Antibiofilm Activity of Novel Silver Complex in Dressings & Gels Against Drug Resistant Microorganisms

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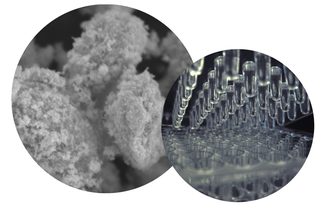


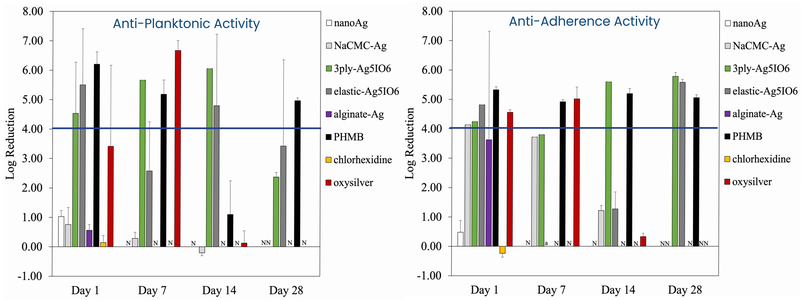
INTRODUCTION

Ag_5IO_6 represents a new class of Ag(I) that is active in the presence of bodily fluids

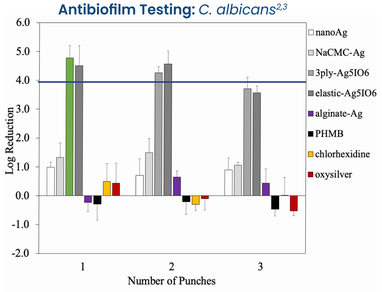
- Grain size=10-40 nm, polycrystalline particles (~1 µm) = ↑ surface area and active species
- Slow-release/equilibrating with good stability in water and saline
- Multiple mechanisms of action from Ag and I → decreased likelihood of resistance; antiviral[1]

Two Ag_5IO_6-Coated Dressings Vs. Commercial Dressings[2,3]

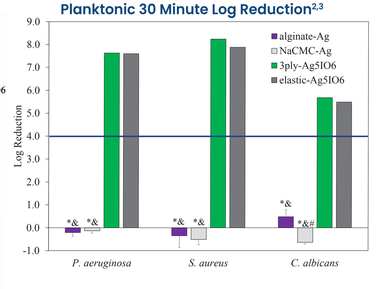
Anti-Adherence Testing: *Candida albicans* ATCC 18804[2,3]



Antibiofilm Testing: *C. albicans*[2,3]



Planktonic 30 Minute Log Reduction[2,3]



Dressings with Gels Containing Ag_5IO_6 Vs. Commercial Silver-Containing Gels

Anti-Adherence Test: (Log_{10} Reductions Relative to Dressings Coated with Ag-Free Gels)

Anti-Planktonic Activity

	Ab1	*Ca*	*Ef*	*Ec*	*Pa1*	*Sa*	*Se*	*Pa2*	*Ab2*	*Cd*
DuoDERM	4.72	4.25	4.38	6.13	4.03	5.29	4.27	5.45	6.94	0.26
CMC	8.12	1.80	6.21	8.33	7.13	3.52	7.23	5.68	5.34	0.10
LubrajelPF	1.15	5.95	2.08	8.72	1.06	5.44	4.88	1.89	5.16	1.43
LubrajelRC	5.94	5.96	4.52	8.66	5.37	6.68	6.47	5.38	8.44	1.36
IntraSite	7.60	5.95	5.78	8.08	2.49	3.43	5.28	5.06	3.21	6.41
SoloSite	7.35	7.01	4.26	8.56	3.16	7.32	4.23	5.52	4.17	8.05
SilverSept	2.91	-0.73	0.64	4.16	0.79	-0.01	1.33	1.78	0.01	0.28
Resta(Elta)	0.63	-0.17	-0.42	-0.11	0.09	-0.69	-1.09	0.45	0.68	2.16
SilvaSorb	0.89	-1.19	-0.01	2.89	0.09	-1.13	-2.14	0.34	0.85	-0.68

Anti-Adherence Activity

Ab1	*Ca*	*Ef*	*Ec*	*Pa1*	*Sa*	*Se*	*Pa2*	*Ab2*	*Cd*
1.22	3.62	5.79	4.35	2.14	4.30	3.56	5.75	5.43	0.26
4.10	0.66	4.62	5.54	3.19	5.24	5.65	4.85	5.42	-0.22
0.34	3.66	3.69	6.88	2.19	2.84	4.12	0.79	4.09	0.93
4.10	2.60	6.23	6.88	3.79	2.79	2.89	3.77	5.97	1.52
1.60	3.79	5.90	6.16	3.05	5.19	5.10	5.62	2.13	6.25
2.02	3.11	5.70	6.70	2.54	5.53	3.23	4.06	4.46	3.87
3.05	-0.03	0.16	3.96	0.81	-0.05	3.54	0.04	0.08	1.00
1.34	-0.08	-0.83	-0.40	0.96	1.66	0.37	0.24	0.00	7.02
-0.41	-0.83	0.21	3.70	1.20	-0.77	-1.58	0.41	0.09	0.51

Mature Biofilms Test: (Log_{10} Reductions Relative to Dressings Coated with Ag-Free Gels)

Anti-Planktonic Activity

	Ab1	*Ca*	*Ef*	*Ec*	*Pa1*	*Sa*	*Se*	*Cd*
DuoDERM	8.93	5.18	9.29	8.84	5.16	8.53	8.82	3.56
CMC	8.70	5.94	4.98	7.69	8.72	8.19	8.35	1.43
LubrajelPF	8.40	4.79	8.28	8.70	9.26	8.44	8.60	4.95
LubrajelRC	9.09	5.55	8.37	8.40	7.72	7.76	7.87	6.40
IntraSite	8.50	6.60	8.70	7.83	6.38	8.28	8.05	1.26
SoloSite	8.72	5.03	8.25	9.00	8.31	7.35	7.97	0.00*
SilverSept	-0.11	-0.81	-0.15	-0.43	-0.17	-0.15	8.28	1.63
Resta(Elta)	0.39	-1.27	0.14	-0.09	-0.13	-0.31	-0.24	-2.01
SilvaSorb	-0.29	0.41	-0.58	-0.50	-0.30	-0.67	-0.27	1.17

Antibiofilm Activity

Ab1	*Ca*	*Ef*	*Ec*	*Pa1*	*Sa*	*Se*	*Cd*
6.87	1.27	7.70	6.03	2.31	4.92	6.02	2.19
7.44	0.36	7.19	1.35	7.18	6.89	6.35	2.39
4.86	2.69	5.15	6.76	6.82	5.22	6.21	3.26
6.35	-1.23	5.87	5.47	5.78	4.39	5.10	3.73
6.84	0.91	5.45	4.90	4.71	4.49	4.97	1.98
6.52	1.42	5.30	2.88	5.79	2.69	4.95	0.00
-0.25	-0.40	-0.05	0.40	-0.52	0.19	-0.12	1.62
-0.01	-1.20	0.88	-0.23	0.02	0.60	0.51	-1.44
0.19	-0.57	1.22	0.42	-0.03	2.43	0.21	-0.26

*No growth on control

Ab = Acinetobacter baumannii ATCC 17978 *(Ab1)* & 19003 *(Ab2)*
Ef = Enterococcus faecalis ATCC 29212
Pa = P. aeruginosa ATCC 27853 *(Pa1)* & 15542 *(Pa2)*
Se = S. epidermidis ATCC 35984
Ca = C. albicans SJ2096870
Ec = Escherichia coli ATCC 25922
Sa = S. aureus 456
Cd = Clostridium difficile ATCC 9689

PURPOSE OF THIS STUDY

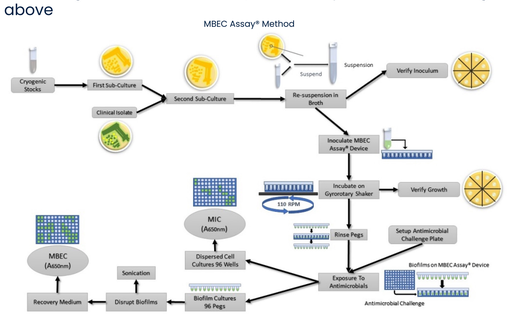
To test Ag_5IO_6-coated dressings against *C. auris*, an emerging antimicrobial-resistant fungus and serious global health threat, and various antimicrobial-resistant bacteria.

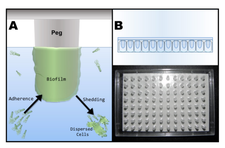
METHODS – ANTI-ADHERENCE TESTING

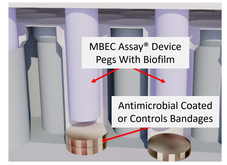
- Ag_5IO_6 (InnovoSIL™-1 silver) coated onto Profore WCL, Comprilan, & Telfa dressings at low, medium, and high concentrations, respectively
- 13x6 mm dressing pieces preconditioned in 2 mL 0.9% saline for 0, 1, 7, 14, or 28 days with 0.9% NaCl changes 3x/week
- Dressings challenged with 2 mL inoculum at 10^5 CFU/mL in 10% MHB (most strains) or 25% SDB (*C. auris*) in 0.9% saline containing 25% human serum (most strains) or 10 g/L bovine serum albumin (*A. baumannii*) for 24h at 35°C and 110 rpm
- Dressings neutralized in Dey-Engley (DE) broth supplemented with 5 g/L L-cysteine + 5 g/L L-glutathione, sonicated for 30 minutes
- Challenge media also neutralized with supplemented DE, followed by planktonic microorganism recovery
- Colony forming units (CFU) of both adhered and planktonic microorganisms enumerated by culture based methods
- Log reductions calculated relative to uncoated control dressings

METHODS – ANTIBIOFILM TESTING

- Dressings coated with Ag_5IO_6 at various concentrations as described above
- Dressings tested against pre-formed biofilms of the same organisms using a modified version of ASTM Method E2799[5] (biofilms formed by adding 150 µL of 10^5 CFU/mL inoculum to 96 well plate, MBEC Assay® lid placed on plate, incubated at 35°C for 24h at 110 rpm, rinsed in 200 µL 0.9% NaCl 1-2min)
- Mature biofilms challenged with 1 or 2 5x5 mm dressing discs in 380 µL 100% TSB (most organisms), 100% SDB (*C. auris*) or 10% TSB in 0.9% NaCl (*A. baumannii*) containing 25% human serum for 24h at 35°C and 110 rpm
- Microorganisms from biofilms, as well as planktonic microorganisms in wells, were recovered as above







RESULTS – ANTIBIOFILM TESTING

Mature Biofilms Test (Log_{10} Reductions Relative to Uncoated Dressings)

	S. aureus		*E. faecalis*		*S. epidermidis*		*A. baumannii*		*C. auris*	
	Plank	Biofilm	Plank	Biofilm	Plank	Biofilm	Plank	Biofilm	Plank	Biofilm
P1	0.88	0.17	0.57	0.07	2.21	1.85	1.81	0.30	0.01	-0.67
C1	2.13	1.35	2.12	0.91	2.28	2.70	4.08	0.46	0.05	-0.51
T1	7.17	4.44	6.25	4.97	6.59	6.19	7.14	0.56	5.20	2.95
P2	0.95	0.26	2.53	1.02	2.83	2.23	2.62	0.97	0.17	-0.75
C2	2.87	1.22	6.08	4.38	3.00	3.22	4.87	2.41	6.45	3.79
T2	8.22	6.02	7.08	5.92	7.52	6.32	7.13	4.14	6.79	2.79

P=Profore WCL, C=Comprilan, T=Telfa; 1=one disc/well, 2=two discs/well

- Clear pattern of increasing activity with increasing concentration
- Ag_5IO_6 is able to penetrate mature biofilms & eliminate the microorganisms within them

RESULTS – ANTI-ADHERENCE TESTING

Anti-Adherence Test (Log_{10} Reductions Relative to Uncoated Dressings)

Day		*S. aureus* (MRSA, USA 400)		*S. epidermidis* (coagulase –ve, ATCC 35984)		*E. faecalis* (VRE, ATCC 51575)		*A. baumannii* (ATCC 17978)		*C. auris* (CDC B11903)	
		Plank	Biofilm	Plank	Biofilm	Plank	Biofilm	Plank	Biofilm	Plank	Biofilm
0	P	6.79	5.75	7.37	3.58	7.63	5.67	6.53	3.08	1.61	1.47
	C	7.31	6.34	4.42	3.26	7.98	6.36	9.09	6.88	6.66	0.00*
	T	4.16	4.39	3.88	0.96	7.97	5.66	6.00	1.92	7.36	0.00*
1	P	7.22	5.98	7.54	4.09	7.68	5.53	6.69	3.73	1.38	0.00
	C	7.70	6.49	3.99	0.00*	7.95	60.6	8.75	6.45	6.27	0.00*
	T	6.55	6.38	3.91	1.96	7.85	5.93	5.61	1.23	6.73	3.27
7	P	7.44	5.61	6.82	3.30	7.60	5.25	6.21	0.00*	0.91	3.13
	C	7.90	6.40	7.23	2.99	7.97	5.89	8.79	6.87	6.65	0.00*
	T	8.05	6.46	5.24	2.83	7.87	6.14	5.12	2.57	7.10	4.93
14	P	3.10	5.85	7.01	4.38	0.02	0.32	3.08	6.27	0.85	5.73
	C	7.19	6.48	7.03	3.60	0.58	1.24	4.08	6.71	6.43	0.00*
	T	7.47	6.16	6.93	5.43	1.60	5.17	6.50	5.03	6.75	0.00*
28	P	0.50	3.16	7.05	5.04	0.05	0.33	1.60	1.60	0.30	4.92
	C	0.30	6.08	6.97	2.44	0.01	0.98	7.04	6.33	6.78	1.47
	T	1.39	6.21	7.30	5.19	2.07	4.60	3.76	0.00*	6.97	1.47

P=Profore WCL, C=Comprilan, T=Telfa; Bold = total kill; * = no growth on controls

- Ag_5IO_6-coated dressings showed strong anti-adherence & anti-planktonic activity out to 7 days; many dressing/conc. combinations showed microbicidal activity up to 28 days

DISCUSSION/CONCLUSIONS

Ag_5IO_6 coated onto non-adhesive wound dressings demonstrated:

- Strong anti-adherence and antibiofilm activity against antibiotic-resistant microorganisms
  - Active in the presence of human serum, media, and saline
- Leached active agent to eliminate planktonic microorganisms shed from mature biofilms
- Eliminated planktonic microorganisms present in media for anti-adherence testing out to 28 days

PRODUCT PROPERTIES

Ag_5IO_6 also has valuable properties for use in medical devices and wound products

- Shelf life of 2+ years as powder and in hydrogels
- Thermally stable to ~440°C
- Photostable
- Ethylene oxide, autoclave, gamma, dry heat, UV, e-beam, x-ray, and VHP compatible
- Cost effective
- Simple to incorporate into/coat onto various materials
- Biocompatibility profile similar to/better than other silvers:
  - Not a sensitizer
  - Hemocompatible
  - No red flags with initial *in vivo* tests
- Rapid broad spectrum anti-planktonic activity (total kill in <5min achieved)

REFERENCES/ACKNOWLEDGEMENTS

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