

Immobilization and Death of Bacteria by Flora Seal® Microbial Sealant

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Abstract: A new advantage of cyanoacrylate surgical microbial sealant, i.e. FloraSeal®, is that organisms die shortly upon contact providing additional protection to the patient.

Keywords: Cyanoacrylate, water activity, immobilization, microbial death, nosocomial infection risk

I. Introduction

Surgical procedures can cause infection to the patient due to a break in aseptic technique causing severe patient distress [1, 2]. Specifically, incised wounds can become infected by the healthcare provider. Cyanoacrylate surgical glues were originally developed to close approximated wounds obviating the need to close wounds with sutures [3]. Now more is known about the additional applications of cyanoacrylate. We report for the first time that FloraSeal®, a combination of 2-octyl cyanoacrylate (80%) and butyl cyanoacrylate, traps, immobilizes bacteria that may cause infection to the patient or healthcare provider and rapidly and powerfully kills clinically relevant gram positive and gram negative bacteria.

II. Methodology

USP Procedures for Water Activity (aW) [4] and Karl Fischer [5] were followed (see Tables 1-2). For the immobilization study, sterile Pig Skin was used to simulate contamination of freshly made surgical incisions. Sterile pig skin was aseptically cut into 4 x 1 cm pieces. Each of two pieces of sterile pig skin/test article was inoculated by delivering 0.1 mL [$\sim 75,000$ colony forming units] of the test organism to a marked ~ 4 cm by 1 cm area of the skin [surgical site]. The incision site was defined by a metric ruler to the depth of the fat layer below the dermis and length of ~ 4 cm. The inoculated skin was placed under a laminar flow hood to allow the inoculum to dry at ambient laboratory temperature. With the supplied applicator, the inoculated skin was painted over the incision site with FloraSeal® and allowed to dry under a laminar flow hood [~ 4 minutes]. With a sterile scalpel an incision [incision site] was made. The incision site was manipulated while wearing sterile gloves by gently squeezing together the incision site four times to simulate surgical trauma.

Organisms were recovered from the incision site. Excess skin was cut away from the incision site with a sterile scalpel. The incision site was irrigated with 0.1 mL of sterile Elution Fluid and the eluate collected. This step was repeated four additional times each time plating the eluate separately. Ten-fold serial dilutions of the eluate were prepared and duplicate pour plate counts and or membrane filtration counts performed. Plates were incubated for 48 to 72 hours at 35 to 37°C. See results in Table 3.

The antimicrobial study was performed with 20 samples/organism. All of the contents of FloraSeal® was aseptically transferred to a suitable container. Twenty (20) containers were prepared per organism. A challenge of greater than or equal to 10^8 CFUs of the test organisms shown in Table 4 was aseptically transferred to the container containing 2.5 mL of FloraSeal® previously just inoculated with the test organism loop. After a three (3) minute contact time, the entire contents of the inoculated sample of FloraSeal® was transferred to 97.5 mL of Sterile USP Purified Water (PW) using a micropipette. The final volume was ~ 100.0 mL. The jar was immediately shaken for 15 seconds. Jar 1 = $\sim 10^8$ cells/100 mL. 1.0 mL from Jar 1 was used to make the next dilution [Tube 2] and the remaining 99 mL from jar 1 was membrane filtered. Ten-fold serial dilutions were made to 10^{-6} . Dilutions were prepared by aseptically transferring 1.0 mL into 9.0 mL of sterile USP PW. Each dilution tube was vortexed for 15 seconds.

III. Discussion

Floraseal® is very anhydrous as demonstrated by its very low Water Activity (Table 1, 0.4 aW) and very low USP Karl Fischer moisture content (0.2%, Table 2). As an immobilization agent, FloraSeal® will mitigate the spread of microorganisms in the surgical field by sealing microorganisms to the skin and preventing possible nosocomial related infection of a wound site. The immobilization effect is broad spectrum against gram positive, gram negative bacteria as well clinically relevant bacteria and yeast including strains resistant to antibiotics. Low water activity is a known limiting factor for microbial survival [6]. Floraseal® completely kills ≥ 8 logs of bacteria after a three minute contact time (Table 4). As per USP <1112>, materials with water activity levels of less than 0.6 are hostile to microbial growth and the specified organisms as described in USP <62>, Microbial Examination of Nonsterile Products: Tests for Specified Microorganisms, are by definition presumed to be absent. The results are equivalent to another form of the product, SurgiSeal [6].

IV. Results

4.1

Table 1 Chemical Measurements of Water USP <1112> Water Activity is Stable

GBL# 0Time	GBL# 24 month	Product Description	0Time – aW Reading	24 month – aW Reading
474289/1	476668/1	FloraSeal® Microbial Sealant	0.424	0.440
474289/2	476668/2	FloraSeal® Microbial Sealant	0.408	0.434
474289/3	476668/3	FloraSeal® Microbial Sealant	0.419	0.432
474289/4	476668/4	FloraSeal® Microbial Sealant	0.408	0.432
474289/5	476668/5	FloraSeal® Microbial Sealant	0.410	0.425
474289/6	476668/6	FloraSeal® Microbial Sealant	0.403	0.426
474289/7	476668/7	FloraSeal® Microbial Sealant	0.397	0.427
474289/8	476668/8	FloraSeal® Microbial Sealant	0.416	0.436
474289/9	476668/9	FloraSeal® Microbial Sealant	0.407	0.435
474289/10	476668/10	FloraSeal® Microbial Sealant	0.405	0.430
Average			0.410	0.432
Sample Standard Deviation			0.008	0.005

4.2

Table 2 Chemical Measurements of USP <921> Moisture Determination is Stable

GBL# 0Time	GBL# 24 month	Product Description	0 Time – % Moisture	24 month – % Moisture
474334/1	476665/1	FloraSeal® Microbial Sealant	0.15	0.20
474334/2	476665/2	FloraSeal® Microbial Sealant	0.16	0.20

4.3

Table 3 FloraSeal® is a Barrier to Ingress of Challenge Organisms to Incised Wound

<i>Staph. epidermidis</i>		MRSA		<i>Coryne-bacterium species</i>		<i>Pseudomonas aeruginosa</i>		<i>C. albicans</i>	
A	B	A	B	A	B	A	B	A	B
3.94	0.0	3.93	0.0	3.91	0.0	3.97	0.0	4.1	0.0

GBL= Gibraltar Laboratories Test Reference # 208671/1-2.43

A = Log Challenge to Incised wound site

B= Log Recovered from Wound Site Treatment with FloraSeal®

4.4

Antimicrobial Kill of FloraSeal®

Summary of Kill After 3 Minute Contact Time [Detail follows below, Table 4]

Organism	Log Reduction
<i>Escherichia coli</i>	8.0
<i>Klebsiella pneumoniae</i>	9.0
<i>Staphylococcus epidermidis</i>	9.0
<i>Staphylococcus aureus subsp. aureus</i> [MRSA]	9.0

Table 4 Quantitative Kill Against Four Clinically Relevant Microorganisms

Control: <i>Escherichia coli</i>		
Dilution	Mock count	
	Replicate 1	Replicate 2
10^0 to 10^{-3}	Not Performed	
10^{-4}	TNTC	TNTC
10^{-5}	>200	>200
10^{-6}	106	117
10^{-7}	11	13
CFU/~10mL	1.1×10^8	
Log ₁₀	8.04 = 8	

Replicate #	Dilution							CFU/~10mL	Log ₁₀	Log ₁₀ Reduction
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
1	0	0	0	0	0	0	0	<1	0	8-0 = 8
2	0	0	0	0	0	0	0	<1	0	8-0 = 8
3	TNTC	TNTC	TNTC	TNTC	>200	22	2	2.2×10^6	6.34 = 6	8-6 = 2
4	0	0	0	0	0	0	0	<1	0	8-0 = 8
5	0	0	0	0	0	0	0	<1	0	8-0 = 8
6	0	0	0	0	0	0	0	<1	0	8-0 = 8
7	0	0	0	0	0	0	0	<1	0	8-0 = 8
8	0	0	0	0	0	0	0	<1	0	8-0 = 8
9	0	0	0	0	0	0	0	<1	0	8-0 = 8
10	0	0	0	0	0	0	0	<1	0	8-0 = 8
11	0	0	0	0	0	0	0	<1	0	8-0 = 8
12	0	0	0	0	0	0	0	<1	0	8-0 = 8
13	0	0	0	0	0	0	0	<1	0	8-0 = 8
14	0	0	0	0	0	0	0	<1	0	8-0 = 8
15	0	0	0	0	0	0	0	<1	0	8-0 = 8
16	0	0	0	0	0	0	0	<1	0	8-0 = 8
17	0	0	0	0	0	0	0	<1	0	8-0 = 8
18	0	0	0	0	0	0	0	<1	0	8-0 = 8
19	0	0	0	0	0	0	0	<1	0	8-0 = 8
20	0	0	0	0	0	0	0	<1	0	8-0 = 8

Legend: CFU = colony forming units, NA = Not Applicable, TNTC = Too Numerous to Count

Control: <i>Klebsiella pneumoniae</i>		
Dilution	Mock count	
	Replicate 1	Replicate 2
10^0 to 10^{-3}	Not Performed	
10^{-4}	TNTC	TNTC
10^{-5}	TNTC	TNTC
10^{-6}	>200	>200
10^{-7}	38	47
CFU/~10mL	4.3×10^8	
Log ₁₀	8.63 = 9	

Replicate #	Dilution							CFU/~10mL	Log ₁₀	Log ₁₀ Reduction
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
1	0	0	0	0	0	0	0	<1	0	9-0 = 9
2	0	0	0	0	0	0	0	<1	0	9-0 = 9
3	0	0	0	0	0	0	0	<1	0	9-0 = 9
4	0	0	0	0	0	0	0	<1	0	9-0 = 9
5	0	0	0	0	0	0	0	<1	0	9-0 = 9
6	0	0	0	0	0	0	0	<1	0	9-0 = 9
7	0	0	0	0	0	0	0	<1	0	9-0 = 9
8	0	0	0	0	0	0	0	<1	0	9-0 = 9
9	0	0	0	0	0	0	0	<1	0	9-0 = 9
10	0	0	0	0	0	0	0	<1	0	9-0 = 9
11	0	0	0	0	0	0	0	<1	0	9-0 = 9
12	0	0	0	0	0	0	0	<1	0	9-0 = 9
13	0	0	0	0	0	0	0	<1	0	9-0 = 9
14	0	0	0	0	0	0	0	<1	0	9-0 = 9
15	0	0	0	0	0	0	0	<1	0	9-0 = 9
16	0	0	0	0	0	0	0	<1	0	9-0 = 9
17	0	0	0	0	0	0	0	<1	0	9-0 = 9
18	0	0	0	0	0	0	0	<1	0	9-0 = 9
19	0	0	0	0	0	0	0	<1	0	9-0 = 9
20	0	0	0	0	0	0	0	<1	0	9-0 = 9

Legend: CFU = colony forming units, NA = Not Applicable, TNTC = Too Numerous to Count

Control: <i>Staphylococcus epidermidis</i>		
Dilution	Mock count	
	Replicate 1	Replicate 2
10 ⁰ to 10 ⁻³	Not Performed	Not Performed
10 ⁻⁴	TNTC	TNTC
10 ⁻⁵	TNTC	TNTC
10 ⁻⁶	>200	>200
10 ⁻⁷	33	43
CFU/~10mL	3.8 x 10 ⁸	

Replicate #	Dilution							CFU/~10mL	Log ₁₀	Log ₁₀ Reduction
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
1	0	0	0	0	0	0	0	<1	0	9-0 = 9
2	0	0	0	0	0	0	0	<1	0	9-0 = 9
3	0	0	0	0	0	0	0	<1	0	9-0 = 9
4	0	0	0	0	0	0	0	<1	0	9-0 = 9
5	0	0	0	0	0	0	0	<1	0	9-0 = 9
6	0	0	0	0	0	0	0	<1	0	9-0 = 9
7	0	0	0	0	0	0	0	<1	0	9-0 = 9
8	0	0	0	0	0	0	0	<1	0	9-0 = 9
9	0	0	0	0	0	0	0	<1	0	9-0 = 9
10	0	0	0	0	0	0	0	<1	0	9-0 = 9
11	0	0	0	0	0	0	0	<1	0	9-0 = 9
12	0	0	0	0	0	0	0	<1	0	9-0 = 9
13	0	0	0	0	0	0	0	<1	0	9-0 = 9
14	0	0	0	0	0	0	0	<1	0	9-0 = 9
15	0	0	0	0	0	0	0	<1	0	9-0 = 9
16	0	0	0	0	0	0	0	<1	0	9-0 = 9
17	0	0	0	0	0	0	0	<1	0	9-0 = 9
18	0	0	0	0	0	0	0	<1	0	9-0 = 9
19	0	0	0	0	0	0	0	<1	0	9-0 = 9
20	0	0	0	0	0	0	0	<1	0	9-0 = 9

Legend: CFU = colony forming units, NA = Not Applicable, TNTC = Too Numerous to Count

Control: <i>Staphylococcus aureus</i> subsp. <i>aureus</i> [MRSA]		
Dilution	Mock count	
	Replicate 1	Replicate 2
10 ⁰ to 10 ⁻³	Not performed	
10 ⁻⁴	TNTC	TNTC
10 ⁻⁵	TNTC	TNTC
10 ⁻⁶	>200	>200
10 ⁻⁷	86	93
CFU/~10mL	9.0 x 10 ⁸	
Log ₁₀	8.95 = 9	

Replicate #	Dilution							CFU/~10mL	Log ₁₀	Log ₁₀ Reduction
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
1	0	0	0	0	0	0	0	<1	0	9-0 = 9
2	0	0	0	0	0	0	0	<1	0	9-0 = 9
3	0	0	0	0	0	0	0	<1	0	9-0 = 9
4	0	0	0	0	0	0	0	<1	0	9-0 = 9
5	0	0	0	0	0	0	0	<1	0	9-0 = 9
6	0	0	0	0	0	0	0	<1	0	9-0 = 9
7	0	0	0	0	0	0	0	<1	0	9-0 = 9
8	0	0	0	0	0	0	0	<1	0	9-0 = 9
9	0	0	0	0	0	0	0	<1	0	9-0 = 9
10	0	0	0	0	0	0	0	<1	0	9-0 = 9
11	0	0	0	0	0	0	0	<1	0	9-0 = 9
12	0	0	0	0	0	0	0	<1	0	9-0 = 9
13	0	0	0	0	0	0	0	<1	0	9-0 = 9
14	0	0	0	0	0	0	0	<1	0	9-0 = 9
15	0	0	0	0	0	0	0	<1	0	9-0 = 9
16	0	0	0	0	0	0	0	<1	0	9-0 = 9
17	0	0	0	0	0	0	0	<1	0	9-0 = 9
18	0	0	0	0	0	0	0	<1	0	9-0 = 9
19	0	0	0	0	0	0	0	<1	0	9-0 = 9
20	0	0	0	0	0	0	0	<1	0	9-0 = 9

Legend: CFU = colony forming units, NA = Not Applicable, TNTC = Too Numerous to Count

V. Conclusion

The important clinical attributes of FloraSeal® are [1] as a surgical microbial sealant that [2] immobilizes bacteria, prevents surgical site contamination, [3] seals any residual bacteria and [4] kills the bacteria from entering the incision site during a surgical procedure.

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