

Evaluation of antimicrobial activity of orthodontic adhesive associated with chlorhexidine-thymol varnish in bracket bonding

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Abstract

Objective: To assess the antimicrobial activity resulting from the association of an orthodontic adhesive with chlorhexidine-thymol varnish. **Methods:** Thirty-two extracted human premolars were used, divided into four groups. In Group 1, the control group, the adhesive used to bond the bracket was not associated with any antimicrobial agent. Groups 2, 3 and 4 were bonded with an adhesive system associated with chlorhexidine-thymol varnish. Groups 3 and 4 were stored in water for 7 days and 30 days, respectively, while the specimens from group 2 were, soon after bonding, placed on agar seeded with *Streptococcus mutans* for 48 hours, at 37° C. **Results:** The experimental groups, with the exception of the control group, showed antimicrobial activity whose action tended to decline commensurately with the amount of time that they remained immersed in water. **Conclusions:** The association of chlorhexidine-thymol varnish with an adhesive system used in orthodontics proved to be advantageous due to its antimicrobial activity.

Keywords: Chlorhexidine. Adhesives. Antimicrobial agents.

INTRODUCTION

Nowadays, the use of orthodontic appliances is widespread. However, these appliances can be associated to difficulty in cleaning. During treatment, retentive areas are created that favor biofilm accumulation and bacterial growth. One of the greatest challenges in orthodontics consists in maintaining proper oral hygiene during treatment. Brackets, bands and other accessories further aggravate these condition by retaining dental plaque, which can lead to gingivitis and enamel

demineralization, causing white spots and caries.⁸

Microbiological studies have established that, after placement of a fixed orthodontic appliance, the number of bacteria rises significantly, particularly lactobacilli and streptococci, subjecting the oral environment to an imbalance and enabling the emergence of diseases. Although dental biofilm is composed of numerous species of bacteria, it is believed that *Streptococcus mutans* is involved in the early development of carious lesions.²⁰

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Thus, orthodontic treatment success lies in correcting occlusion in the best possible manner without, however, affecting the pre-existing health of teeth and supporting tissues. Otherwise, treatment benefits may be questioned.³⁰ Orthodontic practice undergoes constant progress with the use of new techniques and materials that benefit both patients and practitioners.² Attempts to inhibit the development of carious lesions in orthodontic patients have been focused on controlling the bacterial biofilm around the brackets.⁸ During therapy, orthodontists are also responsible for caries prevention.³⁰

In order to reduce the appearance of decalcified areas around the brackets, authors have suggested the use of orthodontic bonding resins which either contain or are associated with antimicrobial agents.^{2,17}

In orthodontics, composite materials are generally used for bonding brackets. These composites can act as a source of nutrition and agglomeration of opportunistic bacteria.⁸ Hahn et al¹³ concluded that microorganisms accumulate around restorative materials. Moreover, it has been previously reported that composites do not exhibit antibacterial activity after polymerization.⁶ It would be convenient to modify existing materials to perform additional functions. Considering that these materials would already be present in the mouth, they could serve as reservoirs or platforms for the dispersal of therapeutic agents.²¹

According to Korbmacher et al,¹⁷ orthodontic bonding systems that release antimicrobial agents to adjacent areas are useful because they reduce the need for patient compliance and can potentially decrease decalcification.

It has been suggested that the incorporation of chlorhexidine could impart antibacterial properties to composites. Chlorhexidine is a cationic chlorophenylbiguanide with antimicrobial properties and affinity for oral structures.

Its bactericidal activity results from coagulation of bacterial cytoplasm with subsequent rupture of cell membrane.²⁴ This agent is considered the gold standard compared to other substances designed to interfere with biofilm formation and development of gingivitis.³ Its spectrum is broad, covering Gram-positive and Gram-negative bacteria, yeasts, dermatophiles and some lipophilic viruses, in addition to having a selective effect on *Streptococcus mutans*.²⁵

However, composite resins are considered clinically insoluble since their components remain trapped inside and experience great difficulty in being released because the resin components restrict their displacement.⁶ Ribeiro and Ericson,²² however, observed antimicrobial activity after combining a composite resin with chlorhexidine to release antimicrobial components, although such activity decreased with time. Ehara et al,¹⁰ however, concluded that resins that release antibacterial agents have certain drawbacks, since their effects are transitory and decrease over time, they also impair mechanical properties and are potentially toxic.⁹

Bishara et al¹ and Damon et al⁷ found that a combination of chlorhexidine and orthodontic resins yielded sufficient shear strength for use in orthodontics, provided that the varnish is pre-mixed with the resin, applied to the etched enamel and cured. Karaman and Uysal¹⁵ agreed that shear strength becomes clinically acceptable when the varnish has been mixed with the resin in a 2:1 ratio, respectively.

The association of orthodontic bonding materials with chlorhexidine is useful as it is an adjunctive method to prevent the appearance of white spot lesions and caries around the brackets. It could play an important role as an auxiliary tool in preventing the demineralization of tooth enamel surfaces, thereby preserving the teeth during orthodontic treatment.

The purpose of this study was to assess the antimicrobial activity resulting from the

association of an orthodontic adhesive system with chlorhexidine-thymol varnish.

MATERIAL AND METHODS

An experimental, transverse laboratory study using 32 human premolars with healthy buccal surfaces, extracted for orthodontic purposes was conducted.

In this research, a modified version of the culture technique described by Ribeiro²³ for verification of growth inhibition was used.

The lingual surfaces of the teeth were flattened, both crown and root, so that the teeth had a buccolingual width of about 7 mm (Fig 1, A), and could be laid on a flat surface with their buccal surfaces facing upward. The mesial and distal surfaces of all teeth were also abraded down to 3.8 mm width, to match the width of the bracket to be bonded. The bonding area was demarcated with adhesive tape so that only the bracket base area was exposed and came into contact with the material being evaluated. The specimens were then subjected to a complete cycle of autoclaving (120°C for 20 minutes). Then, the brackets (MorelliTM, Brazil) were bonded in aseptic conditions and in a laminar flow, after sterilization of the bonding materials. The center of the buccal surface of each tooth was etched with phosphoric acid at 37% for 15 seconds, then washed with pyrogen-free sterile water and the etched enamel was dried with sterile filter paper.

This bonding protocol was described by Martinez,¹⁸ who achieved adequate shear strength with this method. In group 1 (control), 9µL of Ortho Primer MorelliTM was applied to each tooth, waiting up to 30 seconds for it to dry off slightly and curing it for 20 seconds. The metal brackets (MorelliTM) with the composite on them (Transbond XT, 3M, USA) were seated on each tooth with the aid of orthodontic tweezers, positioned and cured for 10 seconds on each side.

In Groups 2, 3 and 4 (Table 1) a mixture of 6µL chlorhexidine varnish (Cervitec, Ivoclar-VivadentTM, Swiss) and 3µL primer (Ortho Primer MorelliTM) at a ratio of 2:1 was used. This ratio is advocated in the literature as providing adequate mechanical properties for clinical use in orthodontics.^{2,7,15,18,22} The mixture was applied to the etched enamel surface and light-cured for 20 seconds. The metal brackets (MorelliTM) with the composite on them (Transbond XT, 3M) were seated on each tooth with the aid of orthodontic tweezers, positioned and light-cured for 10 seconds on each surface (Fig 1, B). After bonding the brackets, the adhesive tapes were removed.

The specimens in group 3 were kept separately in sterile pyrogen-free water for seven days in airtight jars. The specimens in group 4 were kept under the same conditions for 30 days and the water was replaced after 15 days. Groups 1 and 2 were bonded and cultured within up to four hours after bonding. All groups were cultured at the same time using the same bacterial culture.

With the purpose of placing each specimen on sterile Petri plates (100 mm diameter by 15 mm height), about 40 mL of Tryptic Soy Agar (TSA - Difco) culture medium were added to each plate. Each specimen was then placed in one of the plates, according to each group, with the flattened lingual surface seated on the bottom of the plate. Consequently, the buccal surfaces were facing upward, so that each bracket and the enamel adjacent to it were kept free from contact with the culture medium (Fig 1, C). After complete solidification, about 15 mL of the same molten culture medium, cooled to 50°C and seeded with *Streptococcus mutans* (ATCC 25175) was added. The inoculum consisted of a suspension of 8.0×10^{12} CFU of *Streptococcus mutans*/mL of medium, with an optical density of 1.6 in DO_{600} , which was added to the plates containing the specimens.



FIGURE 1 - A) Abrasion of palatal surface. B) Bracket bonded to an area bounded by adhesive tape. C) Tooth fixed in agar without contact with bracket tie-wings.

TABLE 1 - Description of study groups.

Group	Storage time in water	Adhesive	Association with chlorhexidine and thymol (Cervitec™)
1	-	Ortho Primer	Absent
2	-	Ortho Primer	Present
3	7 days	Ortho Primer	Present
4	30 days	Ortho Primer	Present

An amount of agar with *Streptococcus mutans* culture that was sufficient to cover the base of the brackets and enamel surface surrounding the bonding area without covering the tie-wings was added to each plate. The medium was let to dry off and then the plates were placed in a bacteriological incubator for 48 hours at 37°C. After culture, the presence or absence of a zone of inhibition of bacterial growth was evaluated. In cases where a zone of inhibition was formed, its diameter was measured with the aid of a bow divider and a millimeter ruler. The results were subjected to statistical analysis of variance (ANOVA) and Tukey's test.

RESULTS

ANOVA demonstrated significant differences in the results obtained by the groups (p value = 0.000). The control group had no evidence of a zone of inhibition. In group 2, the mean value

was 4.125 mm, with standard deviation of 0.991. The mean value found in group 3 was 3 mm, with standard deviation of 0.756. In group 4, the mean value was 2.625 mm, with standard deviation of 0.518 (Table 2). Statistically significant differences were found between the results obtained in group 1 and other groups and between group 2 and other groups. Between groups 3 and 4, however, no statistically significant difference was found, although, as can be seen in Figure 2, values exhibited a declining trend.

DISCUSSION

A gold standard method is not yet available for in vitro evaluation of antimicrobial agents in bonding agents.²⁶ Several in vitro studies have evaluated the antimicrobial effectiveness of bonding materials by the agar diffusion method.^{14,16} The agar diffusion test is an acceptable method for differentiating the antimicrobial activity of substances at an early stage.²⁹ The zones of growth inhibition are dependent on the toxicity of the material used against the bacteria and the diffusibility of the material inside the culture medium.¹¹ In this study, it was used the agar diffusion method to observe the activity of the agent against one of the most common bacteria associated with caries: *Streptococcus mutans*. These bacteria also feature considerable affinity for composite resins.¹⁹

TABLE 2 - Obtained results ($p = 0.000$).

	Group 1	Group 2	Group 3	Group 4
Zone mean (mm)	0.000	4.125	3	2.625
SD	0.000	0.991	0.756	0.518

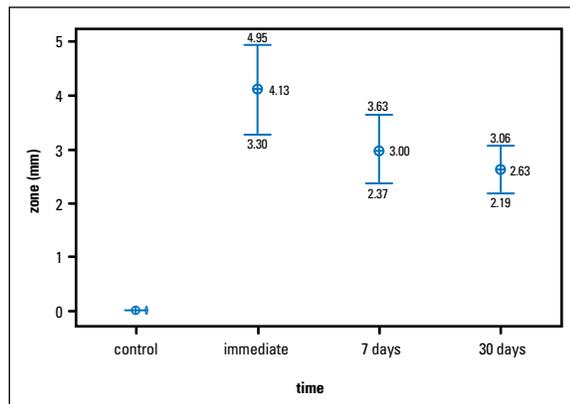


FIGURE 2 - Reduction tendency of the obtained zone when evaluated by the mean for each group.

Cervitec varnish was used because it is a compound widely used as a source of chlorhexidine in many studies.^{1,7,18,23}

This study further disclosed the antimicrobial action of chlorhexidine, whose effectiveness is well established in dentistry,⁵ where it is associated with bonding resins used in orthodontics. However, further *in vitro* and *in vivo* studies are needed to determine the clinical significance and duration of antimicrobial properties on a variety of oral cavity microorganisms involved in the pathogenicity of bacterial biofilms and caries.

Similarly to Ribeiro's²³ findings, it appears that given the formation of a zone of inhibition the combination of chlorhexidine varnish and orthodontic bonding material enabled antimicrobial activity by releasing the antimicrobial substance into the culture medium,

thereby inhibiting *in vitro* bacterial growth in areas surrounding the bracket. It is likely that a small amount of chlorhexidine was released from the portion below the bracket since only a thin layer of adhesive associated with varnish was exposed to the culture medium.⁴

The reduction in the effects of chlorhexidine over time may be due to a reduction in the release rate or a reduction in the actual amount of material present. According to Couto Júnior et al,⁶ although component release seems larger at first, the decrease in this rate indicates that the components in the outer layer are depleted or dissolved in the water. On the other hand, the components trapped inside the resin mass are released with immense difficulty because resin components restrict such displacement.⁶ The literature reports the sustained release, in aqueous environment, of compounds initially located within orthodontic adhesive resins for 150 days,²⁸ or even up to two years.¹²

Often, the therapeutic agents of dental biomaterials are released from materials and exhibit a decreasing release rate. The water in the oral cavity diffuses into the resin matrix. The agent trapped in the adhesive dissolves and disperses in ever smaller concentrations. Over time, the agent is released and extracted from an increasingly deeper matrix layer, which means that the time needed for diffusion to the external environment increases as the rate of release declines.²¹ This may also explain the absence of statistically significant differences between the antimicrobial activity of the group that was stored in water for seven days and the group stored for thirty days. However, we observed a significant reduction in antimicrobial activity between the group that was never stored in water and the group stored for seven days. It has been reported that immersion in water in the first three hours causes 50% of releasable components to be released from the resin.²⁷

There is no way of telling how long the system will display antimicrobial activity, mainly in the oral environment. It is clear, however, that this is an association whose antimicrobial effects display a decreasing trend, although it is probably an inexhaustible source of chlorhexidine. Therefore, these benefits do not last throughout the orthodontic treatment and changes may occur in mechanical properties after the release of the substance. However, it is likely that this activity will last through the most critical period of biofilm accumulation, when proper oral hygiene is a key issue. This period spans from the time of orthodontic appliance installation through the following four months,²⁰ thus justifying its benefits.

Damon et al⁷ and Bishara et al¹ found that a combination of chlorhexidine and orthodontic adhesives yielded sufficient shear strength for use in orthodontics when applied to the etched enamel and cured. Karaman and Uysal¹⁵ agreed that shear strength becomes clinically acceptable when the varnish has been mixed with the

adhesive in a 2:1 ratio, respectively. Ribeiro²³ and Martinez,¹⁸ after evaluating the bond strength of bonding systems whose adhesives had been pre-mixed with Cervitec chlorhexidine varnish, concluded that there was no statistically significant change in bond strength compared with adhesive alone. Further studies are needed to evaluate mechanical strength after the release of chlorhexidine, color stability, local and systemic cell and tissue compatibility, before the use of an adhesive/varnish combination in daily clinical practice is fully warranted.

CONCLUSIONS

Based on this study, it is possible to conclude that the association of chlorhexidine varnish with an orthodontic adhesive showed antimicrobial activity in vitro, even after immersion in water for seven or thirty days. It was also possible to notice a decreasing trend in antimicrobial activity with the increase of immersion time in aqueous media.

REFERENCES

1. Bishara SE, Vonwald L, Zamtua J, Damon PL. Effects of various methods of chlorhexidine application on shear bond strength. *Am J Orthod Dentofacial Orthop.* 1998 Aug;114(2):150-3.
2. Bishara SE, Damon PL, Olsen ME, Jakobsen JR. Effect of applying chlorhexidine antibacterial agent on the shear bond strength of orthodontic brackets. *Angle Orthod.* 1996;66(4):313-6.
3. Bowen WH. Wither or whither caries research? *Caries Res.* 1999;33(1):1-3.
4. Chan DC, Swift EJ Jr, Bishara SE. In vitro evaluation of a fluoride-releasing orthodontic resin. *J Dent Res.* 1990 Sep;69(9):1576-9.
5. Cleghorn B, Bowden GH. The effect of pH on the sensitivity of species of *Lactobacillus* to chlorhexidine and the antibiotics minocycline and spiramycin. *J Dent Res.* 1989 Jul;68(7):1146-50.
6. Couto MP Jr, Nagem H Filho, Nagem HD, Couto MGP. Determinação da taxa de flúor liberado por cinco resinas compostas. *Rev Facul Odontol Bauru.* 2000 jan-jun;8(1/2):65-69.
7. Damon PL, Bishara SE, Olsen ME, Jakobsen JR. Bond strength following the application of chlorhexidine on etched enamel. *Angle Orthod.* 1997;67(3):169-72.
8. Derks A, Katsaros C, Frencken JE, van't Hof MA, Kuijpers-Jagtman AM. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances. *Caries Res.* 2004 Sep-Oct;38(5):413-20.
9. Ebi N, Imazato S, Noiri Y, Ebisu S. Inhibitory effects of resin composite containing bactericide-immobilized filler on plaque accumulation. *Dent Mater.* 2001 Nov;17(6):485-91.
10. Ehara A, Torii M, Imazato S, Ebisu S. Antibacterial activities and release kinetics of a newly developed recoverable controlled agent-release system. *J Dent Res.* 2000 Mar;79(3):824-8.
11. Estrela C, Estrela CRA, Moura J, Bammann LL. Testing calcium hydroxide antimicrobial potential by different methods. *J Dent Res.* 2000;79:529 (IADR Abstract 3081).
12. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. *Dent Mater.* 1990 Oct;6(4):282-7.

13. Hahn R, Weiger R, Netuschil L, Bruch M. Microbial accumulation and vitality on different restorative materials. *Dent Mater*. 1993 Sep;9(5):312-6.
14. Herrera M, Carrión P, Bravo M, Castillo A. Antibacterial activity of four dentin bonding systems. *Int J Antimicrob Agents*. 2000 Aug;15(4):305-9.
15. Karaman AI, Uysal T. Effectiveness of a hydrophilic primer when different antimicrobial agents are mixed. *Angle Orthod*. 2004 Jun;74(3):414-9.
16. Karanika-Kouma A, Dionysopoulos P, Koliniotou-Koubia E, Kolokotronis A. Antibacterial properties of dentin bonding systems, polyacid-modified composite resins and composite resins. *J Oral Rehabil*. 2001 Feb;28(2):157-60.
17. Korbmacher HM, Huck L, Kahl-Nieke B. Fluoride-releasing and antimicrobial self-etching primer effects on the shear bond strength of orthodontic brackets. *Angle Orthod*. 2006 Sep;76(5):845-50.
18. Martinez TP. Avaliação da resistência ao cisalhamento de bráquetes, colados com sistemas adesivos associados a diferentes agentes antimicrobianos. [dissertação]. Salvador (BA). Faculdade de Odontologia, Universidade Federal da Bahia; 2006.
19. Pedrini D, Gaetti-Jardim E Jr, Vasconcelos AC. Retention of oral microorganisms on conventional and resin-modified glass-ionomer cements. *Pesqui Odontol Bras*. 2001 jul-set;15(3):196-200.
20. Petersson LG, Maki Y, Twetman S, Edwardsson S. Mutans streptococci in saliva and interdental spaces after topical applications of an antibacterial varnish in school children. *Oral Microbiol Immunol*. 1991 Oct;6(5):284-7.
21. Rawls HR. Preventive dental materials: sustained delivery of fluoride and other therapeutic agents. *Adv Dent Res*. 1991 Dec;5:50-5.
22. Ribeiro J, Ericson D. In vitro antibacterial effect of chlorhexidine added to glass-ionomer cements. *Scand J Dent Res*. 1991 Dec;99(6):533-40.
23. Ribeiro JLO. Avaliação da resistência adesiva e da atividade antimicrobiana de diferentes sistemas de colagem de bráquetes associados à clorexidina e ao flúor. [dissertação]. Salvador (BA): Universidade Federal da Bahia; 2006.
24. van Rijkom HM, Truin GJ, van 't Hof MA. A meta-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment. *J Dent Res*. 1996 Feb;75(2):790-5.
25. Rosa OPS, Rocha RSS. Clorexidina e cárie dentária. *CECADE News*. 1993 jan-ago;1(1/2):1-24.
26. Schmidlin OA, Zehnder M, Schmidlin PR. Effectiveness of dentine bonding agents against cariogenic bacteria in vitro: a comparison of two methods. *Oral Microbiol Immunol*. 2003 Jun;18(3):140-3.
27. Tanaka K, Taira M, Shintani H, Wakasa K, Yamaki M. Residual monomers (TEGDMA and Bis-GMA) of a set visible-light-cured dental composite resin when immersed in water. *J Oral Rehabil*. 1991 Jul;18(4):353-62.
28. Thompson LR, Miller EG, Bowles WH. Leaching of unpolymerized materials from orthodontic bonding resin. *J Dent Res*. 1982 Aug;61(8):989-92.
29. Tobias RS. Antibacterial properties of dental restorative materials: a review. *Int Endod J*. 1988 Mar;21(2):155-60.
30. Zimmer BW, Rottwinkel Y. Assessing patient-specific decalcification risk in fixed orthodontic treatment and its impact on prophylactic procedures. *Am J Orthod Dentofacial Orthop*. 2004 Sep;126(3):318-24.

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