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1	Caries Inhibition of Pit and Fissure Non Cavitated Lesions in
2	Children by Low-Level Lasers-A Clinical Study
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6 Abstract

7 Background: The incipient non-cavitated carious lesion are characterized by subsurface

⁸ dissolution which have potential to remineralize. Traditional remineralization protocols have

⁹ varying results. An effective remineralizing protocol thus would be one which brings about a

¹⁰ change in enamel crystal and makes it more resistant to acidic challenges of oral

¹¹ cavity.Material Methods: 102 patients of either sex, between age of 6 -18 years having

¹² incipient pit and fissure caries on first or second mandibular molar were selected and its

¹³ respective contralateral tooth type served as control. Occlusal scanning for both groups was

¹⁴ done by LASER fluorescence method (DIAGNOdent) and this was baseline value. Group B

¹⁵ (Test) was irradiated with 810 nm AlGaAs low level LASER for 30 seconds followed by

¹⁶ application of CPP-ACP F remineralizing paste. In Group a (Control) only remineralization

¹⁷ paste was applied. The LASER fluorescence values were recorded after 7 days and the

¹⁸ treatment protocol repeated. 18 months follow up at 6 monthly intervals included LASER

¹⁹ fluorescence serial scanning. Fall in values from baseline indicates remineralization. Statistical

- 20 analysis was done.
- 21

22 Index terms— dental caries, non cavitated lesions, remineralization, wattage, time.

23 1 Introduction

24 Since over a century, fluoride has been the cornerstone caries preventive strategy. Topical fluoride application 25 had varying success rate. Oral fluoride administration in the form of tablets though effective has documented 26 instances of overdose leading to dental fluorosis, nausea, diarrhea, and abdominal cramps. 5,6 Safe drinking water is a constitutionally guaranteed right in India and millions of rupees are invested in water sanitization, however, 27 centralized systems of water supply and monitoringare ineffective. Inappropriate fluoride levels in drinking water 28 have led to dental and skeletal abnormalities. 7, ?? Thus, an effective preventive regime would be one that brings 29 about a change in enamel crystal and makes it more resistant to acidic challenges of the oral cavity. One such 30 modality is the inclusion of LASERs in the prevention of caries. High powered LASERs are discontinued for caries 31 inhibition owing to their high cost, bulky equipment and lack of any evidence-based therapeutic application. 9,10 32 Low powered LASER has many soft tissue oriented clinical applications. Today they are being explored as an 33 alternative to high powered LASERs in hard tissue applications. 11 Hence this study has been designed to explore 34 the possibility of using a low-level LASER as caries preventive and inhibitory tool in caries prone population. 35 36 The findings of the analysis shall help in making standard treatment guidelines, suitable recommendations for 37 caries prevention and inhibition, to benefit children and help in reducing the caries burden. 38 Abstract-Background: The incipient non-cavitated carious lesion are characterized by subsurface dissolution which have potential to remineralize. Traditional remineralization protocols have varying results. An effective

which have potential to remineralize. Traditional remineralization protocols have varying results. An effective remineralizing protocol thus would be one which brings about a change in enamel crystal and makes it more acidic challenges of oral cavity.

42 Material & Methods: 102 patients of either sex, between age of 6 -18 years having incipient pit and fissure 43 caries on first or second mandibular molar were selected and its respective contralateral tooth type served as 44 control. Occlusal scanning for both groups was done by LASER fluorescence method (DIAGNOdent) and this was $_{45}$ baseline value. Group B (Test) was irradiated with 810 nm AlGaAs low level LASER for 30 seconds followed by

application of CPP-ACP F remineralizing paste. In Group a (Control) only remineralization paste was applied.
 The LASER fluorescence values were recorded after 7 days and the treatment protocol repeated. 18 months follow

⁴⁸ up at 6 monthly intervals included LASER fluorescence serial scanning. Fall in values from baseline indicates

49 remineralization. Statistical analysis was done.

50 In vitro studies were conducted on enamel samples prior clinical study to ascertain the optimum wattage and 51 time.

Results: Control group had 14.7 failure rate where as Test group had no failures. The optimal wattage and time of irradiation was 0.5 watts and 30 secs respectively.

54 Conclusions: LASER irradiation leads to caries inhibition as indicated by fall in LASER fluorescence values 55 after irradiation.

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57 caries is a dynamic, progressive disease with varying phases of demineralization and increased remineralization.

58 [1][2][3] To harness the remineralization potentiality of an incipient non-cavitated lesion, it becomes imperative

59 that carious lesions are detected and diagnosed well in time and evaluated regularly for any change in the lesion

60 activity. The carious process is a continuum disease process and to monitor it at a specific point will give an 61 unfounded, inaccurate and unrealistic result.

⁶² 2 Procedure for Clinical Study

Ethical clearance and informed consent were undertaken. Patients of either sex, between the age of 6 years to 18 63 years reporting to OPD having initial pit and fissure caries on first or second mandibular molar were included 64 in the study, and its respective contralateral tooth served as control. The sample size was 102 in both groups. 65 66 Oral prophylaxis was done. Suspected incipient site1 size 0 lesions, as per Mount and Hume classification, were shortlisted. Patients who scored V0, V1 or V2 in Ekstra and criteria for visual scoring for dental caries and 67 0, 1 or 2 in ICDAS index and R0 or R1 in the Ekstra and criteria for radiographic scoring were included in 68 the study. But the diagnosis was more conclusive by a more sensitive method of caries diagnosis that is the 69 LASER fluorescence method. The LASER fluorescence pen (DIAGNOdent pen 2190, KAVO, Birbech Germany) 70 scanned the area of interest on the tooth surface. A diagnostic readout as per Lussi criteria served as a baseline 71 reading. The LASER fluorescence method values were recorded for both test and control. The test group teeth 72 -(Group B) were irradiated with 810 nm AlGaAsdiode LASER of 0.5 watts (WhitestarTM, Creation, Verona, 73 Italy) for 30 seconds, followed by the application of remineralizing paste CPP-ACP F (GC -Tooth Mousse). The 74 contralateral tooth type served as a control-(Group A), in which, after recording LASER fluorescence device 75 values, only remineralization paste had been applied. (Fig 1 & 2) The values were then noted on the seventh 76 day, the test tooth was irradiated again, and the remineralizing paste was applied. After that, the follow up 77 at six-monthly intervals included serial scanning with LASER fluorescence pen and comparison with baseline 78 reading. The readings determined if caries had been inhibited, arrested, or progressed. A radiograph was then 79 taken to assess if caries has progressed at the end of the study. (Fig3) The results were then tabulated and 80 computed. Statistical analysis was carried out. (Graph 1) 81 82 IV.

⁸³ 3 Procedure to Ascertain Optimal Wattage

Thirty freshly extracted teeth, which were cariesfree and without any structural defect, had been selected. They 84 were then sectioned mesiodistally to obtain 60 samples, and the tooth section was coated with nail varnish to 85 obtain windows of 3mm X 3mm on the facial surface, which aided in standardizing the sample's dimensions. 86 The samples were divided into ten groups for each power setting which was being evaluated. Each group had 87 six samples each. The power settings range from 0.1 to 1 watts was selected. For each sample, the preoperative 88 value of the exposed tooth section was evaluated by LASER fluorescence method, and this served as control. The 89 tooth section was surface treated with 37% acid etchant gel for 20 seconds. The LASER fluorescence device's 90 values were then noted. Then they were irradiated with aluminium gallium arsenide LASER of 810nm for 30 91 sec, and each group were then individually evaluated for different power settings power setting from 0.1 watts to 92 1 watt. The power setting which were evaluated were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1. After irradiation 93 for 30 seconds, the LASER fluorescence method reading was again noted. The results were then tabulated and 94 computed. Statistical analysis was carried out. (Graph 2) 95

 $_{\rm 96}$ $\,$ V. Procedure to Ascertain the Time of Irradiation VI.

97 4 Summary of Results

The results are being summarized in the graphs (1-3) and tables (1). In this clinical study, the caries preventive and caries inhibitory potentiality of low-level LASERs was evaluated and was compared with the conventional method, i.e, application of remineralization paste. LASER fluorescence was then used for monitoring the effect of the LASER, and remineralizing on incipient non-cavitated carious lesions. Based on the result it was finally concluded that the parameters of the low-level LASER of 810 nm, i.e wattage and time, which brings about the optimal results, were 0.5 watts and 30 secs, respectively. In the Thirty-six samples surfaces were

prepared as before and evaluated with a LASER fluorescence device to obtain the baseline values. The samples 104 were then surface treated for twenty sec with 30 % phosphoric acid, to simulate surface demineralization. The 105 LASER fluorescence device was then used to record the values of demineralized samples. The samples were then 106 107 divided into six groups of six samples each. The sectioned surfaces of the samples were thereafter irradiated 108 with aluminium gallium arsenide LASER of 0.5 watts for 5 secs, 10 secs, 15 secs, 30 secs, 45 secs, and 60 secs, respectively. The LASER fluorescence device recorded values post-irradiation. Statistical analysis was carried 109 out. The exposure time in which the post-irradiation values came closest to preoperative values was then recoded 110 as the optimal time of exposure. (Graph 3) test group, there was a 100 percent success rate. The control group 111 had 15 failures. The results were statistically significant. 112

¹¹³ **5 VII.**

114 6 Discussion

Dental caries is the most prevalent oral pandemic disease of dental hard tissues. Acevedo et al. observed that 33 115 116 percent of children had non-cavitated lesions between age 11-15 years. 12 SK Jain et al. in an epidemiological 117 study, found that currently, there is a change in pattern and trend of dental caries, and thus, there is an increase in the number of non-cavitated lesions as compared to the cavitated carious lesion, and it is more common at the 118 119 age of 14 years. 13 The tooth which is most prone to dental caries is the first mandibular molar, especially young 120 permanent molars. Hence in this study, the sample population was drawn from six years to eighteen years, and categorically, the first and second molars were included in the study. The sample population was equidistributed 121 amongst both genders. The test and the control are in the same person, and test tooth is the contralateral tooth 122 type of that of the control; thus, making challenges of the oral environment similar. 123

The performance of caries detection methods will be assessed by considering two fundamental parameters: 124 reproducibility and validity. The visual and tactile technique depends on the clinical acuity of the clinician, and 125 126 it is not reproducible even for the same clinician leave alone for other observers. 30 to 40% percent of mineral loss 127 must take place before any carious lesion can be detected on radiograph. 14 Hence a sensitive diagnostic adjunct is required. Current diagnostic aids use the alteration in LASER fluorescence, reflectance, electrical conductance 128 129 or impedance, and ultrasound transmittal properties of enamel concerning demineralization and remineralization during the continuum of a carious lesion over a lengthy period. 14 Studies by Lussi et al., Novaes TF, Atrill, 130 Fung L, Burin et al., have all inferred that LASER fluorescence method is a predictive diagnostic tool and is more 131 sensitive than the traditional method of visual and radiographic examination and. It not only detects early and 132 133 hidden caries but also aids in longitudinal assessment and monitoring of caries activity. [15][16][17][18][19] Hence in this study LASER fluorescence was selected as an assessment tool to study the effect of low-level LASER on 134 135 the enamel surface

136 At the ultrastructural level, the enamel is formed of closely apposition edhydroxylapatite crystals. Outer 50-137 100 microns of enamel layer are heavily impregnated with fluoride. Below this zone, there is more concentration of carbonate ions and less of fluoride ion concentration. The super saturation at the surface layer chokes out 138 fluoride from permeating into subsurface layers. The incipient carious lesion and non-cavitated lesions are thus 139 characterized by intact surface layer due to more fluoride in the 50-100 microns of the tooth's outer surface and 140 subsurface dissolution due to decreased fluoride ion concentration. 1, [5][6][7] ??8]20 The advancement of caries 141 may be impeded or foreshortened using risk modifiers. Fluoride, an efficacious therapeutic risk modifier, present 142 in saliva and biofilm does not affect the biofilm formation and sugar metabolism but reduces demineralization 143 by concurrent precipitation of fluoro-hydroxyapatite, a phase more stable than hydroxyapatite at any given pH. 144 Thus, remineralization is a consequence of fluoride application, not an effect of it. 1,[5][6][7] ??8]20 Ten cate et 145 al. have inferred that to remineralize subsurface lesions 150microns deep 5000ppm of fluoride is required, which 146 is not practical and excessive doses can lead to toxic side effects and even be fatal. 20 Hence an alternative 147 remineralizing paste that could substantiate the effect of fluoride is sought. Casein phosphopeptide amorphous 148 calcium phosphate plus fluoride (CPP-ACP F), has been shown to increase fluoride's uptake into plaque and 149 subsurface enamel by providing bio-available calcium ions, phosphate ions and fluoride ions in the correct molar 150 ratio to form fluorapatite. 21 Hence in this study, we have used CPP ACP F as remineralizing paste both in test 151 and control group. 152

The limitations of remineralizing studies are that most of the evidence-based studies are invitro and is very 153 different when translated to the complex dynamic oral milieu. The other deterrents for remineralization to occur 154 within the body of a subsurface lesion are calcium and phosphate ions must penetrate the surface layer of the 155 enamel. The highly mineralized and charged nature of the surface layer poses a challenge for ion penetration. To 156 infiltrate to subsurface carious layer a very high molar concentration, of approximately 5000 ppm of fluoride ions, 157 158 are needed. 1,20 CPP ACP products if, ingested in significant quantities, will cause side effects. The potential 159 risk increases with patients who have allergic diathesis. The effectiveness of CPP ACP in the remineralizing subsurface lesion is questionable. 5, [7] ??8][20][21][22][23] The other drawback of incorporating a paste based 160 remineralization strategy is that a regular and repetitive replenishment of paste to overcome the loss of calcium 161 and phosphate ion. Thus, an effective remineralizing protocol would be one that brings about a change in enamel 162 crystal and makes it more resistant to acidic challenges of the oral cavity. 163

LASERs, since its introduction in the 1960s, have been studied and piloted to bring about caries prevention

on inhibition by increasing acid resistance. [9][10][11] Ana et al deduced that under specific conditions LASER 165 irradiation can change crystallographic properties of apatite crystals and increasing the acid resistance of lased 166 tooth as well as increased fluoride Sant'anna has conducted an FT Raman spectroscopy study to evaluate low-level 167 diodes as a caries inhibitory tool. 27 Thus, in this clinical study, we have evaluated low-level LASER therapy as 168 a caries inhibitory tool. Comparing test and control it was inferred, to begin with, that the preoperative LASER 169 fluorescence values for all were in the same range. LASER fluorescence values over time have increased in control 170 group thereby indicating demineralization but decreased in test group thereby indicating remineralization, and 171 thus it can be deduced that LASER irradiation brings about changes in the crystallographic properties of enamel 172 apatite and increases the uptake of CPP ACP F paste much more than when the paste is used alone as in control. 173 Thus, our study is confirmation with other reviewed in vitro studies and short term pilot studies. [24][25][26][27]29 174 (Graph 1) 175

Today, aluminium gallium arsenide LASER is currently being investigated as an alternative to high powered LASERs. The hypothesized mechanism of action is that these wavelengths selectively target and remove carbonate ions from hydroxyapatite crystals which results in increased acid resistance of enamel. Additionally, the altered mineral has greater uptake of topically applied fluoride and leads to remineralization of non-cavitated lesions. 11 Thus, in this study, we have selected low-level LASER to increase the acid resistance and the remineralizing potentiality of incipient noncavitated lesions.

182 For any clinical procedure using a LASER, its optical interaction with enamel and dentin must be thoroughly 183 understood. The LASER interaction with dental hard tissues depends upon irradiation parameters such as wavelength, pulsed or continuous emission, pulse duration, repetition rate, beam spot size, and delivery method. 184 All reviewed studies agreed upon the caries inhibitory role of LASERs, but none ascertained the parameters to 185 bring about the desired result. [26][27][28][29][30] Thus, in vitro studies were first conducted to determine an 186 optimum wattage and exposure time. The least amount of deviation there is from control i.e. the closest the 187 LASER fluorescence value to that of the control, the more successful is the treatment. Thus 0.5 watts and 30 secs 188 is the most optimal value as it has shown LASER fluorescence values closest to the corresponding control. The 189 results are statistically significant. (Graph 2& 3) Another challenge in this study was to determine the sequence of 190 the protocol of LASER and remineralizing paste application. Carounanidy has opined that the LASER activated 191 fluoride uptake method can enhance the remineralization potential of an acid challenged tooth. 3 Further, the 192 scientific world is divided on whether fluoride application should precede or follow LASER irradiation. [28][29] 193 In the present study, on the test group, the fluoride application succeeds the LASER treatment. There has been 194 a fall in the LASER fluorescence values in the test group (Group A)which was irradiated by LASER followed by 195 fluoride application. This decrease in LASER fluorescence numerics was seen throughout the follow-up period. 196 However, in the control group (Group B) in which only CPP ACP F was applied, the digital numerics initially 197 fell and then again increased. (Graph1) Thereby, indicating that LASER irradiation followed by remineralizing 198 paste is more effective than remineralizing paste used alone. 199

Each study has its limitations, and no research is flawless, nor should it be expected, as is envisaged in this 200 study. The most reliable way to assess the clinical impact of a novel technique is through its endpoint, such as 201 reversal of carious lesion or survival of the tooth after LASER caries inhibition. However, this standard may be 202 impractical for the evaluation of new therapies, because long periods with large sample size are required for these 203 clinical endpoints to be objectively achieved. A surrogate endpoint can expedite the trial process. A surrogate 204 endpoint is identified as a biological marker that is designed to proxy for a clinical termination. Clinical endpoints, 205 on the other hand, are distinct, direct measurements that reflect how a patient responds to treatment. Clinical 206 endpoints are the most credible characteristics used in the assessment of the benefits and risks of a therapeutic 207 intervention. In this study, we have used a clinical endpoint over a long follow up of eighteen months. 30 Since 208 dental caries is a dynamic disease, a surrogate endpoint should be determined to evaluate the validity of this 209 novel technique. 210

Another limitation is that enamel chemistry studies should also be included to support promising results. The synergism of the low-level LASER and remineralizing paste should be studied at a molecular level.

Thus, this novel concept of combining low-level LASERs with remineralizing paste seems like a promising 213 preventive and inhibitory modality for noncavitated initial caries provided that they are diagnosed early. For 214 early detection, a LASER fluorescence device is found to be a reliable and accurate tool. uptake. 24 The LASERs 215 which have been investigated in the past for caries inhibition are CO2, Nd:YAG, Er:YAG, ErCr:YSGG. These 216 LASERs had innumerable encumbrances like that of exorbitant price tag, unwieldy, heavy cumbersome and 217 besides, the result obtained was also highly debatable. Additionally, the majority of the experiments were lab-218 based and further the diode LASER had never been evaluated as a caries inhibitory technique. Clinical studies 219 at best have been pilot studies or short term. [9][10][11][24][25][26] inhibitory potentiality of low-level LASERs 220 clinically were 0.5 watts and 30 seconds respectively, in the age group 6 years to 18 years. 2. The test group, in 221 which the teeth had been irradiated with low-level LASERs followed by application of remineralizing paste i.e. 222 CPP ACP F, had a 100 % success rate. The control group, in which only remineralizing gel had been applied, 223 had a failure rate of 14.7 %, which necessitated operative intervention. 3. The low-level LASERs increased the 224 uptake of remineralizing paste in irradiated teeth. Thus, LASERs followed by remineralizing paste seems to be 225 the appropriate sequence to be followed. 4. Remineralizing paste alone did not have sufficient long term caries 226 inhibitory potentiality. 5. The low-level LASERs can be used as a viable option to the conventional method, as 227

an accepted modality in preventive and caries inhibitory regimen of dental caries in routine dental practice. 6.
For subsurface incipient carious lesions, the LASER fluorescence device is found to be a reliable and accurate
diagnostic tool. ? Conflict of interest statement: The authors declare there has been no conflict of interest.

231 ? Ethical Approval: Institutional Ethical clearance has been obtained.

232 ? Informed Consent: Informed consent was obtained from all individuals participating in the study. There is 233 an inverse correlation between increase in power and laser fluorescence i.e Laser fluorescence method values of 234 irradiated surface. Indicating that the increase in wattage alters the tooth and makes it closer in its configuration 235 in tooth structure to that of control, thus the values of control and treated group i.e laser irradiated by laser.

Beyond 0.5 watts there is not much difference between control and laser irradiated values by laser fluorescence SC Control; SD -Demineralized; ST -Treated

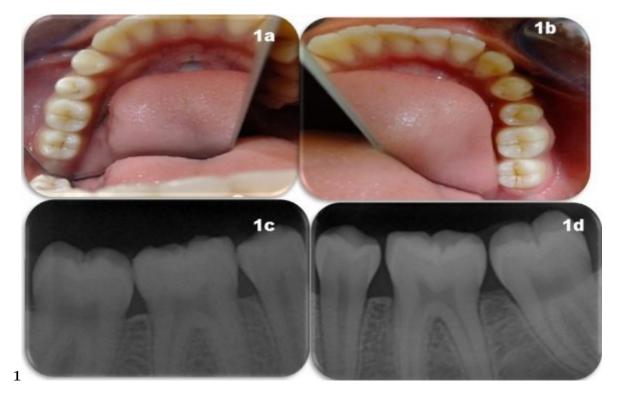


Figure 1: 1.J

237



123

Figure 2: Fig. 1 : Fig. 2 : Fig. 3 :

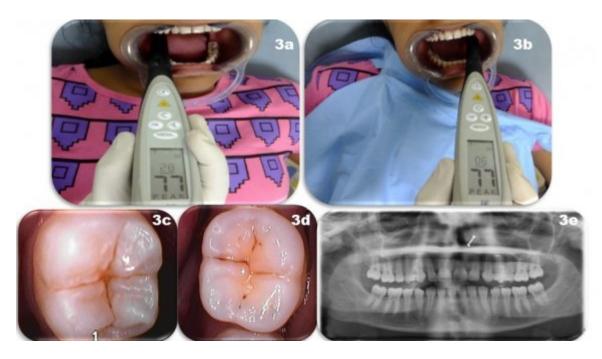


Figure 3:

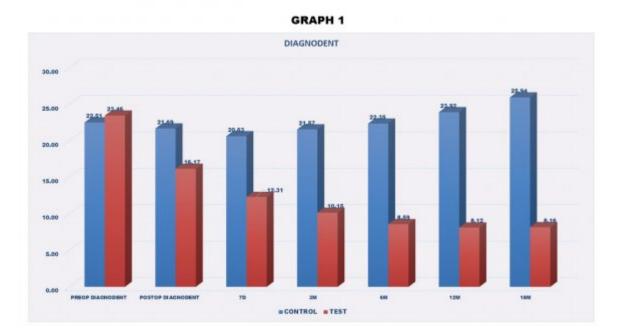


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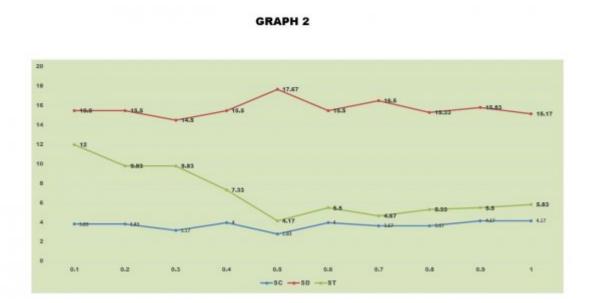


Figure 5:



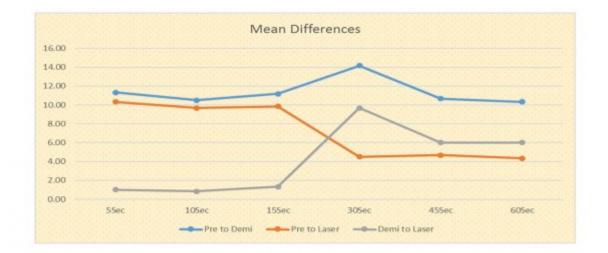


Figure 6:

²³⁸ .1 Acknowledgement: Statistician Mr SS Tanwar

The percentage of success in control is 85.3 percent and failure is 14.7 percent. The sample population is equally represented by both the genders. Correlation of gender and failure shows that 14 failures are seen in females and

1 failure in males. This inference is statistically significant. Age has no bearing on the outcome. The comparison

²⁴² for test group is not computed as there are no failures in test group.

243 .2 GRAPH 2: Optimum Wattage

²⁴⁴ .3 GRAPH 3: Optimal Time of Irridiation

There is significant difference between control demineralized samples and treated and demineralized samples. The treated samples have laser fluorescence values closer to that of control. The time duration of laser irradiation which brings about optimal changes is 30 sec.

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