



# A BRIDGE BETWEEN *IN VITRO* SCREENS AND ANIMAL MODELS FOR THE STUDY OF ANTI-BIOFILM EFFICACY

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A bridge between microtiter-based biofilm models and animal models is described—giving wound care device and drug makers a tool that can effectively screen their wound care products prior to transitioning towards *in vivo* studies—saving money and reducing time to market.

Chronic wounds are a significant problem requiring development of new technologies to promote healing. These wounds have stalled in their healing process, lasting 12-13 months and recurring 60-70% of the time, on average<sup>1</sup>. An estimated 2% of people are affected by chronic wounds—leading to a cost of \$25 billion per year in the United States<sup>2</sup>. Research into chronic wound causes has shown that 60-90% of chronic wounds are associated with biofilms<sup>3,4</sup>—complex microbial communities of bacteria existing in a protective matrix of proteins, nucleic acids and sugars. Biofilm-associated bacteria are significantly more tolerant to antibiotics compared to those in a free-floating state<sup>5</sup>. Removal of biofilm is an important and significant challenge in chronic wound healing that requires a regimen of serial debridement, cleaning, dressing and treating the wounds with antimicrobials. This is a time-consuming and costly process with a significant impact on the patients' quality of life<sup>5</sup>.

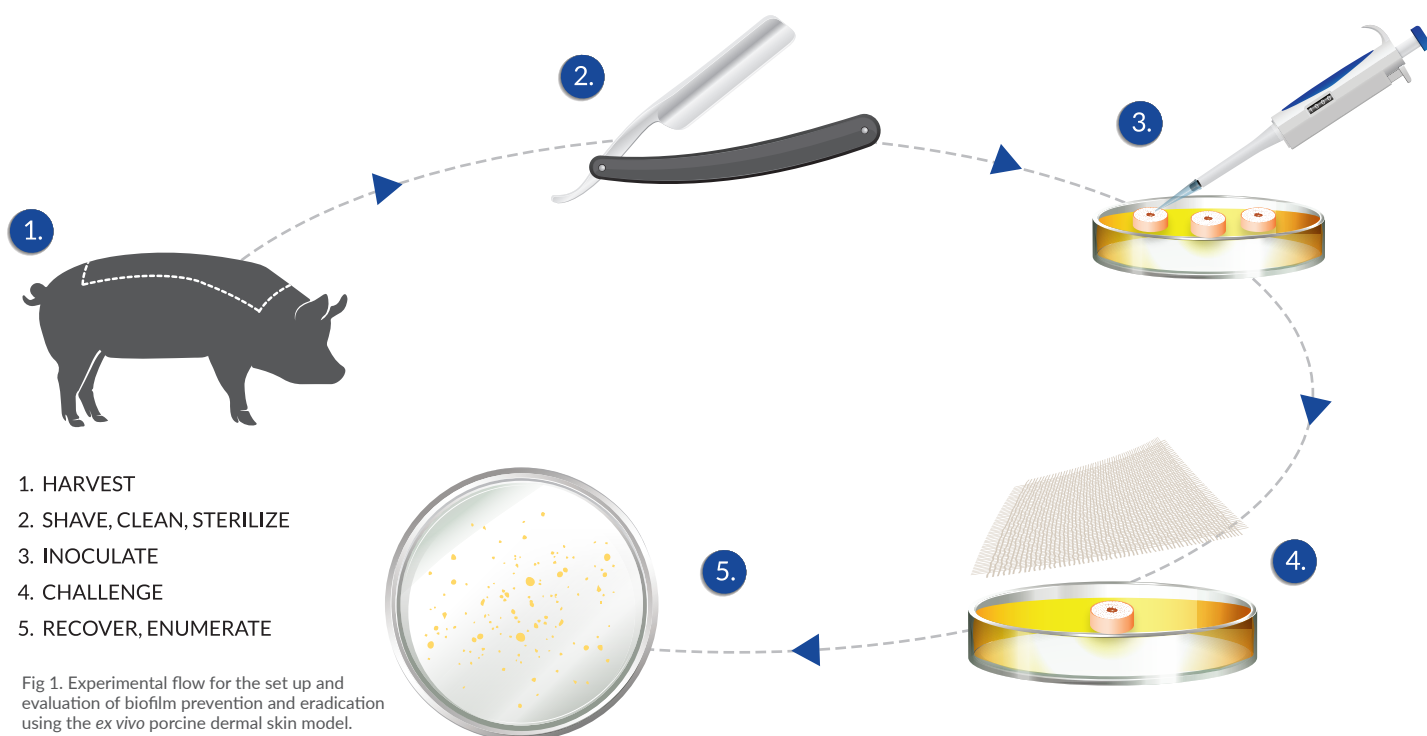
Development of new technologies to combat biofilms and promote healing of chronic wounds is important from a public health standpoint and also has an economic incentive: the global wound care products market is estimated to be \$15 billion per year<sup>3</sup>. However, organizations developing products intended to disperse, destroy, or impede biofilm formation often have few tools at their disposal to assess the efficacy of those products:

- Traditional antimicrobial testing—optimized to measure efficacy against free-floating bacteria

- *In vitro* test methods, such as the MBEC assay<sup>5</sup>—established screening method to measure efficacy of antibiotics on biofilms, performed on rigid plastic surface
- *In vivo* animal models<sup>7</sup>

iFyber has adopted an *ex vivo* porcine dermal model of mature biofilm as a bridge between standard *in vitro* antimicrobial and anti-biofilm assays and *in vivo* animal studies. The model is suitable for evaluation of both biofilm prevention and eradication, and is useful for assessing the efficacy of a variety of products, from antibiotics to medical devices. Information generated with the model is more relevant to the developer than traditional *in vitro* studies—biofilms formed on the soft tissue dermal matrix more closely represent an actual wound bed compared to a rigid polystyrene matrix. Animal models, on the other hand, are costly and not amenable to screening or optimization of dosing, formulation, and dressing format parameters.

This model, developed in the laboratory of Gregory Schultz at the University of Florida, uses sterilized pig skin explants with “wounds” in the dermis that support growth of mature biofilms within a wound-relevant matrix<sup>8</sup> (Fig. 1). Explants can be treated with a variety of dressings before or after inoculation and growth of a mature biofilm. Success is evaluated through recovery and enumeration of the bacteria remaining on the explant, and coupled with confocal microscopy for visual confirmation of the effects against biofilm, if desired. The *ex vivo* model is not intended for understanding the immunological aspects of wounds in living systems; however, it does isolate the biofilm component of a chronic wound, which is important for conducting controlled studies on biofilm management. This screening method is commercially available for pre-clinical studies in chronic wounds, acute bacterial and skin suture infections (ABSSSI), chronic acne, and fungal skin infections.



# APPLICATION NOTE: EVALUATION OF ANTI-BIOFILM EFFICACY OF VARIOUS TREATMENTS USING THE EX VIVO PORCINE DERMAL MODEL

## EFFICACY OF NITRIC OXIDE (NO)-RELEASING POLYMER ON PRE-FORMED *P. AERUGINOSA* BIOFILMS

To assess the anti-biofilm efficacy of a NO-releasing polymer material, antibiotic-tolerant mature *P. aeruginosa* biofilms were produced on porcine dermal explants. The general flow of the experimental set-up is depicted in Figure 1. After tissue harvest, cleaning, cutting, wounding, and sterilizing, porcine dermal explants were inoculated with bacteria and incubated for 72 hours at 37°C on agar plates supplemented with an antibiotic to contain bacterial proliferation within the "wound". Prior to treatment with test articles, the biofilm explants were submerged in media containing high concentrations of antibiotic to kill both planktonic and immature biofilm-associated bacteria, leaving a biofilm subpopulation attached to the explant. Using this experimental set up, iFyber has assessed efficacy of a series of compounds, including NO-releasing polymer (PAN/NO), antibiotic ciprofloxacin, and silver ions, alone and in combination treatments. While individual treatments alone had little to no effect on the *P. aeruginosa* biofilm (Fig. 2), combination treatment of PAN/NO and either ciprofloxacin or silver ions significantly decreased the overall bioburden, in some cases leading to almost complete biofilm eradication.

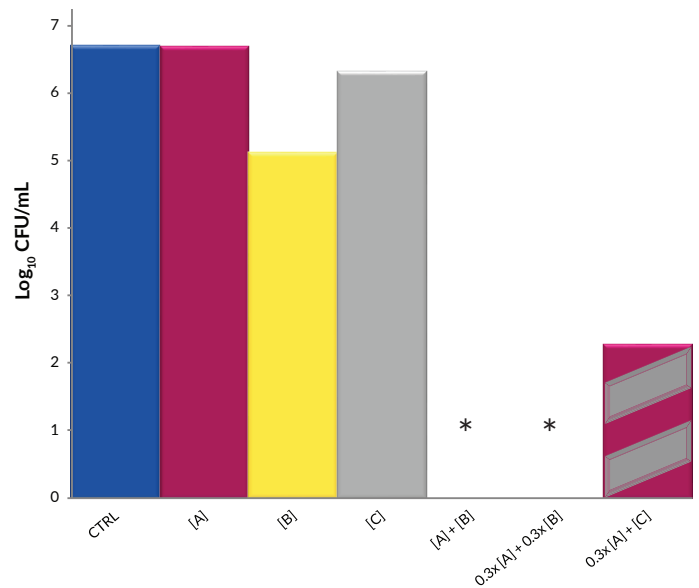


Fig 2. Anti-biofilm efficacy assessment of various treatments on pre-formed 72 h *P. aeruginosa* biofilm. A: 10% PAN/NO B: 30 µg/mL ciprofloxacin C: 200 µg/mL AgNO<sub>3</sub> \*Values below detection of limit (200 CFU/mL).

## EFFICACY OF A COMMERCIAL DRESSING IN PREVENTING FORMATION OF MATURE *P. AERUGINOSA* BIOFILMS

Extending beyond testing the efficacy of solution-based treatments against existing, mature biofilms, iFyber has implemented the *ex vivo* porcine dermal model to evaluate efficacy of different commercial wound dressings on biofilm prevention. Fig. 3 shows representative data for *P. aeruginosa* biofilm prevention using IODOFLEX<sup>®</sup> cadexomer iodine dressings. In this experimental set-up the explants were inoculated with *P. aeruginosa* and incubated for 2 hours without any treatment to allow bacteria time to colonize the explants. Explant "wounds" were covered with either a cadexomer iodine dressing or a simple cotton gauze control. The explants were incubated at 37°C in a humidified atmosphere up to 72 hours, without dressing replacement, and the bacterial growth was evaluated every 24 hours. While the total number of bacteria on the control explants remained the same, approximately 10<sup>9</sup> CFU/mL throughout the experiments, explants treated with the cadexomer iodine dressing reduced the total bacterial load by nearly 8 log units within 3 days of treatment (Fig. 3).

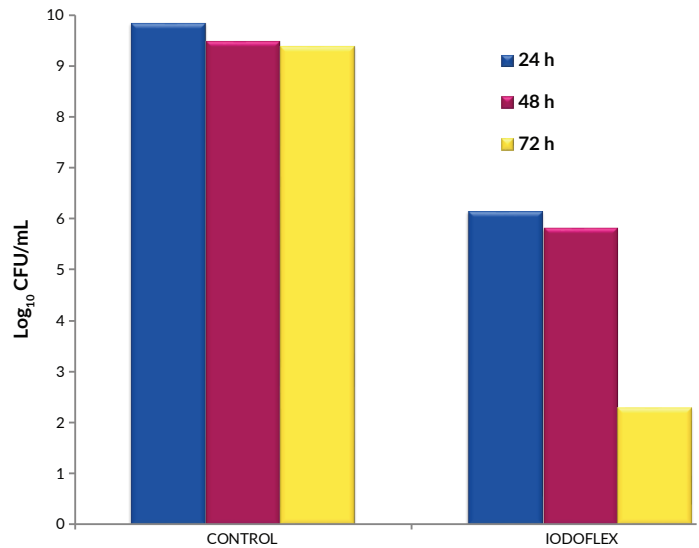


Fig 3. Assessment of commercial dressing IODOFLEX<sup>®</sup> (cadexomer iodine pad) efficacy against *P. aeruginosa* biofilm formation.

## CONCLUSIONS

iFyber has successfully implemented an *ex vivo* porcine dermal model for evaluation of various treatments geared towards either preventing formation of mature biofilm or eradication of pre-existing biofilm. This model is suitable for screening and allows for rapid evaluation of a number of variables for a particular technology, making this model a valuable tool in pre-clinical testing, at a fraction of the cost of animal studies.

## TYPICAL STUDY PARAMETERS

- Bacterial strains to date: *S. aureus*, *P. aeruginosa*
- Biofilm eradication on a 2 to 5 day old biofilm
- Inhibition of explant colonization and biofilm formation
- Time-kill studies
- Combination treatments
- Dressing replacement
- Works with solutions, solid and semi-solid wound dressings

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