Influence of Glucose Concentration on Biofilm Development by *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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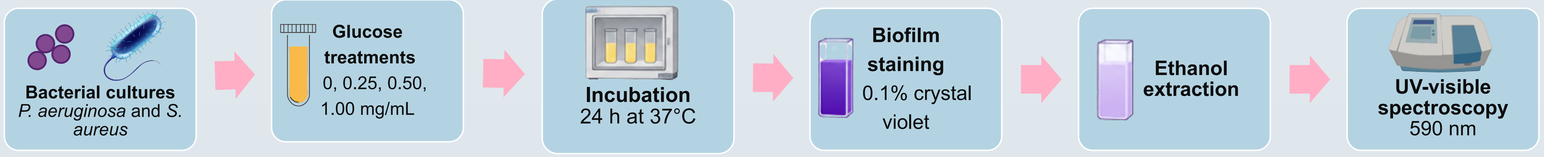
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Introduction

Bacterial biofilms are problematic in healthcare because they can develop on medical devices used in diabetes management, including insulin pump infusion sites and continuous glucose monitoring devices, and they are also important in infections associated with diabetic wounds where treatment and healing may be more difficult (Banu et al. 2015; Patel and Priefer 2021).
Understanding how glucose concentration affects biofilm formation may help explain factors that contribute to biofilm growth in diabetes related environments.
The hypothesis of this study is that increasing glucose concentrations of 0, 0.25, 0.50, and 1.00 mg/mL will result in greater biofilm formation in both *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Materials & Methods

S. aureus and P. aeruginosa were grown in nutrient broth (NA) containing four glucose concentrations: 0, 0.25, 0.50, and 1.00 mg/mL. Three replicates were prepared for each glucose concentration and bacterial species and incubated for 24 hours at 37°C. After incubation, the cuvettes were washed and stained with 0.1% crystal violet, followed by ethanol extraction. Biofilm formation was measured using ultraviolet-visible spectroscopy at 590 nm, with greater absorbance representing greater biofilm formation.



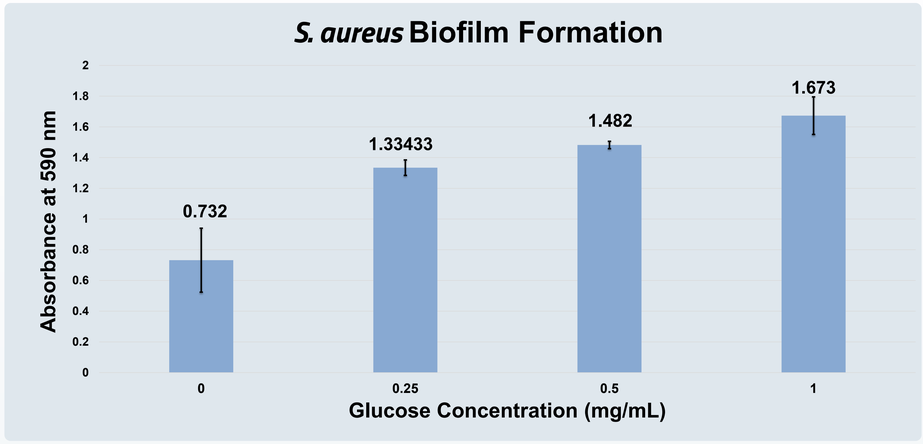


Figure 1. Biofilm formation by *S. aureus* after 24 hours with varying glucose concentrations (absorbance at 590 nm ± SD, n = 3).

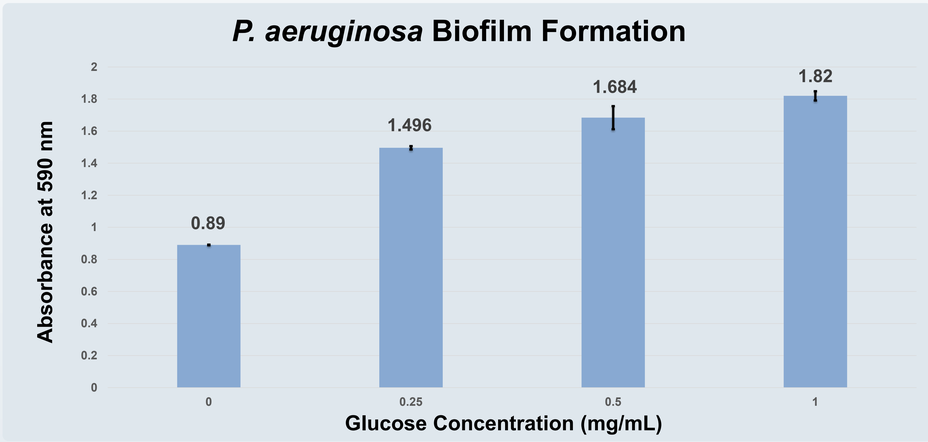
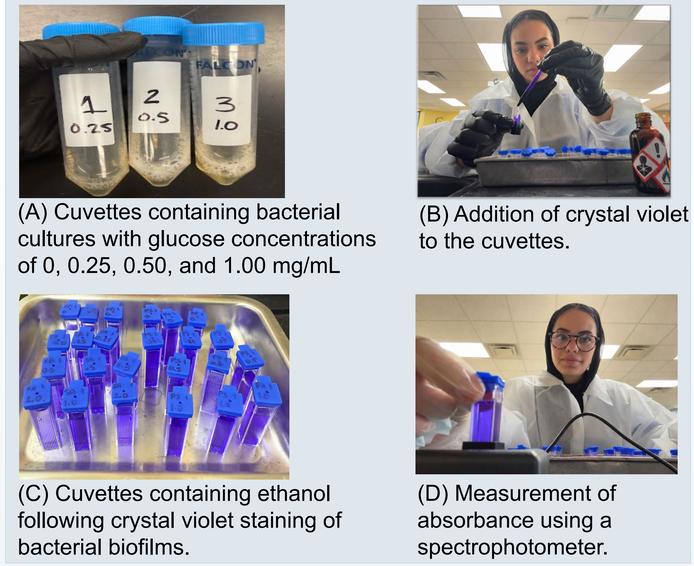


Figure 2. Biofilm formation by *P. aeruginosa* after 24 hours with varying glucose concentrations (absorbance at 590 nm ± SD, n = 3).

Results

The absorbance was significantly different among P. aeruginosa cultures exposed to glucose concentrations of 0, 0.25, 0.5, and 1 mg/mL (Figure 2, single-factor ANOVA, $F_{3,8} = 329.37$, $p < 0.001$). The absorbance was significantly different among S. aureus cultures exposed to glucose concentrations of 0, 0.25, 0.5, and 1 mg/mL (Figure 1, single-factor ANOVA, $F_{3,8} = 32.32$, $p < 0.001$).



(A) Cuvettes containing bacterial cultures with glucose concentrations of 0, 0.25, 0.50, and 1.00 mg/mL

(B) Addition of crystal violet to the cuvettes.

(C) Cuvettes containing ethanol following crystal violet staining of bacterial biofilms.

(D) Measurement of absorbance using a spectrophotometer.

Figure 3. Experimental procedure for biofilm analysis.

Discussion

The data support the hypothesis that increasing glucose concentrations increase biofilm formation in both *S. aureus* and *P. aeruginosa*. These findings are consistent with Waldrop et al. (2014), who reported increased *S. aureus* biofilm formation with increasing glucose, and She et al. (2019), who found that added glucose promoted *P. aeruginosa* biofilm formation. These results suggest that increased glucose availability may contribute to greater biofilm formation in diabetes related environments, which is especially relevant to people with type 1 diabetes who rely on devices such as insulin pumps and continuous glucose monitors and face additional risks from device associated or wound related infections.

Literature Cited

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