Initial Characterization of a Dynamic Culture System for Modeling Staphylococcus aureus Joint Infection

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Introduction

- **Periprosthetic joint infection (PJI)** is one of the most serious complications of total joint arthroplasty (TJA).
- Biofilm-forming pathogens, particularly *Staphylococcus aureus*, are difficult to eradicate with conventional antibiotics, often requiring multiple surgeries and prolonged antimicrobial treatment.
- Current *in vitro* and animal models do not fully replicate the physiological environment of PJI, limiting the evaluation of new therapeutics.
- Microphysiological systems (MPSs) offer a promising approach for modeling infection under clinically relevant conditions.

Objective

Develop a four-chamber microphysiological bioreactor incorporating titanium implants, ex vivo bone and soft tissue, and physiologic fluid flow to model *S. aureus* PJI and evaluate bacterial dissemination and therapeutic response.

Hypothesis

A bioreactor incorporating clinically relevant tissues and fluid flow will better mimic PJI than conventional models, resulting in increased bacterial dissemination and demonstrating the limited efficacy of vancomycin against biofilm-associated infections.

Methods

- **Bioreactor Design:** A four-chamber bioreactor incorporating titanium rods, a GelMA semipermeable membrane, and dual-flow circulation was developed to model PJI. Three flow rates (0, 2, and 8 µL/min) were evaluated.
- **Biofilm Model:** Titanium rods were inoculated overnight with *Staphylococcus aureus* (JE2) and placed in Well 1 to establish the infection source.
- **Tissue Explant Model:** Mouse bone and soft tissue explants were incorporated into Wells 3 and 4 to evaluate bacterial dissemination under physiologically relevant conditions.
- **Vancomycin Treatment:** Vancomycin (0×, 10×, or 100× MIC) was administered at multiple intervention timepoints to assess antibiotic efficacy against biofilm-associated bacteria.
- **Outcome Measures:** Bacterial burden was quantified by colony-forming unit (CFU) assays, biofilm morphology was assessed by electron microscopy, and vancomycin concentrations were measured using ELISA.

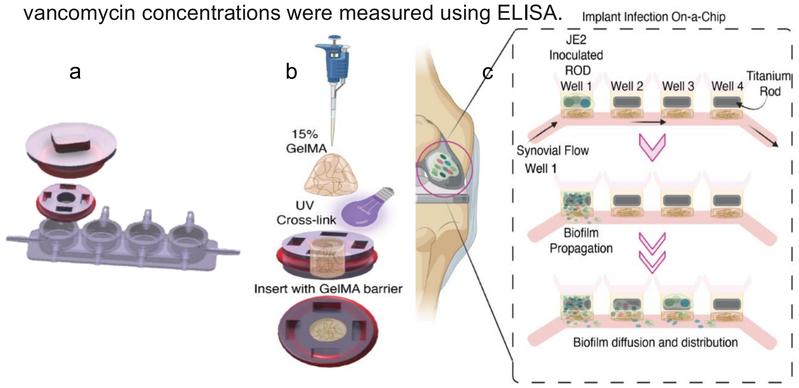

Figure 1. Four-chamber bioreactor model of *S. aureus* PJI. An inoculated titanium rod in Well 1 served as the infection source, allowing bacterial dissemination through the GelMA partition and shared flow channel to downstream wells over 48 hours.

Results

Flow Rate Increases the Spread of Infection

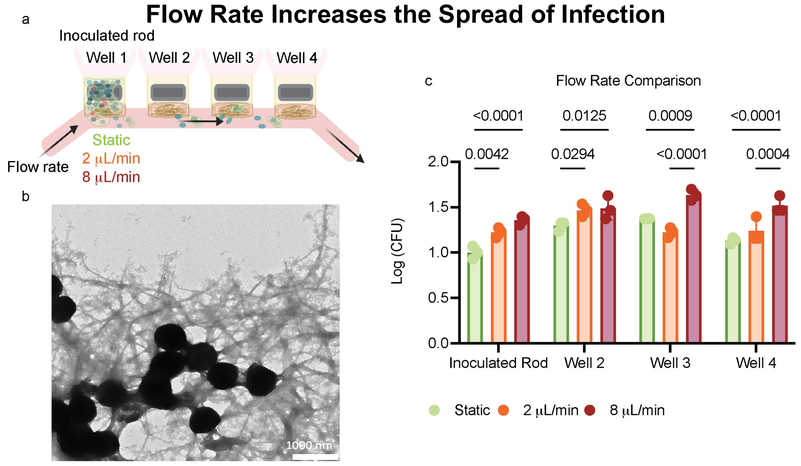

Figure 2. (A) Experimental design comparing bacterial dissemination under static (0 µL/min) and dynamic (2 and 8 µL/min) flow conditions. **(B)** SEM imaging confirmed biofilm formation on the inoculated titanium rod prior to bioreactor culture. **(C)** CFU analysis demonstrated successful downstream migration of *S. aureus* under all flow conditions, with the greatest bacterial burden observed at **8 µL/min**.

Mouse Tissue Explants Increase Bacterial Burden

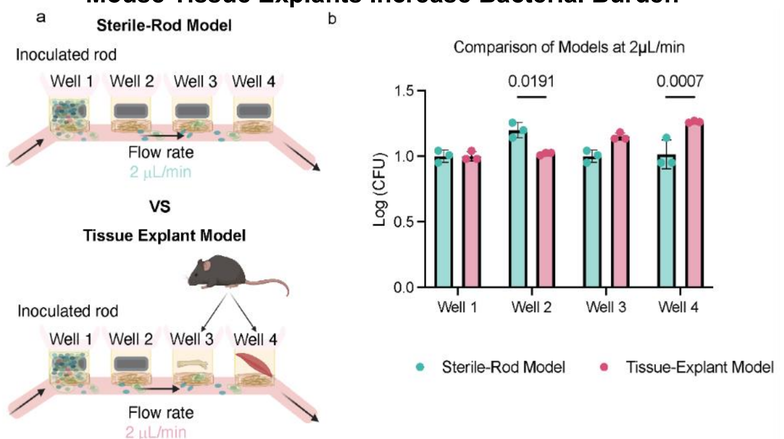

Figure 3. (A) Experimental design comparing the sterile-rod and tissue-explant bioreactor models under **2** µL/min flow. **(B)** CFU analysis showed increased bacterial burden in the presence of bone and soft tissue explants, with soft tissue exhibiting significantly greater *S. aureus* colonization than the corresponding sterile-rod control.

Vancomycin Does Not Reduce Bacterial Load

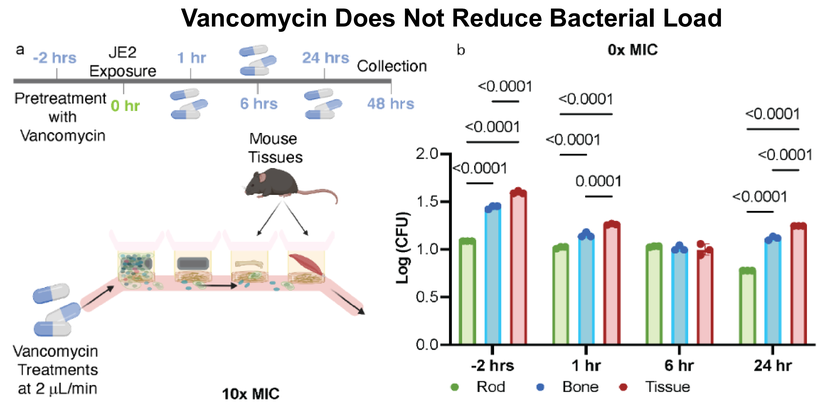

Figure 4. (A) Experimental design for evaluating vancomycin treatment at varying concentrations and intervention timepoints. **(B)** In the absence of vancomycin (0× MIC), high bacterial burdens persisted across all wells. **(C–D)** Treatment with 10× and 100× MIC vancomycin demonstrated the greatest reduction in bacterial burden when administered 2 hours prior to inoculation, whereas post-inoculation treatment resulted in persistent bacterial survival.

Conclusion

- A four-chamber bioreactor model was successfully developed to model *S. aureus* PJI.
- Incorporation of flow and bone/soft tissue explants promoted bacterial dissemination and better recapitulated key features of clinical PJI.
- Vancomycin was most effective when administered prior to infection but demonstrated limited efficacy against established biofilm, consistent with the antibiotic tolerance observed in clinical PJI.
- This bioreactor provides a physiologically relevant platform for evaluating novel therapeutics and may support the future development of personalized treatment strategies for PJI, including combination therapies and bacteriophage-based approaches.

Acknowledgements

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