Surface Antibacterial Properties of Four Tooth-Colored Restorative Materials

F. Shirani, A. Havaei, M. Malekipour, M. Sharafi

Abstract:
Objective: This study investigated the antibacterial properties of an ion-releasing resin composite (Degufill), a hybrid resin composite (InTen-S), a compomer (Compoglass F) and a resin-modified glass ionomer (Vitremer) against streptococcus mutans.

Materials and Methods: The bacteria were derived from the dental plaque and cultured on blood agar plates. Eppendorf tubes were filled by unset restorative materials. A narrow conical cavity was created in the center of each material, prior to curing and the bacterial suspension was placed into each cavity. Each tube was incubated for the selected time periods of 8, 24, 48 hours and 5 days and the procedure was repeated five times. After the incubation period, the suspensions were removed and the number of viable bacteria was evaluated. The data were analyzed using two-way ANOVA, one-way ANOVA and Tukey HSD tests.

Results: After the incubation periods of 8, 24 and 48 hours, all restorative materials except InTen-S showed significant growth inhibition when compared to the control group. There was a significant difference in the number of bacterial colonies in different incubation periods. The interaction between the materials and time intervals was also significant (P<0.05).

Conclusion: The method used in this study was almost successful in ranking restorative dental materials according to their antibacterial effects. InTen-S showed no inhibitory effect on bacterial growth, while other materials, especially Vitremer, showed considerable antibacterial effects.

Key Words: Dental Materials; Anti-Bacterial Agents; Dental Caries; Composite Resins; compoglass flow; glass ionomer

INTRODUCTION
Tooth-colored restorative materials such as composite resins, compomers and resin-modified glass ionomers are increasingly demanded by patients and applied by clinicians. In spite of routine use of posterior tooth-colored restorative materials, poor marginal adaptation of composite resins, mostly due to the polymerization shrinkage, is still considered a major reason for the failure of these restorations. Microleakage and recurrent caries are another major clinical problem associated with direct posterior composite restorations. Bacterial penetration into the prepared cavity can lead to secondary caries, which is the main reason for failures in dental restorations [1-4]. Remaining caries after cavity preparation is another source of bacteria [5] and there are no
standard methods to evaluate the microbial condition after cavity preparation [6,7].
As the etiology of secondary caries is primarily a streptococcus mutans-associated disease [8,9], it is important to evaluate the interaction of tooth-colored restorative materials with these bacteria. Several in-vitro studies have so far demonstrated the antimicrobial effects of glass ionomers [10,11]; while, fewer have been carried out to evaluate the antimicrobial properties of composite resins and compomers, shown to have less antibacterial effects compared to glass ionomers [12,13].
The agar diffusion test has been used as the standard assay in most of the mentioned studies despite its known limitations. Problems associated with this method include its quantitative nature, ability to evaluate only soluble materials, inability to distinguish between bacteriostatic and bactericidal effects, difficulties in comparing a large number of samples and a large number of variables [14]. Thus, Weiss et al [15] have introduced a direct contact test between the microorganism and the material regardless of the solubility and diffusability of their components.
In the present study, using the direct-contact test, bacterial interactions of four tooth-colored restorative materials were evaluated in different incubation periods.

**MATERIALS AND METHODS**

Four tooth-colored restorative materials were selected: a hybrid composite resin (InTen-S; Ivoclar Vivadent, Schaan, Liechtenstein), an ion-releasing composite resin (Degufill Mineral, Degussa, Frankfurt, Germany), a compomer (Compoglass F; Ivoclar Vivadent AG, Leichtenstein) and a resin-modified glass ionomer (Vitremer; 3M ESPE, Minn, USA). All materials were handled and polymerized with strict compliance to the manufacturer's instruction. The microorganism used in this study was streptococcus mutans. Bacterial plaque was collected from different parts of the patient’s mouth, immediately cultured on blood agar and kept in an incubator at 37 °C for 24 hours; afterwards, streptococcus mutans was isolated from other bacteria using differential tests. A Mac-Farland pipette of 0.5 turbidity, standard pipettes by which microbial suspension dilution is characterized, was prepared by adding sterile saline and brain-heart infusion broth to the new and fresh strain. The turbidity of 0.5 Mac-Farland pipette is equal to 1.5 × 10^8 bacteria per milliliter.
Five Eppendorf pipette dispensers (0.5 ml) were used for each material and incubation period. One gram of each restorative material was placed in each pipe. Before curing the materials, a conical cavity (100 ml volume) was prepared in the center of specimen using the Eppendorf pipette edge. The specimens were then cured for 100 seconds using Coltolux light-curing device (Coltolux 50, Coltene/Whaledent Inc, USA). They were autoclaved afterwards at 121 °C and 15 psi for 20 minutes and then 100 ml of the microbial suspension was placed in each prepared cavity in each pipette. Twenty pipettes containing microbial suspension were used for each sample. The bacterial cells were distributed equally in each cavity.

**Table 1.** Mean and standard deviation (SD) of colony count in different groups and times (CFUs/ml).

<table>
<thead>
<tr>
<th>Materials</th>
<th>Mean (SD)</th>
<th>n</th>
<th>Mean (SD)</th>
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<th>Mean (SD)</th>
<th>n</th>
<th>Mean (SD)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg</td>
<td>74.6000 (5.2726)</td>
<td>5</td>
<td>58.0000 (5.0498)</td>
<td>5</td>
<td>33.6000 (5.8566)</td>
<td>5</td>
<td>55.4000 (18.1336)</td>
<td>15</td>
</tr>
<tr>
<td>is</td>
<td>69.6000 (4.2190)</td>
<td>5</td>
<td>52.0000 (4.0000)</td>
<td>5</td>
<td>26.0000 (4.6368)</td>
<td>5</td>
<td>49.2000 (18.9594)</td>
<td>15</td>
</tr>
<tr>
<td>deg</td>
<td>30.4000 (4.5607)</td>
<td>5</td>
<td>24.4000 (3.7815)</td>
<td>5</td>
<td>10.4000 (2.7019)</td>
<td>5</td>
<td>21.7333 (9.3462)</td>
<td>15</td>
</tr>
<tr>
<td>vitr</td>
<td>15.2000 (4.6583)</td>
<td>5</td>
<td>2.6000 (0.5477)</td>
<td>5</td>
<td>3.6000 (1.1402)</td>
<td>5</td>
<td>7.1333 (6.4572)</td>
<td>15</td>
</tr>
<tr>
<td>compo</td>
<td>21.6000 (4.7223)</td>
<td>5</td>
<td>14.6000 (4.3932)</td>
<td>5</td>
<td>15.4000 (4.2190)</td>
<td>5</td>
<td>17.2000 (5.2400)</td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>42.2800 (25.7448)</td>
<td>25</td>
<td>30.3200 (22.1128)</td>
<td>25</td>
<td>17.8000 (11.5902)</td>
<td>25</td>
<td>30.1333 (22.7675)</td>
<td>75</td>
</tr>
</tbody>
</table>

**cg=** control group, **is=** InTen-S, **deg=** Degufill, **vitr=** Vitremer, **compo=** Compoglass F; **CFUs=** Colony Forming Units
cubation period, no bacterial growth was seen in any of the specimens, including the control group. The type of the restorative material and the duration of the incubation period, had a significant effect on the amount of the bacterial growth. In addition, the interaction between the material and the duration was significant (P<0.05).

Tukey HSD results (Fig 1) showed significant differences in bacterial growth in specimens containing different restorative materials at various time intervals (P<0.05).

One-way ANOVA and Tukey HSD analysis showed both significant and non-significant differences in the inhibitory effects of different restorative materials at different incubation periods. Multiple comparisons by the Tukey HSD test showed significant differences between Degufill, Compoglass F, Vitremer, and the control group (P<0.05).

All the restorative materials except InTen-S, significantly suppressed the bacterial growth at different time intervals; Vitremer and InTen-S showed the strongest and the weakest antibacterial effect respectively. The antibacterial effect of Degufill and Compoglass F was slightly less than Vitremer but significantly more than InTen-S and the control group.

**RESULTS**

The descriptive results were obtained and recorded (Table 1). In all different incubation periods a gradual decrease in the bacterial count in all the specimens, including the control group, was observed. After the 5-day incubation period, no bacterial growth was seen in any of the specimens, including the control group.

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**DISCUSSION**

The effect of direct contact between a clinical isolated of streptococcus mutans and four tooth-colored restorative materials was evaluated. In this study, an assay was established to determine viable bacterial counts after incubation of a small amount of bacterial suspension within the materials, designed to avoid dilution of components released from the materials [16]. This method is more reliable than other methods and its biggest advantage is the direct contact between the bacteria and the restorative material, which is a simulation of how it is in the mouth; whereas, a method like the liquid
culture method is limited to testing water-soluble components of the restorative material instead of the whole material [16,17]. Direct-contact test has so far been widely used for evaluating antibacterial activity of different dental materials [16,18-23]. The bacterial strain used in this study was streptococcus mutans. Streptococcus Mutans is known to be the primary etiologic factor for carious lesions, and therefore has a routine use in testing the antimicrobial activity of restorative materials [14]. Matalon et al [17] showed that none of the composite resins used in their study could affect the bacterial growth immediately after polymerization; accordingly, in our study the inhibitory effect of materials were evaluated after the eight hour incubation period. The fifth group was excluded from the present study as no colonies were observed in the experimental and the control groups. It seemed that they did not grow due to the toxic and lytic materials produced by the bacteria and lack of sufficient nutrients in the culture media; that condition affected the microbial colonies after 5 days in all the groups; the same thing that had been encountered in previous studies as well. Though, it seems that this method is more suitable for evaluating the short-term antimicrobial activity.

Statistical analysis revealed that there were significant differences between different materials in all the incubation periods (P<0.05) as follows: Vitremer group showed the strongest antibacterial effect. The relationship between the antibacterial effect of Vitremer and its fluoride release has been mentioned in a large number of studies [9,12,13,24,25]. Some studies are concerned about the lower pH of this material [11,26] and others have shown that HEMA (hydrophilic monomer), which exists in resin modified glass ionomers, has an inhibitory effect on the bacterial growth [10,13]. As the inhibitory action was associated with fluoride release of GIC (140±25 ppm is required to inhibit S. mutans), in this study as well, we suggest a correlation existing between the antimicrobial effect of Vitremer cement and the fluoride release. However, pH did not seem to have any antibacterial effect as the inhibitory effect was not associated with changes in the pH after setting of the material [12].

The present study depicts the lack of antibacterial properties of InTen-S hybrid composite which is in accordance with previous experiments [8,12]. Matalon has previously showed in his study that no antibacterial activity is attributed to polymerized composite resins, although the antibacterial activity of single components, which is part of the formulation, is seen [17]. Some investigations have concluded that ion-releasing composite resins can also have an antimicrobial effect and prevent secondary caries [26,27]. The hybrid composite resin used in this study had large amounts of fluoride in the form of ytterbium tri-flouride. According to some studies, fluoride release from composite resins enriched with ytterbium trifluoride is less than that from glass ionomer cements [13,27,8]. This might explain InTen-S composite resin lacking antimicrobial activities in the present study. No antibacterial activity for composite resins has been advocated before as packable composite materials in our study showed no antibacterial properties as well [17].

Another restorative material, also known as a smart or an ion-releasing composite resin, is Degufill. It is claimed to have pH dependant ion-releasing properties (PO_4^{3-}, Ca^{2+}, OH^-, F^-), and being able to prevent secondary caries [10]; a claim confirmed by Boeckh et al [16] in their study. In the present study, bacterial colonies in Degufill suspension group showed a decrease in number. Acidic pH in conical cavities, as a result of the growth of fermented streptococcus mutans, might cause ion-release in this composite resin [16]. Provided that an ion-releasing composite resin is assumed to
stop the bacterial growth, an increase in the antimicrobial effect is expected to be seen in it (Degufill group) after 24 hours (Fig 1).

In this study, the antibacterial effect of Compoglass F was assumed; whereas in previous studies slight or no antibacterial effect had been found for compomers [12,13]. This discrepancy can be the result of different test methods used in different studies. If fluoride is assumed to be an antimicrobial agent, Compoglass F is expected to have antibacterial effects as well. In several studies, Compoglass F has shown more fluoride release in comparison to other compomers [28,13]. In a study by Yap et al [13] the amount of fluoride released by Compoglass F was greater than resin-modified Glass Ionomer (Fuji II LC) over a 35-day period. According to several reports, the maximum amount of fluoride release occurs during the first 24 hours [29]. The decrease in the antibacterial effect of both Vitremer and Compoglass F can be attributed to the reduction in their fluoride release after 24 hours.

Statistical analysis showed that colony numbers had significant differences in different incubation periods. In the study of Bocckh et al [16] the number of colonies decreased in both the experimental and the control groups after longer incubation periods. However, it appears that undesirable environmental and nutritional conditions was likely to cause bacterial death during the 5-day period, making this method suitable for short-term evaluation of antibacterial activity only.

In general, this study and method used in it seemed almost successful in ranking the restorative dental materials involved in it with regards to their antibacterial activity.

CONCLUSION
In this study, InTen-S had no antibacterial effect. Whilst as expected, the strongest antimicrobial effect was assigned to Vitremer. Antimicrobial activities in Degufill and Compoglass F groups were found to be less than the Vitremer group, and significantly more than the control and the InTen-S groups.

The authors would also suggest the use of different ranges of oral bacteria, and long-term clinical trials in order to determine whether antimicrobial effects of dental materials, confirmed in the lab are sufficient to increase the longevity of dental restorations.

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REFERENCES


