

ORIGINAL RESEARCH

In situ Assessment of Effects of the Bromide- and Fluoride-incorporating Adhesive Systems on Biofilm and Secondary Caries

¹Suyane Maria Luna Cruz de Vasconcelos, ²Mary Anne Sampaio de Melo, ³Joao Paulo Marques Saraiva Wenceslau
⁴Iriana Carla Junqueira Zanin, ⁵Haroldo Cesar Pinheiro Beltrao, ⁶Carlos Augusto Oliveira Fernandes
⁷Paulo Cesar de Almeida, ⁸Lidiany Karla Azevedo Rodrigues

ABSTRACT

Aim: This *in situ* study assessed the effects of adhesive systems containing or not fluoride and/or the antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) on the microbiological composition of dental biofilm and enamel demineralization.

Materials and methods: During two phases of 14 days, ten volunteers wore intraoral palatal appliances containing two slabs of human enamel according to a double-blind, crossover design. The slabs were randomly restored using a composite resin and one of the following adhesive systems: All-Bond SE™ (self-etch, fluoride/MDPB free adhesive, AB) and Clearfil Protect Bond (self-etch containing fluoride and MDPB adhesive, CB). The biofilm formed on the slabs was analyzed with regard to total and mutans streptococci and lactobacilli counts. Demineralization represented by integrated area of hardness × lesion depth Delta S (ΔS) was determined on enamel by analysis of cross-sectional microhardness, at 20 and 70 μm from the restoration margin. Data were analyzed by ANOVA.

Results: No statistically significant difference was found either in enamel demineralization or in the microbiological composition of dental biofilm.

Conclusion: All adhesive systems containing or not fluoride and/or MDPB tested were unable to inhibit secondary caries in the *in situ* model used in the present research.

Keywords: Secondary caries, Adhesive systems, *In situ* model, Antibacterial agents, MDPB, Fluoride.

How to cite this article: de Vasconcelos SMLC, de Melo MAS, Wenceslau JPMS, Zanin ICJ, Beltrao HCP, Fernandes CAO, de Almeida PC, Rodrigues LKA. *In situ* Assessment of Effects of the Bromide- and Fluoride-Incorporating Adhesive Systems on Biofilm and Secondary Caries. J Contemp Dent Pract 2014;15(2):142-148.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Contemporary adhesives systems present satisfactory bonding to enamel and dentin.¹ However, caries development adjacent to restorations has been considered the main cause of clinical restoration replacement.² Additionally, dentin adhesive systems with high bond strength have been reported to be incapable of preventing the occurrence of microgaps between the tooth and restoration.^{3,4} Therefore, dentin bonding systems with anticariogenic activity, even after applied in the cavity, would be beneficial to eliminate the harmful effect caused by residual bacteria in the cavity or bacterial microleakage.^{5,6}

Previous *in vitro* studies have reported that incorporation of the antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB), is an effective method of providing dentin primer with antibacterial activity before and after curing.⁷⁻⁹ MDPB is a monomer synthesized by combining an antibacterial agent and a methacryloyl group and shows antibacterial activity against oral bacteria.¹⁰ The main advantage of MDPB is its capacity to copolymerize with other resin monomers being immobilized within the polymer matrix, which assigns safety and prolonged antibacterial action to this agent. This characteristic also ensures a good survival rate for the restoration, since MDPB, different

¹Student, ²Assistant Professor, ³Undergraduate Student
⁴⁻⁸Associate Professor

¹Post-graduation Program in Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceara, Ceara Brazil

²Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Dental School, Baltimore MD, USA

³Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceara, Ceara, Brazil

⁴The School of Dentistry of Sobral; Professor of the Post-graduation Program in Dentistry, Federal University of Ceara Ceara, Brazil

^{5,6}Department of Operative Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceara, Ceara Brazil

⁷Department of Public Health, Ceara State University, Ceara Brazil

⁸Department of Operative Dentistry, Faculty of Pharmacy Dentistry and Nursing; Professor of the Post-graduation Program in Dentistry, Federal University of Ceara, Ceara, Brazil

Corresponding Author: Lidiany Karla Azevedo Rodrigues Associate Professor, Department of Operative Dentistry Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceara, Ceara, Brazil, e-mail: lidianykarla@yahoo.com

from soluble antibacterial agents, is not deleterious to the physical and mechanical properties of materials to which it is incorporated.^{7,10-12}

Besides, fluoride plays an important role in the control of secondary caries, since it may interfere with physico-chemical¹³ and microbiological¹⁴ processes, not only reducing the caries progress rate but also allowing the arrestment of active lesions.¹⁵ Thus, with the intention of providing fluoride to the specific site at risk of secondary caries, fluoride-releasing restorative materials were developed, since they may induce an anticariogenic activity by increasing the dentin resistance to acids present in the oral cavity.¹⁶

Although the benefits of MDPB-containing adhesive systems in inactivating *in vitro* residual bacteria inside the cavity have been previously reported,¹⁷⁻¹⁹ the potential for *in situ* and *in vivo* enamel and dentin caries inhibition of these systems remains unknown. Furthermore, many of the cariostatic effects of fluoride-containing adhesive systems have been previously evaluated only by *in vitro* caries models. The purpose of this study was to assess the anticariogenic activity of adhesive systems containing or not fluoride and/or MDPB on the microbiological composition of dental biofilm and on enamel demineralization by using

an *in situ* model which bridged the gap between laboratory and clinical research, improving the clinical relevance of the data.

MATERIALS AND METHODS

Ethical Aspects

The study was approved by the local Research and Ethics Committee (# 138/2006). Ten volunteers (mean age 24 years-old) who met the inclusion criteria (normal salivary flow rate, good general and oral health, not using fixed or removable orthodontic appliances, not having used antibiotics during the 2 months prior to the study, ability to comply with the experimental protocol) were invited to take part in this study and those who agreed to participate signed an informed written consent form.

Experimental Design

The study involved a cross-over design for caries induction by biofilm accumulation and sucrose exposure, conducted in two distinct phases of 14 days each²⁰⁻²² with a 7-day wash-out period. Each group comprised 20 restored enamel slabs in duplicates of 10 experimental units (n = 10). The volunteers wore a palatal appliance containing two enamel slabs (Fig. 1),

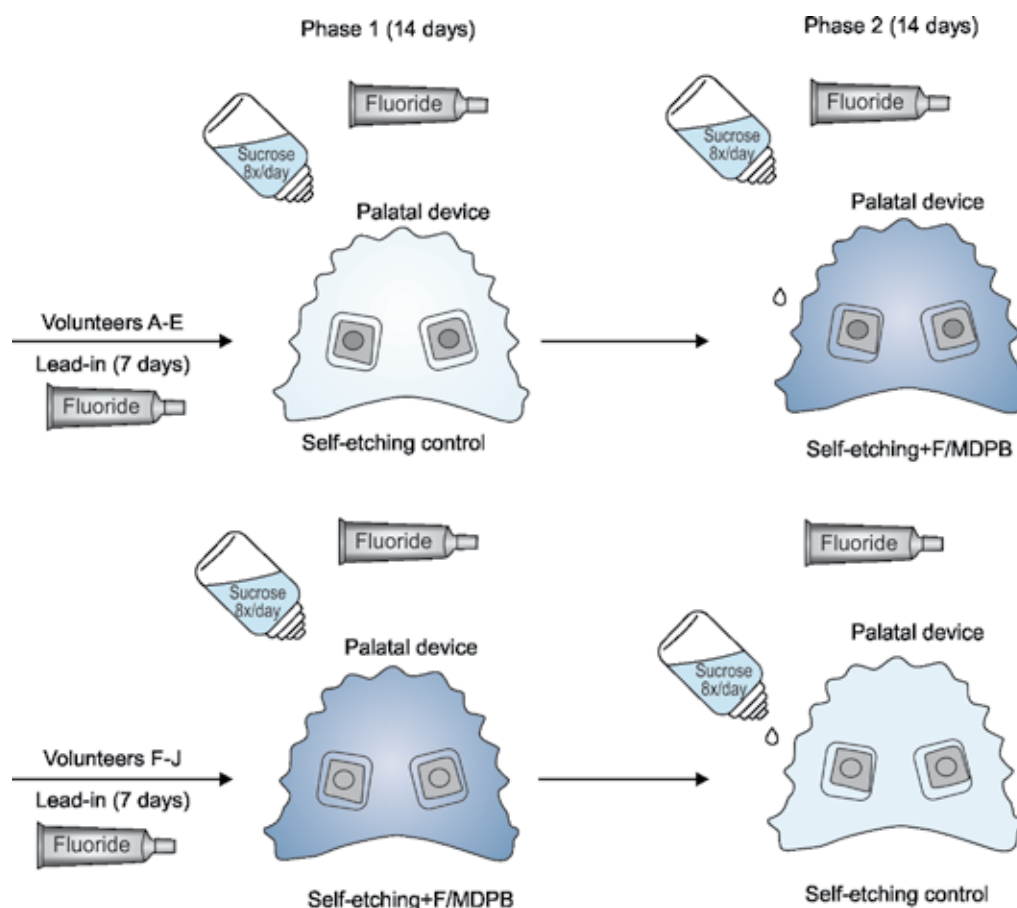


Fig. 1: Representation of the cross-over design used in the study

which were extraorally restored using a composite resin (Filtek™ Z250, shade A1, 3M ESPE Dental Products, St. Paul, USA) and one of the following adhesive systems: All-Bond SE™ (Bisco, Shaumburg, USA), self-etch adhesive (control), AB; and Clearfil Protect Bond (Kuraray Medical Inc, Okayama, Japan), self-etch containing fluoride/MDPB adhesive, CB. The slabs were randomly assigned to the 10 volunteers, who were considered as experimental blocks. In phase 1, five volunteers wore appliances with specimens of group AB and five with group CB. In phase 2, volunteers that had worn appliances with specimens of group AB wore appliances loaded with specimens of group CB and *vice versa* (Fig. 1).

Specimen Preparation

Freshly extracted, sound third molars with more than 2/3 of root formation were cleaned of gross debris, stored in supersaturated 0.1% thymol solution and maintained under refrigeration until they were used, approximately 1 month later. Twenty enamel slabs ($4 \times 4 \times 2 \text{ mm}^3$) were cut using a water-cooled diamond saw and a cutting machine (IsoMet™ Low Speed Saw, Buehler, Lake Bluff, USA) and were randomly assigned to each phase and treatment (Fig. 1). Circular standardized cavities ($\pm 1.8 \text{ mm}$ diameter and $\pm 1.5 \text{ mm}$ depth), were prepared at the center of each slab with a cylindrical diamond bur (# 2294, KG Sorensen, São Paulo, Brazil, replaced after 10 preparations) that provides a stop to limit the depth of penetration, used in high-speed turbine with air-water spray cooling. Afterwards the slabs were autoclaved²³ (121°C , 20 min), randomly divided into four groups and restored in duplicate for each adhesive system, according to the manufacturers' instructions. Cavities were restored in one increment of composite resin (Z100, 3M ESPE, St Paul, MN, USA) (batch # 0T098397BR, expiration date December 2010) and light-cured using a quartz-tungsten-halogen (QTH) light curing unit (Optilux 400- Demetron Research Corp, Danbury, USA). The light output was tested ($480 \pm 20 \text{ mW/cm}^2$) before each use with a Demetron Model 100 radiometer (Demetron Research Corp, Danbury, USA). After finishing and polishing with a sequence of abrasive disks (Sof-Lex – 3M ESPE Dental Products Division, St Paul, USA) applied for 15 seconds each, all specimens were analyzed using a stereomicroscope (Leica Microsystems, Wetzlar, Germany) at $40\times$ magnification to ensure that there was no excess material overlying the restoration/tooth interface.

Palatal Appliance Preparation

Acrylic custom-made palatal appliances were made with two sites ($5 \times 5 \times 4 \text{ mm}$), in which the dental slabs were

positioned and fixed with wax.²⁴ In order to allow plaque accumulation and to protect it from mechanical disturbance, a plastic mesh was fixed to the acrylic resin, leaving a 1 mm space above the surface of the specimen.^{24,25} Within each side of the palatal appliance, the positions of the specimens of each group were randomly determined.

Intraoral Phase

Volunteers followed a 1 week lead-in period before inserting the palatal appliances. During this period and throughout the experimental phases, they brushed their teeth with a silica-based dentifrice (Colgate, Máxima proteção anticáries, Colgate-Palmolive, Ind. Com. LTDA, São Bernardo do Campo, SP, Brazil), containing monofluorophosphate (MFP; 1,450 ppm F). The cariogenic challenge was provided by dripping a 20% sucrose solution onto all slabs 8 times a day (at 8.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 22.00 hours). Before replacing the palatal appliance in the mouth, a 5 minute waiting time was allowed for sucrose diffusion into the dental biofilm.²⁴ Tooth brushing with the fluoride dentifrice was performed after the main mealtimes, 3 times a day (7.30, 12.30, 22.30 hours). Volunteers were instructed to use a pea-size amount of dentifrice and to start brushing the buccal surface of maxillary teeth with the appliance still in the mouth.²⁵ After the slurry of dentifrice and saliva reached the plastic mesh over the specimens, the appliance was removed and kept without rinse until the volunteers finished their routine oral hygiene. After that, the device was washed in tap water, removing all dentifrice/saliva slurry, and re-inserted in the mouth. Volunteers were instructed to wear the intraoral appliances for the whole intraoral phase, except during meals. At these times, the appliances were kept moist in boxes that were provided. Volunteers lived in an optimally fluoridated city and drank and consumed foods prepared with this water. No restriction was made with regard to the volunteers' diet.

Microbiological Analysis

On day 14 of the intraoral phase, approximately 10 hours after the last exposure to the sucrose and dentifrice, the volunteers stopped wearing the intraoral appliance. The mesh was removed and the biofilm formed was collected with a sterilized plastic stick. The biofilm was weighed in sterile pre-weighed microcentrifuge tubes and 0.9% NaCl solution was added (1 ml/mg biofilm). The tubes were agitated during a 2 minutes period in a Disrupter Genie Cell Disruptor (Precision Solutions, Rice Lake, USA) with three glass beads (0.6 mm diameter) to disperse bacterial cells. Afterwards, the suspension was serially diluted (1:10, 1:100, 1:1000, 1:10000) with 0.9% NaCl solution and three drops of $10 \mu\text{l}$ were inoculated in mitis salivarius agar (MS-DIFCO, BD, New Jersey,

USA) added 20% sucrose and prepared according to company specifications to determine total streptococci (TS); in mitis salivarius agar plus 0.2 units of bacitracin/ml to determine mutans streptococci (MS); and in Rogosa agar to assess the number of lactobacilli (LB). The plates were incubated for 48 hours at 37°C using a candle-extinguish jar, obtaining a 5 to 10% carbon dioxide atmosphere. Representative colonies with typical morphology of MS, TS and LB were counted using a colony counter. The results were expressed in CFU/mg dental biofilm (wet weight) and the percentage of mutans streptococci group (%MS) in relation to total streptococci was also obtained.

Microhardness Analysis

The enamel specimens were removed from the appliance and longitudinally sectioned in their central area. One half of each enamel slab was randomly selected and embedded in acrylic resin (Arotec, São Paulo, Brazil), exposing the cut surface,²⁰ for subsequent flattening and polishing with Al₂O₃ paper grit 100, 400, 600 and 1200 and polishing cloths with 1 µm diamond paste (Buehler, Lake Bluff, USA), respectively. Microhardness was measured using a Knoop indenter with 25 gm load for 5 seconds and a microhardness tester (Future-Tech FM Corporation, Kawasaki, Japan) coupled to the software FM-ARS. Two rows of twelve indentations each were made at depths: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160 and 180 µm from the outer enamel surface. The means of KHN in depths higher than 100 µm were used as measure of the integrated hardness profile of inner sound enamel, making each slab its self-control. The distance from the first row to the restoration margin was 20 µm, and the second distance was 70 µm.

The mean Knoop hardness number (KHN) values, at each position from the surface and at 20 and 70 µm distances from the enamel-restoration interface were obtained. Thus, KHN was plotted against depth for each specimen and the integrated hardness profile of demineralization was calculated relative to the underlying sound enamel. The mean sound enamel values of KHN for computation of demineralization were obtained from the inner sound enamel under the lesion in the same tooth. To compute ΔS (integrated demineralization), the hardness profile of demineralization

was subtracted from the value obtained for sound enamel. Data were expressed in Knoop hardness number (kg/mm²) to calculate ΔS.²⁷

Statistical Analysis

In order to assess the effect of treatments, the dependent variables TS, MS, LB counts and ΔS parameter were analyzed; the assumptions of equality of variances (Levene Test) and normal distribution (Koulmogorov-Smirnov Test) were tested. Normal distributions were found for all variables on the equality of variances. The paired t-test was applied to UFC counts and ΔS ($p < 0.05$). The values of KHN were analyzed with a split-plot ANOVA statistical design followed by Tukey's test, considering adhesive and depth as factors. The software Biostat 5.0 (Mamirauá Sustainable Developing Institute; Tefe, AM, Brazil) was used and the significance limit was set at 5%. The relationship between microhardness and depth values was also checked by linear regression analysis ($p < 0.01$).

RESULTS

With regard to microbiological composition of the biofilm formed on slabs restored using the different adhesive systems, no significant differences were found between treatments (Table 1), although a trend to lower values had been found mainly for CB group in relation to total streptococci and lactobacilli.

For the ΔS parameter, no significant differences were found between treatments (*see* Table 1), even though the self-etch MDPB-fluoride free adhesive system showed the highest demineralization in most depths at both distances. The relationship between microhardness and depth showed significant correlation, being directly proportional ($p = 0.0001$) (Graph 1).

DISCUSSION

Antibacterial activity is a desired property for adhesive systems, especially for self-etch adhesive systems, since the prepared dentin is treated with an acidic primer, without being previously etched and washed. These antibacterial properties would discourage the presence of bacteria inside

Table 1: Microbiological analysis of dental biofilm and demineralization (ΔS), for each treatment at studied distances (Mean values with their standard deviation and p-value for each analysis)

Micro-organisms	Groups		p-value	
	AB	CB		
Total streptococci (CFU/mg × 10 ⁷)	1.19 ± 0.98	0.81 ± 0.74	0.13	
Mutans streptococci (CFU/mg × 10 ⁵)	5.20 ± 3.59	5.71 ± 2.87	0.67	
Lactobacilli (CFU/mg × 10 ⁷)	1.93 ± 1.57	0.39 ± 0.31	0.23	
Distance from the restoration margin	20 µm	12004.90 ± 7688.33	9038.57 ± 6992.42	0.35
	70 µm	9375.88 ± 7253.54	8455.41 ± 7428.5	0.69

CFU: colony-forming units; AB: All-Bond SE™ (Bisco, Shaumburg, USA); CB: Clearfil Protect Bond (Kuraray Medical Inc, Okayama, Japan)

the dentinal tubules,⁷ decreasing the possibilities of caries recurrence. Besides, occurrence of microgap formation has been reported between the adhesive resin and the primed dentin surface, as well as between the adhesive resin and the hybrid layer, which implies that a great deal of the formed space is to be surrounded by adhesive resin, thus most invading bacteria would have contact with cured adhesive.⁵ In this way, MDPB-containing adhesives may inhibit the growth of invading bacteria, consequently inhibiting bacterial leakage even when marginal sealing is not complete or destroyed after restoration. However, the effects of these antibacterial adhesive systems on the inhibition of secondary caries are still unknown.

The present study used an *in situ* caries model to simulate a caries challenge to test the anticariogenic effect of adhesive systems, given that this model appears to be more analogous to *in vivo* conditions than chemical-based and bacterial *in vitro* caries models.^{13,28} The *in situ* caries model used in the current study, based on biofilm accumulation and sucrose exposure, was previously reported to be cariogenic to human dental enamel.^{21,29} This was confirmed by our linear regression analysis, which showed that hardness increased significantly and progressively with the enamel depth. Fluoride-containing dentifrice was chosen, since over 95% of all dentifrices sold in the US, Brazil and Western Europe contain fluoride.³⁰⁻³² Additionally, it has been demonstrated that, in the presence of fluoridated toothpaste, demineralization is evident with a frequency of carbohydrate consumption equal or higher than 7-times/day.³³

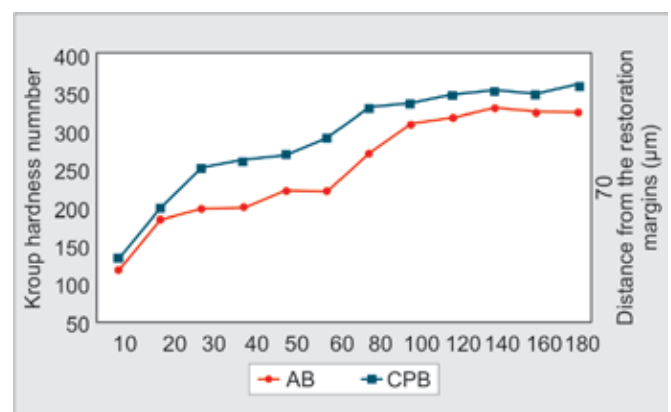
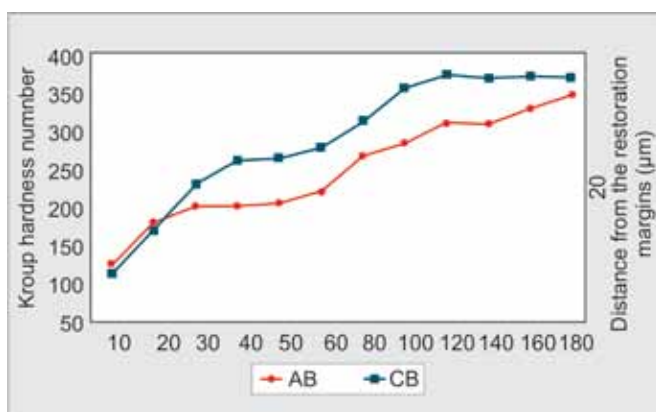
In the present study, the tested self-etch adhesive containing MDPB/Fluoride were not able to inhibit secondary caries, since the microhardness results showed no statistical differences between groups. To our knowledge, no other *in situ* studies have evaluated the anticariogenic effects of these types of adhesives on enamel demineralization inhibition or on biofilm formed over restorations. However, our results corroborate those found by Lobo et al³⁴ which

used an *in vitro* microbiological caries model to evaluate the anticariogenic potential of a fluoride-containing adhesive system including adhesive containing MDPB/F (Clearfil Protect Bond).

Conversely, the present microbiological results neither showed statistical differences between groups, nor confirmed earlier *in vitro* studies^{5,7,8,11,12} that found bacterial inhibition by MDPB. One possible explanation may be due to experimental differences between these studies, which did not evaluate the antibacterial potential of MDPB in a microbiological caries model, but only its capacity of inhibiting bacterial growth on surfaces with MDPB-containing materials. Moreover, MDPB incorporation in a composite resin may be favorable to decrease bacterial growth over restorative materials,⁸ since a bigger contact area with the microorganisms is obtained by means of MDPB immobilization in the polymeric matrix. This fact is not observed in adhesive systems, because MDPB is restricted only to the resin-tooth interface.

The fluoride released from CB group (Clearfil Protect Bond) was expected to reduce carious lesions formation, because minimal amounts of fluoride have been shown to reduce demineralization and enhance remineralization.³⁵ However, this was not evidenced by the microhardness results. It can be suggested as it were used in small amounts, the fluoride released might not have been sufficient to control the cariogenic challenge, as previously demonstrated by *in vitro* studies.^{34,35}

The microhardness values, though not significant, for the AB (All Bond SETM) group were lower than the other group in most evaluated depths at both distances from the restoration margin. This inferior performance can be partially explained by the presence of MDPB and/or fluoride in the CB adhesive, the latter presenting a trend to inhibit demineralization around restorations. Another possible reason is that the incomplete polymerization of the acidic monomer might increase the demineralization.



Graph 1: Relationship between microhardness and depth values. AB $y = 124.6 \pm 18.8 \times r^2 = 0.681$; CPB $y = 140.4 \pm 22.6 \times r^2 = 0.698$ (at 20 µm); AB $y = 130.9 \pm 18.5 \times r^2 = 0.618$ CPB $y = 171.6 \pm 18.0 \times r^2 = 0.620$ (at 70 µm)

One of limitations of the current study was the lack of evaluation of presence of wall lesions, since microhardness analysis was performed to access enamel demineralization. Another one is the impossibility to study the characteristics of biofilms formed within the interfacial spaces adjacent to restorations, due to the difficulties of sampling procedures for such microspaces. This is an important point because the major benefit from antibacterial adhesive systems over secondary caries could be the inhibition of wall lesion formation. Itota et al¹⁶ using a microbial caries system and microradiography, studied the effect of fluoride present in an adhesive system on secondary caries inhibition. The authors observed that, instead of wall lesions, an acid-resistant layer was formed adjacent to the restoration, but there was only a modest inhibition of the outer lesion formation.

In the condition of this *in situ* study, the following conclusion may be drawn: the incorporation of fluoride or MDPB to adhesive systems was not able to inhibit enamel demineralization around composite resin restorations, neither to kill cariogenic bacteria in the biofilm formed over restorations. However, further clinical studies are necessary to evaluate the impact of the anticariogenic efficacy of MDPB or fluoride incorporation to adhesives systems on secondary caries development, mainly in patients with low compliance with prophylactic measures or limited access to other sources of fluoride.

ACKNOWLEDGMENTS

We thank the volunteers for their valuable participation. The first and third authors received scholarships during this study from FUNCAP (0455/07) and FUNCAP/PIBIC (62558862391). This paper was based on a thesis submitted by the first author to School of Pharmacy, Dentistry and Nursing of the Federal University of Ceará, in partial fulfillment of the requirements for an MSc degree in Dentistry. The authors especially thank the Oral Biochemistry Laboratory of Piracicaba Dental School for the use of their microhardness tester. The # 2294 cylindrical diamond burs were donated by KG Sorensen, São Paulo, SP, Brazil.

REFERENCES

- De Munck J, Van Meerbeek B, Inoue S, Vargas M, Yoshida Y, Armstrong S, Lambrechts P, Vanherle G. Microtensile bond strengths of one-and two-step self-etch adhesives to bur-cut enamel and dentin. *Am J Dent* 2003;16:414-420.
- Mjor IA. Clinical diagnosis of recurrent caries. *J Am Dent Assoc* 2005;136:1426-1433.
- Irie M, Suzuki K, Watts DC. Immediate performance of self-etch versus system adhesives with multiple light-activated restoratives. *Dent Mater* 2004;20:873-880.
- Chigira H, Yukitani W, Hasegawa T, Manabe A, Itoh K, Hayakawa T, Debari K, Wakumoto S, Hisamitsu H. self-etch dentin primers containing Phenyl-P. *J Dent Res* 1994;73:1088-1095.
- Imazato S. Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 2003;19:449-457.
- Imazato S, Kuramoto A, Takahashi Y, Ebisu S, Peters MC. In vitro antibacterial effects of the dentin primer of Clearfil Protect Bond. *Dent Mater* 2006;22:527-532.
- Imazato S, Kinomoto Y, Tarume H, Torii M, Russell RRB, McCabe F. Incorporation of antibacterial monomer MDPB into dentin primer. *J Dent Res* 1997;76:768-772.
- Imazato S, Imai T, Russell RRB, Torii M, Ebisu S. Antibacterial activity of cured dental resin incorporating the antibacterial monomer MDPB and an adhesion-promoting monomer. *J Biomed Mater Res* 1998;39:511-515.
- Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. *Dent Mater* 2003; 19:313-319.
- Imazato S, Torii M, Tsuchitani Y, McCabe JF, Russell RRB. Incorporation of bacterial inhibitor into resin composite. *J Dent Res* 1994;73:1437-1443.
- Özer F, Ünlü N, Karakaya S, Ergani O, Hadimli HH. Antibacterial activities of MDPB and fluoride in dentin bonding agents. *Eur J Prosthodont Dent* 2005;13:139-142.
- Imazato S, Ehara A, Torii M, Ebisu S. Antibacterial activity of dentine primer containing MDPB after curing. *J Dent* 1998; 26:267-271.
- Benelli EM, Serra MC, Rodrigues-Jr AL, Cury JA. In situ anticariogenic potential of glass ionomer cement. *Caries Res* 1993;27:280-284.
- Bradshaw DJ, Marsh PD, Hodgson RJ, Visser JM. Effects of glucose and fluoride on competition and metabolism within in vitro dental bacterial communities and biofilms. *Caries Res* 2002;36:81-86.
- Nyvad B, Fejerskov O. Active root surface caries converted into inactive caries as a response to oral hygiene. *Scand J Dent Res* 1986;94:281-284.
- Itota T, Nakabo S, Iwai Y, Konishi N, Nagamine M, Torii Y. Inhibition of artificial secondary caries by fluoride-releasing adhesives on root dentin. *J Oral Rehabil* 2002;29:523-527.
- Imazato S, Imai T, Ebisu S. Antibacterial activity of proprietary self-etch primers. *Am J Dent* 1998;11:106-108.
- Imazato S, Torii Y, Takatsuka T, Inoue K, Ebi N, Ebisu S. Bactericidal effect of dentin primer containing antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB) against bacteria in human carious dentin. *J Oral Rehabil* 2001; 28:314-319.
- Gondim JO, Duque C, Hebling J, Giro EMA. Influence of human dentine on the antibacterial activity of self-etch adhesive systems against cariogenic bacteria. *J Dent* 2008;36:241-248.
- Rodrigues LK, Nobre Dos Santos M, Featherstone JD. In situ mineral loss inhibition by CO₂ laser and fluoride. *J Dent Res* 2006;85:617-621.
- Hujoel PP, Deroguen TA. Validity issues in split-mouth trials. *J Clin Periodontol* 1992;19:625-627.
- Yamamoto K, Arai K, Fukazawa K, Fukui K, Nagamatsu K, Kato K, Nakagaki H, Robinson C. Effect of Plaque Fluoride Released from a Glass-Ionomer Cement on Enamel Remineralization in situ. *Caries Res* 2005;39:157-160.

23. Hara AT, Queiroz CS, Paes Leme AF, Serra MC, Cury JA. Caries progression and inhibition in human and bovine root dentine in situ. *Caries Res* 2003;37:339-344.
24. Cury JA, Rabelo MA, Del Bel Cury AA, Derbyshire MT, Tabchoury CP. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. *Caries Res* 2000;34:491-497.
25. Kielbassa AM, Wrbas KT, Schulte-Mönting J, Hellwig E. Correlation of transversal microradiography and microhardness on in situ-induced demineralization in irradiated and non-irradiated human dental enamel. *Arch Oral Biol* 1999;44:243-248.
26. Tenuta LM, Ribeiro CC, Goncalves NC, Del Bel Cury AA, Aires CP, Tengan C, et al. The short-term in situ model to evaluate the anticariogenic potential of ionomeric materials. *J Dent* 2005;33:491-497.
27. Pecharki GD, Cury JA, Paes Leme AF, Tabchoury CPM, Del Bel Cury AA, Rosalen PL, et al. Effect of Sucrose Containing Iron (II) on Dental Biofilm and Enamel Demineralization in situ. *Caries Res* 2005;39:123-129.
28. Cury JA, Tenuta LM, Ribeiro CC, Paes Leme AF. The importance of fluoride dentifrices to the current dental caries prevalence in Brazil. *Braz Dent J* 2004;15:167-174.
29. Domenick ZT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. *BMC Oral Health* 2006;6:1-13.
30. Mullen J. History of water fluoridation. *Br Dent J* 2005;8:1-4.
31. Duggal MS, Toumba KJ, Amaechi BT, Kowash MB, Higham SM. Enamel demineralization in situ with various frequencies of carbohydrate consumption with and without fluoride toothpaste. *J Dent Res* 2001;80:1721-1724.
32. Lobo MM, Gonçalves RB, Pimenta LAF, Bedran-Russo AKB, Pereira PNR. In vitro evaluation of caries inhibition promoted by self-etch adhesive systems containing antibacterial agents. *J Biomed Res B Appl Biomater* 2005;75:122-127.
33. Rose RK, Turner SJ. Fluoride-induced enhancement of diffusion in streptococcal model plaque biofilms. *Caries Res* 1998;32:227-232.
34. Rolland SL, McCabe JF, Robinson C, Walls AWG. In vitro biofilm formation on the surface of resin-based dentine adhesive. *Eur J Oral Sci* 2006;114:243-249.
35. Peris AR, Mitsui FHO, Lobo MM, Bedran-Russo AKB, Marchi GM. Adhesive systems and secondary caries formation: Assessment of dentin bond strength, caries lesions depth and fluoride release. *Dent Mater* 2007;23:308-316.