Efficacy of Iocide® Disinfectant Solution on *Pseudomonas aeruginosa* Biofilms

Pasmore M.[1], Bhatt B.[2], Warren W.[2], Siegel P.[2], and Siegel G.[2]

[1]Center for Biofilm Engineering, Bozeman, MT, USA, [2]Biomedical Development Corporation, San Antonio, TX, USA

ABSTRACT

Objective: Iocide® disinfectant solution was investigated for treatment of *Pseudomonas aeruginosa* biofilms using the ASTM method E2196-02, with minor modifications.

Methods: *Pseudomonas aeruginosa* biofilms were grown on 13 mm diameter x 1 mm thick, sterilized, hydroxyapatite disks (coupons), under the conditions of constant fluid-shear and temperature (37°C). The conditions of this experiment are similar to those of other standardized disinfectant efficacy tests (i.e., AOAC). At the initiation of each experiment bacteria were allowed to attach to the coupons for 2 hours, prior to the initiation of media flow and shear. The stir plate was then initiated to provide mixing and shear force. The biofilms were allowed to form for a period of 48-hours, obtaining thicknesses of approximately 80 micrometers. Following biofilm formation, the colonized hydroxyapatite coupons were harvested and briefly soaked for 1 minute in sterile dH_2O to remove unattached bacteria. The reactor coupons were then subdivided into two sets for treatment, with one set of coupons challenged with undiluted Iocide® for 8 hours at 22±1°C. The second set of coupons was left untreated to serve as controls.

Results: The results of three separate experiments showed that undiluted Iocide® treatment for 8 hours caused an average 7.2 $\log_{10}$ reduction in CFU/cm^2 compared to controls. These experiments showed that over the time tested, Iocide was very effective at treating the biofilm.

Conclusion: Iocide® treatment shows significant efficacy in killing biofilm bacteria.

INTRODUCTION

Iocide® is a novel antimicrobial agent developed for commercial applications, specifically for treatment of biofilm associated with hemodialysis and dental waterlines (Dentacide®). In addition to having antimicrobial and anti-biofilm properties, Iocide is non-toxic, non-carcinogenic, and not harmful to the environment.

To evaluate Iocide against *Pseudomonas aeruginosa* biofilm, a rotating disk reactor was assembled to generate biofilms in a continuous flow and fluid-shear environment. Test materials were used in the form of small, round "coupons" that were placed within a reactor rotor. Six coupons on which biofilms were formed were placed in the reactor rotor and submerged in the continuous-flow culture. The reactor was then incubated according to the appropriate conditions to support biofilm growth.

METHODS

BACTERIA:

- *Pseudomonas aeruginosa* PAO1.

COUPON MATERIAL:

- Sterilized **hydroxyapatite** (HAP) (Clarkson Chromotography, South Williamsport, PA)
- 13 mm diameter x 1 mm thick disks

Figure 1. Hydroxyapatite disks used in the rotating disk reator system.

***In vitro* MODEL FOR BIOFILM FORMATION:**

- Media: Jordon's media[1]
- Conditioning layer was allowed to form for 1 hour on the coupons prior to inoculation (Jordon's media[1]) .
- An overnight inoculation culture was prepared with 6 ml of sterile Jordan's broth and 1 ml of the frozen stock culture and incubating at 37°C.
- 3 ml of overnight culture was used to inoculate the reactor.
- The bacteria were allowed to attach for 2 hours.
- The stir plate was then initiated at approximately 250 RPM.
- The biofilms formation occurred over a 48-hour period.
- The HA coupons were then harvested and briefly soaked (1 minute) in sterile deionized water to remove unattached bacteria.

TREATMENT

- Reactor coupons were subdivided into two sets
- Set 1 challenged with undiluted Iocide
- Set 2 challenged with sterile dionized water
- Both sets were treated for 8 hours
- Temperature: 22+1°C

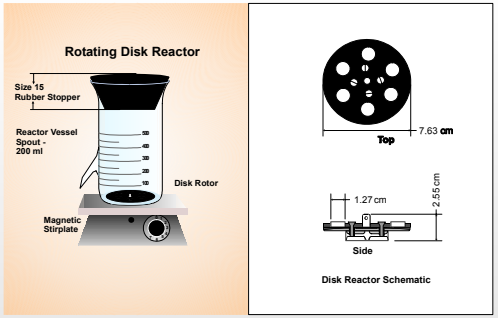

Figure 2. Schematic of the dripflow reactor.

ANALYSIS

- Prior to analyses samples were rinsed with dH_2O for 1 minute.
- CFU analyses employing the "Scrape and Plate" method were performed.
  - Homogenization was used for disaggregation.
  - Six 10-fold dilutions were plated.
  - On TSA plates.
  - And incubated for 24-48 hr at 37°C.

RESULTS

Undiluted Iocide treatment for 8 hours caused an average of 7.2 $\log_{10}$ reduction in CFU/cm^2 compared to controls. These experiments showed that over time, Iocide was very effective at treating biofilm.

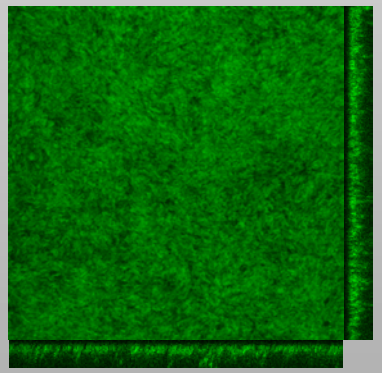

Figure 3. Images of Pseudomonas aeruginosa biofilm grown in rotating disk reactor, including top (xy) and both side (xz, yz) views.

Table 1. Data from the triplicate testing of Iocide treatment of PAO1 biofilms versus untreated control.

Controls	CFU/cm^2
1	5.79E+08
2	3.95E+08
3	5.26E+08
Average	**5.00E+08**
Treated samples	
1	0
2	0
3	0
Average	**0**
Difference	**5.00E+08**
Log Reduction	**7.2**

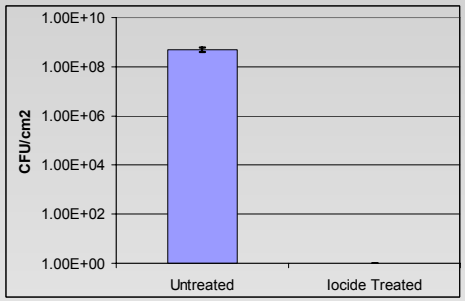

Figure 4. Comparison of colony forming units for treated and untreated PAO1 biofilm samples.

CONCLUSIONS

Iocide is an effective treatment for *P. aeruginosa* biofilms. In this study, treatment with Iocide reduced *P. aeruginosa* biofilm from 7.2 $\log_{10}$ CFU/cm^2 to zero.

REFERENCES

1. Pan P, Barnett ML, Coelho J, Brogdon C, Finnegan MB. Determination of the in situ bactericidal activity of an essential oil mouthrinse using a vital stain method. J. Clin. Periodontol 2000. 27: 256-261.

ACKNOWLEDGEMENTS

National Institutes of Health / National Institute of Diabetes and Digestive and Kidney Diseases. SBIR# 2R44DK056587.

Presented at the ASM Conference on Biofilms 2003 Victoria, British Columbia, November 1-6, 2003