THE *EX VIVO* PORCINE DERMAL MODEL OF MATURE BIOFILM AS A METHOD FOR EVALUATING SUPERCRITICAL CO_2 STERILIZATION

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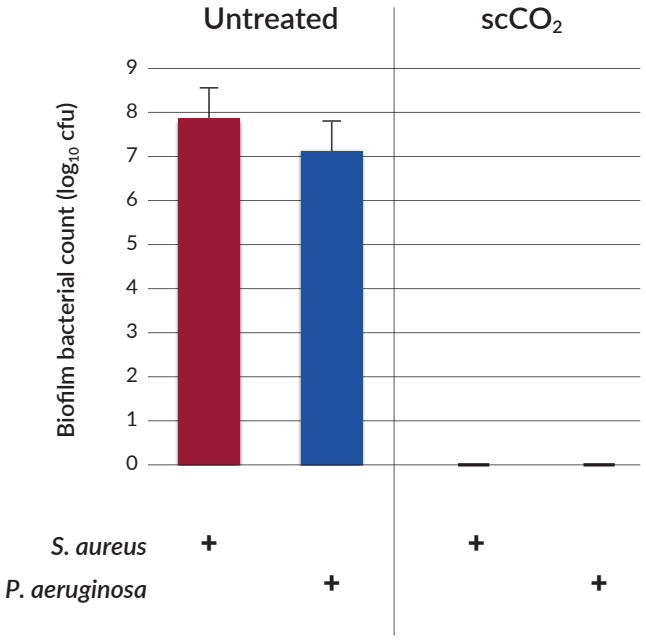
Mature biofilms grown within natural soft tissue matrices are challenging to eradicate and represent an emerging focus of allograft sterilization efforts. Here, we describe the inactivation of biofilms in an *ex vivo* porcine dermal model using the NovaSterilis supercritical carbon dioxide ($scCO_2$) sterilization process. These data show that the *ex vivo* model can be used to evaluate the efficacy of tissue sterilization methods in removing mature biofilms.

It is estimated that over one million allografts[1] are transplanted each year, and the risk of disease transmission due to microbial contamination is a significant concern. Skin allografts are particularly problematic as the tissue is colonized by normal microflora at the time of harvest and many of the bacteria are known to form biofilms[2]. Microbial biofilms are sessile communities of one or more microorganisms that reside within a self-produced extracellular matrix. These cellular aggregates can form on both living and non-living surfaces and have severe implications for human health. As a result, sterilization of tissue allografts is necessary to reduce the risk of microbial transmission. Appropriate test methods are therefore required to evaluate the ability of these techniques to eradicate established biofilms.

iFyber has adopted an *ex vivo* porcine dermal model[3,4] for the evaluation of treatments geared towards either preventing the formation of mature biofilm or eradicating pre-existing biofilm. Specifically, this model uses porcine dermal explants with artificial wound beds, which support biofilm populations that exhibit strong tolerance to antibiotics and disinfecting agents[3,5]. By using a defined amount of inoculum and incubation conditions, we can obtain a consistent level of microbial biofilm within the tissue which allows for quantitative assessment of the efficacy of different treatments. With these characteristics in mind, the *ex vivo* model is an ideal candidate for assessing the efficacy of cleaning and sterilization methods intended for allograft tissues.

Here, mature *Pseudomonas aeruginosa* or *Staphylococcus aureus* biofilms were established over a 3-day-period on porcine dermal explants[4] and exposed to a 45-minute supercritical CO_2 ($scCO_2$) cycle and 8 mL NovaKill™ additive using a commercial Nova2200™ sterilizer. Following treatment, recovery of bacterial survivors was performed by immersing explants in a recovery solution and executing a series of sonication steps. The recovery solution for each sample was then subjected to a 10-fold serial dilution and plated for enumeration. The limit of detection (LOD) for this method of enumeration is 50 CFU/mL. Our results show that $scCO_2$ treatment decreased mature *P. aeruginosa* and *S. aureus* biofilms by over 7 log_{10} in all replicates **(Figure 1)**.

FIGURE 1: BIOFILM ERADICATION ON AN *EX VIVO* PORCINE DERMAL MODEL USING THE NOVASTERILIS $scCO_2$ PROCESS



CONCLUSION

iFyber has successfully implemented the *ex vivo* porcine dermal model of mature biofilm for evaluation of the NovaKill™ $scCO_2$ sterilization process. These results demonstrate that the *ex vivo* model can be used to evaluate biofilm eradication and tissue sterilization via supercritical CO_2 and is amenable to method development efforts aimed at assessing sterilization techniques and processes.

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