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An In Vitro Study of Plaque Deposition and its Comparative Effect on Bond Strength

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AN IN VITRO STUDY OF PLAQUE DEPOSITION AND ITS COMPARATIVE EFFECT ON BOND STRENGTH

by

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Each person whose signature appears below certifies that this thesis in their opinion is adequate, in scope and quality, as a thesis for the Master of Science degree in Orthodontics.

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ABSTRACT

AN IN VITRO STUDY OF PLAQUE DEPOSITION AND ITS COMPARATIVE EFFECT ON BOND STRENGTH

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Treatment with fixed orthodontic appliances can change the environment in the oral cavity by creating stagnant areas that facilitate bacterial colonization and substance retention. The purpose of this study was to examine in vitro plaque formation associated with bonded orthodontic appliances and how this could affect bonding strengths.

Ortho Concise and Transbond XT were the composite resin materials used in this study. Mandibular incisor metal brackets were bonded to bovine mandibular anterior teeth. Each composite resin was studied in two groups: plaque growth and without plaque growth, and their bond strengths were compared at time intervals of 3,45,and 180 days post bonding.

The plaque and control groups were sterilized by steam autoclave and placed in the Streptococcus mutans culture media or sterile saline. All samples

were "debonded" by using an Instron Universal Testing machine, simulating shear failure.

Bond strength was significantly reduced in the 45 and 180 day plaque samples for both adhesives. The 180 day plaque sample had bond strengths similar to the 45 day sample, therefore, the length of time to cause this effect could be clinically brief. Plaque also affected the adhesive fracture site at debonding. This study has proven a relationship between plaque deposition and bond strength, but clinically this relationship may not result in "spontaneous" debonding of brackets.

INTRODUCTION

The presence of fixed orthodontic appliances during treatment changes the environment in the oral cavity by creating stagnant areas that allow increased bacterial colonization and substance retention.[1] These same appliances also interfere with effective oral hygiene methods and often cover considerable parts of the tooth surfaces with metal, ceramic and composite materials. Experiments have shown that changes in the oral environment have been characterized by a local drop in pH and an increase of carbohydrate, streptococci and lactobacilli.[2,3]

In the orthodontic patient not only is there is an apparent increase in plaque, but there is also a higher concentration of bacteria and carbohydrates in each mg of plaque.[2] The enamel surface can decalcify more readily due to the acid-producing bacteria found in this plaque surrounding the bracket. Demineralization and gingivitis have been shown to occur predominately around the gingival portion of bonded/banded orthodontic appliances.[4,5] It is important to note that the inflammatory status of the marginal gingiva has an important effect on early supragingival plaque accumulation.[6] The tooth surface immediately adjacent to the gingival margin is the site of initial deposition of oral bacteria as well as the main zone of plaque growth.[6] The increase in plaque mass over time has been highly associated with multiplication of bacteria rather than continued deposition.[6] Efforts to minimize gingival irritation are possible by using brackets with small bonding bases, and the careful clean up of any excess adhesive around the bases.[5,7,8]

A correlation has been shown between the ability of the bacteria to attach to a surface and then to colonize. Bacteria have shown to have a highly developed recognition system that is capable of recognizing and interacting with specific macromolecules to oral tissue surfaces.[9] The initial attachment mechanism of bacteria is an important factor governing further colonization. This mechanism may be different between the oral cavity environment with or without orthodontic appliances. It has been shown that restorative materials, such as amalgam and composite, may influence not only the adhesion of plaque bacteria, but also the concentration of Streptococcus mutans in plaque.[10] Therefore, it is probable that orthodontic appliances with similar materials in bonding agents produce comparable bacteria responses.

The purpose of this study is to examine in vitro plaque formation associated with bonded orthodontic bracket appliances, and how this could affect bond strength. There are three major requirements for the formation and permanency of an adhesive bond: 1) intimate contact between the liquid adhesive and the solid adherend (wetting), 2) minimal stress concentration at the interface, and 3) minimal attack on the interface by environmental factors.[11] Plaque is believed to affect some of these major requirements, altering the shear/peel strength of the appliance-adhesive tooth bond.

MATERIALS AND METHODS

Composite Resin/Brackets

Two commercially available composite resin materials were used for this study; Ortho Concise (3M Unitek) and Transbond XT (3M Unitek). Stainless steel mesh based mandibular incisor brackets from GAC, with the Omni-Roth prescription, were the brackets used in this study. The different composite resin groups had two groups; plaque or without plaque (control), with a sample size of 25. Bond strengths for each resin were compared at time intervals of 3, 45, and 180 days respectively.

Orthodontic brackets were bonded to bovine enamel because it creates a larger surface in which to grow quantifiable amounts of plaque.[4] The enamel surface was cleaned and polished to closely represent human enamel.[4] Eight bovine mandibular teeth were mounted onto a 3 inch diameter denture acrylic disk to provide a base for testing. The acrylic disks were modified with a metal hook to help transfer the disks into the sterile containers for plaque growth.

The enamel surface was etched for 15 seconds with 37% phosphoric acid (in the area of bracket placement), rinsed for 60 seconds under running water, and then air dried. The manufacturer's recommended bonding protocol was followed for each composite resin system and performed by one person for was followed for each composite resin system and performed by one person for all samples. A force gauge was used in bonding all brackets, to exude comparable amounts of excess bonding material, leaving a similar adhesive thickness for all teeth. After bonding and curing, the samples were placed in distilled water for 24 hours to allow further polymerization and to aid in the removal of any uncured components.[4] The plaque and control groups were sterilized by steam autoclave, before they were placed in the bacterial culture media or sterile saline.

It was determined by a pilot study that gas sterilization (ethylene oxide) was not effective in sterilizing the bovine teeth samples that were mounted in the acrylic disks. Imperfect sterilization leads to significant problems with contamination of the samples. An additional study was conducted analyzing the bond strength of bonded appliances sterilized by steam autoclave or gas sterilization, versus a control group of bonded brackets without sterilization. Concise and Transbond XT were both evaluated. Steam autoclave did an acceptable job of sterilizing the bovine teeth samples and had similar bond strength values as the control without sterilization. Therefore, steam autoclave was the method of choice for sterilization of all plaque and control (non plaque) bovine teeth samples.

Plaque Accumulation

Bacterial cultures of Streptococcus mutans (ATCC 25175) were prepared following the method of Dummer and Harrison with a modification from Pack.[4,12] Trypticase soy broth enriched with 5% sucrose served as the culture media. Following Pack's design, the acrylic disks for the plaque samples were placed into individual sterile containers with the culture broth and incubated at 37° C (Figure 1). The acrylic disks were then transferred into a containers of freshly inoculated medium weekly throughout the duration of the study. The control samples were also incubated at 37° C and transferred into sterile saline containers bi-monthly.

At the completion of the time period studied, the plaque deposits were removed from the enamel surface surrounding the appliance. A sterile swab was used to remove the plaque deposits which were then placed in tubes containing 5 ml of sterile media. Each tube combined plaque deposits from 5 teeth. The tubes were centrifuged for 20 seconds. The suspensions were then serially diluted over a range between 10^{-2} and 10^{-6}. Aliquots of 0.1 ml were plated on two trypticase soy agar plates, and incubated at 37° C for 48 hours. The Streptococcus mutan colonies were counted for each plate. Those colonies too numerous to count were assigned the number 500 for statistical evaluation. An average colony-forming unit (CFU) per tooth was calculated for each of the composite groups and the respective time periods. CFU provides a mathematical number to examine Streptococcus mutan colonies found on each bovine incisor.[4]

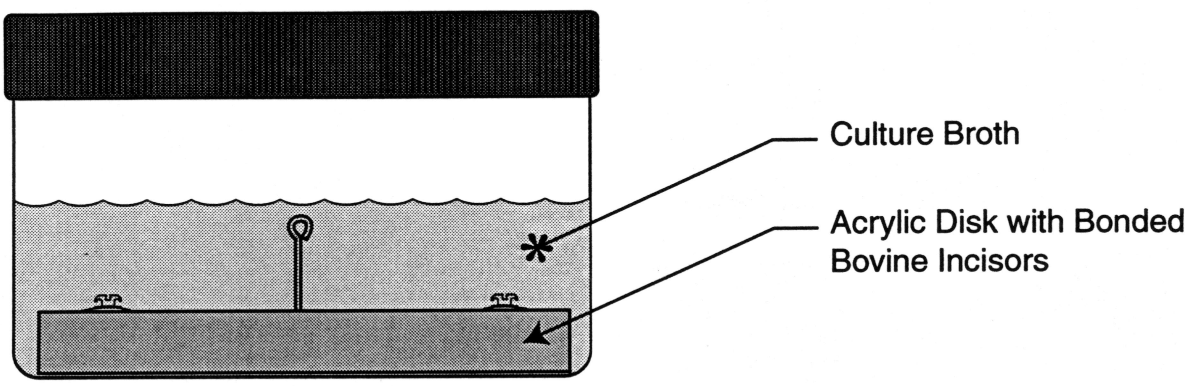

Figure 1. Schematic representation of the plaque samples placed into individualize containers.

Bond Strength

All samples, those with plaque and without, were analyzed for bond strengths by performing a shear/peel test with an Instron Universal Testing machine (# 4204, Los Alamitos, CA.). The vertical axis of the testing machine was set parallel to the tooth surface and shear/peel force was measured.[13]

Figures 2 and 3 illustrate the Instron Universal Testing machine and how tension produced shear and peel forces. The Instron crosshead speed was 5.0 mm/minute for all samples, with the resultant number converted to MPa. At the time of "debonding", the location of failure of the bracket/composite bond was recorded. The notation was as follows: T= all of the composite material remained on the tooth surface; B= all of the composite material remained with the bracket; and BT= composite is found on both the tooth and bracket. This was done in order to determine if a relationship existed between the fracture site and the effects of plaque deposition.

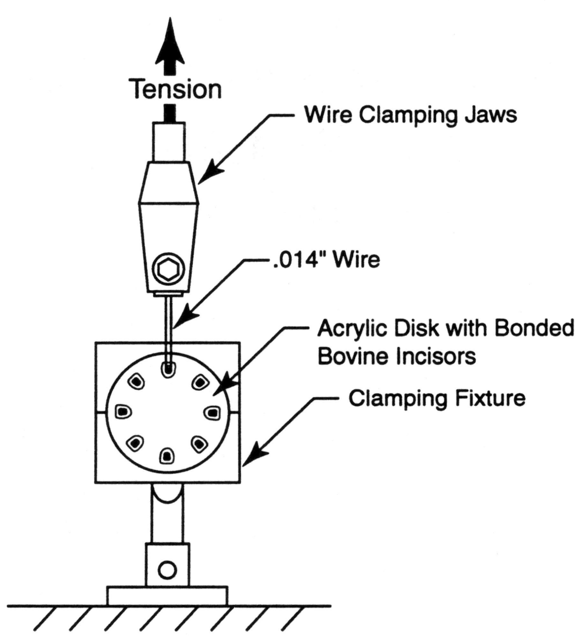

Figure 2. Bonded sample mounted in Instron Universal Testing machine.

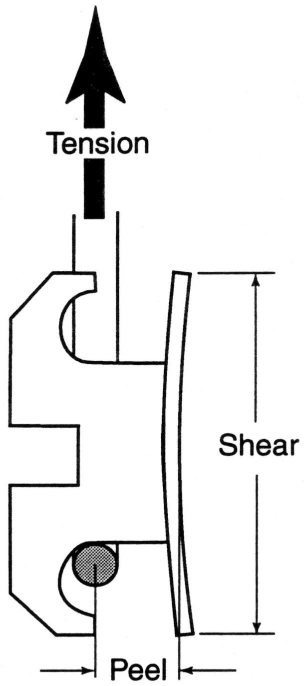

Figure 3. Diagram showing tension producing shear and peel forces.

DATA ANALYSIS

To interpret the data for this study, several statistical analyses were done using the SPSS statistical package with a p-value of 0.05 being significant. Means, standard deviations (SD), standard errors (SE), and confidence intervals were calculated for the bond strengths recorded. Three-way, two-way, and one-way ANOVAs compared bond strengths for the different time periods, any difference among the adhesives, and the presence or absence of plaque. A t-test compared the mean bond strengths for the different adhesive types, in the presence or absence of plaque, for each time period. A Chi Square analysis compared the two different adhesives at each time period, with or without plaque, and for the location of the fracture site at debonding.

RESULTS

Bond strengths were compared for each adhesive and the presence or absence of plaque over time using a three-way ANOVA. There was no three-way interaction (p=0.58), or two-way interaction between adhesive and time (p=0.92) or adhesive and presence or absence of plaque (p=0.21). This indicates that both adhesives reacted similarly over time and plaque affected both adhesives in a similar fashion. There was a significant interaction between time and the presence of plaque (p=0.004).

To evaluate the effect of plaque over time, bond strengths for the combined adhesive samples were compared using a one-way ANOVA. In the plaque samples, bond strength was significantly effected by time (p=0.001) with the 3 day samples having a significantly higher mean bond strength, however bond strength was not significantly different between the 45 and 180 days. In the control samples (absence of plaque), there were no significant changes in bond strengths over the three time periods.

The two adhesives were compared, with and without plaque, for each time period. It was found that at 3 days there was a significant diffference in bond strength between Concise and Transbond XT (p=0.04), and plaque did not affect this strength for either adhesive. The mean bond strength was higher for Concise than Transbond XT at 3 days (Table 1). At 45 days and 180 days, there was no significant difference in bond strength between the adhesives, but plaque affected this strength (45 days p=0.008, 180 days (p=0.026). There was similar variability for the combined adhesive bond strengths in the control (without plaque) and plaque samples, therefore plaque does not make the bond strength more variable.

The confidence intervals in Table 1 give the 95% range of values that the mean bond strength could be for the adhesive group studied. The confidence interval for each adhesive group with the lowest value was the 45 day plaque samples. The Concise/plaque 45 day confidence interval was 14.68-19.06 MPa, and Transbond XT/plaque 45 day was 14.79-18.04 MPa.

GROUP	TIME	MEAN	SD	SE	CONFIDENCE INTERVALS
Concise Control	3 DAYS	20.1	5.7	1.1	17.9-22.3
	45 DAYS	21.2	6.7	1.4	18.5-23.8
	180 DAYS	21.0	6.0	1.2	18.7-23.4
Concise Plaque	3 DAYS	20.8	4.3	0.9	19.1-22.5
	45 DAYS	16.9	5.5	1.1	14.7-19.1
	180 DAYS	18.0	6.5	1.3	15.5-20.6
Trans.XT Control	3 DAYS	17.7	3.9	0.8	16.1-19.2
	45 DAYS	18.1	5.1	1.0	16.1-20.1
	180 DAYS	19.9	5.4	1.1	17.7-22.0
Trans.XT Plaque	3 DAYS	19.4	4.3	0.9	17.7-21.1
	45 DAYS	16.4	4.1	0.8	14.8-18.0
	180 DAYS	17.7	4.9	1.0	15.7-19.6

Table 1. Descriptive statistics for shear/peel bond strength in MPa for mean bond strength, standard deviation, standard error, and confidence intervals.

The mean bond strengths of Concise was higher than Transbond XT at all control time periods (Figure 4). A t-test showed that the mean bond strength, combined over time, for Concise control was significantly greater than Transbond XT (p=0.02). With plaque, the mean bond strengths of the adhesives were not significantly different (p=0.37). Concise's initial higher bond strength diminished significantly with plaque, and then by 45 days was more like Transbond XT.

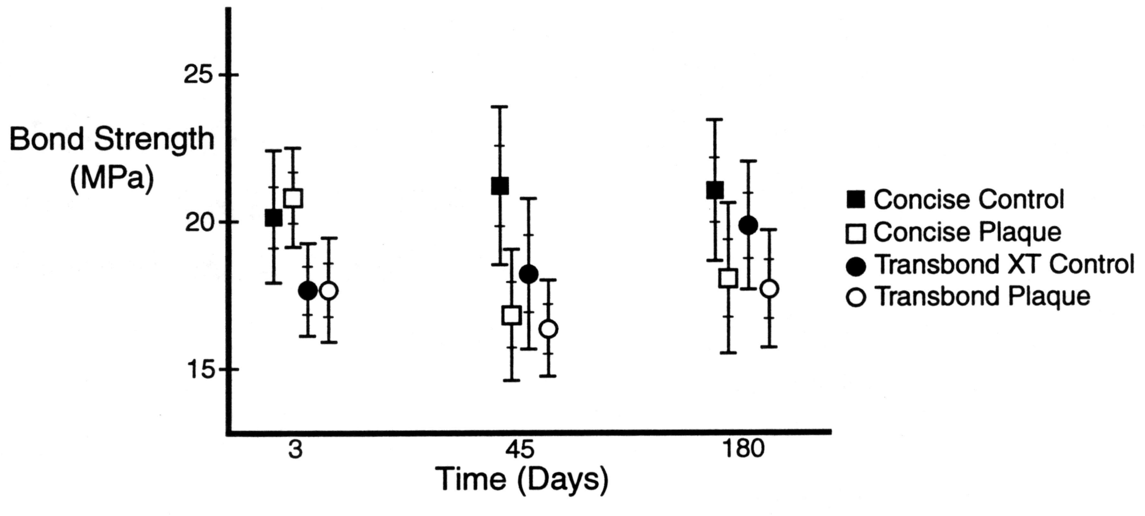

Figure 4. Comparison of the mean bond strengths for Concise and Transbond XT. Heavy outside bars are confidence intervals and the thin inner bars are standard error.

A Chi Square analysis compared the two adhesives for the location and occurrence of the fracture site after debonding for each time period. Figures 5 and 6 indicate where the composite was left after debonding. The majority of all control samples (for both adhesives at all time periods) fall into the category of BT, that is the bracket and tooth both have composite material at the time of debonding. At 45 days, Transbond XT showed a significant change in location of the fracture site (p=0.000). The percentage of BT dropped from the control to the plaque samples, and the bracket (B) and tooth (T) groups increased in percentage with plaque. At 180 days, Concise had a significant change in location of fracture site occurrence (p=0.006). The plaque samples had a decrease in the percentage of BT, and an increase in the B and T groups.

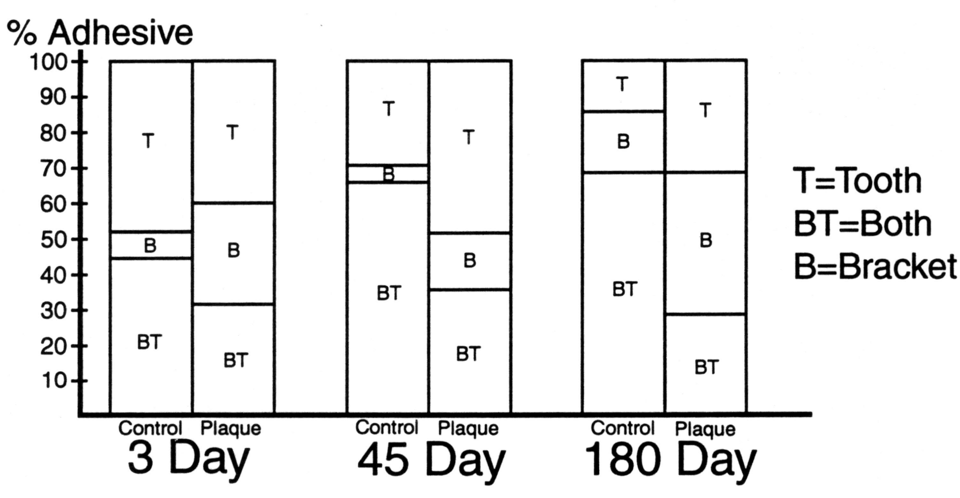

Figure 5. Comparison of percentage of fracture site location for Concise adhesive at all time periods.

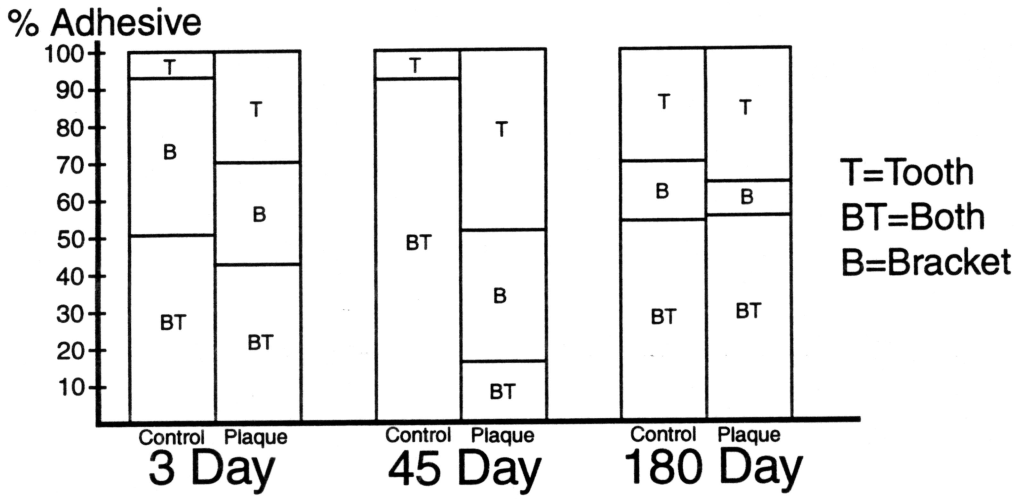

Figure 6. Comparison of percentage of fracture site location for Transbond XT adhesive at all time periods

Concise and Transbond XT were subjected to the same protocol for plaque growth. Each week the acrylic disks on which the 8 bovine mandibular incisors were mounted, were transferred to sterile containers with freshly inoculated bacterial media. An average colony-forming unit (CFU) was calculated for each of the composite types at the completion of the sample time. At 3 days, Concise and Transbond XT had similar amounts (Concise = 40×10^{-7} CFU, and XT = 46.7×10^{-7} CFU). As time passed, Concise accumulated more colonies of plaque per tooth than XT. At 180 days, (Concise = 259.6×10^{-7} CFU, and XT=138×10^{-7} CFU).

Different sterilization methods were tested by comparing bond strengths to a control that was not sterilized. Both Concise and Transbond XT were exposed to gas sterilization (ethylene oxide) and steam autoclave. At the completion of the sterilization procedure, the samples were debonded with the use of the Instron Universal Testing machine with a crosshead speed of 5 mm/min. The control samples that weren't sterilized were also debonded at the same time. The bond strength values were then converted to MPa. The control (without sterilization) mean bond strength for Concise was 18.6 MPa which is very similar to the steam autoclave mean of 18.7 MPa. Gas sterilization for Concise was much higher at 22.7 MPa. The control mean bond strength for Transbond XT was 18.3 MPa which was in between the steam autoclave mean of 17.7 MPa and gas sterilization of 19.1 MPa.

DISCUSSION

Numerous studies have been performed to evaluate the presence of plaque in association with orthodontic appliances. The presence of a bracket and any resin flash around it predisposes to plaque accumulation, with increased risk of demineralization of the surrounding enamel.[5]

This study has provided information on the presence of plaque (Streptococcus mutans) and its influence on bond strength. In comparing samples of the adhesives, with and without plaque for each time period, the 3 day plaque samples for both adhesives were the only ones to have mean bond strength values similar to the control. The 45 and 180 day plaque samples for both adhesives had significantly lower bond strengths than the control. Therefore, being associated with plaque for up to 45 days appeared to be sufficient time to significantly lower the bond strength values and no further effect was found with additional passage of time.

Concise was found to have a higher mean bond strength for all of the control samples and the 3 day plaque sample. In the 45 and 180 day plaque samples, Concise dropped significantly in bond strength and was affected more by plaque than Transbond XT. Concise and Transbond XT have similar strength values in the presence of plaque. This could be related to the results of the CFU count of Streptococcus mutans for the adhesive groups at each time period. The 45 and 180 day CFU counts revealed that Concise had more plaque colonies than Transbond XT. However, the amount of plaque growth for this laboratory study is probably higher than one would find clinically. The protocol for plaque growth of Streptococcus mutans was designed to resemble an orthodontic patient with poor oral hygiene and an abundance of plaque growth. Even though the bond strengths at 45 and 180 days were lower when associated with plaque, clinically this may not be apparent. It is important to encourage good oral hygiene, but plaque growth will not result in "spontaneous" debonding of brackets. The significance of plaque growth and the orthodontic patient may be more evident esthetically, with enamel decalcification and gingivitis, than with a weaker bond strength.

Scanning electron micrographs (SEM) were attempted for both adhesives in the control and plaque samples at each time period. A visual change in the texture of the fracture site for the adhesive between plaque and control groups was anticipated, but the SEM quality was inadequate to display any change in the adhesive structure at any time period.

The location and occurrence of the fracture site at debonding does have clinical relevance. The majority of control tooth samples had a fracture location site with composite found on the tooth surface and the bracket (BT). With plaque, the fracture site decreases in the BT group and increases with more composite staying only on the bracket (B) or on the tooth (T). When composite is found only on the bracket and not on the tooth surface, damage to the enamel during the debonding procedure could be anticipated. If all of the composite is left on the tooth surface then consideration needs to be taken with the different types of finishing procedures to avoid potential damage to the enamel during this process.

Proper sterilization of the bovine teeth samples after the bonding procedure, was of major concern. The teeth had to be sterilized after bonding to minimize any contamination before plaque growth of Streptococcus mutans. The control samples had to follow the same criteria so that these samples could be compared to the plaque groups. The gas sterilization (ethylene oxide) was not effective in sterilizing the teeth in the acrylic disks, and contamination was evident. Steam autoclave did an acceptable job in properly sterilizing all teeth samples and bond strengths for the samples were very similar to the control study without steam autoclave.

The results of this study gave additional information regarding bracket bond failure and Streptococcus mutans in the oral cavity. Plaque accumulation and retention around orthodontic appliances is a significant problem, and this study indicates that plaque growth of 45 days or less, can also affect the bond strength of these appliances. Successful orthodontic treatment will be enhanced with better oral hygiene care and instructions for the orthodontic patients.

Recommendations for future study would include: 1) to evaluate the bond strengths affected by plaque deposition at closer time periods, 2) to examine the surface physics of adhesion, 3) further study into anti-bacterial adhesives or sealants to prevent plaque deposition and its affect on bond strength, 4) to evaluate plaque by-products and how this affects bond strength.

CONCLUSIONS

1. Streptococcus mutans does affect bond strength for both adhesives, within 45 days of plaque deposition the bond strength was lowered.
2. Concise had a higher mean bond strength value for the control samples and the 3 day plaque sample, but then dropped significantly in the 45 and 180 day samples. Concise had similar bond strength values as Transbond XT for the 45 and 180 day plaque samples.
3. Plaque growth affects the adhesive fracture site at debonding. In the presence of plaque, more samples had composite remaining on the bracket or tooth surface and not a combination of both.
4. Plaque deposition has a significant effect on the bond strength of orthodontic appliances, but the effect plaque has on the oral enivironment may be clinically more evident with enamel decalcification and gingivitis, than with a weaker bond strength.

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