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Comparing Antimicrobial Effect of CO2 Laser with Halita in Oral Infection Control

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ABSTRACT

Because of increase in antibiotic resistance, finding alternative treatments for controlling infections in oral cavity is critical. In this study we aimed to compare use of halite with CO_2 laser radiation for controlling infections by *S.aureus* and *P.aeruginosa*. *Staphilococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) were used as standard strains. The effect of CO_2 Laser was evaluated 5, 10, and 15 seconds after exposure to the standard suspension of bacterium with energy density of 12.5 J/cm2 at a distance of 17mm. halite (Chlorhexidine digluconate 0.05%, Cetylpyridinium chloride (CPC) 0.05% and Zinc lactate 0.14%) was examined in the same condition. The average number of microbes was lower in the Halita group than CO_2 laser group before 15 second (P-value <0.001). But after 15 second, No growth observed in CO_2 laser group in contrary with Halita group (P-value <0.001). Average time for complete infection removal for Halita was 60 second and for CO_2 laser was 15 seconds. findings of the present study showed that CO_2 laser radiation is valuable tools for infection control in oral cavity infections. Also halite was successful for infection remove after 60 seconds. Using CO2 laser radiation in combination of halita mouthwash can help for complete eradication of infections from oral cavity.

Key words: halita**,** Co2 laser**,** chlorohexidine, Cetylpyridinium chloride, Infection control.

INTRODUCTION

Oral cavity infections are one of the most important medical problems and increasing drug resistance infections causes hardships in their treatment[1]. Because of increase in antibiotic resistance, finding alternative treatments is critical. Use of these alternative methods especially against infections such as *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) is very important. The most important infections by these microbes are angular cheilitis, bacterial sialoadenitis in salivary glands in patients with septic arthritis of joints, necrotizing ulcerative gingivitis lesions (NUG), pneumonia and chronic suppurative otitis media[2]. Also these infections can play role as a source of heart valve endocarditis[3]. Another common problem by these microbes in oral cavity is inflammation around the implant which can causes destroy of supportive bone of implant (peri implantitis)[4]. Microbial biofilm is the main pathogenesis mechanism of these microorganisms[5]. It shows importance of biofilm remove for infection control of oral pathogens. In implant failures, biofilm remove and infection control is too important and several therapeutic methods are developed to control these infections[6-7]. Mechanical debridement is the most common

method to remove biofilms from implants, especially by set of plastic court to avoid the scratch on the surface of implant[5], but this method is unable to remove bacteria from all porous of implant. Use of chlorhexidine gluconate (CHG) or different antibiotics (such as tetracycline) is another common way for infection control in these patients[8]. Several studies indicated usefulness of Chlorhexidine in the infection control and treatment of peri-implantitis infections[9]. But chlorohexidine has some adverse effects such as Brown teeth, changes in sense of taste, increased mass production and ulceration of the mucous[10]. Cetylpyridinium chloride (CPC) is a Quaternary ammonium compound used as mouthwash with wide range of antimicrobial effect[11]. Halita is a combination of CHG, CPC and lactate to reduce adverse effect of each component and synergy effect for treatment of bacterial infections. There are several studies on anti fungal and anti enterococci effect of halite but no study against *S. aureus* and *P. aeruginosa*.

Recently uses of different laser systems have been developed for infection control and treatment of peri-implantitis. They are recommended to be used in combination with the traditional tools and therapies. Developing these methods have several advantages such as deep penetration and complete removal of microorganisms. In this study we aimed to compare use of halite with CO_2 laser for controlling infections by *S.aureus* and *P.aeruginosa*.

MATERIALS AND METHODS:

All standard strains containing *Staphilococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) were collected from Iranian national Microbial collection (PTCC.irost.org). To evaluate antimicrobial effect each strain were cultured in the liquid medium of Brain Heart Infusion (BHI) (Merck KGaA, Darmstadt, Germany) . Antimicrobial test were done according to previously described (9, 12). In brief, overnight culture of strains were provided by culture at 37 ° C in optional anaerobic conditions to logarithmic phase of bacteria. For getting logarithmic phase, strains were subcultured and their optimal density (OD) were obtained by spectrophotometry (620nm, OD=0.6). Organisms of logarithmic phase were centrifuged for 15 minutes at g 3000 and the liquid surface was removed. The pellet was washed using sterile phosphate buffer saline (PBS) for 2 or 3 times. Sterile buffer was added and the final concentration of cell suspension (approximately CFU/ml 10^9) was prepared. For laser experience, 1 microliter of prepared strains suspension were poured in 1.5 mL eppendorf tubes, then the CO_2 laser radiation was assessed for every 5, 10 and15 seconds at final intervals of 24 hours and 48 hours. For CO_2 laser radiation, wavelength of 10.6 µm and energy density of 12.5 J / cm2 through the tapered humeral head and lack of focus with 5 mm diameter were used at distance of 17mm. All experiences were done in triplicate and suspensions were diluted and were spread at Brain hear infusion agar plates. After 24 hour incubation at 35-37° C their effect were subjected by colony counting.

Halita is the combination of Chlorhexidine digluconate 0.05%, Cetylpyridinium chloride (CPC) 0.05% and Zinc lactate 0.14% prolongs the antiseptic action of the two components for greater bacterial control and reduction of malodorous gas production. For antimicrobial evaluation of halita, 9ml of commercially available solution of halita was added to 1 ml of each microbial suspension (approximately CFU / ml). For time intervals, after every 5, 10, 15 and 60 seconds of exposure, solutions were subjected for culturing in Brain heart infusion agar plates. All plates were incubated 24 at 37 ° C and their bactericidal effect was evaluated colonies counting[13].

Descriptive analysis were used for statistical analysis and Kruskal-Wallis test was used by SPSS ver 17 (IBM, United States) because of Non-normal distribution of data.

RESULTS

Colony count of stains after treatment with Halita and CO_2 laser are presented in Table 1. The main differences are shown in 5 and 10 second intervals. However, CO_2 laser and Halita showed completely different manner on infection removal. The average number of *S. aureus* was lower in the Halita group than CO_2 laser group before 15 second (P-value <0.001). But after 15 second, No growth observed in CO_2 laser group in contrary with Halita

group (P-value <0.001). The same results were obtained after extending incubation time to 48 hours. Average time for complete infection removal for Halita was 60 second and for CO2 laser was 15 seconds (Table 1).

Table 1. Antimicrobial effect of Halita and CO_2 laser against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in different time intervals

Time intervals	*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
(seconds)	Halita	CO2 laser	Halita	CO_2 laser
5 sec	3.7 ±1.2	10000/0±1600/0	104.3± 48.5	1246/67±1501/11
10 sec	11.6 ± 4.7	5533/3±503/3	56.8± 23.4	8400/0±1708/80
15 sec	8.5± 2.9	0	55.9± 17.3	0
30 sec	7.4± 3.1	0	20± 9.4	0
60 sec	0	0	1.5±.46	0
P value*	P<0.01	P<0.01	P<0.01	P<0.01

DISCUSSION

Findings of the present study indicated that CO2 laser successfully reduced bacterial count after short time intervals. This method significantly increased bacteria after 15 seconds which can be used as a conventional method for oral infection control. For halita required time was 60 seconds for complete removal effect. However this time is enough when patient use it as mouthwash but by considering its low penetration power and infection conditions, CO2 laser seems to be more effective for control of these infections. *S. aureus* and *P. aeruginosa* bacteria play important roles in development of various diseases such as peri implantitis in the oral cavity. These bacteria are important in other sites of the body and can cause range of infections[14-16].

Bacterial biofilm production and surfaceadhesion are the key pathogenic factors in pathogenesis of peri implantitis and the inflammation process concluded to destruction of soft and hard tissue around the implant[17]. Colonization of bacteria such as *S. aureus* and *P. aeruginosa* in the failed implants is the most important problem; this problem will be emphasized when isolate is resistance to different antibiotics. Therefore, treatment of peri implantitis should be associated with the infection control and prevention of disease progression[18].

Laser optimization including optimized wavelength and energy output level is important and excessive radiation can damage the materials of the surface due to high temperature[19]. In the present study we used optimal laser wavelength and energy output for less possible damage and high efficacy against microbes. According to our finding this power can successfully remove all microbes after 15 second of radiation.

S. aureus is the major responsible pathogen for angular chilitis, sialoadenitis in salivary glands while it is the most common bacteria involved in bacterial septic arthritis TMJ joints that previously had arthritis[1]. *P aeruginosa* has critical role in the development of Necrotizing Ulcerative Gingivitis (NUG) associated with chronic suppurative otitis media, and pneumonia[1].

In an experience by Hauser- Grspach et al, demonstrated that CO2 laser with low energy (2100 j/cm) reduce the number of *Porphyromonas gingivalis* and *Streptococcus sanguis* bacteria which from the surfaces of zirconia discs (20). Kato et al used CO2 laser 286 and 245 j / cm^2 against *S.sanguis* and *P.gingivalis* and results showed acceptable infection control[21]. The results of this study showed that 100% of *S. aureus* and *P. aeruginosa* were killed 15 seconds after CO_2 laser radiation which is consistent with the results of the previous studies another experiences demonstrated the antibacterial effects of CO_2

against *Streptococcus* and *Actinomyces* species[22]. In a study with energy density of 7.5 and 12.5 j/cm^2, 99.9% of *P. gingivalis* bacteria and more than 99% of *A. actinomycetemcomitans* bacteria were killed successfully[23]. There is no study on effect of halita against *S.aureus* and *P. aeruginosa* but its main components are Chlorohexidine with demonstrated antimicrobial effect. Several studies demonstrated antimicrobial effect of chlorohexidine and Cetylpyridinium chloride. Albequerque et al demonstrated anti-staphylococcus effect of chlorohexidine and Cetylpyridinium chloride[24]. Also, a study by witt et al. demonstrated synergy effect of Cetylpyridinium chloride against microbes and plaque formation[25]. Findings of the present study showed effective time of 1 minute for complete microbial remove in *S. aureus* and *P. aeruginosa* infections.

In conclusion findings of the present study showed that CO_2 laser radiation is valuable tools for infection control in oral cavity infections. Also halite was successful for infection remove after 60 seconds. Using CO_2 laser radiation in combination of halita mouthwash can help for complete eradication of infections from oral cavity.

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