2022

The effect of Er:YAG, Nd:YAG and CO2 laser combined with hydrogen peroxide, sodium hypochlorite, chlorohexidine or fluoride on reducing oral bacterial count implicated in root caries

nitya reddy
Virginia Commonwealth University

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

By
Nitya Reddy, DDS
University of Virginia, May 2012
Virginia Commonwealth University, May 2017

Thesis advisor: Janina Golob Deeb, DDS MSD
DEPARTMENT OF PERIODONTICS

Virginia Commonwealth University
Richmond, Virginia
April 25, 2022

Acknowledgements

I would like to acknowledge and thank my thesis advisor, Dr. Janina Deeb, who helped to make this project possible. Her guidance and advice carried me through all stages of researching and writing. I would also like to thank my committee members, Dr. Todd Kitten, Dr. Thomas Waldrop, Dr. Caroline Carrico and Dr. Kinga Grzech-Lesniak for their expertise and encouragement. Additionally, this project could not have been done without the support of Dr. Kitten's lab and lab manager, Seon-Sook An. And finally, I would like to thank VCU School of Dentistry and the Alexander Fellowship for their grant.

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Abstract

THE EFFECT OF ER:YAG, ND:YAG, AND CO_2 LASER COMBINED WITH HYDROGEN PEROXIDE, SODIUM HYPOCHLORITE, CHLOROHEXIDINE OR FLOURIDE ON REDUCING ORAL BACTERIAL COUNT IMPLICATED IN ROOT CARIES

By: Nitya Reddy, DDS
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Thesis Advisor: Dr. Janina Golob Deeb, DDS, MsD
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Purpose: Lasers have been used for treatment of dentinal hypersensitivity and for various bacterial reduction indications in periodontology. Their effectiveness in killing oral bacteria is not well known. The compounding effect of the combination of a laser treatment and adjunct antimicrobial agent use on bacterial viability is still evolving. The purpose of this *in vitro* study was to evaluate the effect of three lasers commonly used in dentistry in conjunction with chlorhexidine (CHX), hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), or sodium fluoride (NaF) on viability of oral bacteria associated with root caries.

Methods: Three bacterial species were used in our study: *Streptococcus mutans* (*Sm*), *Streptococcus sanguinis* (*Ss*), and *Enterococcus faecalis* (*Ef)*. Bacteria were grown in BHI broth and incubated at 37°C. Bacterial samples were irradiated with the Er:YAG, Nd:YAG and CO_2 lasers for 30 secs. The experiment was repeated three times for each treatment modality. Treatment groups consisted of: 1: no treatment, 2: 0.5% H_2O_2 alone, 3: 0.5% NaOCl alone, 4: .12% CHX alone, 5. 2% NaF alone, 6: Laser irradiation alone, 7: Laser irradiation with 0.5% H_2O_2, 8: Laser irradiation with 0.5% NaOCl, 9: Laser irradiation with .12% CHX, 10: Laser irradiation with 2% NaF for all three lasers. Microbial viability was determined through plating and colony counts. Viable colonies were counted, converted into CFU/ml and transformed into log form for statistical analysis. Statistical analysis was done using a two-tailed paired t-test.

Results: The use of CO_2, Nd:YAG, and Erb:YAG lasers alone failed to show statistically significant antibacterial activity against any of the bacteria. The only effective mono-treatment with irrigation solutions was CHX for *Sm*. The combined treatment of 0.5% NaOCl with Erb:YAG and Nd:YAG showed the greatest and most significant reduction of all three bacterial viability compared to any other treatment group.

Conclusion: The combination of irradiation with Nd:YAG or Erb:YAG laser with the addition of 0.5% NaOCl resulted in the largest reduction of bacterial survival when compared to monotherapies with antimicrobial solutions or lasers.

Introduction

The progression and treatment of periodontal disease can result in attachment loss, gingival recession and root exposure. Gingival recession is defined as the displacement of the gingival margin apically from the cementoenamel junction (CEJ) or from the former location of the CEJ, can be localized or generalized, and be associated with one or more surfaces. The resulting root exposure is aesthetically unpleasing and may lead to sensitivity and root caries.[1] Periodontitis is a chronic inflammatory disease with a bacterial etiology in a susceptible host. These destructive processes are initiated by bacteria but are propagated by host cells resulting in tissue destruction and development of the periodontal pocket.[2] Surgical and non-surgical periodontal treatments which aid in reducing periodontal pockets, also result in recession as a consequence of lost clinical attachment. Root exposure can also occur independent of periodontal disease and treatment. Gingival recession can occur in patients with aggressive toothbrushing habits, thin gingival phenotype and thin bone of alveolar housing and has been associated with aberrant frenal attachments, mucogingival deficiencies, orthodontic therapy, positional characteristics of teeth[3]. Gingival recession is also associated with natural aging.[4]

Exposed root surfaces are very susceptible to developing caries. Root caries commonly present as a progressive lesion found on a tooth root surface that has become exposed to the oral environment due to some degree of periodontal attachment loss.[5] Demineralization is twice as rapid on the root surface as it is on the enamel.[6] A systematic review conducted in 2018 found the pooled prevalence of root caries to be 41.5%, and that the prevalence is increasing due to increasing life span of humans and longevity of dentition.[7] Treating root caries can be very

challenging for the restorative dentist due to isolation, access, adhesive properties of root surface and lack of retention in preparations due to root form and anatomy. Additionally, the microflora of root caries varies from what is found in dentinal caries.[8] The main etiology for the onset and progression of root caries are the presence of bacteria and fermentable carbohydrates on the root surface.[9] Bacteria metabolize sugar into acids, which initiate root surface demineralization by removing calcium and phosphate ions from the surface apatite crystals. For enamel, this process starts when the pH reaches the critical value of 5.5. However, a pH 6.4 is enough for cementum and dentin demineralization which cover the exposed roots. This is due to their lower degree of mineralization, which makes root caries initiation and progression considerably faster.[10] *Streptococcus mutans* (*Sm*), *Streptococcus sanguinis* (*Ss*), and *Enterococcus faecalis* (*Ef)* are three bacteria that have been implicated in the etiology of root caries.[8]

LASERS

Laser is an acronym for "light amplification by stimulated emission of radiation" and has gained significant popularity in dentistry since the 1990s.[11,12] There are many types of lasers available on the market and one of the main differentiating characteristic is the active medium and wavelength of the laser.[12] The most established and commonly used lasers in dentistry are the CO_2 (Carbon Dioxide) , Nd: YAG (Neodymium-doped Yttrium Aluminum Garnet), Er, Cr:YSGG (Erbium plus Chromium–doped Yttrium-Scandium-Gallium-Garnet), Diode, and Er:YAG (Erbium-doped Yttrium Aluminum Garnet) lasers.[12] Chromophores, or the targets within tissue that absorb the laser energy, vary depending on the wavelength. Lasers that produce shorter wavelengths, such as diode and Nd:YAG, tend to be absorbed by melanin and hemoglobin and are able to penetrate tissues more deeply. Whereas lasers that produce longer

wavelengths, such as CO_2 and Er:YAG, tend to be absorbed by water and hydroxyapatite and cannot penetrate tissues as deeply.[13] When laser energy comes in contact with tissue surfaces, it can either be reflected, scattered, absorbed or transmitted to surrounding tissues.[14] Lasers are commonly used for teeth whitening, soft tissue cutting, caries removal and hard tissue ablation. They have also been shown to have a bactericidal effect, which was initially found as a side effect, but has been further explored and became very beneficial.[15]

The Er:YAG wavelength coincides with the absorption peak of water and has been shown to have good bactericidal effects even at low energy outputs. Due to its wavelength at 2940 nm, the Er:YAG laser has the most optimal absorption in water molecules and has been used as an effective alternative to traditional periodontal scaling and root planing.[15] It has been shown to remove the smear layer on root surfaces without any apparent heat damage.[16] There are many in vitro studies that have examined the biocompatibility of this laser as an adjunct to SRP and found it to be favorable. [17,18] Several in vitro studies have reported the Er:YAG laser to have bactericidal potential. One study by Folwaczny examined the antimicrobial effects of this laser on extracted teeth. Specifically, Root surfaces inoculated with *Escherichia coli, Staphylococcus aureus, A.a., Eikenella corrodens and Peptostreptococcus micros* experienced a decrease in bacterial load ranging from 5-22% following laser irradiation with 105 laser pulses without adding any chemical irrigants.[19] This laser has also been shown to decrease endotoxins and lipopolysaccharides on root surfaces as well as increase the rate of growth and adherence of gingival fibroblasts compared to scaling.[15] This may aid in the reattachment of gingival tissues to the root surface. In addition to scaling and root planing, Er:YAG laser is also an effective laser for ablation of both hard and soft tissues.

The Nd:YAG laser has been shown to exert photo-thermal effects capable of killing bacteria by evaporation, destruction and denaturation resulting in their devitalization or inactivation.[20,21] Nd:YAG uses a quartz optic fiber optic tip with diameters of 200-320 μm, allowing access into the periodontal pocket[13] and can penetrate up to 5mm[22] to target pigments (chromophores) but they have little effect on non-pigmented tissues. It is also considered a non-surgical laser due to the type of interaction with gingival tissues. Its wavelength of 1064 nm scatters rather than ablates pigmented tissues. Ablation is what is needed for tissue removal. Additionally, this laser creates a much wider zone of tissue necrosis compared to the Er:YAG and CO_2 lasers, which create a more narrow and precise zone for tissue removal. Due to its ability to target pigmented and inflamed gingival tissues, the Nd:YAG laser can be effective in the treatment of periodontal disease and an adjunct to traditional flap and osseous resective surgery.

Laser-assisted new attachment procedure (LANAP) is an FDA approved laser for treating periodontal disease. It uses Nd:YAG laser (1064 nm) to remove the pocket epithelium and necrotic epithelium. However, the connective tissue is spared, allowing healing and possible regeneration to occur. Their protocol involves ultrasonic scalers to remove surface accretion, bone modification at time of surgery and occlusal adjustments as needed in combination with laser penetration to target diseased tissue. Evidence exists based on histologic analysis to suggest that teeth treated with LANAP protocol undergo periodontal regeneration with new cementum, periodontal ligament, and alveolar bone. In a study by Nevins, 10 teeth were treated and following en block resection, one tooth had new attachment and new cementum and inserting collagen fibers and four teeth healed via long junctional epithelium.[23]

The CO_2 laser was first developed in 1964 as one of the earliest gas lasers but did not reach popularity in dentistry until the 1990s.[24] It is a gas-active medium laser that incorporates a sealed tube containing a gaseous mixture with CO_2 molecules pumped through an electrical discharge current. The light energy is delivered through a hollow tube-like waveguide.[25] A CO_2 laser generates a beam of infrared light with the wavelengths at 9300 nm, 9600 nm, 10,300 nm, and 10,600 nm, with 10,600 nm being the most commonly used in dentistry.[24] The CO_2 laser is widely considered the best surgical laser for coagulation during and after surgery.[25] The chromophore or target of the CO_2 laser is water, which is similar to the Erb:YAG, but differs in that the CO_2 laser targets water inside soft tissues and does not rely on an external water source as the Er:YAG. The absorbed CO_2 laser energy causes the water in the tissue to vaporize and cause tissue removal through ablation.[26] The combination of the energy not absorbed by water and the heat energy by water evaporation leaves a zone of thermal necrosis. The zone of thermal necrosis caused by CO_2 laser in gingival tissues is about 0.15 to 0.33 mm depending on laser settings used.[27] Capillaries are effectively sealed and coagulated with ablation, resulting in minimal bleeding and a clearly visible operating field, which can reduce treatment time. The resulting time that is saved by not suturing or managing hemostasis during surgery makes it a good choice for soft tissue procedures such as gingivectomies, frenectomies, vestibuloplasties.[24]

ANTIMICROBIAL AGENTS

Antimicrobial agents, such as chlorhexidine gluconate (CHX), and chemical irrigants such as sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) have wide antimicrobial activity and are commonly used in dentistry. Applications of antimicrobial agents are used to control the

supragingival plaque. Chlorhexidine (CHX) digluconate is a potent allopathic reagent that is considered one of the most frequently used compounds for chemical plaque control. It has been used as a potent broad-spectrum antiseptic agent since 1950 with a pronounced antimicrobial effect on Gram-negative and Gram-positive bacteria, fungi and some viruses. It has the ability to inhibit the formation and development of bacterial plaque for several hours.[28,29]

Hydrogen peroxide (H_2O_2) has been used in dentistry for more than 70 years.[30] H_2O_2 is an oxidizer that has been shown to possess a wide spectrum of antimicrobial activity since it is active against bacteria, yeasts, fungi, viruses and spores.[31] The use of 3% H_2O_2 resulted in pocket depth reduction of more than 4 mm, with no effect on bleeding and other gingival indices.[32] Administration of CHX and H_2O_2 resulted in reduction of the gingival index and the pocket depth in a clinical trial.[33]

Sodium hypochlorite (NaOCl) has broad antimicrobial activity, fast bactericidal action and non-toxicity at application concentration.[34] Its use in endodontics is known as one of the main canal irrigants.[35] Sodium hypochlorite combined with curettage has been used in periodontics and has shown histologically to be effective in reducing soft tissue inflammation.[36] Sodium hypochlorite achieves predictable chemolysis of the soft tissue wall in the periodontal pocket with minimal effect on the adjacent tissues while not impeding the healing phase.[36] The use of 0.1% NaOCl during periodontal surgeries could potentially improve the healing and regeneration of the connective tissue.[37] The American Dental Association Council on Dental Therapeutics proposed using dilute sodium hypochlorite (0.1-0.5%) for oral irrigation as an antiseptic mouth rinse for its rapid bactericidal action, relative non-toxicity at used concentrations, no color, no staining, very low cost, and no known contraindications.[38]

Sodium fluoride (NaF) applied to tooth surfaces is well established and commonly used to prevent caries. Fluoride reduces demineralization and promotes remineralization of the enamel and has been shown to inhibit bacterial acid production.[39] NaF in combination with CO_2 laser has been shown to inhibit demineralization in in vitro studies.[40]

There has been limited research examining the effect of irrigants and lasers on the bacterial counts of strains implicated in root caries. The purpose of this in vitro study is to evaluate the effectiveness of using chemical irrigants, H_2O_2, NaOCl, CHX, NaF, in conjunction with the Er:YAG, Nd:YAG and CO_2 laser on the bacterial viability of *Streptococcus mutans (Sm), Streptococcus sanguinis (Ss),* and *Enterococcus faecalis (Ef*) in an effort to identify if they could be adjunctive therapies in the prevention of root caries. We hypothesize that the combination of an irrigant with a laser will yield a greater bactericidal effect on *Ss, Sm*, and *Ef* than either the irrigant or laser alone.

Methods

Culture conditions:

Three oral bacterial species implicated in the etiology of root caries were used in this study: *Streptococcus mutans (*UA159*), Streptococcus sanguinis (*SK36*),* and *Enterococcus faecalis (Ef*). They were individually grown but treated in parallel. Freezer stock (-80°C) of the bacterial species was obtained and 5 microliters of the aliquot were used to inoculate 5mls of Brain Heart Infusion (BHI) broth. The inoculum was incubated overnight in an aerobic environment at 37°C. The optical density (OD) of the cultures was measured with a spectrophotometer (Genesys 150, Thermo Fisher Scientific) at 660nm (OD660) and normalized to an OD of 0.5. These cultures were then aliquoted into a 96-well plate. A 150µL of each of the cultures was aliquoted to ten wells for each bacterial strain of the 96-well plate to facilitate ten treatment groups per experiment. All treatments were done under a sterile biological safety hood. The chemicals used were added in the form of concentrated stock solutions (H_2O_2: 3%, NaOCl: 5.25%, CHX: 2%, NaF: 75%) directly to the bacteria cultures in the plate. The stock solutions were diluted with sterile water to the concentrations specified per study group. Laser irradiation with each laser was performed on the appropriate samples. Chemical treatments and laser irradiation were done one well at a time so each bacterial culture was in contact with the chemical agent and/or laser for 60 seconds. Following laser irradiation, each treated well was diluted at 1:50 into fresh BHI broth. Additional dilutions were performed and inoculated onto BHI plates. The plates were incubated for 24-48 hours aerobically at 37°C. Viable colonies were counted for each plate, calculated into CFU/ml, and converted into log form for statistical analysis.

Laser irradiation Parameters:

The Er:YAG, Nd: YAG, and CO_2 laser was set to normal periodontal clinical settings. The samples were irradiated by Er:YAG laser at 2940nm (LightWalker, Fotona, Slovenia), using a 400µm Varian fiber tip of cylindrical quartz at parameters: 40mJ; 40Hz; 1.6W for 30 seconds with the 300µs short pulse duration in contact mode. The Nd:YAG laser (LightWalker, Fotona, Slovenia) irradiated at 1064 nm, using a 300 µm Varian fiber tip (H300) of cylindrical quartz at parameters: 150mJ, 20 Hz, 3W for 30 seconds with the operation mode MSP mode (100µs pulse duration) in direct contact mode. UltraSpeed Smart CO_2 laser at 10,600nm (DEKA, Implant Direct, USA) irradiated using a contra-angle attachment and mirrored attachment at parameters: 50 Hz, 2.9W for 30 seconds with 0.3-sec pulses in direct contact mode. Irradiation was performed with a disinfected aluminum foil barrier to isolate treated wells from contamination.

Study groups:
Group 1a-c: bacteria alone (*Sm, Ss*, or *Ef* alone)
Group 2a-c: bacteria (*Sm, Ss*, or *Ef* alone) + H_2O_2 (0.5%)
Group 3a-c: bacteria (*Sm, Ss*, or *Ef* alone) + NaOCl (0.5%)
Group 4a-c: bacteria (*Sm, Ss*, or *Ef* alone) + CHX (.12%)
Group 5a-c: bacteria (*Sm, Ss*, or *Ef* alone) + NaF (2%)
Group 6a-i: bacteria (*Sm, Ss*, or *Ef* alone) + laser (Er:YAG, Nd:YAG, or CO_2) alone
Group 7a-i: bacteria (*Sm, Ss*, or *Ef* alone) + laser (Er:YAG, Nd:YAG, or CO_2) + H_2O_2 (0.5%)
Group 8a-i: bacteria (*Sm, Ss*, or *Ef* alone) + laser (Er:YAG, Nd:YAG, or CO_2) + NaOCl (0.5%)
Group 9a-i: bacteria (*Sm, Ss*, or *Ef* alone) + laser (Er:YAG, Nd:YAG, or CO_2) + CHX (.12%)
Group 10a-i: bacteria (*Sm, Ss*, or *Ef* alone) + laser (Er:YAG, Nd:YAG, or CO_2) + NaF (2%)

Each experiment was performed three times.

Results

There were significant differences in bacterial growth based on use of irrigant, laser, and bacteria for all combinations. A summary of the models is presented in **Table *1***. **Table *2*** presents the pairwise comparisons for the use of irrigants with and without the laser (laser+irrigant vs irrigant alone). **Table *3*** presents the pairwise comparisons for the use of each laser with and without the irrigant (i.e. laser+irrigant vs laser alone).

Table 1: Model Results

	CO_2		Nd:YAG		Er:YAG	
	F	**P-value**	**F**	**P-value**	**F**	**P-value**
Bacteria	8.09	0.0005	12.91	<.0001	22.64	<.0001
Irrigant	96.47	<.0001	170.92	<.0001	132.87	<.0001
Laser (Y/N)	41.55	<.0001	134.98	<.0001	42.1	<.0001
Bacteria*Irrigant	23.74	<.0001	7.71	<.0001	22.87	<.0001
Bacteria*Irrigant*Laser (Y/N)	7.62	<.0001	11.22	<.0001	11.25	<.0001

Table 2: Pairwise Comparisons for the Effect of Irrigants With and Without a Laser

Laser Comparison	**Bacteria**	**Irrigant**	**Estimated Change with Addition of Laser**	**SE**	**P-value**	**Adj P**
CO_2 vs No Laser	*Ef*	CHX	-0.06	0.78	0.9341	1
		H_2O_2	-0.81	0.78	0.2983	1
		NaF	-1.22	0.78	0.1175	0.9993
		NaOCl	-5.74	0.78	<.0001	<.0001
		None	-1.59	0.78	0.0419	0.9655
	Sm	CHX	0.57	0.78	0.4637	1
		H_2O_2	-0.64	0.78	0.4103	1
		NaF	-0.85	0.78	0.276	1
		NaOCl	-2.20	0.78	0.0052	0.5227
		None	-1.09	0.78	0.1616	0.9999
	Ss	CHX	0.21	0.78	0.7832	1
		H_2O_2	1.57	0.78	0.0451	0.9713
		NaF	-0.56	0.78	0.4721	1
		NaOCl	-6.41	0.78	<.0001	<.0001

		None	-0.55	0.78	0.4783	1
Nd:YAG vs No Laser	*Ef*	CHX	-5.23	0.81	<.0001	<.0001
		H_2O_2	-2.06	0.81	0.0118	0.7396
		NaF	-0.03	0.81	0.9675	1
		NaOCl	-5.74	0.81	<.0001	<.0001
		None	0.08	0.81	0.9242	1
	Sm	CHX	-0.26	0.81	0.7517	1
		H_2O_2	-3.27	0.81	<.0001	0.024
		NaF	0.06	0.81	0.9407	1
		NaOCl	-6.63	0.81	<.0001	<.0001
		None	-0.03	0.81	0.9738	1
	Ss	CHX	-5.24	0.81	<.0001	<.0001
		H_2O_2	-1.52	0.81	0.0618	0.9885
		NaF	0.05	0.81	0.9502	1
		NaOCl	-6.41	0.81	<.0001	<.0001
		None	-0.08	0.81	0.9167	1
Er: YAG vs No Laser	*Ef*	CHX	0.63	0.76	0.407	1
		H_2O_2	0.09	0.76	0.9033	1
		NaF	-0.93	0.76	0.2249	1
		NaOCl	-5.74	0.76	<.0001	<.0001
		None	-0.97	0.76	0.2074	1
	Sm	CHX	-0.26	0.76	0.7378	1
		H_2O_2	-0.58	0.76	0.4449	1
		NaF	-0.36	0.76	0.639	1
		NaOCl	-6.63	0.76	<.0001	<.0001
		None	-0.28	0.76	0.7128	1
	Ss	CHX	0.17	0.76	0.8237	1
		H_2O_2	1.68	0.76	0.0288	0.9233
		NaF	-0.19	0.76	0.8048	1
		NaOCl	-5.77	0.76	<.0001	<.0001
		None	-0.03	0.76	0.9688	1

*SE=Standard Error; Adj P is Tukey's adjusted p-value

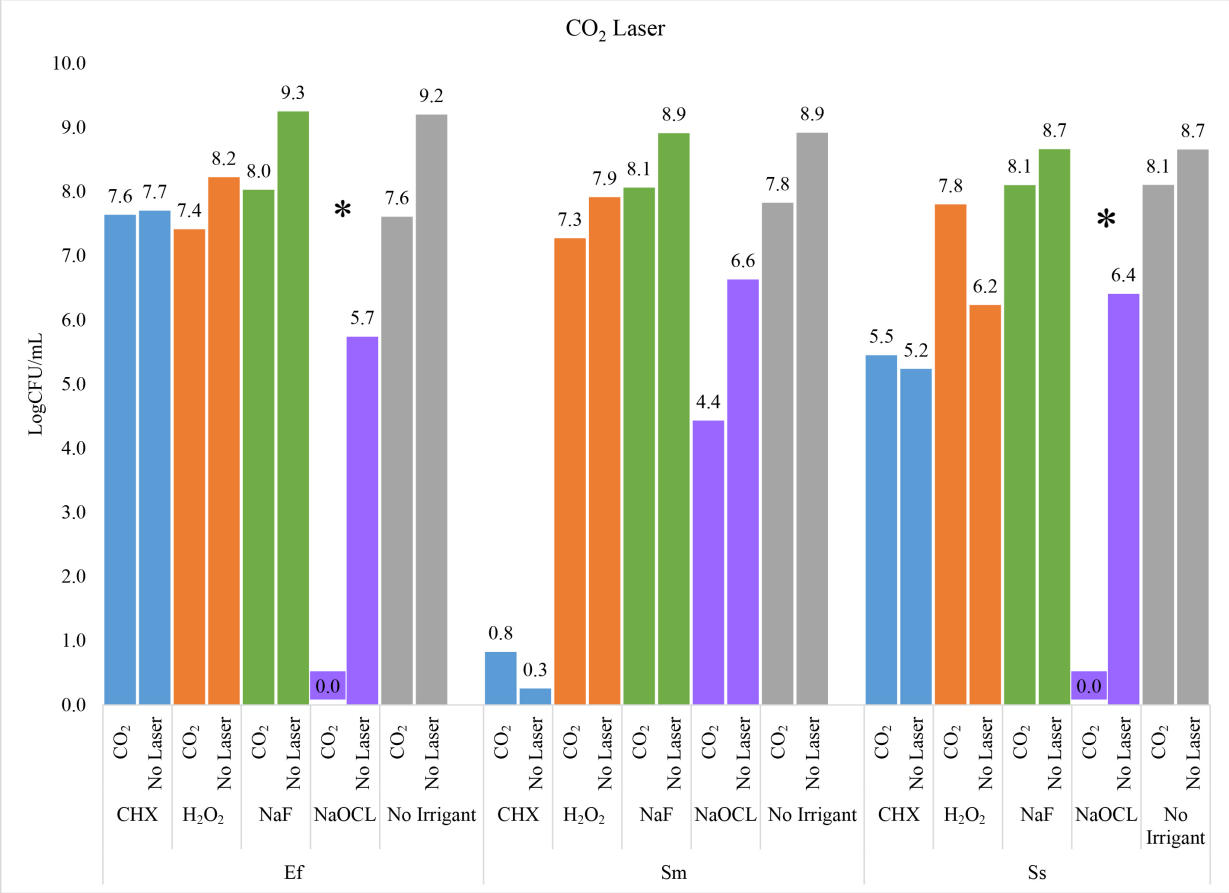
Table 3: Pairwise Comparisons for Effect of Laser With and Without an Irrigant

Laser	Bacteria	Irrigant		Estimated Change of Irrigant vs No Irrigant	SE	P-value	Adj P
CO_2	*Ef*	CHX	vs. No Irrigant	0.03	0.95	0.9753	1
		H_2O_2		-0.20	0.95	0.8376	1
		NaF		0.42	0.95	0.6595	1
		NaOCl		-7.61	0.95	<.0001	<.0001
	Sm	CHX		-7.00	0.95	<.0001	<.0001
		H_2O_2		-0.55	0.95	0.5619	1
		NaF		0.24	0.95	0.8045	1
		NaOCl		-3.39	0.95	0.0005	0.1051
	Ss	CHX		-2.65	0.95	0.0059	0.5554
		H_2O_2		-0.30	0.95	0.75	1
		NaF		0.00	0.95	0.9972	1
		NaOCl		-8.10	0.95	<.0001	<.0001
Nd:YAG	*Ef*	CHX	vs. No Irrigant	-6.81	0.99	<.0001	<.0001
		H_2O_2		-3.11	0.99	0.002	0.2959
		NaF		-0.06	0.99	0.9508	1
		NaOCl		-9.28	0.99	<.0001	<.0001
	Sm	CHX		-8.89	0.99	<.0001	<.0001
		H_2O_2		-4.24	0.99	<.0001	0.0101
		NaF		0.08	0.99	0.9362	1
		NaOCl		-8.89	0.99	<.0001	<.0001
	Ss	CHX		-8.57	0.99	<.0001	<.0001
		H_2O_2		-3.86	0.99	0.0001	0.0388
		NaF		0.14	0.99	0.8879	1
		NaOCl		-8.57	0.99	<.0001	<.0001
Er:YAG	*Ef*	CHX	vs. No Irrigant	0.10	0.93	0.9145	1
		H_2O_2		0.08	0.93	0.9315	1
		NaF		0.09	0.93	0.9275	1
		NaOCl		-8.24	0.93	<.0001	<.0001
	Sm	CHX		-8.64	0.93	<.0001	<.0001
		H_2O_2		-1.31	0.93	0.1639	0.9999
		NaF		-0.08	0.93	0.9278	1
		NaOCl		-8.64	0.93	<.0001	<.0001
	Ss	CHX		-3.22	0.93	0.0007	0.1465

	H_2O_2	-0.71	0.93	0.4481	1
	NaF	-0.15	0.93	0.8689	1
	NaOCl	-7.99	0.93	<.0001	<.0001

*SE=Standard Error; Adj P is Tukey's adjusted p-value

Figure 1: CO_2 Laser



The CO_2 laser as seen in Figure 1 demonstrated a synergistic effect with NaOCl by killing significantly more *Enterococcus faecalis* than with NaOCl alone (-5.7, adj p-value<0.0001) and significantly more than the CO_2 laser alone (-7.6, adj p-value=<0.0001). None of the other laser and irrigant combinations were effective at reducing *E. faecalis*.

The CO_2 laser also demonstrated a synergistic effect with NaOCl by killing significantly more *Streptococcus sanguinis* than with *S. sanguinis* alone (-6.4, p-value<0.0001) or the CO_2 laser

alone (-8.1, p-value<0.0001). None of the other laser and irrigant combinations were effective at reducing *S. sanguinis*.

For *Streptococcus mutans*, the only effective treatment was chlorhexidine and there was no additional benefit with the CO_2 laser (adjusted p-value=1.00).

Figure 2: Nd:YAG

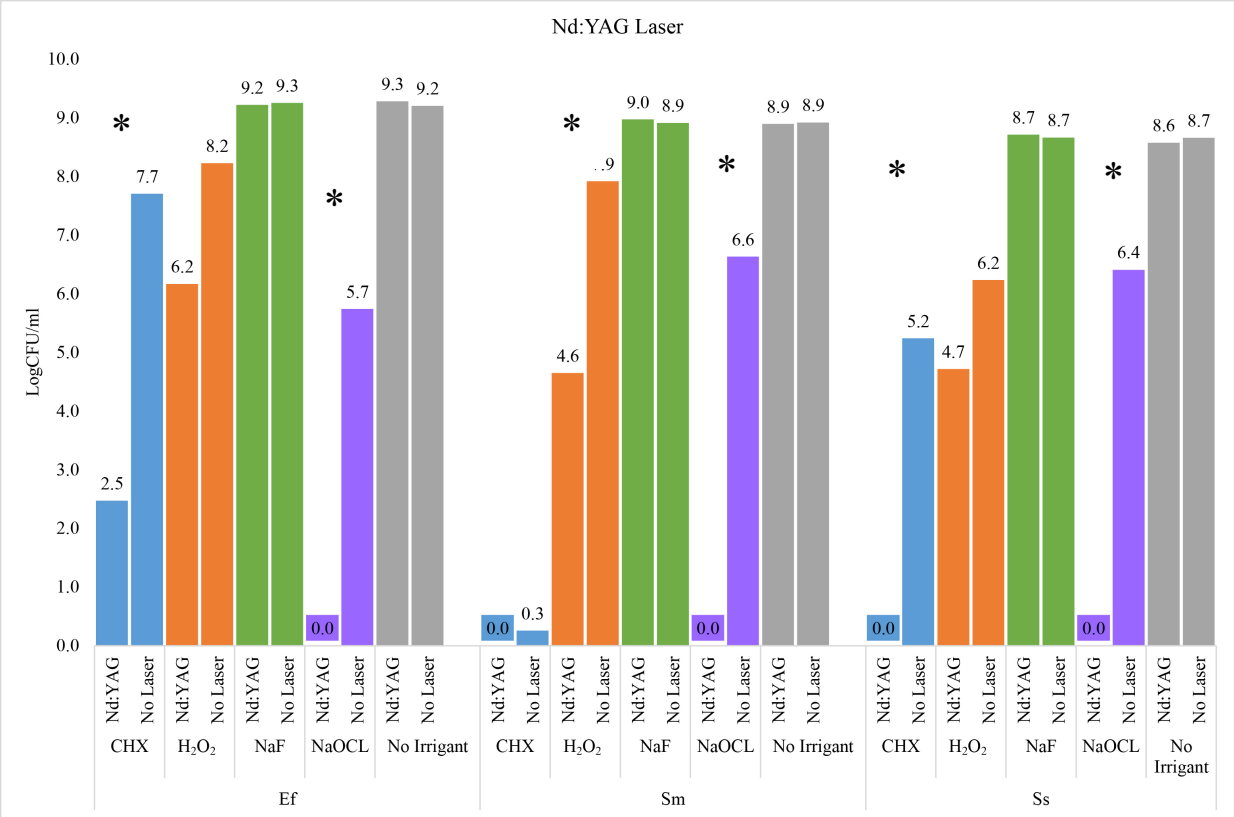
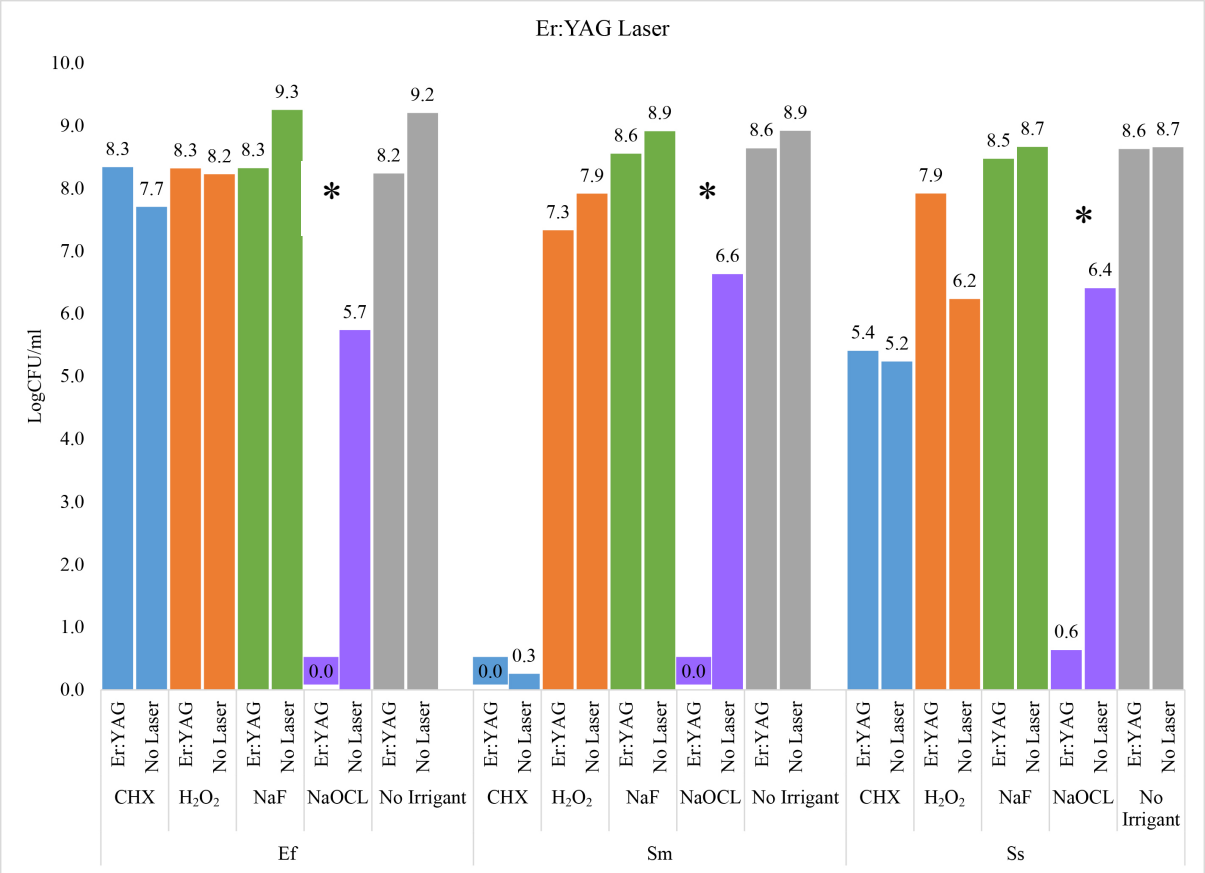


Figure 2 shows that the Nd:YAG laser demonstrated a synergistic effect with NaOCl by killing significantly more *E. faecalis* than with NaOCl alone (-5.7 logCFU, adj p-value<0.0001) or Nd:YAG laser alone (-9.3 logCFU, adj p-value<0.0001) and also by killing significantly more *S. mutans* than NaOCl alone (-6.6 logCFU, adj p-value<0.0001) or the Nd:YAG laser alone (-8.9 logCFU, adj p-value<0.0001), and more *S. sanguinis* than NaOCl alone (-6.4 logCFU, adj p-value<0.0001) or the Nd:YAG laser alone (-8.6 logCFU, adj p-value<0.0001).

The Nd:YAG laser was also synergistic with H2O2 by killing significantly more *S. mutans* than H2O2 alone (-3.3 logCFU, adj p-value=0.0240) and more than the Nd:YAG laser alone (-4.2 logCFU, adj p-value=0.0388). However, the combination of Nd:YAG and NaOCl was more effective than Nd:YAG and H_2O_2 for *S. mutans* (-4.6 logCFU, adjusted p-value=0.0020).

The Nd:YAG laser was synergistic with Chlorhexidine by killing significantly more *S. sanguinis* than with Chlorhexidine alone (-5.2 logCFU, adj p-value<0.0001) or with the laser alone (-8.6 logCFU, adj p-value<0.0001). The same was true with the Nd:YAG with CHX combination killing significantly more *E. faecalis* than Chlorhexidine alone (-5.2 logCFU, adj p-value<0.0001) or the laser alone (-6.8 logCFU, adj p-value<0.0001). For *S. mutans*, chlorhexidine was effective alone with no additional benefit with the Nd:YAG laser (adjusted p-value=.3).

Figure 3: Er:YAG



The Er:YAG laser demonstrated a synergistic effect with NaOCl by killing significantly more *E. faecalis* than with NaOCl alone (-6.6 logCFU, adj p-value<0.0001) and significantly more than with the Er:YAG laser alone (-8.2 logCFU, adj. p-value<0.0001) as seen in

Figure *3*.

There was also a synergistic effect between the Er:YAG laser and NaOCl for *S. mutans*, with the combination killing significantly more *Sm* than with NaOCl alone (-6.6 logCFU, adj p-value<0.0001) or the laser alone (-8.6 logCFU, adj p-value<0.0001). Chlorhexidine reduced *S. mutans* to undetectable levels independent of the Erb:YAG laser (-0.3 logCFU , adj p-value=1). For *S. sanguinis*, there was a synergistic effect between Er:YAG laser and NaOCl with significantly less *Ss* growth combined than with laser alone (-8.0 logCFU, adj p-value<0.0001) or with NaOCl alone (-5.8 logCFU; adj p-value<0.0001).

Chemical Reagents

Flouride did not have any effect alone or in combination with any laser on bacterial reduction for *Ss, Sm* or *Ef*. H_2O_2 was only statistically significant in reducing bacterial growth for *Sm* in conjunction with the Nd:YAG laser, but still didn't eliminate all bacteria (-3.2 logCFU, adj p-value=0.0240) . CHX was effective as a monotherapy for *Sm* reducing the bacterial count to undetectable levels with and without a laser (adj p-values=1) and was statistically significantly more effective on *Ss* (-5.2 logCFU, adj p-value<0.0001) and *Ef* (-5.2 logCFU, adj p-value<0.0001) when used in combination with the Nd:YAG laser. NaOCl proved to have the most extensive effect. NaOCl in combination with Er:YAG and Nd:YAG resulted in a

synergistic effect for all three bacterial species. NaOCl with the CO2 laser was effective for *Ef* (-5.7 logCFU, adj p-value<0.0001) and *Ss* (-6.4 logCFU, adj p-value<0.0001) but not *Sm* (-2.2 logCFU, adj p-value=0.5227).

Bacterial strains

The most effective treatment for *Ef* was NaOCl in combination with any of the three lasers, all of which resulted in undetectable amounts of *Ef* after treatment. Either treatment, NaOCl or the laser alone, was unable to achieve the same level of bacterial reduction as the combination showing the synergistic effects of an antimicrobial with laser treatment.

The most effective treatment for *Sm* was CHX alone or Nd:YAG and Er:YAG with NaOCl. All three combinations resulted in undetectable amounts of bacterial recovery following treatment. While the lasers provided a synergistic effect in combination with NaOCl, CHX by itself also produced the same result.

The most effective treatment for *Ss* was CHX or NaOCl with Nd:YAG, NaOCl with Er:YAG, and NaOCl with CO_2 laser. All of these combinations resulted in undetectable levels of *Ss* after treatment.

Discussion

In this investigation we were able to show the effectiveness of the CO_2, Nd:YAG, and Er:YAG lasers in conjunction with antimicrobials in reducing the bacterial viability of *Ef*, *Sm*, and *Ss* in

vitro confirming our hypothesis. Based on this data NaOCl in combination with Nd:YAG or Er:YAG would provide the most superior outcome in terms of reducing growth to undetectable levels for all three bacteria. The three lasers as singular treatments did not reduce the bacterial load for any of the three tested species (*Sm*, *Ss*, and *Ef*). This is of important clinical significance since many manufacturers of lasers tend to make claims on the direct bactericidal effect of the laser. Therefore, it is important for clinicians to evaluate the literature and examine which bacterial species were studied to determine the bactericidal efficacy of the laser.

Previous studies have confirmed the bactericidal and synergistic effect of the Nd:YAG and Er:YAG lasers when combined with NaOCl, H_2O_2, or CHX on reducing periodontal pathogens, specifically *P. gingivalis* and *F. nucleatum*. [41,42] Similar laser settings were used in both investigations which could be employed in a maintenance protocol. he results from this investigation add to the data to support a non-invasive treatment that can be implemented during periodontal maintenance protocols in high caries risk patients with attachment loss following periodontal disease and therapy.

CHX is a commonly used antimicrobial mouth rinse following periodontal treatment for its bacteriostatic effect. While it is not indicated for continued long term use due to its side effects, such as altered taste and staining of teeth, it has been shown to be an effective agent in reducing bacterial load when used twice daily.[43] CHX used pre-operatively can have a 97% reduction in bacterial load as measured by CFU.[44] CHX worked very effectively as a monotherapy for *Sm* in this study and worked synergistically in combination with Nd:YAG for *Ss*, reducing the bacteria count to undetectable levels.

Hydrogen peroxide rinses have been used for many years to help control plaque and oral infections. A recent systematic review evaluated the effect of H_2O_2 on oral microbial control, plaque and gingival inflammation compared to CHX and a placebo solution. They found H_2O_2 had higher antiplaque efficacy, decreased gingival inflammation and oral bacteria than the placebo but was not superior to CHX.[45] No side effects were observed using the H_2O_2 rinses. The concentration of H_2O_2 used in most studies was 1.5% which is lower than what was used in the present study. However, higher concentrations, such as 3%, did not cause any mucosal irritation in an animal model with a maximum contact time of seven minutes and is the concentration that is most commonly available over the counter.[46] This animal study went on to examine the hydroxyl radicals generated by H_2O_2 photolysis on oral microbes and found that they are a powerful oxidizing agent capable of inducing oxidative damage to oral bacteria. The results from the present study support these findings that the Nd:YAG laser combined with H_2O_2 had the an increased bactericidal effect on *Sm* compared to either treatment alone.

The lasers used in this study have different clinical indications. The Er:YAG and CO_2 lasers are considered surgical lasers while the Nd:YAG is used primarily for non-surgical procedures. A prospective randomized control trial evaluated scaling and root planing (SRP), Er:YAG, and Nd:YAG laser treatment and found significant reduction in *Aggregatibacter actinomycetemcomitans (Aa)* in the laser groups. Er:YAG group resulted in 85% reduction of *Aa*; and the Er:YAG with Nd:YAG had a 100% reduction compared to only 46% reduction in the SRP alone group. Both laser treatments significantly reduced the red complex bacteria compared to SRP. The best improvements in terms of CAL gain and reduction in bacterial load were found in patients treated with the combination of Nd:YAG and Er:YAG lasers.[47] This was one of the first papers clinically evaluating the effect of using both the Er:YAG and Nd:YAG laser for non-

surgical treatment of chronic periodontitis. The current study builds upon already established evidence of the benefits of these two lasers in improving microbiological and clinical outcomes in non-surgical therapy. The present study has shown in vitro the bactericidal and synergistic effects of combining Nd:YAG or Er:YAG with NaOCl to reduce the viability of *Ef*, *Ss*, and *Sm*.

While a meta-analysis in 2015 reported that the Nd:YAG provided no additional clinical benefit beyond what traditional SRP can achieve alone individual studies have shown its benefits.[48] A clinical study evaluating the Nd:YAG laser as an adjunct to SRP found a greater reduction of P. intermedia in the laser treated group which was maintained for two months.[49] This supports the findings from the present study in that Nd:YAG with NaOCl resulted in a reduction in all three bacteria and with CHX resulted in reduction of *Sm*.

NaF had no effect alone or when combined with any of the lasers on bacterial reduction. Previous studies examining the effect of various concentrations of fluorides on *Pg* and *Sm* cultured on titanium disks also did not find a significant decrease in bacterial growth.[50] In fact, one study found a slight increase in bacteria growth when a 1% gel concentration was used compared to the control.[51] NaF when applied to a tooth surface reduces demineralization and promotes remineralization of the enamel. Fluoride treated teeth exhibit higher pH values by inhibiting bacterial acid production, which contributes to its antimicrobial effect, rather than having a direct bactericidal effect.[39] The present study also did not find any bacteria reducing effect with NaF when used as a monotherapy or in conjunction with any of the lasers.

Ravald *et al*. studied the incidence of root caries longitudinally and examined the main reasons for tooth loss in a population of periodontally treated patients at 4, 8, 12 and 14 years of

maintenance periodontal therapy.[52–55] He found that during the first 4 years of follow-up, approximately two-thirds of patients developed root caries and this incidence of new root caries was confirmed during all observation periods further confirming the importance of oral hygiene instructions, nutrition counseling and educating patients that they are more susceptible to caries formation with root exposure which may occur for natural aging or periodontitis and treatment.[52–55] Although we did not find fluoride to have a direct bactericidal effect, it can be used as an adjunctive treatment to aid in the remineralization of tooth surfaces. Root caries prevention is of significant importance to periodontists. In a cross-sectional study, Fadel et al reported a high prevalence of root caries and high caries risk rates in 20% of patients referred for periodontal treatment suggesting this is a significant problem periodontists should be addressing and acknowledging.[56] The findings of this study suggest that use of the Nd:YAG or Er:YAG laser with low concentration NaOCl can be an effective treatment protocol during maintenance appointments in high caries risk patients, particularly since there is established use of laser treatments with the present settings and use of chemical irrigants in dentistry.[14] Clinical application could involve having patients rinse with a chemical solution or use a syringe to apply the solution along the gingival margin into the sulcus and over exposed root surfaces immediately prior to using the laser on the root surface as part of traditional scaling and root planing and maintenance therapy.

While we don't fully understand the mechanism underlying the synergistic effect found when combining laser treatment with NaOCl, there is evidence that thermal energy can potentiate the effect to NaOCl. Intracanal heating of NaOCl in endodontic therapy has been shown to increase bacterial reduction compared to ultrasonic and non-heated agitation techniques.[57] We speculate that the thermal effects from the laser contributed to NaOCl's enhanced bactericidal effect.

There are several limitations to the present study. The determination of the bactericidal effects of the laser with chemical irrigants was performed in vitro, therefore, its clinical significance in periodontal therapy remains unclear. Additionally, only three bacterial species were evaluated with a small sample size.

Conclusion

Periodontal disease is primarily a chronic inflammatory condition with a bacterial etiology. The main component clinicians treat is removing the bacterial etiology. Therefore, if lasers combined with low concentration chemical reagents commonly used in dentistry, can provide additional benefit by reducing the bacterial load of species implicated in both periodontal disease as well as in root caries as seen in this study, it would be of important clinical benefit. In summary, the combination of irradiation with Nd:YAG or Er:YAG laser with the addition of 0.5% NaOCl resulted in the largest reduction of bacterial survival when compared to monotherapies with antimicrobial solutions or lasers.

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