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Effect of light-activation on the antibacterial activity of dentin bonding agents

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Abstract

Aim: This study evaluated the effect of light-activation on the antibacterial activity of dentin bonding systems. **Methods:** Inocula of Streptococcus mutans and Lactobacillus casei cultures were spread on the surface of BHI agar and the materials were applied and subjected or not to light-activation. Zones of bacterial growth inhibition around the discs were measured. **Results:** Excite, Single Bond and the bond of Clearfil SE Bond (SE) and Clearfil Protect Bond (CP) did not show any antibacterial activity. The strongest inhibitory activity was observed for the primers of CP and Prompt (PR) against S. mutans and the primers of SE and PB against L. casei. **Conclusion:** Light-activation significantly reduced or suppressed the antibacterial activity of the initially active uncured dentin bonding systems.

Keywords: dentin bonding systems, antibacterial activity, Streptococcus mutans, Lactobacillus casei

Introduction

In spite of recent advances in restorative dentistry, the formation of microgaps at tooth-restoration interfaces has been considered a significant problem related to the polymerization shrinkage of composite resins, especially at enamel-free margins[1]. The influx of bacteria and their byproducts through these microgaps may be involved in the development of secondary caries which has been considered the most frequent reason of failures in resin composite restorations[2-3]. In addition, after the removal of carious dentin, residual microorganisms can remain on the cavity floor or inside the dentinal tubules and cause pulp inflammation[4]. An impervious sealing of the cavity margins would be ideally desirable to prevent these bacteria from receiving nutrients and maintaining their metabolism and proliferation.

Composite-enamel bonding has been proven predictable, adequate and reliable by the application of the adhesive technique. However, since dentin adhesive systems are incapable of providing an unfailing and consistent dentinal sealing, despite showing high bond strengths[1], it is enviable that these materials present short- and long-term antibacterial effect. It has been shown that some adhesive systems present inhibitory activity against different oral bacteria. Such activity seems to be dependent on their acidity and chemical composition[5-6]. However, this activity can be suppressed after light-activation[7].

The incorporation of an antibacterial monomer such as 12-methacryloylixydodecylpyridinium bromide (MDPB) has been proven effective in granting antibacterial effect to a dentin primer before and after the curing process[5,8-9]. The ability of a MDPB-containing primer to penetrate artificially demineralized lesions and to kill bacteria in dentin, preventing the progression of root-surface caries has been recently demonstrated[10-11].

Since tooth-restoration interfaces do not provide a hermetic sealing against the diffusion

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of microorganisms and/or their byproducts, it would be beneficial if the restorative materials could exert some antibacterial activity as long as the restoration is in function in the oral cavity. Therefore, the aim of this study was to evaluate the effect of light-activation on the inhibitory activity of five contemporary dentin bonding systems against Streptococcus mutans and Lactobacillus casei. The null hypothesis was that light-activation does not affect the antibacterial performance of the dentin bonding systems.

Materials and methods

The following dentin bonding systems were evaluated in this study: Single Bond (SB - 3M ESPE), Excite (EX - Vivadent) – two-step total-etch systems; Clearfil SE Bond (SE - Kuraray), Clearfil Protect Bond (CP - Kuraray) – two-step self-etch systems; Adper Prompt L-Pop (PR – 3M ESPE) – one-step self-etch system. Their composition is shown in Table 1. For Clearfil SE Bond and Protect Bond, the two components (primer and bond) were tested separately. However, since the primer of Clearfil Protect Bond does not contain photoinitiators, 10 μL of the bond were also applied.

The antibacterial activity of each material was evaluated against Streptoccocus mutans (ATCC # 25175) and Lactobacillus casei (ATCC #193) using the agar plate diffusion test. Chlorhexidine gluconate at 0.2% was used as the control group.

Indicator strains were grown in brain heart infusion broth (BHI™, Difco Laboratories, Detroit, MI) for 48 h at 37°C, according to the physiological characteristics of each microorganism. The resultant inoculum was again placed in 5 mL BHI for 24 h at 37°C. The turbidity of the bacterial suspension was adjusted to a 0.5 McFarland standard (approximately 10^8 cfu/mL, according to previous bacterial count). In Petri dishes, base layers containing 15 mL of BHI agar were prepared and 250 μL of each inoculum was spread on their surface. Sterilized paper discs with 5-mm diameter and 1.5-mm thickness were impregnated with 20 μL of each material and subjected to one of the following conditions of light-activation: 1 - without light-activation, 2 - direct light-activation (DLA) after placement of the uncured specimens on the culture medium, or 3 - indirect light-activation (ILA) performed previously to the placement of the specimens on the culture medium. Each material was irradiated for 10 seconds using a pre-calibrated (400 mW/cm^2) light-activation unit (2500 Curing Light, 3M ESPE, St. Paul, CT, USA). Tests were performed six times for each material, bacteria and condition of light-activation. The plates were kept for 2 h at room temperature for diffusion of the materials and then incubated at 37° C for 24 hours.

Table 1. Chemical composition of the dentin bonding systems used in this study.

Materials	Manufacturer	Composition	pH	Batch No.
Single Bond (SB)	3M ESPE, St. Paul, MN, USA	Bis-GMA, HEMA, dimethacrylates, water, ethanol, polyalkenoic acid, acid copolymer, photoinitiator	5.0	1105
Excite (EX)	Vivadent Ets, Schaan, Liechtenstein	Bis-GMA, HEMA, phosphoric acid acrylate, glycerin dimethacrylate, initiators, stabilizers, ethanol, high dispersed silica	1.7	E30108
Clearfil SE Bond (SE)	Kuraray Medical Inc., Okayama, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylates, camphorquinone, N, N-Diethanol-p-toluidine, water.	1.9	00416A
		Bond: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylates, camphorquinone, N, N-Diethanol-p-toluidine, silanated colloidal silica	2.8	00555A
Clearfil Protect Bond (CP)	Kuraray Medical Inc., Okayama, Japan	Primer: HEMA, hydrophilic dimethacrylates, MDP, MDPB, water.	1.9	00002A
		Bond: silinated colloidal silica, sodium fluoride, Bis-GMA, HEMA, hydrophilic dimethacrylates, MDP, camphorquinone, N, N-Diethanol-p-toluidine.	2.8	00005A
Adper Prompt L-Pop (PR)	3M ESPE, St. Paul, MN, USA	Methacrylated phosphoric acid ester, water, photoinitiator (bis-2,4,6-trimethylbenzoyl) phenylphosphine oxide (BAPO), stabilizer, fluoride complex with zinc, parabenes	0.8 (mixed)	168535

Abbreviations: Bis-GMA: Bisphenol A diglycidylmethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide

Table 2. Median and range of the inhibition zones (mm) obtained against *Streptococcus mutans*.

Material		Light-activation		
		Without	Direct light-activation (DLA)	Indirect light-activation (ILA)
Single Bond (SB)		0	0	0
Excite (EX)		0	0	0
Clearfil SE Bond (SE)	Primer	15.5 (15.0-17.5) a,A	15.5 (12.0-17.0) a,A	0
	Adhesive	0	0	0
Clearfil Protect Bond (CP)	Primer	20.0 (17.0-23.5) b,A	18.8 (15.5-26.0) b,A	12.0 (8.0-14.5) a,B
	Adhesive	0	0	0
Adper Prompt (PR)		18.0 (16.0-23.0) b,A	19.5 (14.0-20.0) b,A	0
Clorhexidine (CH)		12.5 (12.0-14.0) c,A	12.5 (12.0-13.0) a,A	11.3 (10.0-13.0) a,A

** Values followed by the same lowercase letter in columns and uppercase letters in the rows are not statistically different (Mann-Whitney, p>0.05)*

Zones of bacterial growth inhibition around the discs were measured (in millimeters) using a digital caliper (Mitutoyo, SP, Brazil). Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the discs. This measurement was repeated three times and the mean was computed for each disc. For statistical analysis of the results, Kruskal-Wallis and Mann-Whitney non-parametric tests were used at a significance level of 5%.

Results

The median values and ranges of the inhibition zones for each material according to the bacteria strain and light-activation condition are shown in Tables 2 and 3. Among the tested materials, no inhibition zones were observed for SB, EX and the bond component of SE and CP, for both bacteria regardless of light-activation.

For the uncured dentin bonding agents, the greatest inhibitory effect against Streptococcus mutans was observed for the primer component of CP and PR, with no statistical difference between them (p>0.05), followed by SE Primer. For Lactobacillus casei, both primer components of CP and SE showed the largest inhibitory zones, without statistical difference (p>0.05), followed by PR. Chlorhexidine gluconate at 0.2% presented the weakest antibacterial activity.

Direct light-activation of the uncured materials on the culture medium did not interfere with the immediate inhibitory activity of the dentin bonding systems against bacterial growth. However, when these materials were light-cured before being placed on the culture medium, the antibacterial activity was suppressed, except for the primer component of CP, for both bacteria and PR only against Lactobacillus casei, although the inhibitory effect of these materials had been significantly reduced.

Discussion

Since most restorations are performed due to caries, microorganisms are present in the cavity walls, left behind intentionally (incomplete caries removal) or not. It is desirable that materials for direct application on the contaminated dentin present some antimicrobial activity to accelerate the inactivation of such microorganisms, mainly represented by S. mutans and lactobacillus. It is also desirable that this antimicrobial activity could last as long as the restoration is in function in the oral cavity to prevent or at least minimize the negative

Table 3. Median and range of the inhibition zones (mm) obtained against Lactobacillus casei.

Material		Light-activation		
		Without	Direct light-activation (DLA)	Indirect light-activation (IDA)
Single Bond (SB)		0	0	0
Excite (EX)		0	0	0
Clearfil SE Bond (SE)	Primer	23.5 (20.0-30.0) a,A	23.5 (20.0-27.0) a,A	0
	Adhesive	0	0	0
Clearfil Protect Bond (CP)	Primer	20.3 (19.0-21.5) a,A	20.8 (20.0-24.0) ab,A	15.5 (14.5-17.0) b,B
	Adhesive	0	0	0
Adper Prompt (PR)		21.5 (16.0-25.0) a,A	20.0 (15.0-22.0) b,A	9.3 (7.5-16.0) a,B
Clorhexidine (CH)		15.5 (15.0-17.0) b,A	16.0 (0-0) c,A	16.0 (14.0-16.0) b,A

* Values followed by the same lowercase letter in the columns and uppercase letters in the rows are not statistically different (Mann-Whitney, p>0.05)

effects of bacteria in the restorative material-dental structure interface. Adhesive systems may act as a antimicrobial material due to some characteristics such as pH, ions release (e.g. fluoride)[5-6] or the inclusion of specific monomers (MDPB)[8,12]. However, it is interesting to investigate if this antimicrobial property has only an immediate effect or a long-term action.

A direct relationship between material acidity and growth inhibition of S. mutans has been reported[13]. However, some oral bacteria, including S. mutans and L. casei, produce organic-acids as the end-product of glycolysis and are able to function at the low pH generated in the surrounding environment. As a consequence, acidogenicity and acidurance are the major physiological traits associated with the virulence of these microorganisms[14]. The sudden exposure of strains of oral streptococci and lactobacilli to pH values between 6.0 and 3.5 results in the induction of an acid tolerance response (ATR) that enhances the survival of these strains at or below pH 3.5[14]. The most rapid adaptative response is exhibited by S. mutans, involving a process that requires protein synthesis within 30 minutes of acid shock[15]. Bacteria that are forewarned by mild acidification can prepare through the induction of a wide range of protective measures, including systems that alter cell membrane composition, extrude protons (H^+), protect macromolecules, alter metabolic pathways and generate alkalis[16].

Based on the above mentioned, it should be considered the possibility that the use of mildly acidic materials could, instead of having a detrimental effect on bacterial growth, be stimulating the generation of more resistant strains. Complete lack of bacterial growth inhibitory activity was seen for SB. This system, in addition to the absence of a specific antibacterial component, has a pH of 5.0, which is not acidic enough to prevent S. mutans and L. casei from maintaining their metabolism. This observation is in line with the results reported by Imazato et al.[10] (2002), Atac et al.[17] (2001) and Baseren et al.[18]. Further studies are necessary to investigate the induction of an acid tolerance response by dentin bonding systems and other dental materials, which enhances the survival of some cariogenic bacteria.

Although presenting a low pH (1.7) due to the presence of phosphoric acid acrylates in its composition, EX did not show inhibitory activity against both bacteria strains for all light-curing conditions. Based on this fact we believe that probably some physical characteristics of the material are important in modulating the antibacterial activity. Although EX has alcohol as solvent, it is presented as a viscous fluid due to the inclusion of filler particles into its composition, which could have prevented this material from diffusing properly in the agar medium. As a consequence, the pH drop was not intense enough to reach the lethal pH values reported for S. mutans (3.5-3.0) or L. casei (2.3)[15]. Despite the negative results obtained for Excite in the present investigation, bacterial growth inhibition using this same filled dentin bonding agent was reported by Çehreli et al.[19]

The bond components of SE and CP are also highly viscous since they do not contain solvents. These substances also did not show inhibitory activity against the cariogenic bacteria used in this study. In addition, when compared to the corresponding primer components, they are considered less hydrophilic despite the presence of MDP and HEMA which are an ionic and a neutral hydrophilic monomer, respectively. Hence, viscosity and hydrophilicity are important characteristics of dentin adhesives concerning their inhibitory effect against bacterial growth[6-7].

Despite the fact that SB and EX did not inhibit bacterial growth, it is relevant to remember that these two-step etch-and-rinse adhesive systems are used after the previous application of phosphoric acid as a way to superficially demineralize the substrate. It has been demonstrated that phosphoric acid has antibacterial activity[20] and causes a significant immediate reduction in the number of microorganisms in carious dentin[21].

In the present study, PR and the primer component of SE and CP had an acidic pH (<2.0) and were antibacterially active even against L. casei, which are more acid tolerant[22]. This means that the bacteria were not able to counter the negative impact of a sudden reduction in cytoplasmatic pH, even possessing constitutive and inducible strategies to survive and function in acid environments. These results may be explained by the entrance of high levels of H^+ protons in the cell cytoplasm that result in loss of activity of the relatively acid-sensitive glycolytic enzymes (which severely affects the ability to produce ATP) and structural damage to the cell membrane and macromolecules such as DNA and proteins culminating with cell death[16]. The low pH of self-etch primers/adhesives could be the result of the presence of polymerizable acidic monomers which are esters originating from the reaction of a bivalent alcohol with methacrylic acid and phosphoric/carboxylic acid[23]. SE, CP and PR contain large amounts of acidic monomers[22] that can interfere on the survival of oral bacteria and could be implicated in the antibacterial activity[18].

Monomers, such as triethylene glycol dimethacrylate (TEG-DMA) and 2-hydroxyethyl methacrylate (HEMA), are major components used in dentin bonding systems as amphiphilic substances to enhance chemical compatibility between the hydrophilic dentin and the hydrophobic composite resin base monomers[24]. However, HEMA, which is a neutral hydrophilic monomer included in the composition of all dentin bonding systems used in this study, does not present inhibitory activity against cariogenic bacteria such as S. mutans, S. sobrinus and L. acidophilus[24]. This fact is sustained by the results of this study since several of the tested adhesive materials, which contain HEMA in their composition, did not show any antibacterial activity.

The resin monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) has been incorporated in the composition of some bonding systems to enhance the antibacterial effect of these materials. The bacterial growth inhibitory activity of this monomer has been demonstrated in several studies[4-5,8-11,25-27]. The progression of root-surface caries lesions in vitro was completely prevented after the application of a MDPB-containing primer through a combination of its antimicrobial activity and sealing of the demineralized dentin[28] Moreover, in vitro[8] and in vivo[12] studies have demonstrated that bonding resins containing MDPB could inhibit bacterial growth, without adversely affecting its bonding characteristics. MDPB is a compound of an antibacterial agent quaternary ammonium with a methacryloyl group, which copolymerizes with other monomers immobilizing the antibacterial agent into the polymer matrix[9]. Among the bonding systems investigated in the present study, this antibacterial monomer is incorporated in the primer component of CP which was active against both S. mutans and L. casei. However, although the inhibitory activity of this particular bonding system was comparable to that of the uncured primer component of SE and PR, it maintained this effect even after polymerization.

The polymerization process negatively affects the antibacterial activity of resinous materials[4]. In this present study, antibacterial activity of dentin bonding systems was evaluated analyzing the influence of photo-activation on this property. The studies using agar

diffusion plate test, including recent publications[25-26,29] that used the same materials tested in our study, Clearfil Protect Bond (CP) and Clearfil SE (SE), did not compare the inhibitory activity of adhesive systems against cariogenic bacteria, with or without photo-activation, only photo-actived. When cured specimens were placed on the agar medium, only the primer component of CP maintained its antibacterial activity against both S. mutans and L. casei, while PR prevented bacterial growth only of L. casei. However, this activity was significantly reduced when compared to the uncured specimens. A dentin primer incorporating MDPB could show antibacterial activity before and after curing against oral bacteria such as S. mutans[5,9]. The antibacterial agent is immobilized in the polymer network by polymerization of MDPB conferring to the materials which incorporate this monomer strong bacteriostatic and small bactericidal effect against cariogenic bacteria[5]. However, since the antibacterial agent is not released from the material, it has been demonstrated that the bacterial growth inhibitory activity of a MDPB-containing material after cured is exerted by direct contact with its surface[4]. This information is conflicting with the results of the present study, since a clear inhibition halo was seen for the primer component of CP, comparable to the inhibition zones observed for chlorhexidine. This indicates that the antibacterial effect exerted by this material occurred even without the direct contact of the microorganisms. As the amount of unpolymerized MDPB released from a cured Bis-GMA-based composite resin was confirmed to be less than the minimum inhibitory concentration (MIC) value for S. mutans[5], it could be speculate that maybe a different component other than the antibacterial agent was leached from the cured material.

Wang and Spencer[30] reported the ability of PR, which utilizes bisacylphosphine oxide (BAPO) as a photoinitiator, in demineralizing the underlying dentin even after polymerized. Based on the micro-Raman spectroscopy results obtained in that study, it was suggested that due to the incomplete polymerization, the acidic characteristics of this self-etching system were retained in the water-presence environment. Transferring this information to the present study, it may be speculated that unconverted acidic monomers present in the oxygen-inhibited layer after light-activation were capable of ionizing when in contact with the hydrophilic culture medium. It has been demonstrated that the layer of air-inhibited, poorly polymerized oligomers produced by PR is unusually thick[31]. This fact could explain the antibacterial effect exerted by PR even after cured, although in a smaller extent. The same was not noticed for the primer component of SE which has a lower concentration of acidic monomers.

The incorporation of fluorides in the composition of dentin bonding systems is an attempt to mainly augment the demineralization protective effect of these materials. In addition to enhancing remineralization in the cyclic demineralization-remineralization caries process, fluoride can act on cariogenic microorganisms by altering their physiological status. The three main microorganism-growth inhibitory mechanisms of fluoride are direct binding of F^-/HF to enzymes and other bacterial proteins, binding of metal F complexes and action as a transmembrane proton carrier[32]. High concentrations of fluoride ranging from 0.16 to 0.3 mol/L can inhibit bacteria growth[33]. Fluoride release from resinous materials has been proven inferior to that from glass-ionomer cements, and appears to play limited role in exhibiting substantial antibacterial effect[4,24]. In the present study, fluoride is incorporated in the composition of PR and the bond component of CP. Despite that, the latter did not show any antibacterial activity, which is in line with the results reported by Özer et al.[4].

Since tooth-restoration interfaces do not provide a hermetic sealing against the diffusion of microorganisms and/or their byproducts, it would be beneficial if the restorative materials could exert some antibacterial activity as long as the restoration is in function in the oral cavity. Unfortunately, the results of the present study demonstrated that adhesive systems do not fulfill that requirement since their antimicrobial activity is significantly suppressed or completely inhibited after curing. Exception should be made to the MDPB-containing adhesive system Clearfil Protect Bond. The positive results for this adhesive indicate that the inclusion of specific monomers in the composition of these materials is an interesting approach to lengthen the inhibitory effect against microorganisms that can infiltrate the tooth-restoration interface, preventing, for instance, the installation of recurrent caries lesions which represent the main factor responsible for the failure of resin restorations.

In conclusion, the null hypothesis was rejected since light-activation reduced significantly or suppressed the antibacterial activity of the initially active dentin bonding systems. Moreover, the inhibitory effect on bacteria growth is material dependent. Some investigators are using other methods to evaluate antibacterial activity of dental materials, such as direct contact test (DCT) that could be important to confirm the results of agar diffusion test[28].

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