Development of a Three-Species Biofilm Model on *Ex Vivo* Porcine Skin Tissues

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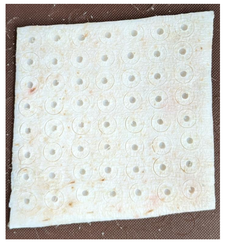
Introduction

Wound infections remain a significant challenge in healthcare, often leading to prolonged healing times and increased morbidity. Despite the availability of numerous antimicrobial treatments, clinical validation of their efficacy is hindered by the lack of suitable preclinical models that accurately mimic the complexity of wound biofilms and the microenvironment. Therefore, we aimed to develop an innovative biofilm model using *Streptococcus mutans* as a primary colonizer.

Methods

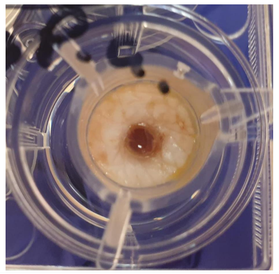
1) Tissue Preparation: Porcine skin explants were obtained from commercial sources and standardized to size. Wounds of 3mm diameter were created, followed by sterilization using supercritical CO2.



2) Experimental Setup: Explants were maintained using a gel-like matrix containing 1% agarose within well-plate inserts, allowing for epidermal exposure to air.

	S1	S2	S3	S4
Bacteria	*S.aureus* USA 300	*P. aeruginosa 15442*	*S.a/P.a*	*S.mutans 25175* <1h>*S.a/P.a*
Inoculation	1.3E+09 (CFU/ml)	1.4E+06 (CFU/ml)	1.3E+09 (CFU/ml) + 1.4E+06 (CFU/ml) *(1:1)*	5.6E+05 (CFU/ml; *S.m*) + 1:1 *S.a/P.a*

3) Bacterial Inoculation: Various combinations of bacteria including *Staphylococcus aureus (S.a)*, *Pseudomonas aeruginosa (P.a)*, and *Streptococcus mutans* were introduced into the wound liquid medium. Both single and mixed-species infections were established (n=3).



4) Data Collection: Bacterial counts and histological analysis were conducted after 3 days to evaluate biofilm formation.

The study was repeated to evaluate biofilm formation after 4 days.

Results

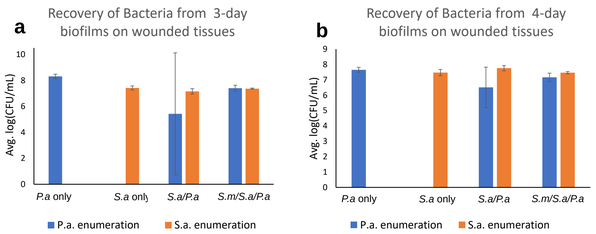
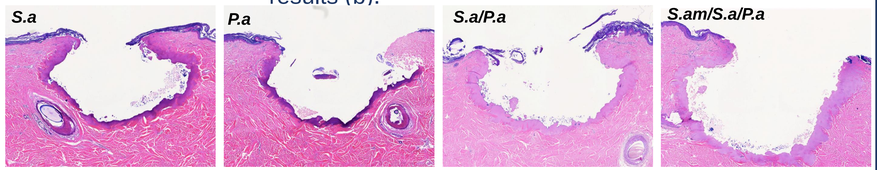
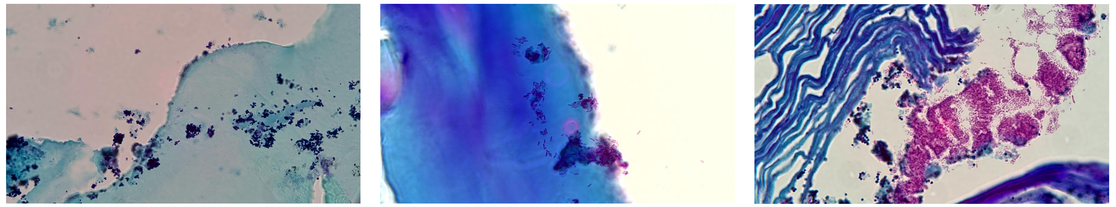


Figure 1. Microbial Growth and Biofilm Formation:

- The average total bacteria recovered from the explants after day 3 (a) ranged between 5.4-8.5 Log10 CFU/ml (n = 3).
- In groups containing a mixture of S.a and *P.a*, a higher proliferation of *P.a* was observed in two replicates, while *P.a* was absent in one replicate (5.4±4.7 log CFU/mL for *P.a* vs 7.4±0.1 Log CFU/mL for *S.a*).
- Groups containing *S. mutans* alongside these two bacteria displayed equivalent survival rates for both strains (7.4±0.2 log CFU/mL for *P.a* vs 7.3±0.04 Log CFU/mL for *S.a*).
- Repeat assays for a 4-day biofilm gave us consistent results (b).

Figure 2. Histological and Gram staining of the models revealed microbial growth on the surface as well as inside the tissues and uniform biofilm formation in the group containing *S.m*.





Conclusion

Using *S. mutans* as a pioneer for biofilm formation helped us to achieve an acceptable survival rate for both bacteria. The ex vivo biofilm model presented in this study provides a valuable platform for assessing the efficacy of antimicrobial wound treatments and advancing our understanding of wound infection dynamics. In future investigations, we aim to include *Candida albicans* alongside *S. aureus* and *P. aeruginosa* to construct a more representative polymicrobial biofilm model of wound infection.

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