Characterization of S. Aureus Growth and Tolerance Development in 2D and 3D in vitro Models

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Introduction
• Fractures are a result of heavy trauma and can lead to infection
• One of the leading cause of death in hospitals is infection
• Current technology is limited in combating tolerant biofilm infections

Objectives
• Understand if tolerance develops over time with various drugs
• Collect data for growth curves and tolerance development
• Investigate bacterial growth with 2D and 3D in vitro models
• Characterize the ability of clinically relevant drugs to eradicate infection when delivered from polyurethane foams

Methods

Results

Growth

Figure 1: The time dependent formation of a mature biofilm from planktonic bacterial cells

Figure 2: Growth of S. Aureus is shown at various seeding densities on a 2x2 well plate. Experiments were repeated at least four times for each sample. Experiments are representative of at least n=3 replicates. Growth is shown at 24 hr. Only one of three replicates is shown for each condition.

Figure 3: 2D Growth Curve of S. Aureus Infection

2D

Figure 4: 3D Convex Growth Curve of S. Aureus Infection

3D Convex

Figure 5: Growth of S. Aureus is shown over time at various seeding densities on a 2x2 well plate containing 3D convex scaffolds. Growth is shown at 24 hr. Only one of three replicates is shown for each condition.

Figure 6: Schematic ofrossover of 3D convex scaffolds. Growth is shown at 24 hr. Only one of three replicates is shown for each condition.

Figure 7: SEM imaging of 3D convex scaffold. Scaffold was 3D printed and cut to size.

Figure 8: SEM Imaging of PLA 3D Convex Scaffold

3D Convex Scaffold

Figure 9: Rifampin treatment aligns with 2D tolerance study

Figure 10: 24 Well Plate Surface

Figure 11: SEM Imaging of PUR Foam

Figure 12: SEM imaging of PUR Foam

Conclusions

Growth
• Regardless of seeding density and geometry at 24 hr, the same amount of S. Aureus cells colonize each substrate
• Cell density as a function of surface area is dependent on initial seeding density and time for growth

Tolerance
• Tolerance communities exist at 24 hr when grown on a 2D surface. We believe these to be biofilm communities
• Bacterial cells grown on a 3D substrate demonstrate a slower kinetic tolerance development profile compared to bacterial cells grown on a 2D substrate

Delivery
• Vancomycin and rifampin delivery aligns with the 2D tolerance profile on the plate surface
• At 6 wt% loading vancomycin is able to inhibit infection from the scaffold unlike rifampin

References

Contact Info

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