# A wounded *ex vivo* human skin model to study microbial biofilm development and anti-biofilm therapeutics

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## Introduction

Biofilm-forming bacteria can adhere to and then colonize acute and chronic wounds. Biofilm formation establishes colonization in wound beds by attachment of pioneer microbes, followed by microcolonies that produce extracellular polymer substances (EPS). The efficacy of antibiotic therapy regimens may be decreased by the presence of biofilms and multidrug-resistant (MDR) bacteria.

The Center for Disease Control and Prevention estimate that 65% of all human infectious diseases are caused by bacteria-containing biofilms, while the National Institutes of Health estimate that the true percentage is closer to 80%.[1] There are over 14 million biofilm infections per year and over 350,000 biofilm-related deaths.[2] Given the preponderance of biofilms in wounds and the associated impact on public health, it is important to explore anti-biofilm therapeutics that can prevent formation and persistence of biofilm in acute wounds, consequently decreasing the incidence of chronic wounds and improving wound-healing recovery times.

***An ex vivo wounded skin model*** - *Ex vivo* human[3,4] and porcine skin[5,6] models have been used to assess the efficacy of antimicrobial and wound-healing therapies. These models bridge the gap between *in vitro* and *in vivo* studies and have certain advantages over both study types. *Ex vivo* models are more predictive of clinical success compared to *in vitro* experiments while remaining more cost effective and an alternative to live animal testing. *Ex vivo* models enable evaluations of the anti-biofilm efficacy and cytotoxicity of therapeutic formulations, dressings, and devices. They may also be used to investigate immune response and wound closure.[7]

## Study Aim

Development of an *ex vivo* wounded human skin model to assess biofilm formation of multidrug resistant organisms (MDROs) with potential for biofilm treatment and disruption by antiseptic technologies.

## Methodology

Wounded explants were placed on transmembrane inserts and wells filled with growth media. Methicillin-resistant *Staphylococcus aureus* Xen30, *Pseudomonas aeruginosa* C3 (clinical isolate), and *Acinetobacter baumannii* QE613 (clinical isolate) were the MDROs inoculated into wound beds for 5 days. Microscopy visualized via bright field at 63X of Hematoxylin & Eosin (H&E), Gram-Twort (GT), and Live/Dead Fluorescence stains were performed to visualize biofilm within the wound bed. Viability of tissue was measured via MTT assay, and adherence of bacteria was counted over duration of the experiment. $Log_{10}$ reduction of bacterial load was enumerated after 5-day infection and 24-hour treatment.

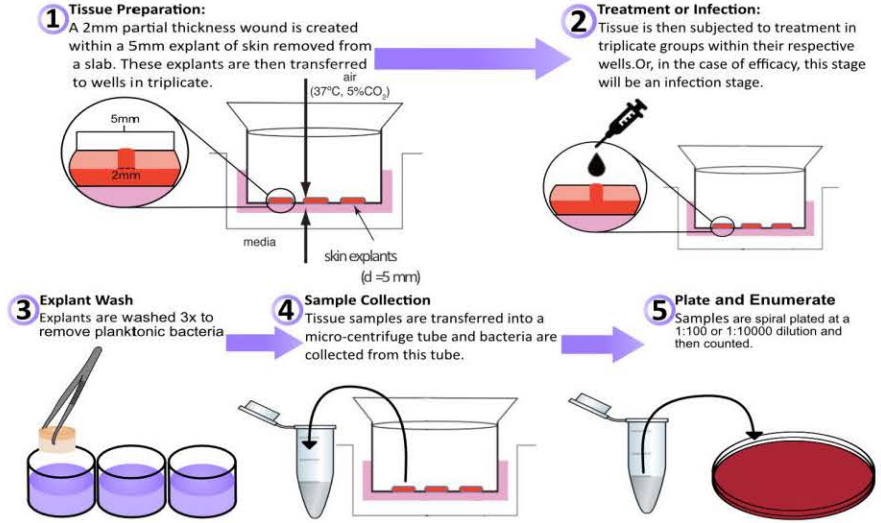


**Figure 1. Method for *ex vivo* human wounded skin model.**

## Results

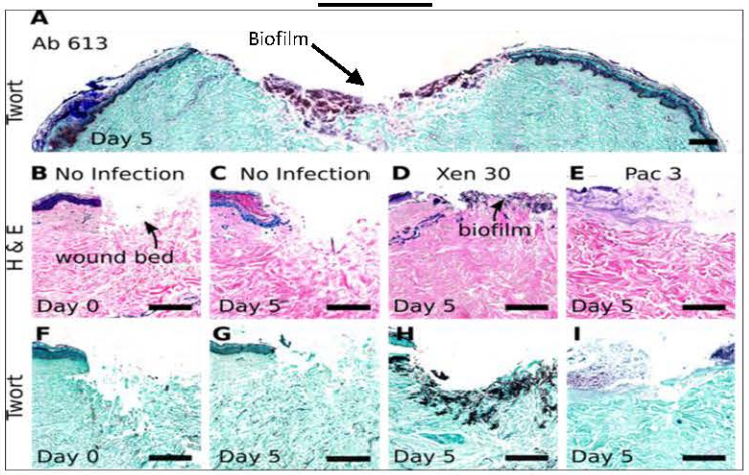


**Figure 2. Histology of H&E and GT staining shows evidence of biofilm formation at day 5 visualized via bright field microscopy at 63X.** Ab: MDR-*Acinetobacter baumannii 613* (clinical isolate); Xen30: MRSA Xen 30; Pac 3: MDR-*Pseudomonas aeruginosa* C3.

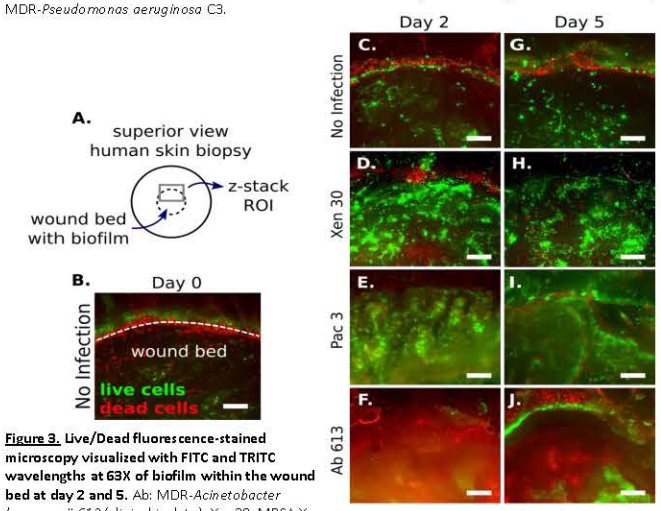


**Figure 3. Live/Dead fluorescence-stained microscopy visualized with FITC and TRITC wavelengths at 63X of biofilm within the wound bed at day 2 and 5.** Ab: MDR-*Acinetobacter baumannii 613* (clinical isolate); Xen30: MRSA Xen 30; Pac 3: MDR-*Pseudomonas aeruginosa* C3.

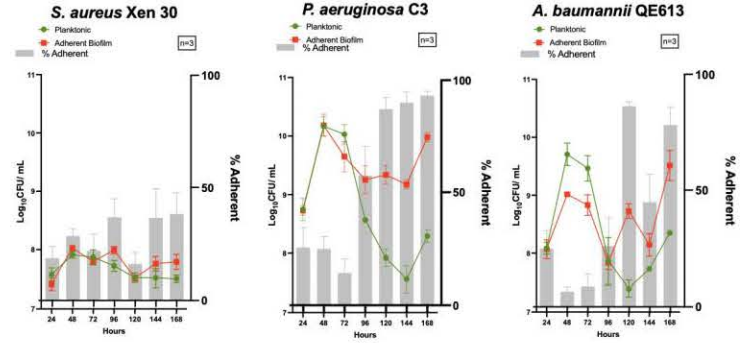


**Figure 4. Characterization of Biofilm formation by MDROs in *ex vivo* wounded human skin.** Wounded explants were infected, and bacteria were allowed to form a biofilm for 7 days. Each day explants were collected and analyzed to quantify the amount of planktonic and adherent bacteria. Error bars represent mean ± SEM.

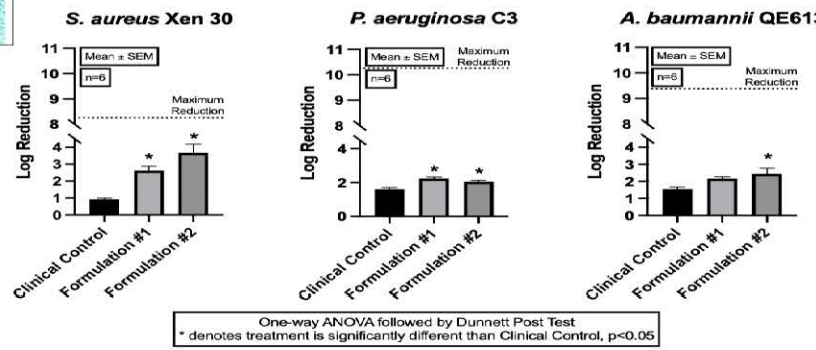


**Figure 5. New antiseptic formulations display efficacy (biofilm disruption) of 5-day biofilms as log reduction from a growth control treated with PBS following a 24-hour treatment.** Formulation #2 was significantly different for all 3 MDROs.

## Discussion and Conclusions

- Biofilm formation of MDROs was established in an *ex vivo* wounded human skin model.
- Using this model, antiseptic efficacy against biofilm wound infections was determined with reproducibility.
- Antimicrobial efficacy for chronic wounds should be explored using ex vivo tissue models as an alternative to live animal testing.

## References

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