ERADICATION OF BIOFILMS USING SUPERCRITICAL CO_2

Application Note Med01-02

The eradication of biofilm is crucial to the reprocessing of reusable medical devices exposed to organic materials. The Nova Process, which uses NovaKill™ with supercritical CO_2, can rapidly eradicate attached bacteria and mature biofilms.

MEDICAL DEVICES AND BIOFILMS

Reusable medical devices and equipment can often be difficult to clean due to small and/or complex geometries. For example, organic contaminants within the tubing, lumen, and elevator chambers of a duodenoscope may be physically out of reach of brushes or specialized equipment. Chemical sterilants including glutaraldehyde, hydrogen peroxide, or peracetic acid have been shown to crosslink biofilm-associated proteins, which may lead to treatment resistance due to ineffective penetration. In contrast, the pressure, temperature, and physical state of supercritical CO_2 ($scCO_2$) enable penetration through and drying of surfaces and cavities within complex medical devices.

BIOFILM INACTIVATION WITH SUPERCRITICAL CO_2 STERILIZATION

NovaSterilis has previously shown that $scCO_2$ sterilization at low temperature (35°C) with the NovaKill™ sterilant additive can inactivate highly resistant endospores. This same process was tested against 1-day-old biofilms grown on plastic surfaces. Specifically, single-species biofilms of *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* were treated using the $scCO_2$ sterilization process with and without the NovaKill additive. As shown in **Figure 1**, $scCO_2$ treatment without additive significantly lowered bacterial counts in all three biofilms within just 10 minutes, with the strongest effect seen against *P. aeruginosa*. The addition of the NovaKill additive to a 10-minute $scCO_2$ treatment was sufficient to eradicate biofilm, resulting in a 7 log_{10} decrease in biofilm-associated bacteria counts for all three species tested.

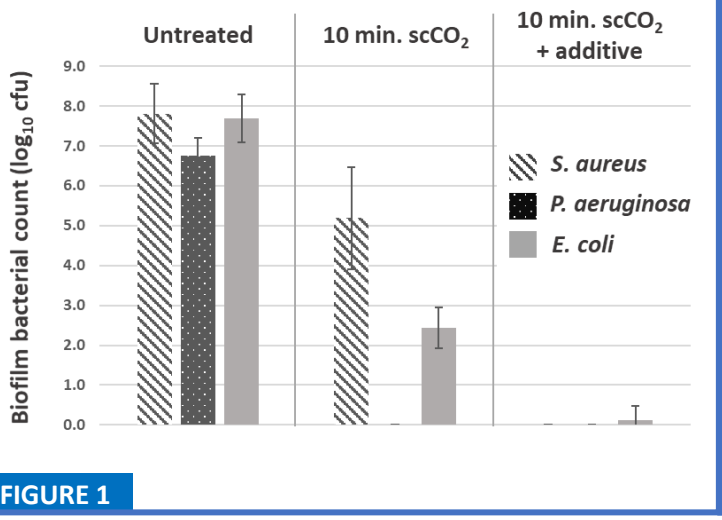

FIGURE 1

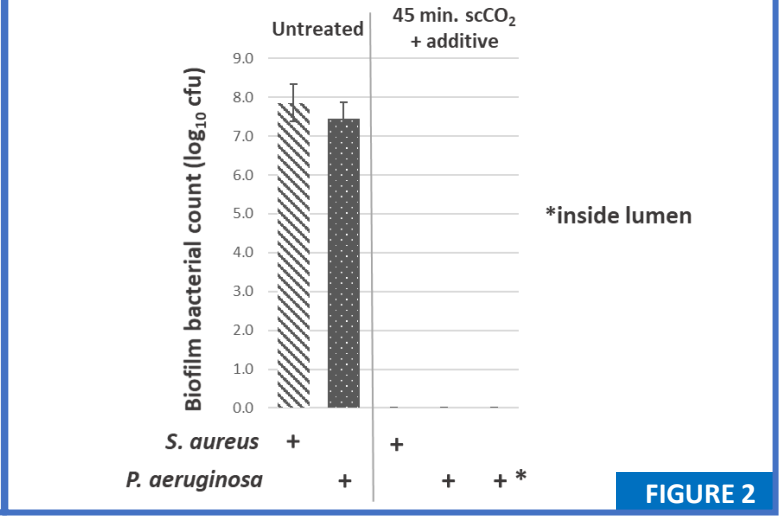

FIGURE 2

The Nova process was also tested against 3-day-old mature biofilms grown on porcine dermal explants. Biofilms grown on natural porcine tissue matrices are significantly more resilient than biofilms grown on a plastic surface. Mature *P. aeruginosa* or *S. aureus* biofilms were exposed to a 45-minute $scCO_2$ sterilization time with NovaKill additive. Both species of mature biofilms were fully eradicated over 7 log_{10} in all replicates (**Figure 2**). To evaluate the efficacy of the Nova Process in a more challenging use case scenario, multiple dermal explants carrying mature *P. aeruginosa* biofilm were placed inside a device positioned between two 1-meter lumens. The purpose of the lumen challenge device is to simulate conditions that may be present with more complex medical devices (refer to Application Note *Med01-01* for details on the lumen challenge device). Consistent with the previous study, a 45-minute $scCO_2$ sterilization time that included the NovaKill additive was sufficient to eradicate all biofilm-associated *P. aeruginosa* within the dermal matrix.

CONCLUSIONS

These studies demonstrate that the Nova Process, which uses NovaKill™ with supercritical CO_2, can sterilize devices that are burdened by biofilms. The short amount of time required to achieve complete biofilm eradication, along with sterilization results showing inactivation of highly resistant bacterial endospores, serve as a strong basis for further development of commercially relevant sterilization solutions using the Nova Process.

ADDITIONAL READING

Qiu *et al.*, *J Biomed Mater Res Pt B: Appl. Biomater*. 2009;91B:572-8.
Setlow *et al.*, *J. Appl. Microbiol.* 2016;120:57-69.
White *et al.*, *J. Biotechnol.* 2006;123:504-15.

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