. Recent research by multiple centers has suggested that the potential of bacteria to form biofilm is moderated by the concentration of glucose. Fluorescence studies suggest that the presence of increasing concentrations of glucose affects the *agr* pathway, leading to the formation of biofilm. It has also been suggested that the pH of the local cellular environment may play a role in this signaling process. Additional studies have found that in controlled conditions, there is a consistent increase in biofilm production across many different strains and bacterial types in media with high (1%) concentrations of glucose. However, the concentrations used to generate biofilms in many of these studies are in a greatly super-pathophysiological range. It is unclear if the authors accounted for the glucose already in the growth media when they additionally supplement the media with glucose. This study seeks to explore the relationship between biofilm formation and glucose concentration in a physiologic to pathophysiological range. This study asks the question: What is the effect of pathological concentrations of glucose on biofilm growth?

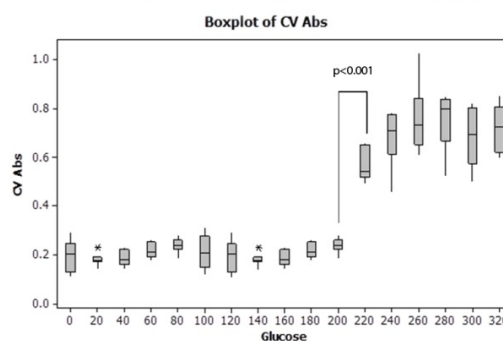
**Methods:** Two standardized strains were selected for this work: *Staphylococcus epidermidis* (ATCC 35984) and *Staphylococcus aureus* (ATCC 49230, UAMS 1). Biofilms were grown in accordance with the procedure documented by Kwasny and Opperman, excepting the use of a different media with varying glucose concentrations. Cells were grown overnight in Lenox Broth. Overnight culture was diluted 100-fold with growth media supplemented with varying glucose concentrations. Glucose concentrations ranged from 0-320 mg/dL at intervals of 20mg/dL. 200  $\mu$ L of the bacterial dilutions were seeded in tissue culture treated 96 well plates. Plates were then incubated at 37°C for 24-48hrs without shaking. Excess liquid culture was removed and biofilm were washed four times by gentle immersion in deionized water. Biofilm was then heat fixed at 60°C for 1 hour. Biofilm was stained with 0.1% crystal violet dye (Best Science Supplies, Big Pine Key, Florida) for 15min. Crystal violet dye was removed and the biofilm washed four times by gentle immersion in deionized water. Finally, crystal violet dye was eluted with 30% acetic acid solution for 15min. 200  $\mu$ L of extracted dye was then transferred to unused wells in the 96 well plate and assayed by Fluostar Omega Multiplate Reader (BMG Labtech, Cary, North Carolina) at 600nm. Samples were intermittently photographed with a Nikon Macro Lens.

### Statistical Analysis:

Amount of biofilm grown over time was analyzed with ANOVA with glucose concentration as a factor. Post-hoc analyses were performed with Tukey's Test for multiple

comparisons to identify similar groups. Standard Normal plots of residuals were constructed to determine if the ANOVA model was well behaved.

**Results:** *Staphylococcus epidermidis* was a much more robust biofilm former than *staphylococcus aureus* at all glucose concentrations ( $p < 0.001$ , ANOVA). Both species tested demonstrated a statistically significant increase in the production of biofilm with increased glucose concentration in the culture media ( $p < 0.001$ , ANOVA). *Staphylococcus epidermidis* showed a consistent increase in biofilm formation with no clear break point. This is in distinction to *Staphylococcus aureus* which did not demonstrate a substantial increase in biofilm production until a threshold was crossed between 200mg/dL and 220mg/dL ( $p < 0.001$ ).



**Figure 1: *Staphylococcus aureus* growth as a function of glucose concentration.** Glucose concentration is reported in mg/dL. There is a significant trend of increased growth as the concentration increases.

**Conclusions:** The rate of biofilm formation of *Staphylococcus aureus* and *Staphylococcus epidermidis* is quite different, at least for the two strains studied. In the same time period, *Staphylococcus epidermidis* produced five times the amount of biofilm produced by *Staphylococcus aureus*. These results support that pathophysiological glucose concentrations would be expected to impact biofilm formation rates *in vivo*. These results suggest that the formation of biofilm *in vivo* is happening over hours to days after exposure, and that the presence of pathophysiological glucose concentrations, depending on other environmental variables and bacterial types, may reduce the time that the body has to respond to the pathogen before it is well established in biofilm. Thus, postoperative glucose management may be critical in controlling the subsequent PJI infection rate.

*In vitro* testing indicates a statistically significant increase in biofilm formation for strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* as glucose concentration increases into the pathologic level.