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Effect of Saliva Contamination on Crown Retention Strengths of Resin and RMGI Cements

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EFFECT OF SALIVA CONTAMINATION ON CROWN RETENTION STRENGTHS OF RESIN AND RMGI CEMENTS

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A THESIS

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ABSTRACT

Background: The most common contaminant in the oral cavity is saliva. Many studies have shown that contamination with saliva negatively affects the strength of the resin-dentin bond. A literature review by Nair et al reported that 64.6% of studies on the topic show a detrimental effect of salivary contamination on adhesive materials. No published studies were found that thoroughly studied the effects of saliva contamination on RMGIs and resin cements with regards to crown retention rates.

Objective: To study the effect of salivary contamination on crown retention strengths of RMGI and resin cements at various stages of bonding and in wet and dry environments.

Methods: 100 extracted human mandibular premolars were centered in Teflon cylinders and embedded in auto-polymerizing acrylic resin. Occlusal surfaces of each specimen were ground flat on a model trimmer. Each specimen was fixed into a lathe for precise uniform reduction and prepared to uniform dimensions (20° total taper and 2 mm height) using a flat end taper diamond bur. Bonding surface areas of the prepared surfaces were calculated under 20X magnification using a Keyence digital microscope. The specimens were scanned using a digital scanner. Cylindrical copings were designed using 3Shape CAD design software. A 50µm cement gap was selected for each crown. The

restorations were designed with a handle placed perpendicular to the long axis of the tooth which was used to attach a wire loop for debonding the crowns. The passive drool technique was utilized to collect unstimulated, whole saliva. In Part 1 of the study, two RMGIs, one Bioactive, one Self-adhesive Resin and one Adhesive Resin Cements were tested with and without dried salivary contamination. In Part 2a, one RMGI cement was tested with dried saliva and wet saliva contamination groups. In part 2b, one resin cement was evaluated with salivary contamination occurring before and after adhesive application and also with dried or wet saliva. The cement was applied to the copings according to manufacturer's IFU. Crown retention was measured by placing the specimens in a universal testing machine. Specimens were loaded in tension at a crosshead speed of 1 mm/min until debonding; the debonding force (N) was recorded. Retention strength was calculated in MPa by dividing the debonding force (N) by the total bonding surface area of the preparation (mm^2). Crown retention strength and force were analyzed with two-way ANOVA for Part 1 and one-way ANOVA for Part 2. Failure mode was examined under 20X on the Keyence digital microscope and classified in one of five categories: cement mainly on prepared tooth (over 75%), cement on both crown and tooth (between 25 and 75%), cement mainly on crown (over 75%), fracture of tooth without crown separation, or fracture of crown.

Results: In part 1, two-way ANOVA comparing crown retention strength for cement type and contamination (clean and saliva contamination) showed no significant interaction ($p = 0.394$) between the two factors; however, the factor "cement type" was significant ($p<0.001$). In Part 2a, one-way ANOVA showed that there was significant difference ($p<0.001$) when cementing crowns with dried or wet saliva and in Part 2b,

one-way ANOVA showed that there was significant difference in crown retention strength when contamination occurred before adhesive application when compared to contamination after adhesive application.

Conclusion: Salivary contamination has a significant effect on the crown retention strength of RMGI cements when cemented over wet saliva and for resin cements when contamination occurs before adhesive application.

Keywords: Crown retention strength, salivary contamination, RMGI, resin cement, zirconia.

DEDICATION

This thesis is dedicated to my family. Everything I have achieved so far has been because of your support, prayers, and steadfast belief in me. There are no words that can adequately describe what you all mean to me.

To my Biomaterials family here at UAB, thank you for giving me some of the best years of my life.

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LIST OF ABBREVIATIONS

RMGI – Resin-modified glass ionomer

10-MDP – 10-methacryloxydecyl dihydrogen phosphate

IFU – Instructions for use

PFM – Porcelain fused to metal

MMP – matrix metalloproteinases

bisGMA – bisphenol A-glycidyl methacrylate

TEGDMA - Triethylene glycol dimethacrylate

1. INTRODUCTION

Shillingburg defined Fixed Prosthodontics as "the art and science of restoring damaged teeth with cast metal or porcelain restorations, and of replacing missing teeth with fixed or cemented prosthesis". In the same text, he also mentioned the principles of tooth preparation - preservation of tooth structure, retention and resistance, structural durability, and marginal integrity. In the past four decades, dentists have tried to stick to his directives and the principles given by other great prosthodontists while simultaneously trying to adapt to the changes that came with advances in dental materials technology. Standards for acceptable dental care began changing with an increase in the number of fixed restorative procedures which in turn was affected by a better understanding of the concepts of retention and resistance, the introduction of new luting cements as well as new restorative materials, and most recently, the development of adhesive technology and highly esthetic full-coverage crowns.

Crown retention testing

Crown retention and the factors that contribute to it have been a subject of study for decades. Worley et al[1]. in 1982 studied the effect of residual zinc oxide eugenol on the retention of crowns cemented with zinc phosphate cement. Brukl et al[2]. studied the crown retention with resin cement on natural teeth and DeWald et al[3] studied the effect of the type of core used and luting agent on crown retention. Zinc phosphate was introduced in the

1800s and is the oldest luting cement known with a high degree of clinical acceptability[4]. Glass ionomer cement was later introduced in 1969 with properties of chemical adhesion and fluoride release and was followed up with resin modified glass ionomer (RMGI) in the 1980s[4]. Subsequently, newer formulations of resin luting agents were also introduced.

Popularization of dental ceramics

Ceramic technology has evolved quickly over the past 10-15 years[5]. The choice of full coverage restorations has shifted from all metal to all ceramics over time. A survey of 1777 dentists by Makhija et al[6] in 2016 found that lithium disilicate is the material of choice for anterior restorations followed by layered restorations while all zirconia followed by porcelain fused to metal are the most commonly used materials for posterior crowns. This preference for ceramic restoration is backed by several studies proving the excellent performance of these restorations. A four-year survival analysis of dentin bonded feldspathic crowns placed in a dental school showed a failure rate of only 6%[7]. Hammoudi[8] et al carried out a randomized clinical trial where 62 adults with excessive tooth wear received 713 crowns, either lithium disilicate or translucent zirconia. After 6 years, both types of crowns had a survival rate of 99.7%. A systematic review by Larsson et al.[9] (survival) evaluated the success of zirconia-based crowns in a clinical setting and reported a five-year survival rate of 95.9% for tooth-supported prostheses, thus making them comparable and similar to PFM crowns.

Cement selection for ceramic crowns

The advancements in ceramics led to the discussion of which luting agent is best for which restoration material. A survey[10] carried out by the NPBRN in 2019 asked dentists whether they preferred to bond or not bond crowns clinically. Of the 3468 single unit crowns evaluated, 38.1 % were bonded whereas 61.9% were not bonded. Of those not bonded, RMGI cement was the luting agent of choice. Dentists also preferred bonding glass-ceramic crowns over all-zirconia or porcelain fused to metal (PFM) crowns. However, while there is a lot of literature available[11,12] about the performance of RMGI as a restorative material, frustratingly little is available about its use as a luting agent. A study evaluating the crown retention strengths of three different RMGI luting agents found all of them to be clinically acceptable with one showing higher values than the others[11]. Another study compared the long-term retention rates of zirconia crowns cemented with automix and hand mixed RMGIs and reported favorably for the latter[13].

It has been suggested that RMGIs provide adequate retention for ceramic restorations and bonding should be considered only when the retention achieved by luting agents is inadequate[14]. A systematic review by Maraulakos[15] found that the crown retention strengths of adhesive and conventional cementation are similar for both lithium disilicate and zirconia. Bonding to zirconia has been a matter of controversy[15] in the past as the techniques of bonding to lithium disilicate could not be applied to zirconia[16]. It has been suggested that adhesive resin cementation leads to an increase in retention of lithium disilicate crowns and fatigue resistance of zirconia crowns[15] and bonding is considered necessary for crowns with poorly retentive forms. The data regarding this is conflicting. A study by Campos[17] reported that adhesively cemented crowns survived fatigue testing longer than conventionally cemented ones. However, a fracture resistance study by Nakamura[18] found that the strength of zirconia crowns is not related to cement used as long as a minimal thickness of 0.5 mm of the material is maintained. On the other hand, there are several studies in the literature evaluating the effect of varying degrees of crown taper on retentive strengths of various cements. One study reported significantly higher bond strength values of resin cements in comparison to glass ionomer and zinc phosphate cement[19]. Another study by Osman et al[20] on preparations with increased taper reported that crown retention testing caused the specimen to fracture before the crowns debonded, suggesting that the adhesive strength was higher than the cohesive strength of dentine irrespective of the taper given. Two other studies also reported higher retentive strengths of adhesive cements as opposed to conventional cements in poorly retentive preparations[21,22]. These papers support the belief that adhesive bonding is beneficial for short, tapered crowns.

Bonding to zirconia

Recent studies have found that the combination of surface air-borne particle abrasion and using 10-MDP monomer is a reliable technique for bonding to zirconia restorations[16]. De Souza et al[23] studied the use of MDP-based materials for bonding to Zirconia and found that using an MDP-containing primer with an MDP-containing adhesive provided the highest 48-hr micro tensile bond strength values. Subsequently, a meta-analysis by Inokoshi et al[24] on the effectiveness of bonding to zirconia ceramics concluded that the combination of mechanical and chemical pre-treatment greatly benefitted the bond of resin cements to zirconia ceramics.

There are several in vitro studies comparing the bond strengths of various cements for zirconia restorations. One study evaluated the effect of air abrasion on zinc phosphate and glass ionomer cements, zinc phosphate, glass ionomer cement and resin cements[25], while another study by Ehlers et al[26] compared the retentive strength of various adhesive cements with glass ionomer cement, RMGI, and zinc phosphate. Ernst et al in one study[27] compared the retentive strengths of self-adhesive cements, self-etching adhesives, and RMGI, while in another[28] compared a compomer, glass ionomer cement, RMGI with a self-adhesive resin. Turker[29] also compared the bond strength of glass ionomer cement, RMGI and an MDP containing resin cement. All of these studies[25-29] found the adhesive cement to be better than their comparison groups while some ambiguity was found within different types of adhesive cements (self-etch vs self-adhesive). However, a clinical trial by Torres et al[30] studied the clinical performance of crowns cemented with either glass ionomer cement or self-adhesive resin cement and found no significant difference between the two. An in-vitro study by Palacios et al[31] also studied the retention strengths between RMGI and two types of adhesive cements and found no difference between the three groups while another by Pilos et al[32] found the retention strength of the RMGI group to be higher than the resin group. As mentioned earlier, comparison between different types of resin cements draws mixed results and no consensus can be reached on the best adhesive strategy[33,34].

Effect of contamination on bonding

An important aspect of adhesion is the response of bonded restorations to any kind of contamination. Saliva and blood are the two most studied contaminants, with more studies with saliva likely due to the constant presence of saliva in the oral cavity. For direct restorations, several measures can be taken to prevent any contaminants from interfering with restoration placement and the use of rubber dams is the accepted standard of care in dental practice today. However, the actual use of dams varies widely, based on the location of practice, socioeconomic factors, and patient-specific circumstances, among many others. In the case of indirect restorations, rubber dams are not typically feasible due to the location of the tooth preparation finish line. Therefore, using an appropriate luting agent is essential in situations where one might anticipate possible contamination. Many clinicians prefer using water-based cements such as RMGIs in such cases. Though contamination is detrimental to any cement, the magnitude is considerably smaller with RMGI. Several studies support this claim. Shimazu et al performed two experiments[35,36] on bovine teeth in which the effect of saliva contamination on bond strengths and microleakage with glass ionomer cements, RMGIs, and composite resins with no significant detrimental effect of contamination on the glass ionomer cements. Additionally, a study by Caccifesta[37] suggests that the shear bond strength of an RMGI increased after salivary contamination.

The opposite seems to hold true for resin-based cements. Several studies[39-42] point to a decrease in bond strengths between adhesive systems and various substrates. This applies to everything from all-in-one adhesives to self-etch adhesives to various kinds of ceramics. A literature review by Nair et al[43] reported that 64.6% of studies show a detrimental effect of salivary contamination on adhesive materials. However, some studies claim that saliva contamination has no significant effect on bond strength and is not detrimental to the longevity of the restoration[44,45]. No published studies were found that thoroughly studied the effects of saliva contamination on RMGIs and resin cements with regards to crown retention rates. This study was designed to account for the different types of forces (shear, tensile) that play a role in the loss of crown retention and how salivary contamination would interact with them to affect the ultimate crown retention strength.

2. OBJECTIVES

The main objective of this study is to measure the retention strength of zirconia copings cemented with different kinds of cements with or without salivary contamination. In order to address the objective of the thesis successfully, the thesis is composed of following sections.

2.1 Retention Strength of Zirconia Copings Bonded with RMGI, Bioactive, Self-adhesive Resin and Adhesive Resin Cements

The objective is to measure and compare the retention strength of zirconia copings to prepared human teeth with RMGI, Bioactive, Self-adhesive Resin and Adhesive Resin Cements with and without salivary contamination.

2.2 Retention Strength of Zirconia Copings Bonded with RMGI and Adhesive Resin Cements with Wet and Dry Saliva Contamination at Different Stages of Bonding

The objective is to measure and compare the retention strength of zirconia copings to prepared human teeth with an RMGI and resin cement with both wet and dry salivary contamination during different stages of bonding.

3. HYPOTHESES

1. There is a difference in the retention strength of zirconia copings cemented with RMGI, Bioactive, Self-adhesive Resin and Adhesive Resin Cements - This will be determined the factor *material* in a 2-way ANOVA.

2. There is a difference in the retention strength of zirconia copings with and without salivary contamination. - This will be determined by the factor *contamination* in a 2-way ANOVA.

3. The difference in the retention strength of zirconia copings with and without salivary contamination will be dependent upon the cement used. - This will be determined the interaction between *material* and *contamination* in a 2-way ANOVA.

4. For RMGI cement, wet or dry saliva contamination will lead to a lower retention strength. This will be determined by completing a 1-way ANOVA and Tukey post-hoc analysis if necessary.

5. For adhesive resin cement, wet or dry saliva contamination at different steps of bonding (before or after adhesive application) will lead to a lower retention

strength. This will be determined by completing a 1-way ANOVA and Tukey post-hoc analysis if necessary.

4. MATERIALS AND METHODS

4.1 Retention Strength of Zirconia Copings Bonded with RMGI, Bioactive, Self-adhesive Resin and Adhesive Resin Cements

Table 1: Trade names and pictorial representation of cements used in the study

Cement	Type	Manufacturer	Image
RelyX Luting Plus	RMGI	3M	
FujiCEM Evolve	RMGI	GC	

Calibra Bio	Calcium aluminate/RMGI hybrid	Dentsply Sirona	
RelyX Universal	Dual-cure resin	3M	
K-0221 High Voltage	Dual-cure self-adhesive resin	Dentsply Sirona	

Table 2: Part 1 study design

	Rely X Luting Plus	**FujiCEM Evolve**	**Calibra Bio**	**RelyX Universal**	**K-0221 High Voltage**
No saliva contamination	Group 1	Group 2	Group 3	Group 4	Group 5

Saliva contamination	Group 6	Group 7	Group 8	Group 9	Group 10

4.1.1 Specimen Preparation

UAB Institutional Review Board (IRB) approval was obtained. 100 extracted, non-carious, non-restored human premolars were collected from the UAB School of Dentistry Oral and Maxillofacial Surgery Department. The teeth were divided into 10 groups (n=10/group). The roots of the selected teeth were notched with a separating disc to provide retention of the specimen in the acrylic resin (Figure 1). The teeth were centered in Teflon cylinders with the help of a surveyor and digital caliper and embedded in auto-polymerizing acrylic resin (Yates Motloid, Chicago, IL) (Figure 2). Occlusal surfaces of each specimen were ground flat on a model trimmer (Figure 3). Each specimen was fixed into a lathe for precise uniform reduction and prepared to uniform dimensions (20° total taper and 2 mm height) using a 846.11.025 HP medium flat end taper diamond bur (Brasseler, Savannah, SC) (Figure 4). This height and taper were selected in order to minimize the resistance and retention form of the tooth preparation and focus on the retention provided by the cement. To standardize the area of the preparation margin to 1mm, the margins were trimmed in the lathe to remove any excess tooth structure (Figure 5). This preparation design allows increased standardization of the preparation; however, it results in some crown margins being placed on dentin. This compromise was deemed acceptable as most of the retention of the crown would be expected to occur from the dentin axial walls of the tooth preparation. The prepared specimens were stored in distilled water prior to any bonding procedures.

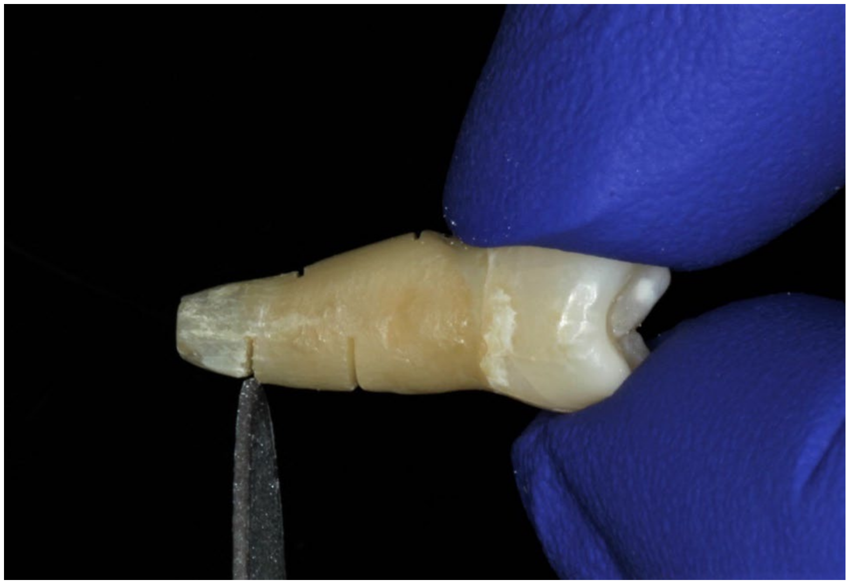

Figure 1. Notching tooth roots

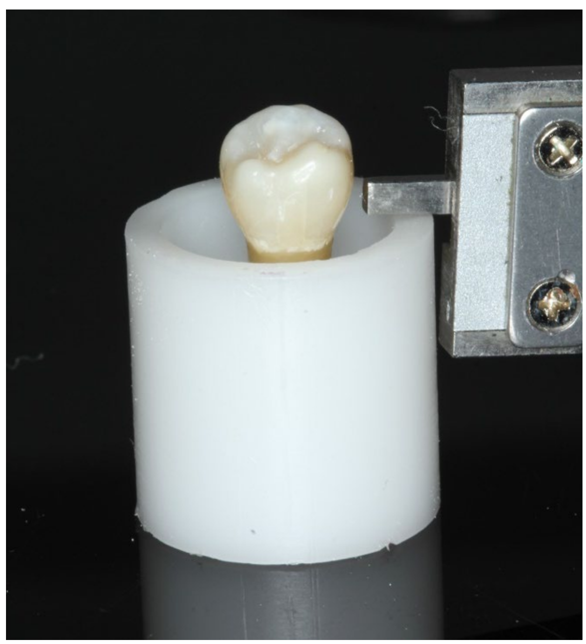

Figure 2. Tooth mounting

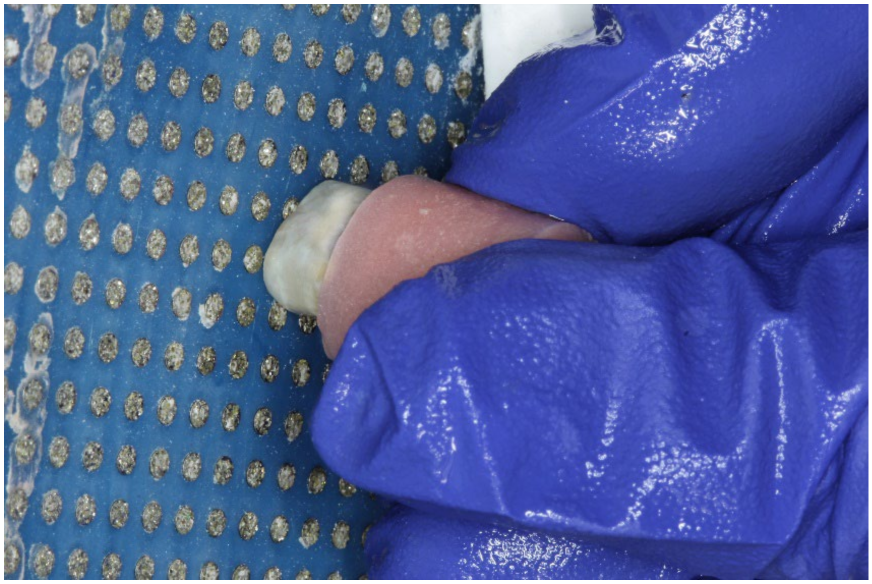

Figure 3. Removing occlusal surface

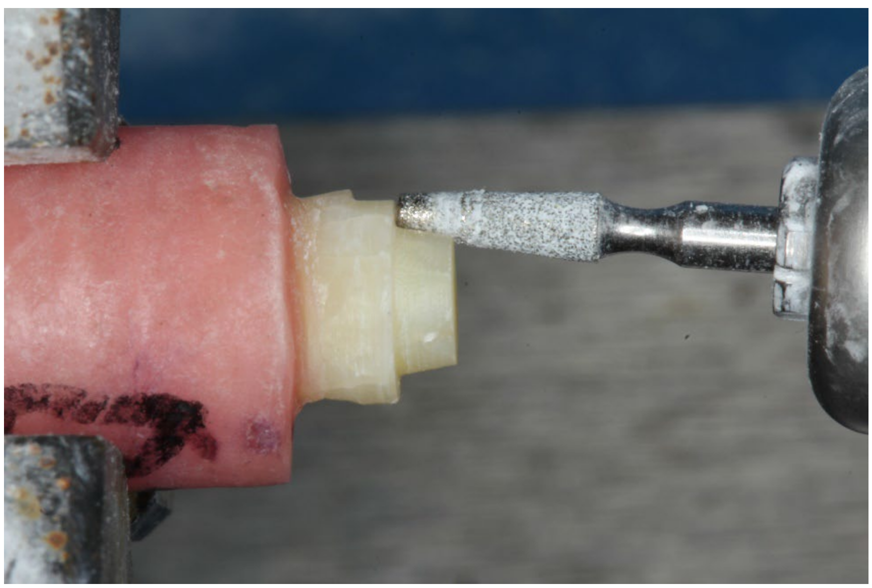

Figure 4. Preparation of axial walls

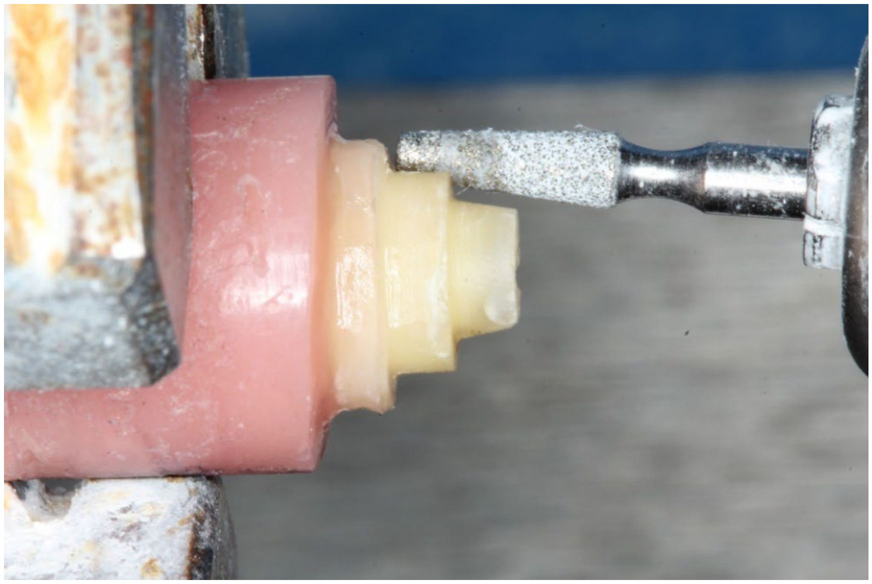

Figure 5. Refining margin

The bonding surface area of the prepared tooth surface was calculated under 20X magnification using a digital microscope (VHX 6000 Series: Keyence, Tokyo, Japan). The lateral walls, occlusal table, and margin width were measured in order to calculate the bonding surface area using the following formula: Total bonding area = Lateral surface area of truncated cone + Area of top circle of truncated cone + Difference between area of base and bottom of the truncated cone

Each individual area mentioned in the equation above was calculated using the following formulae:

Lateral surface area of truncated cone = π (R+r) S=$\boldsymbol{\pi}$ **R**+**r** $\boldsymbol{S}$

Area of top circle of truncated cone =πr2=$\boldsymbol{\pi r 2}$

Area of bottom circle of truncated cone =πR2=$\boldsymbol{\pi R 2}$

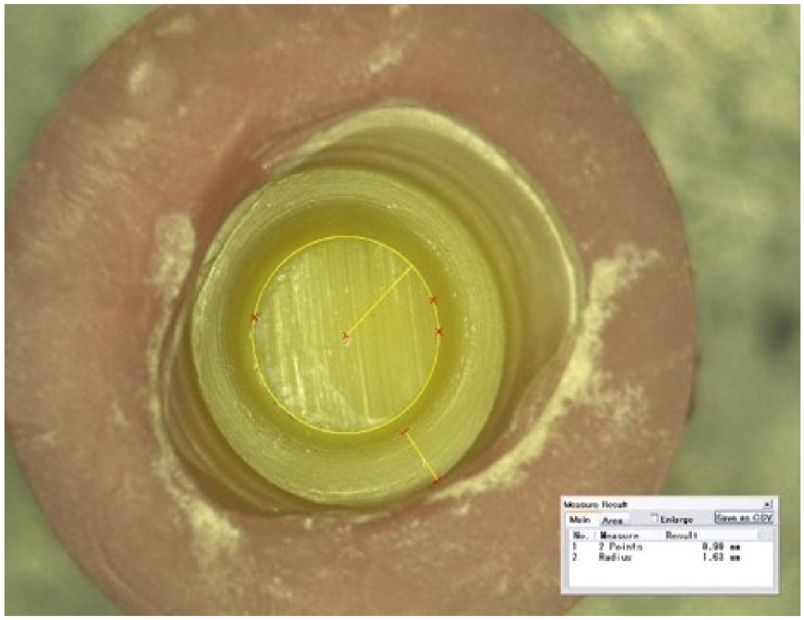

Figure 6. Measurements from occlusal view

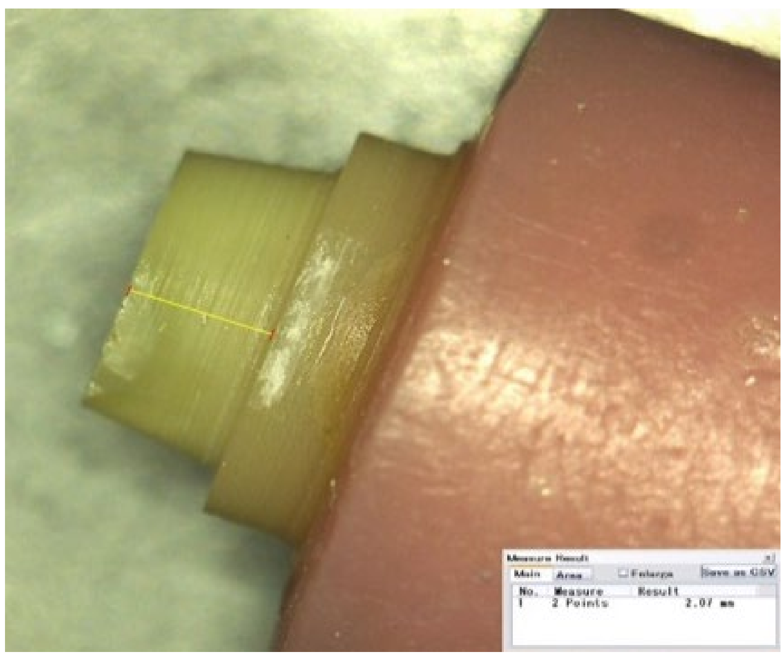

Figure 7. Measurements from axial view

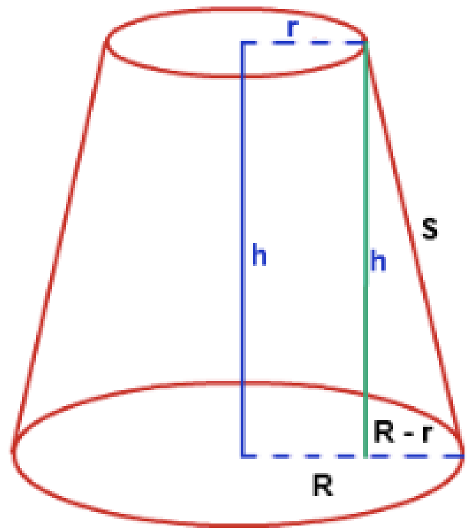

Figure 8. Terms used for surface area calculation

The specimens were scanned using a digital scanner (True Definition Scanner, 3M ESPE, St. Paul, MN) and STL files of the scans were exported. Cylindrical copings were designed using a laboratory CAD design software (Design Studio, 3Shape, Copenhagen, Denmark). A 50µm cement gap was selected for each crown. The shape of the crowns was designed by starting with an autogenerated crown form. The crown form was elongated vertically, and the occlusal portion of the crown was cropped to produce a flat top. The restorations were designed with a handle placed perpendicular to the long axis of the tooth. The handles were used to attach a wire loop for debonding the crowns. The STL files of the designed crowns were exported and sent to a dental laboratory. The laboratory milled and sintered the zirconia (Cerec Zirconia+, Dentsply Sirona, York, PA) copings according to the manufacturer's instructions for use (IFU).

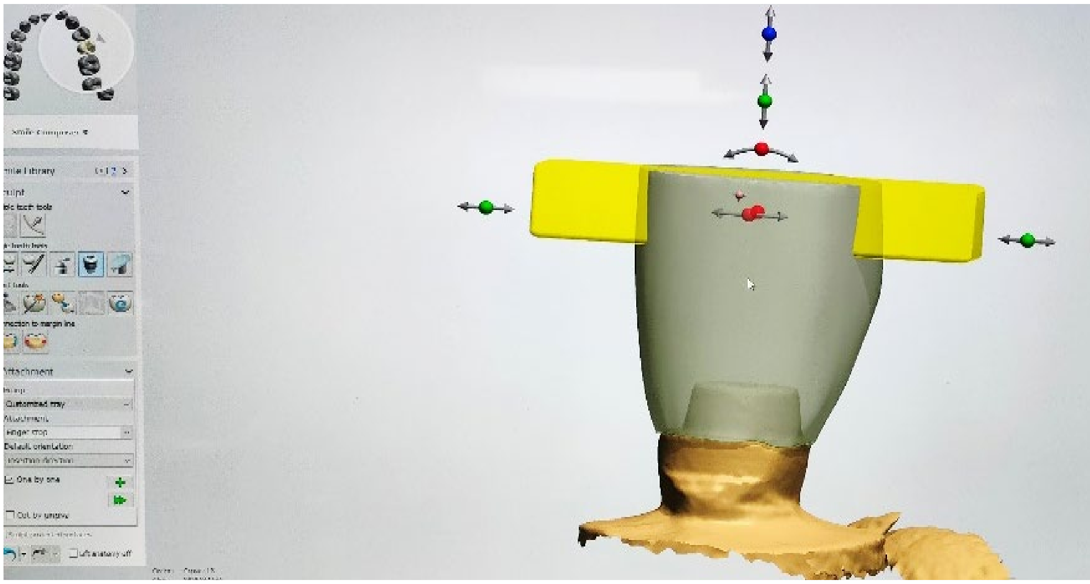

Figure 9. Designing crown in 3shape software

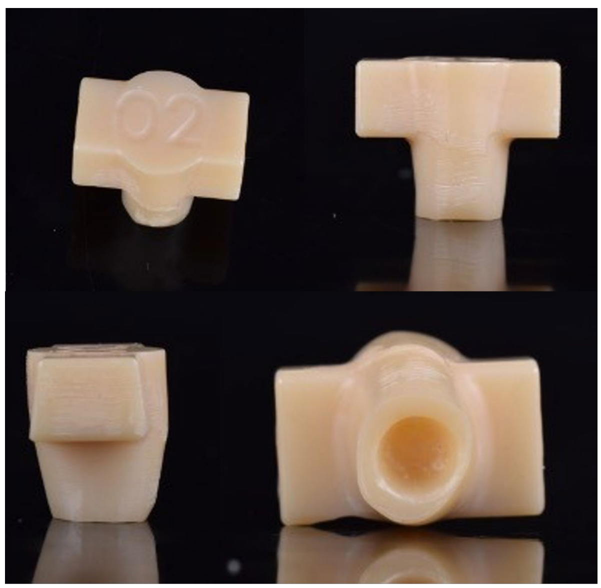

Figure 10. Zirconia coping

4.1.2 Collection of fresh whole saliva

UAB Institutional Review Board (IRB) approval was obtained for collection of saliva. Whole saliva was collected from one participant. The passive drool technique[46] was utilized to collect unstimulated, whole saliva in order to maintain consistency in the type of sample collected. The participant was not allowed to eat, drink coffee or caffeinated soft drinks, or consume dairy products one hour before collecting saliva samples. Furthermore, five minutes prior to saliva collection, the participant was asked to rinse her mouth with clear water to avoid contaminants. The participant allowed the saliva to collect on the floor of her mouth for 1 minute. The saliva was then collected by placing a 5mL polystyrene tube against their bottom lip, tilting her head forward, and allowing passive flow of saliva into the tube. A total of 15 mL of saliva was collected (12.5 mL was required for the test). All saliva was collected no more than 24 hours prior to use, and was stored in a covered container at 4°C.

Figure 11. Test tube of collected saliva

4.1.3 Cementation of copings

All tooth preparations were thoroughly cleaned with distilled water and blotted dry prior to cementation. The copings were airborne particle abraded with 50 µm alumina at 2 bar pressure for 10 seconds by the manufacturer. Half of the specimens had 0.25 mL of fresh whole saliva applied to the preparation with a microbrush for 10 seconds and then left to dry on the tooth preparation prior to bonding. In group 9, saliva was applied and allowed to dry on the tooth first before adhesive application. Scotchbond Universal (3M ESPE, St. Paul, MN) was used as a primer on the copings in groups 4 and 9. The cement was applied to the copings according to the manufacturer's instructions. The copings were then seated with finger pressure to ensure complete seating. Excess cement was carefully removed with a microbrush in an uncured stage while the coping is being held fixed. They were immediately placed under a 2.5 kg load. All copings were self-cured as per the time reported in their IFU.

4.1.4 Storage and thermocycling

The copings were stored in a moist zip lock bag for 24 hours at 37°C and then thermocycled for 10,000 cycles from 5-55^{0}C with a dwell time of 30 secs. Afterward, samples were stored in distilled water at 37°C for 30 days.

4.1.5 Retention testing

Specimens were placed in a custom-positioning fixture (UAB Research Machine Shop, Birmingham, AL) and stabilized and centered by set screws on the sides of the positioning fixture. The positioning fixture was mounted in a universal testing machine (model no. 5565, Instron, Canton, MA). The handles on the coping were grasped by a wire loop. The specimens were then loaded in tension at a crosshead speed of 1 mm/minute until the coping was separated from the tooth. The maximum debonding force (N) was recorded and the retention strength calculated using the following formula:

Retention (MPa) = Debonding force (N) / Total bonding surface area (mm2)

Figure 12. Crown in retention test

4.1.6 Mode of failure analysis

Mode of failure was analyzed under 20× magnification on the digital microscope (VHX 6000 Series: Keyence) at the intaglio surfaces of the copings and the external surface of the tooth preparations. Failures were classified into one of five different categories as summarized in Table 3.

Table 3. Modes of failure

Types	***Description***
Type 1	Cement mainly on prepared tooth (over 75%)
Type 2	Cement on both crown and tooth (between 25 and 75%)
Type 3	Cement mainly on crown (over 75%)
Type 4	Fracture of tooth root without crown separation
Type 5	Fracture of Crown

4.1.7 Statistical analysis

The crown retention strengths were compared with a 2-way ANOVA for factors *material* and *contamination status* using SPSS software (IBM, Armonk, NY). A p-value of <0.05 was considered significant. To test the hypothesis, the interaction between factors *material* and *contamination status* was examined and individual t-tests to compare the effect of *contamination status* for each *material* were performed if the interaction was found to be significant.

4.2 Retention Strength of Zirconia Copings Bonded with RMGI and Adhesive Resin Cements with Wet and Dry Saliva Contamination at Different Stages of Bonding

Table 4. Part 2a study design

	Control	**Dry saliva**	**Wet saliva**
RelyX Luting Plus	Group 1	Group 2	Group 3

Table 5. Part 2b study design

Stage of contamination		
Control		Group 1
Before adhesive	Saliva dried + Adhesive dried	Group 2
	Saliva wet + Adhesive	Group 3
After adhesive	Adhesive dried + Saliva dried	Group 4
	Adhesive dried + Saliva wet	Group 5

4.2.1 Specimen Preparation

After retention testing and failure analysis of the specimens and copings in Part 1 of the study, the residual cement on the specimens and copings was cleaned off by airborne particle abrasion with glass beads at 2 bar pressure. They were then rinsed off with distilled water and the specimens were stored in distilled water before further use. The collection of saliva was the same as described in Part 1 of the study.

4.2.2 Cementation of copings

All specimens were blotted dry prior to cementation.

For Part 2a: RMGI cement, the control group (Group 1) was cemented according to the IFU provided. For groups 2 and 3, 0.25mL of saliva was pipetted onto the tooth and spread around with a microbrush. In group 2, the saliva was allowed to dry as per the protocol in Part 1 and then the copings were cemented. For group 3, the copings were cemented onto the specimen immediately after saliva application. Thus, the copings were cemented under wet conditions.

For Part 2b: Resin cement, in the control group, adhesive was applied to the tooth and agitated for 20 seconds followed by air-drying for 10 seconds. This protocol for adhesive application was followed for the subsequent groups as well. Coping was then cemented as per the protocol used in Part 1 of the study. In group 2, the protocol was similar to the one used for group 9 of Part 1 of the study. Saliva was pipetted onto the tooth, spread around, and left to air-dry. Adhesive was applied as per the protocol described above and the coping was cemented. Group 3: Saliva was pipetted onto the tooth. A drop of adhesive was immediately placed on the wet saliva with a microbrush and spread around. The coping was then cemented immediately under wet conditions. Group 4: Adhesive was applied to the tooth as per the described protocol. 0.25mL of saliva was pipetted on top of the adhesive layer and left to air-dry. Coping was cemented as described above. Group 5: Adhesive was applied to the tooth as per the described protocol. 0.25mL of saliva was pipetted on top of the adhesive layer and the coping was cemented immediately. No primer was used for the copings in any of the groups. The copings were seated with finger pressure to ensure complete seating. Excess cement was carefully removed with a microbrush in an uncured stage while the coping is being held fixed. They were immediately be placed under a 2.5 kg load and self-cured as per the time reported in their IFU.

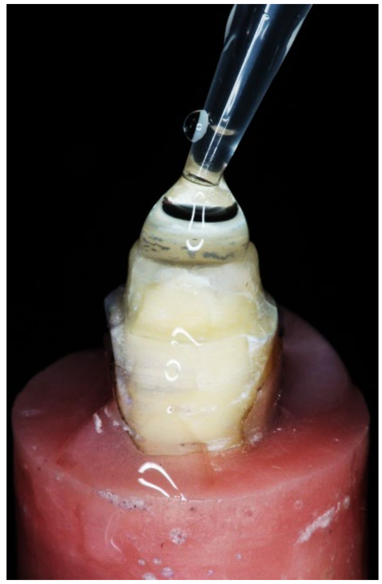

Figure 13. Application of saliva to the tooth

Figure 14. Seating coping on a wet tooth

4.2.3 Storage and thermocycling

The copings were stored in distilled water in a zip lock bag for 15 days at 37°C. They were not thermocycled.

4.2.4 Retention testing

Specimens were placed in a custom-positioning fixture (UAB Research Machine Shop, Birmingham, AL) and stabilized and centered by set screws on the sides of the positioning fixture. The positioning fixture was mounted in a universal testing machine (model no. 5565, Instron, Canton, MA). The handles on the coping were grasped by a wire loop. The specimens were loaded in tension at a crosshead speed of 1 mm/minute until the coping was separated from the tooth. The maximum debonding force (N) was recorded and the retention strength was calculated using the following formula:

Retention (MPa) = Debonding force (N) / Total bonding surface area (mm2)

4.2.5 Mode of failure analysis

Mode of failure was analyzed under 20× magnification on the digital microscope (VHX 6000 Series: Keyence) at the intaglio surfaces of the copings and the external surface of the tooth preparations. Failure was classified into one of five different categories as summarized in Table 3.

4.2.6 Statistical analysis

For Part 2a: RMGI cement, the crown retention strength was compared with 1-way ANOVA and Tukey's post-hoc analysis, if required. Significance (α) was set at 0.05 for all groups. Analysis was performed using SPSS software (IBM, Armonk, NY)

For Part 2b: Resin cement, the crown retention strengths were compared with 1-way ANOVA and Tukey's post-hoc analysis, if required. Significance (α) was set at 0.05 for all groups. Analysis was performed using SPSS software (IBM, Armonk, NY)

5. RESULTS

5.1 Retention Strength of Zirconia Copings Bonded with RMGI, Bioactive, Self-adhesive Resin and Adhesive Resin Cements

5.1.1 Crown Retention Strength

The average crown retention strengths (and standard deviation) for all cements and contamination status in Part 1 are presented in Table 6 and Figure 15.

Table 6: Crown retention strength mean values for Part 1

Retention Strength (MPa)					
	RelyX luting plus	**FujiCEM Evolve**	**Calibra Bio**	**RelyX Universal**	**K-0221 High voltage**
No saliva contamination	2.05 ± 0.76	3.82 ± 1.29	3.05 ± 1.04	4.99 ± 1.96	2.81 ± 2.13
Saliva contamination	1.42 ± 0.95	2.27 ± 1.61	3.04 ± 0.95	4.69 ± 1.26	2.67 ± 0.89

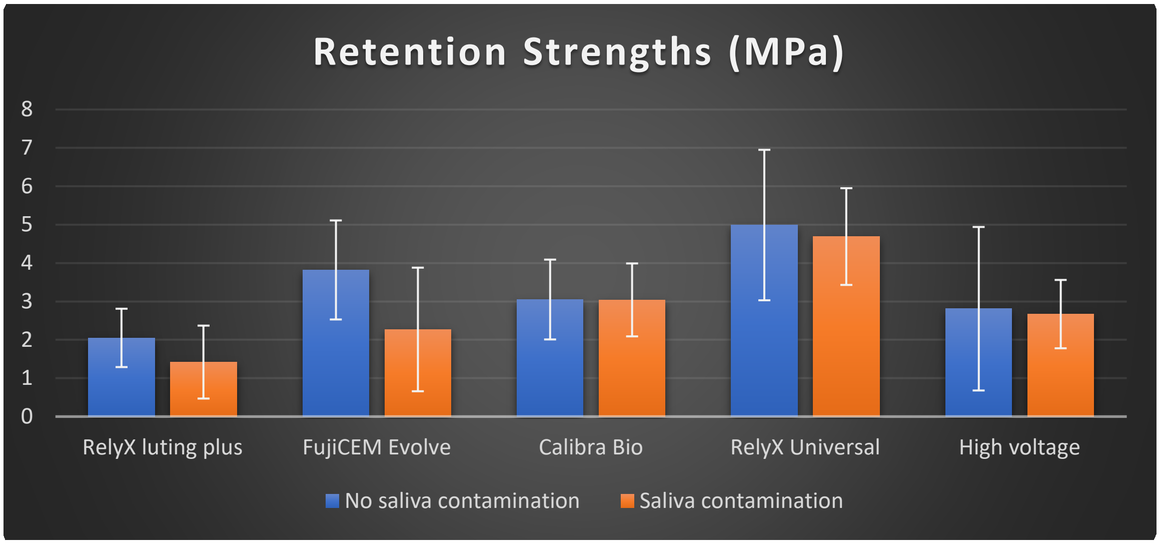

Figure 15. Crown retention strengths for Part 1

5.1.2 Statistical Analysis

2-way ANOVA was performed for factors *material* (RelyX luting, fujicem evolve, calibra, relyx universal and high voltage) and *contamination status* (contaminated and non-contaminated) and the interaction of both. The factor "contamination (p=0.056)" and the interaction between 'cements' and 'contamination (p=0.394) was found to be non-significant. However, the factor 'cements' was found to be significant ($p < 0.001$) (Figure 16).

Tests of Between-Subjects Effects

Dependent Variable: Retention

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	116.187[a]	9	12.910	7.004	<.001
Intercept	959.761	1	959.761	520.679	<.001
Contamination	6.898	1	6.898	3.742	.056
Cements	102.368	4	25.592	13.884	<.001
Contamination * Cements	7.628	4	1.907	1.035	.394
Error	167.739	91	1.843		
Total	1238.053	101			
Corrected Total	283.926	100			

a. R Squared = .409 (Adjusted R Squared = .351)

Figure 16. Two-way ANOVA table for Part 1 of the study

Retention

Tukey HSD[a,b,c]

Cements	N	Subset 1	Subset 2	Subset 3
RelyX Luting plus	21	1.7489		
High voltage	20	2.7438	2.7438	
FujiCEM Evolve	20		3.0486	
Calibra Bio	20		3.0501	
RelyX Universal	20			4.8426
Sig.		.145	.952	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.843.

a. Uses Harmonic Mean Sample Size = 20.192.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Figure 17: Tukey's post-hoc analysis for Part 1 of the study

5.1.3 Failure Analysis

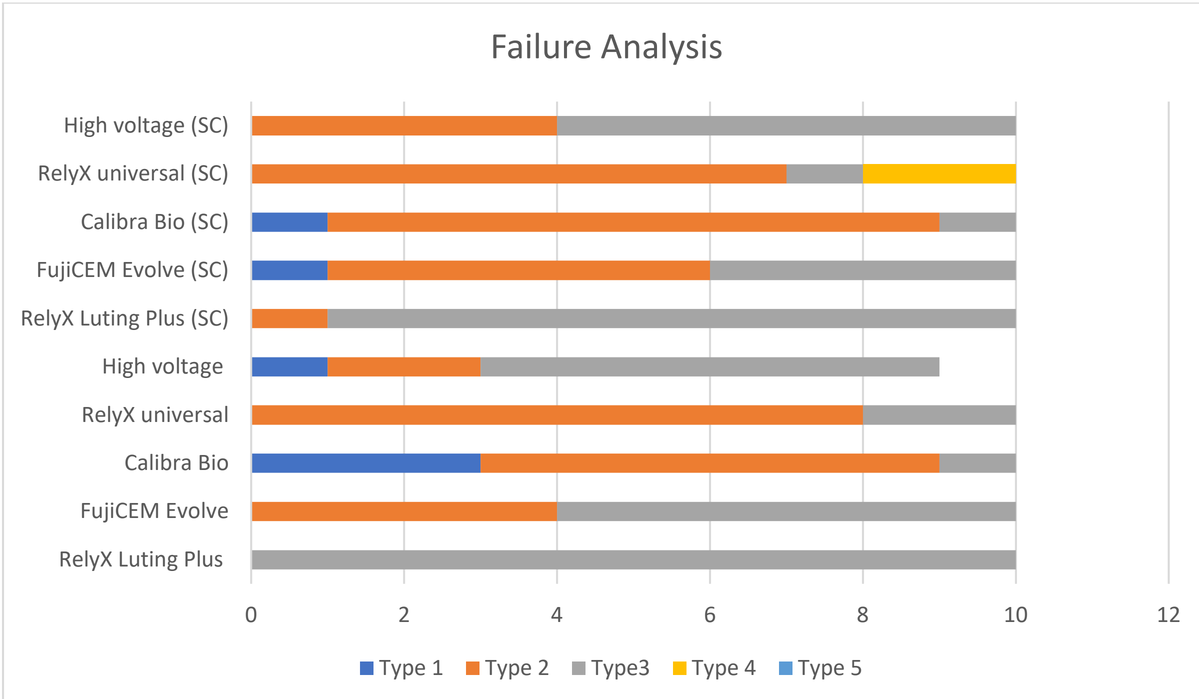

Figure 18. Failure analysis for Part 1 of the study

5.2 Retention Strength of Zirconia Copings Bonded with RMGI and Adhesive Resin Cements with Wet and Dry Saliva Contamination at Different Stages of Bonding

5.2.1 Crown Retention Strength

The average crown retention strength (and standard deviation) for all cements and contamination status in Part 2a and Part 2b are presented in Tables 7 & 8 and Figures 19 & 20, respectively.

Table 7: Crown retention strength mean values for Part 2a

	Control	**Dry saliva**	**Wet saliva**
RelyX Luting Plus	Group 1	Group 2	Group 3
Retention strength (MPa)	2.92 ± 0.86	2.49 ± 0.70	1.40 ± 0.22

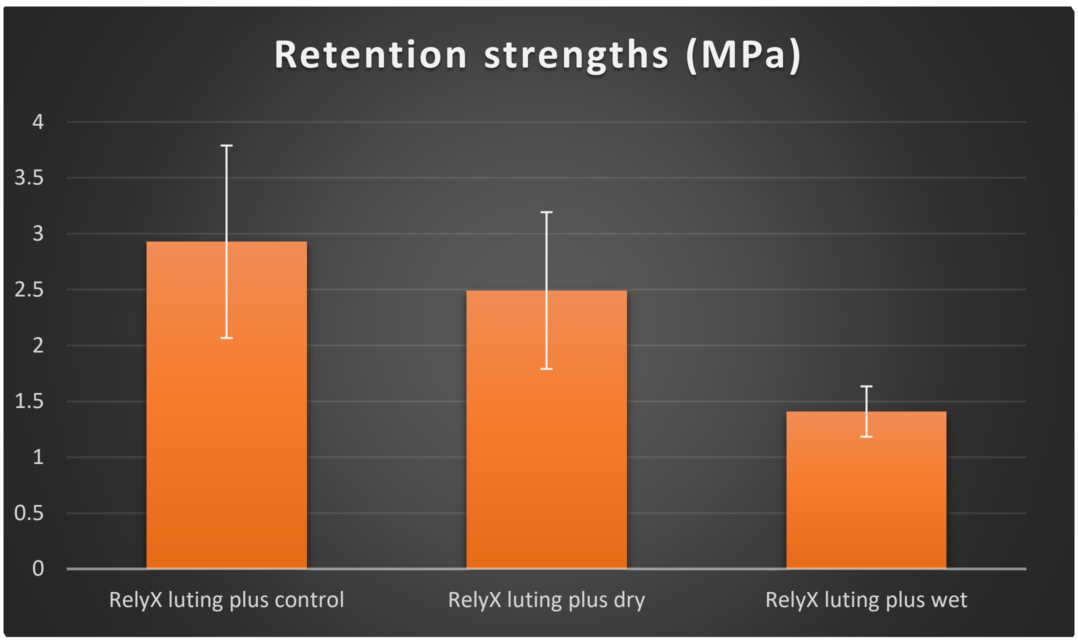

Figure 19: Crown retention strengths for Part 2a

Table 8: Crown retention strength mean values for Part 2b

Stage of contamination		Retention strengths (MPa)
Control		7.66 ± 1.01
Before adhesive	Saliva dried + Adhesive dried	2.97 ± 0.64
	Saliva wet + Adhesive	3.19 ± 0.87
After adhesive	Adhesive dried + Saliva dried	7.53 ± 0.78
	Adhesive dried + Saliva wet	6.05 ± 2.04

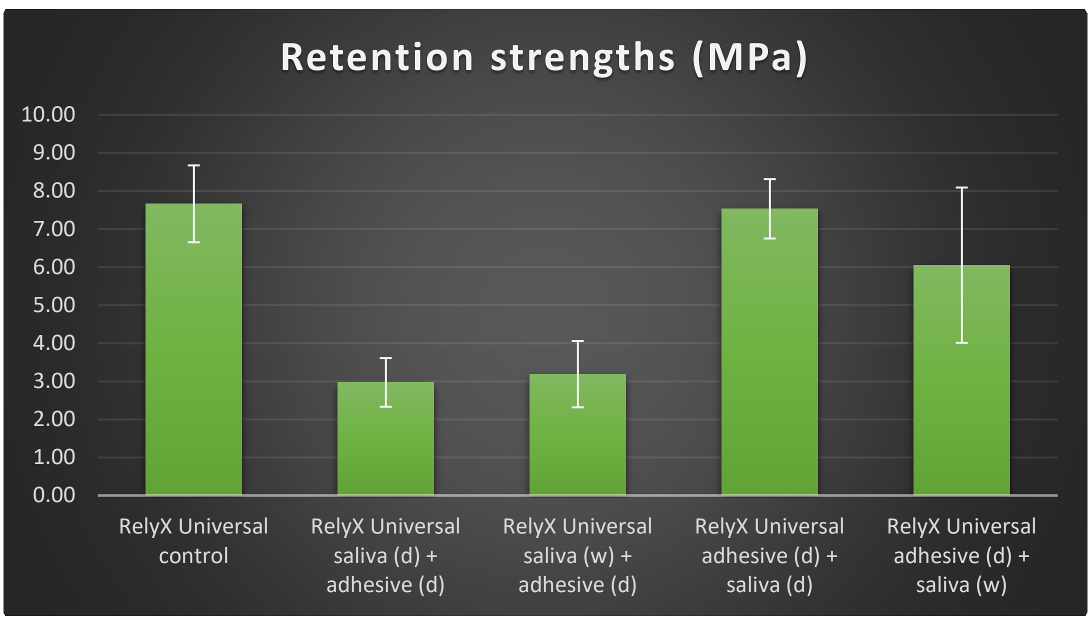

Figure 20. Crown retention strength for Part 2b

5.2.2 Statistical Analysis

For Part 2a, a 1-way ANOVA was performed with Tukey's post-hoc analysis. The difference between the groups was found to be statistically significant ($p<0.001$) (Figure 21). Tukey's post-hoc analysis separated groups into 2 significantly different groups (Figure 22).

ANOVA

Data

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.663	2	6.332	14.409	<.001
Within Groups	12.304	28	.439		
Total	24.967	30			

Figure 21: One-way ANOVA table for Part 2a of the study

Data

Tukey HSD[a,b]

		Subset for alpha = 0.05	
RMGI	N	1	2
wet	10	1.4086	
dry	10		2.4920
Control	11		2.9288
Sig.		1.000	.308

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.313.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Figure 22: Tukey's post-hoc analysis for Part 2a of the study

For Part 2b, a 1-way ANOVA was performed and groups were determined to be significantly different (p<0.001)" (Figure 23). Tukey post-hoc analysis separated groups into 2 significantly different groups (Figure 24).

ANOVA

data

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	162.209	4	40.552	16.800	<.001
Within Groups	106.207	44	2.414		
Total	268.415	48			

Figure 23. One-way ANOVA Table for Part 2b of the study

data

Tukey HSD[a,b]

group	N	Subset for alpha = 0.05 1	2
saliva (d) + adhesive (d)	9	2.7854	
saliva (w) + adhesive	10	3.9324	
adhesive (d) + saliva (w)	10		6.0494
control	10		7.1476
adhesive (d) + saliva (d)	10		7.5309
Sig.		.485	.235

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.783.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Figure 24: Tukey's post-hoc analysis for Part 2b of the study

5.2.3: Failure analysis

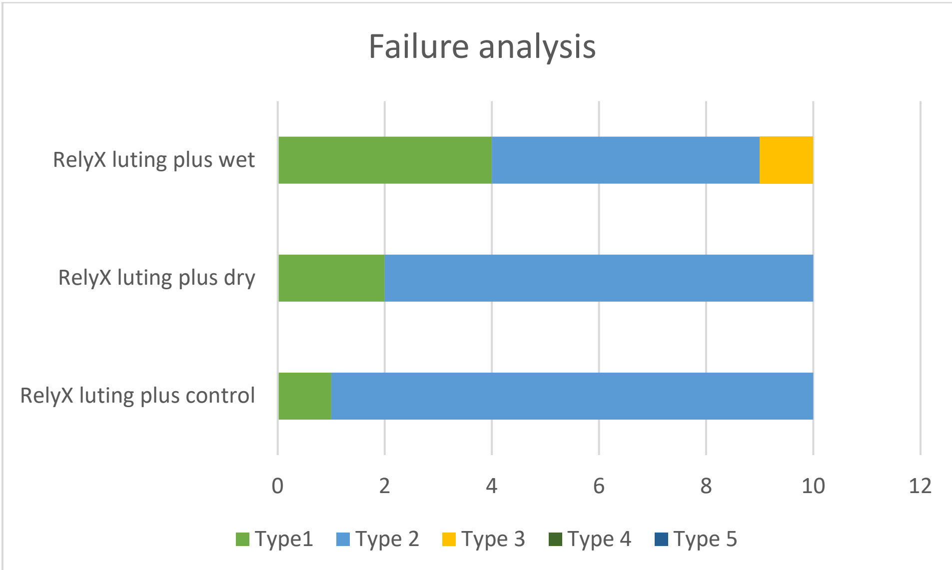

Figure 25. Failure analysis for Part 2a of the study

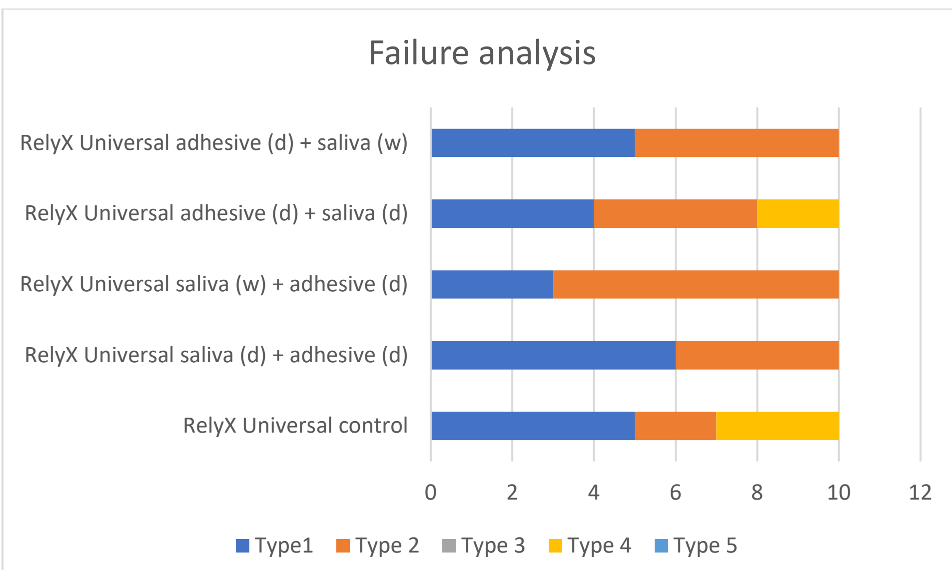

Figure 26. Failure analysis for Part 2b of the study

6. DISCUSSION

The proposed plan for this study originally consisted of only Part 1 of the methodology described above. However, after testing retention strengths, the data obtained led this author to reconsider both the study design and the objectives outlined. Several aspects of the data did not align with the expected results or the known behavior of these materials. The wide variation in retention strengths of the two RMGI cements, the poor performance of RMGI cements in the saliva contaminated groups, and the lack of statistically significant differences between saliva contaminated and non-contaminated groups (especially for resin cement), all warranted a re-evaluation of the study design and a re-assessment of the test itself.

Several factors were considered for Part 2 of the study. When saliva contamination is discussed with regards to any cementation, dilution of the material with watery saliva is considered one of the main reasons for failure. This effect was not seen in Part 1 as the copings were bonded in a dry environment. Additionally, when resin cements are bonded, questions of when contamination occurs is critical (before or after application of adhesive on the tooth). Lastly, we cannot truly compare RMGI and resin cements, not when the additional layer of adhesive and the role of contamination before or after bonding are accounted for. Keeping all this in mind, we tried to come up with a protocol for Part 2 which would fairly evaluate these conditions. To keep the study relatively simple, only one RMGI and one resin cement were used; conditions of dry and wet saliva and contamination during various stages of bonding were also studied.

In Part 2a, no statistically significant difference was found between the control group and copings cemented on dried saliva while both were statistically different from specimens cemented on wet saliva. This aligns with the statement above and provides us with an acceptable solution; if saliva gets in the way while cementing a zirconia crown with an RMGI cement, drying the saliva off and continuing with cementation is a viable approach.

In Part 2b, the results were more varied. This section was designed to answer two questions: how detrimental salivary contamination is when it occurs before the adhesive is applied versus after adhesive application, and what difference does a dry versus wet environment during bonding make. The obtained data establishes a clear distinction in the retention strengths with respect to the stage of contamination. Crown retention strengths were the lowest in the groups where saliva was applied and dried before adhesive was applied (group 2) or where the adhesive was mixed/diluted with saliva (group 3). No statistically significant difference was seen with respect to the environment they were bonded in (wet or dry). On the other hand, if adhesive was applied first and then saliva was allowed to dry on top of it (group 4), the crown retention strength was almost the same as the control (group 1). Even bonding in a wet environment (group 5) did not significantly affect the retention strengths if the adhesive was already on the tooth. A simple one-way ANOVA showed that there was no statistical difference between the control group (group 1) and groups where contamination occurred after bonding (groups 4 and 5), while groups with contamination before bonding were significantly different (groups 2 and 3).

Human saliva contains several enzymes that affect dentin-resin bond stability. Not only do MMPs and cysteine cathepsins[47] lead to degradation of the dentinal hybrid layer, but some salivary esterases are also capable of hydrolyzing bisGMA and TEGDMA monemers[48,49]. Thus, the organic components of saliva are capable of interfering with adhesive bonding as well. This could well explain the low crown retention strengths seen in group 2 of Part 2b, where the saliva is allowed to dry before adhesive application. Deposition of these enzymes in the dentinal tubules could lead to an ill-formed hybrid later and degradation of the monomers in the adhesive, ultimately leading to a poorly retentive crown. When a layer of adhesive is present before contamination, these enzymes do not get the chance to interact with the dentin and have a negligible effect on retention strength. An interesting phenomenon observed by the authors was that in groups where cementing or bonding occurred in a wet environment, a significant amount of saliva escaped along with the excess cement when the coping was placed on the tooth. It can be hypothesized that the denser cement pushed the watery saliva away when the cementation was taking place. How this affects retention strength is a potential topic for further study.

While this study has several strengths, like those of analyzing different types of cements separately, testing contamination at various stages of bonding and under different environments, there are several limitations as well. To avoid complicating the study, only one RMGI and one resin cement were tested. Whether different cements follow the results seen with their representative material remains to be seen. The adhesive protocol followed led to the bonding agent being used in self-etch mode and the resin cement to be used without a zirconia primer. Dozens of different combinations using different etching modes, different modes of the cement, presence or absence of ceramic primers and multiple bottle systems can be made, and testing those was beyond the bounds of this study. The use of a bioactive hybrid cement in Part 1 of this study was an interesting addition and more testing is required to see if it is a viable option for cementation. Lastly, in-vitro studies have several limitations by themselves. A larger sample size is ideally needed to reliably extrapolate the findings to a clinical scenario. Systematic reviews are difficult to assemble with in-vitro studies because of the lack of homogeneity seen in such studies. Perhaps, an ISO standard related to crown retention testing would help to standardize protocols. The nature of the topic makes it impractical to carry out a clinical trial on it. Thereby, in-vitro studies become a clinician's best source of knowledge on the topic, no matter how heterogenous it may be.

7. CONCLUSION

7.1 Hypotheses

1. There is a difference in the retention strength of zirconia copings cemented with RMGI, Bioactive, Self-adhesive Resin and Adhesive Resin Cements - This will be determined the factor *material* in a 2-way ANOVA - ***ACCEPT***

2. There is a difference in the retention strength of zirconia copings with and without salivary contamination. - This will be determined by the factor *contamination* in a 2-way ANOVA - ***REJECT***

3. The difference in the retention strength of zirconia copings with and without salivary contamination will be dependent upon the cement used. - This will be determined the interaction between *material* and *contamination* in a 2-way ANOVA - ***REJECT***

4. For RMGI cement, wet or dry saliva contamination will lead to a lower retention strength. This will be determined by completing a 1-way ANOVA and Tukey post-hoc analysis if necessary - ***ACCEPT***

5. For adhesive resin cement, wet or dry saliva contamination at different steps of bonding (before or after adhesive application) will lead to a lower retention strength. This will be determined by completing a 1-way ANOVA and Tukey post-hoc analysis if necessary - ***ACCEPT***

7.2 Within the limitations of this study, the following conclusions can be drawn:

1. The effect of salivary contamination differs between RMGI and resin cements, based on the environment in which cementation took place and for resin cements, the stage at which contamination occurred.
2. For RMGI cements, salivary contamination has no significant effect on crown retention strength if the saliva is dried off before proceeding with cementation. However, cementing on wet saliva is detrimental to the retention strength of the crown.
3. For resin cements, contamination that occurs before adhesive application is more detrimental to the crown retention strength than if contamination occurs after adhesive application, irrespective of whether cementation took place in a dry or wet environment.

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APPENDIX A

INSTIUTIONAL REVIEW BOARD APPROVAL

tional Comments:

Lab research on de-identified extracted molars.