Biofilm Longevity Assessment of *E. coli*, *S. aureus*, and *C. albicans* in Nonnutritive Phosphate Buffered Saline

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ABSTRACT

The Modified Robbins Device (MRD) remains an important tool in the study of biofilms and is routinely used to assess the antimicrobial properties of novel agents on biofilms[1,2]. As such, the natural lifespan of biofilms (e.g. nutrient depletion, osmotic pressure, etc.) must be controlled for when testing for biofilm reduction caused by exposure to anti-biofilm agents using this testing apparatus. We assessed the longevity of three mono-culture biofilms of different host organisms (*Escherichia coli* (ATCC 25922), *Staphylococcus aureus (*ATCC 6538), and *Candida albicans* (ATCC 10231)) in nonnutritive phosphate buffered saline (PBS) incubated in 6-port static testing devices. The biofilms were established in a MRD on sterile PVC plastic sampling coupons. After 22 hours of incubation at 37°C ± 3°C in half-strength Tryptic Soy Broth (TSB; bacterial test systems) or half-strength Yeast Dextrose Broth (YD/B; *C. albicans*) circulating at 60 mL/hour, via a peristaltic pump, the sampling coupons were transferred from the MRD to a 6-port static sampling device filled with PBS. Coupons were then sampled to identify the concentration of viable microbes remaining on the coupon. At 7 days (168 hours), the survival rates of these microorganisms varied widely and not in an easily predictable fashion. These results show the importance of testing biofilm survival independent of test articles since there is a large range in biofilm longevity for different microbial species.

METHODS

Each microbe was inoculated from frozen bead culture into 20 mL of TSB (bacterial test systems) or YD/B (*C. albicans*) and grown overnight at 37°C ± 3°C. The biofilms were established in a Modified Robbins Device (MRD) on PVC plastic sampling coupons by combining the 20 mL inoculum with 1 L of half-strength TSB or half-strength YD/B. This was circulated for 6 hours at 60 mL/hour at 37°C ± 3°C to establish the initial biofilm. The inoculated media was then removed from the system and replaced with appropriate fresh half-strength media. The fresh media flowed through the MRD and into a waste container at 60 mL/hour for 22 hours at 37°C ± 3°C. The sampling coupons were then transferred from the MRD to a 6-port static sampling device filled with PBS and incubated at 37°C ± 3°C. Coupons were then sampled at 0, 6, 24, 30, 48, 72, 144, and 168 hours to identify the number of colony forming units (CFUs).

REFERENCES

1) Antibiotic resistance of Pseudomonas aeruginosa colonizing a urinary catheter in vitro. Nickel JC et al. Eur J Clin Microbiol. 1985 Apr;4(2):213-8.
2) Growth of bacterial biofilms on Tenckhoff catheter discs in vitro after simulated touch contamination of the Y-connecting set in continuous ambulatory peritoneal dialysis. Dasgupta MK, et al. Am J Nephrol. 1990;10(5):353-8.

RESULTS

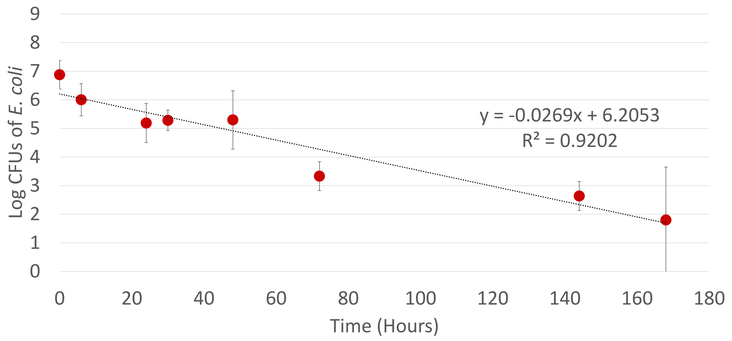

Figure 1. *E. coli* Recoverable CFUs. The graph above shows the average recoverable log CFUs per sampling coupon at 0, 6, 24, 30, 48, 72, 144, and 168 hours. There was a total of a 5 log reduction in the number of recoverable CFUs over 168 hours.

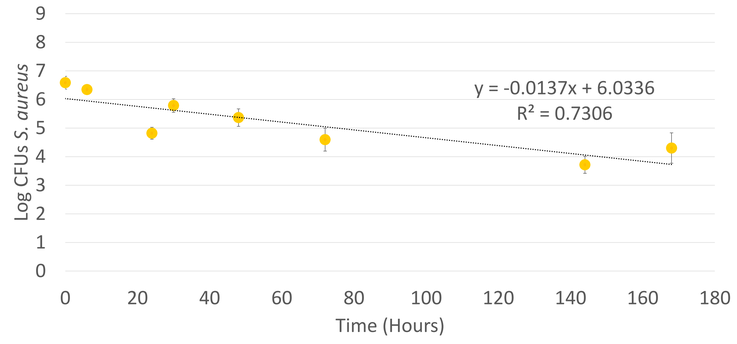

Figure 2. *S. aureus* Recoverable CFUs. The graph above shows the average recoverable log CFUs per sampling coupon at 0, 6, 24, 30, 48, 72, 144, and 168 hours. There was a total of a 2 log reduction in the number of recoverable CFUs over 168 hours.

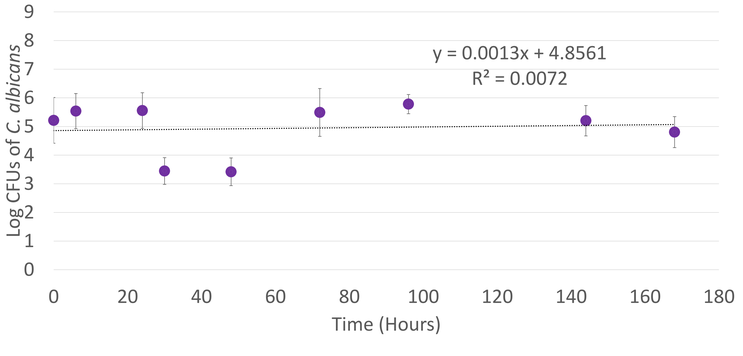

Figure 3. *C. albicans* Recoverable CFUs. The graph above shows the average recoverable log CFUs per sampling coupon at 0, 6, 24, 30, 48, 72, 96, 144, and 168 hours. There was an ~0.5 log reduction in the number of recoverable CFUs over 168 hours.

DIAGRAMS

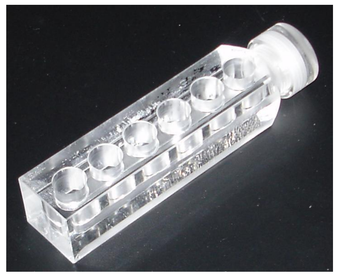

Figure 4. Static Testing Device. This static testing device allows for up to 6 sampling plugs to be immersed in a liquid medium.

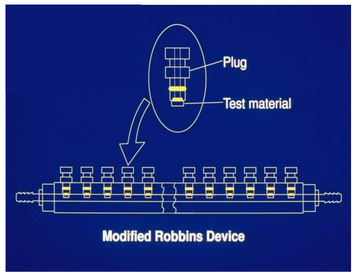

Figure 5. Diagram of a Modified Robbins Device. The Modified Robbins Device allows up to 25 individual biofilms to be created on individual sampling plugs.

CONCLUSIONS

These tests show that biofilms incubated in the static testing devices are suitable models for testing longer acting anti-biofilm agents. In particular, the ability of *C. albicans* to survive in a nonnutritive environment for at least 168 hours in the static testing device shows that this in vitro method may be a good model for testing novel anti-biofilm technologies. The difference between the log reduction in recoverable CFUs of *S. aureus* and *E. coli* in mono-culture biofilms is counter to prior work with co-culture biofilms in which *S. aureus* had greater log reductions than *E. coli* when co-cultured together with *Pseudomonas aeruginosa*. This conclusion shows that there is variability in biofilm longevity that cannot always be predicted, and therefore must be measured through experimentation.

Continued work to lengthen the duration of the incubation of the biofilms in a the static testing devices may determine a possible terminal point at which the biofilms are no longer able to sustain themselves. It may be particularly interesting to see the limits to which biofilms are able to survive in stressful, nonnutritive environments, and the potential changes in metabolism that result from it. It would also be useful to apply this experimental method to more microbial species and co-culture biofilms.

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