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# Evaluation of efficacy of different surface treatment protocols by laser fluorescence: an in vitro study

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## Abstract

**Background**: Demineralization and remineralisation of enamel are occurring concomitantly in oral cavity. Fluorides and other remineralizing pastes alone or along with high powered lasers to harnesses the remineralization potentiality of the tooth have been used with varying results. Low powered lasers like aluminium gallium arsenide lasers are being evaluated to bring about caries inhibition.

**Aim**: The aim of the study was to evaluate caries inhibitory potentiality of aluminium gallium arsenide laser.

**Material and method**: 54 extracted intact posterior teeth were selected. They were sectioned mesio - distally and coated with nail varnish so to obtain windows of 3mm X 3mm.The samples were then divided so that each group and sub group had 6 samples each. Group 1: Control; Group 2 A:Laser irradiation with 2 watts for 30 secs; Group 2 B: Laser Irradiation with 3 watts for 30 secs; Group 3: Enafix Paste for 1 hour; Group 4: CPP-ACPF remineralizing paste for 1hour; Group 5 A: Laser Irradiation 2 watts for 30 secs followed by application of Enafix for 1 hour; Group 5 B:Laser Irradiation 3 watts for 30 secs followed by application of Enafix for 1 hour; Group 6 A: Laser Irradiation 2 watts for 30 secs followed by application of CPP-ACPF for 1 hour; Group 6 B: Laser Irradiation 3 watts for 30 secs followed by application of CPP-ACPF for 1 hour. The control group had no treatment done on it and baseline values recorded by laser fluorescence device. All Groups except control group were demineralised by 30% phosphoric acid for 20 secs and laser fluorescence values noted. The groups were surface treated and the laser fluorescence readings were recorded.

**Results**: The data was collected and statistical analysis was done by paired 't' test to compare control and test goup and one way analysis of variance ANOVA test. A significant difference between three or more sample means was revealed by an ANOVA, hence for multiple comparison Tukey HSD Post hoc Tests were undertaken.

**Conclusion**:The laser irradiation of 2 watts followed by CPP-ACPF application was observed as the most effective surface treatment modality.

## Introduction

Current knowledge of the caries is based on understanding the caries continuum process. Dental caries is a dynamic progressive disease of tooth with varying phases of demineralization and remineralization. Thus, the thrust in caries management has moved away from restorative phase to detecting caries at an early non-cavitated stage and risk assessment to determine appropriate preventive, intervention and recall frequency[1-3].

Primary prevention aims at preventing caries before it occurs clinically. It is most effectively done by preventing exposure to what causes the disease, by caries risk assessment and modifying unhealthy behaviours and by increasing resistance to the disease. In addition to addressing the goal of primary prevention of caries, secondary preventive modalities cannot be ignored. Secondary prevention comes into play when caries has progressed to a stage which is clinically detectable i.e. non-cavitated lesion[4,5].

The immediate need based preventive care of a specific incipient lesion once it is detected and assessed is to inhibit the progression of the lesion to a stage of surgical intervention. For secondary prevention many modalities like fluoride has been used alone or in combination with non-fluoride remineralizing agents like CPP-ACP (Casein phosphopeptide-amorphous calcium phosphate)[4-6].

The aim of this study was to evaluate the efficacy of different surface reminerlizing protocols.

## Material and methods

1. Laser fluorescence device (DIAGNOdent pen 2190 KaVo, Biberach, Germany)

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2. Aluminium Gallium Arsenide Laser (White star ™, Creation, Verona, Italy)
3. Casein phosphopeptide-amorphous calcium phosphate fluoride
4. Calcium sucrose phosphate (ENAFIX) paste
5. 30 % phosphoric acid gel

## Procedure

54 freshly extracted intact teeth were selected, disinfected and sectioned mesiodistally. They were coated with nail varnish so as to obtain a window of 3mm × 3mm.

6 samples each were assigned to the following groups and then surface treated as follows

**Group 1**: Control.

**Group 2 A:**Laser irradiation with 2 watts for 30 secs.

**Group 2 B:** Laser Irradiation with 3 watts for 30 secs.

**Group 3:** Enafix Paste for 1 hour.

**Group 4:** CPP-ACPF remineralizing paste for 1hour.

**Group 5 A:** Laser Irradiation 2 watts for 30 secs followed by application of Enafix for 1 hour**.**

**Group 5 B:** Laser Irradiation 3 watts for 30 secs followed by application of Enafix for 1 hour.

**Group 6 A:** Laser Irradiation 2 watts for 30 secs followed by application of CPP-ACPF for 1 hour.

**Group 6 B:** Laser Irradiation 3 watts for 30 secs followed by application of CPP-ACPF for 1 hour.

All groups and sub groups were initially assessed by Laser fluorescence and baseline values noted. All the test groups and subgroups were demineralized by 30 percent phosphoric acid and the values were noted by Laser fluorescence.

After demineralization, treatment groups were again surface treated as already quoted above.

The laser fluorescence values were then recorded for each group and subgroup.

The data was collected and statistical analysis was done by paired 't' test to compare control and test goup and one way analysis of variance ANOVA test. A significant difference between three or more sample means was revealed by an ANOVA, hence for multiple comparison Tukey HSD Post hoc Tests were undertaken(Tables 1& 2).

## Results

**Graph 1A: Comparision of all groups**

GROUP 6A i.e 2 watts laser followed by application of CPP-ACPF has shown to give optimum results. All groups in which laser treatment has been done gives a better result as compared to the paste application when used.

**Graph 2: Difference in surface treatment**

GROUP 6A that is 2 watts laser followed by application of CPP-ACPF has shown to given best results. All groups in which laser treatment has been done gives a better result as compared to the paste application when used alone.

## Discussion

Caries prevention and management strategies are directed towards making enamel more resistant towards dissolution by acidic challenges of oral cavity. The caries progression may be halted or reduced by the use of risk modifiers. Fluoride is an effective therapeutic risk modifier and preventive agent for dental caries. Fluoride has played an important role in the prevention of dental caries since the introduction of water fluoridation in the 1940s. The reduction of demineralization and enhancement of remineralization is due to the basic fluoride ions irrespective of the vehicle[7-8].

Jayarajan J, *et al.* conducted an invitro comparative study of the remineralizing potentiality of CPP-ACP and CPP-ACPF. Due to inclusion of NaF in CPP-ACPF, it showed better remineralizing potential than CPP-ACP alone [9]. Preethee T, Kandaswamy D observed that CPP-ACPF had superior remineralizing potential than that of CPP-ACP and Novamin [10]. Chole *et al.* reviewed the contemporary remineralizing agents available for remineralization and their implementation in clinical practice. CPP-ACPF, Bioactive glass, NovaMin and Clinpro were reviewed and authors inferred that amongst this CPP-ACPF is best remineralizing material because it incorporated fluoride and stabilizes ACP, which is very unstable when used alone[11].

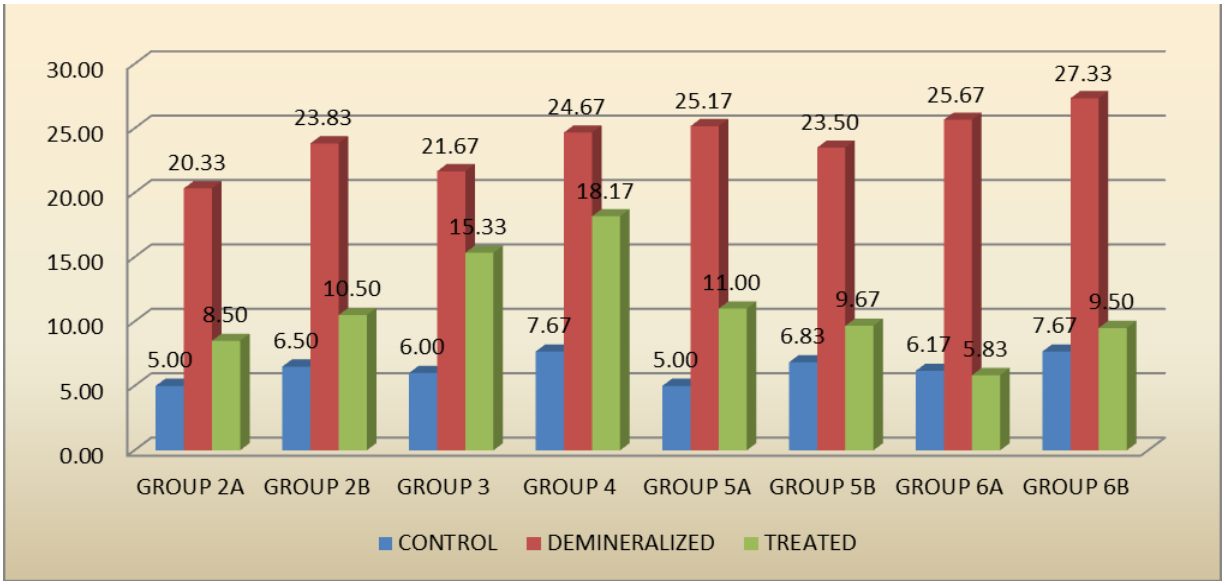
Fluoride ions require a high concentration of 5000 ppm to penetrate to subsurface layer hence its role in remineralizing sub surface lesions is doubtful[12]. CPP-ACP products if ingested in significant quantities will cause side effects. The effectiveness of CPP-ACP in remineralizing subsurface lesion is questionable. Another limitation is that repeated application of remineralizing paste is needed to maintain and replenish a constant supply of the lost remineralizing ions [13]. Hence a surface treatment protocol is needed which gives the optimum results by bringing about a change in enamel crystal.

Since 1960, when the first laser was developed by Maiman, lasers have been experimented with the aim to improve acid resistance [14]. Niazy MAin an in vitro study observed that there was a synergistic effect of CO2 laser and CPP-ACP on enamel and root caries[15].Powell GL reviewed different lasers used in prevention of dental caries. The lasers he analysed were Er: YAG, Er: YSGG, Nd: YAG, Diode, $CO_2$, Argon. He inferred that lasers per se have some role in inhibiting caries but their efficacy increases when they are supplemented with remineralizing solutions. The argon laser seems to be the most widely used laser in caries inhibition[16].

Lasers like $CO_2$, Nd: YAG, Erbium: YAG have all said to have a caries inhibitory role. These lasers had disadvantage of high cost, being bulky and result obtained was debatable. Further, most of the studies evaluating the high-power lasers are invitro studies[14,17,18]. Aluminium gallium arsenide laser has been hypothesized to increase acid resistance and the advantage is low cost and portability[17].Thus, in this study different surface treatment were compared.Lasersof 2 wattage followed by application of CPP-ACPF has shown to give best results. The results were statistically significant. The next best results were observed with Lasers of 3 wattage followed by application of CPP -ACPF. Thereafter it was the laser irradiation done alone groups which showed promising results. The Laser irradiation followed by Enafix application came next. Remineralizing paste when used alone was the least effective surface treatment modality(Graphs 1 & 2).

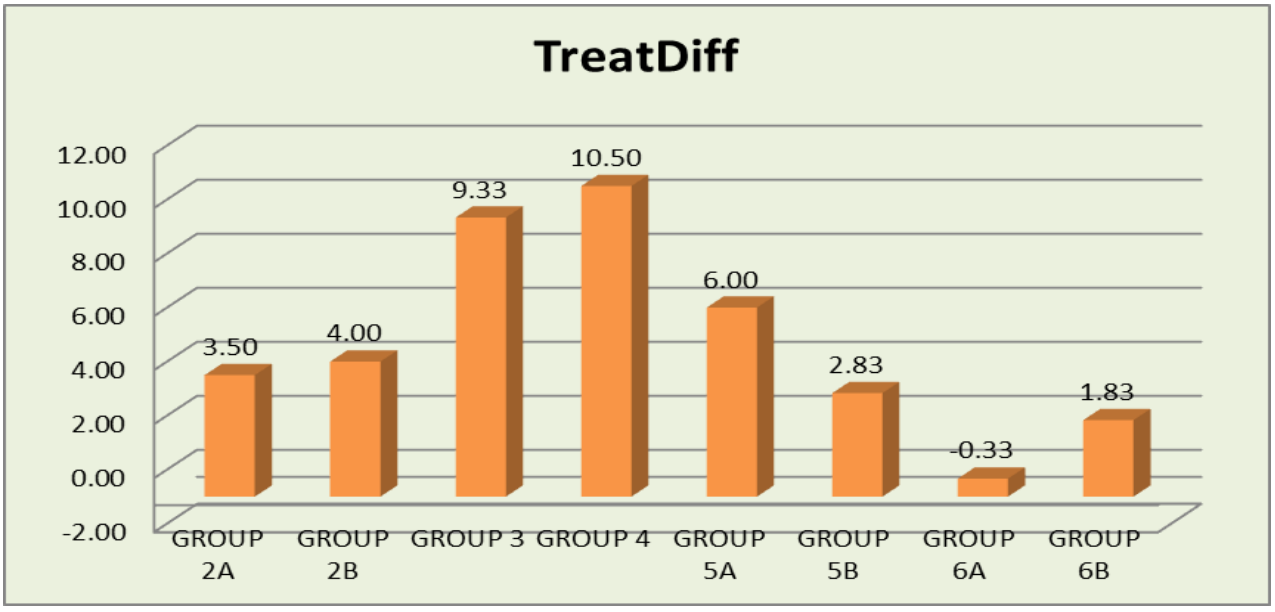
**Table 1.** Between treated groups there is a significant difference at 99 % confidence level hence post hoc test undertaken.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| | | Sum of Squares | df | Mean Square | F | Significance |
| CONTROL | Between Groups | 21.563 | 4 | 5.391 | 1.878 | 0.132 |
| | Within Groups | 123.417 | 43 | 2.87 | | |
| | Total | 144.979 | 47 | | | |
| DEMINERALIZED | Between Groups | 155.729 | 4 | 38.932 | 1.467 | 0.229 |
| | Within Groups | 1141.25 | 43 | 26.541 | | |
| | Total | 1296.979 | 47 | | | |
| TREATED | Between Groups | 586.312 | 4 | 146.578 | 16.565 | <0.001 |
| | Within Groups | 380.5 | 43 | 8.849 | | |
| | Total | 966.812 | 47 | | | |




GROUP 6A i.e 2 watts laser followed by application of CPP-ACPF has shown to give best results. All groups in which laser treatment has been done gives a better result as compared to the paste application when used.

**Graph 1A.**Comparison of all groups.




GROUP 6A that is 2 watts laser followed by application of CPP-ACPF has shown to given best results. All groups in which laser treatment has been done gives a better result as compared to the paste application when used alone.

**Graph 2.**Difference in surface treatment.

**Table 2. Post Hoc Tests.**

| Tukey HSD Multiple Comparisons | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dependent Variable | (I) Group | (J) Group | Mean Difference (I-J) | Standard Error | Significance | 95% Confidence Interval | |
| | | | | | | Lower Bound | Upper Bound |
| TreatDiff | GROUP 2A | GROUP 2B | -0.5 | 1.59513 | 1 | -5.5988 | 4.5988 |
| | | GROUP 3 | -5.83333* | 1.59513 | 0.015 | -10.9322 | -0.7345 |
| | | GROUP 4 | -7.00000* | 1.59513 | 0.002 | -12.0988 | -1.9012 |
| | | GROUP 5A | -2.5 | 1.59513 | 0.766 | -7.5988 | 2.5988 |
| | | GROUP 5B | 0.66667 | 1.59513 | 1 | -4.4322 | 5.7655 |
| | | GROUP 6A | 3.83333 | 1.59513 | 0.268 | -1.2655 | 8.9322 |
| | | GROUP 6B | 1.66667 | 1.59513 | 0.964 | -3.4322 | 6.7655 |
| | GROUP 2B | GROUP 2A | 0.5 | 1.59513 | 1 | -4.5988 | 5.5988 |
| | | GROUP 3 | -5.33333* | 1.59513 | 0.035 | -10.4322 | -0.2345 |
| | | GROUP 4 | -6.50000* | 1.59513 | 0.005 | -11.5988 | -1.4012 |
| | | GROUP 5A | -2 | 1.59513 | 0.91 | -7.0988 | 3.0988 |
| | | GROUP 5B | 1.16667 | 1.59513 | 0.995 | -3.9322 | 6.2655 |
| | | GROUP 6A | 4.33333 | 1.59513 | 0.147 | -0.7655 | 9.4322 |
| | | GROUP 6B | 2.16667 | 1.59513 | 0.87 | -2.9322 | 7.2655 |
| | GROUP 3 | GROUP 2A | 5.83333* | 1.59513 | 0.015 | 0.7345 | 10.9322 |
| | | GROUP 2B | 5.33333* | 1.59513 | 0.035 | 0.2345 | 10.4322 |
| | | GROUP 4 | -1.16667 | 1.59513 | 0.995 | -6.2655 | 3.9322 |
| | | GROUP 5A | 3.33333 | 1.59513 | 0.439 | -1.7655 | 8.4322 |
| | | GROUP 5B | 6.50000* | 1.59513 | 0.005 | 1.4012 | 11.5988 |
| | | GROUP 6A | 9.66667* | 1.59513 | 0 | 4.5678 | 14.7655 |
| | | GROUP 6B | 7.50000* | 1.59513 | 0.001 | 2.4012 | 12.5988 |
| | GROUP 4 | GROUP 2A | 7.00000* | 1.59513 | 0.002 | 1.9012 | 12.0988 |
| | | GROUP 2B | 6.50000* | 1.59513 | 0.005 | 1.4012 | 11.5988 |
| | | GROUP 3 | 1.16667 | 1.59513 | 0.995 | -3.9322 | 6.2655 |
| | | GROUP 5A | 4.5 | 1.59513 | 0.118 | -0.5988 | 9.5988 |
| | | GROUP 5B | 7.66667* | 1.59513 | 0.001 | 2.5678 | 12.7655 |
| | | GROUP 6A | 10.83333* | 1.59513 | 0 | 5.7345 | 15.9322 |
| | | GROUP 6B | 8.66667* | 1.59513 | 0 | 3.5678 | 13.7655 |
| | GROUP 5A | GROUP 2A | 2.5 | 1.59513 | 0.766 | -2.5988 | 7.5988 |
| | | GROUP 2B | 2 | 1.59513 | 0.91 | -3.0988 | 7.0988 |
| | | GROUP 3 | -3.33333 | 1.59513 | 0.439 | -8.4322 | 1.7655 |
| | | GROUP 4 | -4.5 | 1.59513 | 0.118 | -9.5988 | 0.5988 |
| | | GROUP 5B | 3.16667 | 1.59513 | 0.504 | -1.9322 | 8.2655 |
| | | GROUP 6A | 6.33333* | 1.59513 | 0.006 | 1.2345 | 11.4322 |
| | | GROUP 6B | 4.16667 | 1.59513 | 0.182 | -0.9322 | 9.2655 |
| | GROUP 5B | GROUP 2A | -0.66667 | 1.59513 | 1 | -5.7655 | 4.4322 |
| | | GROUP 2B | -1.16667 | 1.59513 | 0.995 | -6.2655 | 3.9322 |
| | | GROUP 3 | -6.50000* | 1.59513 | 0.005 | -11.5988 | -1.4012 |
| | | GROUP 4 | -7.66667* | 1.59513 | 0.001 | -12.7655 | -2.5678 |
| | | GROUP 5A | -3.16667 | 1.59513 | 0.504 | -8.2655 | 1.9322 |
| | | GROUP 6A | 3.16667 | 1.59513 | 0.504 | -1.9322 | 8.2655 |
| | | GROUP 6B | 1 | 1.59513 | 0.998 | -4.0988 | 6.0988 |
| | GROUP 6A | GROUP 2A | -3.83333 | 1.59513 | 0.268 | -8.9322 | 1.2655 |
| | | GROUP 2B | -4.33333 | 1.59513 | 0.147 | -9.4322 | 0.7655 |
| | | GROUP 3 | -9.66667* | 1.59513 | 0 | -14.7655 | -4.5678 |
| | | GROUP 4 | -10.83333* | 1.59513 | 0 | -15.9322 | -5.7345 |
| | | GROUP 5A | -6.33333* | 1.59513 | 0.006 | -11.4322 | -1.2345 |
| | | GROUP 5B | -3.16667 | 1.59513 | 0.504 | -8.2655 | 1.9322 |
| | | GROUP 6B | -2.16667 | 1.59513 | 0.87 | -7.2655 | 2.9322 |
| | GROUP 6B | GROUP 2A | -1.66667 | 1.59513 | 0.964 | -6.7655 | 3.4322 |
| | | GROUP 2B | -2.16667 | 1.59513 | 0.87 | -7.2655 | 2.9322 |
| | | GROUP 3 | -7.50000* | 1.59513 | 0.001 | -12.5988 | -2.4012 |
| | | GROUP 4 | -8.66667* | 1.59513 | 0 | -13.7655 | -3.5678 |
| | | GROUP 5A | -4.16667 | 1.59513 | 0.182 | -9.2655 | 0.9322 |
| | | GROUP 5B | -1 | 1.59513 | 0.998 | -6.0988 | 4.0988 |
| | | GROUP 6A | 2.16667 | 1.59513 | 0.87 | -2.9322 | 7.2655 |

*. The mean difference is significant at the 0.05 level.

There is significant difference with 99% confidence level for GROUP 6A and 6B. The inference drawn is that laser irradiation of 2 watts and 3 watts followed by CPP -ACPF application give the best result. GROUP 5B, 3 & 4 show significant difference with 95 % confidence level.

Moriyama et al. in an in vitro study evaluated the effectiveness of fluorescence based methods to detect insitu demineralization and remineralization on smooth surfaces. The devices compared were DIAGNOdent, DIAGNOdent Pen, Vista Proof Camera. It was concluded that DIAGNOdent and DIAGNOdent pen were more effective diagnostic tool to detect insitu demineralization and remineralization on smooth surfaces[19]. Guerrieridiscusses the various caries detection method which are available for detection of caries and stressed that diagnostic devices like laser fluorescence can monitor the regression or progression of early carious lesions[20]. Patil N in an invitro studyused laser fluorescence device to evaluate the remineralizing potentiality of CPP-ACP, CPP-ACPF and tricalcium phosphate fluoride. Remineralization efficacy was greatest in TCP-F followed by CPP-ACPF and least in CPP-ACP[21].Thus laser fluorescence device was used for evaluating and monitoring demineralization and remineralization in samples. The laser fluorescence values of control were noted as baseline. The demineralized samples show an increase in laser fluorescence values. After surface treatment the group which came closest to that of control values was the most effective surface treatment modality.

## Conclusion

1. The laser irradiation of 2 watts followed by CPP-ACPF application was observed as the most effective surface treatment modality.

2. Laser irradiation per se brought the laser fluorescence values closer to the preoperative control values.

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