LASER DISRUPTION AND KILLING OF MRSA BIOFILMS

Yosef Krespi, M.D., Victor Kizhner, M.D., Laura Nistico, Ph.D., Luanne Hall-Stoodley, Ph.D., Paul Stoodley, Ph.D.

NEW YORK HEAD & NECK INSTITUTE

ABSTRACT

Objective: To study the efficacy of two different lasers in vitro, in disrupting biofilm and killing planktonic pathogenic bacteria.

Materials and Methods: Biofilms of S. aureus Xen 31, a stable bioluminescent clinical MRSA construct, were grown in a 96 well microtiter plate for three days. The study included seven arms; a) control, b) ciprofloxacin (0.3 mg/L,) alone, c) SW laser alone, d) NIR laser alone e) SW laser and ciprofloxacin, f) SW and NIR lasers, g) SW, NIR lasers and ciprofloxacin. The results were evaluated with an IVIS biophotonic system (for live bacteria) and optical density (for total bacteria).

Results: With no antibiotics there was a 43% reduction in OD ($P<0.05$) caused by the combination of SW and NIR suggesting that biofilm had been disrupted. There was an 88% reduction ($P<0.05$) in live biofilm. Ciprofloxacin alone resulted in a decrease of 28% of total live cells (biofilm remaining attached and disrupted planktonic cells) and 58% of biofilm cells (both $P>0.05$). Ciprofloxacin in combination with SW and SW + NIR lasers caused a decrease of over 60% in total live biomass and over 80% of biofilm cells, which was significantly greater than ciprofloxacin alone ($P<0.05$).

Conclusions: SW and NIR Laser combination is a powerful alternative for control or eradication of MRSA biofilms.

CONTACT

Yosef Krespi, M.D.
St. Luke's Roosevelt Hospital
Email: hnsg@aol.com
Phone: 212-262-4444
Website: www.nyhni.org

STUDY SPONSOR

Valam Corporation
www.valam.com

INTRODUCTION

Chronic rhinosinusitis (CRS) is the most commonly treated upper respiratory tract infection. *S. aureus* as one of the most common organisms causing CRS (18.6–36.6%), with 9.22% incidence of MRSA-causing CRS. Furthermore, the recovery rate of MRSA in CRS nearly doubled in the past years. Combined with a rising primary FESS failure biofilms are considered more as a failure cause. [1,2] Biofilms are organized, surface adhering, microbial communities. Due to multiple causes biofilms are more resistant to conventional therapy necessitating to look for new treatment modalities. Our intent was to find one such modality of treating MRSA biofilm[3].

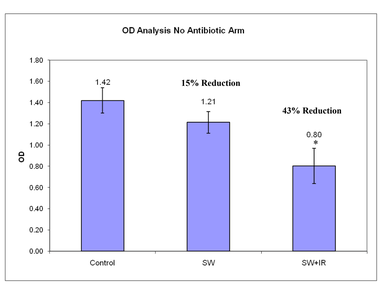
METHODS AND MATERIALS

MRSA biofilms of *S. aureus* Xen 31 grown in a 96 well microliter plate for 48 hours were treated according to the following seven arm treatments. A Q-switched Nd-YAG laser (SW) set with a frequency of 1 pulse per second at a wavelength of 1,064 nm while the output energy for the experiment's laser system was between 8 and 12 mJ. The biofilm was exposed to 10-20 pulses of shockwave placed in each of the tested wells. A 940nm diode (NIR) laser was applied with an energy level of 3W with a distance between the well and the probe set constantly to cover the entire well diameter of 0.7cm diameter thus eliciting a power density of 7.8W/cm². Taking constant time equaling to 180 seconds, energy density was total of 1400 joule/cm². The included seven arms are as follows: a) control, b) ciprofloxacin (0.3 mg/L,) alone, c) SW laser alone, d) NIR laser alone e) SW laser and ciprofloxacin, f) SW and NIR lasers, g) SW, NIR lasers and ciprofloxacin. Results were read with optical density (OD) and IVIS analysis for total bacteria. IVIS is a light-based analysis using bacteria which have been genetically engineered to produce light. Only live, actively metabolizing bacteria produce light.

RESULTS

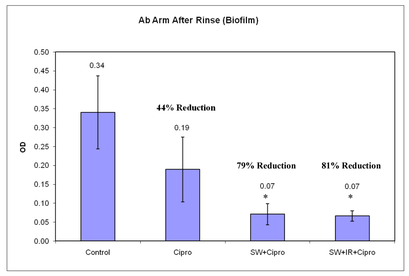
The reduction in bacteria for the different arms using OD and IVIS respectively are as follows b-44%,58%, c-15%, 8%, d-20% increase, e-79%, 81% ($P<0.05$), f-43%, 88% ($P<0.05$), e-81%, 85% ($P<0.05$). Parenthesis show only statistically significant results.

MRSA – No Antibiotic Arm
(OD analysis for Total Bacteria)

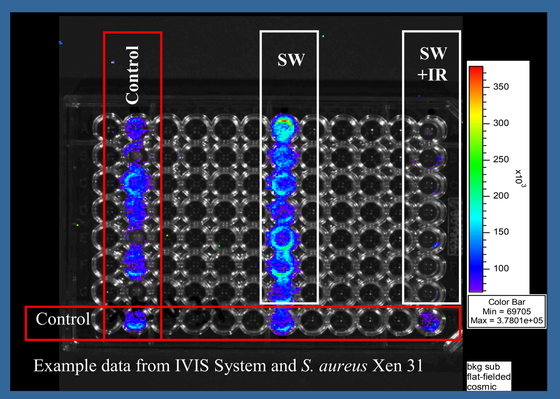


Optical Density = Planktonic + Biofilm
* = Significantly different

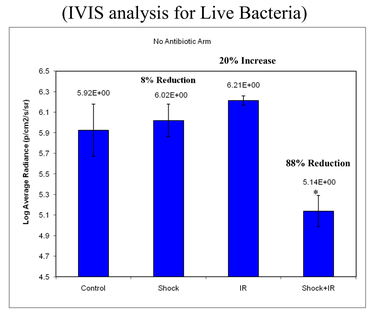
MRSA –Antibiotic Arm
(After Rinse OD analysis for Total Bacteria)



After rinse = Biofilm
* = Significantly different
Confidential
3

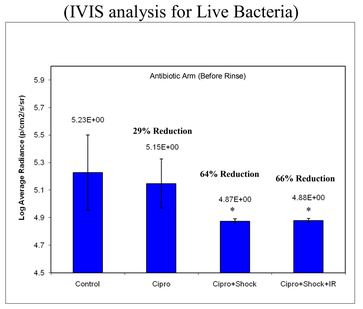


MRSA – No Antibiotic Arm
(IVIS analysis for Live Bacteria)



IVIS = Live Bacteria
* = Significantly different

MRSA – Antibiotic Arm
(IVIS analysis for Live Bacteria)



Before rinse = Planktonic + Biofilm
* = Significantly different

In-vitro – multi-arm biofilm destruction and IR radiation study

TX Arms	Total Bacteria Reduction (OD Analysis)		Live Bacteria Reduction (IVIS Analysis)		
Antibiotic TX	NO	CIPRO (post-rinse)	NO	CIPRO (pre-rinse)	CIPRO (post-rinse)
Antibiotic Alone	X	44%	X	29%	58%
Shockwave Alone	15%	79%	8%	64%	81%
Infrared Alone	nt	nt	-20%	nt	nt
SW + IR	43%	81%	88%	66%	85%

% reduction compared to Control
statistically significant
X=not applicable
nt = not tested

DISCUSSION

Biofilms are emerging as an integral part of CRS pathology. Bacteria in biofilm communities display significantly greater resistance to traditional antimicrobial therapies than their planktonic counterparts. Current therapies targeting biofilms are multiple. Some are merely mechanical while other use combination therapy trying to enhance antimicrobial treatment. A previous successful study of disrupting biofilm i.e. removing the biofilm shield with a SW laser gave way to the next step which is killing the biofilm[4]. It was already shown that low level diode laser can take an active role in photodynamic therapy with as high as 99.9% killing rate possible after enhancing with a photosensitizer as shown by Wilson[5]. Thus laser-generated shockwave exposed the bacteria to a floating state, thus enabling a second strike. The second strike was inflicted by a diode laser with 940nm wave length with several arms enhanced by cipro. Arms that contained diode followed by the shockwave or either laser alone were not of significant killing power.

A possible explanation for the slight significance of ciprofloxacin containing arm versus the laser only arm is temperature rise over 44C° rendering the antibiotic less active. The temperature rise may be a possible explanation for the bactericidal effect of the laser as shown by Yeo previously[6].

Several limitations to our current study are using in vitro model that does not predict biofilm formation in vivo and not measuring the temperature rise whenever the NIR was used. These encouraging results can set the basis for further investigation regarding biofilm forming bacteria killing without the need for systemic antibiotic treatment. Measuring the effect on cilia function as already undertaken in burns and chronic wounds, elaborating a safe energy output level with tolerable heating effect, combining photosensitizers or enhancing with topical antibiotic are the next steps.

CONCLUSION

We have demonstrated an effective non pharmacologic treatment method for MRSA biofilm disruption and killing using two different lasers. The preferred treatment sequence is a shockwave laser followed by NIR laser. Treatment optimization of biofilm may be improved further possible with the addition of ciprofloxacin in higher concentrations consistent with sinus mucosa levels.

REFERENCES

1. Jiang RS, Hsu CY. Revision functional endoscopic sinus surgery. Ann Otol Rhinol Laryngol. 2002 Feb;111(2):155-9.
2. Jiang RS, Jang JW, Hsu CY. Post-functional endoscopic sinus surgery methicillin-resistant Staphylococcus aureus sinusitis. Am J Rhinol 1999;13:273–277.
3. Prince AA, Steiger JD, Khalid AN, Dogrhamji L, Reger C, Eau Claire S, Chiu AG, Kennedy DW, Palmer JN, Cohen NA. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. Am J Rhinol. 2008 May-Jun;22(3):239-45.
4. Krespi YP, Stoodley P, Hall-Stoodley L. Laser disruption of biofilm. Laryngoscope. 2008 Jul;118(7):1168-73.
5. Omar GS, Wilson M, Nair SP. Lethal photosensitization of wound-associated microbes using indocyanine green and near-infrared light. BMC Microbiol. 2008 Jul 1;8:111.
6. Yeo CB, Watson IA, Stewart-Tull DE, Koh VH. Heat transfer analysis of staphylococcus aureus on stainless steel with microwave radiation. J Appl Microbiol. 1999 Sep;87(3):396-401.