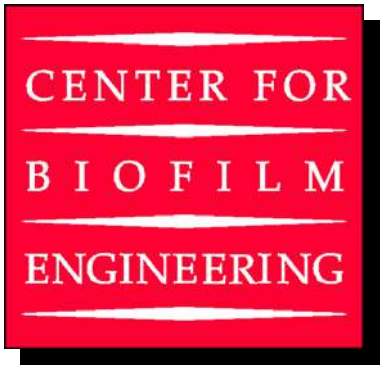

Center for Biofilm Engineering Proposal

FINAL REPORT

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Evaluation of an Ionic Silver Solution against Dual Species In-Vitro Wound Biofilms: Results of Biofilm Experiments

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Introduction

EnzySurge Ltd. tasked the Center for Biofilm Engineering with the evaluation of the efficacy of its ionic silver solution, SilverStream, against dual species biofilms *in-vitro*. This study assessed the effects of the ionic silver solution against biofilms using viable plate counts and confocal scanning laser microscopy (CSLM).

Experimental Design

This project used an *in-vitro* model system, the drip flow reactor (DFR), to evaluate ionic silver solution efficacy against established dual species biofilms grown on hydroxyapatite (HA) - coated glass slides (Figure 1).

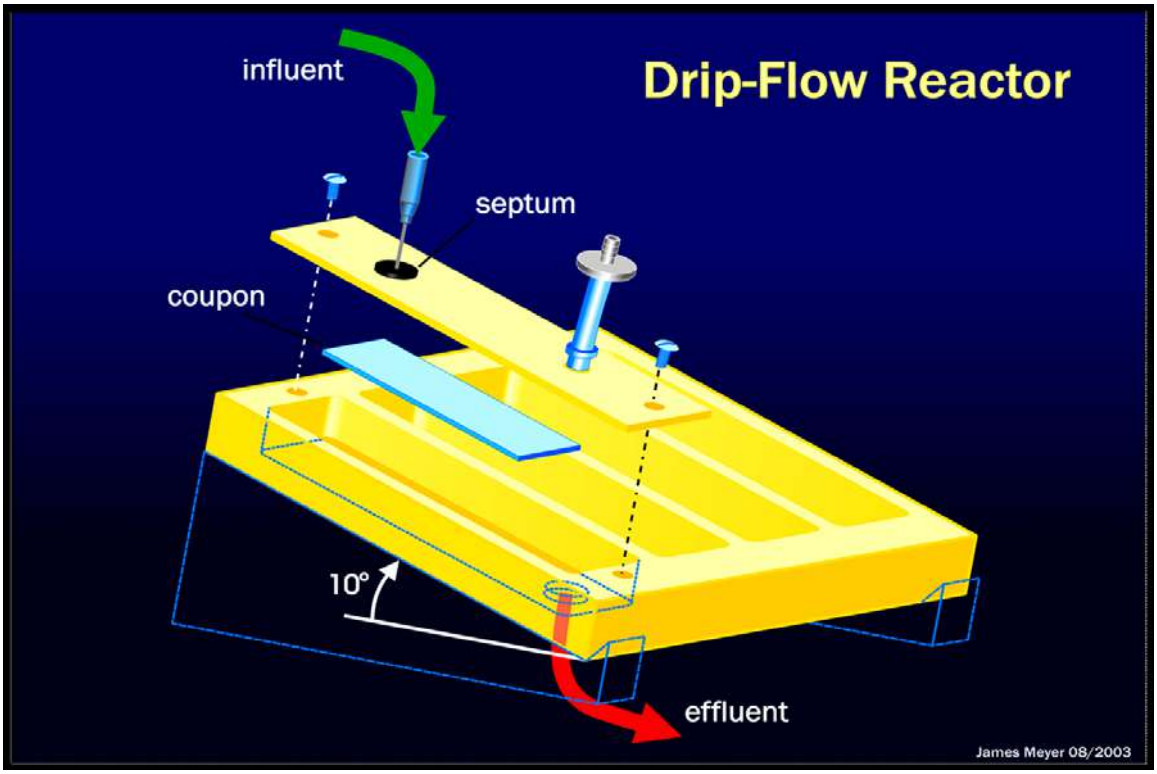

Figure 1. Schematic of Drip Flow Reactor

Inoculum and Biofilm Growth

The test strains for this project were *Staphylococcus aureus* SWRWCC #10943 (MRSA) and *Pseudomonas aeruginosa* SWRWCC #215. These isolates were collected from chronic wounds at the South West Regional Wound Care Center, supplied to the CBE and are maintained in the Medical Biofilms Laboratory (MBL) as frozen stocks at -70°C. Overnight cultures were grown

from frozen stocks at 37°C in Brain Heart Infusion broth (Table 1) supplemented with 5% bovine serum (BHI).

Table 1. Ingredients list for Brain Heart Infusion

Ingredient	**Grams per liter**
Brain heart infusion from solids	3.5
Peptic digest of animal tissue	15.0
Sodium chloride	5.0
Dextrose	2.0
Pancreatic digest of casein	10.0
Disodium phosphate	2.5

The DFRs were operated in a 37°C incubator under aerobic conditions. Prior to inoculation, sterile medium (10%-strength Brain Heart Infusion broth supplemented with 5% bovine serum) was dripped-in and allowed to collect over the HA-glass slides. Each channel of the reactor was inoculated with 1.0ml of a 50:50 mixture of the overnight cultures. The reactor was set at a 10° angle and sterile medium dripped through the reactor at a rate of 40 ml/hr total (10 ml/hr per coupon). The reactor was run for a period of 3 days.

Treatment

Treatments were applied in either a static or dynamic mode. For static treatment, the chamber was filled with 40 ml of treatment solution for 5 minutes. For dynamic treatment mode, a 30ml syringe, with a 19G needle was used to apply 20 ml total treatment volume over 5 minutes and the JetOx ND debrider was used to deliver 20 ml treatment solution over a 5 minute time period. Silver Stream treatments were compared to matched sterile saline controls.

Viable Plate Counts

After treatment and prior to processing for viable plate counts, the slides were neutralized with a 1.45% sodium thiosulfate and 1% sodium thioglycolate solution.

Plate counts were performed by removing the biofilms from the slide surface and disaggregating the bacteria using a sequence of vortex, sonicate, and vortex in a phosphate buffered saline (PBS) (Table 2) to produce a bacterial suspension. The suspension was serially-diluted with PBS and plated on either Pseudomonas Isolation Agar (PIA) and Staphylococcus Medium 110 (SIA) for the isolation and enumeration of *P. aeruginosa* and *S. aureus,* respectively, or plated on Tryptic Soy Agar (TSA) in which the colonies formed by the different species were differentiated visually. The plates were incubated at 37°C for 24 hours and the number of colony forming units (CFU) counted. Based on the dilution and surface area of the slide, the number of CFU per unit area was calculated.

Table 2. Ingredients list for Phosphate Buffered Saline

Ingredient	Grams per 2.0 liters
NaCl	16
KCl	0.4
Na_2HPO_4	2.3
KH_2PO_4	0.4

Experiments and Results

Biofilm Experiment: Effects of Increased Treatment Soak Times

The effects of increased treatment soak time were evaluated on biofilms grown in the DFR. All slides received a 5 minute JetOx treatment and soak time began after that 5 minute treatment. Time 0 indicates that the slides were treated using the JetOx for 5 minutes but were not subjected to a soak treatment. Log Reductions (LR) were calculated by comparing to Time 0 data. Samples were not rinsed prior to treatment but were neutralized prior to processing for viable plate counts. All samples were soaked in SilverStream except for one sample at 355 minutes which was soaked in saline.

Table 3. Results of Increased Soak Time.

Soak Time	*P. aeruginosa*	*S. aureus*	Log Reduction Pa	Log Reduction Sa
Minutes	Log CFU/cm^2			
0	6.96	6.13		
10	2.98	2.7	3.98	3.43
25	2.5	2.58	4.46	3.55
55	1.68	0.6	5.28	5.53
355 (saline)	5.43	3.9	1.53	2.23
355 (SilverStream)	0.57	0.57	6.39	5.56

These results (Table 3, Figure 2) indicate that the soak time did make a difference in the efficacy of Silver Stream. All slides received a 5 minute JetOx treatment and soak time began after that 5 minute treatment. Time 0 indicates that the slides were treated using the JetOx for 5 minutes but were not subjected to a soak treatment. Log Reductions (LR) were calculated by comparing to Time 0 data.

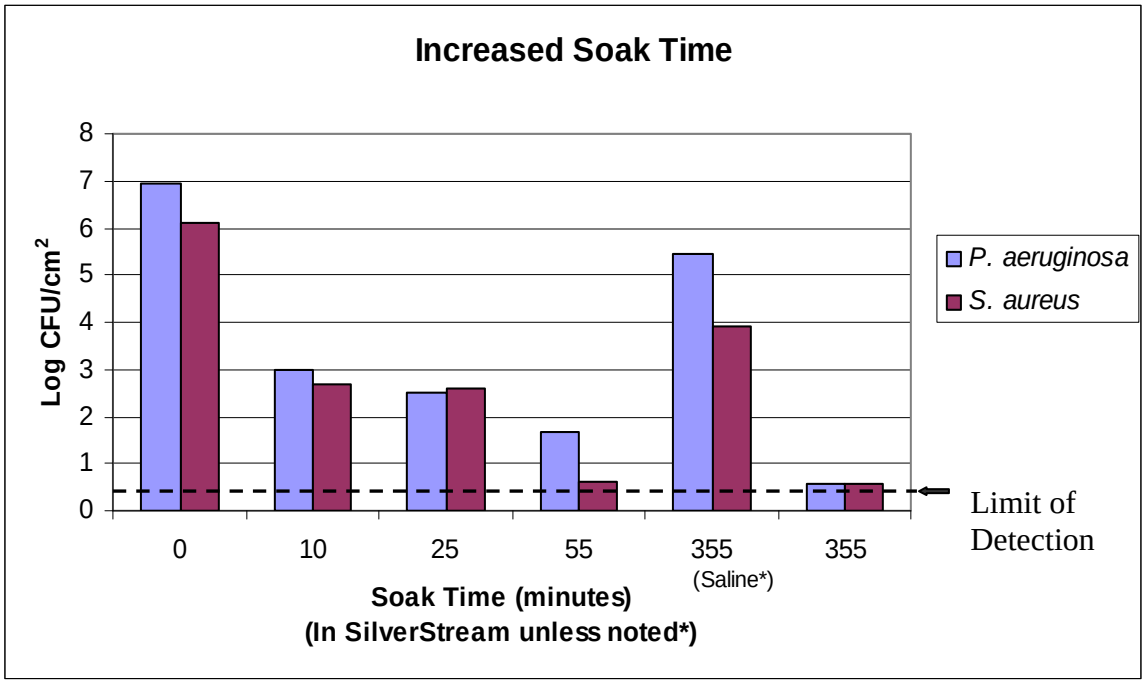

Figure 2. Results of Increased Soak Time.

In order to maximize the number of treatments, an untreated control was not used in this experiment. Log Reductions (LR) were calculated in Table 5 by comparing treatments to the Time 0 treatment (i.e. Time 0 was the control).

Increased soak time in SilverStream did reduce cell viability. At the 355 minute soak time point, the SilverStream treatment resulted in a 6.39 LR in *P. aeruginosa* and a 5.56 LR in *S. aureus* compared to Time 0).

Increased soak time in saline slightly reduced cell viability. At the 355 minute soak time point, the saline treatment resulted in some reduced cell viability (1.5 LR in *P. aeruginosa* and a 2.2

LR in *S. aureus* compared to Time 0).

Biofilm Experiments

Solutions were applied using the needle and syringe for 5 minutes instead of the JetOx system and total treatment times were 1 hour and 6 hours. While treatment with saline alone resulted in an approximately 1.6 log reduction (LR) at one hour and an approximately 2 LR at 6 hours for both species tested, SilverStream treatments resulted in a 4.84 LR for *P. aeruginosa* and a 5.44 LR for *S. aureus* at one hour and a 7.44 LR for *P. aeruginosa* and a 7.32 LR for *S. aureus* at 6 hours (Table 4).

Table 4. Results from Biofilm Experiments

		Control	**5 minutes needle + 55 minute soak in Saline**	**5 minutes needle + 55 minute soak in SilverStream**	**5 minutes needle + 5hrs 55 minute soak in Saline**	**5 minutes needle + 5hrs 55 minute soak in SilverStream**
		Log CFU/ml	**Log CFU/ml**	**Log CFU/ml**	**Log CFU/ml**	**Log CFU/ml**
P. aeruginosa	Run 4	9.50	8.29	4.45	7.79	1.87
	Run 5	9.90	8.09	5.42	7.96	2.80
		9.94				
	Average	**9.78**	**8.19**	**4.93**	**7.88**	**2.34**
	LR		**1.59**	**4.84**	**1.90**	**7.44**
S. aureus	Run 4	9.07	7.53	3.86	7.11	1.91
	Run 5	9.77	8.16	4.26	7.87	2.46
		9.66				
	Average	**9.50**	**7.84**	**4.06**	**7.49**	**2.18**
	LR		**1.66**	**5.44**	**2.01**	**7.32**

Conclusions

One hour treatment of biofilms with SilverStream resulted in a 4.84 LR in *P. aeruginosa* and 5.44 LR in *S. aureus*.

Six hour treatment of biofilms with SilverStream resulted in a 7.44 LR in *P. aeruginosa* and 7.32 LR in *S. aureus*.

Submitted to EnzySurge Ltd by:

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