The Antibiofilm Efficacy and Depth Penetration of Prototype Wound Dressings Using Wounded *Ex Vivo* Human Skin

Jessica Sanders[1], Isabel Laubach[1], Marnie Peterson[1], Nora Bastida Agote[2], Markus J Kettel[2]

Introduction

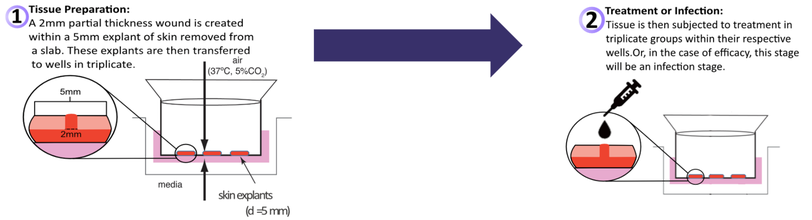
Biofilms are the leading cause in the stalling of wound healing in clinical settings as they can penetrate deep into tissue and evade topical antimicrobial treatment. Ex vivo human skin models allow for moderately high throughput screening while remaining clinically relevant by using a host-derived substrate, allowing for multiple experimental outputs and data types. **This study aimed to determine the biofilm disruption and antibiofilm depth penetration properties of two new prototype wound dressings, using a wounded skin model.**

Prototypes

Superabsorbent wound dressings (Prototype Dressings 1 based on Zetuvit plus®) and ointment impregnated wound contact layers (Prototype Dressings 2 based on Atrauman®) were coated with a Mixed Metal Complex (MMC). The metal complex is prepared by complexation of ionic Silver (Ag) and Zink (Zn) with Ethylenediaminetetraacetic acid (EDTA). Dressing concentrations of 3% MMC for Prototype Dressing 1 and 1.8% MMC for Prototype Dressing 2 were used to design new antimicrobial wound dressing prototypes with antibiofilm properties against deep wounds infected with biofilm.

Methodology

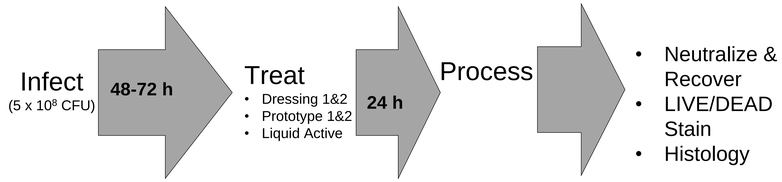
Intact human skin tissue (HST) explants (5 mm) were produced with 2 mm partial thickness wounds by biopsy punch. Explant wound beds were inoculated with Pseudomonas aeruginosa or methicillin-resistant *Staphylococcus aureus* (Xen30) and incubated to establish 48 or 72 hour biofilms.



Wounds beds were washed to remove planktonic bacteria and treated with liquid 3% MMC active solution (100 µL/treatment), commercially available Zetuvit plus (Dressing 1)*and non-medicated wound contact layer Atrauman (Dressing 2)#, or respective new prototype dressings (Prototype Dressing 1 or Prototype Dressing 2).

After 24 hours of treatment, explants were sonicated, vortexed, and released bacteria plated for enumeration. Biofilm viability was assessed by Live/Dead staining of one explant replicate and imaged with an Olympus BX63 fluorescent microscope. Two explants from each treatment were reserved for histological evaluation with hematoxylin and eosin (H&E) and Gram-Twort staining.

Results



Organism	Log Reduction from Growth Control (Mean±SD)				
	Liquid Active	Dressing 1*	Prototype Dressing 1	Dressing 2#	Prototype Dressing 2
P. aeruginosa (ATCC®700888™)	>3.35 ± 0.30	0.36 ± 0.05	3.56 ± 0.42	0.39 ± 0.08	1.46 ± 0.07
MRSA (Xen30)	>2.98 ± 0.96	0.67 ± 0.02	0.98 ± 0.25	0.30 ± 0.02	0.62 ± 0.08

Table 1: Average log reduction (CFU/explant) from growth control (n=3, Mean±SD). *P. aeruginosa* 72h growth control = 8.28 ± 0.01 CFU/explant. MRSA 96h growth control = 7.41 ± 0.07 CFU/explant. *Zetuvit® Plus, Hartmann, 2023. # Atrauman® , Hartmann, 2023.

Newly formulated Prototype Dressing 1 demonstrated the greatest overall percent reduction for both organisms, 99.97% reduction from *P. aeruginosa* 48 hour controls and 89.47% reduction from MRSA 96 hour controls.

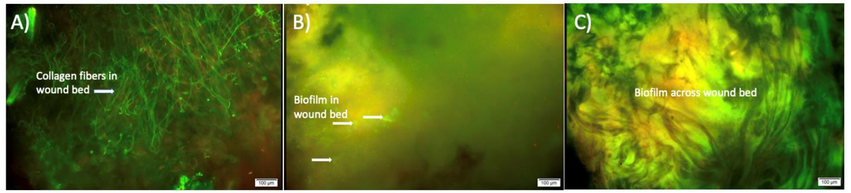


Figure 1: Live/Dead fluorescent images of wounded human skin tissue. A) Uninfected HST wound bed after 72 hours of incubation. B) Wound bed 72 hours post-infection with ***P. aeruginosa* ATCC® 700888™** (end of treatment period). C) Wound bed 96 hours post-infection with ***S. aureus* Xen 30** (end of treatment period). (Green: SYTO 9 stain, alive cells; red: propidium iodide, dead cells).

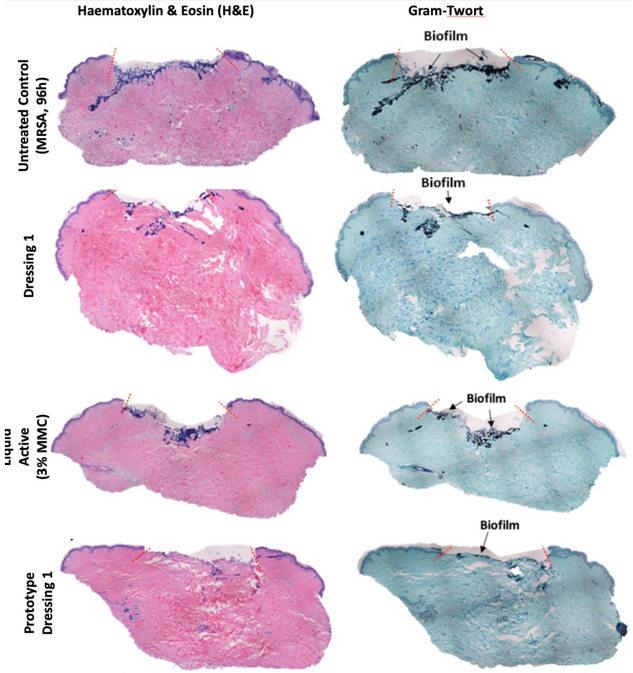


Figure 2: Haematoxylin & Eosin and Gram-Twort staining images of wounded HST infected with MRSA Xen 30, with and without treatment.

Discussion and Conclusions

- Greater efficacy for Prototype Dressing 1 compared to legacy Dressing 1* was supported by log CFU/explant recovered post treatment as well as visibly less biofilm remaining in wound beds of tissue explants as observed by histology. Prototype Dressing 2 was likewise more effective than legacy impregnated Dressing 2#.
- **Newly designed prototype dressings demonstrated greater antibiofilm efficacy than their existing commercial products.** Both prototype dressings were outperformed only by the liquid active solution alone.
- H&E and Gram-Twort stained histology showed that the bacteria, although killed, remained in the wound bed when treated with liquid active alone as compared to any of the physical dressings assessed.
- Physical wound dressings may function to physically remove bacteria from the wound bed when discarded post treatment.

This project was carried out by Perfectus Biomed Group Now Part of NAMSA® and funded by Paul Hartmann.

[1]Perfectus Biomed Group, Techspace One, Sci-Tech Daresbury, Keckwick Lane, Cheshire, WA4 4AB, UK, Email: info@perfectusbiomed.com; [2] Paul Hartmann AG, Paul Hartmann Str. 12, 89522 Heidenheim, Germany, markus.kettel@hartmann.info