Tufts University

School of Dental Medicine

Master of Science

The Effects of Different Concentrations of Ethyl Alcohol as Drying Agent on Pit and Fissure Sealant Treatment

Thesis submitted in partial fulfillment of the requirement for the degree of Master of Science

Tufts University School of Dental Medicine 2012

Thesis submitted by

Basma I. Tamasas

Principal Advisors

Gerard Kugel, D.M.D., M.S., Ph.D., Professor and Associate Dean for Research, Tufts University School of Dental Medicine.

Masly Harsono, B.D.S, M.S., School of Dental Medicine, Tufts University.

Committee Members

Cheen Y. Loo, B.D.S., M.P.H., Ph.D., Associate Professor and Director of the postdoctoral program Pediatric Dentistry Department, Tufts University School of Dental Medicine.

Matthew Finkelman, Ph.D., Tufts University School of Dental Medicine, Department of Public Health and Community Service.

Abstract

Objective: Moisture contamination is considered one of the major causes for pit and fissure sealant failure. Post etchant drying agent has been proposed for use in dentistry to improve the retention of the sealant by evaporating the residual moisture. Therefore, the purpose of this *in vitro* study is to evaluate the microleakage of sealant placed after the application of ethyl alcohol (50% once, 99% once and 99% twice), as a post etchant drying agent. The effect of salivary contamination on microleakage will also be evaluated.

Materials and Methods: Sixty four human molar teeth were randomly divided into eight groups of eight each. The treatment groups were defined by the combination of different drying agents (no drying agent, 50% ethyl alcohol once, 99% ethyl alcohol once and 99% ethyl alcohol twice) and salivary contamination condition (contaminated, not-contaminated). All teeth were sealed with Ultraseal XT Plus sealant. The teeth were thermocycled (2500 cycles at 5˚C-55˚C) and were then immersed in 50% silver nitrate. Mikroleakage was examined. Statistical analysis was done using one way ANOVA and independent samples t- test.

Results: Sealant placed on teeth after the utilization of 50% ethyl alcohol showed significantly less microleakage than the other tested two drying agents and the control group ($P < 0.001$). Saliva contamination did significantly increase the microleakage. ($P=0.001$).

Conclusions: Under the conditions used in this in-vitro study, the use of 50% ethyl alcohol as post etchant drying agent, under both contaminated and non-contaminated conditions, significantly reduced sealant microleakage.

Acknowledgments

In the name of Allah, most gracious, most merciful praise be to Allah.

My deepest thanks to my lovely parents, my sister, and my brothers. Your inspiration, prayers, and belief in me have sustained me through some of the hardest times. Also, my heartfelt thanks go to my husband Adel for his love and support throughout these years.

I express my deepest gratitude for my supervisors, Dr. Kugel and Dr. Harsono for introducing me to the world of dental research. I have been privileged to benefit from their expertise in this field, their inspiring attitude and interest in this work.

Besides my advisors, I am profoundly grateful for my thesis committee members Dr. Loo and Dr. Finkelman for their guidance, support and endless help.

I offer special thanks to Dr. Stark, for giving me the opportunity to be part of the research family in the dental school.

This project would not have been possible without the help of some of very special staff who worked extra hard to help me finish my project and especially Ms. Stephanie John.

Special thanks also to all my graduate friends, especially Dr. Zletni for sharing the literature and invaluable assistance. I would like to extend special thanks to my lovely neighbors Amy and Kurt Biewald for their valuable advice and help throughout the study, and to all my best friends who have always been there for me.

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Introduction

The prevention of dental caries has improved in the last few decades. Different preventive protocols have been used such as water fluoridation and topical fluoride application. However, these methods have been found to be less effective in occlusal caries prevention.[1] It is well cited in literature that the major type of caries found in children, adolescents, and young adults is occlusal caries.[2, 3] The American Dental Association recommended the use of pit-and-fissure sealants as an effective preventive method for controlling occlusal pit-and-fissure caries.[4] The sealant acts as a barrier between the enamel surface and the surrounding environment of food and acids produced by the bacteria of dental plaque; furthermore, it seals the lesion and prevents the occlusal dental caries from progressing further.[5, 6]

Due to these facts, pit and fissure sealant should have a high micro mechanical retention to be effective in caries prevention. Therefore, if any portion of a sealant is lost, the benefits of the sealant are also lost. Additionally, if micro-leakage occurs around the sealant the effectiveness will be affected. Therefore, many researches have been conducted to try to discover ways of improving sealant retention and reduction in microleakage of pits and fissure sealants through the use of various materials and application techniques.

Moisture contamination is a common problem during sealant application. Because it is hard to control it can also happen at any time during sealant placement. For example, moisture contamination can occur either from (1) salivary contamination which can happen when working with children and uncooperative patients, from (2) gingival crevicular fluid contamination which mainly can happen during sealing partially erupted teeth,[7] and also from (3) the rinse water that becomes entrapped in the fissure depth and which air drying does not evaporate. If the etched enamel gets contaminated the microspores will occlude which results in poor bonding between the sealant and the enamel surface.[8, 9] Therefore, moisture contamination is considered one of the major causes for sealant failure.[4, 10]

Differing techniques have been used to control salivary contamination. A report from the American Dental Association Council, as of 2008, recommended the use of the four handed technique when possible for isolation. The application of bonding agent under the sealant has been proposed to reduce the effect of salivary contamination. Although studies have reported contradictory results regarding bonding agent effectiveness,[11, 12] the American Dental Association Council reported that use of one bottle of bonding agent after acid etching may enhance sealant retention.[4]

Another example of controlling salivary contamination consists of the use of drying agents because the resin based sealants commonly used in dentistry are hydrophobic materials. Therefore, with resin sealants, the moisture can restrict the wetting of the enamel surface.

The application of drying agents has been proposed to volatize moisture from the occlusal surface of a tooth prior to sealant placement and to improve sealant penetration depth in pits and fissures which enhances the potential for sealant bonding.[13] However the literature is still sparse when it comes to studies investigating the use of post etchant drying agent prior to sealant placement and the existing works have shown controversial results.[7, 14-16] To the best of our knowledge, no study has yet been done to evaluate the effects of the use of different concentrations of ethyl alcohol as post etchant drying agent before sealant placement. Most of the previous studies utilize 99% ethyl alcohol as a drying agent which contains a high alcohol percentage that makes it evaporate very quickly and provides less time for the ethyl alcohol to volatilize the moisture during the sealant placement procedure.

Literature Review

Dental caries, one of the most common chronic preventable childhood diseases, is an infectious disease. Usually bacterial in origin, caused mainly by Streptococcus mutans and Lactobacillus found in the biofilm (dental plaque). These bacteria form weak organic acids by hydrolysis of food debris presented on the tooth surface and the surrounding environment. When enough acid is produced that the local pH falls below 5.5 the acid will start to dissolve the hydroxyapatite which is the main component of tooth enamel. This process is known as demineralization of the tooth tissue.[17-19]

Tooth decay is a site-specific disease that exhibits primarily in pits and fissures systems. These structures usually have complex anatomy. They are deep, narrow, and difficult to access for self-cleaning or cleaning with conventional methods such as tooth brushing. Pit and fissure morphology is considered an important indicator for caries risk.[20] Molars are more frequently affected than premolars; and, mandibular molars are more frequently decayed and restored than maxillary molars.[21-23] Among the populations of the United States and Europe twenty percent of children experience 60% to 80% of the cases of dental caries.[24] 90% of late adolescents and young adults in this population have dental caries.[25, 26] Over 80% of all carious lesions in young permanent teeth involve pits and fissures of the occlusal surface which is considered the major type of caries in children, adolescents, and young adults.[1, 3, 27, 28]

Dental caries, the primary pathological cause of tooth loss in children, is often not a self-limiting disease. Without proper care dental caries can progress until the tooth is destroyed. Moreover dental caries is associated with different adverse outcomes, including: substantial pain, bacteremia, compromised chewing ability, toxic overdose of analgesics (such as acetaminophen administered during the early stages of decay), reduced sleep capacity, decreased ability to eat some foods, social embarrassment, and lowered self-esteem.[29-31] Therefore dental caries remains a major public health concern in most industrialized countries. This has led to several different preventive protocols being used to reduce its incidence.

Fluoride Use in the Prevention and Treatment of Dental Caries

Fluoride is one of the most important and effective components of preventive dental programs in children. Fluoride ions interfere with the demineralization process of tooth decay by forming a fluorapatite-like remineralized veneer over the demineralized enamel structure. This veneer is less soluble in acids than the hydroxyapatite of the tooth structure.[32] Fluoride also inhibits bacterial metabolism by blocking enzymes involved in the bacterial glycolytic pathway, thus inhibiting the process by which cariogenic bacteria metabolize carbohydrates to produce acid, and that affects adhesive polysaccharide production by this bacteria.[33] Fluoride is applied topically to the teeth using toothpaste mouth- rinse, gels, or varnishes, while systemic delivery involves water fluoridation and fluoride supplements (tablets, lozenges, liquids).

Combined use of multiple sources of fluoridation offers additional protection above that provided by only one source of fluoridation. Several *in vitro* studies found that the effectiveness of fluoride in caries prevention depends on its concentration. A rapid rise in its effectiveness was observed when the fluoride concentration increased from 0.03 ppm to 0.1 ppm.[34, 35] However fluoride was found to be effective in smooth caries prevention but not the prevention of the occlusal type of caries.[1] Pit and fissure sealant application has been found to be an effective procedure for occlusal caries prevention.[2, 12, 36] A systematic Cochrane review of four trials was done to compare the effectiveness of fluoride varnish vs. pit and fissure sealant in occlusal caries prevention. Results in two studies found that pit and fissure sealant application was more effective in the prevention of occlusal caries than fluoride varnish applied each 6 months, at 23 months, 4 years and 9 years. One split mouth study found significantly more caries in fluoride varnished teeth than in teeth that were both sealed and varnished with fluoride. The fourth study found no significant difference between the two preventive techniques. Based on this finding the authors reported the superiority of sealant over fluoride varnish in occlusal caries prevention.[37]

Pit and Fissure Sealant

Pit and fissure sealant is a material used in preventive dentistry to act as a mechanical barrier against lodgment of food and bacteria of dental plaque in pits and fissures of occlusal surface of the teeth to protect these highly susceptible areas from caries. In 1955 Buonocore[38] in an *in vitro* study, anticipated the ability of sealants to prevent caries by using phosphoric acid to etch pits and fissures for sixty seconds, followed by application of a resin material. Later, in 1967, Drs. Buonocore and Cueto[39] in the first clinical study verified the first successful application of methyl cyanoacrylate material as a sealant, to the pits and fissures of teeth. After the introduction of pit and fissure sealants to the dental field the occlusal caries incidence was reduced by about 76.3%,[40] while the fluoride varnish, applied by a dental hygienist or a trained assistant, found to prevent about 45% of cavities.[41]

Pit and Fissure Sealant Efficacy

The efficacy of fissure sealants in caries prevention depends on caries risk of the individual,[42] the prevalence of decay in the country,[43] marginal integrity and how long the sealant stays intact. However, clinical retention and longevity have been considered to be the major measurements of sealant success.[44] The literature contains many studies that reported the effectiveness of pit and fissure sealant in caries prevention. A Cochrane systematic review of 16 trials reported that sealed first permanent molar teeth had 78%, 60%, less caries on occlusal surfaces after 2 years and 4years respectively, when compared to unsealed molars.[12] Other systematic review found that the caries-preventive effect of sealants was affected by sealant replacement, whereas studies used a sealant replacement strategy had high reductions in caries risk.[22]

Reports on the effectiveness of pit and fissure sealants in the prevention of caries in the primary dentition involve a split mouth trial of children aged 4 to7 years which showed complete sealant retention in primary molars of 95% with flowable composite, and 77.5% where resin-based sealant was used after one year.[45] Another randomized clinical trial which included two years of follow up reported that primary molars sealed with resin based sealant have over a 70% retention rate.[46] These finding agree with the ADA Council of Scientific Affairs' recommendation that if a child's teeth are considered to be at risk for developing dental caries sealants should be place on his/her primary teeth.[4]

The evidences for the effectiveness of resin-based and glass-ionomer sealants in caries prevention conflicts. The Cochrane review compared resin based with glass-ionomer sealants for up to 84 months. Out of the eight studies included three reported better caries-preventive effect for resin based sealants, and two of the studies reported better effect for glass-ionomer. The other three studies showed no significant difference.[12] While Beiruti and colleagues[47], who included 19 studies, concluded that there is no difference between both sealant types as regards to caries preventive effects.

Several studies reported the efficacy of the pit and fissure sealant in managing carious lesion by reporting in its effect on bacterial levels in the lesions. They concluded that sealants stop the progression of carious lesion if they retained on the tooth surface.[6, 48]

The systematic review by Oong *et al.* searched the evidence for the effectiveness of fissure sealant on stabilizing or reducing bacteria levels in caries lesions, found that placement of fissure sealants over caries reduced the mean number of viable bacteria by 100 to 1,000 fold. On average, 47% of sealed lesions had viable bacteria compared with 89% of unsealed lesions, two weeks to five years after placement. This data supports the Council of Dental Research's policy that sealants can be used effectively to prevent the initiation and progression of dental caries.[4]

Pit and Fissure Sealant Retention

It is very important for dentists to consider that, the effectiveness of dental sealant as a caries prevention technique based on the sealant's ability to retain on the tooth.[49] Previous studies have found different percentages of sealant loss to occur annually.[50,51] Houpt *et al.*[52] in a six year study found that teeth with partial or complete sealant loss have the same chance of caries incidence as unsealed teeth. The Cochrane Database of Systematic Reviews reported the complete retention of resin sealants ranged from 79% to 92% of cases at 12 months, 71% to 85% at 24 months, 61% to 80% at 36 months, 52% at

48 months, 72% at 54 months, and 39% at 9 years. It was clear that the sealant retention rate decreased over time.[12] Therefore, a regular check of sealed teeth is mandatory to maintain any sealant that has been fractured, lost, or developed an o open margin.[22] Whereas, 80% to 90% sealant success after 10 years or more have been reported by studies incorporated routine recall and maintenance visits.[53, 54]

The application of pit and fissure sealant is technique-sensitive, requiring attention to detail during all steps. However there is limited evidence available which can be used to identify the best technique for each stage of the process of application. Different application techniques have been used with the intention of enhancing the effectiveness of etching the enamel surface and improving sealant retention. These techniques may include: the use of different sealant material, different methods of surface cleaning, using different etching systems, and using different methods of isolation and surface treatment to control the moisture.

Factors Affecting Sealant Retention

1-Type of Sealant

The sealants can be classified according to their composition into two predominant types: Resin based fissure sealants and Glass ionomer cements (GICs). The glass ionomer sealants evolved from GICs are considered less technique sensitive, as it does not require acid etching of the tooth surface,[4] whereas the basic bonding mechanism of the cements is chemical through ionic attraction between two carboxyl (COO-) groups in the cement to calcium (Ca++) contained in enamel and dentin. However; it has been shown that the application of phosphoric acid etching before glass ionomer-based sealant placement significantly improved their retention rate.[54] GICs are not as moisture sensitive as the resin based sealants and do not require extra provisions for consistent retention or adhesion as they adhere directly to dental hard structure even if humidity present. Moreover, they release fluoride ions into adjacent enamel, and take in fluoride from other sources, such as fluoride toothpastes and mouth rinses, and thereby act as a rechargeable, slow-releasing fluoride tool.[54, 55] Nevertheless, studies have shown different results in terms of their retention ability. Vranic *et al*.[56] found that the retention rate of glass ionomer sealant treated with heat during the setting time was significantly lower than that of conventional composite resin. Another study by Subramaniam *et al*.[57] evaluated the retention of glass ionomer sealant compared to a self-cure resin-based sealant. They concluded that the retention of the resin sealant was superior to that of the glass ionomer sealant. In fact, the recent report of the ADA Council of Scientific Affairs recommended that Resin-based sealants are the first choice for dental sealants and GICs may be used in cases where moisture contamination is a big concern.[4]

Resin-based sealants are based on bis-phenol a glycidyl dimethacrylate (Bis-GMA) or urethane with the addition of diluents such as triethylenglycol dimethacrylate (TEGDMA) and/ or two-hydroxyethyl methacrylate (HEMA). Resin based sealant classified based on their content to filled and unfilled sealant. The amount of filler can range from a small percentage up to 75% of fillers. A higher percentage of filler content would increase sealant viscosity and affect its penetration into the etched enamel surface thus compromising sealing and retention.[58] Studies have demonstrated that low viscosity (unfilled) sealants penetrate deeper to ensure a good marginal seal when compared to high viscosity (filled) sealants. Therefore the unfilled resin may show better retention.[10,59] Barrie *et al*.[60] over a two year clinical study reported that unfilled dental sealants were retained in teeth at 88 percent when compared with filled dental sealants, which were retained at 81%.

According to their polymerization, resin based sealants may be initiated chemically (auto polymerization) or by light. They are classified as auto-polymerized (ARBS), light-cured RBS with fluoride (FRBS) or without (LRBS). A systemic review by Muller *et al.* found no significant difference in retention rate when using either auto polymerizing resin-based sealant or light cured sealant regardless of what the follow-up times were.[61] Additionally, a systemic review by Locker and colleagues[51] concluded that fluoride-containing visible light cured sealants have the same retention rates as auto polymerizing and conventional light cured sealants at the equivalent follow-up periods. However, two studies found that FRBS had a lower retention rate when compared with LRBS after a follow-up of 48 months.[10, 62]

Low-viscosity resin-based restorative materials (flowable composites) have also been used as fissure sealants. Flowable resins have a lower volumetric filler percentage. Gillet[63] concluded that using flowable composites as sealant materials gave almost full penetration to the depth of the fissure and may offer better long term effectiveness than traditional sealants.

Another way resin based sealants are classified is according to their color. They are classified as colored, transparent, or translucent. The advantage of the colored sealant over the uncolored sealant is that it is easier to examine during placement and follow up procedures.[7] It is much faster to assess retention with a white sealant than with a clear sealant at follow up intervals. Also, confirmation of retention over long time periods is easier with a colored sealant.[64] Rock *et al.*[65] assessed the advantages of clear vs. colored (opaque) sealant. Significant differences were found in the combined identification error rate. For opaque resin it was 1%, while it was 23% for clear resin. Significant differences were also found in how the three dentists identified each type of resin accurately.

2-Surface Cleaning and Enamel Preparation

Many studies have tried to improve dental-sealants' penetration by modifying pit and fissure cleaning and preparation techniques. Different methods have been used, like; pumice prophylaxis, brushing with non-fluoridated tooth paste, and various forms of mechanical enamel preparation, such as air polishing, air abrasion, and modification with a bur (enameloplasty). Although some dental clinics prefer the use of pumice slurry in a low speed hand piece to clean the enamel surface for the optimal acid etching, Pope *et al*.[66] found that pumice is not an effective method for cleaning and totally removing the debris and organic matter (especially if located in the deep pits and fissures). A recent systemic review comparing different surface cleaning methods found no difference in sealant retention when surfaces were cleaned with a hand piece and prophylaxis brush with pumice when compared with surfaces cleaned by running an explorer through the fissures and cleaning with an air-water syringe. [61] The air polishing system (utilizing sodium bicarbonate particles) used before acid etching as one of the enamel preparation techniques and leads to high bond strength between the sealant and tooth surface.[7]

Recently preparation methods that provide good result in terms of microleakage reduction are air abrasion, bur preparation (invasive technique), and Er:YAG laser. Enameloplasty has also been found to increase sealant penetration and provides more surface area for bonding of resin based sealant.[67] Many studies concluded that better sealant efficiency occurs when using this invasive technique.[68, 69] Several studies reported that bur preparation followed by acid etching resulted in less marginal leakage than the conventional method preparation.[70, 71] Although, Mazzoleni *et al*.[72] when using different cleaning methods of mechanical brushing, air abrasion with 27 µm to 50 µm, and intensive bur preparation, found that air abrasion provided the best results for microleakage reduction after sealant placement. The same results regarding the superiority of the air abrasion technique has been found by Hatibovic *et al*.[15] when they compared the 27 micron alpha-alumina particles with bur and acid etch, or acid etch alone. The authors concluded that the removal of intact enamel is not necessary while we can get the same or better retention from other non-invasive techniques. The Er:YAG laser *in-vitro* studies found that laser alone could not replace acid etching based on microleakage and that there was no statistically significant difference when they use either laser with acid etching or acid etching alone were used.[73, 74]

In addition, a recent study by Vijayaraghavan and colleagues[89] found that fluoride released sealant placed with acid etching showed significantly lower microleakage than sealant placed with Er: YAG laser.

3- Self-Etching vs. Acid- Etching

The essential mechanism of adhesion of resin to enamel is the micromechanical retention from the formation of resin tags on the irregular surface created by acid etching. From what Dr. Buonocore[38] had discovered three decades earlier, one important way to gain high micromechanical retention is by maximizing the amount of surface area available for bonding between the sealant and tooth surface. This can be done by conditioning the surface of the enamel with phosphoric acid. To this day, conditioning surface enamel with a 35%-37% phosphoric acid is still the standard method for preparing enamel pits and fissures prior to sealant placement.[4] The Application of 37% phosphoric-acid etchant from 15 to 60 seconds leads to the formation of microscopic retentive areas in the surface of the enamel of approximately 27 microns in depth.[75] The sealant material will then be able to flow into the clean porous etched enamel, and once cured, the formed resin tags keep the sealant in place.

Unlike the total etching technique self-etch does not require the application of acid etching in one single step. Self- etching technique has been developed to simplify bonding procedures and reduce adhesive technique sensitivity since separate rinsing and drying steps of the total etching are eliminated.[76] Instead, a primer in the self-etch adhesive primes the enamel and produces a layer that incorporates minerals into the hybrid zone. [77] Many studies have investigated the use of self-etching adhesives systems prior to sealant placement and have found contradicting results. Multiple studies have reported low resin-enamel bond strength when the self-etch adhesives have been used.[78-81] Additionally, when self-etch or total-etching adhesives were used before sealant placement on salivary contaminated teeth the total failure rates were around 60% for both adhesives equally.[82]Two additional trials compared sealant retention by comparing use of a self-etch adhesive system with sealant vs. an acid-etch technique plus adhesive. Both studies found that sealant retention was improved in the acid-etch plus adhesive group.[83, 84] The ADA Council of Scientific Affairs, as of 2008, does not recommend the use of a self-etching bonding agent as it may provide less retention than an acid and rinse.[4]

4-Salivary Contamination

Pit and fissure sealant application is a highly sensitive technique. Moisture contamination is considered one of the major causes for sealant failure because it is difficult to control during the sealant placement.[4, 10] The factors influencing moisture control vary from patient to patient and relate to the following: the eruption state of the tooth,[85] the patient's ability to co-operate,[21] the materials and equipment available for isolation, and/or a combination of these factors. When working with children, this contamination can happen quickly and easily (for example, from a child moving his/her tongue causing salivary fluid to be moved onto the etched enamel).

Other sources for contamination may come from the gingival crevicular fluid, especially when we work with partially erupted teeth where opercula of the gingival tissue extend over the distal marginal ridge of partially erupted teeth.[86] It has been reported that sealants placed soon after tooth eruption are more likely to need replacement. Moreover, tooth position in the mouth found to be an important determinant for adequate isolation.[85]

Once the etched enamel surface gets contaminated, no matter the length of time of contamination, the microspores will occlude which results in poor bonding between the sealant and the enamel surface,[8, 9] thus minimizing the effectiveness of pit and fissure sealants in reducing the caries incidence in teeth that have been sealed.[12, 87]Tandon *et al*.[88] indicated that even a one second exposure to saliva can cause the formation of a protein layer that resisted the 30 seconds of vigorous irrigation, and they suggested that it would be necessary to repeat the etching procedure to get adequate bonding of a resin material. Additionally, it has been documented that rinsing of the contaminated etched surface with water is not sufficient to remove the organic debris and protein left by saliva.[89]Therefore, different aids and techniques have been used to control moisture contamination, for example: the four-handed technique that helps keep the field dry by retracting the tongue and cheeks through use of a high-volume vacuum. Griffin *et al*. in a systemic review of 11 studies concluded that the four handed delivery of resin based sealant may increase the retention.[90] and to ensure the quality of the cotton roll isolation during sealant placement in community settings four-handed dentistry is needed.[91]

A systematic review of sealant retention included 3 studies that evaluated the use of a rubber dam as an isolation aid over the cotton roll, the review reported no difference in retention between the two methods of isolation at 24 months.[61] The same systematic review found that when the 124 studies identified in the review were evaluated the cotton roll was the main type of isolation utilized in most of the studies. The rational for this finding may be due to (1) the discomfort during a dam clamp placement, (2) the need for a local anesthetic, (3) difficulty in placing a clamp onto a partially erupted tooth, and (4) need for sterilization of the instrument. All of which lead to preferring use of the cotton roll for isolation.[91]

Other methods have been proposed to reduce the effect of salivary contamination, the bonding agent being one prominent example. They suggest that placement of bonding agent under sealants allows for better penetration into the pits and fissures than sealant alone. This increases the wettability of the enamel and enhances bonding potential which can be of great importance when trying to increase sealant retention.[92, 93] Feigal *et al*.[87] found that the use of a dentine bonding agent on the enamel under the sealant can reduce the effect of salivary contamination upon sealant microleakage significantly ($P < 0.0001$).

A recent systemic review by Feigal and Donly[87] reported that a low-viscosity, hydrophilic bonding layer placement under the sealant has been found to enhance sealant effectiveness and retention. However, other systematic reviews and clinical studies found that there is no significant change in microleakage when sealant placement included the use of a dentine bonding agent. [12, 94]

Another example of a method used to control salivary contamination consists of use of a drying agent. It has been suggested that a drying agent be used on etched enamel to improve the retention of the sealant by evaporating the residual moisture thus increasing surface tension and the wettability of the sealant. In addition, the drying agent increases the bonding potential between the hydrophobic sealants and the etched enamel by displacing the moisture and providing a more hydrophobic environment which promotes potential for sealant bonding.[13, 95] However studies have found contradicting results regarding the effect of the drying agent on sealant treatment. Rex *et al*.[16] in a one- year clinical study found no significant difference in retention of sealant placed with, and without, drying agent. Although, they reported that the use of post etchant drying agent enhanced sealant retention. Duangthip *et al.*[86] concluded that sealant placed with post etchant drying agent was not more effective than sealant placed without it, in term of sealant penetration. Controversially, a different study by same authors reported that the use of post etchant drying agent improved the sealant quality significantly.[14] Hatibovic *et al*.[15] compared the microleakage of three sealants(two filled and unfilled one) with different enamel preparations techniques. They found that the filled sealant applied with the drying agent was the most effective sealant in microleakage prevention. However, the 99% ethyl alcohol which was commonly used in the previous studies is technique sensitive (High alcohol percentage content will make it evaporates very quickly). Therefore, we hypothesized that two applications of 99% ethyl alcohol and a lower concentration of ethyl alcohol with single application may provide more time for ethyl alcohol to volatilize the moisture during the sealant placement procedure.

Microleakage Test

Different ways have been used to evaluate the sealant retention, included, bond-strength tests, scanning electron micrograph (SEM) evaluations, and microleakage distance measuring. Microleakage is defined as the movement of oral fluids between the tooth and dental restoration's interface. The fluid may include bacteria and toxic substances which may affect the tooth's structure as well as the pulp.[96] It has been proposed that microleakage increases the development of caries lesions.[97] In -vitro microleakage studies have been used to evaluate the marginal sealing ability of dental sealants.[98] Additionally, microleakage measurement in *in vitro* studies has been used to evaluate the effectiveness of different conditioning procedures for the retention of fissure sealants.[5, 99]

Evaluation of the penetration of a dye is the most simple and widely used methodology for microleakage evaluation. Various solutions and dyes with different concentrations have been used to study the microleakage, such as radioactive isotopes, methylene blue dye, basic fuchsin, erythrosine, silver nitrate, alcohol gentian violet, and rhodamine. In addition, different methods have been used to measure dye penetration through the tooth sealant interface, such as measuring the percentage, or the distance in microns, of dye penetration along the enamel-sealant interface. However most of the previous studies used a ranked scale to assess the microleakage. For example, the one described by

Grande *et al*. [100] used the following 0 = no dye penetration; 1 = dye penetration only on the superficial margins; 2 = dye penetration restricted to lateral interface, and 3 = extensive dye penetration to the bottom of the sealant. In our study microleakage will be used as measurable criteria to evaluate the longevity of sealant retention.

Aging by Thermocycling

Dental materials in the oral cavity are constantly exposed to heat and pH changes. Due to differences in thermal contraction and/or expansion coefficient between the tooth structure and the dental material crack propagation along the bonded interface and subsequent marginal gaps can occur.[101] The thermal contraction/expansion coefficient of resin materials (25-60 ppm/°C) are more than that of enamel (11.4ppm/°C) and dentin (8 ppm/°C).[102] Therefore thermocycling is a widely used thermal fatigue method to evaluate the durability of dental material which is bonded to tooth structure in *in vitro* studies. This method tries to produce the same thermal changes that occur in the oral cavity which are caused by breathing, drinking, and eating.[103]

Studies reported the use of different thermocycling regimes, for example: cycling numbers ranging from 100 to 50,000 cycles, temperature extremes ranging from 4°C to 15°C in cold bath and up to 45°C to 60°C in hot bath, and dwell time of 15, 30, or 60 seconds. Based on the hypothesis that each cycle may happen 20 to 50 times per day, 10,000 cycles has been estimated to simulate one year of clinical function.[103]

Specific Aims and Hypothesis

Aim

The purpose of this in vitro study is to evaluate microleakage of sealant placed after the application of ethyl alcohol (50% in single application and 99% in both single and double applications), as a post etchant drying agent applied before using the commonly utilized resin based sealant Ultraseal XT Plus. The effect of salivary contamination on microleakage will also be evaluated.

Hypothesis

1. The sealant placed on teeth after the use of 50% ethyl alcohol will show a lower microleakage mean than the single and double applications of 99% ethyl alcohol. Double applications of 99% ethyl alcohol will show a lower microleakage mean than the single application.

2. The sealant placed on salivary contaminated teeth will show a higher microleakage mean than the sealant applied on dry teeth.

Research Design and Methods

- *Sample Size Calculation*

A power calculation was conducted using nQuery (Version 7.0). Assuming a variance in means of 3.8 for the different concentration of ethyl alcohol, and a within-group standard deviation of 6.67, a sample size of n=8 per group is adequate to achieve a type I error rate of 5% (α =0.05) and power of 99% to detect a difference between different ethyl alcohol concentrations.

- *Sample Preparation*

Sixty four extracted human third molars teeth, free of caries, hypomineralization, fluorosis, fissure sealant and restoration were selected for this study. They were stored in 0.5% Chloramine T solution after the extraction. The teeth were selected by visual inspection. The occlusal surface of each tooth was cleaned with non-fluoridated paste (Zircon; L. D.Caulk, Milford, DE, USA) using a bristle brush for 15 seconds, rinsed with air water syringe for 20 seconds, and then dried with oil free compressed air for 15 seconds.[14]

- *Etching Procedure*

Occlusal fissures were conventionally etched with 35% phosphoric acid gel (Ultra Etch, Ultradent Products Inc., USA) for 30 seconds with the help of a disposable brush, rinsed with distilled water for 15 seconds, and then dried with oil free compressed air for 15 seconds.[104] After that, teeth were numbered from one to sixty four and then randomly divided into four groups by using a randomization program on website (www.random.org).

- *Salivary Contamination*

The occlusal surfaces of groups 1 through 4 were salivary contaminated with artificial saliva prepared in the lab using the ingredients illustrated in table 2. All ingredients were dissolved in one liter of distilled water, and the pH of saliva was adjusted by buffers to be 6.2, and measured by pH electrode (ROSS Ultra® 8102 BN, Thermo Scientific, MA, USA). The saliva was placed on the etched pit and fissure surfaces of the teeth with a micro-brush for 10 seconds, and then air dried. The other four groups (group 5 through 8) were not contaminated.

- *Ethyl Alcohol Application*

1. Groups 1, 5 - No drying agent was applied prior to sealant placement (as control groups)

2. Groups 2, 6- Prior to sealant placement 50% ethyl alcohol (Ethyl Alcohol 50% (1:1) Solution, Aqua Solutions, Fisher Scientific Inc., USA) was applied on the etched occlusal surfaces with a micro-brush for 10 seconds. Then the surfaces were air dried with a moisture-free and oil free compressed air for 5 seconds.

3. Groups 3, 7- Prior to sealant placement 99% ethyl alcohol (Primadry, Ultradent Products Inc, USA) was applied on the etched occlusal surface, using a black micro brush tip for 10 seconds, Then the surfaces were air dried with a moisture-free and oil free compressed air for 5 seconds.

4. Groups 4, 8- Prior to sealant placement 99% ethyl alcohol (Primadry, Ultradent Products Inc, USA) was applied on the etched occlusal surface for 10 seconds, the

surfaces were dried using a moisture free - oil free compressed air for 5 seconds, and Primadry was reapplied for 10 seconds and then dried for 5 seconds.

- Sealant Application

Teeth in the eight groups were sealed with opaque filled sealant material (Ultraseal XT plus syringe, Ultradent products, Utah, USA) using an Inspiral brush tip. A very thin layer of sealant was applied and left for one minute to penetrate. Finally, the sealant was light cured for 20 seconds using a standard light curing unit (Demintron, Kerr, Germany). The teeth were then restored into normal saline at room temperature for twenty-four hours.

- Thermocycling

After that all sealed teeth were subjected to 2500 thermocycles alternating between hot water (55^0C) and cold water (5^0C) with 30-seconds immersion time in each of the water baths (Thermocycling test apparatus, Sabri Dental Enteprises, Inc, Downer Grove, IL). [105, 106] After being subjected to thermocycling (figure 2), the teeth were coated with two layers of nail polish (with the exception of restorations as well as a 1mm rim of tooth structure around the restorations).

- Microleakage Testing

The teeth were immediately immersed in 50% (V/W) silver nitrate ($AgNO_3$) solution (Sigma-Aldrich Co, St. Louis, Mo) in the dark for 3 hours. Excess $AgNO_3$ solution was rinsed off with tap water. The teeth were placed in a developing solution under fluorescent light for 24 hours.

The teeth were embedded in self-curing acrylic resin (figure4). The resin blocks were sectioned with two parallel cuts in the bucco-lingual direction using a low-speed diamond wheel precision saw machine (Isomet 1000, Buehler, Germany) (figure5) yielding 3 surfaces per tooth for analysis. The image of the sealant/enamel interface were captured with a stereomicroscope (Olympus SZ16) at 60x magnification with attached CCD digital camera (Ueye, IDS, Germany) (figure 6) and stored in digital format. The length of silver nitrate dye penetration was presented as a dark line on the image at the sealant/tooth interface and measured in microns using Omninet 9.0 software (Buehler, Germany). A calibration slide was used to check the software measurement's accuracy.. Two consecutive measurements were taken from each surface: A) the sum of microleakage distances throughout the sealant-tooth interface. B) The total length of sealant- tooth interface. For each surface we calculated the proportion of microleakage {(A/B)*100} (figure7). These measurements resulted in our having three percentage of microleakage for each tooth. We calculated the mean of these three measurements to create a single number indicating the proportion of microleakage of each tooth.

A total of 192 sectioned surfaces were examined for this study. A calibration slide was used to check the software measurement's accuracy.

Statistical Analysis

All analyses, including initial descriptive statistics, were calculated using SPSS version 19. Originally a two-way ANOVA with ethyl alcohol concentration and salivary contamination as independent variables, and proportion of microleakage as a dependent variable was run but because the interaction was statistically significant, ethyl alcohol concentrations were compared at each level of salivary contamination using one way ANOVA with post hoc tests (Tukey's HSD). Likewise the salivary contamination condition was compared at each level of ethyl alcohol concentration using independent samples t-test. The level of significance was set at $P < 0.05$.

Results

The percentage of the samples without microleakage in each group was as follows: Group 1= 8.3%, Group 2= 23.3%, Group 3= 16.7%, Group 4= 12.5%, Group 5= 30.1%, Group 6= 32.4%, Group 7= 30.5%, Group 8= 30%. The mean (and SD) microleakage proportion of all groups are presented in Table 3 The application of ethyl alcohol as drying agent prior to sealant placement (Groups 2, 3, 4, 6, 7, and 8) showed considerably lower microleakage proportion than the control groups (Groups 1 and 5). The lowest microleakage mean was obtained when 50% ethyl alcohol concentration was used as drying agent on both contaminated and non-contaminated teeth.

Figure 8 revealed stereomicroscopic images showing microleakage between the enamel surface and the fissure sealant on each representative group. Sample groups contaminated with saliva had greater microleakage when compared to the non-contaminated groups. Box plot diagram was also plotted to graphically illustrate the distribution of microleakage proportion of the different experimental groups (Figure 9).

Statistical analysis with one-way ANOVA showed that there were significant differences between samples in both salivary and non-salivary contamination groups ($p<0.001$). Post hoc comparison using Tukey's HSD in salivary contamination groups indicated that sealant placed utilizing 50% ethyl alcohol showed significantly less microleakage than sealant placed with either 99% (once or twice) or the sealant placed without the prior use of ethyl alcohol ($p<0.001$). In addition, sealant placed utilizing 99% ethyl alcohol once and twice showed statistically significant less microleakage than sealant placed without the prior use of ethyl alcohol ($p<0.001$). However, there was no significant difference in microleakage between the use of 99% once and twice ($p= 0.16$) (groups 3 and 4).

Post hoc comparison using Tukey's HSD in non-salivary contamination groups indicated that 50% ethyl alcohol group had significantly less microleakage mean ($p<0.001$) compared to sealant placed with the other ethyl alcohol concentrations or the control group (without drying agent). In addition, sealant placed utilizing 99% ethyl alcohol once and twice showed significant less microleakage than the control group ($p<0.001$). However, there was no significant difference in microleakage between the use of 99% once and twice ($p= 0.21$) (groups 7 and 8).

When samples were grouped either as "contaminated" or "non-contaminated" and compared using the independent samples t- test, significant differences in microleakage were found between salivary contaminated and non-contaminated groups. The microleakage proportion of mean values in contaminated groups showed significantly increase compared to non-contaminated groups ($p<0.001$) for all possible concentrations.

Discussion

Microleakage and different ethyl alcohol concentrations as drying agent

The retention of pit and fissure sealant is a micromechanical process established by resin tag formation into the micro porosity created in the etched enamel surface.[38] However, different percentage of sealant loss still can be expected annually.[51] Moisture contamination is generally accepted as the most critical aspect during sealant placement. A variety of fissure treatment and isolation methods have been studies in an attempt to control the moisture contamination to successfully reduce microleakage and improve sealant retention. The use of ethyl alcohol as drying agent has been recommended by the manufacture of Ultraseal XT plus and it is thought to improve sealant retention by evaporating the residual moisture in fissures, so that the hydrophobic sealants could penetrate more into the etched enamel. The drying agent may further improve the ability of sealant to wet the etched enamel resulting in superior penetration and more resin tag formation which results in reduced microleakage. However, the published studies have revealed contradicting results, which sometimes makes conclusions difficult.

Our study results showed significant differences in the microleakage mean among the groups of sealant placed with the use of different ethyl alcohol concentration and control groups. The result are similar to the study reported by Duangthip *et al.*[14] who concluded that the use of post etchant drying agent improves the quality of pit and fissure sealant by volatilizing the moisture content of fissures. Also, the results were similar to Rex *et al*[16] who reported that the use of post etchant drying agent enhanced sealant retention, although they found no significant difference in retention of sealant placed with and without drying agent.

Further, the present study had partially similar results to two studies in which the drying agent was used before sealant placement. The first study compared the microleakage of three sealants (filled with drying agent, filled without drying agent and unfilled sealant) with different enamel preparations techniques. They found that the filled sealant applied with the drying agent was the most effective sealant in microleakage prevention.[15] The other study reported that the application of acetone as drying agent prior to sealant placement could improve the penetration depth of resin into the fissuers.[13]

On the other hand, a study done by Duangthip *et al*.[86] had contradicting results. Their study investigated the effect of different drying agent (no drying agent, 99% alcohol, 99.5% acetone) used before sealant placement on microleakage and penetration of pit and fissure sealant and they concluded that use of post etchant drying agent didn't decrease the microleakage or improve the sealant penetration significantly. The difference in findings may be due to the use of different methods, parameters and variables. First, our study was done under the ambient room conditions while the mentioned study was conducted in a humidity chamber which may affect the ability of the drying agent to volatilize the moisture. Secondly, the fissure morphology which was found to be the greatest influence on the penetration in the Duangthip *et al.* study was not evaluated in our study. Therefore the contradicting result may be explained by the confounder affect. Lastly, the thermocycling protocol and the sealant type were different in both studies. So for all of the previous reasons it was unclear if the comparison between the two studies is even valid.

In the present study, under both salivary contamination and non-contamination condition, the use of 50% ethyl alcohol as post etchant drying agent yielded significantly better results in term of microleakage reduction. One possible explanation for the smaller amount of microleakage following the use of 50% ethyl alcohol fissure treatment before sealant placement would be that the lower concentration of the ethyl alcohol could provide more working time for the drying agent to volatilize the moisture before sealant placement while the 99% is a highly volatile structure due to the high ethyl alcohol concentration which make it very hard to control and to apply. This also may explain the reason why, even when we apply the 99% ethyl alcohol twice, it was not better than its application once which is again due to its highly volatile properties which make it hard to control.

Microleakage and salivary contamination

The retention of resin sealants is a micromechanical process established by the infiltration and polymerization of the sealant into the microporosity network created by the acid etchant on enamel surfaces. Some factors, however, may undermine the bonding of occlusal sealants and hence compromise their long-term clinical success. One such factor is control of contamination of enamel after acid etching. The literature showed that one second exposure to saliva can cause the immediate formation of a protein layer that resisted the 30 seconds of vigorous irrigation.[88] Therefore, when resin tag formation is disturbed by moisture contamination during the sealing procedures, poor adhesion and sealant failure should be expected. One objective of this study was to look at how salivary contamination affected the level of microleakage. This was done by comparing etched, dried and sealed teeth to those that were contaminated with artificial saliva after the etching process. Contaminated teeth were dried until no moisture could be detected on the tooth. Our results agreed with those of the previous studies in which the overall microleakage mean of saliva-contaminated enamel was markedly higher than that recorded under dry non-contaminated condition. The finding that salivary contamination reduces bond strength and increases the microleakage is not new.[107-109] However, it is good for clinician to be always aware of this, and to see that this true for new sealant materials as well.

Our study has some limitations. First, there was difficulty in measuring Microleakage distances due to the lack of a definite starting point on the occlusal surface. To control for this limitation in our study, we had three measurements from each tooth and we took the mean of these three measurements. Secondly, the lack of reported studies using the same method and materials tested in the present study is a limitation to declaring a reliable comparison with outcomes of previous studies. Finally, this study was an initial investigation of the use of different ethyl alcohol concentrations as a drying agent on sealant treatment which is limited by several laboratory factors that may affect the accuracy of the results. Therefore, more in vivo studies are needed to draw more clinically applicable conclusions under conditions of oral environment. Further studies should assess the ultimate influence of post etchant drying agent on the quality of the adhesion bond strength

Conclusion

Within the limitation of our study we can conclude that:

1. Ethyl alcohol application as post etchant drying agent prior to sealant placement significantly improves the microleakage of sealant in both salivary contamination and non-contamination conditions.
2. The use of 50% ethyl alcohol as drying agent yielded significantly lower microleakage mean in both salivary contamination and non-contamination conditions.
3. Salivary contamination significantly increases mean of microleakage.

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Table 1: Materials used in the study

Materials In Study	Manufacturer
Ultradent Etch Gel.	Ultradent Products Inc., South Jordan, Utah
Ultraseal XT Plus Sealant	Ultradent Products Inc., South Jordan, Utah
PrimaDry (99% ethyl alcohol) Agent.	Ultradent Products Inc., South Jordan, Utah
50% Ethyl Alcohol(1:1) Solution	Fisher Scientific, Pittsburgh, PA
50% Silver Nitrate Solutions	Sigma-Aldrich Co., Pittsburgh, PA

Table 2: Artificial saliva ingredients

Calcium Chloride ($CaCl_2$)	0.077 g
Magnesium Chloride Hexahydrate ($MgCl_2$ $6H_2O$)	0.049 g
Potassium Dihydrogen Phosphate (KH_2PO_4)	0.544 g
Potassium Chloride (KCl).	2.23 g
Sodium Azide (NaN_3).	0.019 g
HEPES (2-hydroxyethyl)-1-Piperazineethanesulfonic Acid.	4.76 g

Table 3: Microleakage percentages by ethyl alcohol concentration.

Contamination Condition	Ethyl Alcohol Concentration (EA)	Microleakage Percentage Mean ± SD	P-Value
Salivary Contamination	No EA	25.24 ± 0.40	<0.001
	50% Once	16.19 ± 0.50	
	99% Once	21.06 ± 1.11	
	99% Twice	20.23 ± 0.84	
No Salivary Contamination	No EA	20.58 ± 2.02	<0.001
	50% Once	8.75 ± 0.79	
	99% Once	12.9 ± 1.30	
	99% Twice	12.61 ± 1.16	

***** Intergroup comparisons were conducted by using Tukey's HSD test which revealed significant differences among all groups under contamination condition except for group 3 and 4 (p= 0.16), and significant differences among all groups under non-contamination condition except for groups 7 and 8 (p= 0.21).

* Independent samples t-test was run for salivary contamination condition at each level of ethyl alcohol concentration and P-value was <0.001 for all groups.

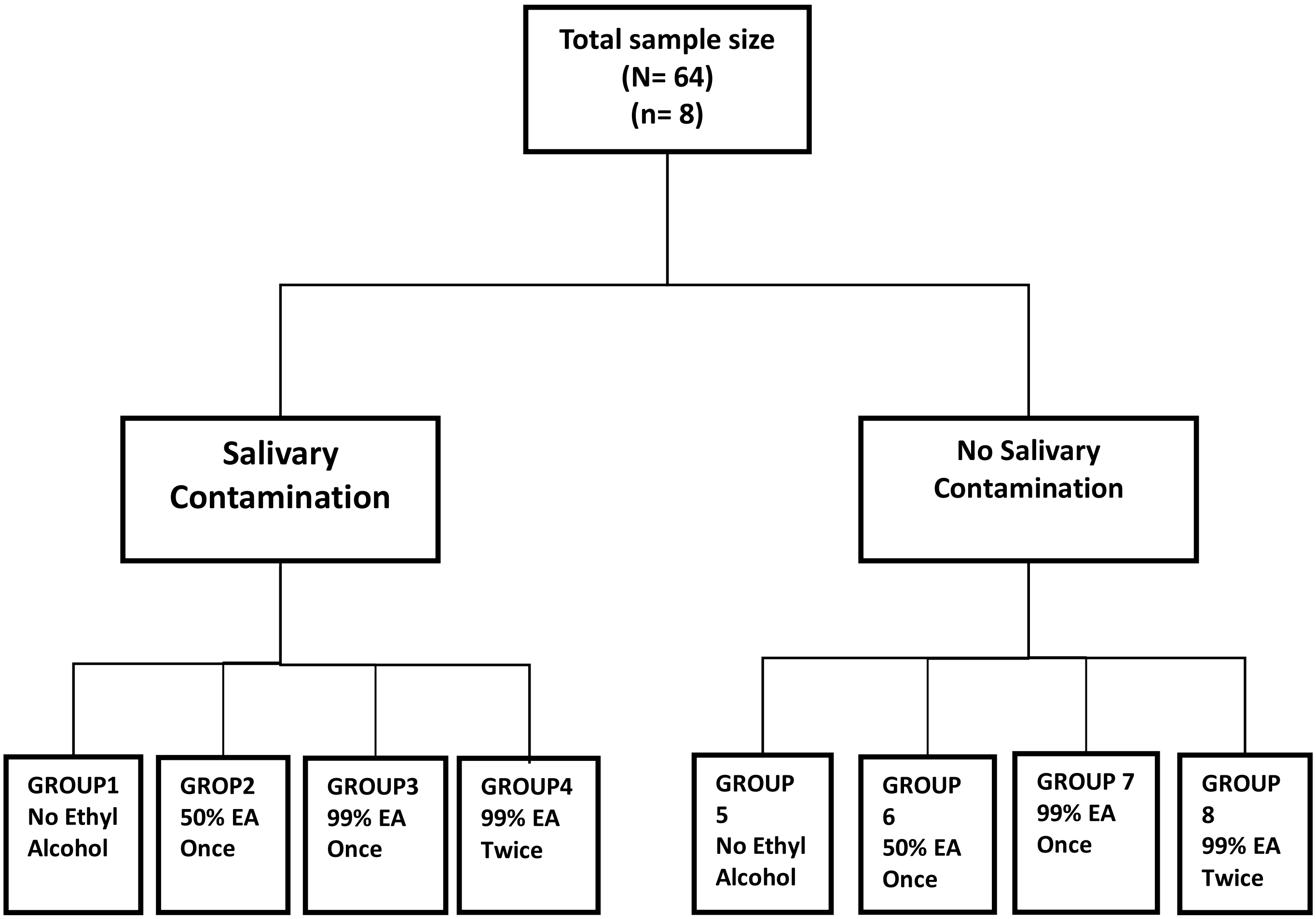

Figure1: Reveals how the eight individual groups of teeth were categorized

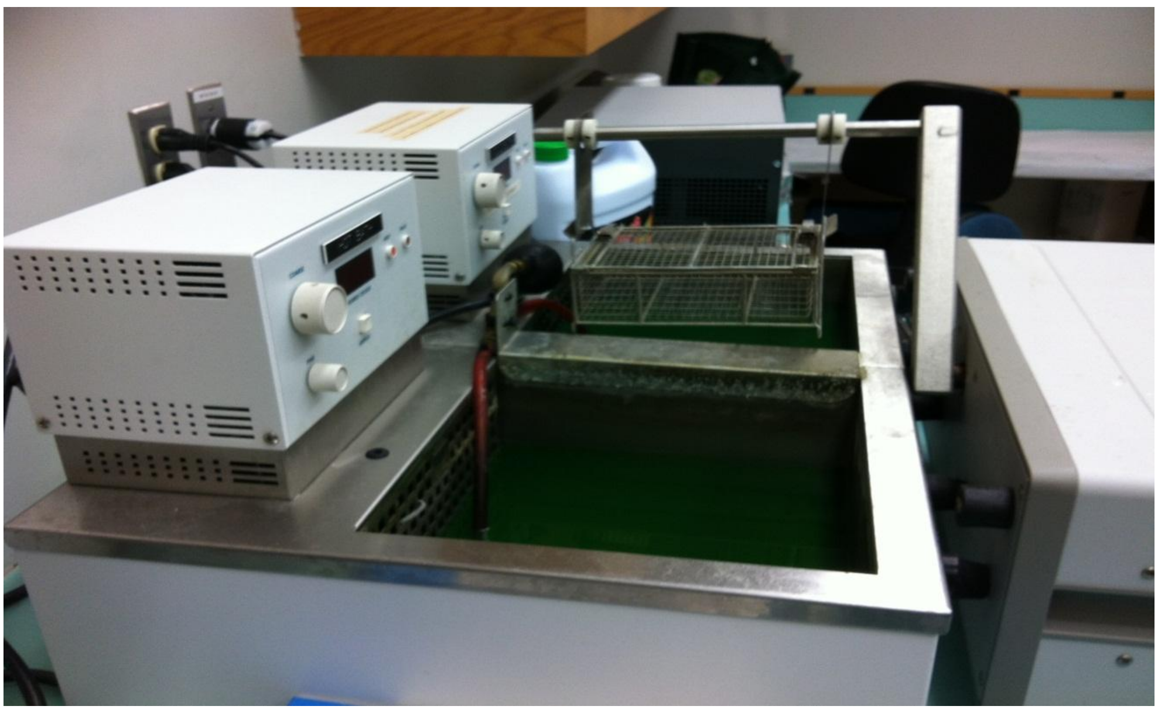

Figure 2: Thermocycling machine.

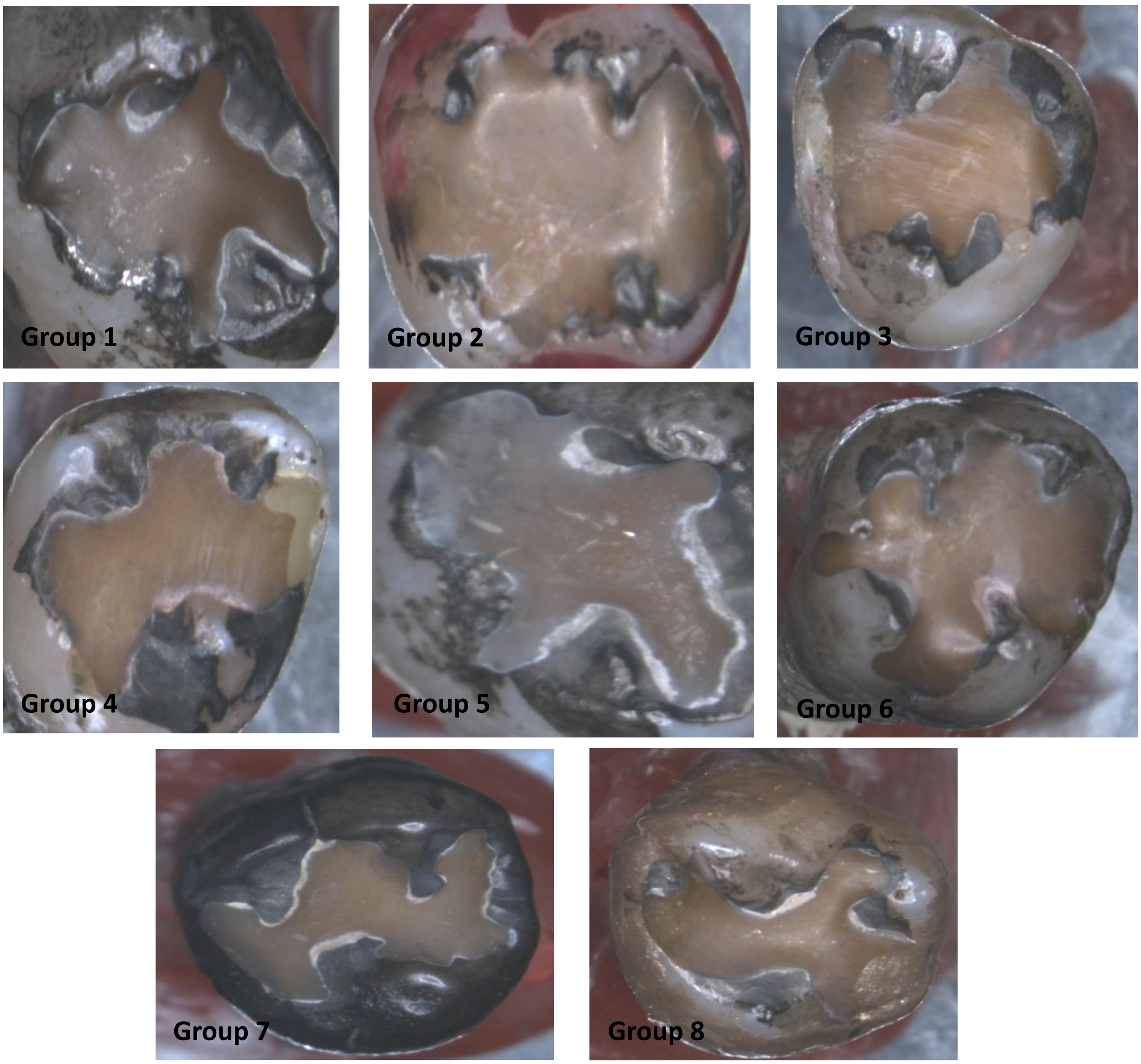

Figure 3: Stereomicroscopic images showing representative examples of teeth after being embedded in 50% Silver Nitrate dye and developing solution.

Figure 4: Specimens were embedded in epoxy resin.

Figure 5: Isomet 1000, Buehler

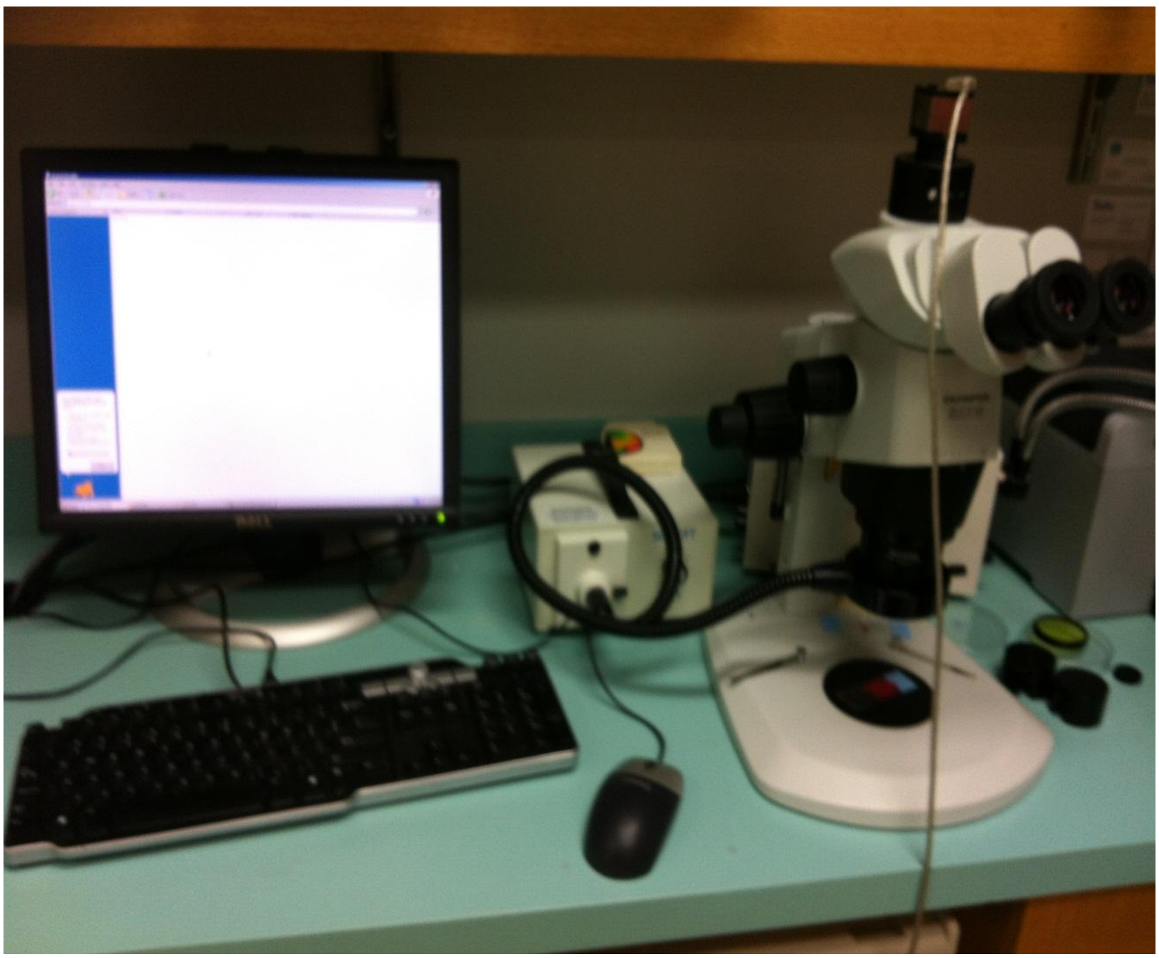

Figure 6: stereomicroscope (Olympus SZ16)

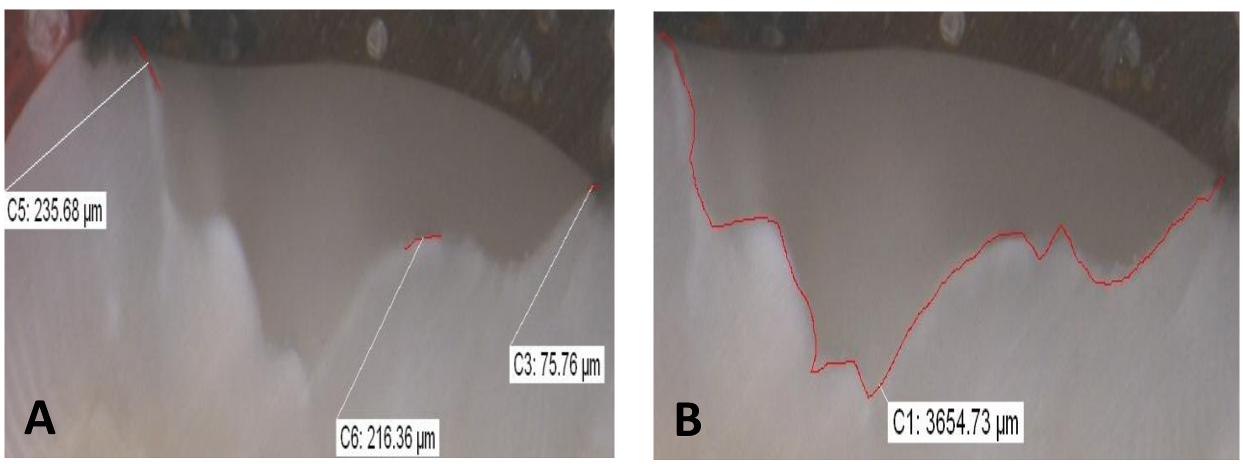

Figure 7: Scoring system used for the evaluation of microleakage: A) the sum of microleakage distance throughout the sealant- tooth interface in micrometers (C3+C5+C6, red lines) using imaging software. (B) Enamel-sealant interface distance (C1, red line) in micrometers.

*The proportion of microleakage= {(C3+C5+C6) / C1}*100

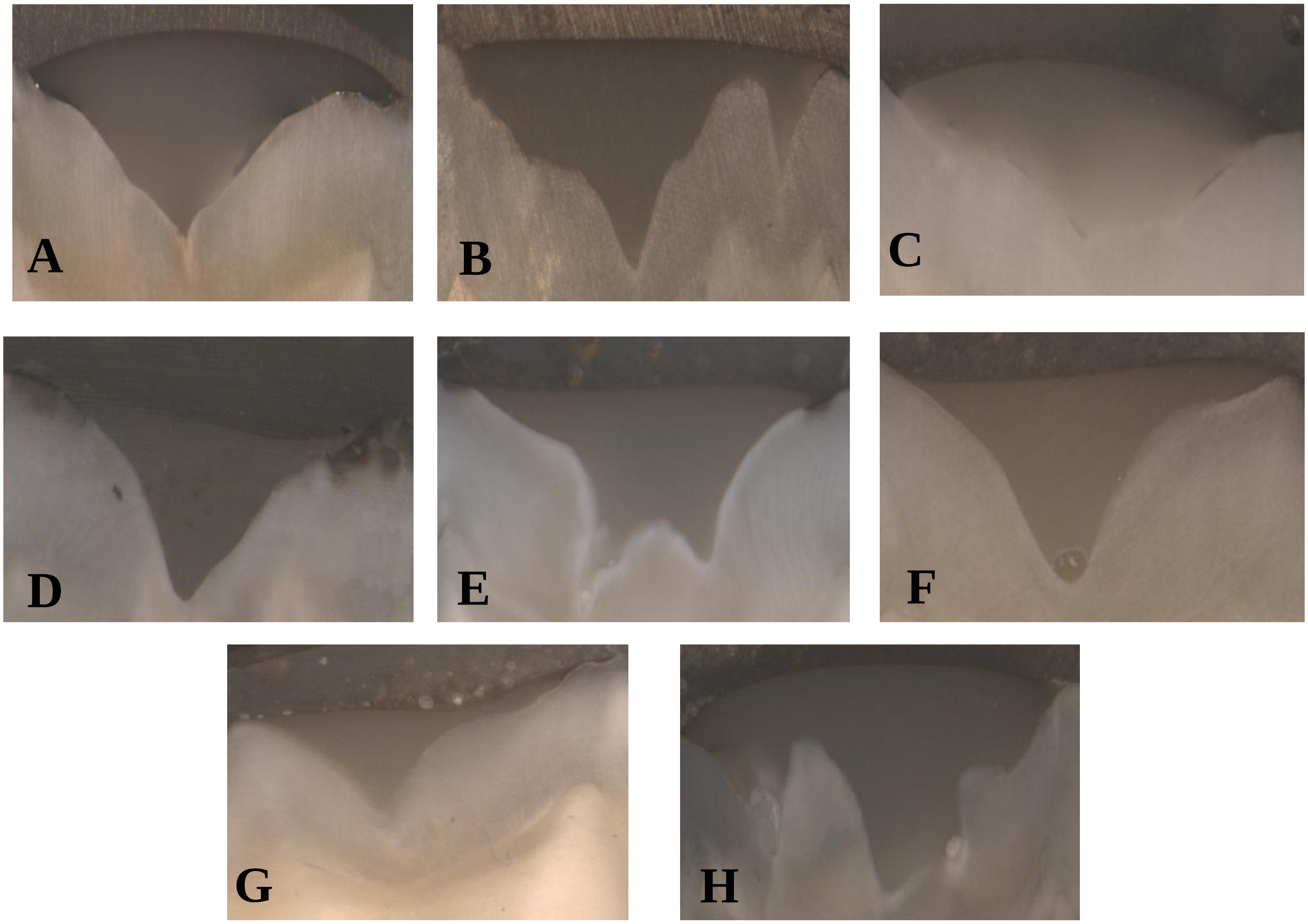

Figure 8: Stereomicroscopic images showing representative examples of microleakage between the enamel surface and fissure sealant of each study group. (A) Group 1: No drying agent, with saliva contamination. (B) Group 2: 50% ethyl alcohol with saliva contamination. (C) Group 3: 99% ethyl alcohol once with saliva contamination. (D) Group 4: 99% ethyl alcohol twice with saliva contamination. (E) Group 5: No drying agent, without saliva contamination. (F) Group 6: 50% ethyl alcohol without saliva contamination. (G) Group 7: 99% ethyl alcohol once without saliva contamination. (H) Group 8: 99% ethyl alcohol twice without saliva contamination.

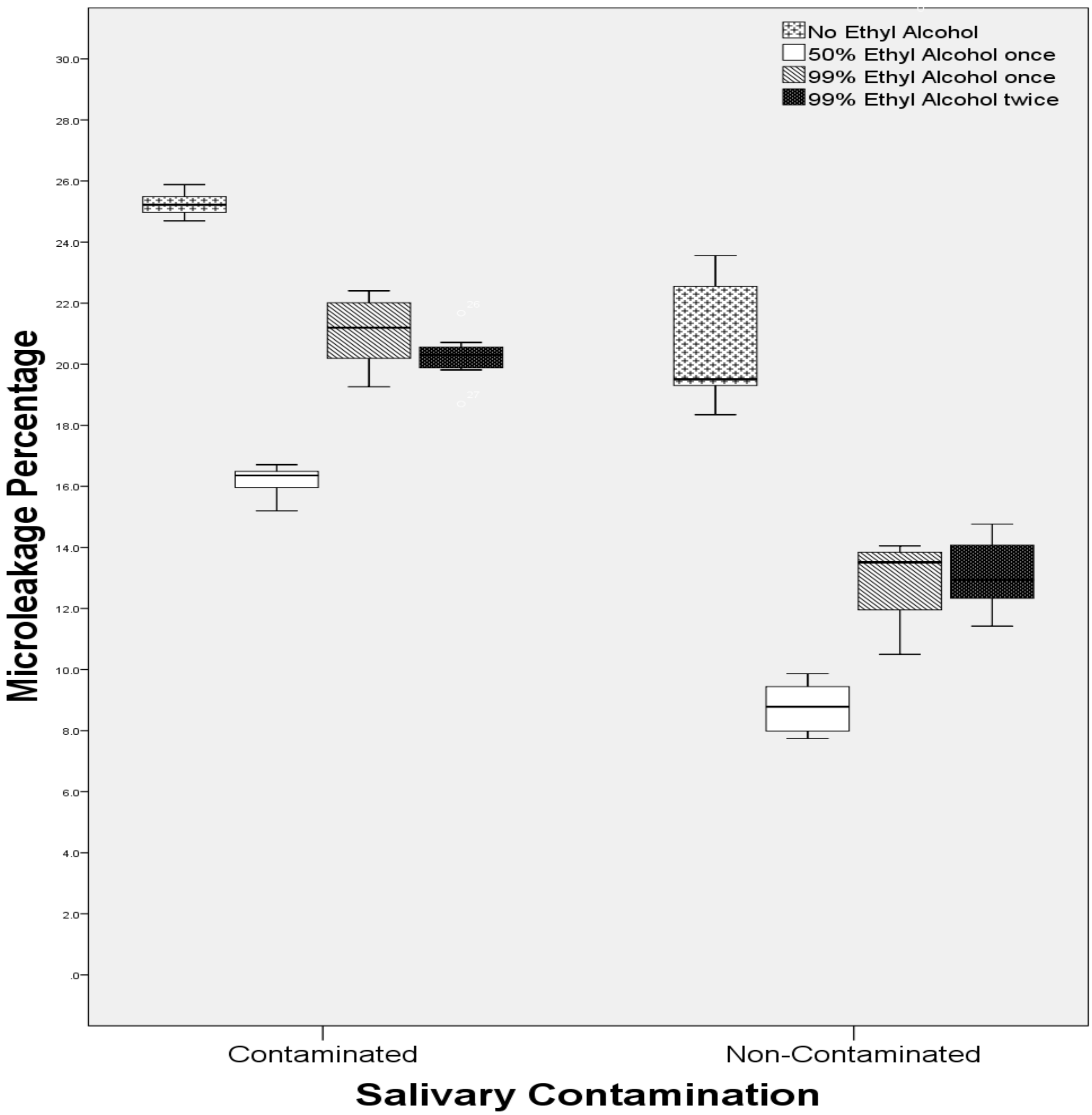

Figure 9. Microleakage proportion in each study group.

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