Effect of different finishing-polishing systems and mouthwashes on biofilm adhesion to composite surfaces: An in-vitro study

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ABSTRACT

Background: The occurrence of bacterial adhesion on the restored teeth with composite resin is becoming the major concern for many dentists worldwide. This study was carried out in order to compare and evaluate S. mutans biofilm adherence on surface of composite resin subjected to Sof-Lex polishing and contouring system and Shofu polishing system and mouthwashes like 0.2% CHX and 7.5% povidone iodine solution (PVI). Materials and methods: Samples (n = 30) of nanohybrid composite resin were randomly divided into three groups for polishing with aluminium oxide disks, Shofu polishing systems and mylar strip group followed by biofilm adhesion of S. mutans on those polished discs. These discs were then randomly treated with the mouthwashes to check for their efficacy against S. Mutans. Results: Smoother surface was generated with Sof-Lex aluminium oxide disks when compared to Shofu polishing systems. There was a statistically significant difference before and after immersion in CHX and PVI (p<0.01). The comparison of all the groups showed the largest decrease in PVI, followed by CHX.

Introduction

The use of composite resins and resin-based materials for anterior and posterior restorations has increased dramatically in the past decade due to the clinical demand for more esthetically acceptable and long-lasting materials.1 Bacterial adhesion to the surface of composite resins and other dental restorative materials is an important parameter in the etiology of secondary caries formation. A polished restorative surface ensures adequate esthetics and significantly reduces the risk of initial bacterial adherence and subsequent colonization.2 Care is required during polishing since inappropriate usage can result in greater surface roughness than that existed prior to polishing.3,4

Streptococcus mutans adhere to the primary colonizers by cell-to-cell interactions. Further bacterial growth on tooth surface leads to the formation of biofilm on the teeth, also called dental plaque.5,6 Among the chemotherapeutic agents used in mouthwashes, chlorhexidine (CHX) is the “gold standard” for comparison with other substances due to its proven efficiency.7 Although effective, 0.2% CHX has certain side effects such as brown discoloration of the teeth, oral mucosal erosion, and bitter taste. Hence, there is need of an alternative mouthrinse that could negate all the side effects of CHX but yet effective equivalent to it.8,9

Povidone iodine (PVI), on the other hand, causes relatively low irritation to the oral mucosa and has a
strong sterilizing effect. It is a mixture of polyvinyl pyridine and iodine, reducing iodine-related irritation, pigmentation, and allergic reactions and exhibiting the antibacterial action of iodine simultaneously. As a topical disinfectant, it is used prophylactically in the oral mucosa and is widely used as a sterilizer and mouthwash due to its oral antibiotic effect.

### Aim

1. To compare and evaluate *S. mutans* biofilm adherence on surface of composite resin subjected to Sof-Lex polishing and contouring system and Shofu polishing system
2. To compare and evaluate *S. mutans* biofilm adherence on surface of composite resin subjected to 0.2% CHX and 7.5% povidone iodine solution

### Materials and Method

Samples (*n* = 30) of nanohybrid composite resin (Filtek Z350 3 M ESPE, St Paul, Minnesota, USA) were prepared in a circular shaped disk – 6.0 mm diameter and 2.0 mm in height. The teflon molds were filled with nanohybrid composite in a single increment and were covered with Mylar matrix strip to obtain a flat surface. Samples were cured for 20 s with a curing unit (Woodpecker LED D Curing Light Curing Unit). Samples were retrieved from the mold using a surgical blade and immersed in dark vials containing distilled water at 37°C for 24 h.

**Polishing treatment**

Randomly selected samples of nanohybrid composite resin were subjected to one of three finishing and polishing techniques:

- **Group A**: Control group — use of Mylar matrix strip with no finishing or polishing procedures (*n* = 10).
- **Group B**: Aluminum oxide disks (Sof-Lex, 3 M ESPE, MN, USA) (*n* = 10).
- **Group C**: Shofu composite polishing system (Shofu Dental Corporation, Japan) (*n* = 10)

### Biofilm adhesion

*S. mutans* (MTCC number 890) were maintained as frozen stock cultures, and cultured anaerobically at 37°C in a CO₂ jar for 2 hours. For the adherence testing in laminar flow chamber. 1.5 ml of broth and 0.1 ml of standardized *S. mutans* suspension was added to each 24-well tissue culture plate. The plates were sealed and incubated at 37°C for 24 h in a CO₂ jar.

Samples were then removed and washed thrice with a sterile physiological solution to dislodge loosely bound material. After 24 h incubation at 37°C and mean values of colony forming units (CFU) were noted. The response variable was the mean CFU/mL present in the *S. mutans* biofilms formed on the composite resin surface.

Data were statistically analyzed by three-way analysis of variance (ANOVA).

### Evaluating Mouthwash Effects on Resin discs

During the 7-day incubation period in heart infusion broth, composite discs were subjected to immersion cycles in the selected mouth rinses

- **Group A**: Control group— distilled water group (*n* = 10)
- **Group B**: 0.2% CHX group (*n* = 10)
- **Group C**: 7.5% Povidone iodine group (*n* = 10)

for 60 seconds once daily. Samples were mildly agitated in the test solutions using a water bath shaker operating at 10 Hz, for 20 seconds, three times, at 1-minute intervals. The amount of *S. mutans* was quantified. The Colony-Forming Units (CFU) were measured and quantified. The difference was calculated to measure the change in CFU.
Table 1: Mean and SD values of the CFU/ml (log10) of *Streptococcus mutans* within the biofilms

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD values</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Mylar strip)</td>
<td>27.2±3.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group B (Sof-Lex discs)</td>
<td>110.6±7.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group C (Shofu system)</td>
<td>123±9.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 2: Mean and SD values of the CFU/ml (log10) of *Streptococcus mutans* within the biofilms

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD values</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Distilled water)</td>
<td>26.8±5.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group B (CHX)</td>
<td>25.7±8.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group C (Povidone Iodine)</td>
<td>29.9±5.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 3: Difference in the readings from table 1 and 2 to check the reduction in *S. mutans* count before and after immersing in the mouthrinse

<table>
<thead>
<tr>
<th>Groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Distilled water)</td>
<td>Less difference</td>
</tr>
<tr>
<td>Group B (CHX)</td>
<td>Major difference but less than group C</td>
</tr>
<tr>
<td>Group C (Povidone Iodine)</td>
<td>Major difference</td>
</tr>
</tbody>
</table>

Results

The Mylar strip group showed least bacterial adhesion than the Sof-Lex and Shofu polishing groups. This difference in the mean values between the groups was found to be statistically significant (*p* < 0.01).

Smother surface was generated with Sof-Lex aluminum oxide disks when compared to Shofu polishing systems, which was seen as higher bacterial adhesion with Shofu polishing system and a significant mean difference was revealed statistically. Mean values of CFU/mL were converted into logarithmic (log10) values and analyzed by three-way ANOVA test for significance (Table 1).

Discussion

There was a statistically significant difference before and after immersion in CHX and PVI (*p*<0.01) (Table 2). There was no great change in the CFU of *S. mutans* in the distilled water group, which was not statistically significant (*p* >0.01). The comparison of all the groups showed the largest decrease in PVI, followed by CHX (Table 3).
The sorption and solubility of composite resins may serve as precursors to a variety of chemical and physical processes that not only create biological concerns but also produce deleterious effects on the structure and function of resin matrix. Although the surface obtained by using the Mylar strip is perfectly smooth, it is rich in resin organic binder. Therefore, removal of the outermost resin by finishing and polishing procedures would tend to produce a harder, more wear resistant, and hence, a more aesthetically stable surface. A composite finishing system is effective if the abrading particles are relatively harder than the filler materials; otherwise, the polishing agent will only remove a soft resin matrix but leave the filler particles protruding from the surface. Hardness difference between silicon carbide and the silica filler particles leads to relatively more aggressive finishing and polishing with the Shofu composite polishing system than the Sof-Lex polishing system resulting in a significantly rougher surface.

The specimens with the undisturbed biofilms were inoculated for an additional 7 days to observe the ability of the two mouthwashes to inhibit the progression of carious lesions. In this study, a water bath shaker was used with a moderate stroke of 10 Hz for 20 seconds three times at 1-minute intervals to simulate the gargling condition created by an adult. One of the reasons for selecting a short exposure time (10 second vortex) of our bacterial suspensions and biofilms to the mouthwashes in this study was to provide evidence that short exposure to these antibacterial agents can be remarkably effective. Short exposure times also minimize chemical biohazard concerns associated with oral antiseptic use. Chlorhexidine, though it is the most widely used and recommended mouthwash, it has it’s own share of disadvantages which led to the search for more efficient and feasible replacement. Povidone iodine fits the criteria perfectly providing all the benefits like reduced growth of bacteria, disinfection and no disadvantages like metallic taste with prolonged usage, burning sensation in mouth and higher prices compared to the other commercially available mouthwashes.

**Conclusion**

The initial adherence and subsequent colonization of bacteria on the surface of composite resins are the key of the pathogenesis of the secondary caries promoted particularly by *S. mutans*. The quality and amount of adhered biofilm are important to the success of the esthetic restorations on a long-term basis. Therefore, this study observed evaluation of S. mutans adherence on polished surface of the most commonly used restorative material as well as displayed that povidone iodine mouthrinse can prove to be a better option compared to chlorhexidine gluconate to avoid secondary caries.

**References**

5. Evans A, Leishman SJ, Walsh LJ, Seow WK. Inhibitory effects of antiseptic mouthrinses on Streptococcus mutans, Streptococcus sanguinis and...


