

# Evaluation of Effects of Irradiation with 980 nm Diode Laser at 0.8 W, 1.2 W and 1.4 W, on Sheep Bone - An In Vitro Histological Study

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## ABSTRACT

### BACKGROUND

Diode laser has been a boon to treat various periodontal diseases in the last decade. Literature cautions that contact of diode laser would be detrimental to bone and leads to bone resorption. However, till date no studies have documented bone damage at different power settings of laser. So, the objective of this study was to evaluate the effects of 980 nm diode laser irradiation on sheep bone under different power settings in continuous wave mode for fixed amount of time.

### METHODS

A fresh femur of sheep devoid of any muscle and soft tissue was obtained. Three markings, each 10 mm long were made for the specimens. The specimens were categorized as sample A, B and C. 980 nm Diode Laser was used to irradiate the specimens with 0.8 W, 1.2 W and 1.4 W at continuous mode as the power settings for sample A, B and C respectively for 10 seconds in direct contact with bone in a brushing like pattern.

### RESULTS

The depth of bone damage was measured using Haematoxylin and Eosin stain. Bone damage was minimum for group 1 followed by group 2 and 3.

### CONCLUSIONS

When the specimens were irradiated by a 980 nm diode laser in direct contact with the bone tissue, damage was seen both clinically and microscopically in all groups.

### KEY WORDS

Laser Therapy, Femur, Diode Lasers, Osteocytes

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## BACKGROUND

Diode lasers have been in the forefront of research with regard to treatment of various dental clinical diseases and to improvise the clinical application to the soft tissues. A zone of thermal necrosis is associated with all laser-tissue interactions with some degree of tissue vaporization.<sup>[1]</sup> This zone of thermal damage should ideally be kept to a minimum, as it may interfere with wound healing and graft take, and reduce tensile strength.<sup>[2]</sup> There are certain variables that determine the initial tissue effect like laser wavelength, laser power, the available laser waveform (continuous wave, chopped, and pulsed beams), tissue optical properties, and tissue thermal properties.<sup>[3]</sup> Lasers have certain properties and should be taken in to account when lasers are applied to the tissues. The outcome depends on the interaction of appropriate use of laser parameters and properties of tissues on which they are irradiated. Laser parameters include wavelength, power, power density, energy, energy density, beam diameter, time of irradiation and frequency of treatment. Operator has control over laser irradiation settings, whereas tissue properties are fixed and cannot be altered. Target tissue properties include colour, consistency, framework, density and pigmentation. Always the clinical end results depend on the interaction between laser and target tissues, which mandatorily require optimal use laser parameters.<sup>[4]</sup> When CO<sub>2</sub> laser is irradiated on soft tissues, there are three histological zones are formed namely thermal zone, tissue necrosis zone and ablative zone from inside to outside. Vaporization of tissues in ablative zone and repair of tissues in the thermal zone with zone of necrosis in between is seen in the histological section of the tissues. Trauma to the underlying tissues depend on penetrative capability of laser and thickness of overlying tissues. Laser application with increased energy density on thin gingiva can cause irreversible damage to underneath bone. The thickness of attached gingiva varies from 1.25±0.43 mm on labial aspect of mandible.<sup>[5]</sup> In these areas thermal effect of usage of different parameters of CO<sub>2</sub> laser in gingival surgical applications have already been documented histologically. So when CO<sub>2</sub> laser is used for gingival surgical procedures in areas where gingiva is thin then the cortical bone may likely to get traumatized. Diode lasers have shown good results in containing the infection and inflammation when used as an additive to nonsurgical periodontal therapy in the treatment of periodontitis. They have also shown success in conditioning the enamel and dentin surfaces and bactericidal effect in root canal treatment.<sup>[6-8]</sup> Although it is known that Diode laser causes bone damage, it's not clear from literature as what time and parameters of laser causes damage to the bone. So, the objective of this double blind Invitro study was to evaluate the effects of diode of 980 nm laser irradiation on sheep bone under different power settings in continuous wave mode for fixed amount of time.

## METHODS

The double blind Invitro Study was carried out in the Dept. of Periodontology, HKE's S. Nijalingappa Institute of Dental Sciences and research. For this study, fresh sheep femurs were used no later than 6 hours after the animals' death. A fresh

femur of sheep devoid of any muscle and soft tissue was obtained. They were divided into 18 separate specimens. These specimens were categorized into A, B and C groups including 6 specimens in each group. Prior to laser exposure 10 mm long markings were made for each specimen on the fresh femur bone. 980 nm Diode Laser was used to irradiate the specimens for 10 seconds in direct contact in a brushing like pattern in the continuous wave mode under three different power settings for group A, B, C (Fig. 1, 2, 3). Group A specimens were exposed at 0.8 W, Group B at 1.2 W and Group C at 1.4 W. Investigator was given specimen randomly and asked to apply laser for 10 seconds without revealing the group names and laser settings. The investigator and pathologist were not aware of the details of specimens and laser settings. The specimens were cut into blocks using handpiece and a bur and stored in the formalin until the laboratory investigations. Then bone specimens of all the groups were subjected for decalcification followed by the Haematoxylin and Eosin staining and preparation of the slides for further histopathological investigation.

## Statistical Analysis

Mean and SD (standard deviation) of viable osteocytes were calculated. Descriptive & inferential statistical analysis was done. Histological findings were analysed by Kruskal-Wallis and Mann-Whitney U test. Kruskal Wallis test to check the significant difference between the three groups. Mann-Whitney U test to check the significant difference between the two groups. With the p value less than 0.05 being considered as significant.

## RESULTS

Light microscopic examination of the laser-treated bone specimens revealed a consistency in morphologic alteration that varied only in degree of change as dictated by increasing energy densities. Macroscopically, the surface target area featured a "trough-like" ablation defect. The ablation defects generally had a rounded base and walls that were either parallel or slightly divergent. On histologic examination Group A specimens exposed at 0.8 W revealed intact interstitial tissue and osteocytes viable showing nucleus (Fig. 1). Group B specimens exposed at 1.2 W revealed intact interstitial tissue and focally damaged osteocytes few present with nucleus and few without nucleus (Fig. 2). Group C exposed at 1.4 W revealed damaged interstitial tissue and a greater number of osteocytes without nucleus and few numbers of viable osteocytes (Fig. 3). Table No. 1, Fig. 1, 2 and 3 Shows increased mean value of viable osteocytes in group A followed by Group B and Group C i.e. with the values of 71.83, 42.83 and 39.17 respectively indicating least damage to group A. Table No. 2 Shows statistically significant difference between the groups A, B, C with the mean values of 14.83, 7.83, and 6.33 respectively with the p value 0.022.

Table no. 3 shows statistically significant difference between group A and group B with the mean value of 8.83 and 4.17 with the p value of 0.024, and between group A and group C with the mean values 9.00 and 4.00, with the p value of 0.016 respectively. Whereas between group B and group C there

were no statistically significant difference with the mean values 7.17 and 5.83, with the p value of 0.520 respectively.

	N	Minimum	Maximum	Median	IQR*
Group A	6	37.00	98.00	69	42.25
Group B	6	29.00	65.00	38	24.75
Group C	6	20.00	30.00	38	17

**Table 1. Median and IQR of Viable Osteocytes**

\*Inter Quartile Range

Groups	N	Mean Rank	p Value
A	6	14.83	
B	6	7.83	0.022 (S)*
C	6	6.33	

**Table 2. Overall Comparison of Groups (Kruskal Wallis Test)**

\*Significant

Groups	N	Mean Rank	p Value
A	6	8.83	
B	6	4.17	0.024 (S)*
A	6	9.00	
C	6	4.00	0.016 (S)*
B	6	7.17	
C	6	5.83	0.520 (NS)**

**Table 3. Pairwise Comparison of Groups (Mann-Whitney U Test)**

\*Significant \*Non-Significant

### DISCUSSION

Laser – tissue interactions can pave a way for better comprehension of beneficial or deleterious effects that a laser can have on the tissues due to the variation of parameters used in the study. This can give better understanding of optimal use of laser parameters for the irradiation. Tissue response depends on the laser beam properties used and greater trauma to adjacent areas have been observed in the constant wave mode compared to pulse mode. Several studies have reported the effects of different laser irradiation on bone. McDavid VG et al<sup>[9]</sup> reported laser irradiation using CO2 and Nd:YAG significantly delayed healing of bone even when used with water coolant. Sasaki KM et al<sup>[10]</sup> in their study reported that the major changes found on bone surface after Er: YAG laser irradiation consisted of micro-cracking, disorganization, reduction and slight recrystallization of the hydroxyapatites of surrounding organic matrix. Lana S et al<sup>[4]</sup> in their in Vitro Study Concerning the effects of the CO2 Laser on oral mucosa and subjacent bone showed all specimens regardless of, energy density, tissue composition, energy, power density exhibited a distinct residual carbonized tissue layer, a zone of thermal necrosis characterized by tissue coagulation, and a zone of tissue exhibiting thermal damage. As noted in the present study, the amount of tissue damage peripheral to the ablation defect was increasing with the increasing wattage in terms of number of viable osteocytes present in the specimen examined under microscope with the Haematoxylin and Eosin staining. And clinically charring was noticed on the group C specimens exposed at 1.4 W. Group A exposed at 0.8 watt showed more no. of viable osteocytes with the mean value of 71.83 whereas group B exposed at 1.2 watt showed mean value of 42.83 and group C exposed at 1.4 watts showed mean value of 39.17 viable osteocytes. In our study, effort was made

mainly to evaluate the effects of diode laser on the fresh sheep bone exposed to the increasing power of 0.8 W, 1.2 W and 1.4 W under continuous wave mode. Diode laser caused bone damage for above parameters. Different bone architecture compared to human bone, staining procedures and small sample size were the limitations of our study.

### CONCLUSIONS

The extent of tissue damage was minimal when bone was exposed to 0.8 W power compared to that exposed at 1.2 W and 1.4 W. Although it is difficult to extrapolate these results to humans, still diode laser should be used cautiously in the vicinity of bone as it causes irreversible damage to bone even at 0.8 W.

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