QUANTITATIVE EVALUATION OF ADHESION OF *STREPTOCOCCUS MUTANS* TO THREE ORTHODONTIC ADHESIVES : AN *IN VITRO* STUDY

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Abstract

The aim of this study was to quantitatively evaluate the affinity of Streptococcus mutans *to three types of orthodontic adhesives. 108 crown-specimens free of caries and other enamel defects were selected and calibrated to 4x3 mm2 with 2mm thickness. Thirty six standard maxillary lateral incisor brackets were bonded to enamel calibrated specimens with Transbond XT™ (control, group1) and compared respectively to two groups of similar samples bonded with "Transbond Plus™ Self Etching Primer and Transbond XT™"- group 2 (Fluor), "Clearfil Protect Bond™with Kurasper F™" -group 3 (Antibacterial with Fluoride). The cellular culture method for counting adhered* S. mutans *was "Kit Dentocult".*

There was a significant difference in reducing bacteria in group 2 releasing fluoride when compared to group 1, and a non-statistically difference in group 3 incorporating antibacterial monomer MDPB.

Transbond Plus™ enhances antibacterial capacity due to the fluoride release pattern called "burst effect". Clearfil Protect Bond™ limits external action around brackets in an inhibitory bacteriostatic effect.

Keywords: *Streptococcus mutans - fluoride - antibacterial agent.*

ÉVALUATION QUANTITATIVE DE L'ADHÉSION DE STREPTOCOQUES MUTANS À TROIS ADHÉSIFS ORTHODONTIQUES : UNE ÉTUDE *IN VITRO*

Résumé

L'objectif de cette étude in vitro est de comparer l'adhésion des *S. mutans* sur trois groupes d'adhésifs différents.

108 couronnes dentaires exemptes de carie et autres défauts amélaires sont choisies et calibrées à 4x3 mm2 avec 2mm d'épaisseur. Un échantillon de 36 brackets collés sur ces facettes d'émail avec du "Transbond XT™" (groupe de contrôle) est comparé respectivement à deux groupes d'échantillons identiques collés avec deux adhésifs différents "Transbond Plus™ Self Etching Primer" avec "Transbond XT™" (fluor) et "Clearfil Protect Bond™" avec pâte "Kurasper F™" (antibactérien et fluor). La méthode de culture cellulaire, Kit Dentocult, est adoptée pour compter les bactéries.

Une différence significative est notée avec le groupe 2 qui dégage le fluor comparé au groupe 1 de contrôle et pas de différence significative avec le groupe 3 immobilisant le monomère MDPB dans sa résine matrice.

Le Transbond Plus™ améliore la capacité antibactérienne due au mode de dégagement de fluor appelé « effet de salve » ou « burst effect ». Le Clearfil Protect™ Bond limite l'action externe autour des brackets par un effet inhibiteur bactériostatique.

Mots clés : *Streptococcus mutans* – fluor - agent antimicrobien.

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Introduction

Patients receiving orthodontic treatment have alterations in their oral cavity, such as drop in ph and creation of additional retentive sites for food and S*treptococcus mutans* (S. *mutans*); these changes increase the levels of these microorganisms in saliva and in dental biofilm [1].

Despite the advances in orthodontic materials and treatment mechan- ics, the placement of fixed appliances is still associated with a high risk of developing white spot lesions, mainly in patients with bad oral hygiene, one month after the placement of the appli- ances. Patients often have difficulty maintaining adequate oral hygiene with orthodontic appliances attached directly to the teeth. The increased plaque accumulation and the concom- itant bacterial acid production result in decalcification by diffusion of calcium and phosphate ions out of enamel [2]. The prevalence of new decalcifications among orthodontic patients with fixed appliances is reported to range from 13% to 75% [3, 4]. The enamel demin- eralization is caused by organic acids produced mainly by S. *mutans*, which have been shown to be the prime caus- ative organisms of dental caries [5, 6]. The increased prevalence of enamel decalcification during fixed appliance therapy is partially due to the irregular

surfaces of brackets, bands, wires and other attachments, which create stagnation areas for plaque, render tooth cleaning more difficult, and limit naturally occurring self-cleaning mechanisms, such as the movement of the oral musculature and saliva [7].

Among the many orthodontic appliances, orthodontic brackets have a significant role in enamel demineralization because they are attached to the dentition continuously during almost all orthodontic treatment period. Their complex design provides a unique environment that impedes access to the tooth surfaces for cleaning [8] thus, creating common sites for demineralization at the junction between the bonding adhesive and the enamel [9].The orthodontic adhesives remaining on the enamel surface around the bracket are known to be risk factors for predisposition to enamel demineralization because the rough adhesive surface can provide a site for the rapid growth of oral microorganisms [10]. The adhesion of bacteria to surfaces forms an important initial stage in dental plaque formation and enamel demineralization.

Preventing these lesions during treatment is an important concern for orthodontists because the lesions are unaesthetic, unhealthy and potentially irreversible [11]. Approaches to inhibit the development of carious lesion in patients with fixed appliances have focused on the control of the bacterial bio-film around the brackets and on the maintenance of a constant fluoride level in the oral cavity [12].The potential advantage of a bracket-bonding material with sustained release of fluoride is that a continuous release of fluoride would be possible adjacent to the bracket, the area at highest risk for decalcification. Furthermore, the need for patient compliance is less than with self-administered delivery of fluoride.

Differences in bacterial adhesion to the different orthodontic adhesives may be expected because of their different characteristics and the release of incorporated fluoride [10]. Fluoride-releasing bonding material showed
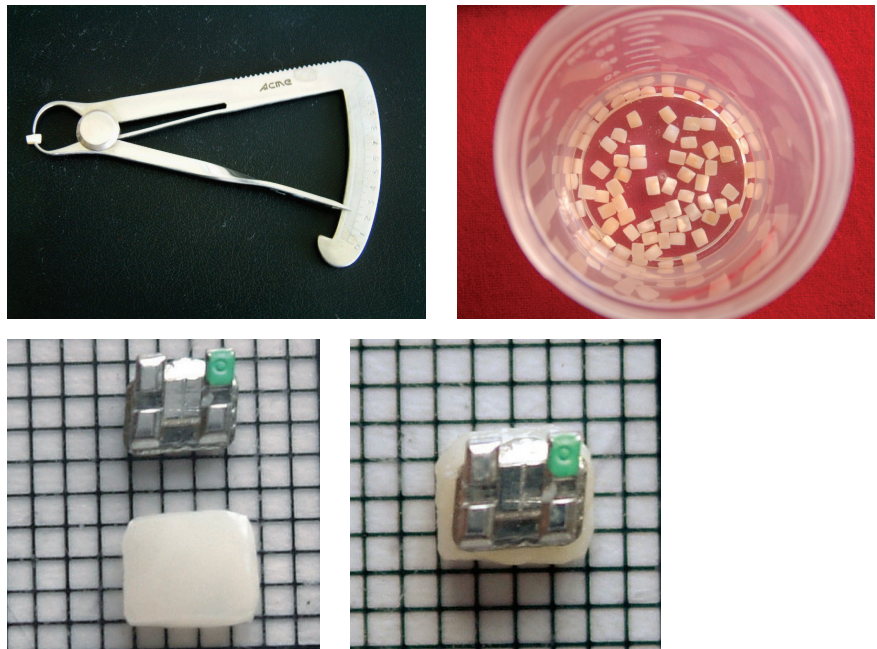

Fig.1: Preparing and calibrating crown specimens.

almost no demineralization-inhibiting effect. It has been suggested that the combined use of antimicrobials and fluoride enhances the cariostatic effect of fluoride [13].

The objective of this study is to quantitatively evaluate the affinity of S. *mutans* to three types of orthodontic adhesives for comparing the effect of fluoride release and the combination of a new antimicrobial primer on the adhesion of these bacteria relative to incubation time.

Materials and methods

Specimens

A total of 54 caries-free human incisors and premolars, stored for a maximum of 3 months in aqueous 1% chloramines-T solution, were used in this investigation. After detachment of two-thirds of the root and elimination of all soft tissue structures, the roots of the teeth were cut off with a cooled, oil-free diamond disk, and the buccal and lingual surfaces were separated to obtain two surfaces per tooth. From these 54 teeth, 108 crown-specimens free of caries and other enamel defects were selected and calibrated to 4x3 mm^2 with 2mm thickness (Fig.1).

All specimen surfaces were polished with a rubber cup and fluoride-free pumice, sprayed with water and dried with a compressed oil-free air stream. All teeth were bonded with low profile brackets (American Orthodontics ™), MBT system, upper lateral incisor left bracket. The average surface of the bracket base was 4x3=12 mm^2 for the upper lateral incisor brackets with a standardized methodology.

Bonding procedures

The teeth were randomly allocated into three groups of 36 specimens each, as follows:

Group 1 (control): the enamel surfaces were etched with 37% phosphoric acid for 30 seconds, rinsed for 10 seconds, and dried with oil-free and moisture-free air until the enamel had a faintly white appearance. Transbond XT™ primer (3M Unitek) was applied to the etched surface in a thin film and light cured for 10 seconds. Transbond XT™ adhesive paste (3M Unitek) was applied to the bracket base, and the bracket was then positioned on the tooth crown and pressed firmly to expel
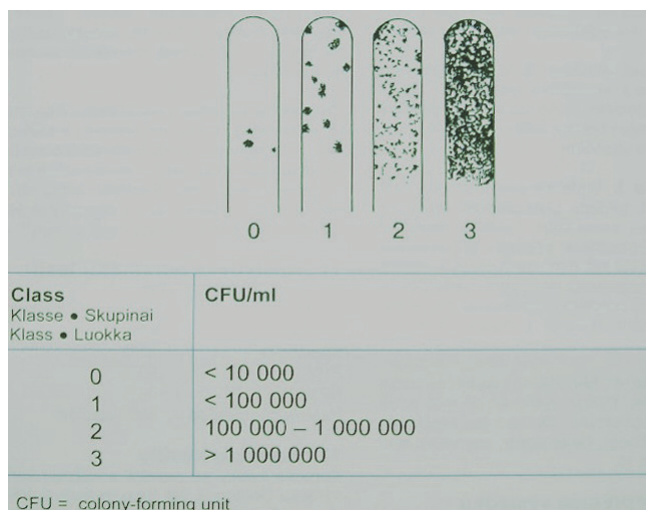
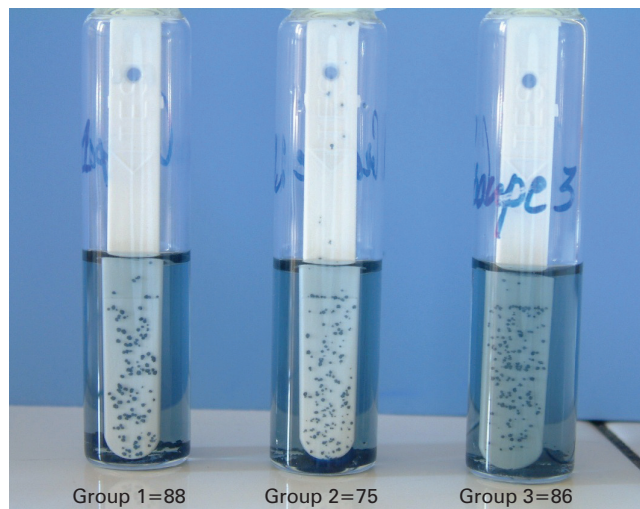Fig.2: Number of adherent bacteria on the strips = the number of colony-forming unit CFU/ml (Kit Dentocult).

Fig. 3: Number of Streptocoque mutans adhered to the strips in the first test.

excess adhesive. The excess adhesive was removed around the bracket base, and the adhesive was light-cured for 20 seconds from the mesial and from the distal sides.

Group 2 (Transbond Plus™ Self Etching Primer): the enamel was treated with Transbond Plus™ Self Etching Primer (3M Unitek), which was gently rubbed onto the surface for approximately 15 seconds with the disposable applicator supplied with the system. A moisture-free air source was used to deliver a gentle burst of air to the primer for 3 seconds. The bracket was bonded with the same bonding resin and light cured as for the control group.

Group 3 (Kurasper F™ with Clearfil Protect Bond™, Kuraray Medical Inc.): a two- step self-etching primer Clearfil Protect Bond™ was applied as suggested by the manufacturer. After a gentle application for 20 seconds, the primer was dried with a mild air flow, then Clearfil Protect Bond™ was applied, gently air flowed, and light cured for 10 seconds. After these steps, the brackets were coated with Kurasper F™ paste, and light cured from both the mesial and distal sides for 20 seconds each [13].

Adhesion of Streptococcus mutans to orthodontic brackets

The 36 brackets of each type of adhesive were collected. We prepared a solution of suspension bacteria, S. *mutans* isolated (ATCC 25175), at a concentration of 0.5McFarland to charge these specimens and control the effects of bacterial adhesion.

Effect of adhesive type

The experiments were done three times to respect the reliability and reproducibility: our 108 specimens, 36 brackets of each type of adhesive, were divided to 12 brackets at each time, for three times.

In a first experiment, 12 brackets of each type were placed in 3 sterile numbered tubes. A 5ml of S. *mutans* was added to each tube. The brackets with the bacterial suspension were aerobically incubated at 37^0C for 90 minutes, with intermittent shaking. Afterwards, the brackets were rinsed twice carefully with NaCl 0.9% to remove any non-adherent bacteria [14].

Culture of adhering bacteria

For each experiment, and after washing with PBS, the brackets with

their adhering bacteria were treated with 2 ml of 0.25% Trypsin /EDTA for 45 minutes in aerobic conditions at 37^0C, for detachment of the adherent bacteria.

The Kit of Dentocult SM Strip Mutans (Orion Diagnostica) was used to detect S. *mutans* for *in vitro* diagnostic only. The method is based on the use of selective culture and growth of S. *mutans* on the test strip. Strips were inserted in these solutions for five minutes. The bacitracin discs were placed in the selective culture vials 15 minutes before, and then strips were transferred to these vials and incubated for 48 hours at 37°C. The final step was the counting of adherent bacteria on the strips, and the number of colony-forming unit (CFU)/ml (Fig. 2).

Calculations and statistics

These experiments were repeated three times, 12 brackets at each time to respect the reliability of results and to obtain a sample of 36 brackets in each group. Parametric tests with descriptive statistics mean and variance for quantitative variables were used in these tests (number of CFU/ml). Counting number of colonies

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	Group 1	Group 2	Group 3
Test 1 n=12 Brackets	88	75	86
Test 2 n=12 Brackets	87	73	85
Test 3 n=12 Brackets	85	76	82
Total N= 36	260	224	253

Table 1. Number of Streptocoque mutans sticked to the three groups of adhesives.

	Group 1	Group 2	Group 3
	N1=36	N2=36	N3=36
Mean	86.66	74.66	84.33
SD	2.33	2.33	4.33

Table 2. Mean and variance of *S. mutans*.

Matching groups	Residual Variance	F	p-value
Group 2 *vs* Group 1	2.33	91.78	0.00012****
Group 3 *vs* Group 1	3.33	2.45	0.156

Table 3. One-Way ANOVA* compare the adherence of *S. mutans* between three groups of adhesives.
* ANOVA indicates analysis of variance.

was done blindly by another operator. Analytic statistic "ANOVA" was used to compare these 3 groups, with a statistical significance $p<0.05$.

Results

Table 1 shows the number of S. *mutans* that were attached to the strips of the three groups of adhesives. Note that the first test showed blue spots representing the number of colonies of S. *mutans* (Fig.3). S. *mutans* were the least observed in group 2 (Table 1).

Table 2 shows higher mean in group 1 and lower mean in group 2. The variance in group 3 (4, 33) was higher than the two other groups. When the adhesion of S. *mutans* on different types of adhesives was tested, ANOVA showed significant difference ($p=0.00012$) between group 1 and group 2, and no statistically significant difference ($p=0.156$) between group 1 and group 3 in spite of a higher mean in group 1 which has not reached a degree of significance (Table 3).

Discussion

Approaches to inhibit the development of carious lesions in patients with fixed appliances have focused on the control of the bacterial biofilm around the brackets and on the maintenance of a constant fluoride level in the oral cavity.

There was a significant difference between fluoride adhesive (Transbond Plus™ Self-Etching Primer) and control group Transbond XT™. No significant difference was found with antimicrobial group (Clearfil Protect Bond™ + Kurasper F™) in spite of a slight difference between the mean of group 3 (84, 333) and the mean of the control group (86, 666).

Difference between fluoride and control group was expected. Transbond XT™ and Transbond Plus™ are composite resin-based cements from the same manufacturer (3M Unitek). Transbond XT™ did not exhibit any antibacterial phenomena, similar to other composite resin materials tested by the same methodology. However, Transbond Plus™ had antibacterial capabilities for at least 24 hours. This might be attributed to components added to the material by the manufacturer, such as fluoride, which is absent in Transbond XT™.

Incorporation of fluorides into dental materials, as well as into orthodontic cements, is based on the concept that fluoride will be released gradu-

ally from the set-material in vivo, thus providing continuous long-acting anti-cariogenic effect which is due primarily to changes in enamel solubility [15].

These fluoride-releasing materials show a "burst-effect" fluoride-release pattern, the greatest amount of fluoride is released within the first day. With a rapid decline to much lower levels, it is important to evaluate the usefulness of these materials as fluoride "reservoirs" during the average orthodontic treatment time of 2 to 3 years. The study of Chatzistavrou et al. [16] confirmed the "burst-effect" more specifically, the initial high fluoride release of the first day of the experiments decreased to almost half in 3 days, and then continued to drop to third in 7 days, seventh in 30 days, and fourteenth in 60 days.

Fluoride from bonding adhesives is delivered to the enamel at the peripheral margin of the bonding adhesive, where it can form the demineralization-resistant Fluor apatite on the enamel surface. Several studies have shown that the therapeutic effect of fluoride released in sustained small doses can protect enamel at the periphery of the orthodontic bracket, where most decalcification occurs clinically in
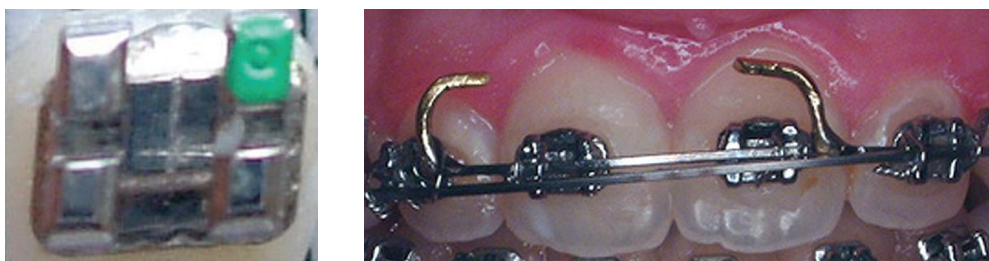
Fig.4: Comparing critical marginal zones around bracket a) *in vitro* and b) *in vivo*.

orthodontic patients. This is also consistent with our results.

The non-significant difference between anti-microbial and control group was unexpected. Furthermore, results obtained from various *in vitro* tests should be carefully evaluated for the methodology used before the findings are interpreted. The two-step self-etching adhesive system used in this study (Clearfil Protect Bond™) contains MDPB, an antibacterial monomer found in antibacterial adhesives. Imazato et al. [17-19] have been conducting investigations on the utilization of MDPB since 1997. Unlike its fluoride-release effect, the antibacterial activity of MDPB may not extend around the bracket, thus producing a potential limitation action against bacteria attacking his surface. We join these findings with our results: the calibrated identical specimens were bonded to standard lateral incisor bracket with the only variable, the bonding material which occupied only 3% of the all surface area immersed in the bacterial suspension. The action of MDPB was only bacteriostatic inhibiting 3% of bacterial growth comparing to control group.

Assumptions of these clinical implications, *in vitro*, need to be transferred *in vivo*. The adhesion of bacteria on brackets and at the periphery of bonding adhesives would seem to be more complicated, in a situation like the oral cavity where interactions between the salivary pellicle, many different bacteria, and bracket's surface characteristics take place [20-23].

When comparing the fluoride group (Transbond XT™ + Self Etching Primer) with the control one (Transbond XT™) our findings join some studies and reject others. The studies by Cohen et al. [24] and Chatzistavrou et al. [16] showed that initial burst of fluoride release material or the burst effect fluoride release pattern is the greatest within the first few days, especially the first 24 hours.

The study of Sug-Joon Ahn et al. [8] showed that fluoride release from the orthodontic adhesive cannot alter the adhesion patterns of cariogenic streptococci. There was no difference in the adhesion amount between fluoride-releasing and non–fluoride-releasing composites. This can be explained by the fact that the orthodontic bonding adhesive may release fluoride at a rate that affects enamel demineralization rather than bacterial adhesion.

When comparing the antibacterial group (Clearfil Potect Bond™ +Self Etching Primer with the control one (Transbond XT™), we have reported findings concerning *in vitro* antibacterial effect corresponding to anterior studies: MDPB has been used since 1995 and incorporated into the self-etching primer and adhesive resin. MDPB copolymerizes with other monomers after curing, and the antibacterial agent is covalently bonded to the polymer network. The immobilized agent does not leach out from the material but acts as a contact inhibitor against the bacteria that attach to the surface. Unlike its fluoride-release effect, the antibacterial activity of MDPB may not extend around the bracket thus, producing a potential limitation effect (Fig.4).

The combined application of Clearfil Protect Bond™ and Kurasper F-Bond™ is claimed to release sufficient fluoride and to have an antimicrobial effect in the micro-environment around the bracket, the region where demineralization lesions occur. In an *in vitro* study, Korbmacher et al. [11] combined two products offered by one manufacturer, Kurasper F-Bond™ with the antimicrobial self-etching primer Clearfil Protect Bond™. In addition to its antibacterial characteristics, Clearfil Protect Bond™ is claimed to release fluoride, but according to the manufacturer, Kurasper F™ has the potential to release four times more fluoride. Therefore, the etched enamel was precoated with Kurasper F-Bond™ because only this component will release sufficient fluoride within a shorter time. We think it was a great advantage combining these two products, but in this situation we are facing a technical concept mistake mixing two different bonding procedures at the same time; one conventional technique with its three classic phases and another two phases self-etching primer technique. Further research is needed to clarify whether these materials are sufficiently effective inhibiting bacterial activity under *in vivo* conditions.

Conclusion

In clinical settings bonding systems are exposed to a number of different intraoral factors. Nevertheless, *in vitro* testing still remains a necessity for the initial evaluation of bonding systems.

Incorporation of fluorides into orthodontic adhesives as Transbond Plus™ enhances the antibacterial capacity the first few days, called "burst effect" fluoride release pattern.

Immobilization of MDPB into Clearfil Protect Bond™ limits his external action around brackets on an inhibitory bacteriostatic effect on the growth of S. *mutans* attacking its small surface which represents 3% of all the surface area.

The surface characteristics of the brackets affect the amount of adhesion which is governed by thermodynamic rules. Stainless steel with a high surface-free energy will attract more bacteria to its surface than a material with a low surface-free energy.

Acknowledgments

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