VISUALIZATION OF BIOFILMS IN AN *EX VIVO* PORCINE DERMAL MODEL

Elizabeth Bevels, Ute Schwab, Ph.D., and Nina Bionda, Ph.D., iFyber LLC, Ithaca NY

WWW.IFYBER.COM

Histological analyses and microscopy techniques for evaluating biofilm prevention and eradication using an *ex vivo* porcine dermal model of mature biofilm are described. Semi-quantitative and qualitative data derived from these models can aid the development of effective device- and drug-based wound care treatments.

iFyber has adopted an *ex vivo* porcine dermal model[1] of mature biofilm as a bridge between standard *in vitro* antimicrobial and anti-biofilm assays and *in vivo* animal studies. This *ex vivo* model uses porcine dermal explants with artificial wound beds that support biofilm populations closely resembling those seen in human chronic wounds. This model is suitable for evaluation of both biofilm prevention and eradication and can be used to assess topical antibiotic treatment efficacy for wound care applications. Treatment results using this model are typically quantified through recovery and enumeration of both total and biofilm-associated bacteria remaining on the explant; however, biofilm formation in the wound bed can be also evaluated by histological analysis and confocal microscopy. These techniques allow for qualitative visual evidence of the effects of anti-biofilm treatments on the structure and morphology of underlying tissues and the prevention or eradication of the biofilm itself.

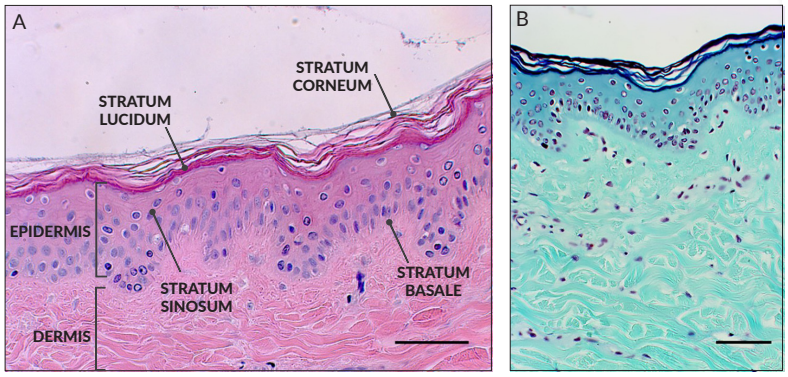
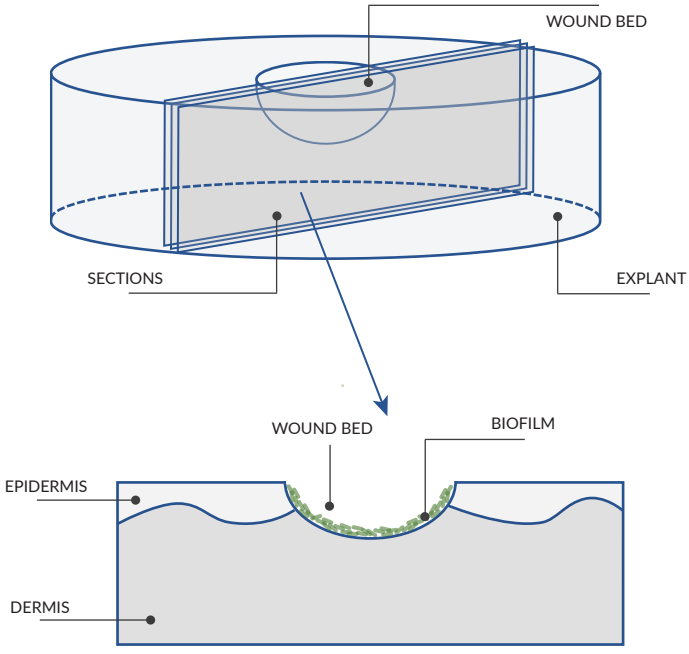


Fig. 2. Cross-sectional view of uninfected porcine explant tissue stained with H&E (A) and Gram (B) stain. Scale bar: 50 µm.

Fig. 1. Schematic of histological sections of *ex vivo* porcine dermal model of mature biofilm



HISTOLOGY

For histological analysis explants are fixed with 4% paraformaldehyde, washed, embedded, sectioned, and consecutive sections of each skin tissue explant are mounted and stained (see **Figure 1**). The sections are examined under a light microscope and images are taken of the wound bed. The images in **Figure 2** highlight the different layers of the native porcine tissue.

When tissue explants are incubated with *Pseudomonas aeruginosa* for three days, a dense and thick biofilm forms in the tissue lining the wound bed[2] (**Figure 3A**). The tissue beneath the biofilm is compromised as evidenced by the separation of the epidermis from the rest of the explant. In some areas, bacteria invade deep within the dermis, as seen in **Figure 3B**.

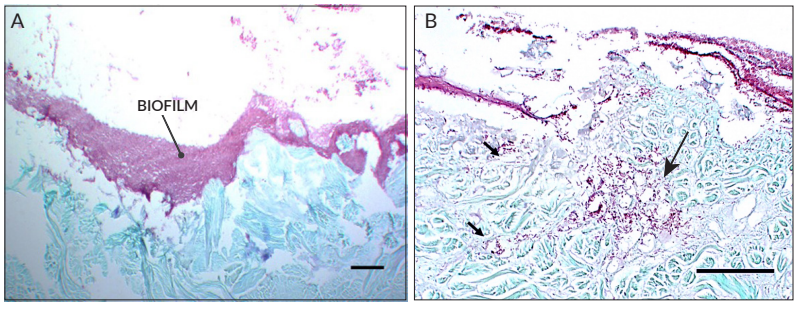


Fig. 3. Cross-sectional view of a biofilm formed in the wound bed of porcine dermal explant tissue (A) and with individual bacteria invading the tissue underneath the biofilm (B). Gram stain; *P. aeruginosa* bacteria appear red; scale bar: 50 µm.

VISUALIZATION OF BIOFILMS IN AN *EX VIVO* PORCINE DERMAL MODEL

WWW.IFYBER.COM

CONFOCAL MICROSCOPY

For visualization of live bacterial biofilms, confocal microscopy is performed on porcine explants containing *P. aeruginosa* PAO1 labeled with green fluorescent protein (GFP) biofilms. Propidium iodine staining can also be used to differentiate live versus dead bacteria. The explant is examined by confocal microscopy using a 20x immersion objective at different focal planes to provide a series of images (referred to as a z-stack). These z-stacks are generated by incrementally stepping through the wound bed from top to the bottom (schematically shown in **Figure 4**).

Representative images are shown in **Figure 5** (**A-F**), indicating that bacterial attachment at the tissue interface results in large biofilm aggregates across the wound surface. At the bottom of the wound bed, a dense biofilm lining the tissue is observed (**Figure 5F**). Much of the biofilm is comprised of live bacteria, as indicated by the green color; however, dead cells are also visible as noted by the bacteria stained red due to propidium iodide uptake. The images of the z-stack can also be reconstructed in three dimensions and then rotated or tilted to view cells or structures that would otherwise by obscured using standard microscopy (data not shown). Collectively, these images provide visual information on not only changes in the distribution of biofilm as a result of different treatments, but also alterations in the number and location of live versus dead bacteria.

CONCLUSION

Beyond the recovery and enumeration of both total and biofilm-associated bacteria, histological and confocal microscopy techniques can be used to qualitatively assess the efficacy of anti-biofilm treatments in an *ex vivo* porcine dermal model. In addition to visualizing the relative numbers and location of live and dead bacteria, imaging can help determine whether the treatment was effective in penetrating the skin and acting on bacteria that have invaded the underlying tissues. Collectively, data obtained from these models can help drive the development of wound biofilm treatments.

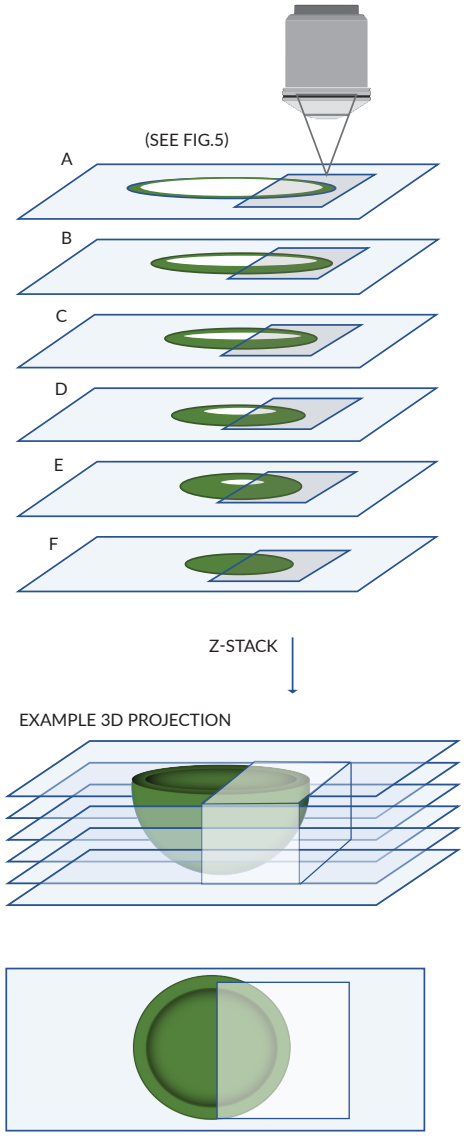
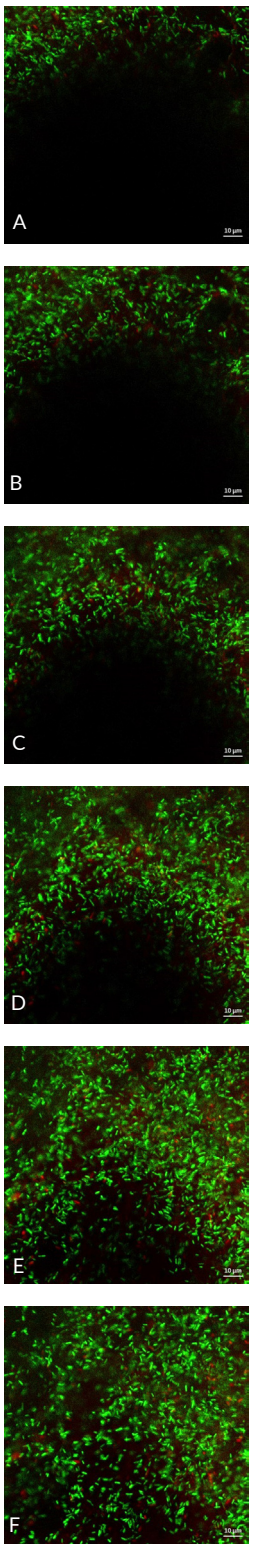


Fig. 4. (Above) Schematic demonstration of acquiring z-stacks using confocal microscopy from the wound bed of infected porcine skin tissue explant and resulting 3D projection (green represents GFP biofilm lining the wound bed). Sections A-F correspond to images shown in Fig. 5.

Fig. 5. (Right) Confocal microscopy Z-scan image series from the top to the bottom surface of the wound bed after incubation with *P. aeruginosa* PAO1-GFP for 3 days in the porcine skin explant. Note: only few bacteria are seen on the top surface being attached to the edge of the wound bed (A) but increasingly more bacteria are attached (B to F) as images proceed deeper into the tissue.



1 Yang, Q, et al. Development of a novel *ex vivo* porcine skin explant model for the assessment of mature bacterial biofilms. Wound Repair Regen. 2013 Sep-Oct; 21(5): 704–714.
2 Bionda, N and Mouchka, G. A bridge between *in vitro* screens and animal models for the study of anti-biofilm efficacy. 2017 iFyber App Note F400-01-03