original article

Microbial contamination and inhibitory effect against Streptococcus mutans from fifth-generation bonding systems

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ABSTRACT: Purpose: The aim of this study was to evaluate microbial contamination and inhibitory effect against Streptococcus mutans (SM) of Prime & Bond (PB), Single Bond (SB) and Excite (EX) bonding systems before use, and after 10 and 20 applications.

Methods: The bonding material was collected by applying a drop of the material directly on broth brain-heart infusion. The samples were homogenized, diluted and seeded on blood agar plates. To evaluate the inhibitory effect on SM, a drop of each bonding material was dispensed on filter discs and placed on blood agar plates. The Cochran statistical analysis was used to evaluate the total amount of viable bacteria among the different bonding systems. Comparisons between the inhibitory effects on SM were made using the Kruskal-Wallis test.

Results: Adhesives SB and EX presented microbial contamination (p<0.05) and inhibitory effect (p<0.05) over SM strains with statistically significant differences concerning PB. SB and EX inhibitory capacity remained after 20 applications.

Conclusions: The monomer's variation in chemical composition, solvent and application technique of the bonding systems had an influence on contamination by the total number of bacteria and on the inhibitory effect on SM. (Journal of Applied Biomaterials & Biomechanics 2010; 8: 52-5)

Key words: Antibacterial, Inhibition, Dentin bonding systems

INTRODUCTION

The philosophy of contemporary restorative dentistry is to preserve the health of the dental structure as much as possible, including the maintenance of the affected dentin (1, 2). However, the literature reports microbial viability in this substrate (3-5). Therefore, the use of materials that present antibacterial and bacteriostatic properties is extremely important in diminishing the residual microbiota.

The maintenance of the aseptic chain during restorative dentistry may reduce the chances of cavity contamination (6, 7). In aiming to make microbiota unavailable after cavity preparation, the literature has professed the incorporation of antimicrobials into bonding systems. Imazato et al (6-14) incorporated the 12-methacryloyloxydodecylpyridinium bromide (MDPB) to the bonding system and observed a significant inhibitory effect on microbiota. Saito et al (15) reported similar results to Imazato’s, but incorporating benzalkonium chloride to the bonding agents.

The presence of viable bacteria after cavity preparation is the main etiological agent of post-surgical sensibility, pulp inflammation and the development of recurring carious lesions. Another aspect that must be considered is the possibility of the contamination of the bonding systems during clinical use.

The fifth-generation bonding systems usually present with the need to apply two layers in the cavity preparation enabling the contamination of those bonding agents. The literature (16, 17) affirms that the presence of monomers such as UDMA, TEG-DMA, Bis-GMA and HEMA in the bonding systems could result in adhesion and microbial growth. Therefore, the objective of this study was to evaluate the microbial contamination and the inhibitory effect of Prime & Bond (PB), Single Bond (SB) and Excite (EX) bonding systems on Streptococcus mutans (S. mutans (SM)) before use, and after 10 and 20 applications.

MATERIALS AND METHODS

This study was approved by the Ethics Committee in Research from PUC-Campinas (Protocol 107/07). The evaluation of the bonding systems PB 2.1 (Dentsply De Trey GmbH D - 78467 Konstanz), SB 2 (3M ESPE, St Paul, MN 55144, USA) and EX (Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein) was performed through sample collection before use and after 10 and 20 applications.
**Microbial contamination evaluation (CFU/ml)**

Thirty-six dental surgeons were randomly chosen to apply the bonding systems in the dental clinic of PUC-Campinas. The labels of the material containing the manufacturers' instructions from each adhesive were provided as the application of the bonding agent occurred. Three collections were performed: one drop before use, one after 10 applications, and one after 20 applications of the bonding systems. The samples were applied directly on 3 ml of the transport medium (brain-heart infusion) and they were homogenized in a tube shaker (Vortex-Wizard, Porto Alegre, Brazil) for 3 min. Dilutions were performed in 4.5 ml of peptone water up to 10⁻³. Three 25 μl aliquots of these decimal solutions were seeded on the plate surfaces containing blood agar (Labcenter, Campinas, Brazil) with a micropipette. The total number of viable bacteria was counted using a colony counter (Phoenix - Campinas, São Paulo, Brazil) by a single calibrated examiner.

**Inhibitory effect on Streptococcus mutans strains - inhibition halos (mm)**

A drop of each bonding system was dispensed on sterile filter discs and placed on blood agar plates previously inoculated with standard strains of *S. mutans* ATCC 25175 standardized in 0.5 MacFarland scale. This scale allowed the bacterial concentration of a suspension to be estimated by its turbidity; 0.5 corresponded to a concentration of 1.5 x 10⁸ (bacterium); 0.5% sodium hypochlorite (Fórmula & Ação, São Paulo, Brazil) served as a positive control and saline solution (Fórmula & Ação, São Paulo, Brazil) served as a negative control. The inhibition halos were measured in millimeters by a single calibrated examiner.

**Cultivation conditions**

Subsequently, the cultures were incubated for 5 days at 37 °C in an 85% nitrogen (N₂), 10% carbon dioxide (CO₂) and 5% hydrogen (H₂) atmosphere, achieved using the generating envelope systems in an anerobiosis jar.

**Statistical analysis**

Cochran statistical analysis was used to evaluate the total number of viable bacteria among the different bonding systems. Comparisons between the inhibitory effects on SM were conducted using the Kruskal-Wallis test.

### RESULTS

**Microbial contamination evaluation (CFU/ml)**

The adhesives SB and EX presented microbial contamination with statistical significant differences concerning PB (p<0.05) (Tab. I and Fig. 1).

**Inhibitory effect on Streptococcus mutans strains - inhibition halos (mm)**

Before using the bonding agents, the bonding systems SB and EX presented a higher inhibitory effect on SM strains with statistically significant differences in relation to PB and SR (p<0.05). After 10 applications, the SB bonding system presented a higher inhibitory effect on SM strains with statistically significant differences in relation to PB and SR (p<0.05). After 20 applications, the SB and EX bonding systems presented a higher inhibitory effect on SM strains with statistically significant differences in relation to PB and SR (p<0.05) (Tab. II and Fig. 2).

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**Table I - Total number of viable bacteria in the bonding systems (log10) – Cochran’s Q**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>0.0000± 0.0000*</td>
</tr>
<tr>
<td>SB</td>
<td>2.2944±1.9880†</td>
</tr>
<tr>
<td>EX</td>
<td>0.7356±1.2741†</td>
</tr>
</tbody>
</table>

Legend: (*,†): p<0.05. PB: Prime & Bond; SB: Single Bond; EX: Excite

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**Fig. 1 - Contamination of the bonding systems. PB: Prime & Bond; SB: Single Bond; EX: Excite.**
TABLE II - INHIBITION HALOS (MM) OF SAMPLE GROUPS

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before Mean ± SD</th>
<th>After 10 applications Mean ± SD</th>
<th>After 20 applications Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>0.0000±0.0000*</td>
<td>0.0000±0.0000*</td>
<td>0.0000±0.0000*</td>
</tr>
<tr>
<td>SB</td>
<td>8.6667±1.5275†</td>
<td>8.0000±1.7321†</td>
<td>6.3333±0.5774†</td>
</tr>
<tr>
<td>EX</td>
<td>4.6667±1.5275†</td>
<td>1.0000±0.0000</td>
<td>3.3333±1.5275†</td>
</tr>
<tr>
<td>SR</td>
<td>0.0000±0.0000*</td>
<td>0.0000±0.0000*</td>
<td>0.0000±0.0000*</td>
</tr>
<tr>
<td>HP</td>
<td>1.0000±0.0000</td>
<td>2.0000±1.0000†</td>
<td>1.3333±0.5774</td>
</tr>
</tbody>
</table>

Legend: (*,†): p<0.05. PB: Prime & Bond; SB: Single Bond; EX: Excite; SR: saline solution; HP: sodium hypochlorite

DISCUSSION

Antibacterial activity of dentin bonding agents depends on several factors including composition and acidity. Adhesion-promoting monomers are considered to be one of the important elements relevant to the antibacterial effect of primers or adhesive solutions (8). The results of this study showed that SB and EX bonding systems presented contamination by the total number of viable bacteria after 10 applications. These results agree with Brambilla et al (16) and Schmalz et al (17) who observed that the monomers Bis-GMA, UDMA, TEGMA and HEMA presented the possibility of adhesion and microbial growth. In addition, TEGDMA can stimulate the proliferation of cariogenic microorganisms such as Streptococcus sobrinus and Lactobacillus acidophilus. Takahashi et al (18) agree with Brambilla et al (16) in respect to the adhesion of microorganisms by resin monomer polymerization forming vesicular structures. However, in contrast to Brambilla et al (16), Takahashi et al (18) did not observe growth in the number of viable bacteria.

The SB bonding system is composed of 10-20% of Bis-GMA (19) and must be applied twice to the dental surface, enabling the dentist to contaminate the bonding agent. This caused a significant microbial growth in the SB bonding system in our results. EX, which is to be applied only once to the dental surface, also presented contamination by the total number of viable bacteria, but in a smaller proportion. Therefore, our results complement Brambilla et al (11) that in addition to the composition of the bonding systems, the application technique influences the possibility of microbial contamination. The PB bonding system did not present microbial contamination after 10 applications, even containing 11% of UDMA. This may be related to the presence of acetone as a solvent, preventing contamination and bacterial growth, agreeing with Walter et al (20) who observed the lack of microbial growth in bonding systems that contained acetone as a solvent in detriment to contamination by bacteria presented in the bonding agents that contained ethanol as a solvent.

After 20 applications, the SB bonding system sustained high microbial contamination, in contrast to EX and PB. The EX bonding system presented no contamination because of the lower contamination probability during clinical use, once this bonding agent presents single application.

Regarding the inhibitory effect on S. mutans strains of bonding systems used in this study, it was possible to observe inhibition halos in the samples containing SB and EX. The inhibitory effect remained the same even after 20 applications. These results agree with Çehreli et al (21), and Atac et al (22) who observed EX's inhibitory zones on S. mutans. Concerning SB, Imazato et al (8) observed inhibition zones on S. mutans strains agreeing with the results of this study, in spite of the fact that others (22, 23) did not observe such effect.

The microbial capacity of SB and EX bonding systems observed on S. mutans strains in this study can be explained by the presence of polyalquenoid acid copolymer in SB and phosphoric acid acrylate in EX (8), once the monomers TEGDMA, HEMA and Bis-GMA (16, 17) and the solvent ethanol did not present an inhibitory effect on S. mutans.

PB did not present an inhibitory effect on S. mutans in this study, results that agree with Herrera et al (24), Imazato et al (8) and Schmalz et al (17). Contrasting with the results of this study and the literature, Atac et al (22) observed that PB contains resin nanofillers that have been incorporated in its formula to reinforce this system's physical properties, with the possibility of releasing these particles to contribute to the inhibitory effect on S. mutans.

The possibility of microbial contamination of bonding systems due to clinical procedures must always be a concern in clinical practice. The use of biosafety protocols...
before inserting bonding agents in the cavity is fundamentally important. The antimicrobial capacity of bonding systems is related to their chemical composition and the bonding agents’ interaction with several microbial strains present in the oral cavity.

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