

Sensitization of Periodontitis Disease Causing Bacteria by Low Power He-Ne Laser Radiation

Akash Tripathi Satsangi^{1*}, Manish Mathur¹, Parul Saxena¹, Guru Prasad^{1*}, Jitendra Nath Shrivastava³,
Jenendra Nath Shrivastava¹, and Sukhdev Roy²

¹Microbiology lab, Department of Botany

²Photonics lab, Department of Physics & Computer science Dayalbagh Educational Institute
(Deemed University), Dayalbagh, Agra-282005 (INDIA)

³Department of Botany D. B. S. College, Kanpur-208006 (INDIA)

The present investigation is an attempt to create an optimized protocol for a bactericidal modality of different powers of He-Ne laser radiation to eliminate periodontitis disease causing bacteria from dental plaques. Periodontitis is most prevalent infectious disease of men and caused by a limited number of Gram negative oral bacteria. *Porphyromonas gingivalis* and *Streptococcus sanguis* are the important bacteria responsible for periodontitis diseases. Effect on periodontitis disease causing bacteria were produced by the exposure of different powers of He-Ne laser light i.e. 9 mW, 17 mW and 26 mW of red colour of wavelength 632.8 nm in two different periods of time i.e. 10 min. and 20 min. in the presence of dye Methylene blue (MB) used as a photosensitizer. The results have been shown in terms of percentage inhibition of colony forming units (cfu.) of bacteria. This study has shown that maximum inhibition of cfu. were observed in Laser+MB-20 min. exposure time. This inhibition was followed by Laser+MB-10 min., but minimum inhibition was seen in Laser only at 10 min. exposure. In case of effect of methylene alone on the cfu. of bacteria, it was seen that MB have not shown more inhibition of cfu. and it had shown that the no. of cfu. are very similar to that of control. The above observation of the present study was seen in case of every 3 different type of used powers of laser for both the bacteria. Maximum percentage inhibition of cfu. were seen in case of 26mW powers of He-Ne laser, which was 67.28% to 61.42% for *Porphyromonas gingivalis* and *Streptococcus sanguis* respectively. So, increasing the power of laser (safe range for dentistry is 3-30 mW) under conditions shows an increased percentage inhibition of cfu. Thus the present investigation may be a useful adjunct with mechanical debridement in the prevention of recolonization of subgingival lesions by pathogenic microorganisms which are harmful and drug resistant.

key words: He-Ne laser, *Porphyromonas gingivalis*, Sensitization, *Streptococcus sanguis*.

INTRODUCTION

Microbial plaques are the primary etiological agent of the inflammatory periodontal diseases. The major purpose of periodontal therapy is to eliminate bacterial deposits from tooth surface [5, 16]. Periodontitis disease is caused by a number of bacteria such as *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Streptococcus sanguis*. The use of systemic antibiotics has been an adjunct in the treatment of periodontitis disease. The over use of antibiotics has been a major culprit in the production of drug resistant organisms [11]. Therefore an alternative method to eradicate bacteria is required. In this field one such approach is Photodynamic therapy (PDT). Recently a series of studies have shown that it is possible to

kill bacteria with a low power laser after sensitizing them with a low concentration of dye, such as Methylene Blue (MB) [7, 9, 19]. In this connection it is necessary to have some knowledge about laser. The word "laser" abbreviates "light amplification by stimulated emission of radiation". It is a device by which an instance monochromatic, collimated, highly coherent light beam can be obtained. Wavelengths of laser light used in laser therapy are of visible and infrared ranges. These ranges are very safe and far away from damaging ultraviolet, X-rays, gamma and cosmic rays. Many people think of lasers as cutting lasers. In order to cut with laser, it is necessary to increase the power density from 300 to 10000 W/cm². Laser do not have a warming effect unless they are operated above 5 W/cm². In present study the different powers of He-Ne laser were used in the ranges of 9 mW, 17 mW and 26 mW (where as safe limit for dentistry is 3-30 mW) and wavelength was 632.8 nm of red color which is visible and nondamaging. According to few studies laser light alone has no effect on bacterial viability in the absence of sensitizer [14]. Thus the

*To whom correspondence should be addressed.

E-mail :

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main aim of the proposed work is to carry out the sensitization of periodontitis disease causing bacteria by low power He-Ne laser radiation.

MATERIALS AND METHODS

The different powers of He-Ne laser (Melles Griot, U.S.A.) i.e. 9 mW, 17 mW and 26 mW (combination of 9mW and 17 mW) of wavelength 632.8nm of red colour (visible and nondamaging range) were used for the exposure of laser light on bacterial suspension. The bacterial strains in this study were *Streptococcus sanguis* and *Porphyromonas gingivalis*, which were identified by the key proposed by Williams *et al.*; 1989 [18]. Bacteria were isolated from the infected teeth of the patient by Cotton swab method [4] and were cultured on nutrient-agar medium {Beef extract(E.merk,Germany) -1 g., Yeast extract(E.merk, Germany)-2 g, Peptone (Qualigens fine Chemicals, GlaxoIndia, Ltd. Mumbai)-5 g, NaCl (E.merk, Germany)-5 g, Agar (Qualigens fine Chemicals,Glaxo India, Ltd. Mumbai)-20g , Distilled water-1000ml}.

Bacterial suspension were made in distilled water and were quantified by a UV-visible spectrophotometer (VIS Spectrophotometer Elico India Ltd., Indraprastha, New Delhi, SL-159, Instalation year-1999 oct.10). A dye Methylene blue (Sigma Aldrich, U.S.A.) was used as a photosensitizer in bacterial suspension with concentration 0.01% wt./vol. Samples of bacterial suspension were taken in micro titration plates for experiment and methodology were followed as in Chan and Lai experiment [3]. The treated bacterial suspension were maintained on nutrient agar medium as a subculture for a time period of 48 hrs. of an anaerobic incubation at 30°C. Samples were distributed in to four groups for testing

1. Negative Controls- Bacterial suspensions were untreated either by laser or photosensitizer.
2. Laser alone- Bacterial suspensions radiated with different powers of laser in absence of Methylene blue as photosensitizer.
3. Photosensitizer only- Bacterial suspension were treated only with photosensitizer.
4. Photosensitizer only- Bacterial suspension were treated with both laser and photosensitizer.

The delivery of laser to each group follows: 3 different powers (each in one time) of used laser applied for two different time periods i.e. 10 min. and 20 min. Statistical analysis of the data were done with respect to standard deviation.

RESULTS

In the present investigation sensitization of periodontitis disease causing bacteria have been studied in terms of percentage inhibition of cfu. in each of the test group, which is tabulated for each of the three different powers of laser used in this study. Table1, 2 and 3 shows the susceptibility of the two bacteria to He-Ne laser of the power 9 mW, 17 mW and 26mW respectively.

It was observed in Table 1 that percentage inhibition of colony forming units were maximum in laser + MB-20 min. exposure for both the bacteria, this inhibition was followed by laser + MB-10 min. exposure. Percentage inhibition of cfu. were seen in laser alone -10min. exposure for both the bacteria but after increasing the exposure time upto 20 min. the percentage inhibition was higher than that of 10 min. exposure of laser beam for both the bacteria. In case of effect of Methylene blue alone on the cfu. it was seen that the MB have not shown more inhibition on cfu. and shows that number of cfu. are very similar to that of control for both the bacteria.

In Table 2, it was also seen that percentage inhibition of cfu. were maximum for the exposure of laser in presence of MB for a time period of 20 min. for both the bacteria, this inhibition was followed by laser+MB-10 min. exposure. But increasing the exposure time upto 20 min. showed more percentage inhibition than that of 10 min. exposure of laser beam. It was interesting to note that inhibition of cfu. may or may not be affected in presence of MB as compared to control. Sometimes the suspension treated with MB alone showed that number of cfu. become less as compared to control e.g. *Porphyromonas gingivalis* , but sometimes it exceeds the no. of cfu as in control e.g. *Streptococcus sanguis* and showed a negative value of percentage inhibition.

In Table 3, the maximum inhibition of cfu. was seen in laser +MB -20 min. exposure for both the bacteria, this inhibition

Table 1. Susceptibility of Oral bacteria to light from He-Ne laser of Wavelength 623.8 and power of 9 mW following treatment with MB

Bacterial Strain	Group-1	Group- 2		Group- 3	Group- 4	
	Control	Laser		Methylene Blue only	Laser + MB	
		10 min.	20 min.		10 min.	20 min.
<i>P.gingivalis</i>	117 ± 3	114 ± 2 (2.56%)	108 ± 1 (7.69%)	113 ± 3 (3.41%)	96 ± 1 (17.94%)	58 ± 4 (50.42%)
<i>S.sanguis</i>	1104	106 ± 5 (3.77%)	97 ± 6 (11.8%)	104 ± 3 (5.45%)	79 ± 3 (28.18%)	66 ± 3 (40.00%)

Note: ± Shows Standard Deviation

Figures in parentheses shows percentage over control

was followed by laser+MB-10 min. exposure. There is less inhibition of cfu. was seen in laser only at 10 min. exposure but increasing the exposure time up to 20 min. once again more percentage inhibition was seen for both the bacteria. But in observing the effect of MB an interesting point was noted that the number of cfu. exceeds the number of cfu. as in control in case of *Porphyromonas gingivalis* and a (-ve) value of percentage inhibition was shown but in case of *Streptococcus sanguis* the effect of MB on cfu. was also very less in terms of percentage inhibition.

A comparison between group 1 and 3 reveals that treatment with photosensitizer in the absence of laser irradiation did not cause any significant reduction in viability of any of the tested bacterial cultures. This demonstrates that there is no direct toxicity with MB as sensitizer at low concentration. In comparing group 2 and 3 reveals that the irradiation with a He-Ne laser for up to 20 min. had no significant effect on the viability of target bacteria in absence of MB as photosensitizer. According to time of exposure both the tested microorganism showed significant decrease in viable colony counts when treated with both sensitizer and laser. Further more there was statistically significant difference in killing between the different powers of laser. A comparison between Table 1, 2 and 3 exposurer of light from both He-Ne laser of power 9mW + 17mW i.e. 26 mW as compared to 9mW and 17 mW separately resulted in a significant increase in percentage inhibition in case of different combinations of laser treatment e.g.laser-10 min., laser-20 min., laser + MB-10 min., laser + MB-20 min. During 10 min. exposure of MB

incorporated microorganisms with a He-Ne laser of 26mW power, the percentage inhibition ranged from 56.42% to 60.74% but when the exposure time increased from 10 min. to 20 min. with same power of laser the percentage inhibition became increased from 61.42% to 67.28%.

DISCUSSION

The present findings show that exposure on bacterial suspension with laser light in the presence of MB as a photosensitizer results in a dose-dependent decrease in viability. The most effective combination was that of MB with a 632.8 wavelength He-Ne laser at 26 mw. This produced a 61.42% to 67.28% kill rate of bacteria. To exclude the possibility that the absorption of laser energy in MB may raise the temperature to kill bacteria, a pretest was done to evaluate the thermals effects. In present investigation it was observed that the temperature was increased up to only 0.5-3°C in all lasing group with or without MB. It is suggested that as a photosensitizer Methylene blue did not convert laser energy into heat that may kill microorganisms under the test conditions. In general, the ability of the laser to inhibit the periodontal pathogens was species-dependent. It appears that *Porphyromonas gingivalis* was more resistant to killing while *Streptococcus sanguis* was least, under the prevailing conditions.

In the beginning of the last century it was recognized that microbes became susceptible to visible light mixed with a photosensitizing compound [8]. More recently the concept of selectively sensitizing cells for targeted killings by safe doses

Table 2. Susceptibility of Oral bacteria to light from He-Ne laser of Wavelength 623.8 and power of 17 mW following treatment with MB.

Bacterial strain	Group-1	Group- 2		Group- 3	Group- 4	
	Control	Laser		Methylene Blue only	Laser + MB	
		10 min.	20 min.		10 min.	20 min.
<i>P. gingivalis</i>	95 ± 4	91 ± 3 (5.39%)	86 ± 3 (10.46%)	87 ± 3 (8.04%)	55 ± 3 (42.10%)	39 ± 3 (58.94%)
<i>S. sanguis</i>	109 ± 4	101 ± 3 (7.92%)	95 ± 3 (14.73%)	115 ± 5 (-5.2%)	74 ± 4 (32.11%)	50 ± 4 (54.12%)

Note: ± Shows Standard Deviation

Figures in parentheses shows percentage over control

Table 3. Susceptibility of Oral Bacteria to light from He-Ne laser of wavelength 623.8 and power of 26 mW following treatment with MB.

Bacterial strain	Group-1	Group- 2		Group- 3	Group- 4	
	Control	Laser		Methylene Blue only	Laser + MB	
		10 min.	20 min.		10 min.	20 min.
<i>P. gingivalis</i>	140 ± 4	128 ± 3 (8.57%)	121 ± 4 (13.57%)	143 ± 2 (-2.14%)	61 ± 4 (56.42%)	54 ± 7 (61.42%)
<i>S. sanguis</i>	107 ± 4	95 ± 3 (11.21%)	86 ± 3 (19.62%)	105 ± 5 (1.90%)	42 ± 4 (60.74%)	35 ± 4 (67.28%)

Note: ± Shows Standard Deviation

Figures in parentheses shows percentage over control

of visible light has become the basis of a new therapeutic modality for the treatment of tumors: photodynamic therapy [1, 9] and attempts to bring the concepts of PDT to the field of dentistry [15, 17]. Numerous research groups have verified the lethal effect of laser radiation on microorganisms associated with dental caries and Periodontitis [2, 10, 15]. Studies have shown that light from both high power and low power laser was effective in killing oral pathogenic bacteria. However the use of low power light has advantages in that a bactericidal effect can be achieved without damaging host tissues [20].

Successful PDT always involves the optimization of a large number of parameters. Obviously selection of an effective photosensitizer is essential for the success of the technique as well as being non-toxic to humans. The ideal photosensitizer needs to absorb a laser beam at the compatible wavelength and has to produce high excitation efficiency. Methylene blue, which belongs to the phenothiazinium family of dyes is a well-known photosensitizer. It is safer than other photosensitizing dyes [12]. Present investigation reveals that oral microorganism in presence of MB were killed by short term exposure of laser light. Control group treated with MB did not reveal any demonstrable killing. Regarding the percentage inhibition our results are supporting the work of Chan *et al.* [3] which has reported that the microorganisms incorporated with low concentration of MB can be killed by short term exposure to laser light. It was believed that the observation of present study demonstrated that this approach could be used practically to kill oral bacterium.

It is obvious that the bactericidal effect was dependent on the doses of power given by laser. These results suggest that the power of the laser light source used in PDT is a crucial point for optimizing the therapeutic effect and is an important factor in assessing the clinical applicability of this potential therapeutic approach. From a practical point of view, this application of lethal photosensitization (safe range for Dentistry is 3-30 mW) to the elimination of microorganisms from a periodontal lesion would seem to be relatively straightforward matter [1, 6]. This is specifically shown from *P. gingivalis* and *Streptococcus sanguis*. Both aggressive periodontal pathogens are particularly susceptible to killing by photosensitization. This might be due to the presence of protohaemin and protoporphyrin. Two compounds present in these species that can absorb red light and therefore might enhance the killing effect [13]. Another advantage to PDT is that the photosensitizer can be applied topically into a periodontal pocket, hence avoiding overdoses. Also, as this is a local therapy, one can reduce the probability of side effects associated with the systemic administration of antimicrobial agents. Following application of a sensitizer, a laser beam could be delivered into the target area precisely via a fiber optic cable. Therefore, disturbances of micro flora at other sites in the oral cavity would not occur.

Although the results of this study have shown that oral pathogens can readily be killed by the appropriate laser-dye

treatment combination, further *in vivo* evaluation is necessary.

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