DENTAL UNIT WATERLINE ANTIMICROBIALS AND THE EFFECT ON BOND STRENGTH OF ORTHODONTIC BRACKETS

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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ABSTRACT

DENTAL UNIT WATERLINE ANTIMICROBIALS AND THE EFFECT ON BOND STRENGTH OF ORTHODONTIC BRACKETS

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Objectives: to evaluate the effects of dental unit waterline (DUWL) antimicrobials on the shear bond strength (SBS) of orthodontic brackets. **Methods**: Fifteen human molar teeth were embedded in acrylic resin and randomly assigned to one of three surface treatment groups 1) Control/Demineralized deionized water; group 2) ICX; 3) Listerine. Each tooth was cleansed with the assigned DUWL antimicrobial or control. A 15 second application of 37% phosphoric acid gel was applied and rinsed with the assigned DUWL antimicrobial or control. A pre-coated Victory MBT bracket was then bonded. Twenty-four hours later the SBS of each bracket was blindly tested using a universal testing machine. **Results:** The pilot study shows no significant difference in the SBS of brackets bonded with DUWL containing ICX and Listerine as compared to the control group. **Conclusions:** Insufficient number of samples in to determine a difference in SBS of orthodontic brackets while using water cleansed with ICX, Listerine, or control.

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LIST OF ABBREVIATIONS

1. DUWL Dental Unit Waterline
2. EPA Environmental Protection Agency
3. SBS Sheer Bond Strength
4. CDC Center of Disease Control
5. ADA American Dental Association

CHAPTER I: INTRODUCTION

In an effort to improve the quality of dental care, infection control protocols have been implemented and continuously improved upon. One aspect of infection control has been the quality of water used during dental treatment. Many studies have been done to assess the amount and the makeup of biofilm accumulated in dental unit waterlines (DUWL) and, as a result, products have been manufactured which aim to reduce biofilm. However, very little research has been done in regards to how these waterline antimicrobials affect the success of restorations placed intraorally. Throughout this literature review, we will discuss the need for DUWL assessment as well as summarize the findings of the research which investigates the effect of these antimicrobials on bond strength.

In 2012, a case study was published which involved an 82-year-old woman that died of Legionnaire's disease. The woman had no underlying disease and investigations into how she acquired such a disease followed. It was found that she had recently visited her dental office. Water samples from the dental office's tap water and DUWL were positive for L. pneumophila serogroup 1, the same as that which was found in the patient's bronchial aspirate. The tap water in the dental office contained 1.5×10^3 CFU/L. The sample from the DUWL was $4x10^3$ CFU/L. The sample from the high speed turbine was $6.2x10^4$ CFU/L (Ricci, M. 2012).

The Environmental Protection Agency (EPA) has long evaluated the quality of our drinking water, and the quality of the water used during dental treatment should, at minimum, meet the EPA standards for drinking water. The maximum amount of colonyforming units (CFUs) is 500 per mL for drinking water and 100 CFUs for purified water (EPA.gov). In 1995, the American Dental Association released a statement that called for dentists to deliver treatment to patients using water with a maximum of 200 CFUs per mL (ada.org). The Safe Drinking Water Act goes on to further define a contaminant as, "any physical, chemical, biological or radiological substance or matter in water." According to the EPA, it can be expected that drinking water will have at least some amount of contaminants, however the aim of these regulations are to keep the amount of these contaminants at levels that are compatible with health (EPA.gov).

CHAPTER II: REVIEW OF LITERATURE

Dr. G.C. Blake first mentioned contaminated waterlines in a dental setting in 1963. At the time, water from reservoirs was used to cool handpieces. Dr. Blake found Pseudomonas pyocyanea from the spray bottles and turbine handpieces (Blake, 1963). Since then, research has been done to further study the type of micro-organisms which can be found within dental water systems. Pseudomonas, Non-tuberculosis mycobacterium, Legionnella, Fungi, Streptococci, and Porphyromonas gingivalis have all been found within dental water systems (Porteous, 2010).

In a study which collected water samples from DUWL immediately following treatment, it was found that oral streptococci was found in 58/81 dental units (Petti et al. 2013). The number of units which tested positive for oral streptococci was much higher than many other studies. However, this study was specifically designed to test for transmission of oral streptococci into the waterlines via fluid retraction. It was also conducted in private practices in which the dental units needed to be at a minimum of one year old. The data from this study suggests that oral fluid retraction into dental unit waterlines is a more common occurrence than previously expected.

Earlier studies placed emphasis on bacteria found in waterlines, however fungal colonization can also pose a health risk. The number of aerobic mesophilic fungi (AMF) found in DUWL can range from 10-813 CFU/mL (Kadaifciler et. al 2013). The highest levels were found in water coming from high speed handpieces followed by air-water syringes. This study determined that fungi were able to attach themselves and become absorbed within biofilm which means that biofilm can actually protect and harbor fungi. Few studies which examine fungi levels have been completed and a standardized method for determining the amount of fungi in water environments has not been established. More research is needed in this area, however this study is a prime example as to why waterline disinfectants are important and should be further studied and investigated.

Currently, there are a number of products on the market to reduce the amount of biofilm in dental unit waterlines. Certain devices aim to treat the source water while others are chemical products which aim to remove and prevent biofilm. Devices which treat source municipal water must have FDA clearance. Chemical products that reduce biofilms within dental water systems must be registered with the EPA. These manufacturers must submit data which proves that their products are effective, however standard efficacy testing methods are not yet established (Porteous, 2010).

A variety of chemicals can be found in dental unit water treatment products. Chlorhexidine gluconate (Blake, 1963; Agahi, 2012), Sodium hypochlorite, hydrogen peroxide, chlorhexidine, chlorine dioxide, iodine, ozone, paracetic acid (Porteous, 2010) and commercial mouthwashes are a few of the recommended chemicals that can be added to DUWLs to reduce biofilm aggregation and formation (Garg, 2012; Mills, 2000). In our efforts to decontaminate water used during our dental procedures, it's important to know how these chemical products might influence the outcome of the certain procedures, especially bonding.

One of the approaches used to control the contamination of DUWL has been to add commercially available mouth rinses. Von Fraunhofer (2004) studied the effects of Listerine on immediate SBS to dentin. Although 1:50 parts Listerine to water was quoted as the recommended dilution, varying dilutions of Listerine were used. Increasing the concentration of Listerine created a more rigorous test to see if the product had an adverse effect on bonding composite to dentin. The results of the study concluded that even with the highest concentration of Listerine, a 1:1 water mixture, there was not a significant difference between SBS of the Listerine groups as compared to the control group which used distilled water as an irrigant. When the specimens were examined under a microscope, it was found that the teeth subject to the varying concentrations of Listerine had a cleaner dentin surface with less surface accumulations following cutting and etching as compared to the control group (Von Fraunhofer, 2004). In contrast to this, Roberts (2000) published a study and found that Listerine had an adverse effect on the SBS to dentin. Examination of the specimens revealed that the Listerine groups failed entirely at the adhesive-dentin interface possibly due to the essential oils.

Upon examination of the materials and methods from the previous two studies, there are differences which may have affected the outcome. First, Roberts' study waited seven days prior to testing shear bond strength, whereas VonFraunhofer tested the shear bond strength immediately following the bonding procedures. Leloup (2001) found that there is a decrease in bond strength in groups tested after twenty-four hours due to the hydrolytic effect of water. Secondly, Roberts (2000) stated that the manufacturer of Bio 2000, a product which contains essential oils as a flavoring agent, recommended rinsing with distilled water prior to placing restorations. Upon further investigation of the manufacturer's instruction, the product information could not be found and may in fact be off the market at this point. The studies did use different bonding agents, however both studied 5th generation adhesives. In addition, the contact time of the waterline antimicrobial differed in the two studies. During Roberts' study, the tooth was only in contact with the waterline antimicrobial for 15 seconds whereas the contact time during Von Fraunhofer's study was 30 seconds. One might expect that increased contact time with the product would accentuate a decrease in bond strength, however in this study it did not. The use of Scope has also been investigated, and two studies found that there is not a significant difference in SBS when compared to the distilled water and municipal water groups (Knight, 2001; Taylor-Hardy 2000).

Essential oils are used as active ingredients in Listerine, however it is also used as a flavoring agent in other DUWL cleaners such as Bio 2000. Bio 2000 was found to lower the bond strength of composite to dentin (Roberts, 2000), as well as bond strengths to enamel (Taylor-Hardy, 2001). However, when a final rinse with tap water was utilized, Bio 2000 did not significantly reduce bond strengths (Taylor-Hardy, 2001).

The use of chlorhexidine gluconate has also been advocated as a waterline antimicrobial. It is also listed as one of the active ingredients in Bio 2000 and it has been discussed that it is the chlorhexidine that is to blame for the reduced bond strength of Bio 2000 (Roberts 2000). In 2012, the use of chlorhexidine as a waterline antimicrobial was studied once again. This time, instead of a proprietary waterline antimicrobial, 0.2% chlorhexidine was drawn into a 50 mL syringe and injected over the tooth surface over a period of 15 seconds. The results of the study showed that 0.2% chlorhexidine had a statistically significant reduction on bond strength to dentin (Sreenvasa et al, 2012). This is in agreement with the results of the Roberts and Taylor-Hardy study which found Bio 2000 to have a significant reduction in bond strength to dentin and enamel respectively.

Sodium hypochlorite has also been utilized as a waterline antimicrobial and the results of its effect on bonding have been mixed. Taylor-Hardy (2001) demonstrated that the use of 5.0 ppm of sodium hypochlorite had an adverse effect on bonding to enamel of bovine teeth when used with the 5th generation adhesive Single Bond. Roberts (2000) demonstrated that the use of 3.0 ppm sodium hypochlorite reduced the shear bond strength of composite to dentin when used with Single Bond, however it did not do so significantly. Further, when the specimens were examined it was found that the failures which occurred were cohesive failures within the dentin only, and not at the adhesive to dentin interface. Ritter (2007) found that 5.0 ppm sodium hypochlorite did not significantly reduce shear bond strength to dentin when used with either a 5th generation adhesive, PQ1 total-etch, or a 6th generation adhesive, Clearfill SE Bond .

ICX is a proprietary DUWL cleaner which utilizes sodium percarbonate, silver nitrate, and cationic surfactants (USAF – Synopsis 2012). It has been shown that this product does not affect the SBS to dentin when used with fifth generation adhesives such as Prime and Bond NT, Optibond Solo Plus, and PQ1 total etch (Ritter 2007 and Von Fraunhofer 2007). When used with a sixth generation adhesive, Clearfil SE bond, ICX once again proved that it did not adversely affect bond strength to dentin (Ritter 2007). It should be noted that ICX does not contain oils or other non-polar chemicals which might otherwise deposit on the dentinal surface. There is some evidence that when water cleansed with ICX is used in combination with Optibond Solo Plus, the SBS is actually enhanced because the oxidizing percarbonate agent, along with the cationic surfactants, lowers the surface tension and promotes penetration of the adhesive into the dentinal tubules (Von Fraunhofer 2004).

Citric Acid can also be utilized in dental waterline antimicrobials. Examples of such products include BioClear and Citrisil. However, these products have both been shown to reduce the bond strength to dentin (Sreenvasa 2012 and Roberts 2000). It was proposed that the silver active ingredient within Citrisil may interact with either the dentinal surface or the bonding agent. Furthermore, the acidity of the citric acid could adversely affect bonding to dentin (Sreenvasa et al 2012).

Iodine is another agent used to reduce biofilm within dental waterlines. Denta Pure is a cartridge that can be installed into a dental unit's reservoir bottle. The active ingredient is elemental iodine. Bishara (2005) preformed a study which investigated the effects of bonding orthodontic brackets when Denta Pure was used as the waterline disinfectant. It was shown that when used with APC Victory Series brackets and Transbond XT adhesive system no significant differences were found in shear bond strengths as compared to control groups which used distilled water.

Alpron is a proprietary product which contains citric acid, sodium-p-toluolsulphonechloramide, ethylenediaminetetraacetic acid (EDTA) and sodium tosylchloramide. Alpron has been studied in regards to its effects on bond strength and found that there is no significant difference in SBS to dentin when used with Single Bond, a fifth generation adhesive (Sreenivasa et.al 2011). This is in agreement with Betke (2005), who also showed Alpron to have no significant effect on bond strength when measured via push-out tests. In this study, a variety of dentin adhesives and resin composites were used and the only negative statistically significant difference in bond strength was found when Prime & Bond NT adhesive was used with Spectrum Composite (Betke, 2005). In another study, the use of Alpron improved tensile bond strength of composite to enamel and dentin as compared to groups which did not have a water additive (Gray, 2007). Alpron was also studied in conjunction with Panavia cement and found that there were no adverse effects of using the product when bonding to either dentin or enamel. However, the highest bond strengths could be found in the enamel/Alpron group and the lowest in the dentine/distilled water group (Patel et.al. 2009).

The number of studies aimed at DUWL antimicrobials and their effect on bonding are few and the number of choices for DUWL antimicrobials are many. The outcomes of the studies show that bonding using water with antimicrobial additives can range from enhancing bond strength to decreasing bond strength. The literature suggests that this may not only be attributed to the waterline antimicrobial used, but also the adhesive and that the combination of the two utilized in the bonding procedure can have an impact on bond strength due to possible chemical interactions (Ritter, 2007).

Authors of previous studies have made suggestions for future studies which may further enhance our understanding of the outcomes and chemistry behind waterline antimicrobials and bonding. Ritter (2007) criticized previous studies because of "their lack of standardization and consistency." Roberts (2000) suggested that in future studies the relative acidity of DUWL antimicrobials could be further investigated. In addition to this, it was suggested that when preparing the specimens, irrigation containing the waterline antimicrobial agent should be used instead of distilled water, which was utilized during this particular study. Of the studies found for this literature review, only one utilized this method when preparing the specimens. Taylor-Hardy (2001) prepared the specimens with a high speed handpiece in which the respective waterline antimicrobial was used in the reservoir system, therefore each specimen was subject to its respective antimicrobial during preparation for approximately one minute. Furthermore, the specimens appear to have gone through the bonding procedure immediately after preparation. All other studies prepared their specimens using distilled water and most prepared the specimens with a carborundum disc or grinding bed. Although Taylor-Hardy utilized a method of preparation most similar to preparation of teeth intraorally, his study included bovine teeth whereas all other studies where done on human teeth.

To our knowledge, there is only one other study which evaluates the effects of DUWL antimicrobials on the bonding of orthodontics brackets. This particular study by Bishara (2005) examined the effects of elemental iodine only. The purpose of this study is to evaluate the effects of two different DUWL antimicrobials on the bond strength of orthodontic brackets to enamel. The DUWL treatments to be studied areICX, and Listerine.

CHAPTER III: MATERIALS AND METHODS

Methodology

Fifteen de-identified, caries-free extracted human teeth were obtained from WRNMMC Oral Surgery Clinic and stored in demineralized deionized water (DDW) since extraction for the duration of the study. Each tooth was positioned in the center of a plastic mold such that the buccal surface of the clinical crown protruding out of the mold. The mold was filled with self-curing acrylic resin and allowed to bench cure. Once cured, the teeth in acrylic blocks were be stored in DDW until conduction of the bonding procedures.

Victory APC Plus MBT orthodontic brackets was used for this study.

Each tooth specimen was assigned to one of three treatment groups (Table 1; n=5 specimens per group). Randomization was determined using a computer program based on random number generation. Group 1 used a 1:10 dilution of Listerine in DDW; Group 2 used DDW as the irrigant and serve as the control group; Group 3 used DDW mixed with ICX per the manufacturer's instructions.

- Control Group (Rinsed with DDW): To prepare the enamel surfaces for bonding, plain pumice was used to clean the buccal surface, followed by a 30 second rinse with DDW. The buccal surface was then be etched for 15 seconds with 37% phosphoric acid gel then rinsed with DDW for 30 seconds. The tooth was dried to a frosty white appearance. With a microbrush, one coat of Assure Universal Bonding Resin was applied and lightly air dried to evaporate the solvent. The pre-coated Victory MBT bracket was placed firmly onto the tooth surface followed by removal of excess adhesive. The appliance was light cured for 5 seconds from the mesial and 5 seconds from the distal with an Elipar S10 LED curing light.

- Other groups followed the same protocol using their respective antimicrobial irrigants.
- 100 ml of solution was applied with a monoject syringe over a 30 second time interval. This procedure was done twice for each specimen.

Immediately following the bonding procedure, all specimens were placed in room temperature DDW for 24 hours. Separate containers for each of the groups were used so as not to cross-contaminate the specimens. In order to blind the person testing the bonding strength, containers were labelled with codes rather than the actual irrigant labels.

SBS testing was conducted using a Universal Testing Machine. Personnel conducting the tests was blinded to the irrigant used and tested the teeth in a random order. . Each acrylic block was secured to the machine and an occluso-gingival load applied to the bracket-tooth interface. A crosshead speed of 1.0 mm/min was applied. All results was recorded in megapascals (MPa).

Data Analysis:

Median (± standard deviation) of SBS values (MPa) for all groups was presented and compared with Kruskal-Wallis test . Statistical analysis was accomplished using Statistical Package for the Social Sciences (SPSS) Version 19 computer software (SPSS, Inc., Chicago, IL). The experimentwise significance level was set at $\alpha < 0.05$.

CHAPTER IV: RESULTS

Pilot Study Results:

The results of the pilot study are presented in Appendix A and Appendix B. The Kruskal-Wallis test was used to analyze the data collected. Results of this test indicate that there is not significant difference (P =.898) in the SBS of the teeth bonded with DUWL containing ICX or Listerine as compared to the Control group (DDW). Due to the wide range in SBS and the limited sample size, the median is preferred over the mean, see Appendix C, Table 1. The median shear bond strength for Group 1 (Listerine) was 109.70 MPa (range 73.5-262.6). The median shear bond strength for Group 2 (DDW) was 143.13 MPa (range 56.0-359.1). The median shear bond strength for Group 3 (ICX) was 158.3 MPa (range 36.1-288.3).

When examining each group individually, group 3 (ICX) exhibited outliers, see Appendix D, Graph 1. The outlier, 36.1 MPa, is represented as a small circle. The extreme outlier, 288.3 MPa, is represented as a star. If all 15 specimens are looked examined collectively, the outliers in Group 3 are no longer considered outliers, see Appendix E, Graph 2. Collectively, the mean SBS for all of groups is 163.7 MPa and the standard deviation is 95 MPa.

CHAPTER V: DISCUSSION

The present findings suggest that there is no significant difference in the SBS of orthodontic brackets bonded with Listerine and ICX DUWL antimicrobials as compared with DDW alone. However, the standard deviation within each group was high and the overall number of samples was low.

If this study were to be repeated, the number of teeth needed would depend on the difference in SBS that is desired. Based on the present data, if a difference of 30 MPa was desired, two groups of 159 teeth would be needed. The volume of teeth needed in order to make this statistically significant makes repetition of this study impractical. However, if there were a smaller standard deviation, fewer teeth could be used to produce a statistically significant outcome.

Possible causes for the high standard deviation could be inconsistent bonding methods, differences in quality of enamel, inexperience working with the universal testing machine, or perhaps problems with the universal testing machine itself. Every effort was made to standardize the bonding and SBS testing methodology in order to reduce variables. Both bonding and SBS testing were done blindly to avoid bias.

To account for operator experience, specimen 1 in each group was tested with the universal testing machine, followed by specimen 2 in each group, and so on. In examining the data, it can be seen that higher SBS values were seen at the beginning and middle of the testing groups while each group exhibited the lowest SBS value for specimen 5, the last specimen tested.

The SBS values collected in this study are far greater than values collected in similar studies. The study by Bishara et. al is most similar to the present study design. In the Bishara study, the mean SBS for the experimental group (iodine disinfectant) and control group (distilled water) were 6.5 MPa and 4.7 MPa respectively. Results produced by Knight et. al had a mean SBS to enamel of 25.06 MPa for the experimental group (Distilled water with Scope), 26.23 MPa for the Distilled water group, 28.39 MPa for the Municipal water group. In the present study, only the 5th specimens in each group have SBS values relatively close to the aforementioned studies.

In addition to collecting SBS values in megapascals, visual and tactile observations were made for each specimen and recorded. In four out of the fifteen specimens, the universal testing machine detected a break which upon visual examination, the bracket remained on the tooth. Closer examination revealed that in these four specimens the brackets were not mobile. Attempts were made to remove the brackets manually with a forcep however the brackets remained firmly bonded. Of these four specimens, three came from group 2 (DDW). If the readings from the universal testing machine were removed from this study and visual/tactile information was used instead, it would seem as though group 2 (DDW) performed the best. In one instance the bond of the bracket to the tooth was so strong that it began to pull the tooth from the acrylic resin prior to detecting a break.

Further visual examination revealed that when brackets de-bonded, there was a mixture of adhesive and cohesive failures. In group 1 (Listerine), the majority of specimens had composite remaining on the tooth. In group 2 (DDW), only 2 specimens had brackets visually de-bond. One specimen had composite remaining on the tooth only while the other had composite remaining on both the tooth and the bracket. In group 3 (ICX), the majority of specimens had composite remaining on the tooth.

Due to the variability in SBS values as well as inconsistency in detecting a break from the universal testing machine, it is recommended that another pilot study be done prior to completion of this study. Further knowledge can be gained by adding a third experimental group to examine the effects of Chlorhexidine on the SBS strength of bonding orthodontic brackets. The pilot study was originally designed to include this group however the solution did not arrive in time.

A cross-head speed of 1.0 mm/min was used in the present study. The overwhelming majority of studies examining the effect of DUWL antimicrobials on bond strength used this particular cross-head speed. However, the Bishara study used a cross-head speed of 5.0 mm/min and is most similar to the present study due to its use of orthodontic brackets. In order to add to the collection of data, a crosshead speed of 5.0 mm/min is recommended for the next study.

CHAPTER VI: CONCLUSION

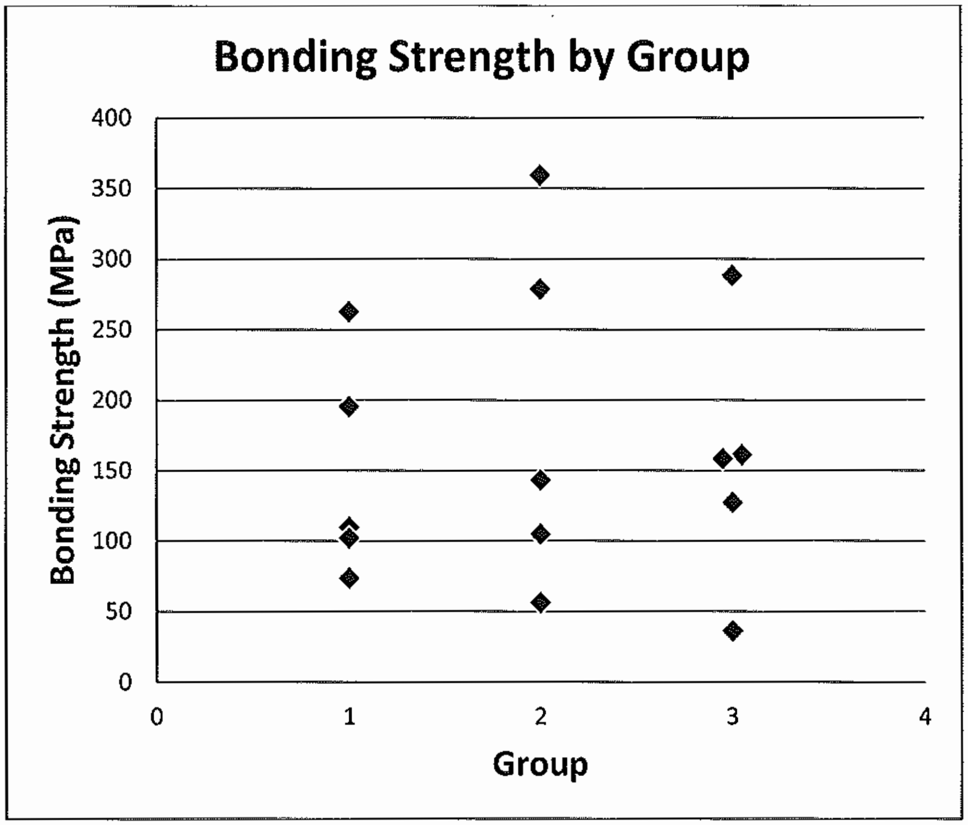
DUWL antimicrobials are necessary to keep biofilm levels at an acceptable level. Results of this pilot study show no significant difference in the SBS of orthodontic brackets with DUWL containing ICX or Listerine as compared to the Control group (DDW). However, more samples will be required to determine whether or not DUWL antimicrobials have different effects on SBS of orthodontic brackets.

APPENDIX A: DATA COLLECTION SHEET – PILOT STUDY

Group (Listerine)	Specimen #	Shear Bond Strength (MPa)	Group (DDW)	Specimen #	Shear Bond Strength (MPa)
1	1	109.7	2	1	359.1
	2	102.0		2	143.13
	3	262.6		3	104.7
	4	195.4		4	278.5
	5	73.5		5	56

Group (ICX)	Specimen #	Shear Bond Strength (MPa)
3	1	127.2
	2	288.3
	3	161.0
	4	158.3
	5	36.1

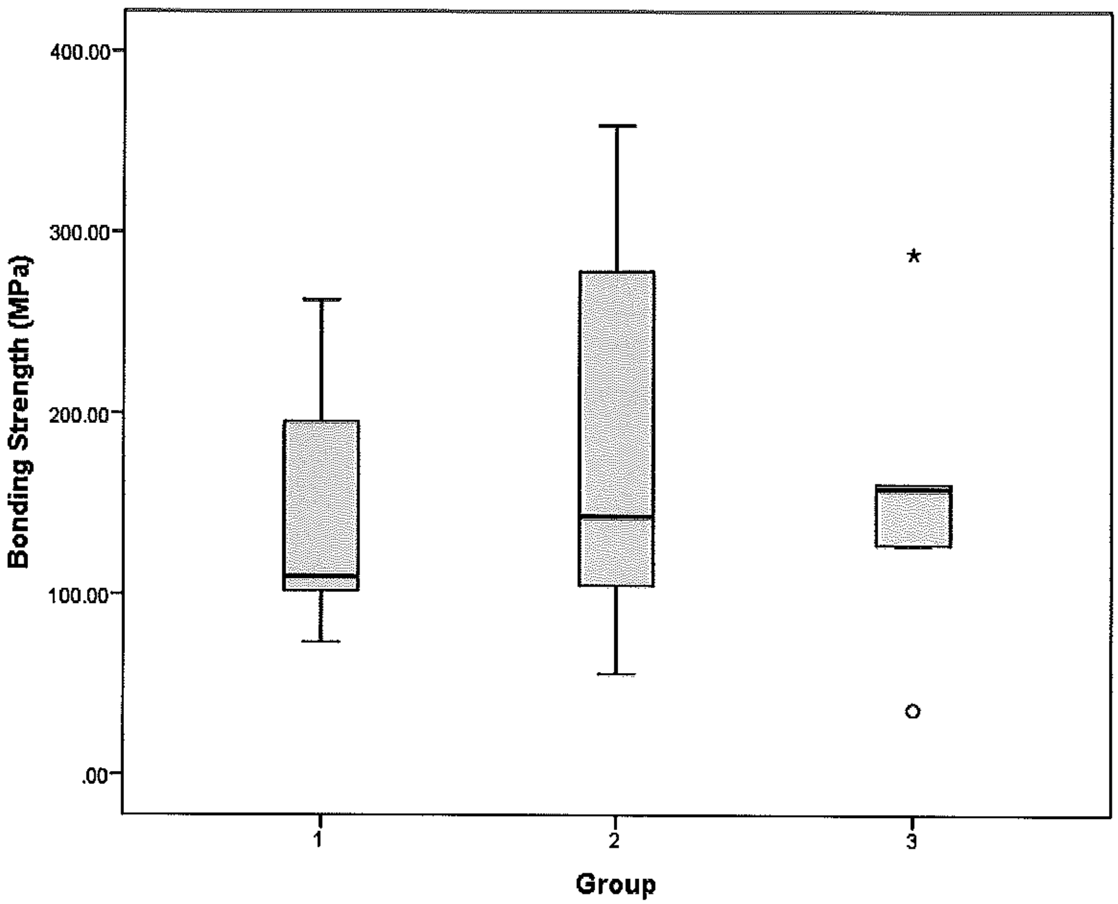
APPENDIX B – GRAPH 1 – RAW DATA



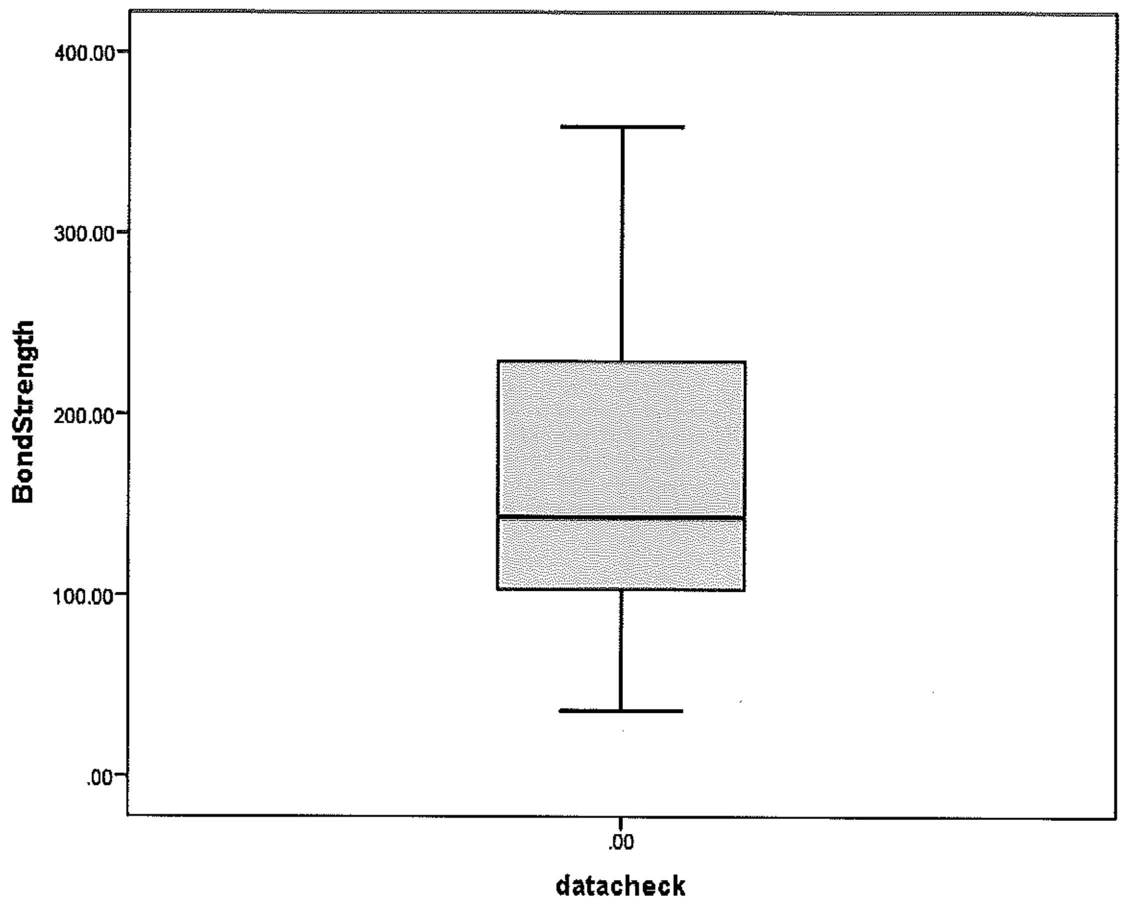
APPENDIX C: Table 1

Group	Minimum	Maximum	Median	Percentile 25	Percentile 75	Mean	Standard Deviation	Valid N
1	73.50	262.60	109.70	102.00	195.40	148.64	78.28	5
2	56.00	359.10	143.13	104.70	278.50	188.29	126.33	5
3	36.10	288.30	158.30	127.20	161.00	154.18	90.45	5

APPENDIX D: Graph 2



APPENDIX E: Graph 3



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