Bacterial Susceptibility and Feasibility of Bacterial Clearance in Polyurethane vs Collagen-Based Dermal Substitutes

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Introduction

Infection is a serious concern for acute wounds, often resulting in delayed wound closure with potential for exacerbation of wound size and partial or total failure of dermal substitutes (DS). Polyurethane biodegradable temporizing matrices (BTM)*, innately resistant to enzymatic breakdown, are considered by some to reduce risk of wound infection and DS failure, despite lacking supportive evidence. Further, *in situ* antibacterial treatment of infected BTM is sometimes employed as a clinical strategy. Here we investigated the difference in susceptibility of BTM in comparison to collagen-based matrices to bacterial infection along with the efficacy of clearing established infection from matrices.

Polyurethane Biodegradable Temporizing Matrix (BTM)

- *Non-degradable upper polyurethane layer to provide immediate coverage and protection to wound, removed during grafting*
- *Intermediate adhesive layer*
- *Underlying 2mm layer of open cell foam intended to act as dermal layer permitting biologic ingrowth*

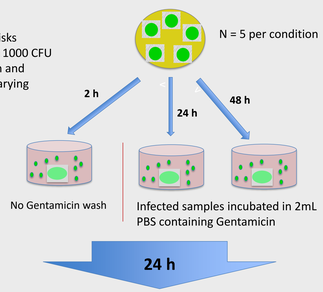
http://polynovo.com/product-btm/

In Vitro Bacterial Susceptibility

Matrices Tested:
- Collagen-Chondroitin-6-sulfate (Coll-C6S)
- Bovine acellular dermal matrix (bADM)
- bADM + ionic silver (bADM-Ag)
- Decellularized fish skin (dFS)
- Polyurethane biodegradable temporizing matrix (BTM)

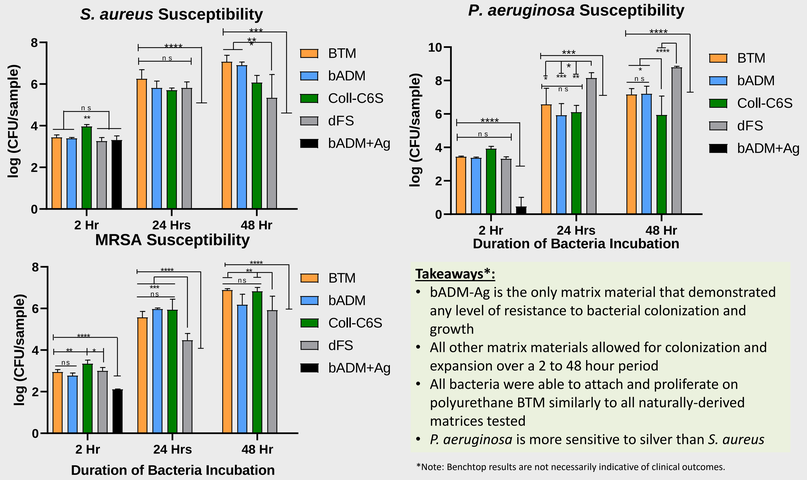
Bacterial Stains:
- *S. aureus*
- MRSA
- *P. aeruginosa*

Protocol:
12mm matrix disks inoculated with 1000 CFU of bacteria each and incubated for varying durations



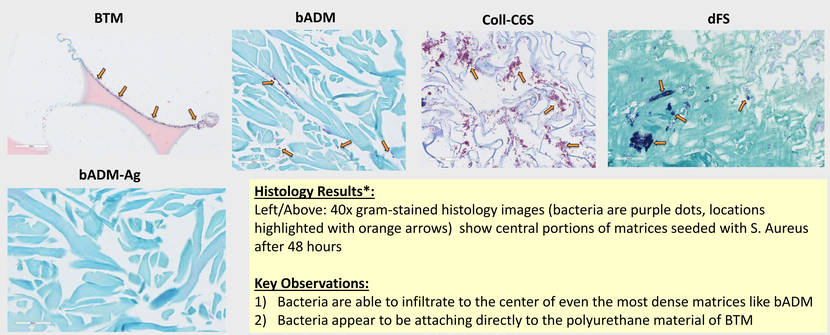
For each bacterial strain and timepoint:
1) Extract bacteria into PBS and quantify overall numbers
2) Fix and process with gram stain to visualize how far into the matrix bacteria have migrated

Bacterial Susceptibility Results



Takeaways*:
- bADM-Ag is the only matrix material that demonstrated any level of resistance to bacterial colonization and growth
- All other matrix materials allowed for colonization and expansion over a 2 to 48 hour period
- All bacteria were able to attach and proliferate on polyurethane BTM similarly to all naturally-derived matrices tested
- *P. aeruginosa* is more sensitive to silver than *S. aureus*

*Note: Benchtop results are not necessarily indicative of clinical outcomes.



Histology Results*:
Left/Above: 40x gram-stained histology images (bacteria are purple dots, locations highlighted with orange arrows) show central portions of matrices seeded with S. Aureus after 48 hours

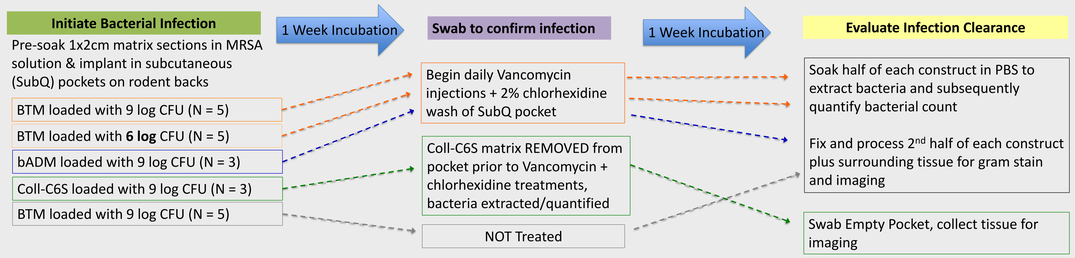
Key Observations:
1) Bacteria are able to infiltrate to the center of even the most dense matrices like bADM
2) Bacteria appear to be attaching directly to the polyurethane material of BTM

*Note: Benchtop results are not necessarily indicative of clinical outcomes.

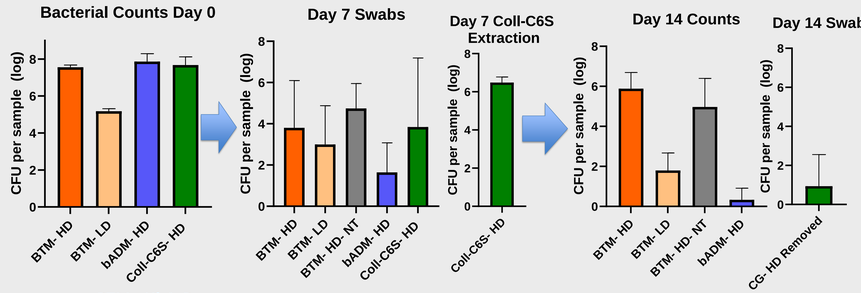
Conclusions

- In benchtop bacterial testing all materials, with the exception of bADM-Ag, allowed for bacterial colonization and infiltration for all strains tested showing that all these materials lack innate resistance to bacterial colonization.
- Infection cannot easily be cleared from BTM if it is left in place within tissue.
- It is unclear if the treatment of BTM had any substantial impact on lowering bacterial burden. Removal of an infected matrix construct (such as with CG) has the greatest chance of allowing full clearance of infection.
- While the BTM material itself can resist degradation from bacteria, allowing it to stay in place in the presence of infection has several downsides:
  - The bacterial infection is likely to persist.
  - An inflammatory and acute immune response is likely to persist.
  - Newly formed granulation tissue or surrounding healthy tissue will likely get degraded.
  - The overall rate of wound healing is likely to be slower.

In Vivo Bacterial Clearance



Bacterial Clearance Results



Takeaways:**

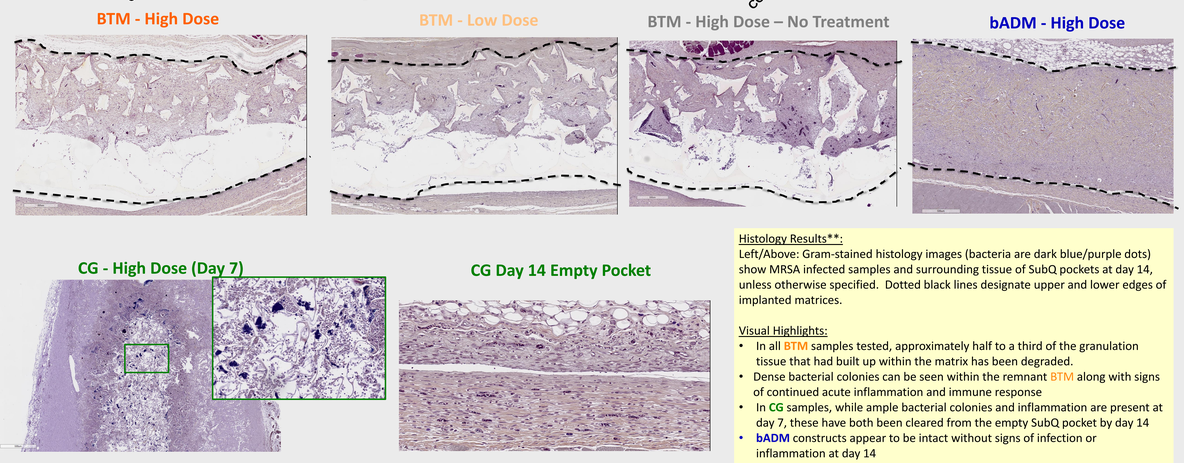
1) Thorough antibiotic treatment of biofilm levels of bacteria in BTM does not seem to have a significant impact on bacterial load compared to no treatment at all

2) Antibiotic treatment of BTM inoculated with lower dose of bacteria (BTM-LD) does lead to a substantial reduction in bacterial presence, but infection still lingers after 1 week.

3) Regular bADM seems to possess innate properties that confer a level of resistance to bacterial colonization, and when subjected to antibiotic treatment, infection can be completely or mostly eliminated.

4) Removing a colonized matrix construct from the SubQ pocket allows for complete or near complete bacterial elimination.

**Note: Pre-clinical results are not necessarily indicative of clinical outcomes.



Histology Results:**
Left/Above: Gram-stained histology images (bacteria are dark blue/purple dots) show MRSA infected samples and surrounding tissue of SubQ pockets at day 14, unless otherwise specified. Dotted black lines designate upper and lower edges of implanted matrices.

Visual Highlights:
- In all BTM samples tested, approximately half to a third of the granulation tissue that had built up within the matrix has been degraded.
- Dense bacterial colonies can be seen within the remnant BTM along with signs of continued acute inflammation and immune response
- In CG samples, while ample bacterial colonies and inflammation are present at day 7, these have both been cleared from the empty SubQ pocket by day 14
- bADM constructs appear to be intact without signs of infection or inflammation at day 14

**Note: Pre-clinical results are not necessarily indicative of clinical outcomes.