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FLUORIDE RELEASE AND RECHARGE CAPACITY OF A GLASS HYBRID MATERIAL WITH AND WITHOUT A RESIN COAT IN PRIMARY TEETH "AN IN VITRO STUDY''

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ABSTRACT

INTRODUCTION: Fluoride-releasing restorative materials act as fluoride reservoirs delivering fluoride to nearby tooth structures and absorbing fluoride from surrounding or external sources. This trait is desirable in caries prevention and eradication.
OBJECTIVES: The aim of this study was to compare the fluoride release and recharge capability of a novel glass hybrid material (Equia Forte Fil) with and without a resin coat (Equia Forte Coat).
MATERIALS AND METHODS: The sample consisted of 68 freshly exfoliated or extracted (for orthodontic purposes) sound primary molars. Standardized buccal class V cavities were prepared in all teeth and then restored with Equia Forte Fil according to manufacturer's instructions. The samples were randomly allocated into two groups (n=34). Group I [Experimental] (n=34): teeth restored with Equia Forte Fil in addition to Equia Forte Coat. Group II[Control] (n=34): teeth restored with Equia Forte Fil only. The samples were immersed in 5ml artificial saliva and fluoride release was evaluated on the 1st, 3rd, 7th, 14th, 21st and 28th days using fluorine ion-specific electrode. After 28 days, each group was further divided into 2 subgroups (n=17). Subgroup A [Experimental (n=17): treated with Enamel Pro Varnish. Subgroup B[Control](n=17): received no fluoride treatment. The amount of fluoride re-release was measured at the same time intervals. Data was analyzed using Independent t test, Repeated measures ANOVA with post hoc Bonferroni test. R Significance level was set at $P \leq 0.05$.
RESULTS: There was a statistically significant difference ($P \leq 0.0001$) in fluoride release and recharge between the 2 groups, where Group II drastically released more fluoride. Time had a significant effect on fluoride release within subgroups IA and IIA on specific days, as the amount of fluoride progressively decreased throughout the study. As for subgroups IB and IIB data was constant.
CONCLUSIONS: The application of the nano-filled resin coat had a dramatic effect in reducing fluoride release and recharge capabilities of Equia Forte Fil.
KEYWORDS: Fluoride release, Recharge, Nano filled coat, Equia Forte Fil, fluoride varnish.
RUNNING TITLE: Fluoride release of Equia Forte Fil with coat.

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INTRODUCTION

The American Academy of Pediatric Dentistry (AAPD), in 2020, defined caries as the most common chronic childhood disease in the US (1). Dental caries is one of the most prevalent chronic diseases affecting humans worldwide. It remains a major concern in most developed countries, affecting around 60-90% of school-aged children and most adults (2). However, a recent decline in dental caries has been observed in these countries due to improved public health programs, rise in fluoride use, better preventative programs, and using newer restorative materials (2). An important characteristic of these materials is their fluoride release and recharging capability inside the oral cavity (3).

Fluoride is both a therapeutic and preventive agent that can be used to prevent caries formation, induce remineralization of partly demineralized lesions, and inhibit plaque formation (4,5).
Among the numerous fluoride- releasing restorative materials; the most popular one used in pediatric dentistry is glass ionomer cements (GICs). Fluoride compounds are incorporated into the material during the manufacturing process (2).
Glass ionomer cements have favorable adhesion to tooth structure, in addition to fluoride release over a prolonged period of time (2). However, the amount of fluoride released from dental materials decreases over time; therefore, it is necessary to intermittently recharge them with an external fluoride source, increasing fluoride re-release following topical fluoride application (6). The source of fluoride for

recharge can be obtained from low concentration daily sources like fluoride dentifrices and mouth rinses or professional topical applications (7,8).

Glass ionomer cements (GICs) are widely used in dentistry since they are biocompatible, adhere chemically and have both protective and re-mineralizing actions. Unfortunately, traditional GICs are considered temporary materials, inadequate in stress-bearing areas, mainly due to their poor physical properties as marginal leakage and poor wear resistance. This led to the development of newer fluoride releasing materials with enhanced physical properties by using optimum acid fluoroaluminosilicate glass ratio, optimum particle size and distribution (5, 7). In addition, to overcome drawbacks of GICs like moisture sensitivity, contamination, and initial desiccation, using a surface coating agent is recommended. Consequently, this led to the development of nano-filled coats. Nevertheless, their effect on fluoride release is not clear (9).

In 2007, a GIC Hybrid technology was presented based on the development of highly viscous GICs by optimizing the polyacid and improving particle size distribution (Equia, GC, Tokyo, Japan). (5,10,11)

Equia Forte Fil (GC, Tokyo, Japan), released in 2015. is a glass hybrid material with ultrafine, highly reactive glass particles with fluoride releasing property, consisting of a highly viscous conventional GIC material Equia Fil with added highly reactive smaller silicate fillers that subsequently increased the matrix cross-linkage (12) In addition, a novel nano-filled resin coat Equia Forte Coat (GC, Tokyo, Japan) (5,11). supplied with the cement, seals its surface providing protection till maturation is completed during the first months. (13,14) This coat improves abrasive wear resistance, fracture strength and marginal integrity of the cement through proper infiltration of the GIC surfaces (5,13,14). Moreover, its glaze effect results in superior esthetics. (13,14)

Equia Forte Fil has a powder and liquid component. The powder is 95% strontium fluoroaluminosilicate glass and 5% polyacrylic acid. While the liquid contains 40% aqueous polyacrylic acid. Strontium was added to substitute calcium; increasing the radiopacity of the material and enhancing fluoride release since strontium fluoride (SrF_2) is more soluble than calcium fluoride (CaF_2) (15,16).

The Equia Forte Coat is made up of 50% methyl methacrylate and 0.09% camphorquinone. This nano-filled coat is hydrophilic, has low viscosity and is light cured (13,14).

Tiwari & Nandlal (2013) (9) stated that the nano-filled coat reduces fluoride release into the oral cavity while releasing fluoride into adjacent cavity walls, creating zones of inhibition of secondary caries and helping in internal remineralization when compared to non-coated groups. **Brzović-Rajić et al (2018)** (5) confirmed that statement. However, they said that the amount of fluoride released remains sufficient for caries prevention, with the added benefit of improved mechanical properties.

Conversely, **Dasgupta et al (2018)** (7) concluded that Equia Forte Fil without addition of its coat showed the highest fluoride release and recharge potential when compared to other tested materials like Equia Fil, a giomer, a compomer, and a nanohybrid composite. Meanwhile**, Jafari et al (2019)** (11) concluded that Equia Forte Fil coated with Equia Forte Coat released less fluoride than an uncoated light cured resin reinforced GIC (Fuji II).

Because of the controversy regarding the effect of surface coating agents on fluoride release and recharging ability of GICs, this study aims to evaluate the effect of the nano-filled resin coat on fluoride release and recharge capacity of glass hybrid Equia Forte Fil. The null hypothesis of the present study was that there would not be any difference in the fluoride release and recharge capability of the Equia Forte Fil, regardless of using its nano-filled coat.

MATERIALS AND METHODS

This in-vitro study was performed at the Pediatric Dentistry and Dental Public Health Department and The Institute of Graduate Studies and Research, Environmental Studies Department, Alexandria University. The study was performed after receiving approval from the Research Ethics Committee, Faculty of Dentistry, Alexandria University. Date: 0102-12/2019 IORG 0008839.

Sample size

The estimated sample size was 34 teeth per group (total sample of 68) implementing a power of 80% and a significance level of Pvalue ≤ 0.05 (5,17) Sample size was based on Rosner's method (18) calculated by Gpower 3.0.10.

Sixty-eight (n=68) sound primary molar teeth [exfoliated or extracted (for orthodontic purposes)] were collected from the outpatient clinics of Alexandria University Hospitals, Ministry of Health Hospitals, and private clinics in Alexandria.

Sample preparation

All teeth were cleaned from blood and debris with fluoride free pumice and a low-speed handpiece, then carefully examined using a magnifying lens. They were chosen if they were free from caries or cracks and free from any developmental defects.

The teeth were then washed, the remaining roots (if present) were cut off at the cemento-enamel junction and any pulp tissue was removed. They were then stored in normal saline until the test started.

Pieces of self-adhesive labels with dimensions 3x2 mm were stuck on the middle third of the labial surface of each tooth for cavity standardization and nail polish was painted on the rest of the buccal surface. Standard non-beveled buccal Class V **(3 mm × 2 mm × 1.5 mm)** cavities were prepared in the middle third of the labial surface of each tooth (n=68) (19). A carbide bur size #330 (SS White Bure, New Jersey) and a high-speed handpiece with a water coolant system were used (19). The depth of the cavity was adjusted to 1.5 mm by inserting the entire head of the bur and was checked using a periodontal probe. A new bur was used with each 10 cavities to avoid dullness (19). A single operator prepared all the cavities. Intra-reliability test was performed to ensure that the operator was consistent.

Sample grouping

All prepared teeth were cleaned with water and gently dried after the nail polish was removed. Each tooth was given a serial number and then randomly allocated to one of two groups, according to the restorative material used, using random allocation software program. **Group I: [Experimental] (n=34)** was restored with Equia Forte Fil and Equia Forte Coat. (GC, Tokyo, Japan) **Group II: [Control]** (n=34) was restored with Equia Forte Fil only (GC, Tokyo, Japan) with no added surface coat. Figure (1)

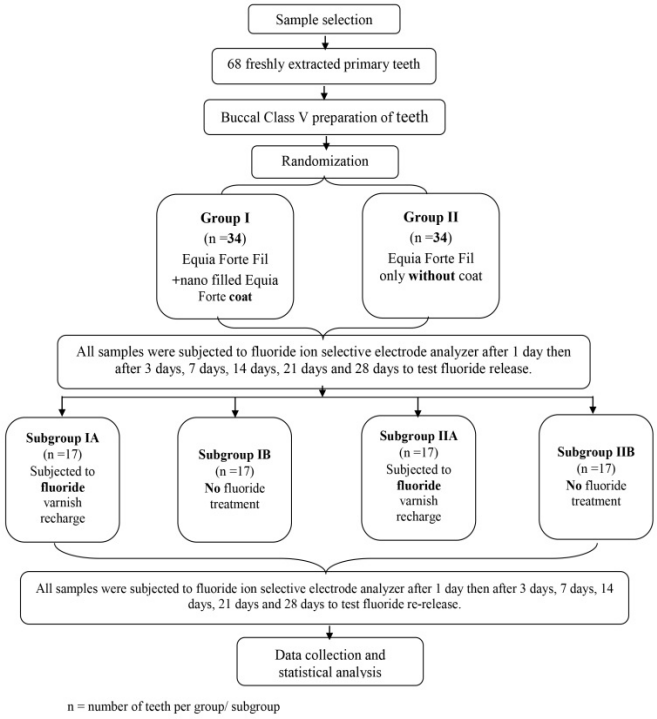

Figure 1 : Flow chart of the study design.

The restorative material was manipulated according to the manufacturer's instructions. As for Equia Forte Coat applied to Group I samples, it was applied to the surface of the restoration using a microbrush without air drying, then light cured (Woodpecker Dte Lux Dental Blue LED Light Cure machine) for 20 seconds, following manufacturer's instructions (5,11). Furthermore, finishing and polishing of the samples was avoided to prevent changes in the materials' surface area (3).

After the restorative procedure was complete, each tooth was placed in 5ml of prepared artificial saliva in labeled plastic vials and stored inside an incubator (VEB MLW Dental FabrikTeffurt, BST5020, Germany) for 24 hours, in a moist environment at 95% humidity and 37^{0}C, to simulate the oral environment (5).

1. Fluoride release analysis

Fluoride ions release was detected in artificial saliva at 1, 3, 7, 14, 21 and 28 days, using fluoride Ion Specific Electrode (ISE) (Fluoride electrode model 94-09BN, Orion Research Inc. products gp.529 Main st Boston MA 02129 USA). Prior to any measurements, the fluoride electrode was calibrated using standard fluoride solution of 0.1,1,10, 100 and 1000 (ppm) concentrations, prepared from 1000ppm standard sodium fluoride solution.

Each collected artificial saliva sample was buffered with equal volume of Total Ionic Strength Adjustment Buffer (TISAB III) (Orion, Thermo Fisher Scientific) solution [ratio 1:1] to control pH of immersion solutions and prevent formation of fluoride complexes. It also frees fluoride ions bound to hydrogen, allowing an accurate measurement of the total fluorine content (6).

Amount of fluoride release was measured using fluorine ion-specific electrode (6). The fluoride release concentrations were automatically displayed on the analyzer as millivoltage (mV) readings (20, 21) Millivoltage readings were then entered into the computer using EXCEL software that mathematically established the part per million (ppm) values through the fluoride slope curve of the standard fluoride solution concentration.

After obtaining fluoride concentration readings, each sample was thoroughly rinsed with deionized water, dried with absorbent paper, and then returned to a clean plastic vial filled with 5ml of fresh artificial saliva and stored in the incubator.

Sample subgrouping

Topical fluoride exposure protocol: 28 days after initial fluoride release, samples from each group were subdivided into 2 subgroups, each had 17 samples.

Subgroup A [Experimental](n=17): in which the specimens were treated by fluoride varnish [Enamel Pro Fluoride varnish (Premier) 5% sodium fluoride (NaF) with Amorphous Calcium Phosphate (ACP)], applied for 4 minutes then washed with copious artificial saliva for 10 seconds and dried with absorbent paper.

Subgroup B [Control](n=17): [No topical fluoride treatment was applied]. Each sample was stored in a labeled plastic vial containing 5ml of fresh artificial saliva and placed in the incubator at 37°C for 24 hours (6).

2. Fluoride re-release analysis

Artificial saliva was analyzed for fluoride re-release on days 1, 3, 7, 14, 21 and 28, using fluoride ISE as previously described (6).

3. Fluoride recharge capacity

Recharge capacity was calculated as the difference in fluoride release between the two subgroups (22).

Statistical analysis

Data was analyzed using IBM SPSS (Statistical Package for Social Science) statistical software (version 25). The collective quantitative data was tested for normality, which was confirmed using Shapiro Wilk test. Descriptive statistics were summarized using mean and standard deviation (SD). Independent t test was used for intergroup comparisons. Repeated measures ANOVA with post hoc Bonferroni test for intragroup was used to compare fluoride release and recharge among the groups, with a P value ≤0.05

RESULTS

1-Fluoride release results

Independent t test was used in comparing fluoride release between group I (Equia Forte Fil +Equia Forte Coat) and group II (Equia Forte Fil only) at different time intervals; days 1, 3, 7, 14, 21 and 28.

Table (1) indicated that there was a statistically significant difference between the 2 groups during the 6-time intervals (P=<0.0001*).

Table 1: Fluoride release in ppm between group I and group II

Time points	Group I (n=34)	Group II (n=34)	P value
	Mean (SD)		
Day 1	1.555 (0.836)[a]	4.802 (1.132)[a]	<0.0001*
Day 3	0.699 (0.270)[b]	2.331 (0.607)[b]	<0.0001*
Day 7	0.270 (0.097)[c]	1.228 (0.541)[c]	<0.0001*
Day 14	0.189 (0.048)[d]	0.760 (0.279)[d]	<0.0001*
Day 21	0.194 (0.0348)[e,d]	0.466 (0.1450)[e]	<0.0001*
Day 28	0.001 (0.0004)[f]	0.006 (0.003)[f]	<0.0001*
P value	<0.0001*	<0.0001*	

*Statistically significant difference at p value≤ 0.05

n = number of teeth per group
Lower case letters indicate statistically significance difference within groups
The amount of fluoride release on day 1 was 1.555±0.836 ppm for group I and 4.802±1.132 ppm for group II. This amount decreased over the 28 days as shown in table (1), reaching 0.001±0.0004ppm on day 28 in group I. It only increased slightly on day 21 to 0.194± 0.0348 and then continued to decline.
Whereas group II continued to display a decrease in the amount of fluoride released throughout the entire duration, reaching an average of 0.006±0.003 ppm on day 28.
Both groups followed similar fluoride release patterns, except on day 21, where there was a slight increase in Group I.
Group II released more fluoride on all days when compared to group I. Uncoated Equia Forte Fil released more fluoride than the coated samples. Figure (2)

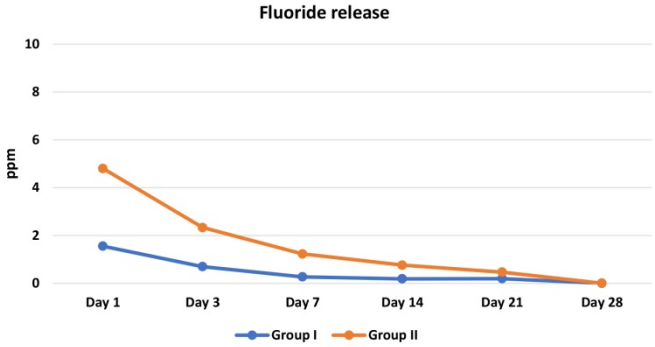

Figure 2: Fluoride release in ppm between group I and group II.

Repeated measures ANOVA with post hoc Bonferroni test for intragroup was used to compare fluoride release concentrations in artificial saliva after teeth were restored with Equia Forte Fil +Equia Forte Coat (group I) in different time intervals: 24 hours, 3 days, 7 days, 14 days , 21 days and 28 days, indicated that there was a statistically significant difference when comparing the fluoride released (P<0.0001*) after 24 days, 3 days, 14 days , 21 days and 28 days, with the exception of comparisons between day 14 to day 21 where no statistically significant difference was observed.
Fluoride release after 24 hours was 1.555±0.836 ppm and declined till it reached 0.001±0.0004ppm on day 28.
The highest fluoride release was on day 1, followed by a sharp decline after day 3 and continued to decrease till day 21 where there was a slight increase, then continued to decrease again till day 28.
As for group II (Equia Forte Fil only), there was statistically significant difference within the group throughout the different time intervals (P<0.0001*) after 24 hours, 3 days, 7 days, 14 days, 21 days and 28 days.
Both groups followed similar fluoride release patterns except on day 21 where there was a slight increase in Group I.

2-Fluoride re-release results

As for subgroups IB (Equia Forte Fil+ Equia Forte coat) and subgroup IIB [(Equia Forte Fil only) with no fluoride recharge, the data remained constant over the entire 28-day duration.
Table (2) shows a comparison between subgroups **IA** (Equia Forte Fil + coat) and **IIA** (Equia Forte Fil only), where both groups received an external fluoride source.

Table 2: Fluoride recharge in ppm between subgroup IA and subgroup IIA

Time points	Subgroup IA (n=17)	Subgroup IIA (n=17)	P value
	Mean (SD)		
Day 1	0.043 (0.019)[a]	0.733 (0.409)[a]	<0.0001*
Day 3	0.032 (0.022)[a,b]	0.560 (0.239)[a,b]	<0.0001*
Day 7	0.022 (0.013)[b]	0.457 (0.318)[b]	<0.0001*
Day 14	0.018 (0.006)[b]	0.253 (0.125)[c]	<0.0001*
Day 21	0.009 (0.004)[c]	0.073 (0.029)[d]	<0.0001*
Day 28	0.005 (0.001)[c]	0.071 (0.012)[d]	<0.0001*
P value	<0.0001*	<0.0001*	

*Statistically significant difference at p value≤ 0.05
n = number of teeth per subgroup
Lower case letters indicate statistically significance difference within groups

Independent t test showed that there was significant fluoride release from subgroup IIA over the 28-day period, after it received fluoride recharge by varnish, when compared to subgroup IA; thus, there was a significant difference in recharge between the 2 subgroups with P<0.0001* on days 1, 3, 7,14, 21 and 28. Figure (3)

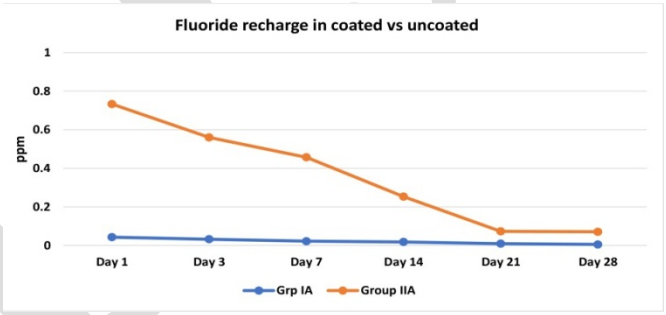

Figure 3: Fluoride recharge in ppm between subgroup IA and group IIA.

Repeated measures ANOVA with post hoc Bonferroni test for **subgroup IA** showed:
Fluoride release after 24 hours was 0.043 ± 0.019 ppm and started to decline till it reached 0.005±0.001 ppm on day 28.
Fluoride release after 24 hours was significantly different form days 7, 14, 21 and 28 (P<0.0001*), while it was not significantly different to day 3.
Fluoride release after 3 days was significantly different from days 21 and 28 (P<0.0001*), while it was not significantly different to days 7 and 14.
Fluoride release after 7 days was significantly different from days 21 and 28 (P<0.0001*), but not significantly different from day 14. Release after 14 days was significantly different from days 21 and 28 (P<0.0001*). Whereas fluoride release after 21 days was not significantly different from day 28.
For **subgroup IIA**: After recharge, fluoride re-release increased then started to decline from 0.733±0.409 ppm on day 1 to 0.071±0.012 pmm (near pre-recharge levels) after 28 days.
Fluoride release after 24 hours was significantly different from days 7, 14, 21 and 28 (P<0.0001*), while it was not significantly different from day 3.

Fluoride release after 3 days was significantly different from days 14, 21 and 28 (P<0.0001*) with no significant difference from day 7.
Fluoride release after 7 days was significantly different from days 14, 21 and 28 (P<0.0001*). And release after 14 days was significantly different to days 21 and 28 (P<0.0001*). Fluoride release after 21 days was not significantly different to day 28.
The fluoride recharge in subgroup IIA declined from 0.733±0.409 ppm on day 1, reaching 0.071±0.012pmm after 28 days.
While recharging, samples not coated with Equia Forte coat were able to uptake and release more fluoride than the coated group samples and followed the same fluoride release pattern before recharge.
Subgroup IIA was able to absorb and release more fluoride than subgroup IA, indicating that the presence of Equia Forte Coat may hinder fluoride release and recharge capacity of Equia Forte Fil.

DISCUSSION

The results of this study showed that Equia Forte Coat hindered Equia Forte Fil's ability to release fluoride and its capacity for recharge by external fluoride sources, consequently influencing the amount of fluoride re-released following recharge.
Equia Forte System is a glass hybrid material, which symbolizes the latest revolution in GICs and resin technologies with packable Equia Forte Fil and nano filled Equia Forte Coat working together (11). Equia Forte Fil consists of ultrafine, highly reactive glass particles, scattered within the conventional GIC structure (7). It is supplied as capsules to avoid manipulative errors.
Equia Forte Coat is a light cured surface coat, containing a low viscosity methyl methacrylate monomer, phosphoric acid and a photoinitiator. It includes single phase dispersed nanofillers giving it a 'micro-lamination effect' with uniform flow and complete wetting of the cement surface; thus, offering a thick protective coat (5,23). This final lamination provides a smooth glossy surface reducing tendency for bacterial adherence, optimizing physical properties and protects the GIC restorations. Moreover, the coat guards against water contamination or drying out during initial cure and provides a dispersion hardened surface. It bonds properly to both tooth and cement surfaces and fills voids effectively sealing GIC surface and dispersing mechanical stresses. In addition, the dispersion of nanofillers reinforces the outer layer, increasing its abrasive wear resistance and guards it against acid erosion (5,11,23). Meanwhile, **Hattab et al**, stated that surface coating agents interfere with microleakage, reducing fluoride release from GIC significantly in both deionized water and artificial saliva (24).
The storage medium used in this study was artificial saliva, to simulate the oral environment, even though the rate and amount of fluoride release in artificial saliva is substantially less when compared to deionized water (20,25).
Ion specific electrode with an ion-analyzer is the most frequently employed technique for measuring fluoride release (8) and was used in this study because it is simple, specific, inexpensive, rapid, and does not necessitate the use of complex laboratory equipment. Additionally, it gives a precise and direct estimate of the free fluoride ions available in solution (7,21).
Fluoride release from GIC takes places through 3 mechanisms: Surface wash out, diffusion through cracks and pores and bulk diffusion (3).
From the results of the present study, we observed that Group II released significantly more fluoride than Group I, however, both groups followed similar fluoride release patterns. Over a 28-day period, fluoride release was initially high and at its highest after 24 hours. Fluoride levels sharply declined by the end of day 3 then continued to progressively decrease until sustained at a lower level till day 28. This was in accordance with previous studies (3,7,8,11). However, on day 21, Group I witnessed a slight increase in fluoride release before continuing to decline till day 28.
Initial fluoride release from GIC is due to an acid-base reaction. Throughout the setting reaction, the polyacrylic acid attacks the glass fillers' surfaces leading to rapid dissolution and release of significantly large amounts of fluoride ions into the surrounding. Furthermore, the ultrafine fillers increase the surface area for the acid–base reaction to occur, enhancing fluoride release, leading to high amounts of release in the first 24 hours. This is called "burst effect phenomenon" and is induced by surface wash out. Moreover, the amount of fluoride released is proportional to the concentration of fluoride ions in the material (3,6-8,11).
The initial burst effect of GICs is crucial for its effect in caries prevention and antibacterial effect. Whereas sustained release of fluoride increases tooth resistance to new carious attacks (2,3,5). Regrettably, the low levels of fluoride release that follow the initial burst release could be insufficient to prevent secondary caries. Consequently, the fluoride recharge and re-release capability of a material has become a vital feature, especially in high caries risk patients (3).
The reduction of the fluoride levels in the subsequent days might be caused by slower particles' dissolution and release through the materials' pores and fractures (3,6,8,11). This happens because fluoride ions do not react chemically during the setting reaction, and since they remain unreacted, they can diffuse down their concentration gradient and become released into the oral environment or be taken up by the glass ionomer if it is subjected to solutions with higher fluoride concentration (5).
Meanwhile, the re-increase observed in day 21 after the drop could be related to the bulk diffusion that takes place during the maturation phase due to contact between GIC and storage medium (3,24).
The present study showed that the non-coated group released significantly more fluoride than the surface coated group in all time periods. These results agreed with previous studies that reported a 60-76% reduction in fluoride release after coating of GICs. It was hinted that the coats prevented the dissolution of the superficial layer of immature GIC, that if left uncoated, is more likely to dissolve and erode quicker (5) Equia Forte Coat occludes the superficial rinse and diffusion through pores mechanisms; consequently, impeding fluoride release (2,3,5,9).

The pattern of fluoride release from the coated group was gradual for 1st week and then decreased to a steady level over the next 2 weeks. This was consistent with other studies (2,9).

The cariostatic effect of fluoride-releasing materials was linked to a sustained release of fluoride. However, fluoride ions leaching out of materials decrease over time and usually declines sharply after 3 days, therefore it is necessary to intermittently replenish fluoride levels in the restorative materials, allowing them to act as an intraoral fluoride reservoir, providing regulated slow fluoride release for sites at risk for recurrent caries. Fluoride release increased considerably after topical fluoride application and recharge profile was similar to release (6,7).

Professionally applied fluoride varnish is a commonly used in pediatric dentistry for caries prevention (26). Enamel Pro varnish was used to recharge the tested samples to mimic the clinical situation. The fluoride re-release that happens instantly after recharge is caused by the superficial effect of topical fluoride, while the release during the following days is attributed to the ability of fluoride to diffuse through the material's pores and to be stored for later re-release (27).

After exposure to external fluoride varnish, fluoride re-release from the tested samples increased. This could be explained by the fact that GICs can uptake fluoride from external sources with high concentrations of fluoride (11).

In this study, subgroups IA and IIA showed the ability to be recharged with fluoride varnish and re-release fluoride ions once again. The highest values were recorded at the end of the 1st day after recharge, which sharply declined by the end of the 3rd day and then continued to gradually decrease reaching near pre-exposure levels by the end of the 28th day. Subgroup IIA was able to take up and re-release more fluoride ions than subgroup IA, indicating that the presence of Equia Forte Coat hinders fluoride release and recharge. These results were in accordance with previous studies (3,7).

The recharging potential of GICs and their re-release of fluoride ions relies greatly on the hydrogel layer structure surrounding the glass particles. Equia Forte Fil has a thick hydrogel matrix phase allowing for better fluoride uptake and re-release. Additionally, the porosity and permeability of GIC are vital for the recharging ability of the material (3,7).

The low fluoride re-release of coated Equia Forte Fil could be explained by the saturation effect theorized by **Freedman and Diefinderfer (2003)** (3), as GICs can no longer take up more fluoride after a certain threshold. This was confirmed by the low fluoride release of the coated samples before recharge.

Arbabzadeh-Zavareh *et al* (2012) (3,5) stated the amount of fluoride release from a GIC material on day 60 was considered the base measurement after the material's exhaustion.

Meanwhile, in this study, the control subgroups IB and IIB that were not replenished by the varnish showed undetected levels of fluoride ions after one month. This may be due to differences in methodology between different studies such as using Teflon mold discs instead of class V cavities, providing larger surface area for fluoride recharge and thus more re-release. However, here, some of the recharged fluoride ions were up taken by the tooth itself. In this study, we chose teeth to better simulate the oral condition. Class V preparation was selected to standardize the cavity size in all teeth as both upper and lower Ds and Es were used, so Class I or II cavity sizes and outlines would have varied between different types and sizes of teeth, also to allow for easier reproducibility.

Furthermore, the amount and type of storage media also had an effect on amount of fluoride release. For instance, deionized water has no existing ions and can give an accurate estimate of fluoride release from a material (7,11), yet it would not accurately reflect the oral condition (6). Moreover, the frequency of changing the storage medium is also influential; if the samples are surrounded by storage media saturated by fluoride ions, they are less likely to promote passive diffusion of fluoride ions down the concentration gradient. Likewise, the pH and temperature of the storage medium should also be considered.

The results of this study suggested that materials with high fluoride release have a high recharging capability, which was in accordance with the findings of other investigators (3,5).

Even though the application of surface coat to GIC is necessary to guard against moisture contamination, desiccation and provide sufficient seal, it has a dramatic reducing effect on fluoride release, before or after recharging. The results showed a significant difference in the fluoride release/recharge ability between the two groups with and without applied coats. Therefore, it can be concluded that the nano filled coat inhibits fluoride release from the GIC material when compared to control samples, but the quantities may be sufficient for caries protection, bearing in mind the advantageous effects of Equia Forte Coat on Equia Forte Fil's mechanical properties. Additionally, most of the fluoride release from the material will be directed towards the walls and floors of the cavity in which the material was placed.

A possible limitation of this study was that the research was in-vitro, where fluoride release was measured in samples immersed in a static medium that cannot replicate the dynamic nature of the oral environment such as salivary flow characteristics, presence of plaque, difference in the temperature and pH, occlusal loading and the oral hygiene and dietary habits utilized by the patient. However, simulation of these conditions could give valuable information. Therefore, it is recommended that further investigations be performed to confirm these findings and to study the valuable remineralizing effects of using Equia Forte Fil as a restoration using microradiography and confocal microscopy.

Within limitations of this study and based on the previous data, the null hypothesis can be rejected as there is a statistically significant difference in both fluoride release and recharge capacities of Equia Forte Fil, with and without the addition of Equia Forte Coat

CONCLUSION

1- Equia Forte Fil had the capacity of fluoride release and recharge. However, this capacity was reduced significantly when Equia Coat was added. There was significant difference between both groups with or without Equia coat in both fluoride release and recharge.

2- Time had a significant effect on the fluoride release within each group on specific days as the amount of fluoride progressively decreased throughout the study. It also had a significant effect on fluoride re-release within subgroups IA and IIA, as they followed the same pattern of progressive decline.

CONFLICT OF INTEREST
The authors declare that there was no conflict of interests.
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