The Effect of Adhesive Systems on Fluoride Release of a Conventional Glass Ionomer:

An In Vitro Study

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ABSTRACT

Objective: The aim of this study was to assess the effect of three different generations of bonding system on fluoride release of a conventional glass ionomer in comparison to uncoated conventional glass ionomer.

Materials and Methods: Forty samples of glass ionomer restorative material in acrylic mold were distributed in four groups: G1: control-uncoated Fuji IX, G2: coated with ScotchBond Multipurpose, G3: coated with Excite F, and G4: coated with L-pop adhesive system. Each specimen was stored in a centrifuge tube containing 4ml of artificial saliva in 37^{o}C. Fluoride release of each sample was measured daily from day 1 through day 10, and on day 14 and 15, by using fluoride ion specific electrode probe. Data were analyzed with one-way ANOVA for the cumulative fluoride release over the span of 15 days, as well as day 1 values. Kruskal-Wallis and Mann-Whitney U tests were used for the fluoride release at other time points, day 2 and day 3.

Results: The Fuji IX coated with L-pop adhesive showed significantly higher amount of fluoride release, followed by the control group, the uncoated Fuji IX. ($p<0.05$). There was no significant difference between the groups coated with ScotchBond Multipurpose and Excite F. The highest amount of fluoride release was shown within the first 24 hours immediately after restoration placement among all four groups.

Conclusion: With consideration to the limitations of an in-vitro study, we conclude that L-pop bonding agent (6th generation adhesive system), increased the amount of fluoride released from the glass ionomer. We also concluded that ScotchBond Multi-purpose (4th generation bonding system) and Excite F (5th generation bonding system) acted as a barrier and reduced the release of fluoride from the glass ionomer.

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It has been well demonstrated that fluoride ions applied topically can reduce the incidence and progression of caries by integrating into the mineral component of enamel and dentin.[1,2] Fluoride induces the arrest or reversal of enamel lesions by inhibiting demineralization and increasing remineralization.[3,4] Fluoride can be provided for the tooth surfaces through different methods such as dentifrices, mouth rinses, and topical fluoride gel or varnishes. In addition, fluoride can be made available by fluoride-releasing restorative material placed in close proximity to tooth surfaces.[5] Therefore, dental practitioners are exposed to several dental materials that claim the benefit of fluoride release,[6] among which glass ionomer (GI) restorative materials have caught attention of many dental practitioners.[7] Glass ionomer cement (GIC) systems have become important dental restorative and luting dental materials for use in preschoolers, children and teenagers.[8] Due to poor physical property and bonding of the GIC, there is a tendency among some practitioners to use bonding agents with GIC to improve the longevity of the restoration.[9,10] However, the bonding agent could have a negative effect on fluoride release of the GICs.[10]

The mineral component of dental enamel is mainly composed of hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$].[11] During cariogenic acid attach, the H^+ decrease the OH^- concentration and protonate phosphate ions $PO4^{3-}$ in the plaque fluid to $HPO4^{2-}$ and $H2PO4^-$. Because the $PO4^{3-}$ concentration decreases when the pH is low, $PO4^{3-}$ and OH^- ions dissolve out of the tooth to maintain the equilibrium. This process leads to the release of calcium from the hard dental tissue and as a result the tooth dissolves.[12] The reaction between hydroxyapatite and low concentration of fluoride has been suggested to be an ionic exchange, in which fluoride replaces the position of hydroxyl ions in the crystal lattice structure. This replacement results in formation of a more stable apatitic structure, the fluorapatite [$Ca_{10}(PO4)_6F_2$]. Fluorapatite is essentially less soluble than hydroxyapatite, even under acidic conditions, subsequently is more resistant to demineralization and dental caries formation. Therefore, fluoride improves the quality of mineralized dental tissue.[11]

In vitro studies have shown that enamel is protected from demineralization by the fluoride released from the fluoride releasing materials which are placed in close proximity to the enamel.[13,14] Secondary caries formation is one of the most common causes of the post-operative failure.[15] Delbem *et al*.[16] reported that the initiation and progression of secondary caries is significantly reduced when glass ionomer cements are used, due to the fluoride release. Qvist and colleagues[5] showed that there is a reduced rate of development and progression of caries on surfaces in contact with fluoride releasing materials such as glass ionomers, resin-modified glass ionomers and compomers compared to surfaces in contact with amalgam. Similar results were found by Derkson *et al*.[17] and Svanberg[18] on the effectiveness of fluoride in inhibiting demineralization in adjacent teeth next to the fluoride releasing restorative material. Since it was noted that secondary caries formation was rarely associated with fluoride-containing silicate cement restorations, increasing attention has been focused on the development of various fluoride-releasing products such as glass ionomers.[19]

Glass ionomers possess certain properties that make them useful and clinically attractive dental restorative materials. Glass ionomer cements are composed of the powder component of calcium fluoroaluminosilicate glass (base) and aqueous solution of an acrylic acid homo or copolymer (acid).[7] Glass ionomer cement components, when mixed together, undergo a setting reaction involving neutralization of the acid groups by the powdered solid glass base.[8] During glass production, fluoride flux is added to prevent oxidation, then fluoride is released after mixing of powder with the polyalkenoid acid and becomes available for absorption by the tooth structure.[20] Fluoride ions are released during the acid-base reaction of the cement and they are not an essential part of the matrix formation; therefore, they are free to move in and out of the matrix formation.[21] Hence, the GIs are able to release, absorb and re-release fluoride and maintain an increased level of fluoride around restorations and reduce the chance of secondary caries formation.[22] They are potentially acting as fluoride reservoir by way of retaining fluoride delivered by toothpastes or topical fluoride treatments at the material surface and then release this fluoride slowly.[23] Glass ionomers were invented in 1969 and reported by Wilson and Kent in the early 1970s,[24] and since then much attention from researchers and clinicians was focused towards this material because it was reported to form chemical bond to the tooth structure.[20,25,26] Beyond use as a liner, luting cement, or in sandwich technique,[27] GI can be used as a restoration itself, from preparation interface all the way to the surface in primary teeth.[28,29] In class II and III restorations in primary teeth, the proximal contact point is a location to take advantage of the unique fluoride-releasing properties of GI.[30,31]

There are several other advantages to this material including adhesion to moist dental structure, anti-cariogenic properties due to fluoride release, thermal compatibility with tooth enamel, biocompatibility and low cytotoxicity.[7] However, the conventional glass ionomer cements have several disadvantageous characteristics such as difficult manipulation, poor esthetics, small mechanical resistance and weak cohesive resistance.[25] The most important limitation that hinders the clinical use of the conventional glass ionomer as a final restorative material is its poor strength and weak bond to tooth structure when subjected to load[32]. In the 1980s, with the goal of creating more durable and stronger GI, one manufacturer added silver amalgam powder (Miracle Mix, GC America) and another combined the glass powder with elemental silver by a process of high heat fusion (Ketac Silver, 3M ESPE)[33]. The addition of silver had the advantage of improving the wear resistance of the silver cermet cement somewhat over traditional glass ionomers; however, fracture resistance and toughness of the metal-modified materials were still too low to recommend them for the stress bearing areas and the gray color precluded routine use of these materials in anterior teeth.[8] In the early 1990s, light-hardened resin-modified glass ionomers (RMGI) were introduced. The liquid of polyacid component includes a photopolymerizable resin which hardens the material when a visible light beam is applied. Addition of the resin component not only reduced the initial setting time and handling difficulties, but also increased the wear resistance and improved physical properties of the GIC.[34]

Even though the light hardening RMGI are very popular and being used by many dentists, the conventional self-cured glass ionomer cements are still being used widely. The new GIC products that have been introduced are cured by acid-base reaction but have much improved physical properties compared to older versions of GICs. Fuji IX GP and Fuji IX GP Fast have a rapid set which significantly reduces early moisture sensitivity. Faster hardening has been achieved by changing the particle size and particle size distribution of the glass powder.[8] An even newer version of this GIC is available, Fuji IX GP Extra, which has faster setting time while maintaining the ample working time of Fuji IXGP Fast.[35] These types of materials are ideal for certain uses in primary teeth, interim restoration in permanent teeth, long-term non-stress bearing restorations in permanent teeth as well as in atraumatic restorative technique (ART). There is much interest internationally in ART for patient populations who lack the advantage of modern dentistry.[36]

Even though it is not a technique indicated by the manufacturers, there is an interest in clinical use of adhesive systems in combination with GICs or RMGIs to increase the retention of the materials.[9,10] In 1994, White[37] suggested the association of adhesive agents to RMGI to increase dentin bond strength. In support of this suggestion, the study done by Pereira *et al*.[38] showed a superior bond strength of RMGI associated with different bonding agents. These results made this association to be more of an interest. In 2011, Zhang *et al*.[39] compared the bond strength of conventional glass ionomer (CGI) with self-etch vs. etch and rinse conventional bonding system, in which they reported higher bond strength between CGI associated with self-etching adhesive. However, this association could also have consequences such as reduction of fluoride diffusion into tooth structure because the bonding agent could act as a mechanical barrier to the fluoride ions.[10] Some past studies have demonstrated reduced fluoride release when an adhesive system was used with glass ionomer materials.[9,10,40] Wang[10] evaluated the short-time fluoride release of a resin-modified glass ionomer cement (Vitremer) coated with two different one-bottle adhesive systems (Single Bond and Prime & Bond). It was reported that all groups released fluoride in a similar pattern, with greater release in the beginning and then decreasing with time. The researcher found that the RMGI coated with either of the adhesive systems released statistically significant less fluoride compared to RMGI without the coating. Similarly, Miranda *et al.*[9] assessed the interference of hybrid layer composition (the adhesive system) with fluoride releasing restorative materials (Vitremer, Heliomolar and Z100). The adhesive system they used was ScotchBond MultiPurpose Plus. Their study showed that the use of a dental adhesive significantly decreased the fluoride release of Vitremere and reduced the fluoride release of Heliomolar to undetectable levels. Nevertheless, both Wang[10] and Castro[40] reported that fluoride release was decreased but not eliminated, which suggests a permeability of these barriers. Some studies showed that single-step, self-etching adhesives behave as permeable membranes after polymerization.[41] Sano *et al*.[42] reported a nano-leakage in the hybrid layer by examining the migration of silver nitrate into the interface between the dentin and five different dentin bonding agents. They suggested that the silver ions penetrated into demineralized dentin of the hybrid layer and fluoride ions which have similar dimensions to silver ions can also penetrate the hybrid layer. Therefore, differences in the permeability of adhesive systems depend on their formulations.[43]

Based on results from several studies, all fluoride-containing dental materials released their greatest amount of fluoride ions during the first 24 hours.[10,44,45] After this time, the amount released drops significantly but they still continued to release low amounts of fluoride for several weeks to several months.[46] After initial drop of the fluoride release, the amount that is released becomes more constant after 3-4 days.

The principles of adhesive dentistry date back to 1955 when Buonocore, using techniques of industrial bonding, suggested that acid could be used as a surface treatment before application of the resins[47]. In the late 1960s, Buonocore[48] suggested that it was the formation of resin tags that caused the principal adhesion of the resins to acid-etched enamel. This idea that resin penetrates the micro porosities of etched enamel and bonds reliably to enamel because of the micromechanical bond is well accepted today.[49] On the other hand, early attempts to bond to dentin resulted in poor bond strength due to the difference in dentin composition (more organic matrix) in comparison to enamel. However, subsequent generations of dental adhesives have dramatically improved the bond strength to dentin and sealing of dentin margins while retaining a strong bond to enamel[49]. Nakabayashi *et al.*[50] were the first to demonstrate true hybrid layer formation in acid-etch dentin.

In the past two decades, dental practitioners have become less interested in the application of etching in a separate step due to the introduction of new generations of dentin boding systems and self-etching bonding agents, which contain acidic monomer in their structure[51]. Studies have shown that less time is needed to apply self-etch systems.[52,53]

One type of bonding agent classification is according to its generation. Dental adhesives have evolved from no-dentinal etch (1st and 2nd generation) to total-etch (4th and 5th generation) to self-etch (6th and 7th generation) systems.[54] The 1st through 3rd generation of bonding systems were introduced in the 1960s to 1980s, but had poor clinical results. The 4th generation of bonding system was introduced in the early 1990s. This system is based on the total-etch technique, simultaneous etching of enamel and dentin. The bonding mechanism of fourth generation system consists of three steps: 1- condition (etch), 2- Prime, 3- bond. An example of a 4th generation bonding system is ScotchBond MultiPurpose Plus. As some practitioners think the three step bonding system is too complicated and time consuming, many manufacturers have attempted to simplify the system by combining certain steps. A common method of simplification is to combine the primer and bonding agent steps to have "one-bottle adhesive".[55] Therefore, the 5th generation bonding system is the "one-bottle adhesives" which were introduced during the mid-1990s. This system requires two steps which include conditioning (etching) the enamel and dentin prior to the application of the primer/adhesive.[55] Excite adhesive in our study is an example of the 5th generation bonding system. To simplify the bonding system steps, manufacturers have introduced single-step self-etching adhesives, which etch, prime and bond tooth surfaces simultaneously. These are

6th generation bonding systems, which are characterized by the possibility to achieve a proper bond to enamel and dentin using only one solution.[49] The 6th generation adhesive was introduced in the late 1990s and early 2000s. The separate etching step is eliminated by incorporating an acidic primer that was placed on the enamel and dentin after tooth preparation[56]. There are two variations of the 6th generation: Type I (self-etching primer and adhesive): includes two bottles, liquid 1-acidic primer, liquid 2- adhesive, and acidic primer applied to tooth, followed by adhesive. Type II (self-etching adhesive): two bottles or unit dose containing acidic primer and adhesive, a drop of each is mixed and applied to the tooth. Adper Prompt L-pop is an example of type II 6th generation adhesive.[49] Simplification of contemporary dental adhesives has occurred at the expense of an increasing incorporation of hydrophilic monomers (i.e., HEMA, BPDM, MDP, Phenyl-P).[57] Polymerized hydrophilic adhesives can behave as permeable membranes that potentially allow outward and inward fluid flow, and hydrophobic adhesives act as less permeable membranes.[58-60]

Fluoride release and uptake associated with glass ionomer systems, while useful for all young patients, are particularly advantageous for those who are highly susceptible to dental caries.[8] The goal of most clinicians when using GICs is to use a type of material that meets the requirements such as sealing the cavity, preventing further damage to tooth destruction, preventing secondary caries or arresting incipient caries, as well as having longevity in the mouth environment.[27] Different bonding generations may have different physical properties and permeabilities which could affect fluoride release from GIC differently. Most of the previous studies have evaluated the fluoride effect on RMGI associated with adhesives; therefore, not only are more studies needed to confirm those findings, but also studies are required to assess the effect of different bonding systems on fluoride release of conventional glass ionomers. In the near future as bioactive dental materials are being more introduced in the market, there will be more popularity amongst them to be used. Releasing fluoride is one of the important characteristics of these materials. However, some of these products are introduced to be used with the bonding system. Therefore, as the cariostatic property of these materials is important, higher amount of fluoride release from the fluoride releasing restorative material is considered favorable.

SPECIFIC AIMS, HYPOTHESES AND OUTCOMES

The aim of this study was to assess the effect of three different generations of bonding system on fluoride release of a conventional glass ionomer in comparison to uncoated conventional glass ionomer.

Hypothesis 1: Bonding agents will inhibit the fluoride release of the glass ionomer.

Hypothesis 2: Bonding agents from hydrophobic group (scotch Bond Multipurpose) will inhibit fluoride release more than the hydrophilic groups (L-Pop and Excite F)

Primary Outcome: The cumulative fluoride release over the span of 15 days.

Secondary Outcomes: The fluoride release at other time points, day 1, 2 and 3.

RESEARCH DESIGN AND METHODS

A) POWER CALCULATION:

A power calculation was conducted using nQuery Advisor (version 7.0). Assuming means of 49.45, 8.74, 7.71[10], and 0.34* (μg/cm^2) for the Fuji IX (F), Fuji IX and L-pop (FL), Fuji IX and Excite F (FE), and Fuji IX and Scotch bond (FS) groups, respectively, as well as a common standard deviation of 6.04 [10], a sample size of n=10 per group was adequate to obtain a Type I error rate of 5% and a power greater than 99%.

* This value assumes that the proportion of fluoride release that occurs within the first 24 hours is constant between F and FS.

B*) EXPERIMENTAL DESIGN*

Materials used in this study:

Fuji IX GP Extra: Product of GC America. Packable, fast setting conventional GI with fluoride release. This product contains a next generation glass filler, SmartGlass. Available in premeasured capsules (Figure 1).

Scotch Bond Multipurpose adhesive: Product of 3M ESPE. 4th generation adhesive system. Total etch adhesive type. It has a water-based primer (Figure 2).

Excite F adhesive: Product of Ivoclar Vivadent. A 5th generation light-activated dentin bonding agent. Excite F is ethanol based (Figure 3).

Adper Prompt L-Pop adhesive: Product of 3M ESPE. Self-etch adhesive system. It has a disposable application system. Water-based adhesive, consisting of two components which are mixed together immediately prior to use (Figure 4).

Components consist of: Liquid 1 (red blister): Methacrylated phosphoric esters, Bis-GMA, Initiators based on camphorquinone, Stabilizers.

Liquid 2 (yellow blister): Water, 2-Hydroxyethyl methacrylate (HEMA), Polyalkenoic acid, Stabilizers.

Groups:

Control group: Fuji IX GP Extra without any bonding agent (F)

Test groups:

Fuji IX GP Extra plus Scotch Bond Multipurpose adhesive (3M ESPE-4th generation) (FS)

Fuji IX GP Extra plus Excite F adhesive (Ivoclar Vivadent-5th generation) (FE)

Fuji IX GP Extra plus L-pop adhesive (3M ESPE- 6th generation) (FL)

Specimen preparation:

Forty disc-shaped acrylic molds (5mm diameter X 2 mm thickness) were prepared. The GI material (FUJI IX GP Extra) was used according to the manufacturer's instructions. Each capsule was activated and then triturated with triturator (Promix Dentsply Caulk) for 10 seconds with the fast (rabbit) speed. The GI then was dispensed in bulk through the applier (GC Fuji Capsule applier) in each disc shape preparation and pressed between two glass slides during the setting time. Fuji IX GP Extra specimens were allowed to set in their molds for a total of 2.5 minutes (based on manufacturer's instruction) before applying adhesives.(Figure 5) The specimens were assigned to 4 groups, 10 in each group: Fuji IX GP Extra without bonding (control), Fuji IX GP Extra plus bonding with Scotchbond (adhesive and primer) (test), Fuji IX GP Extra plus bonding with Excite F (test), Fuji IX GP Extra plus bonding with L-pop (test). In test groups, one coat of designated adhesives was added to GIC and was used according to the manufacturer's instructions. Each surface of the specimen was light cured for 20s, using quartz-tungsten-halogen light-curing unit (3M ESPE). In group 2 preparation, first a layer of scotchbond adhesive was coated on the GI, light cured, and followed by a layer of Scotchbond primer. Each specimen was stored in a centrifuge tube containing 4ml of artificial saliva in 37^{o}C. The artificial saliva contained the following composition (mmols/L): $CaCl_2$ (0.7), $MgCl_2.6H_2O$ (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer (20).[61] Every 24 hours for 15 days, the discs were transferred to new 4ml artificial saliva. Each specimen was removed from each tube by a shaped orthodontic wire, rinsed with air water spray and then distilled water and transferred to a new artificial saliva tube.

C) *FLUORIDE ANALYSIS AND MEASUREMENT:*

The fluoride release was measured from each group after 1, 2, 3, 4, 5, 6, 7, 14 and 15 days. A fluoride ion specific electrode probe was used for the measurement of the fluoride ions released in saliva solution connected to ion analyzer (Thermo scientific, No 0809, Beverly, MA, USA) (Figure 6). The fluoride electrode was used according to manufacturer's directions, and the electrode was calibrated with three standard fluoride solutions with different ionic concentrations (1ppm, 100 ppm and 1000 ppm F) in the very beginning and every session before measurement calibration was checked with 2 ppm fluoride concentration. The artificial saliva was exchanged 24 hours prior to each measurement. The artificial saliva was kept in the refrigerator and it was mixed for 10 minutes prior to each exchange. Measurement of fluoride was carried out by buffering the artificial saliva with TISAB II with the ratio of 1:1 ml of artificial saliva to TISAB II (Total ionic strength adjustment buffer, 940906, Orion Research, Inc, Beverly MA, USA), then the two solutions were mixed for 3 minutes before the measurement. Fluoride amount released (ppm) was divided by the area of the disc to give recorded units in $\mu gF/cm^2$ for the statistical analysis. The total surface area (both sides) of each specimen was 0.3925 cm^2.

Exchange of the samples: The artificial saliva was stored in the refrigerator and when needed for use, it was placed on the mixer for 10 minutes prior to the transfer to the sample tube. Each specimen was removed from the tube, rinsed with water spay first, then rinsed with distilled water and then transferred to a new centrifuge tube and 4 ml of artificial saliva was added to the each new tube. Before making fluoride measurements, a centrifuge tube containing 1:1 ratio of artificial saliva and TISAB II was tested to determine baseline fluoride concentration of each set of artificial saliva. The baseline concentration amount was deducted from the fluoride concentration of each sample.

pH measurement:

pH measurement was performed using pH probe before TISABII was added for Fluoride measurement (Thermo Scientific Orion Dual Star pH/ISE).

D) ***STATISTICAL ANALYSIS:***

Descriptive statistics (means, medians, standard deviations and inter quartile ranges) were computed for each group. For the primary outcome (cumulative fluoride release over the span of 15 days), as well as day 1 values, statistical significance was assessed via one-way ANOVA. Tukey's HSD was used for post-hoc tests. P-values less than 0.05 were considered statistically significant. For day 2 and day 3, the Kruskal-Wallis test was used instead of one-way ANOVA, and a p-value less than 0.05 was considered statistically significant. Kruskal-Wallis test was used instead of one-way ANOVA because of the p-value of Levene test was less than 0.05. The Mann-Whitney U test with Bonferroni correction was performed for post-hoc tests of the secondary outcome. A p-value less than 0.008 was considered statistically significant. SPSS version 22 was used in the analysis.

Results

All 40 samples were measured for their fluoride release over the span of 15 days.

Group 4, Fuji IX coated with L-pop adhesive, showed the highest amount of fluoride release among all four groups followed by the control group, non-coated Fuji IX GI. The total fluoride released (mean (SD); unit $\mu g/cm^2$) during the span of 15-day period was 74.68 (9.88), 46.86 (12.33), 39.56 (6.16) and 138.84 (11.38) for groups 1, 2, 3 and 4 respectively (Table 1). All four groups released the greatest amount of fluoride in the first 24 hours and the fluoride release decreased substantially in the subsequent days. For analyzing the primary outcome, the one-way ANOVA revealed a statistically significant difference in cumulative fluoride release ($p<0.001$) among the groups. Tukey's HSD showed statistically significant differences between all groups ($p<0.001$), except groups 2 and 3 ($p>0.05$) (Table 2).

For the secondary outcomes, the fluoride release on days 1, 2 and 3 were compared among the groups. On day one, all groups had their greatest amount of fluoride release of the 15-day period. Group 4 had the greatest fluoride release among all groups followed by group 1. Groups 2 and 3 had similar amount of fluoride release but both had lower release than groups 1 and 4. One-way ANOVA indicated a statistically significant difference among groups ($p<0.001$). Tukey's HSD found significant differences between groups 1 and 2, 1 and 3, 1 and 4, 2 and 4, and groups 3 and 4 ($p<0.001$). There was no significant difference between groups 2 and 3 ($p=1.0$).

On day 2, Group 4 had the greatest fluoride release among all groups followed by group 1. Groups 2 and 3 had similar amount of fluoride release. The Kruskal-Wallis test indicated statistically significant difference among the groups ($p<0.001$). Mann-Whitney U test revealed a statistically significant difference among all groups ($p<0.008$), except groups 2 and 3 ($p=0.165$).

On day 3, Group 4 had the greatest fluoride release among all groups followed by group 1. The Kruskal-Wallis test showed a statistically significant difference between the four groups ($p<0.001$). Through Mann-Whitney U test it was shown that there was a statistically significant difference on fluoride release between groups 1 and 3, 1 and 4, 2 and 4, and groups 3 and 4 (all $p<0.001$). However, there was no statistically significant difference between groups 1, 2 ($p=0.052$) and 2, 3 ($p=0.019$) when the Bonferroni correction was used.

Despite the differences among the groups, all groups showed a similar pattern of fluoride release. The greatest amount was released during the first 24 hours, followed by a noticeable decrease. Following this period, a constant and continuous release was shown from day 5 to day 15 (graph 1).

The pH analysis indicated lower pH level (more acidic condition) in group four in comparison to all other groups in the first 5 days, and thereafter, the pH for all groups was in the same range (pH=5.11). Average pH in day 1 was 5.1, 4.9, 4.8 and 3.3 for groups 1, 2, 3 and 4, respectively.

Discussion

Despite the fact that the use of adhesive system is not indicated by manufacturers, some dental practitioners might be using bonding to improve the retention of the Glass ionomer restorations. White[37] and Pereira et al.[38] associated the resin modified glass ionomer cement to different bonding agents and obtained superior bond strength which led this association to be of an interest in clinical use. However, this positive result could be accompanied with negative results such as reduction in fluoride release from the GI restorative material. Previous studies[9,10] showed that the bonding agent could act as a physical barrier and lower the amount of fluoride ion released. Hence, this study was conducted to evaluate the effect of three different boding agents on fluoride release of conventional GI. The importance of fluoride release from restorative materials in reducing caries progression and induction has been well established in the literature. In the near future as the bioactive restorative materials are being more introduced in the market, there will be more popularity amongst them to be used. One of the characteristics of these materials is releasing fluoride ions. However, there are some products that are introduced to be used with the bonding system. Therefore, it is important to know the effect of bonding agents on the release of fluoride.

In our study, the glass ionomer restoration specimens coated with Scotch Bond MultiPurpose (4^{th} bonding system generation) and Excite F (5^{th} bonding system generation) showed significantly lower fluoride release over the 15 day period, but the fluoride release was not eliminated. These results are comparable with the results from studies by Wang[10] and Miranda.[9]

Mixing of the glass ionomer powder and liquid generates an acid-base reaction beginning with partial dissolution of the surface of the glass particles by the acid. Fluoride ions are released from the glass particles and released into saliva and become available for uptake by the adjacent teeth. There is a tendency in the earliest stages for the GI material to uptake additional water.[62] It has been shown that ion migration within or through any material can occur in the presence of water. Therefore, the high levels of fluoride release observed in the days immediately following the restoration placement indicates the release of fluoride from the exposed glass particles at the outer surface of the restoration.[63,64] Our results corresponded with this fact, and similar to several other studies,[9,10,44] the most amount of fluoride released measured was in the first 24 hours after the restoration placement. Bonding agents can act as a physical barrier and lower the exposure surface of the restoration material when solubility of the material is highest. In the above study it was also shown that the GI restoration coated with Scotch Bond Multipurpose and Excite F had lower fluoride release compared to control group.

Differences in the permeability of adhesive systems depend on their formulations.[43] In the self-etch adhesives, particularly all-in-one adhesives, their acidic functional monomers forms highly hydrophilic interfacial structures making them more susceptible to water absorption.[65] Theoretically, due to their composition, hydrophobic dentin adhesives would have less permeability; therefore, less water absorption in comparison to hydrophilic adhesives.[66] Due to this property, 4th generation dental adhesives, such as Scotch bond Multipurpose, could impede the release of fluoride ions from the GI restoration bonded with this type of adhesive system. This result was also evident in our study. It was also evident that L-pop adhesive system (6th generation, hydrophilic) promoted a higher fluoride release, probably due to its composition and lower pH, when compared to the other bonding systems, Scotch Bond Multipurpose adhesive and Excite F, or the control group, Fuji IX GI. Fluoride improves the quality of dental tissue by being incorporated in the mineralized component of the tooth. When hydroxyapatite is changed into fluorapatite, the tooth structure is more resistant to acidic changes and caries progression. When fluoride releasing materials such as GI is placed in freshly cut enamel, the fluoride ions will penetrate the tooth structure, especially in the first 24 hours when GI has its highest fluoride release. Therefore, the higher amount of fluoride release is very beneficial. According to our results, clinicians may consider coating a fluoride releasing sealant with one of the bonding agents that reduced the fluoride release. This can be helpful in keeping the fluoride release internally rather than it being released externally to the saliva.

Evidence of fluoride release reduction by 4^{th} and 5^{th} generation bonding agents used in this study call for further investigation before indication of this association in clinical use. Even though using L-pop bonding system showed superior results, more studies are needed to confirm these findings. Further lab and clinical studies should be conducted to determine the bond strength of L-pop bonding system used with GICs as well as its effect on pulpal tissue as it creates a more acidic condition around the restoration.

Limitations of this study are:

- In-vitro studies are unable to fully simulate actual conditions of the oral cavity.
- The differences in viscosity and components between artificial saliva and natural human saliva.
- Using extracted teeth would simulate oral cavity conditions better.

CLINICAL IMPLICATION:

Cariostatic property of glass ionomer is associated with the amount of fluoride released. Therefore, in the case of using glass ionomer with adhesive system, higher amount of fluoride release from the restorative material is considered favorable. Clinicians can take into consideration the above results when using a bonding agent with fluoride releasing materials. They may want to choose a bonding agent which would not lower the beneficial effect of a fluoride releasing material.

FUTURE STUDIES:

Future studies should involve extracted teeth instead of acrylic molds to simulate better oral conditions. Clinical studies should be conducted to confirm the benefit of the association of glass ionomers and adhesive systems. Also, more studies are required to confirm our findings.

CONCLUSION

With consideration to the limitations of an in-vitro study, we conclude that L-pop bonding agent (6^{th} generation adhesive system), increased the amount of fluoride released from the glass ionomer. We also concluded that ScotchBond Multi purpose (4^{th} generation bonding system) and Excite F (5^{th} generation bonding system) acted as a barrier and reduced the release of fluoride from the glass ionomer.

Appendix A

Tables and Graphs

Table 1. Mean, Median, SD and Inter Quartile Range of fluoride release in four groups

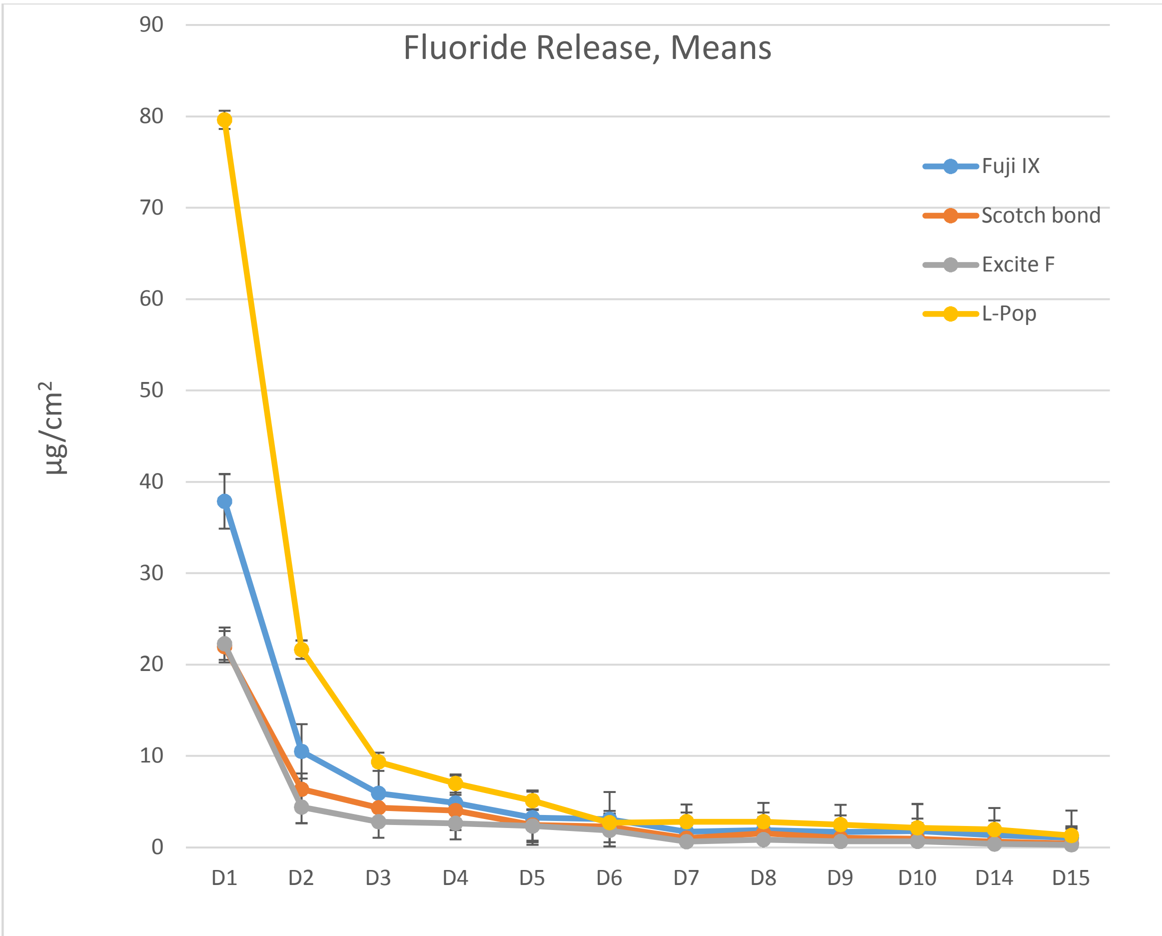
		Un-coated FUJI IX	Scotch Bond Multipurpose	Excite F	L-Pop
DAY 1	**Mean**	37.87	21.95	22.28	79.62
	Median	37.99	21.95	21.23	77.23
	SD	4.42	10.66	5.83	7.46
	IQR	6.69	19.42	10.85	12.29
DAY 2	**Mean**	10.50	6.35	4.31	21.63
	Median	9.85	5.82	3.99	20.31
	SD	2.91	3.20	0.75	3.57
	IQR	3.62	3.77	1.25	6.73
DAY 3	**Mean**	5.77	4.32	2.80	9.35
	Median	5.66	4.35	2.80	9.71
	SD	1.16	1.51	0.51	9.71
	IQR	1.69	2.93	0.83	2.06
SUM	**Mean**	74.68	46.86	39.56	138.84
	Median	75.90	49.85	38.64	138.29
	SD	9.88	12.33	6.16	11.38
	IQR	11.41	16.51	8.40	20.18

Table 2. Tukey HSD Results for cumulative fluoride released in 15-days

Group	Group	P-value *
1	2	<0.001
2	4	<0.001
3	1	<0.001
4	3	<0.001
1	4	<0.001
2	3	0.392

*** P-values ≤ 0.05 were considered statistically significant**

Graph 1. Mean fluoride release (μg/cm²) per day, per group



Appendix B

Figures

Figure. 1 Fuji IX GP Extra GI

Figure. 2 ScotchBond Multi Purpose Adhesive

Figure.3 Excite F Adhesive

Figure. 4 Prompt L-pop Adhesive

Figure 5. Specimen preparation

Figure. 6 fluoride ion specific electrode probe

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