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Full Length Research Paper

Antibacterial influence of Omega diode laser exposure durations on *Streptococcus mutans*

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The oral cavity with plaque contained *Streptococcus mutans* and other bacteria considered to be the major aetiological agents of caries due to their presence in relatively high numbers in plaque, prior to the appearance of carious lesion and its ability to rapidly degrade carbohydrates and induce a tolerance to low level pH environments. The aim of this study was to evaluate the bactericidal effects of omega diode laser on *S. mutans* with intervals exposed time. *S. mutans* isolated from patients with high caries risk were exposed to different interval time (5, 10, 15, 20 and 25 s) and then swabbed to the rabbit teeth. The bactericidal effect was shown in the exposure time of 15, 20 and 25 s without any curiosity sign on the rabbit teeth, the caries sign appeared on the low time exposed (5 and 10 s). A diode laser can eliminate the *S. mutans* when irradiated above 10 s.

Key words: Antimicrobial, diode laser, *Streptococcus mutans*, dental caries.

INTRODUCTION

Dental caries is still reported as the single most common chronic childhood disease (U.S. Department of Health Human Services, 2000). In developing countries, more than 80% of caries prevalence has been reported in epidemiological studies (Stephen, 1993). The biofilms formation with acid end-products through the metabolism of carbohydrates by acidogenic microorganisms within these biofilms is an important factor in the development of dental caries (Svensater et al., 2000). The essential process involves demineralization of the tooth structure by high concentrations of organic acids (Van Houte, 1994). *Streptococcus mutans* has been implicated as the primary aetiological agent because of its relatively high numbers in plaque prior to the appearance of carious lesions, due to ability to degrade carbohydrates rapidly with the formation of abundant acid and its ability to induce a tolerance to low pH environments (Svensater et al., 2001). Severe early childhood caries (S-ECC), a particularly aggressive form of dental caries affecting young children, is strongly linked to *S. mutans* and *Streptococcus sobrinus* (Caufield et al., 1993; Berkowitz, 1996; Becker et al., 2002; Beighton et al., 2004).

Many of the medical and biological applications of lasers due to the use of high-power beam of laser radiation to coagulate various tissues, that is, to produce a small scar, or cut tissue. Photocoagulation by light occurs due to the change of light energy into heat energy when the laser light is absorbed. This is absorbed by the various pigments normally present in the tissue (Wilson et al., 1992). The interaction of the laser radiation with tissue produces predominantly thermal response, the cell will be destroyed. The normal body temperature is about 37°C but tissue normally can withstand temperature of up to 700°C for a duration of less than 1 s. Protein in the cell is usually damaged by the temperature, and the tissue will be destroyed and scar formed. This process is called photocoagulation (Dass et al., 2007).

Laser killing time is a modification of what is called

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thermal killing time (the time in minutes to kill a suspension of bacteria or spores at a prescribed temperature and under specific conditions (Frank, 1986), therefore the description of “laser Death time” can be the time in seconds to kill a suspension of bacteria or spores at prescribed laser power and under specific condition as explained before. The aim of this study was to evaluate the bactericidal effects of omega diode laser used with different exposure intervals on *S. mutans*

MATERIALS AND METHODS

Study design

The S. mutans used in this study was isolated from patient diagnosed and suffering from dental caries that attended Dental Teaching Hospital in Khartoum-Sudan. The experimental work was conducted at the Laser Institute in Sudan University of Sciences and Technology Khartoum-Sudan. During a period between August 2008 – April 2009

Laser Irradiation

A diode laser (Omega Xp mobile, UK) Setup was done to provided a constant beam of coherent, continuous monochromatic light with a power of 30 mw and Pulsing of 146Hz was used in this study(Figure 1). A light-emitting diode (LED) (670 nm, 5 KHz) was used as an aiming device and the laser beam was delivered through a 10cm optic fiber with a straight hand piece in continuous mode. Before the laser irradiation, the laser energy was carefully calibrated with a power meter (Coherent; Morita Mfg. Corp., Tokyo, Japan) to control the output energy from the fiber tip within the desired irradiation condition. The calibration of laser energy with a power meter after laser irradiation was also performed

Bacterial strain

S. mutans which was used in this study were isolated from carious lesions of patient's teeth on the Mitis-salivarius Bacitracin Agar (MSBA) (Oxoide-England). Supplemented with bacitracin antibiotic solution at 200 unit/ml and followed the methods that were previously described (Harolod, 1994; Spratt and Pratten, 2003). One colony of *S. mutans* can be cultivated in Brain Heart infusion Broth (BHIB) (Oxoide-England) for 24 h and was inoculated in small tubes containing Tryptone soya broth (TBS) (Oxoid Ltd) and supplemented with 15% glycerol.

S. mutans irradiation

S. mutans was maintained by subculturing on blood agar every 7 days. One colony or more grown on BHIB for 18 h at 37°C in CO_2 environment, the growth suspensions were centrifuged at approximately 3500 r.p.m for 10 min, the supernatant removed and bacterial sediment culture should be re- suspended by using phosphate buffer saline. This suspension was vortexed to get high homogenous suspension. This suspension was diluted by using physiological saline to get a concentration suspension at 1×10^5 CFU/ml. 1 ml of this suspension was distributed in six Eppendorf tubes and exposed to diode laser light at different interval time (5, 10, 15, 20, 25 s), except one of these Eppendorf tubes was not exposed to laser light, in order to keep it as a control positive and also tube number seven containing physiological saline without bacteria suspension exposed to laser light as negative control). After that the suspension in Eppendorf tubes was cultured on (Mitis-Salivarius Agar) and were incubated anaerobically for 24 h at 37°C. The colony forming unit (CFU) was counted.

Rabbits

Seven rabbits under environmental control habitat were used, which were the same species, weight, age and male sex. Five of them were tested groups and two rabbit were used as a control groups. Teeth X-ray was done for the rabbits before and after being swabbed with *S. mutans* bacteria which was irradiated by the omega diode laser.

RESULTS

A diluted bacterial suspension in 5 tubes contained 1 ml volume which was exposed to the laser light at different times (5, 10, 15, 20, and 25 s) was shown as in Table 1. The observations of the colony growth were shown, the numbers of colony were decreased according to length of irradiation exposed to bacterial suspension.

Irradiation of the rabbit’s teeth

Radiographic film of the 7 rabbit's teeth showed no symptom and sign of cariousity before being swabbed with the *S. mutans* (Figure 2). After irradiation of the *S. mutans* in 5 tubes and control by omega diode laser swabbed and applied on the rabbits teeth for the aforementioned period , were observed after clinical examination of rabbit teeth after 21 days that there was disparity, for caries percentage from the tubes [1] and [2] and which was exposed to a period of [5] and [10], but those tube samples which were exposed for a period of [15], [20] and [25] s had a 100% death due to complete absence of the caries in the rabbit teeth. The X-rays which were done for the rabbit teeth showed that, no sign or appearance of the curiosity in the rabbits teeth which were swabbed from the tubes [3], [4] and [5] (Figure 3).

DISCUSSION

Dental plaque in oral cavity is the term commonly used for the biofilm that is formed on the tooth surface and consists of a complex microbial community embedded in a matrix of polymers of bacterial and salivary origin (Regan and Parish, 1982). The causative organisms include *S. mutans, S. sobrinus, Lactobacillus casei* and *Actinomyces viscosus* (Marsh and Martin, 1992). *S. mutans* possesses the ability to adhere to pellicle-coated tooth surfaces and to form acids, characteristically associated with the cariogenicity of this micro-organism (Hamada et al., 1984). In this study, a high number of *S. mutans* were isolated from caries lesion of the patient that attended the Dental Teaching Hospital in Sudan.

Table 1. Results showed the effects of laser irradiation exposed to the 5 tubes with *Streptococcus mutans.*

Time of the laser irradiation exposed	Designation of tube	Pulse/ Rate	Probe (mm)	Results
5	T1	146	30	Growth
10	T2	146	30	Growth
15	T3	146	30	No growth
20	T4	146	30	No growth
25	T5	146	30	No growth

Key: T: Tube, s: second.

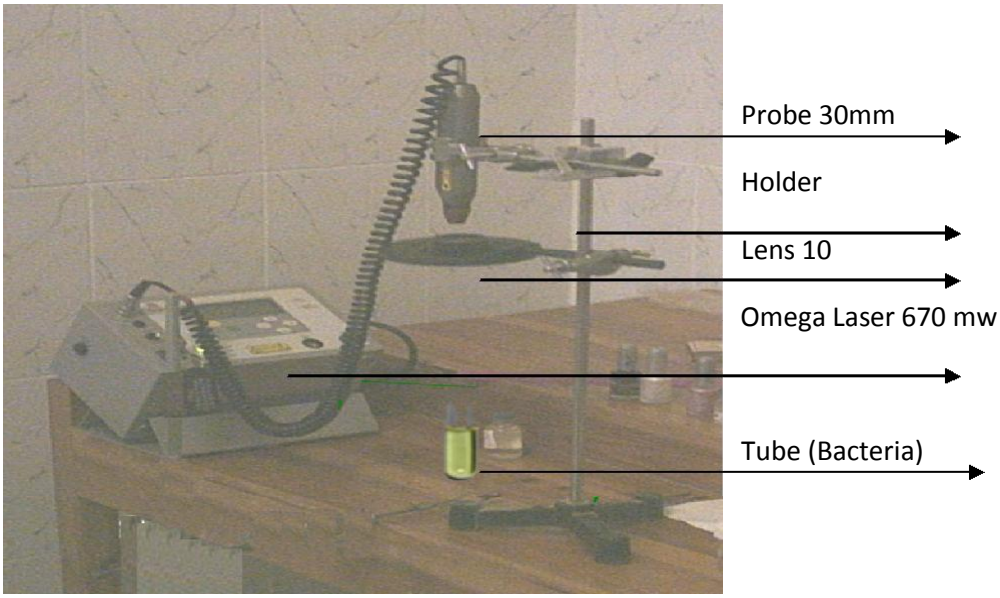
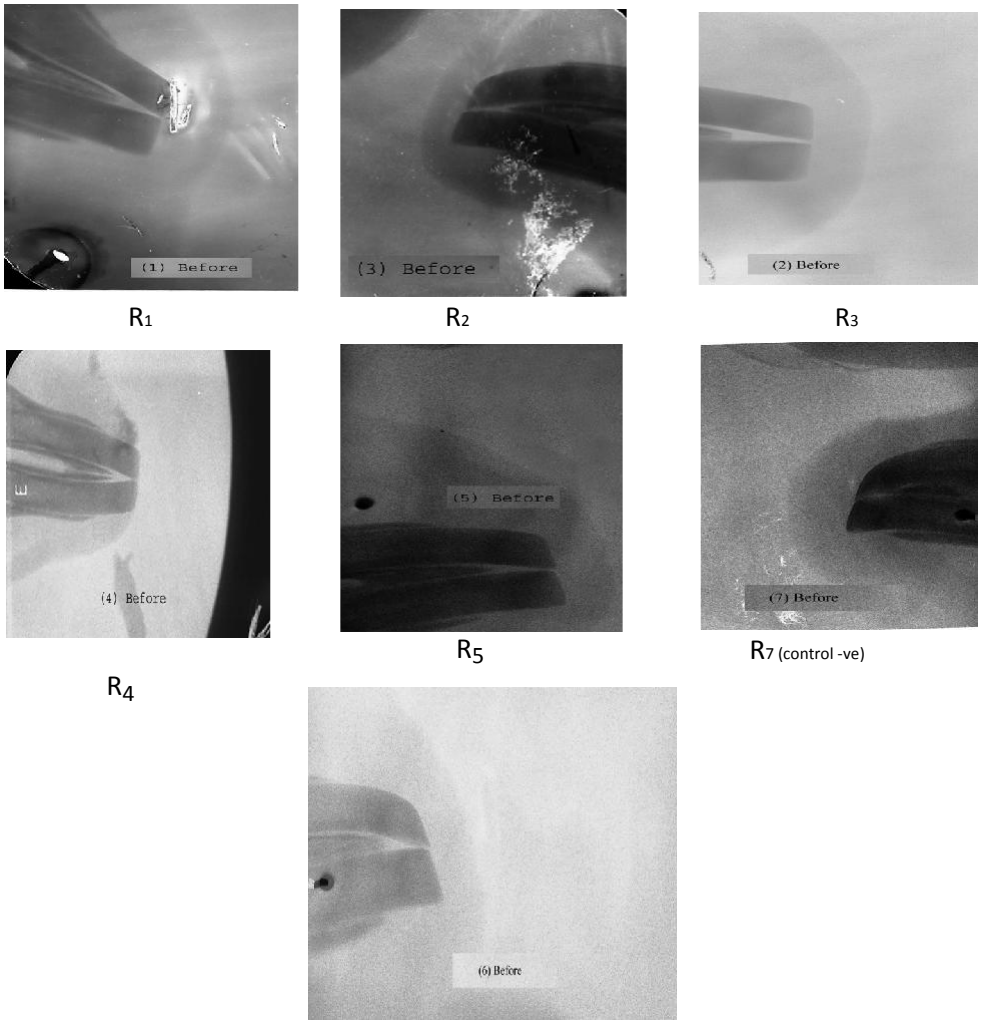


Figure 1. Omega diode laser system of experimental work

Previous studies done by Wilson et al. (1992, 1993), have shown that cariogenic bacteria, and other plaque-forming organisms; can be killed by low power laser light in the presence of a suitable photosensitizer. The possible mechanisms regarding the antibacterial effect of diode laser are summarized in the following. Thermal and photo-disruptive effects were considered the principal reasons for the laser to eliminate the bacteria (Ando et al., 1996). Immediate cell death might not occur during laser irradiation, but sublethal damage inhibited cell growth after exposure to laser irradiation (Dworkin, 1958). The sublethal damage included destruction of cell wall integrity and possibly the accumulation of denatured protein. Integrity of cell wall is intimately related to the mechanical stability of gram-positive bacteria. The damage of cell wall will cease the cell growth and successive cell lysis. (Elmros et al., 1976).

On the other hand, the cellular protein is highly sensitive to thermal changes. The laser irradiation might produce denatured protein and induce the cell to create new proteins to compensate the denaturation (Rosenberg et al., 1971). Some proteins such as IDG-60 immuno-dominant glycoprotein are indispensable for maintaining the integrity of the cell wall and the structure uniformity of cell shape (Chia et al., 2001). The stress on the cells to prevent the accumulation of denatured protein debris could also cause cell death (Dworkin, 1958). In our study, the decrease in viability of *S. mutans,* as shown by the CFU, is exposure duration dependant of light energy of Omega Diode Laser; and the bactericidal effect was at the long length of the irradiation. Bor-Shiunn et al. (2006) reported that the percentage of killing CFU (%) depend upon irradiation of the diode laser through 500 mm thickness of dentin specimens. Comparable with chlorhexidine whose antibacterial activity was reduced bacterial viability to 54%, using the same thickness of dentin disks (Schmalz, 2004). The efficiency of diode laser is prominently higher.

In addition, another antibacterial monomer, 12-Methacryloyloxydodecylpyridinium bromide (MDPB) was claimed to have effective antimicrobial activity and inhibition of root caries progression (Kuramoto et al., 2005).



R_1 R_2 R_3

R_4 R_5 R_7 (control -ve)

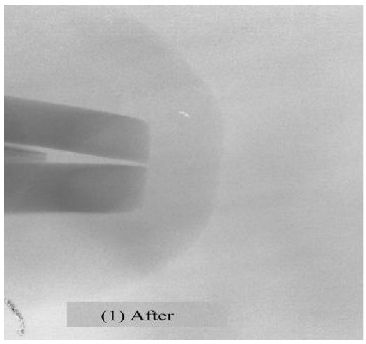
R_6 (control +ve)

Figure 2. The radio graphical films of rabbits teeth before application with *Streptococcus mutans* irradiated with Omega Diode Laser. Key: R_1 to R_7: rabbit teeth.

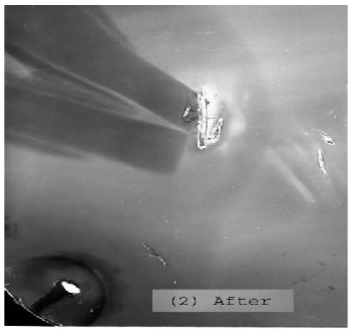
However, MDPB could only penetrate 140 mm into the demineralized lesion (Imazato et al., 2002), that was less than the effective depth of diode laser. Moritz et al. (1998) reported that the diode laser has a bactericidal effect. It was stated that the application of laser irradiation lead to reduction of bacterial strains. The irradiation at 1.5 W and 15 pps through dentin revealed significant cell damage. Dental caries results from interactions over time between bacteria that produce acid, a substrate that the bacteria can metabolize, and many host factors that include teeth and saliva. Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms (Featherstone, 2004; Fejerskov, 2004). The mechanisms of the caries process are similar for all types of caries endogenous (Featherstone, 2004; Scheie and Peterson, 2004) bacteria (largely mutans streptococci).

In this study, the radiographic film taken before and after application of the rabbit teeth with *S. mutans* exposure to different doses of diode laser irradiation the rabbit teeth R1 and R2 (low time exposure) showed the carious lesions after a period of 21 days. Walter (1986) reported that in many studies on *S. mutans*, based on epidemiological studies, showed that *S. mutans* accounted for 74 to 100% of the mutans streptococcus in diverse populations, which harbored *S. mutans,* which cause the caries-active in infants (Caufield and Griffen, 2000), furthermore, it was the first mutans streptococci (MS) to colonize among infants, shortly after their teeth erupt and in another study done by Alaluusua (1983), the only MS isolated from caries-active infants was shown. Also, van Houte (1994) reported that the predominant bacteria in carious lesions were *S. mutans*.

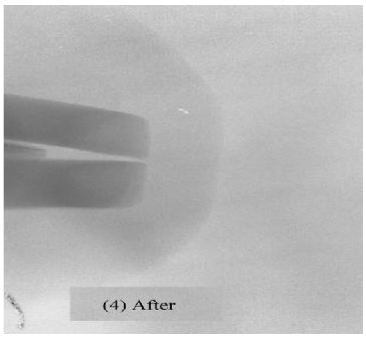
In conclusion, *S. mutans* was the main causative agent



R 1:5 s (+ve)

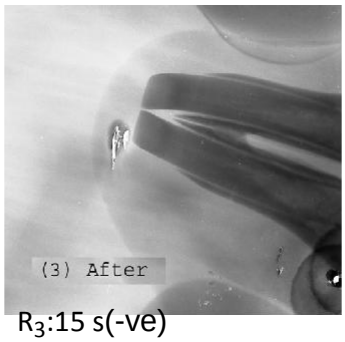


R 2:10 s (+ve)

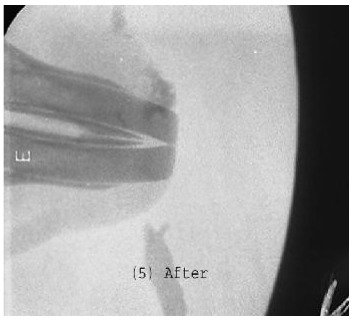


R 4:20 s (-ve)

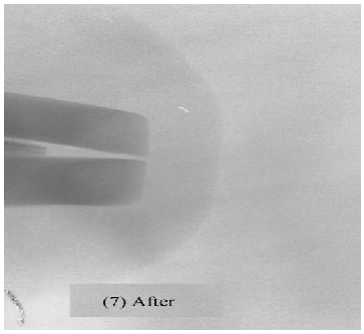
R_6: Control (+ve)



R_3:15 s(-ve)



R_5: 25 (-ve)



R_7: Control (-ve)

Figure 3. The radiographical films of the rabbits teeth after application with *Streptococcus mutans* irradiated with Omega Diode Laser, to show the curiosity sign in the rabbit's teeth (R_1 and R_2). Key: R_1-R_5: rabbits under tested, R_6 and R_7: Control test.

of dental caries and the diode laser irradiations at a light-emitting diode (LED: 30 m/w and 670 nm, 5 KH2) and have a lethal effect on *S. mutans* when the time of its exposure was above 10 s.

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