Tufts UNIVERSITY | School of Dental Medicine

Hydrochloric Acid Cyclic Exposure Effect on Microleakage of Resin Cements

A Thesis

Presented to the Faculty of Tufts University School of Dental Medicine

in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Dental Research

by

[Sarah Nassief]

[April 2018]

THESIS COMMITTEE

Thesis Advisor

Ala Ali, B.D.S, M.Sc. D.Sc., D.M.D

Assistant Professor

Department of Prosthodontics

Tufts University School of Dental Medicine

Committee Members

Vasiliki Tsakalelli, D.M.D, M.Sc.

Assistant Professor

Department of Prosthodontics

Tufts University School of Dental Medicine

Aikaterini Kostagianni, D.D.S., M.S.

Assistant Professor

Department of Prosthodontics

Tufts University School of Dental Medicine

Sarah Pagni, Ph.D., M.P.H.

Assistant Professor

Department of Public Health and Community Service

Tufts University School of Dental Medicine

ABSTRACT

Introduction: Marginal microleakage is one of the major complications associated with bonding to dental porcelain. The extent of microleakage is unpredictable and differs according to the types of cement used. Research has evaluated the effect of common acidic media on the oral cavity and dental restorations. However, little is known about the effect of hydrochloric acid from the stomach in gastroesophageal reflux patients on the microleakage of resin cements under ceramic restorations.

Objective: To evaluate microleakage of different resin cements after immersion of zirconia-reinforced lithium silicate crowns (ZLS) in hydrochloric acid (HCl) solution.

Materials and Methods: 45 extracted non-carious human molars were prepared for porcelain crowns. Teeth were randomized into three different types of resin cement: RelyX™ UniCem (3M, ESPE), Variolink® Esthetic Dual-cure (Ivoclar Vivadent) and Panavia™ 21 (Kuraray America Inc.). Five replicates were used as a control for each resin cement. ZLS crowns were designed and milled from Celtra® Duo blocks using CEREC CAD/CAM system and then cemented to the prepared teeth using the assigned cement according to the manufacturers' guidelines. Samples were thermal cycled between water temperatures of 5℃ and 55℃ for 5,000 cycles with a 15 second dwell-time after each temperature. In order to mimic one year of clinical time in a patient's mouth, samples of the experiment group were subjected to 91 one hour cycles in HCl (pH 2) followed by one hour in artificial saliva, however samples of the control group were submersed in artificial saliva for 91 hours. After the 91 cycles were completed, specimens were submerged in 50% silver nitrate solution for 24 hours followed by a developer solution for eight hours. The samples were embedded in clear epoxy resin and sectioned in a buccolingual direction at 0.5 mm. Sections were analyzed by a stereomicroscope at a magnification of 10X. Proportion of microleakage was calculated by dividing the total length of the dye penetration by the total length of the restoration. Data were analyzed with the Kruskal-Wallis test and Mann-Whitney U test with the Bonferroni correction.

Results: There was a statistically significant difference in median microleakage between the experiment and the control groups ($p < 0.05$) However, there was no statistically significant difference between the three types of cement in the experiment group.

Conclusion: Within the limitations of this in-vitro study, the results indicated that all of the resin cements tested in the study exhibited microleakage to some degree, especially when they get exposed to the hydrochloric acid solution.

DEDICATION

I dedicate my master thesis to my mother Faten for her unconditional love and support.

To my father, Saeed who will be always in my heart.

To my husband, Mohammed for his usual support and inspiration.

To my sister and brothers Housam, Shereen, and Faris for being always with me.

To my son, Abdulrahman for making my life much happier.

This journey would

not have been possible without all of you being in my life.

ACKNOWLEDGMENTS

My great appreciation and gratitude to Dr. Ala Ali for his guidance and support during this project.

Dr. Vasiliki Tsakalelli for dedicating her knowledge, time and help for this thesis.

Dr. Aikaterini Kostagianni for her time and support.

I would like to thank Dr. Sarah Pagni for her statistical support and comments.

I would like to thank Mr. Jeffrey Daddona for guiding me in the Gavel lab.

Finally, I am also thankful to Umm Al-qura University in Saudi Arabia for sponsoring my postgraduate education.

TABLE OF CONTENTS

DEDICATION v

ACKNOWLEDGMENTS vi

TABLE OF CONTENTS vii

LIST OF FIGURES viii

LIST OF TABLES ix

LIST OF ABBREVIATIONS x

LIST OF SYMBOLS xi

Introduction 2

Aim and Hypothesis 13

Materials and Methods 14

Sample Size Calculation 19

Statistical Analysis 19

Results 20

Discussion 21

Conclusion 26

References 27

APPENDICES 33

Appendix A: Tables 34

Appendix B: Figures 37

LIST OF FIGURES:

Figure 1: Handpiece connected to a surveyor.

Figure 2: Prepared tooth.

Figure 3: CEREC CAD/CAM machine**.**

Figure 4: Scanned model with the finish line in the CAD/CAM machine.

Figure 5: Design of crown in the CAD/CAM machine.

Figure 6: Experimental and control groups of the study.

Figure 7: Resin cements used in the study.

Figure 8: Crown seating with 50 N occlusal load.

Figure 9: Samples inside the incubator at 37℃.

Figure 10: Sectioned sample.

Figure 11: Stereomicroscope.

Figure 12: Total length of restoration in a sectioned tooth under stereomicroscope.

Figure 13: Extent of dye penetration in a sectioned tooth under stereomicroscope.

Figure 14: Boxplot of microleakage scores in all the groups.

LIST OF TABLES:

Table 1: Composition of resin cements used in this study.

Table 2: Modified United States Public Health Service criteria (USPHS/CDA)

USPHS – Marginal Adaptation.

Table 3: Median microleakage scores and interquartile ranges of all cements.

LIST OF ABBREVIATIONS

CAD/CAM: Computer-assisted design/computer-assisted manufacturing.

ZLS: Zirconia reinforced lithium silicate.

HCl: Hydrochloric acid.

IQR: Interquartile range.

LIST OF SYMBOLS

℃: Degree Celsius.

N: Newton.

μm: Micrometer.

Hydrochloric Acid Cyclic Exposure Effect on Microleakage of Resin Cements

Introduction

Ceramic restorations have continuously increased in popularity due to their superior aesthetics and biocompatibility with oral tissues; additionally, they demonstrate reliability and effectiveness as an outstanding restorative material.[1] However, certain functional challenges associated with the nature of the oral environment are still present.[2, 3]

Ceramic is an inorganic brittle material whose favorable characteristics have kept it in use for over one hundred years.[3, 4] Several types of porcelain are traditionally used to restore anterior and posterior teeth, such as feldspathic porcelains, aluminous porcelains**,** pressable or injection-molded ceramics, lithium silicate and lithium disilicate glass ceramic. The need to improve mechanical features associated with fracture resistance has led to the introduction of new materials like zirconia and zirconia-reinforced lithium silicate/zirconia (Celtra Duo).[5]

The types of porcelain that are currently in use have different compositions.[5] For example**, feldspathic porcelain**, which is comprised of a combination of potassium feldspar and glass, are used primarily as a veneering material for metal ceramic restorations, porcelain veneers, and single crowns. However, it is not recommended to be used for posterior high-load bearing regions.[6, 7]

Aluminous porcelains have a slightly different mixture than feldspathic porcelains, with a larger total amount of oxide crystals (40-50 wt.%).[7] Aluminous porcelains are indicated as being suitable for laminate veneers, veneering for all ceramic restoration, inlays, and onlays. Both aluminous porcelains and feldspathic porcelains are highly esthetic restorative materials and demonstrate an excellent compressive strength.[8-10]

Pressable or injection-molded ceramics are provided in the form of ceramic ingots, which are subsequently melted and pressed into a mold using the lost wax technique. IPS Empress Esthetic for instance has 40-50 wt.% of leucite crystals.[11] Pressable molded ceramic is a high-strength porcelain.[12] **Fluorapatite porcelain** exhibits a unique pattern among all other existing dental porcelains. It is composed of dispersed fluorapatite crystals ($Ca_{10}(PO_4)_6F_2$) in a feldspathic glassy matrix.[13] Fluorapatite crystals have a needlelike morphology. This is the only type porcelain that can be found in the human body, in natural bone and teeth; that feature gives the porcelain its unique optical properties including translucence and opalescence.[11]

Zirconium dioxide (zirconia) is a polymorphic material that exists in three solid forms under cooling circumstances: cubic (2680°C to 2370°C), tetragonal (2370°C to 1170°C), and monoclinic (1170°C to below room temperature).[14] Three to four percent of volume expansion is expected during the transformation from the tetragonal to the monoclinic phase. This feature could be an advantage when resisting progression of crack.[15, 16] **Celtra Duo** is classified as a zirconia-reinforced lithium silicate (ZLS). It is composed of 58% silica (SiO_2), lithium metasilicate, disilicate, and phosphate crystals, in addition to 10% zirconia crystals.[17-19] ZLS is a CAD/CAM restorative material that joins the excellent mechanical features of zirconia with the highly esthetic appearance of glass-ceramics.[20]

CAD/CAM Technology

Ceramic restorations can be invented either by conventional laboratory techniques or by using a dental CAD/CAM system. The CAD/CAM machinery system, which stands for computer-assisted design/computer-assisted manufacturing, has been effectively introduced to the market in the last decade.(21) The first CAD/CAM restoration was an inlay which was produced in 1985 utilizing a feldspathic ceramic block.(22) The CAD/CAM system is a good alternative for the laboratory technique since the latter is considered to be time-consuming and sensitive to handling.(14)

The CAD/CAM system includes several components. A scanner analyzes data from the oral cavity and registers that to the computer. There is unique design software that allows the user to view data and to create new data and reports. The system also serves as a production device, fabricating ceramic restorations.(23)

CAD/CAM ceramic restorations have demonstrated a significantly higher fracture resistance than crowns fabricated by the traditional method.(24) After 21 years of clinical monitoring, ceramic inlays produced by a CAD/CAM system have demonstrated a success rate of 90% after 10 years and 85% after 12 and 16 years, according to a clinical prospective study.(23, 25, 26) After 1991 several types of ceramic blocks with some enhanced mechanical properties emerged in the market for the CAD/CAM machine such as Dicor™, Vita™ Mark II, ProCAD™, and Empress™ .(27)

Resin Cement

Resin cement consists of a mixture of an organic matrix through which phosphoric acid methacrylate reacts with the inorganic fillers from the environment. However, in the oral cavity the phosphoric acid methacrylate interacts with the hydroxyapatite that presents in the tooth structure. Several resin-based dental cements have been introduced in the last few years, such as RelyX UniCem, Panavia and Variolink II.(28, 29) In addition to providing a sound and durable bond between tooth surface and ceramics, resin cements can improve the appearance of ceramic restoration and produce a higher ceramic strength.(30)

Resin cements are the preferred type of cement in terms of resisting microleakage. They have demonstrated some superior characteristics over the other luting agents such as zinc phosphate, polycarboxylate, and glass ionomer cements. Resin cement could maintain an acceptable margin which reduces the amount of leakage, caries, and restoration failure.(31) Resin cements have several shades that can improve the appearance of ceramics, so final restorations would appear more natural in comparison to the opaque classic cements.(32) Extensive tooth preparation for retention mean is no longer needed with resin cements because of their unique adhesion properties, which can maintain a sound tooth structure.(33) In addition to these characteristics, resin cement bonds well to the tooth surface. Existing literature has reported resin cement to have high bond strength, high tensile and compressive strength. In addition, it shows low solubility and high flexural properties which are essential to prevent the debonding process.(34)

There are several types of resin cements; **RelyX Unicem 2 Automix** is a dual-cure, self-adhesive universal resin cement. Even though it does not necessitate any bonding or conditioning of tooth surface, it bonds to it as well as the multi-step luting agent does.[(35)] **Variolink Esthetic,** which replaces Variolink II, is a dual-curing, color stable adhesive luting system. This type of cement is indicated for permanent cementation of highly esthetic ceramic and composite resin restorations since it comes in five different shades that make it suitable for all of the cases.[(36)] **Panavia 21** is an advanced universal self-cure adhesive resin cement; the paste involves universal and catalyst pastes. It does require mild conditioning for enamel and dentin to enhance the bond strength and to accelerate polymerization. Panavia 21 is available in three shades.[(37)] The composition of each resin cement is listed in Table 1.

Proper cementation technique is a crucial step in maintaining a successful indirect restoration. Longevity of ceramic restorations in the oral cavity mainly relies on several mechanical characteristics of cement such as compressive, flexural strength, and film thickness. Masticatory forces in the mouth directly affect the ceramic restorations. Therefore, the selected cement must have a good compressive strength to withstand these forces. Since ceramic is a brittle material, the high compressive strength of cement will increase the fracture resistance of the restoration.[(38)] Moreover, cement must have a sufficient flexural strength to spread the stresses between the tooth and the restoration without fracture.[(39)]

In a study done by Piwowarczyk et al., they compared the compressive strength of different types of luting cements after 24 hours and 150 days. Variolink II light-cured reached the highest score of all the cements examined with 303.5 MPa after 24 hours and 325.8 MPa after 150 days. Followed by RelyX ARC light-cured with 284.5 MPa after 24 hours and 288.9 after 150 days then Panavia F light-cured with 244.2 MPa after 24 hours and 257.8 MPa after 150 days and finally RelyX Unicem light cured with 240.6 MPa after 24 hours and 194.5 MPa after 150 days.[40]

Typically, the cement space for all ceramic restorations is larger than the one needed for porcelain fused to metal or full metal crowns. Studies have indicated that the clinically acceptable marginal gap for ceramic restorations ranges from 20 to 150 µm.[41-43] If the space between the restoration and the margin of the prepared tooth is too large, plaque accumulation, gingival inflammation, and secondary caries could occur.[44, 45]

Bonding to Tooth Structure

Pretreatment of Tooth Surface

Establishment of a successful bond between ceramic restoration and enamel surface needs a micromechanical mean of retention that can be obtained by roughening the cavity preparation.[46] However, at the dentin level, creation of a hybrid layer and resin tags is the key behind the formation of an effective bond.[47] Establishment of such a strong bond can be reached by complete dissolution of the smear layer and demineralization of the intertubular and peritubular dentin with the acid etch, which exposes the collagen matrix, giving way to resin to be infiltrated.[48]

For alumina ceramics, zirconia, and leucite reinforced glass ceramics, silica coating technique was reported as the best surface treatment.[49, 50] However, lithium disilicate ceramics require airborne particle abrasion and acid etch to produce the maximum tensile bond strength to the resin.[49, 51] On the other hand, etching alumina reinforced feldspathic ceramic with hydrofluoric acid followed by an application of silane coupling agent produce greater bond strength than the combined treatment of airborne particle abrasion with aluminum oxide or silicon oxide followed by silanization.[51] Another study done by Panah et al., reported that the most effective surface treatment to improve the bond strength of lithium disilicate ceramic to a resin includes silane coating after airborne particle abrasion and acid etching method.[52]

According to literature, Phosphoric acid is the recommended acid etch to use for tooth surface with a concentration of 30% to 40% and etching time ranges from 15 to 30 seconds.[53, 54] With this 15 seconds of acid etching, enamel will be etched enough however, dentin will be demineralized 5 to 8-μm, which makes the infiltration of resin easier to be done.[55]

Adhesive steps of etching, priming, and bonding should be regularly applied on the enamel and dentin for the hybrid layer to be created and the micromechanical retention to be obtained. The hybrid layer is defined as an extremely organic layer characterized by being hydrophobic, acid resistant, and tough.[56]

The hybrid layer aids in increasing the bond strength between cement and the tooth surface.[56] Numerous types of resin cements are usually offered with their own adhesive system to satisfy the compatibility between them.[57]

There are different types of adhesive systems; selected types of cement are provided as a total etch resin cement (etch and rinse adhesive system). However, some other cements are available as self-etch resin cement (adhesives contain self-etch primers). The latest generation of resin cements excludes the need for any pretreatment before the cement itself by combining the monomers and adhesives with the cement (self-adhesive resin cement).[57]

Pretreatment of the Restoration

Cement acts as an attachment between the tooth surface and the restoration. Establishment of a successful bond between ceramic restoration and the tooth structure requires a pretreatment of the internal surface of the restoration as well.

This pretreatment will:

- Increase the surface area for bonding by roughening the internal surface of the restoration; and
- Form chemical bonds between the ceramic, the fillers, and the cement increasing the wettability of cement to the restoration.[57]

Using hydrofluoric acid etch surface conditioning before the resin cement builds a micromechanical retentive surface to ensure a durable bond between ceramics and the tooth surface.[58, 59] This step is usually followed by the application of a silane coupling agent to form a chemical covalent and hydrogen bond. Usually, the final step is to apply the luting agent.[55]

Roughening the internal surface of the restoration could be accomplished by air abrasion, sandblasting, or etching with a hydrofluoric acid if the final restorations are either ceramic or composite; however, for restorations with a metal subsurface, application of an alloy primer is recommended.[57] Increasing the wettability of the cement to the restoration is usually achieved through the procedure of silanization of the etched porcelain or composite.[60] Silane agents create a chemical bond between the resin and the ceramic or composite restoration. The organic group from the silane bonds chemically to methacrylate-based resins. They also have an Inorganic group which reacts with the hydroxyl group from the ceramics.[61]

Microleakage Development

Although no restoration can reach the optimal marginal adaptation, successful restorations have been associated with a degree of marginal adaptation and microleakage for several years.[62] The extent of microleakage is unpredictable and differs according to the types of cement used with the restoration.[31]

Microleakage, the penetration of bacteria, liquids, molecules, or ions between the restorative material and the abutment tooth, is one the most serious complications associated with the use of porcelain; it can cause secondary caries, marginal staining, pulpal hypersensitivity, and ultimately, the need for replacement.[63-65] Several factors contribute to microleakage, including the difference between the coefficient of the thermal expansion of the dental restorative material and the restored tooth, shrinkage of the luting agent, and inadequate marginal adaptation.[66]

Crown adaptation is determined by the extent of the marginal and internal gap of the restoration. Internal gap could be defined as the vertical distance that lies between the axial wall of the prepared tooth to the internal surface of the ceramic restoration. However, the marginal gap is described as the vertical distance from the inner surface of the ceramic restoration to the finish line of the prepared tooth.[67]

This marginal adaptation performs an essential role for restoration success. Cement degradation could happen whenever there is an increase in the marginal or internal gap, which eventually leads to microleakage, caries, restoration fracture, and periodontal diseases.(68, 69)

Comparing between different types of cements, zinc phosphate demonstrates more evidence of microleakage among glass ionomer and resin cements, this phenomenon can be attributed to the fact that it exhibits a higher level of solubility than glass ionomers and resin cements.(70, 71)

Gastroesophageal Reflux Disease

The effect of commonly used acidic media on the oral cavity and dental restorations has been frequently evaluated. Among the most common media of interest is the hydrochloric acid that is present in the gastric fluid of patients who have gastroesophageal reflux,(72-74) which occurs when an individual has no control over the passage of gastric contents.(75) As the gastric enzymes are very acidic and typically have a pH below 1, the frequent presence of gastric acid in the mouth can potentially turn the oral cavity into a constant acidic environment in which damage to the hard and soft tissues of the oral cavity might occur.(76, 77) A study by Kumar et al. found that females are more susceptible to this type of disease than males, with 63.1 % of females in the study being affected.(78)

Dental erosion, an extra-esophageal complication, is an irreversible loss of the tooth elements, usually found in the palatal and lingual surfaces of maxillary teeth.(79, 80) Dental erosion is a gradual process which takes many years to develop and It is usually not adequately detected until it is in the advanced stage.[81]

In long-term circumstances, dental erosion might affect the occlusal surface as well, causing loss of tooth structure, loss of the vertical dimensions, and severe damage to the whole masticatory system.[82] In a study done by Sujatha et al., dental erosion was found in 41% of patients with gastroesophageal reflux.[80, 83]

Although gastroesophageal reflux patients need comprehensive medical treatment, they also require an interdisciplinary dental treatment for oral complications. The treatment required for tooth wear and erosion may involve composite restorations, root canal treatment, veneers, full coverage crowns for the affected teeth and possibly full mouth rehabilitation.[84]

As hydrochloric acid from the gastric contents has a massive destructive effect on the teeth, the subsequent longevity of ceramic restorations may also be affected by the presence of acid in the oral cavity. Ceramic breakdown may occur as a result of prolonged exposure to this acid. Chemical dissolution of ceramics from this exposure leads to selective ion leaching, which can result in surface roughness, decreased stability and flexural strength, increased plaque retention, and possible abrasion of the opposing tooth.[85, 86] However, there is no sufficient information in literature about the effect that hydrochloric acid from the stomach has on the microleakage of resin cements under ceramic restorations.

Aim

The purpose of the present in vitro study is to evaluate the effect of hydrochloric acid on microleakage of RelyX UniCem, Panavia 21 and Variolink Esthetic cements after immersion of lithium silicate crowns in hydrochloric acid solution.

Hypotheses

Primary hypothesis: There is a difference in microleakage among the three types of resin cements Panavia 21, RelyX Unicem and Variolink Esthetic when utilized on lithium silicate crowns after immersion in hydrochloric acid solution.

Secondary hypothesis: Exposed lithium silicate crowns to hydrochloric acid solution will exhibit higher microleakage score than the non-exposed group.

Materials and Methods

This study is a laboratory study that has been conducted at the Prosthodontics Lab at Tufts University School of Dental Medicine (TUSDM).

Forty-five extracted intact human molars were obtained from the oral and maxillofacial surgery clinics and the Gavel Research Lab at TUSDM. Extracted teeth were collected in a de-identified manner meaning that they were not being able to be connected to the patient they came from. Teeth were stored in an aqueous solution of 0.5% sodium hypochlorite (Bleach, Olinchloralkali, Cleveland, Tennessee) at room temperature until the beginning of the study. The teeth were free from any developmental defects, cracks, restorations, and caries. Any stains or surface debris were removed from the surface of the teeth using an ultrasonic scaler (Cavetron SPS, Dentsply, York, PA).

For adequate retention, a notch was created into the roots of all of the teeth before they were vertically fixed in place with the cemento-enamel junction higher than the top of a mounting template by 1 mm. To lock the teeth in place, orthodontic acrylic resin (Caulk Orthodontic Resin, Dentsply Caulk, Milford, DE) was used to fill the mounting templates. After teeth mounting were completed, the occlusal surface of all of the specimens were trimmed to a flat surface 4 mm above the surface of acrylic resin using a model trimmer (Whip Mix, Louisville, KY). Tooth preparation of a full-coverage crown was performed using a high-speed handpiece (Midwest Dentsply, Des Plaines, IL) that was connected to a surveyor (Degussa F1; DeguDent, Hanau, Germany) and water coolant system (Figure 1).

A diamond bur (847-16; Henry Schein, Melville, NY) was prepared at a six-degree angle from the vertical axis of the tooth to achieve a total convergence angle of 12 degrees. A custom jig (Lab-Putty, Coltene, Switzerland) was used to secure the mounted teeth vertically and to hold them immovably in the surveyor base.

The mounted sample was turned against the running bur to achieve the axial cutting. The cervical margin was prepared as a shoulder finish line all around with rounded internal angles. The axial surface was cut to 4.0 mm height and the depth of the axial surfaces was reduced by 1.5 mm to create a shoulder finish line by utilizing copious water irrigation. For sample smoothing, a fresh diamond bur (KD7W6 Brasseler, Savannah, GA) was used for each sample. After the teeth preparations were completed (Figure 2), CEREC Optispray (Dentsply Sirona, Long Island, NY) was sprayed on each prepared tooth before scanning. Afterward, an optical impression was taken for each prepared tooth using the E4D CAD/CAM machine (CEREC, Blue-cam, Bensheim, Germany) (Figure 3). To achieve a high-determination picture, the scanner used optical imaging of the tooth from various headings.

Each crown had a wall thickness of 2.0 mm in the contact area, thickness at the central fossa was 1.5 mm and 2.5 mm at the cusp tip. All crowns were designed such that they had a cement thickness of 100 µm. The crowns were milled from zirconia-reinforced lithium silicate porcelain blocks (Celtra Duo, Dentsply international, Milford, DE) using the inLab MC XL milling unit (CEREC, Bensheim, Germany) (Figures 4,5).

Prior to crown cementation, two trained calibrated prosthodontists evaluated the marginal adaptation using Modified United States Public Health Service criteria (USPHS/CDA) found in USPHS – Marginal Adaptation.[87] Samples classified under R and S criteria were the only accepted samples. Crown quality criteria is listed in Table 2. Some modifications were made to specimens that needed adjustments to improve the seating of the crown by carefully grinding the internal surface of the crown using a high-speed handpiece (Midwest Dentsply, Des Plaines, IL)) and a small round diamond bur (6801; Brasseler, Savannah, GA).

After milling was completed, every specimen was assigned a number using a Sharpie permanent marker. Specimens were divided and allocated randomly into three experimental groups and three control groups (Figure 6). Randomization was performed using R (Version 3.1.2).

Crowns were cemented to their corresponding prepared teeth using three types of resin cement, (Figure 7) which were utilized in accordance with the respective manufacturers' guidelines. Teeth and restorations were cleaned from any debris with water then dried with a gentle air. The internal surface of each crown was acid etched with a 5% solution of hydrofluoric acid (Ivoclar Vivadent, Schaan, Liechtenstein) for 20 seconds. A silane coupling agent (The Micro Dose, Premier, Plymouth Meeting, PA) was applied to the internal surface of each restoration for 60 seconds to increase the bond strength of cements then dried with a gentle air. After that the enamel surface of each prepared tooth was treated with a 37% solution of phosphoric acid for 15 seconds rinsed with water and dried. Cement was dispensed from each tube and applied to the internal surface of the crowns, then positioned on the respective tooth with a finger pressure.

The combined teeth and crowns were then set in a loading machine (Instron, Model 5566; Canton, Mass), and each one was exposed to a seating power of 50 N for the predefined setting time of five minutes to take into account room temperature polymerization (Figure 8). Every spaceman was light cured for 20 seconds on each surface. Excess cement was removed from all margins using a dental explorer No.23. Cemented copings were stored in tap water at 37℃ for 24 hours before thermocycling. Specimens then thermocycled (Model:1156, VWR, Germany) between water temperatures of 5˚C and 55˚C for 5,000 cycles with a 15 second dwell time at each temperature. Exposed surfaces of the teeth were coated with a layer of nail varnish to seal the open dentinal tubules. The experimental group was subsequently immersed in a hydrochloric acid solution at a pH of 2 (DR. Clark digestive power, Chula Vista, CA).

Samples were subjected to 91 cycles in HCl, each lasting one hour, and another one hour in artificial saliva (pH=6.7). About five cycles were completed manually per day. The amount of time has been calculated according to the assumption that 91 hours equates to one year intraorally.(88) However, samples in the control group were immersed in artificial saliva for 91 hours. During the cycling procedure, samples were placed in a jar and kept inside an incubator at 37℃ (Figure 9).

After all the cycles have been completed, the specimens were submerged in 50% silver nitrate solution (Salt Lake Metals, Salt Lake, UT) for 24 hours, rinsed extensively with water, and placed in a freshly mixed developer solution (Eastman Kodak Co, Rochester, NY) for eight hours in a dark room. All of the samples were embedded in clear epoxy resin (EpoKwick Resin, Buehler, Lake Bluff, IL), then sectioned in a buccolingual direction utilizing a 0.5 mm thickness and an eight-inch diameter diamond wheel (Isomet 1000, Buehler Ltd, Evanston, IL) (Figure 10). Both sections were analyzed and all margins were evaluated under a stereomicroscope (SZX16, Olympus, Pennsylvania) at a magnification of 10X (Figure 11).

The specimens were scored by the percentage of color infiltration along the dentinal walls. The proportion of microleakage was calculated by dividing the total length of the dye penetration by the total length of the restoration (Figures 12,13).

Sample Size Calculation:

The software nQuery version 7.0 was used for the power calculation with the following assumptions: a type I error rate of 5% and a maximum type II error rate of 20%. A sample size of n=5 per group was found to have 98% power to detect a difference in microleakage between the different resin cements.

Statistical Analysis

Descriptive statistics (medians and interquartile ranges for continuous variables) were calculated. Differences in microleakage between cement groups first were determined by using one-way ANOVA because there was one categorical factor associated with a continuous outcome, and outcomes are not related to each other; however, the assumption of normally distributed data was violated. So, a Kruskal-Wallis test was performed to compare the microleakage score between groups. A Mann-Whitney U test together with the Bonferroni correction was used for post-hoc tests. Normality was assessed graphically and with a Kolmogorov-Smirnov test. The software SPSS version 24 was used for the statistical analysis.

Results

The study sample was divided into two groups; the experimental group included 30 molars (n=10), and the control group which is composed of 15 molars (n=5). After the procedure of teeth sectioning; two halves were obtained from each of the 45 specimens and an average of both sides was taken.

The Kruskal-Wallis test showed statistically significant differences in median microleakage among all the groups tested in the experimental group ($p < .05$). Post-hoc tests after Bonferroni correction that were conducted ($p < .008$) revealed the differences between these groups:

RelyX Unicem in control and experimental group ($p = .001$), and between Panavia 21 in the control and the experimental group ($p = .001$).

Experimental group: Panavia 21 showed the highest median microleakage score of 59.24% (IQR=25.88), Followed by RelyX UniCem and Variolink Esthetic which measured 54.95% (IQR=8.48) and 45.83% (IQR=36.11) respectively.

Control group: Variolink Esthetic median microleakage score was the highest among the control group at 32.82% (IQR=18.41). However, the median microleakage score for RelyX UniCem was the lowest at 21.81% (IQR=15.82) and the microleakage score for Panavia 21 was 25.49% (IQR= 10.50).

Median microleakage scores and interquartile ranges are presented in Table 3.

Discussion

It is well-known that the type of cement that used for bonding has a strong impact on the microleakage value.[89, 90] In the meantime, resin cements have been widely used because of their enhanced physical properties and their promising marginal seal.[91]

The objective of the current study was to evaluate the effect of hydrochloric acid on the microleakage of three different resin cements; RelyX UniCem, Panavia 21 and Variolink Esthetic after the immersion of lithium silicate (Celtra Duo) crowns in hydrochloric acid solution. The results of this study support the second research hypothesis, that all of the experimental groups exposed to the HCl exhibited a higher microleakage value than the control groups. Nevertheless, the first null hypothesis could not be rejected since there was not a statistically significant difference between the three experimental groups tested.

This is the first study that evaluates the effect of hydrochloric acid on microleakage of ceramic crowns cemented using resin cements. Evidence on this matter is crucial to the dentist in order to select the most appropriate restorative material to use with these types of patients.

Even though this in-vitro study did not demonstrate the actual situation of the in-vivo effect of acid exposure. However, in the present study the most applicable method was used to stimulate the oral cavity pH buffering process in patients' mouth by subjecting the samples to 91 one-hour cycles in HCl (pH 2) followed by one hour in artificial saliva which represents one year of clinical exposure. This time interval was calculated and reported by Harryparsad et al.[88] and was formulated according to the theory that the gastroesophageal patient has an average of three purges per day, each of them lasting approximately five minutes.[92, 93]

Based on the literature, gastroesophageal reflux patients have a low salivary pH which sometimes falls below 2, an impaired salivary flow and reduced buffering capacity.[94-97] All of these complications might exaggerate the effect of acid in the oral cavity and cause tooth demineralization, caries and erosion, which in consequence might make the dental treatment very challenging for the dentist. Most of these patients will need an extensive treatment such as full coverage crown, veneers, onlays, or even full-mouth rehabilitation.[98] This makes the HCl one of the most common media of interest to investigate.

All experimental groups demonstrated higher microleakage scores than the control groups for all types of cement. This finding might explain the effect of the acid exposure on the microleakage, as the hydrochloric acid solution increased the microleakage value with all the types of cement.

There was no statistically significant difference among the experimental groups. However, Panavia 21 revealed the highest median microleakage score among all the groups with 59.24% while Variolink Esthetic showed the lowest median score of 45.83%.

Similar results were reported in a study done by Cal et al., who compared RelyX Unicem, Variolink II and some other resin cements in class V restorations. That study reported no statistically significant difference between Variolink II and RelyX Unicem at the gingival margin, which agrees with the current study. However, Variolink II showed the lowest microleakage score among the other groups at the occlusal margin.[99]

As in the experiment groups, there were no statistically significant differences between the resin cements in the control groups. However, Panavia 21 and RelyX Unicem showed the lowest microleakage scores in the control groups with 25.49% and 21.81% respectively.

Under a stereomicroscope most of the samples in the experiment groups revealed extensive dye penetration, which was extended out to the axial walls in both sides of the tooth. Conversely, samples of the control groups displayed lighter dye penetration only around the margins, which was in agreement with the final results.

These findings in the experimental group coincide with those reported in a previous study by Weng et al., who compared Variolink II and RelyX Unicem in class V restoration. The study showed that the microleakage of Variolink II and RelyX Unicem at enamel and dentin was not significantly different.[100]

Although, Uludag et al. found a significant difference between Variolink II and Panavia 21, it was in agreement with this study that Panavia 21 had a higher microleakage score than Variolink II for both dentin and enamel margins.[101] This result was not in concordance with a study done by Naumova et al., which reported that the Panavia F 2.0 showed the lowest microleakage score in comparison with RelyX Unicem and other types of resin cements.[102] Panavia F 2.0 and Panavia 21 are resin cements with the same composition; they only differ in the mode of curing and the fluoride release.

RelyX Unicem revealed the lowest microleakage score in the control group. However, Variolink Esthetic exhibited the lowest microleakage score in the experimental group. This observation has some similarity with another in-vitro study done by Piwowarczyk et al. These researchers examined the microleakage of RelyX Unicem and Panavia F after four weeks of storing the specimen in distilled water. That study reported a significant difference between the cements, and Panavia F had a higher microleakage score than RelyX Unicem, a result that agrees with this study.[103]

The literature supports the idea that CAD/CAM restorations have a larger marginal gap than crowns fabricated by the conventional method.[104],[105] This might have contributed to the amount of microleakage we found in the present study. Thus, resin cement would have a greater influence in reducing the amount of microleakage.

Differences in microleakage scores between the different self-adhesive resin cements might be due to their different chemical compositions and their functional monomers. In addition, the variable pH scores might influence the etching capability of these cements to tooth surface, possibly causing unfavorable adhesion and microleakage.[106] Apparently, resin cement that reveals a low microleakage result can be accomplished by producing a successful seal between the restoration, luting agent, and tooth surface without the need of pretreatment.

RelyX Unicem cement is composed of specific multifunctional phosphoric-acid methacrylates, which react with the tooth in various means by forming a complex compound with calcium ions or through a physical interaction such as a hydrogen bond or a dipole to dipole interaction. This range of interactions allows RelyX Unicem to produce self-adhesion to the dental tissues; subsequently a proper effective seal is going to take place in the tooth–cement interface.(103)

There were some differences in results from previously published studies. These differences might be attributed to the different ceramic materials, different cementing agents used in each study, and the test conditions.

The limitations of the present study are varied. The standardization method might be affected by some human errors. Additionally, because it is an in-vitro study, it does not stimulate the actual situation of the oral cavity. Another limitation is the limited types of cement examined. All efforts have been made to mimic gastroesophageal reflux patients, but the real situation of those patients cannot be reached in the lab since saliva and enzymes would affect the test conditions.

Future studies should focus on investigating other types of ceramics and including different cements. Future prospective in-vivo studies are recommended to stimulate the actual situation of patients with gastroesophageal reflux disease.

Conclusions

Within the limitations of this in-vitro study, the results indicate that;

- None of the resin cements investigated in the study produced microleakage-free restorations.
- Microleakage value increased when specimens become exposed to the hydrochloric acid solution.
- Acid exposure increases resin-based cement degradation.

References:

1. Naenni N, Bindl A, Sax C, Hammerle C, Sailer I. A randomized controlled clinical trial of 3-unit posterior zirconia-ceramic fixed dental prostheses (FDP) with layered or pressed veneering ceramics: 3-year results. J Dent. 2015;43(11):1365-70.
2. Haselton DR, Diaz-Arnold AM, Hillis SL. Clinical assessment of high-strength all-ceramic crowns. J Prosthet Dent. 2000;83(4):396-401.
3. Pjetursson BE, Sailer I, Zwahlen M, Hammerle CHF. A systematic review of the survival and complication rates of all-ceramic and metal-ceramic reconstructions after an observation period of at least 3 years. Part I: single crowns. Clin Oral Implan Res. 2007;18:73-85.
4. Sailer I, Pjetursson BE, Zwahlen M, Hammerle CHF. A systematic review of the survival and complication rates of all-ceramic and metal-ceramic reconstructions after an observation period of at least 3 years. Part II: fixed dental prostheses. Clin Oral Implan Res. 2007;18:86-96.
5. Milleding P, Haraldsson C, Karlsson S. Ion leaching from dental ceramics during static in vitro corrosion testing. J Biomed Mater Res. 2002;61(4):541-50.
6. Kelly JR, Nishimura I, Campbell SD. Ceramics in dentistry: historical roots and current perspectives. J Prosthet Dent. 1996;75(1):18-32.
7. McLean Jw Fau - Hughes TH, Hughes TH. The reinforcement of dental porcelain with ceramic oxides. 1965(0007-0610 (Print)).
8. Giordano R, Cima M, Pober R. Effect of surface finish on the flexural strength of feldspathic and aluminous dental ceramics. Int J Prosthodont. 1995;8(4):311-9.
9. Giordano RA, 2nd, Pelletier L, Campbell S, Pober R. Flexural strength of an infused ceramic, glass ceramic, and feldspathic porcelain. J Prosthet Dent. 1995;73(5):411-8.
10. Lohbauer U, Muller FA, Petschelt A. Influence of surface roughness on mechanical strength of resin composite versus glass ceramic materials. Dent Mater. 2008;24(2):250-6.
11. Holand W, Rheinberger V, Schweiger M. Control of nucleation in glass ceramics. Philos T Roy Soc A. 2003;361(1804):575-88.
12. Lee HH, Kon M, Asaoka K. Influence of modification of Na2O in a glass matrix on the strength of leucite-containing porcelains. Dent Mater J. 1997;16(2):134-43.
13. Sinmazisik G, Ovecoglu ML. Physical properties and microstructural characterization of dental porcelains mixed with distilled water and modeling liquid. Dent Mater. 2006;22(8):735-45.
14. Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials. 1999;20(1):1-25.
15. Kosmac T, Oblak C, Jevnikar P, Funduk N, Marion L. The effect of surface grinding and sandblasting on flexural strength and reliability of Y-TZP zirconia ceramic. Dent Mater. 1999;15(6):426-33.
16. Chevalier J, Cales B, Drouin Jean M. Low‐Temperature Aging of Y‐TZP Ceramics. Journal of the American Ceramic Society. 2004;82(8):2150-4.
17. Seghi RR, Denry IL, Rosenstiel SF. Relative fracture toughness and hardness of new dental ceramics. J Prosthet Dent. 1995;74(2):145-50.

18. Tinschert J, Natt G, Mautsch W, Augthun M, Spiekermann H. Fracture resistance of lithium disilicate-, alumina-, and zirconia-based three-unit fixed partial dentures: a laboratory study. Int J Prosthodont. 2001;14(3):231-8.
19. Mitra SB, Wu D, Holmes BN. An application of nanotechnology in advanced dental materials. J Am Dent Assoc. 2003;134(10):1382-90.
20. Vita Zahnfabrik BS. VITA SUPRINITY®, Technical and scientific documentation. Germany,2014.
21. Miyazaki T, Nakamura T, Matsumura H, Ban S, Kobayashi T. Current status of zirconia restoration. J Prosthodont Res. 2013;57(4):236-61.
22. Mormann WH, Bindl A. All-ceramic, chair-side computer-aided design/computer-aided machining restorations. Dent Clin North Am. 2002;46(2):405-26, viii.
23. Beuer F, Schweiger J Fau - Edelhoff D, Edelhoff D. Digital dentistry: an overview of recent developments for CAD/CAM generated restorations. 2008(1476-5373 (Electronic)).
24. Bindl A, Luthy H, Mormann WH. Strength and fracture pattern of monolithic CAD/CAM-generated posterior crowns. Dent Mater. 2006;22(1):29-36.
25. Reiss B. Clinical results of Cerec inlays in a dental practice over a period of 18 years. Int J Comput Dent. 2006;9(1):11-22.
26. Sjogren G, Molin M Fau - van Dijken JWV, van Dijken JW. A 10-year prospective evaluation of CAD/CAM-manufactured (Cerec) ceramic inlays cemented with a chemically cured or dual-cured resin composite. Int J Prostho- dont. 2004(0893-2174 (Print)):241-6.
27. Li RWK, Chow TW, Matinlinna JP. Ceramic dental biomaterials and CAD/CAM technology: State of the art. Journal of Prosthodontic Research. 2014;58(4):208-16.
28. Behr M, Rosentritt M, Regnet T, Lang R, Handel G. Marginal adaptation in dentin of a self-adhesive universal resin cement compared with well-tried systems. Dent Mater. 2004;20(2):191-7.
29. Technical data sheet, 3M-E.

. 2016.
30. Addison O, Marquis PM, Fleming GJ. Quantifying the strength of a resin-coated dental ceramic. J Dent Res. 2008;87(6):542-7.
31. Gu XH, Kern M. Marginal discrepancies and leakage of all-ceramic crowns: influence of luting agents and aging conditions. Int J Prosthodont. 2003;16(2):109-16.
32. Paul SJ, Pliska P, Pietrobon N, Scharer P. Light transmission of composite luting resins. Int J Periodontics Restorative Dent. 1996;16(2):164-73.
33. el-Mowafy O. The use of resin cements in restorative dentistry to overcome retention problems. 2001(0709-8936 (Print)).
34. Stamatacos C, Simon JF. Cementation of indirect restorations: an overview of resin cements. Compend Contin Educ Dent. 2013;34(1):42-4, 6.
35. RelyX™ Unicem [package insert]. St. Paul, MN.U.S.A.: 3M ESPE; 2002.
36. Variolink® Esthetic [package insert]. Schaan, Liechtenstein: Ivoclar Vivadent AG,; 2016.
37. Panavia 21™ [package insert]. New York, NY: Kuraray Medical Inc.; 2011.
38. John M. Powers JWF, Kathy L. O’Keefe, Brent Kolb, Gytis Udrys. <Guide to All Ceramic Bonding>. Kuraray America. 2013.

39. Li ZC, White SN. Mechanical properties of dental luting cements. J Prosthet Dent. 1999;81(5):597-609.
40. Piwowarczyk A, Lauer HC. Mechanical properties of luting cements after water storage. Oper Dent. 2003;28(5):535-42.
41. McLean JW, von Fraunhofer JA. The estimation of cement film thickness by an in vivo technique. Br Dent J. 1971;131(3):107-11.
42. Karlsson S. The fit of Procera titanium crowns. An in vitro and clinical study. Acta Odontol Scand. 1993;51(3):129-34.
43. Molin MK, Karlsson SL, Kristiansen MS. Influence of film thickness on joint bend strength of a ceramic/resin composite joint. Dent Mater. 1996;12(4):245-9.
44. Santos GC, Jr., Santos MJ, Rizkalla AS. Adhesive cementation of etchable ceramic esthetic restorations. J Can Dent Assoc. 2009;75(5):379-84.
45. Orstavik D, Orstavik J. In vitro attachment of Streptococcus sanguis to dental crown and bridge cements. J Oral Rehabil. 1976;3(2):139-44.
46. Farmakis ETK, K. Kontakiotis, E.G Nikolaos, K. Effect of Er,Cr:YSGG laser on human dentin collagen: a preliminary study. J Laser Dent. 2008;16:15-20.
47. Craig G.R. PJM, and O'Brien W.J. Bonding to dental substrates. In Restoration Dental Material, 11th ed: St. Louis: The C.V. Mosby Co.; 2006. p. p. 264.
48. Giray FE, Duzdar L, Oksuz M, Tanboga I. Evaluation of the Bond Strength of Resin Cements Used to Lute Ceramics on Laser-Etched Dentin. Photomedicine and Laser Surgery. 2014;32(7):413-21.
49. Kim BK, Bae HE, Shim JS, Lee KW. The influence of ceramic surface treatments on the tensile bond strength of composite resin to all-ceramic coping materials. J Prosthet Dent. 2005;94(4):357-62.
50. Blum IR, Nikolinakos N, Lynch CD, Wilson NH, Millar BJ, Jagger DC. An in vitro comparison of four intra-oral ceramic repair systems. J Dent. 2012;40(11):906-12.
51. Goia TS, Leite FP, Valandro LF, Ozcan M, Bottino MA. Repair bond strength of a resin composite to alumina-reinforced feldspathic ceramic. Int J Prosthodont. 2006;19(4):400-2.
52. Panah FG, Rezai SM, Ahmadian L. The influence of ceramic surface treatments on the micro-shear bond strength of composite resin to IPS Empress 2. J Prosthodont. 2008;17(5):409-14.
53. Buonocore MG. Retrospections on bonding. Dent Clin North Am. 1981;25(2):241-55.
54. Silverstone LM. Fissure sealants: the enamel-resin interface. J Public Health Dent. 1983;43(3):205-15.
55. Zhu JJ, Tang AT, Matinlinna JP, Hagg U. Acid etching of human enamel in clinical applications: a systematic review. J Prosthet Dent. 2014;112(2):122-35.
56. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27(1):1-16.
57. M. Sunico-Segarra AS. Resin Cements: Factors Affecting Clinical Performance. A Practical Clinical Guide to Resin Cements: Springer, Berlin, Heidelberg
; 2015.
58. Sorensen JA, Engelman Mj Fau - Torres TJ, Torres Tj Fau - Avera SP, Avera SP. Shear bond strength of composite resin to porcelain. 1991(0893-2174 (Print)).

59. Chen JH, Matsumura H, Atsuta M. Effect of different etching periods on the bond strength of a composite resin to a machinable porcelain. J Dent. 1998;26(1):53-8.
60. Horn HR. Porcelain laminate veneers bonded to etched enamel. Dent Clin North Am. 1983;27(4):671-84.
61. Kern M. Resin Bonding to Oxide Ceramics for Dental Restorations. Journal of Adhesion Science and Technology. 2009;23(7-8):1097-111.
62. Alptekin T, Ozer F, Unlu N, Cobanoglu N, Blatz MB. In vivo and in vitro evaluations of microleakage around Class I amalgam and composite restorations. Oper Dent. 2010;35(6):641-8.
63. Kidd EA. Microleakage: a review. J Dent. 1976;4(5):199-206.
64. Bergenholtz G, Cox CF, Loesche WJ, Syed SA. Bacterial leakage around dental restorations: its effect on the dental pulp. J Oral Pathol. 1982;11(6):439-50.
65. Goldman M, Laosonthorn P, White RR. Microleakage--full crowns and the dental pulp. J Endod. 1992;18(10):473-5.
66. Craig RG. Restorative dental materials 8th ed. St Louis : Mosby, . 1989.
67. Holmes JR, Bayne Sc Fau - Holland GA, Holland Ga Fau - Sulik WD, Sulik WD. Considerations in measurement of marginal fit. 1989(0022-3913 (Print)).
68. Peumans M, Van Meerbeek B, Lambrechts P, Vanherle G. Porcelain veneers: a review of the literature. J Dent. 2000;28(3):163-77.
69. Toh CG, Setcos JC, Weinstein AR. Indirect dental laminate veneers--an overview. J Dent. 1987;15(3):117-24.
70. Gerdolle DA, Mortier E, Loos-Ayav C, Jacquot B, Panighi MM. In vitro evaluation of microleakage of indirect composite inlays cemented with four luting agents. J Prosthet Dent. 2005;93(6):563-70.
71. Goodacre CJ, Bernal G, Rungcharassaeng K, Kan JY. Clinical complications in fixed prosthodontics. J Prosthet Dent. 2003;90(1):31-41.
72. Bartlett DW, Evans DF, Anggiansah A, Smith BGN. A study of the association between gastro-oesophageal reflux and palatal dental erosion. Brit Dent J. 1996;181(4):125-31.
73. Bishop K, Deans RF. Dental erosion as a consequence of voluntary regurgitation in a jockey: A case report. Brit Dent J. 1996;181(9):343-5.
74. Gandara BK, Truelove EL. Diagnosis and management of dental erosion. J Contemp Dent Pract. 1999;1(1):16-23.
75. Deschner WK, Benjamin SB. Extraesophageal manifestations of gastroesophageal reflux disease. Am J Gastroenterol. 1989;84(1):1-5.
76. Cazzonato Junior H, Bernasconi G, Pedrazzoli Júnior J. Gastroesophageal reflux and oral lesions: is the acid that bad? GED gastroenterol endosc dig. 2003;22(2):42-6.
77. Myklebust S, Espelid I, Svalestad S, Tveit AB. Dental health behavior, gastroesophageal disorders and dietary habits among Norwegian recruits in 1990 and 1999. Acta Odontologica Scandinavica. 2003;61(2):100-4.
78. Kumar S, Sharma S Fau - Norboo T, Norboo T Fau - Dolma D, Dolma D Fau - Norboo A, Norboo A Fau - Stobdan T, Stobdan T Fau - Rohatgi S, et al. Population based study to assess prevalence and risk factors of gastroesophageal reflux disease in a high altitude area. 2011(0975-0711 (Electronic)).

79. Tolia V, Vandenplas Y. Systematic review: the extra-oesophageal symptoms of gastro-oesophageal reflux disease in children. Aliment Pharmacol Ther. 2009;29(3):258-72.
80. Barron RP, Carmichael RP, Marcon MA, Sandor GK. Dental erosion in gastroesophageal reflux disease. J Can Dent Assoc. 2003;69(2):84-9.
81. DeVault KR. Extraesophageal symptoms of GERD. Cleve Clin J Med. 2003;70 Suppl 5:S20-32.
82. Ranjitkar S, Smales RJ, Kaidonis JA. Oral manifestations of gastroesophageal reflux disease. J Gastroenterol Hepatol. 2012;27(1):21-7.
83. Sujatha S, Jalihal U, Devi Y, Rakesh N, Chauhan P, Sharma S. Oral pH in gastroesophageal reflux disease. Indian J Gastroenterol. 2016;35(3):186-9.
84. Van Roekel NB. Gastroesophageal reflux disease, tooth erosion, and prosthodontic rehabilitation: a clinical report. J Prosthodont. 2003;12(4):255-9.
85. Anusavice KJ. Degradability of dental ceramics. Adv Dent Res. 1992;6:82-9.
86. Matsou E, Vouroutzis N, Kontonasaki E, Paraskevopoulos KM, Koidis P. Investigation of the influence of gastric acid on the surface roughness of ceramic materials of metal-ceramic restorations. An in vitro study. Int J Prosthodont. 2011;24(1):26-9.
87. Bayne SC, Schmalz G. Reprinting the classic article on USPHS evaluation methods for measuring the clinical research performance of restorative materials. 2005(1432-6981 (Print)).
88. Harryparsad A Fau - Dullabh H, Dullabh H Fau - Sykes L, Sykes L Fau - Herbst D, Herbst D. The effects of hydrochloric acid on all-ceramic restorative materials: an in-vitro study. 2014(1029-4864 (Print)).
89. White SN, Sorensen JA, Kang SK, Caputo AA. Microleakage of new crown and fixed partial denture luting agents. J Prosthet Dent. 1992;67(2):156-61.
90. Shortall AC, Fayyad MA, Williams JD. Marginal seal of injection-molded ceramic crowns cemented with three adhesive systems. J Prosthet Dent. 1989;61(1):24-7.
91. Jaberi Ansari Z, Kalantar Motamedi M. Microleakage of two self-adhesive cements in the enamel and dentin after 24 hours and two months. J Dent (Tehran). 2014;11(4):418-27.
92. Hetherington MM, Altemus M, Nelson ML, Bernat AS, Gold PW. Eating behavior in bulimia nervosa: multiple meal analyses. Am J Clin Nutr. 1994;60(6):864-73.
93. McLaren EA CP. Ceramics in dentistry - part 1: classes of materials. Inside Dentistry. 2009.
94. Mihailopol CF CCPG, Topoliceanu C, Ghiorghe CA. Correlations between dental erosion severity and salivary factor in patients with gastroesophageal reflux disease. Romanian Journal of Oral Rehabilitation
. 2001;3(4):63-6.
95. Moazzez R, Bartlett D Fau - Anggiansah A, Anggiansah A. Dental erosion, gastro-oesophageal reflux disease and saliva: how are they related? 2004
(0300-5712 (Print)).
96. Campisi G, Lo Russo L, Di Liberto C, Di Nicola F, Butera D, Vigneri S, et al. Saliva variations in gastro-oesophageal reflux disease. J Dent. 2008;36(4):268-71.
97. Larsen MJ. Chemical events during tooth dissolution. J Dent Res. 1990;69 Spec No:575-80; discussion 634-6.

98. Schwarz S, Kreuter A, Rammelsberg P. Efficient prosthodontic treatment in a young patient with long-standing bulimia nervosa: A clinical report. J Prosthet Dent. 2011;106(1):6-11.
99. Cal E, Celik EU, Turkun M. Microleakage of IPS empress 2 inlay restorations luted with self-adhesive resin cements. Oper Dent. 2012;37(4):417-24.
100. Weng WM, Zhang Xy Fau - Zhang F-q, Zhang FQ. [Microleakage of various cementing agents for casting ceramics]. 2009(1006-7248 (Print)).
101. Uludag B, Ozturk O, Ozturk AN. Microleakage of Ceramic Inlays Luted with Different Resin Cements and Dentin Adhesives. Journal of Prosthetic Dentistry. 2009;102(4):235-41.
102. Naumova EA, Valta A, Schaper K, Arnold WH, Piwowarczyk A. Microleakage of Different Self-Adhesive Materials for Lithium Disilicate CAD/CAM Crowns. Materials. 2015;8(6):3238-53.
103. Piwowarczyk A, Lauer Hc Fau - Sorensen JA, Sorensen JA. Microleakage of various cementing agents for full cast crowns. 2005(0109-5641 (Print)).
104. Anadioti E, Aquilino Sa Fau - Gratton DG, Gratton Dg Fau - Holloway JA, Holloway Ja Fau - Denry I, Denry I Fau - Thomas GW, Thomas Gw Fau - Qian F, et al. 3D and 2D marginal fit of pressed and CAD/CAM lithium disilicate crowns made from digital and conventional impressions. 2014(1532-849X (Electronic)).
105. Reich S, Uhlen S, Gozdowski S, Lohbauer U. Measurement of cement thickness under lithium disilicate crowns using an impression material technique. Clin Oral Investig. 2011;15(4):521-6.
106. Abo T, Uno S, Sano H. Comparison of bonding efficacy of an all-in-one adhesive with a self-etching primer system. Eur J Oral Sci. 2004;112(3):286-92.

APPENDICES

Appendix A: Tables

Appendix B: Figures

Appendix A: Tables

Resin Cement	Manufacture	Composition	Type
RelyX Unicem	3M ESPE, St. Paul, Minn	Base paste: Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, silanated fillers, initiator components, stabilizers Catalyst paste: Methacrylate monomers, alkaline (basic) fillers, silanated fillers, initiator components, stabilizers, pigments	Self-adhesive resin cement
Panavia 21	Kuraray Co Ltd, Osaka, Japan	Bis-GMA, MDP, quartz, benzoyl peroxide, initiators, phosphate, monomer, amine, sulfine, stabilizer	Resin cement
Variolink Esthetic	Ivoclar Vivadent, Schaan, Liechtenstein	Base paste: Bis-GMA, UDMA, TEGDMA, filler Catalyst paste: Bis-GMA, UDMA, TEGDMA, filler	Resin cement

Table 1: Composition of resin cements used in this study.

R – Excellent / Ideal	Explorer does not catch; continuous adaptation and indistinguishable margins.
S - Acceptable	Explorer detects but cannot penetrate marginal area.
T – Acceptable w/ modifications	Explorer detectable and penetrates marginal area.
V – Unacceptable	Explorer detectable, gross marginal discrepancies upon explorer examination.

Table 2: Modified United States Public Health Service criteria (USPHS/CDA) USPHS – Marginal Adaptation.

	Resin Cement	**Median**	**IQR**
Experimental Groups	RelyX Unicem	54.95^{A}	8.48
	Panavia 21	59.24^{A}	25.88
	Variolink Esthetic	45.83^{AB}	36.11
Control Groups	RelyX Unicem	21.81^{C}	15.82
	Panavia 21	25.49^{C}	10.50
	Variolink Esthetic	32.82^{BC}	18.41

Table 3: Median microleakage scores and interquartile ranges of all cements.

Note: Shared letters indicate no significant difference at the 0.8% level of significance (based on the Bonferroni correction).

Appendix B: Figures

Figure 1: Handpiece connected to a surveyor

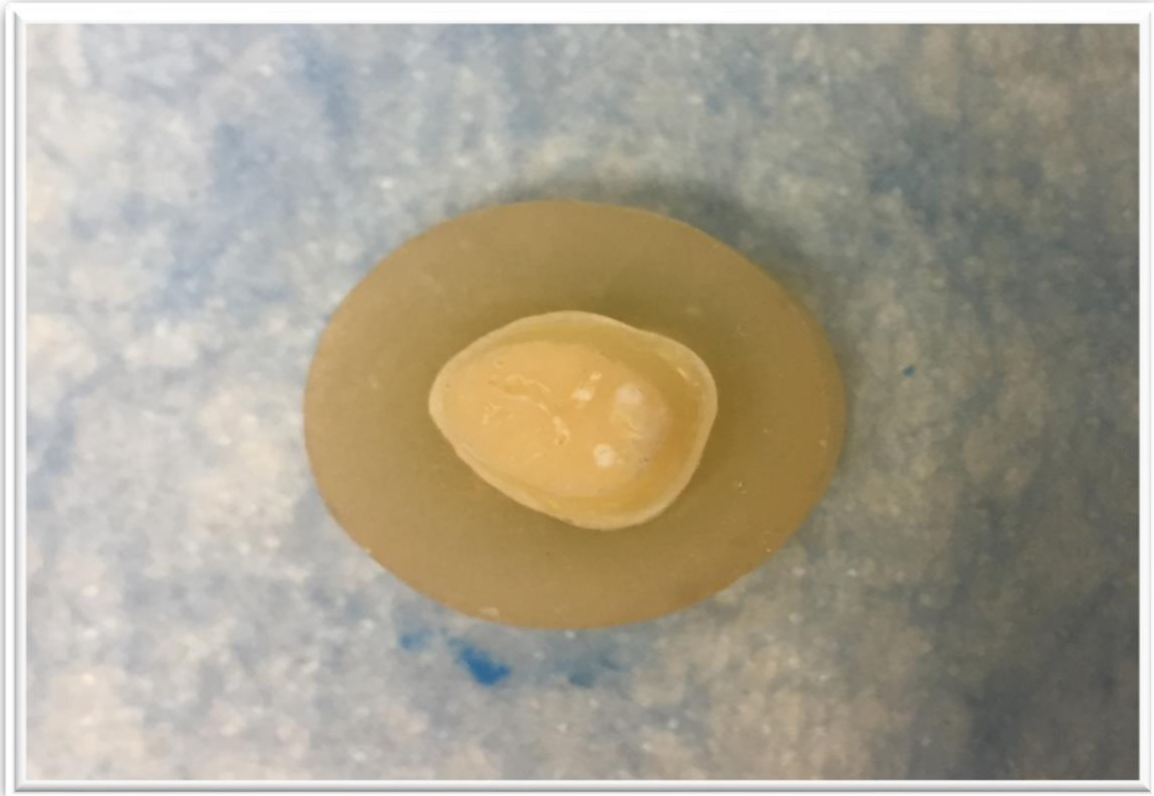

Figure 2: Prepared tooth

Figure 3: CEREC CAD/CAM machine**.**

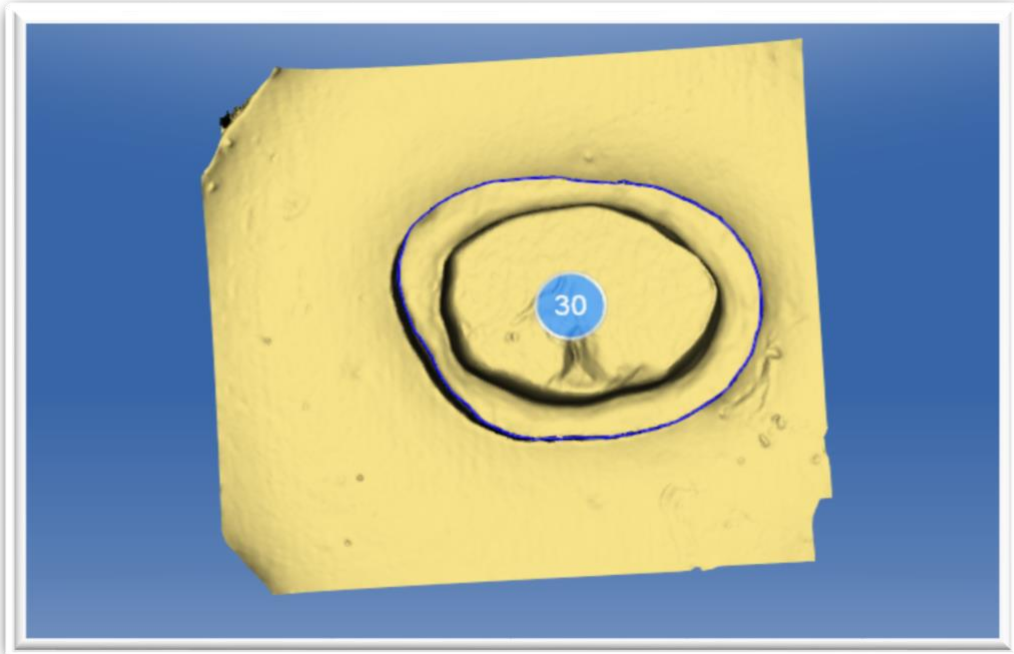

Figure 4: Scanned model with the finish line in the CAD CAM machine.

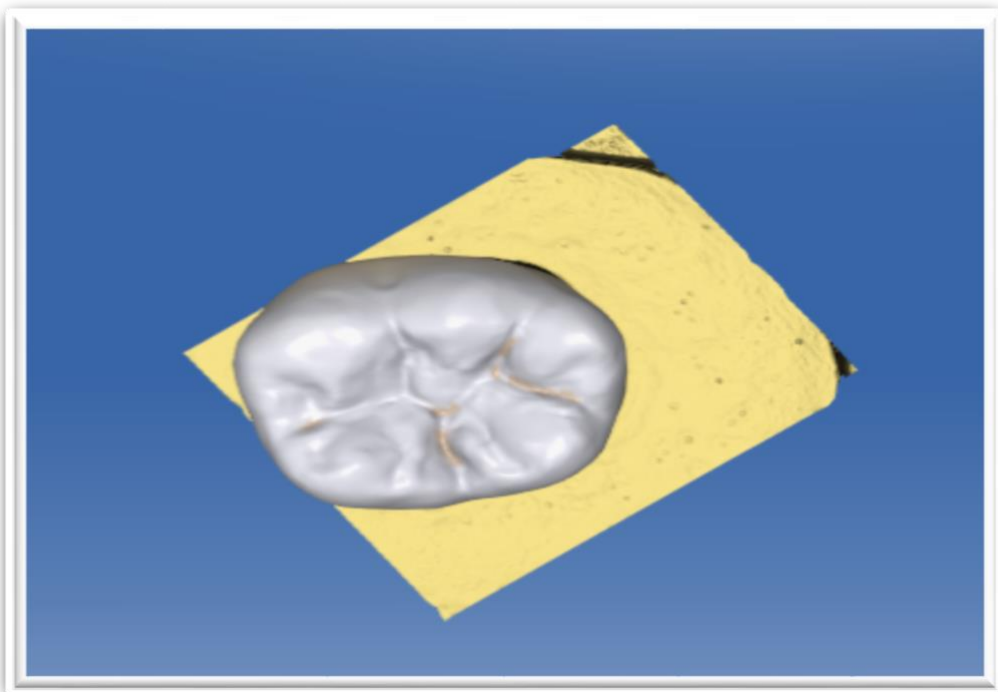

Figure 5: Design of crown in the CAD CAM machine.

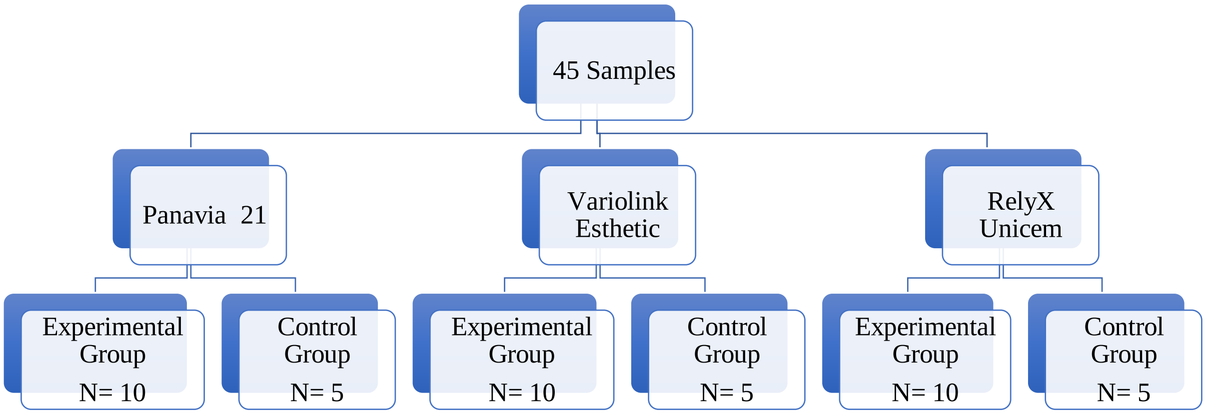

Figure 6: Experimental and control groups of the study.

Figure 7: Resin cements used in the study.

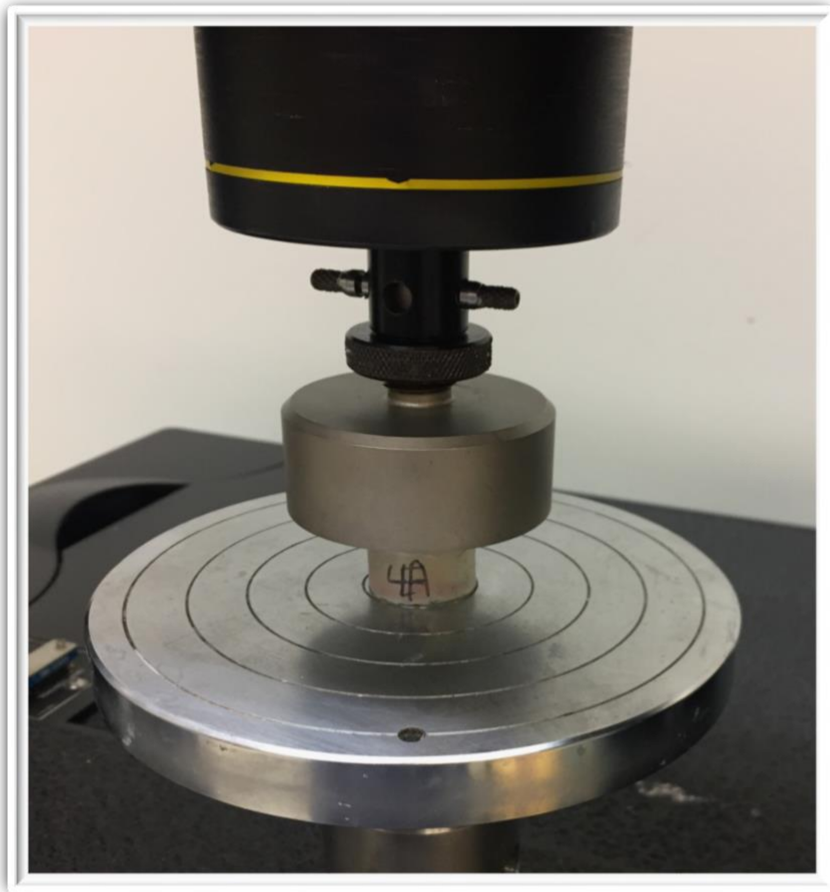

Figure 8: Crown seating with 50 N occlusal load.

Figure 9: Samples inside the incubator at 37℃.

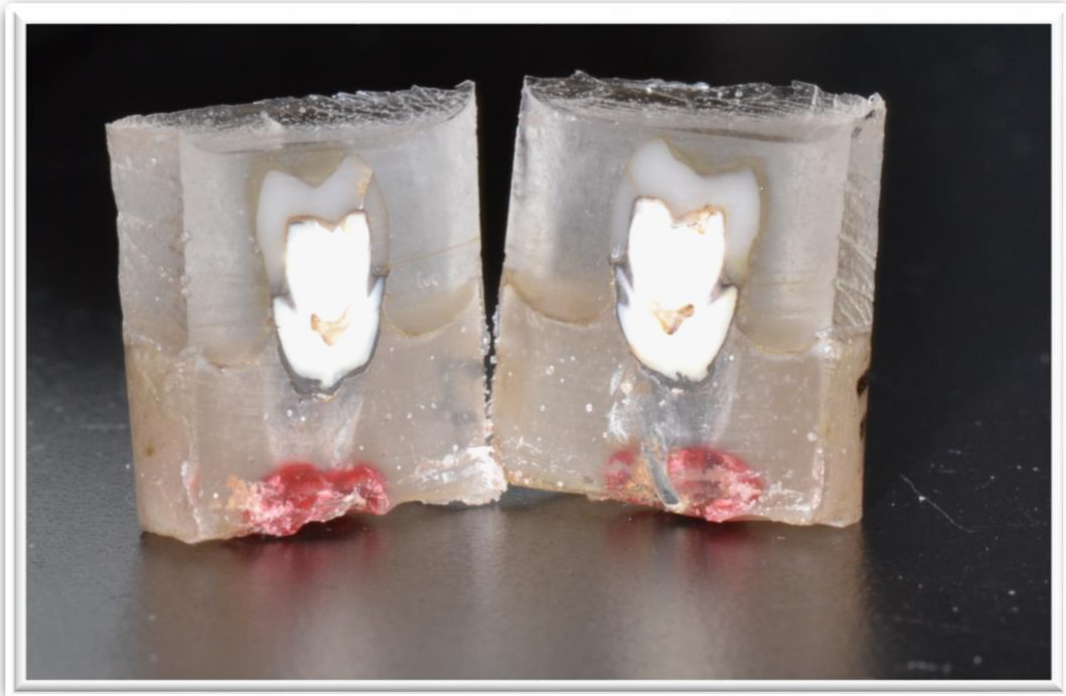

Figure 10: Sectioned sample.

Figure 11: Stereomicroscope.

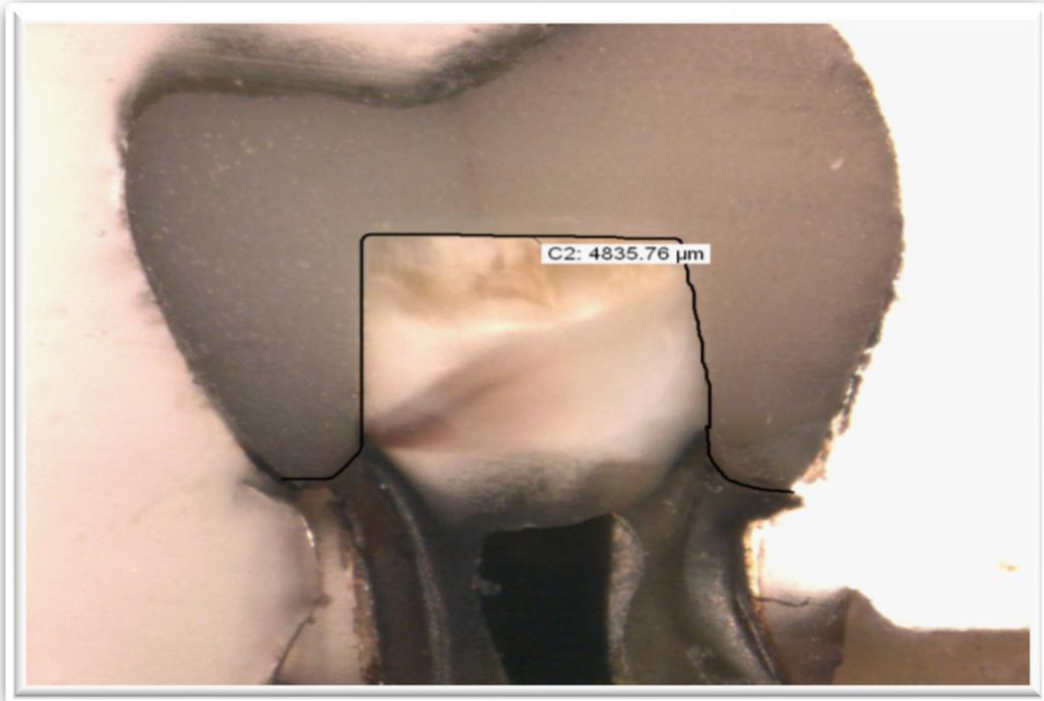

Figure 12: Total length of restoration in a sectioned tooth under stereomicroscope.

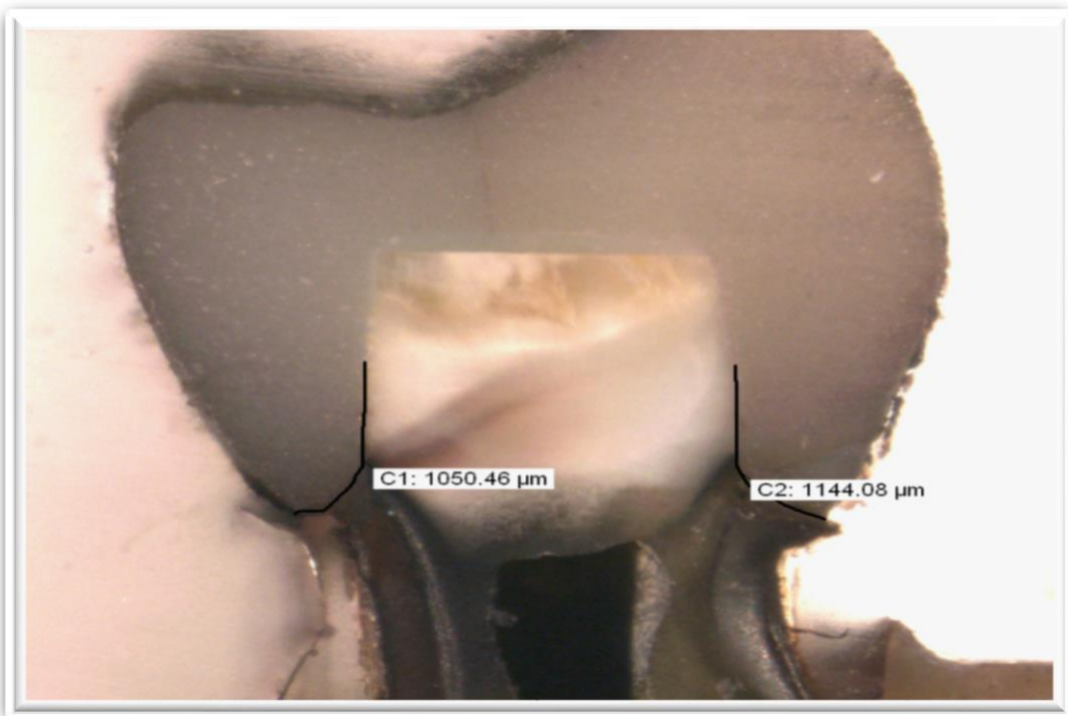

Figure 13: Extent of dye penetration in a sectioned tooth under stereomicroscope.

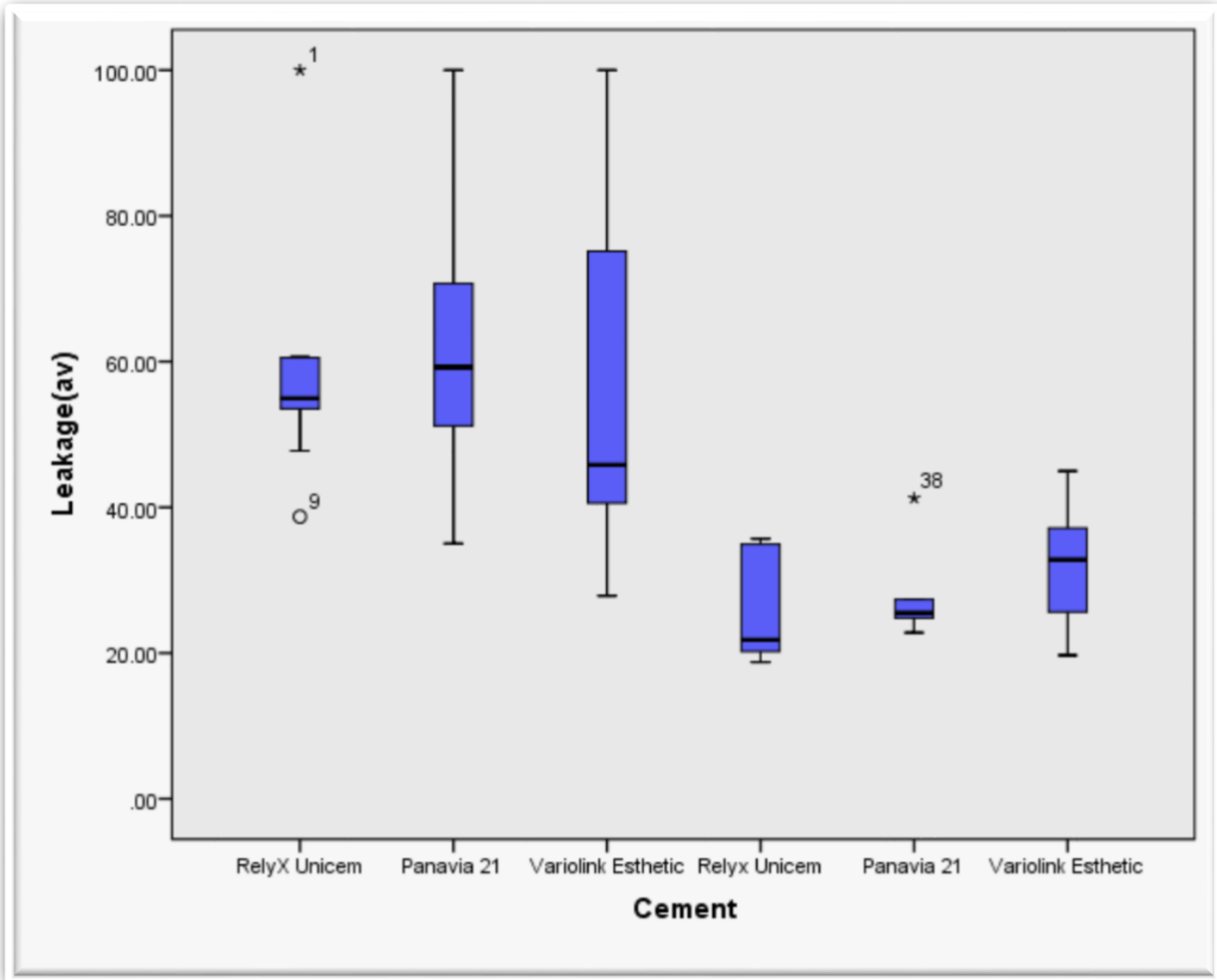

Figure 14: Boxplot of microleakage scores in all the groups.