Effect of Fluoride-releasing Elastomers on Mutans Streptococci in Dental Plaque: An In Vivo Study

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ABSTRACT

Aims: The purpose of this study was to evaluate the effect of fluoride-releasing elastomeric modules in the control of Streptococcus mutans levels in the oral cavity.

Materials and methods: The study consisted of 30 patients, with two experimental periods of three weeks and a three-week washout period between experimental periods. At the first visit, fluoridated modules were placed around brackets on 12 11 33 and non-fluoridated ones on 21 22 43. During the 2nd visit, the modules were removed and sent for a microbiological analysis. Non-fluoridated modules were placed on all brackets for one visit to allow for a washout period. At the 3rd visit, fluoridated elastomeric modules were placed around brackets on 21 22 43 and non-fluoridated on 12 11 33. At the 4th visit, the procedures at the 2nd visit were repeated.

Results: A mean comparison between bacterial counts of fluoride-releasing and non-fluoridated elastomeric modules in both the trials were done by the Mann–Whitney U test, which showed the result to be significant (p < 0.001). A comparison between bacterial counts between fluoride-releasing and non-fluoridated elastomeric modules in a specific area was done by the Wilcoxon signed rank test, which showed the result to be significant (p < 0.001). A comparison between bacterial counts of fluoride-releasing elastomeric modules in both trials were done by the Mann–Whitney U test, which also showed a significant result (p < 0.001).

Conclusion: The sustained-release fluoridated elastomeric modules are effective in reducing the levels of Streptococcus mutans in dental plaque around the brackets for a time period of 21 days.

Clinical significance: The sustained-release fluoridated modules were effective in reducing the CFU of S. mutans and are also stable at the end of 21 days of the experimental period. But the action of fluoride released from the modules is localized, temporary, and requires constant maintenance of these modules over the brackets throughout the treatment period.

Keywords: Demineralization, Elastomeric modules, Fluoride, Streptococcus mutans, Sustained release.

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INTRODUCTION

Orthodontic treatment with fixed appliances predispose to accumulation of plaque, resulting in carious lesions around brackets because of the intense demineralization around the brackets.¹,² There will be also a significant increase in the number of Streptococcus mutans (S. mutans), which is associated with the development of white spots and a later development of carious lesions.¹

During orthodontic treatment, preventive measures such as giving instructions for maintenance of oral hygiene, mechanical removal of plaque, encouraging the daily usage of fluoride mouth wash rely on participation of the patient.³,⁴ Hence, methods that do not require patient cooperation such as application of sealing material, fluoride collagen materials, fluoride cements, and fluoride varnishes around the bracket can be employed to prevent demineralization. But these methods are technique-sensitive and time-consuming.¹,⁴,⁵

Elastomeric ligatures are being used regularly as a part of routine orthodontic therapy for ligating the arch wire to the brackets. Hence, the use of fluoride-releasing elastomeric modules aids in the local delivery of fluoride and is more beneficial.⁵ But, one of the main disadvantages is that the fluoride is released only for a shorter duration; however, it can be resolved by using the sustained fluoride-releasing system, which has an advantage of releasing constant levels of fluoride for a longer duration.¹

Some studies⁶,⁷ reported contradictory results stating that no anticariogenic effect has been found for fluoride-releasing elastomeric ligatures because of inconsistencies in the release of fluoride. In contrast, controlled delivery systems permit the release of fluoride consistently over a time frame. For this reason, a biocompatible and non-inflammatory polymer, polyethylene co-vinyl acetic acid deriviation (PEVA), is utilized as delivery vehicles.⁸ A new method is used to fuse fluoride into PEVA to give a controlled release of fluoride ions, which would be helpful to prevent the advancement of white spot lesions in orthodontic patients.⁹

Hence the present study was conducted to evaluate the efficacy of fluoride-releasing elastomeric modules in the control of S. mutans levels in the oral cavity and to quantify the reduction in the amount of S. mutans levels in the dental plaque.
Materials and Methods

The study was carried out on 30 patients (14 males and 16 females) who were undergoing orthodontic treatment. Ethical clearance was obtained from the institutional ethical committee (Reference No: 19/CIR/13) and the informed consent was obtained from all the participants before the onset of the study. All information is kept confidential and can only be accessed by researchers.

The sampling frame was based on the following inclusion and exclusion criteria. Both male and female patients of any age group undergoing an orthodontic treatment with good general health were included in the study. In contrast, patients with any systemic medical condition that could interfere with planned treatment (e.g., uncontrolled diabetes and current pregnancy at the time of treatment), under antibiotics or using any antimicrobial mouthwash, or under treatment with self-ligating brackets were excluded from the study.

Materials Used (Fig. 1)

For this study, sustained fluoride-releasing elastomeric modules (colored and custom-made) were used as experimental group and conventional (non-fluoridated) elastomeric modules (Leone Orthodontics, Sesto Fiorentino, Firenze, Italy) as control group. A ligature gun is used for engaging these modules to the brackets; after the experimental period, these modules were collected in microcentrifuge tubes. The selective medium for S. mutans is prepared by adding 0.2 units/mL bacitracin to mitissalivarius agar base and poured into a petri dish.

Preparation of Custom-made Sustained Fluoride-releasing Elastomeric Modules

Polyethylene co-vinyl acetate (PEVA) at an amount of 4.2 grams was taken and dissolved in about 20 mL of methylene chloride along with 0.40 grams of Naf powder for effective and safer release of fluoride concentration within therapeutic levels—i.e., about 1.43 mg F2/ring/day as given by Baturina et al.9 This mixture was shaken vigorously for about 2–3 minutes on a vortex machine and later followed by a 10 minutes treatment in the sonicator for a homogenous distribution. This mixture was poured in to the moulds to create the ring shape of the elastomeric modules. It was then benchdried at room temperature for a night.

Another solution of PEVA/methylene chloride was prepared as above and was poured into another mould containing previously prepared fluoride module so that this freshly prepared solution forms an overcoat on the previously prepared fluoride modules.

Methodology

The study design was a randomized clinical trial, employing a split mouth, crossover design. It consisted of two experimental periods of three weeks with a washout period of 3 weeks in between. During the first visit, fluoride-releasing elastomeric modules were placed around brackets on 12 11 33, and non-fluoridated elastomeric modules were placed around brackets on 21 22 43 (Fig. 2). Non-fluoridated toothpaste was also advised.

During second visit, sterile microcentrifuge tubes containing 2 mL of reduced transport fluid (RTF) were prepared. The fluoride-releasing and the non-fluoridated elastomeric modules were removed and placed aseptically in two separate sterile microcentrifuge tubes containing 2 mL of reduced transport fluid (RTF) and were labelled. A total of 90 fluoride-releasing modules were collected in this first trial from 30 patients. These samples were then sent for microbiologic analysis. After finishing these procedures, Non-fluoridated elastomeric modules were placed around all brackets for one visit (i.e., for 3 weeks) to allow for a washout period (Fig. 3).

During the third visit, i.e., the next experimental visit, the conventional elastomers that were placed around brackets on 12 11 21 22 33 43 during the second visit were removed and discarded by the same operator. Later elastomeric modules were applied to the teeth that are exactly contralateral to those that received elastomeric modules during the first visit—i.e., fluoride-releasing elastomeric modules were placed around the brackets on 21 22 43 and nonfluoridated elastomeric modules around brackets on 12 11 33 (Fig. 4). On the fourth visit, procedures during visit 2 were repeated. A total no. of 90 fluoride-releasing modules was collected in this second trial from 30 patients. These samples were then sent for microbiologic analysis.

Microbiologic Process

The samples were vortexed for 30 seconds using a Vortex mixer (Cyclo Mixer, RemiEquipments PVT LTD., India) and serially diluted up to $10^{-2}$ dilution in distilled water. From the diluted sample,
The efficacy of fluoride-releasing modules was evaluated using a 100 μL sample plated onto the mitis salivarius sucrose bacitracin (MSSB) agar (Fig. 5). All petri dishes were then incubated in a 5%-CO2-enriched anaerobic incubator at 37°C for 48 hours (B.O.D Incubator, Inco instruments and Chemicals Pvt. LTD, India). After 48 hours, the petri dishes were removed from the incubator and total numbers of colony forming units (CFU) were counted to estimate the growth of S. mutans (Fig. 6).

All the statistical analysis was done in Statistical Package for Social Sciences software (version 15, SPSS Inc., Chicago, USA). Means and standard deviations for bacterial count in fluoride-releasing and non-fluoridated elastomeric modules for trials 1 and 2 were done and the associated p values were listed in Tables 1 to 4.

The mean comparison between the effects of fluoride-releasing and non-fluoridated modules on the S. mutans count in both the trials and the mean comparison between bacterial counts of fluoride-releasing elastomeric modules in different trials were analysed by the Mann–Whitney U test, and a comparison of bacterial counts between fluoride and non-fluoride modules in a specific area (i.e., fluoride modules on 12 11 33 in first trial and non-fluoride modules on 12 11 33 in second trial, and also non-fluoride modules on 21 22 43 in first trial and fluoride modules on 21 22 43 in second trial) to evaluate the efficacy of the fluoride modules were analysed by the Mann–Whitney U test.

### Results

The results were depicted in Tables 1 to 4 and Figures 7 to 10. The results and statistics showed that the fluoride-releasing elastomeric modules are more efficient in reducing the S. mutans count when compared with the conventional modules for a time period of 21 days. Even though the individual sample results showed not much reduction when compared to the control results, the obtained result was statistically significant (p < 0.001).

### Discussion

During the orthodontic treatment by the fixed appliance therapy, there would be an increase in the plaque accumulation around the brackets, resulting in the development of white spots and later development of carious lesions.2 Thus, prevention of white spot formation is important for the orthodontist to consider. Although previously published reports10,11 have indicated that the molars are...
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more susceptible to white-spot formation because of difficulties in maintaining plaque control, the 6 maxillary anterior teeth were considered independently for two reasons: the frequency of white-spot formation has been accounted to be high in this area and the orthodontic patients are, to a great degree, careful about the appearance of the front teeth.

During the 1st trial, the comparison of *S. mutans* levels between fluoride-releasing and non-fluoridated elastomeric modules showed a significant reduction in the number of CFU of *S. mutans* with the fluoride-releasing modules and was statistically significant (*p* < 0.001). In the same way, when the levels of *S. mutans* between the fluoride and non-fluoride modules after the second trial were

**Table 1:** A mean comparison between bacterial counts (CFU/mL) in fluoride-releasing and non-fluoride elastomeric modules in trial 1 and trial 2

<table>
<thead>
<tr>
<th>Trials</th>
<th>Clinical parameters</th>
<th>Mean</th>
<th>SD</th>
<th>Mean ± SD</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st trial</td>
<td>Fluoride</td>
<td>408.73</td>
<td>86.68</td>
<td>171.24 ± 33.45</td>
<td>5.131</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Non-fluoride</td>
<td>579.97</td>
<td>120.13</td>
<td></td>
<td>Significant</td>
<td></td>
</tr>
<tr>
<td>2nd trial</td>
<td>Fluoride</td>
<td>392.73</td>
<td>82.07</td>
<td>174.10 ± 53.60</td>
<td>5.317</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Non-fluoride</td>
<td>566.83</td>
<td>135.67</td>
<td></td>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis: Mann–Whitney *U* test. Statistically significant if *p* < 0.05

**Table 2:** A mean comparison between bacterial counts (CFU/mL) of specific area in fluoride-releasing and non-fluoride elastomeric modules in different trials

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trials</th>
<th>Mean</th>
<th>SD</th>
<th>Mean ± SD</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride</td>
<td>1st trial</td>
<td>408.73</td>
<td>86.68</td>
<td>158.10 ± 48.99</td>
<td>4.618</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-fluoride</td>
<td>2nd trial</td>
<td>566.83</td>
<td>135.67</td>
<td></td>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis: Wilcoxon signed rank test. Statistically significant if *p* < 0.05

**Table 3:** A mean comparison between bacterial counts (CFU/mL) of specific area in fluoride-releasing and non-fluoride elastomeric modules in different trials

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trials</th>
<th>Mean</th>
<th>SD</th>
<th>Mean ± SD</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride</td>
<td>2nd trial</td>
<td>392.73</td>
<td>82.07</td>
<td>187.24 ± 38.06</td>
<td>4.762</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-fluoride</td>
<td>1st trial</td>
<td>579.97</td>
<td>120.13</td>
<td></td>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis: Wilcoxon signed rank test. Statistically significant if *p* < 0.05

**Table 4:** A mean comparison between bacterial counts (CFU/mL) in fluoride-releasing elastomeric modules in different trials

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trials</th>
<th>Mean</th>
<th>SD</th>
<th>Mean ± SD</th>
<th>Z value</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Fluoride</td>
<td>1st trial</td>
<td>408.73</td>
<td>86.68</td>
<td>16 ± 4.61</td>
<td>0.5777</td>
<td>0.564</td>
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<tr>
<td>Fluoride</td>
<td>2nd trial</td>
<td>392.73</td>
<td>82.07</td>
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</tbody>
</table>

Statistical analysis: Mann–Whitney *U* test. Statistically significant if *p* < 0.05

**Fig. 7:** A mean comparison between fluoride and non-fluoride bacterial counts in the 1st trial and 2nd trial

**Fig. 8:** A mean comparison between fluoride and non-fluoride bacterial counts in different trials
analysed, there was also a significant reduction in the CFU of *S. mutans* in the dental plaque around fluoride-releasing modules.

These findings are in agreement with the study done by Wilson and Gregory, where there was a significant reduction in the *S. mutans* levels after a time period of 1 week. But this effect was not significant by the 2nd week through week 13 in the same study. Previous research states that concentrations of less than 0.05 ppm are reported to be beneficial in reduction of caries.

The past studies propose that the clinical utilization of fluoride-releasing elastomeric modules might be compelling in anticariogenic prophylaxis from the principal day of bonding and past the main week, even up to 6 months. It has been prompted that for ideal clinical advantage, elastomeric ligature ties ought to be replaced month to month.

One report, however, indicates that the elastomeric modules would need to be replaced even sooner. Replenishment of fluoride-releasing elastomeric ties would reintroduce a monthly high dose of fluoride release that would once again benefit calcium fluoride formation in the enamel surrounding the brackets. Moreover, it has been reported in the studies that the frequency of fluoride application (not merely the level of concentration), is important in anticariogenicity, highlighting the importance of slow-release properties of fluoride from materials.

Some studies showed that the fluoride-releasing elastomeric modules released the fluoride with an initial burst effect, and later the release was negligible. It was also suggested that replenishing the fluoride supply in the oral cavity at regular intervals helps maintaining the fluoride concentrations in the oral cavity and helps in the prevention of white spot lesions. In order to achieve this, apart from these modules, additional fluoride supplements have to be prescribed, which may again depend on compliance. Replacing the fluoride modules at regular appointments may help achieve this goal.

As per the studies, polyethylene co-vinyl acetic acid derivation (PEVA) could discharge the safe therapeutic fluoride concentration. PEVA incorporated with NaF powder coated with a thin layer of pure PEVA polymer were able to release fluoride into the surrounding medium in a favorable profile. These sustained-release fluoride modules were placed for about 21 days in each patient in each trial, respectively.

A mean comparison between fluoride and non-fluoride bacterial counts in different trials was also done in this study to see the efficacy of these sustained-release fluoride modules. When the bacterial counts of fluoride-releasing elastomeric modules in the first trial were compared with bacterial counts in non-fluoridated modules of the second trial, there was a mean difference of 158.10 CFU/mL with a standard deviation of 48.99 CFU/mL.

This result suggests that even though the CFU of *S. mutans* was reduced in number by the effect of fluoride released from the fluoridated modules, the number get increased when non-fluoride modules were placed in the place of fluoridated modules during the 2nd trial. This proves that the effect of the fluoride from the fluoride-releasing elastomeric modules is localized and is for a short period of time until these fluoride modules are in place or even though if some of the fluoride may be present in those areas after the removal of fluoridated modules in trial 1, during the washout period of 21 days, the remnant fluoride may have been washed out by the salivary flow and new microorganisms may have been accumulated owing to the presence of conventional elastomeric modules on those teeth throughout the washout period.

When the teeth with non-fluoridated modules of the first trial were replaced with fluoridated modules in the 2nd trial, the mean difference observed was 187.24 CFU/mL with a standard deviation of 38.06 CFU/mL. It shows that as soon as the fluoridated modules have been placed, there was a reduction in the number of CFU of *S. mutans*. The above two comparisons prove that the action of fluoride released from the fluoride-releasing elastomeric modules is localized, temporary, and requires constant maintenance of these modules over the brackets throughout the treatment period.

Another comparison was also done between the fluoride-releasing elastomeric modules of both the trials to see whether any external factors such as the dominant-side tooth brushing had an influence on the number of CFU of *S. mutans*. The fluoride-releasing elastomeric modules of the 2nd trial showed less number of CFU of *S. mutans* when compared to the fluoride-releasing elastomeric modules of the 1st trial, with a mean difference of 16 CFU/mL and standard deviation of 4.61 CFU/mL, which is statistically not very significant (p = 0.564).

Hence, these sustained-release fluoridated modules were effective in reducing the CFU of *S. mutans* and were also stable at
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...the end of 21 days of the experimental period without any swollen, unesthetic appearance intraorally.

As orthodontic patients are seen every 21–45 days for their routine appointments, it would be better to have a continuous fluoride release between the appointments. In the present study, each trial was done only for 21 days. Hence, further studies are required to prove the in vivo efficacy of this sustained fluoride-release system for a time period of more than 21 days.

The daily recommended supplemental fluoride intake to prevent the demineralization of enamel is 0.024–0.05 ppm, which corresponds to a fluoride release rate of 1.2–2.8 μg F/ring/day, assuming 28 elastomeric rings per patient. But in the present study, each trial was done with only three sustained fluoride-release modules in a time period of 21 days. Hence, further in vivo studies are required to measure whether there is a constant fluoride release rate at therapeutic levels.

**Conclusion**

The sustained-release fluoride modules are effective in reducing the *S. mutans* levels in dental plaque around the brackets for a time period of 21 days. There was a reduction of about 30% of CFU of *S. mutans* levels.

**References**