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[1]
- Current technology is limited in combating tolerant biofilm infections[2]

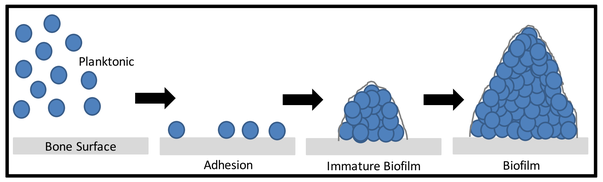
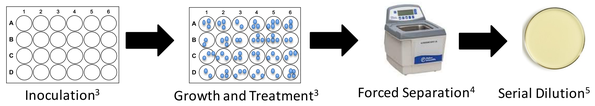


Figure 1: The time dependent formation of a mature biofilm from planktonic bacterial cells

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



Schematic 1: Experimentation is done in a sterile environment. First the inoculation of a strain of *S. Aureus* is done in a 24 well plate. Growth is allowed and then bacteria is either harvested or treated with a clinically relevant drug. When harvested the well plate is washed with PBS to get rid of planktonic bacterial cells. Adherent bacterial cells are separated from substrate surface using sonication. Bacterial cells are serial diluted and quantified by CFU count using TSA plates.

Surfaces of Study	***Treatments of Study***	***Delivery Vehicle***
24 Well Plate	Vancomycin (Vanc)	Polyurethane (PUR) Foams
Poly(lactic acid) (PLA) Scaffold	Rifampin (Rif)	

Future Directions

Extension of 2D Rifampin Treatment S. Aureus Infection

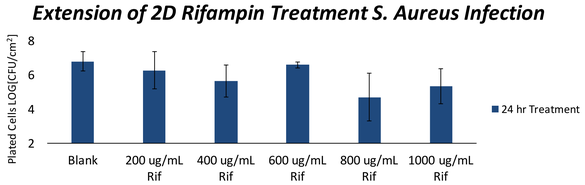


Figure 13: 2D rifampin treatment of *S. Aureus* infection was extended to understand how high dosages of rifampin will affect an infection.

3D Convex Growth and Tolerance of S. Aureus Infection

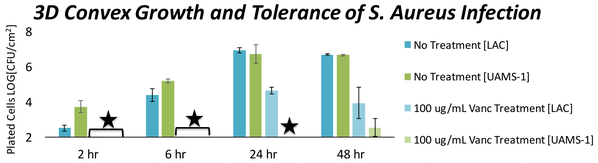


Figure 14: *S. Aureus* strain UAMS-1 is known for its biofilm formation capabilities. LAC, another strain of *S. Aureus*, is easy to manipulate but its biofilm capabilities were unknown. A vancomycin treatment was done on the LAC strain to understand the drugs effect. Both strains were tested at 10^5 CFU/cm² seeding density, n=3. Stars indicate infection below limit of detection.

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References

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Results

2D

Growth

2D Growth Curve of S. Aureus Infection

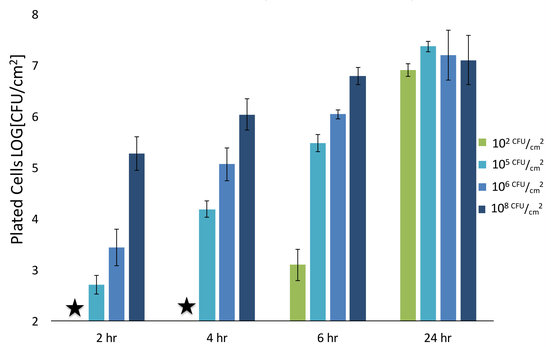

Figure 2: Growth of *S. Aureus* UAMS-1 strain over time at various seeding densities on a 24 well plate. Data is normalized to account for surface area, 4.5 cm². Stars indicate data below limit of detection. n=3

- Regardless of seeding density all bacterial cells grow to approximately the same amount at 24 hr
- 10^5 CFU/cm² has gradual development of bacterial cell community

Tolerance

Vancomycin Treatment of S. Aureus Infection

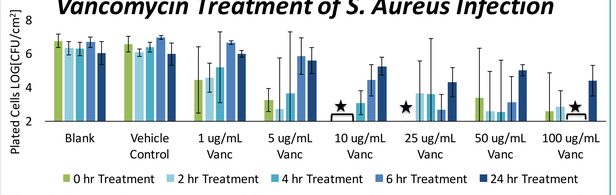

Figure 3: Vancomycin was used to treat a *S. Aureus* infection on a 24 well plate at varying growth times. The treatment of vancomycin was for 24 hours. Stars indicate data below detection limit. n=3

- Limited tolerance developed after 0h
- After 24 hr, tolerance has developed

Rifampin Treatment of S. Aureus Infection

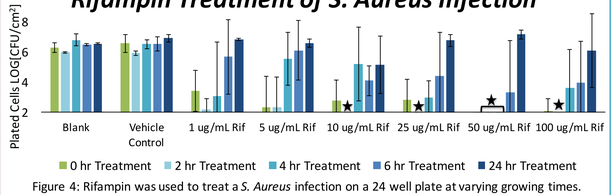

Figure 4: Rifampin was used to treat a *S. Aureus* infection on a 24 well plate at varying growing times. The treatment of rifampin was for 24 hours. Stars indicate data below limit of detection. n=3

- Rifampin is also incapable of eradicating infection after 24 hr

Delivery

Vancomycin Treatment of S. Aureus Infection

Scaffold Surface

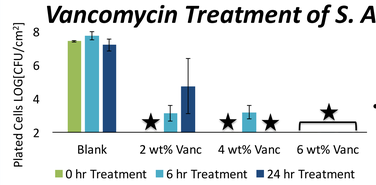

Figure 8: Sterile PUR foams were analyzed after treatment to investigate if the infection spread to the foams. Stars indicate data below detection limit. n=3

- At high loading infection is prevented from spreading to scaffold

24 Well Plate Surface

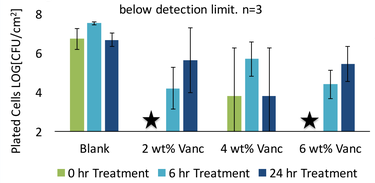

Figure 9: PUR foams were loaded with vancomycin and used to treat established *S. Aureus* infection for 24 hr at varying growth times. Stars indicate data below detection limit. n=3

- Delivery of vancomycin treatment aligns with 2D tolerance study

Rifampin Treatment of S. Aureus Infection

Scaffold Surface

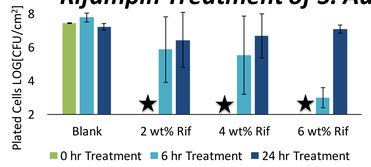

Figure 10: Sterile PUR foams were analyzed after treatment to investigate if the infection spread to the foams. Stars indicate data below detection limit. n=3

- Unable to inhibit infection from spreading to scaffold

24 Well Plate Surface

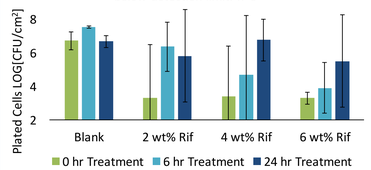

Figure 11: PUR foams were loaded with rifampin and used to treat established *S. Aureus* infection for 24 hr at varying growth times. n=3

- As in 2D limited capability for eradication of infection

SEM Imaging PUR Foam

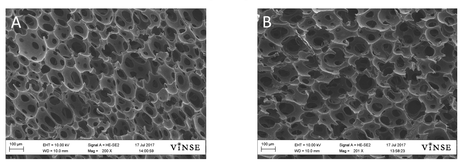

Figure 12: SEM imagine of 4 wt% A) Rifampin B) Vancomycin PUR foams.

- Pores range from 100-300 um
- Foams are approximately 95% porous
- Pores are open and allowed for expedient delivery

3D Convex

Growth

3D Convex Growth Curve of S. Aureus Infection

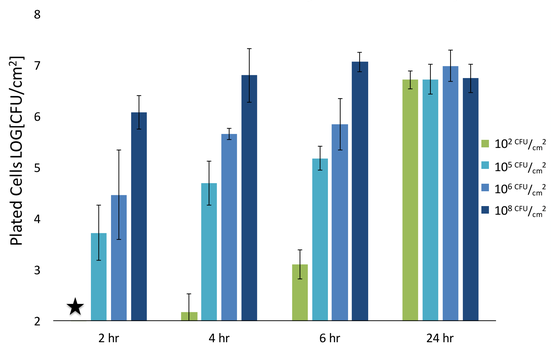

Figure 5: Growth of *S. Aureus* UAMS-1 strain over time at various seeding densities in a 24 well plate containing 3D convex scaffolds. Data is normalized to account for surface area, 9.7 cm². Stars indicate data below limit of detection. n=6

- Regardless of seeding density all bacterial cells grow to approximately the same amount at 24 hr
- 10^5 CFU/cm² has gradual development of bacterial cell community

Tolerance

Vancomycin Treatment of S. Aureus Infection

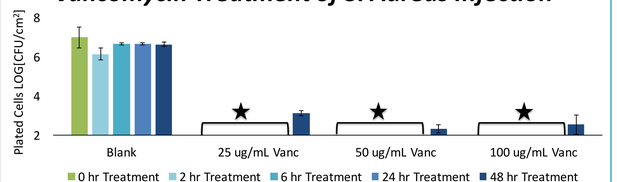

Figure 6: Vancomycin was used to treat a *S. Aureus* infection on 3D convex scaffold at varying growth times. The treatment of vancomycin was for 24 hours. Stars indicate data below detection limit. n=3

- At 24 hr eradication of infection below limit of detection is still possible
- At 48 hr infection was reduced by 4 $\log_{10}$(CFUs/cm²)

SEM Imaging of PLA 3D Convex Scaffold

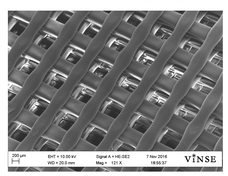

Figure 7: SEM imaging of PLA 500 um scaffold. Scaffold was 3D printed and cut to size.

- Uniform 3D convex PLA scaffold
- 500 um pores
- Surface area of 9.7 cm²

Conclusions

Growth

- Regardless of seeding density and geometry at 24 hr, approximately 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

- Tolerant 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

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