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Evaluation of Flexural Strength, Micro-Tensile Bond Strength and Fluoride Release of a Glass Hybrid Restorative Material

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Abstract
The aim of the study was to evaluate flexural strength (FS), micro-tensile bond strength (μTBS) and fluoride release (FR) of a newly introduced glass-ionomer cement based on glass-hybrid technology (EQUIA Forte Fil) compared with conventional cement (Fuji IX GP). Flexural strength was determined by a Universal Testing Machine using three-point bending test. Micro-tensile bond strength to sound dentin was measured using the Universal Testing Machine. Fluoride release was evaluated at 1,3,5 and 7 days in distilled water using an ion specific electrode connected to an ion analyzer. Data were statistically analysed and compared using unpaired Student's t-test ($\alpha = 0.05$). Flexural strength results showed that EQUIA Forte exhibited insignificantly higher values compared with Fuji IX. EQUIA Forte exhibited significantly higher μTBS than Fuji IX. Fluoride release results showed that after 24 h from storage there was no significant difference between the two cements, while a significant difference was noted on the other incubation periods. EQUIA Forte showed better mechanical properties, good bond strength with sound dentin and higher FR compared with the conventional cement, which could be a promising step in the development of GIC.

Keywords
Flexural strength, Bond strength, Fluoride release, Glass ionomer cements

INTRODUCTION

Glass ionomer cements (GIC) were developed by Wilson and Kent in 1972 (Wilson & Kent, 1972). Their application in clinical dentistry has been driven by several properties such as fluoride release and chemical adhesion to tooth. In addition, they have negligible setting shrinkage, and coefficient of thermal expansion close to tooth and low creep. However, they are vulnerable to early exposure to moisture due to the slow setting characteristics. In the past two decades, dental materials scientists have worked diligently to produce glass-ionomer cement systems that overcome the three chief drawbacks of the conventional GIC; difficult handling properties, low wear resistance, and low compressive strength. High powder/liquid ratio conventional GICs were introduced, alternatively termed packable or high viscosity GICs. The success of this formulation encouraged manufacturers to develop fast-set GICs with better physical properties (Fagundes et al., 2006). High viscosity glass ionomers are also a good alternative for clinical application since it is reported that their physical properties are improved, condensable, at the same time provided better esthetics due to smaller particle sizes and have fast setting reaction (Markovic et al., 2008). The enhancement of the chemical formula incorporated reduction in the size of the glass fillers have led to development of glass hybrids (GHs) (Al-Abdi et al., 2017). These are reinforced glass ionomers, with a second, smaller, and more reactive silicate particle and higher-molecular-weight acrylic acid molecules, which supposedly increase matrix cross-linking. This innovative glass hybrid technology is supposed to increase the ion availability and builds a much stronger matrix structure with greater physical properties, wear resistance and fluoride release (Pitel, 2017).

Therefore, the aim of this in-vitro study was to evaluate the newly introduced glass-ionomer cement based on glass hybrid technology comparing it with a conventional one, regarding; flexural strength, micro-tensile bond strength to sound dentin and fluoride release.

MATERIALS AND METHODS

Two types of glass ionomer restorative cements were used in this study: glass hybrid-based (EQUIA Forte Fil) and a conventional one (Fuji IX GP). For each test, ten specimens were prepared regarding the type of studied cements. Each material was manipulated according to the manufacturer's instructions described in Table1.

Table 1 Materials used in the study

Materials	Composition	Method of application
EQUIA Forte Fil (Glass hybrid-based GIC)	Powder: 95% strontium fluoro-alumino-silicate glass, including the newly added highly reactive small particles, and 5% polyacrylic acid powder. Liquid: 40% polyacrylic acid, 50% distilled water and 10% polybasic carboxylic acid.	Capsule mixed for 10 secs, applied to denin surface or mold and left for 20 mins for complete setting.
Fuji IX GP (Conventional GIC)	Powder: alumino-fluoro-silicate glass. Liquid: polycarboxylic acid, tartaric acid and water.	Capsule mixed for 10 se cs, applied to **denin surface or** mold and left for 20 mins for complete setting.
EQUIA Forte Coat (Light-cured, low-viscosity nanofilled surface coating resin)	50% Methyl methacrylate, colloidal silica, 0.09% camphorquinone.	Applied using a micro-brush on the surface and then immediately light cured for 20 secs
Fuji Varnish (Resin Protective coating)	Isopropyl acetate, acetone, cornmint oil and cinnamaldehyde.	Applied using a micro-brush on surface
Cavity Conditioner	77% distilled water, 20% polyacrylic acid, 3% aluminumchloride hydrate.	Applied on dentin surface for 20 secs and then rinsed

I. Evaluation of Flexural Strength

A total number of twenty bar-shaped specimens have been prepared in a custom made split-Teflon rectangular mold (20 mm length, 2.5 mm width and 2.5 mm thickness) (Arita et al., 2003). The specimens have been divided into two groups each of ten specimens according to the type of the cement used. The encapsulated GIC material were prepared according to the manufacturer instructions. After activation and mixing of the capsule of each type of GIC for 10 sec in the amalgamator, the mixed cement was applied to the mold using the cement applier. After 20 min, the specimen was removed from the mold. All the specimens were wet polished in sequence using #600, #800, #1000 and #1200-grit silicon carbide sandpapers (Fuji Star, Sankyo, Japan) to remove any excess material. After polishing, all Fuji IX specimens were coated from all sides by a layer of varnish (GC Fuji Varnish), while EQUIA Forte fill specimens were coated from all sides by (EQUIA Forte Coat). The specimens were stored in distilled water at 37º C for 24 hours (Arita et al., 2003).

Three-point bending testing

Before testing, the dimensions of each specimen were measured to check its accuracy using a graded micrometer screw gauge (Mitutoyo, Kawasaki, Japan)**.** Each specimen was loaded in a three-point bending test using a Universal Testing Machine (Autograph AGS-H, Shimadzu, Japan), running at a crosshead speed of 1 mm/min until fracture. The readings were recorded with the help Bluehill 2 modular software (Instron) (Khoroushi & Keshani, 2013), and flexural strength (FS) was calculated using the following formula:

$$FS = \frac{3PL}{2bd^2}$$

where **P** is the load at fracture, **L** is the distance between the two supports (10 mm), **b** is of the width and **d** is the depth of the specimen (A. Moshaverinia et al., 2010).

II. Evaluation of Microtensile Bond Strength (μTBS)

Teeth selection and dentin preparation

Ten freshly extracted caries-free human permanent molars (extracted after approval no. D2013-022 by the Ethical committee of Tokyo Medical and Dental University) were washed with 10% buffered formalin solution and cleaned with periodontal curettes and stored in deionized water at 3-4° C. Afterwards, the teeth were then classified into two main groups each of five teeth according to the type of cement used. The occlusal dentin surface was exposed by cutting perpendicular to the long axis of the tooth using a low-speed diamond disc (Isomet, Buchler, USA) under water irrigation. The prepared dentin surface was examined using a stereomicroscope (Olympus model SZ-PT, Tokyo, Japan) at x40 magnification to detect any remaining enamel. The teeth were hand polished using #600 silicon carbide sandpapers under running water to expose a flat dentin surface with standardized smear layer (Saad et al., 2017). Each tooth was embedded in self-cured acrylic resin (Acrostone, Anglo-Egyptian Company, Cairo, Egypt) in plastic cylinders with the flat dentin surface exposed.

Specimen preparation

The exposed dentin surface was washed with deionized water, dried with compressed air, conditioned by dentin conditioner (Cavity Conditioner, GC, Corp, Tokyo, Japan) for 20 secs according to the manufacturer instructions, then rinsed with water, and dried by cotton pellet to avoid desiccation of the dentin surface. The materials were prepared according to the manufacturer instructions, the encapsulated cement was applied to the prepared dentin surface through a customized Teflon cylindrical mold (5-mm height and 6-mm diameter) (Saad et al., 2017). The specimens were left until complete hardening, afterwards Fuji IX GIC group was coated by a layer of GC Fuji varnish, while specimens of EQUIA Forte Fil GIC group were coated by EQUIA Forte Coat (Schwendicke et al., 2017). The specimens were stored in distilled water at 37^{o}C for 24 hours. Serial sectioning was done in bucco-lingual direction then rotated 90^{o} clockwise and sectioned in mesio-distal direction under water cooling. A final horizontal cut at the level of the cemento-enamel junction was done to obtain beams with a cross sectional area of approximately 1 mm^{2}.

Measurement of μTBS

Total number of 50 beams (five from each tooth) were used for this test. The exact dimension of each beam was measured individually using a graded micrometer screw gauge (Mitutoyo, Kawasaki Japan). Each beam was fixed using a jig to mount beams onto the Universal Testing Machine (Instron model 3345 England). Each beam was aligned in the central groove of the jig and glued in place by its ends using cyanoacrylate-based glue (Zapit, DVA Inc, USA). Before application of the cyanoacrylate, a layer of Vaseline to prevent cyanoacrylate intervention at the interface during its application coated the bonding interface of each beam. The jig was then mounted into the Universal Testing Machine. A tensile force was applied to each beam at a crosshead speed of 1 mm per min until bond failure occurs. The data were recorded using a computer software BlueHill (Instron) (Saad et al., 2017). Micro tensile bond strength was derived by dividing the imposed force at the time of fracture by the bonded area in mm^{2} and was expressed in MPa as shown in the following formula (El Zohairy et al., 2004): **μTBS= F/A**

Where **F** is the force at failure and **A** is the cross-sectional area of each beam.

Failure mode examination

After debonding, specimen fragments were carefully removed from the jig with a scalpel and stored in their corresponding labelled plastic cones until examination of failure mode. The fractured sites of two representative debonded sticks from each tooth were observed using a stereomicroscope (Olympus model SZ-PT, Tokyo, Japan) at x40 magnification to identify the mode of failure. The failure mode was classified as either cohesive within the GIC or dentin, adhesive between GIC and dentin or mixed (a combination of adhesive failure along the dentin surface and cohesive failure in the GIC or dentin) (Choi et al., 2006).

Representative specimens were prepared for evaluation of the mode of failure under Scanning Electron Microscope (SEM) (JSM-6510LV, Jeol, Tokyo, Japan) at x2000 magnification. It was done at the electron microscope unit, Faculty of Agriculture, Mansoura University. The specimens were cleaned with 96% ethanol in an ultrasonic bath for two minutes and then air-dried. Afterwards, specimens were mounted on metallic stubs, gold sputter-coated (SPIMODULETM, SPI Supplies, USA), and evaluated under a SEM to recognize the pattern of failure.

III. Evaluation of Fluoride Release

Specimens preparation

A total number of twenty cylindrical specimens were prepared using split Teflon cylindrical molds each with dimensions of 8 mm diameter and 3 mm thickness (Mousavinasab & Meyers, 2009). The specimens have been divided into two main groups each of ten specimens according to the type of cement used. The encapsulated GIC material were prepared according to the manufacturer instructions. After activation and mixing of the capsule of each type of GIC for 10 sec in the amalgamator, the mixed cement was applied to the mold using the cement applier. After overfilling the mold, the material was covered from the top surface by a glass plate to condense the material in the mold and the material was

allowed to harden for 20 min. After hardening, the specimens were removed from the mold and transferred for 1 h to a wet atmosphere by using cotton gauge (Mousavinasab & Meyers, 2009). Each sample was then immersed separately in a polyethylene tube containing 7 mL of deionized water. The specimens were stored in an incubator with a constant temperature of 37°C.

Measurement of released fluoride

After different incubation times (24 hr, 3 days, 5 days and 1 week), the specimens were rinsed with 1 ml of deionized water and transferred to a new 7 ml of deionized water. The released fluoride concentration was measured for each period using an ionmeter (F-53, Horiba, Kyoto, Japan) attached to fluoride specific electrodes and reference electrodes (2060A and 8010, respectively Horiba). The ionometer was calibrated using five standard fluoride solutions containing 1,5,10, 20 and 50 ppm (Ei et al., 2018). Before measurement, 0.1 ml of (Total Ionic Strength Adjustment Buffer) TISAB II (Orion Research, Cambridge, MS, USA) (Orion Research, Cambridge, MS, USA) was added to each solution to a 10% concentration to provide constant background ionic strength, decomplex fluoride and adjust pH (Ei et al., 2018; Mousavinasab & Meyers, 2009). The fluoride electrode readings for each specimen solution were recorded and converted to ppm concentration. The final results were reported as released fluoride amount ($\mu g/cm^2$), using the following equation (Mousavinasab & Meyers, 2009):

$$\mu g/cm^2 = \frac{ppm}{Ax\ ML}$$

where **ppm** (particle per million) is the fluoride value produced by each specimen, **A** is the surface area of each specimen (1.76 cm^2), and **ML** is the amount of storage solution (7 ml).

IV. Statistical Analysis

Means and standard deviations of flexural strength, microtensile bond strength and fluoride release were calculated for each of the studied cements. The results of each test were statistically analyzed and compared using unpaired student t test ($\alpha = 0.05$). All statistical analyses were performed using software (SPSS V21.0; IBM Japan, Inc). Shapiro-Wilk test was used to diagnose the normal distribution of data. The data showed normal distribution and scores were described as mean and SD.

RESULTS

I. Flexural Strength

Table 2 shows Means and standard deviations of flexural strength (MPa) for EQUIA Forte Fill and Fuji IX restorative materials. These values were 21 ± 7 for EQUIA Forte Fil and 16 ± 4 for Fuji IX. The results of the Student's t-test showed that there was no statistically significant difference in flexural strength ($P > 0.05$) between the two studied restorative cements.

Table 2 Means, standard deviations and results of t-test for flexural strength (MPa) and micro-tensile bond strength test (MPa) of the studied restorative materials

	Material	Mean (MPa)	SD	t-value	P-value
Flexural strength (MPa)	EQUIA Forte	21	7	1.862	0.083
	Fuji IX	16	4		
Micro-tensile bond strength test (MPa)	EQUIA Forte	16.6	3.5	4.999	0.000*
	Fuji IX	10.4	2.2		

* P-value < 0.05 is considered statistically significant

II. Micro-Tensile Bond Strength

Figure 1 represents the means and standard deviations of micro-tensile bond strength values (MPa) for each of the studied cements. The results of t-test showed a statistically significant difference in micro-tensile bond strength ($P < 0.05$) between EQUIA Forte (16.6 ± 3.5) and Fuji IX (10.4 ± 2.2).

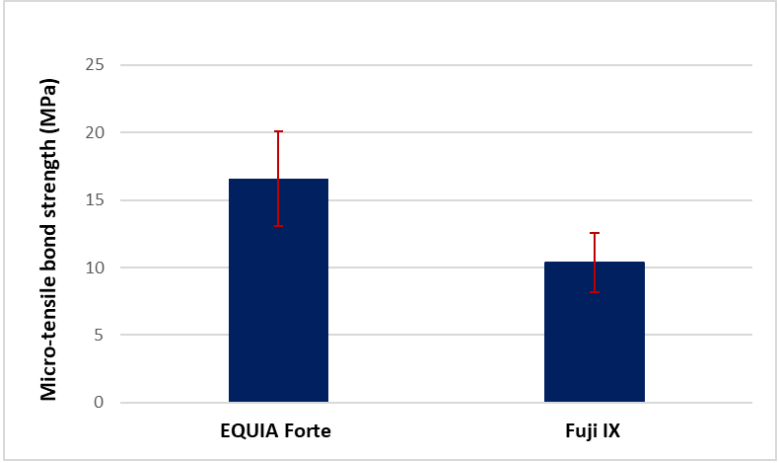

Fig. 1 Means, standard deviations and results for micro-tensile bond strength test (MPa) of the studied restorative materials

Mode of Debonding

10 representative sticks (2 sticks from each tooth) were investigated for the mode of failure. Streomicroscopic examination of the debonded sites showed only two patterns of debonding. These included: A. adhesive mode of failure (at the interface between GIC and dentin surface). B. cohesive failure within the glass ionomer restorative material. These modes of failure varied between the two restorative materials, where the number of cohesive failure mode was predominant for EQUIA Forte Fil (9 cohesive failures, 1 adhesive failure) than Fuji IX (6 cohesive failures, 4 adhesive failure). Scanning electron micrographs of the dentin surfaces revealed adhesive and cohesive mode of failure. In case of adhesive mode of failure in dentin, the GIC was pulled out from the dentinal tubules as shown in Figure 2. In the cohesive mode of failure, GIC remained attached to the dentin surface as shown in Figure 3.

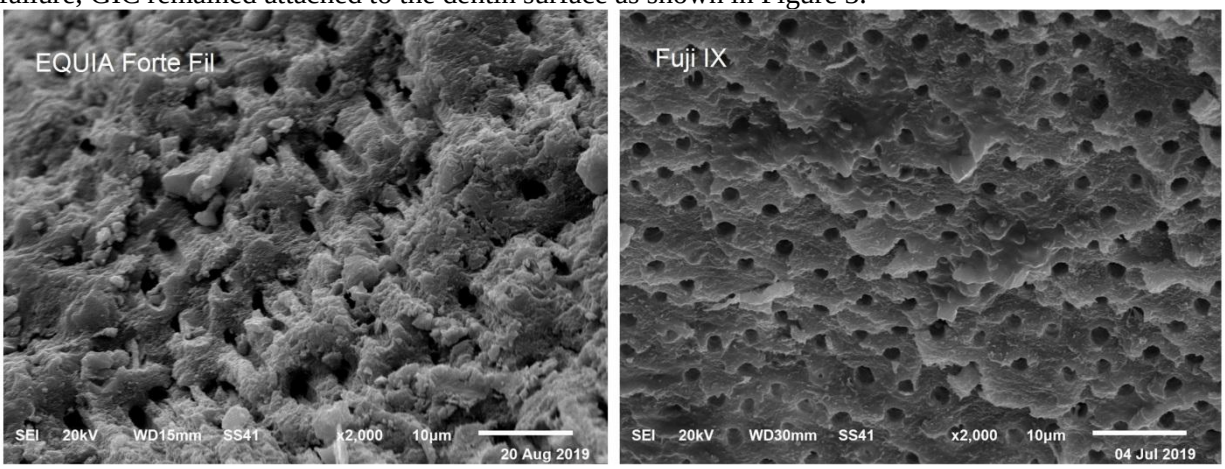

Fig. 2 Scanning Electron Micrographs showing: adhesive failure at dentin surface for the studied restorative materials (x2000)

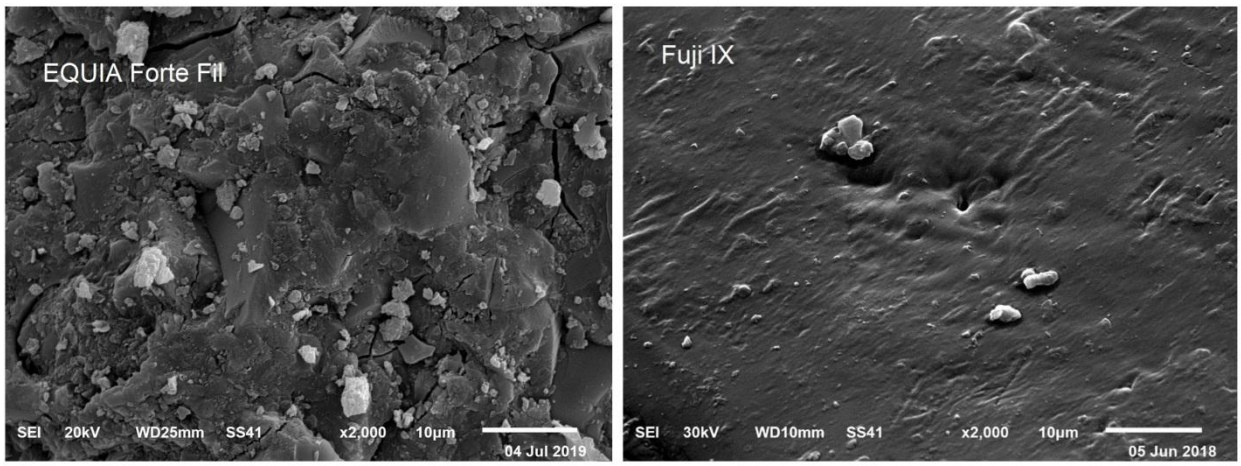

Fig. 3 Scanning Electron Micrographs showing cohesive mode of failure for the tested restorative materials (x2000)

III. Fluoride Release

Means and standard deviations of fluoride release values (µg/cm^2) after storage of each specimen of both types of cements in distilled water for 1, 3, 5, and 7 days are represented graphically in Figure 4. After 1 day, the results of t-test showed no statistically significant difference in fluoride release ($P > 0.05$) between F uji IX (34.3 ± 11.4) and EQUIA Forte (40.4 ± 3.0). On the other hand, after 3, 5, and 7 days, Fuji IX released statistically significantly lower fluoride values compared with EQUIA ForteFil ($P < 0.05$).

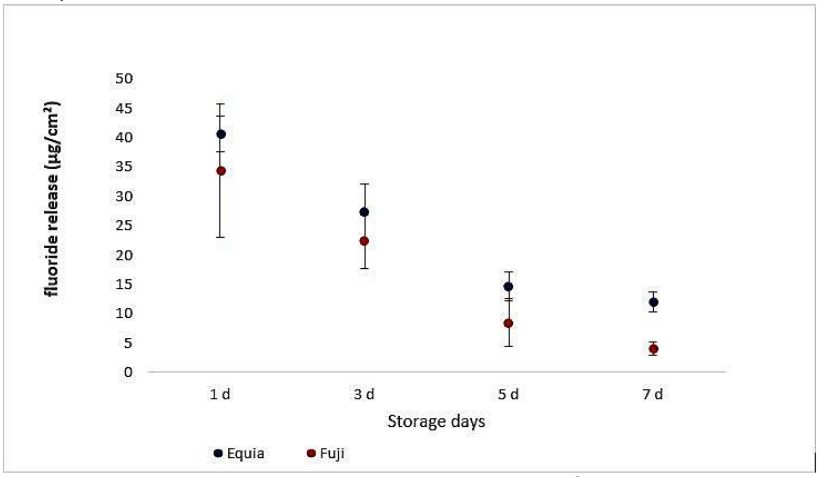

Fig. 4 Means and standard deviations of fluoride release (µg/cm^2) for each of the studied cements after 1, 3, 5, and 7 days storage in distilled water

DISCUSSION

The flexural strength's mean value of EQUIA Forte Fil was higher than that for Fuji IX. These findings were in agreement with a recent study done by Moshaverinia et al. (2019), who had compared EQUIA Forte Fill with Fuji IX glassionomer using flexural strength test which revealed a significant difference between both materials. Additionally, a study at 2021 has been done to investigate flexural strength of three recently developed self-adhesive bulk-fill materials including EQUIA Forte Fil which had showed high flexural strength value comparing to others (François et al., 2021). However, in the current study, the results showed that there was no statistically significant difference in flexural strength between both restorative cements. The higher flexural strength value of EQUIA Forte Fill compared with Fuji IX, can be related to the high molecular weight of the polyacrylic acid within the EQUIA Forte Fill liquid structure. This may help to provide more availability of carboxylic acid groups and increase polysalt bridge formation and crosslinking within the structure of the set cement due to enhanced acid-base reaction (Moshaverinia et al., 2019). It can also be related to the presence of ultrafine and highly reactive second smaller glass particles distributed throughout the structure of the glass powder. The presence of highly reactive glass may help reinforce the mechanical properties of the set cement (Pitel, 2017). It can also be related to the nanofilled self-adhesive light cured EQUIA Forte coat used to cover the surface of the restoration, as it infiltrates the surface of the glass ionomer, providing long lasting protection, good marginal integrity and increasing the strength and wear resistance of the GIC (François et al., 2021; Lohbauer et al., 2011; Schwendicke et al., 2017). It should be noted that for this test, both materials were coated. And although coating is not expected to affect the number of internal porosities, it will prevent water loss and presumably improve flexural strength for both materials. (Thongbai-On & Banomyong, 2020)

Measuring the flexural strength only after 24 hours is a limitation in this study, and further studies comparing flexural strength over extended time intervals - which may allow further maturation of the setting reaction and achievement of final maximum strength- are recommended.

Non-trimming microtensile test was used in this study, as one tooth can produce more than 25 to 30 beams (Shono et al., 1999). Small size of specimens had led to more favorable stress distribution, so bond failure will be closer to true ultimate strength.

In the current study, μTBS of EQUIA Forte Fil glass ionomer cement was evaluated and compared with Fuji IX. The results showed that there was a statistically significant difference between microtensile bond strength of EQUIA Forte Fil and Fuji IX glass ionomers. High μTBS of EQUIA Forte Fil can be related to, the presence of highly reactive small particles of fluoro-alumino-silicate (FAS) micronsizedfillers (< 4 μm) within the powder structure (Pitel, 2017). These small micron fillers may lead to more ions availability, more ions release after glass and polyacrylic acid reaction. Consensually, more chelation with the tooth structure occurs, and increase the ion exchange layer (mineral zone) thickness at the interface between the tooth structure and GIC, through ion exchange of the calcium ions of hydroxyapatite and the phosphate ions of the cement, which helps in improving the bond strength. It can also be related to the high molecular weight polyacrylic acid molecule (Pitel, 2017). This high (MW) may lead to availability of more carboxylate groups after acid-base reaction that may help to improve the bond strength, through the ionic exchange between carboxylic groups and calcium ions in enamel and dentin afterwards ionic bond is formed between them (Khoroushi & Keshani, 2013). It should be mentioned that the predominance of cohesive failure type may occur when the GIC bond to the dentin surpasses its cohesive strength be due to that GICs may have big number of porosities and air inclusions that act as stress-raisers, at which the fracture which initiate (Burrow et al., 2002). The results of the failure mode examination showed that cohesive failure patterns were observed more with EQUIA Forte Fil than Fuji IX specimens. This indicates its better bond strength to the dentin (Kucukyilmaz et al., 2017).

Long term fluoride release is one of the main advantages of glass ionomers (Forsten, 1990). Low incidence of recurrent caries has been associated with restorative material capable to release fluoride ions (Wiegand et al., 2007). Three mechanisms of fluoride release from GICs were reported by Kuhn and Wilson: superficial rinse, diffusion through pores and microfracture and then mass diffusion (Preston et al., 1999). Different methodologies were used in the studies to measure fluoride release, including specimen size, media used to measure, quality of media are responsible for high numerical differences between studies (Gandolfi et al., 2006). Analysis of fluoride release of materials into an aqueous solution can be done by different methods (Preston et al., 1999). Ion Selective Electrode method was used in this study to evaluate fluoride release from both restorative cements. This method is an accurate and established method of determining fluoride release, it also gives a direct estimation of the free fluoride present. Media used to evaluate the fluoride release from dental materials are deionized water, saliva or pH-cycling models (Karantakis et al., 2000). Although saliva or pH-cycling models could better simulate the oral environment, deionized water is a medium that reflects well the fluoride release of the materials, without the confounding influence of minerals or organic molecules which might be presented in saliva or pH-cycling solutions. In the present study, specimen geometry, surface treatment, environmental temperature, the type and pH of the storage medium, the experimental design, analytic method, and finishing technique were standardized for both materials. The current study has evaluated the fluoride release from EQUIA Forte Fil and compared it with Fuji IX after (24 hr, 3 days, 5 days, 1 week). The results showed that both cements released the maximum amount of fluoride on the first day, there was no statistically significant difference between them (Ruengrungsom et al., 2020). While on the other time intervals (3 days, 5 days and 1 week) EQUIA Forte Fil released significantly higher fluoride than Fuji IX. Similar findings were obtained by Lígia et al. (2019) (Bueno et al., 2019), who evaluated fluoride release of 18 types of glass ionomer restorative materials. It was noted that the Equia Forte specimens

released more fluoride ions than some other GICs. Another study in 2019 (Attar & Turgut, 2003), in which EQUIA Forte Fil showed the highest fluoride release than other types of the glass ionmers that was in agreement with this study. The amount of released fluoride from both GICs was greater during the first day of immersion, then declined on the third day, and gradually decreased with until the seventh storage day. These results were in concordance with most of studies that evaluated the fluoride release from GICs (Dasgupta et al., 2018). The high level of fluoride release from GIC materials on day 1 was probably due to an initial "burst" of fluoride release from the glass particles. The burst release of fluoride ions is related to the acid-base setting reactions of the polyalkenoic acid and the fluoride‐containing glass particles and also to the rapid dissolution of fluoride from the outer surface into the solution (Attar & Turgut, 2003). While the slower release of fluoride during the following days has been related to the slower dissolution of glass particles through cement pores and fractures. The reason of high EQUIA Forte fluoride release could be due replacement of calcium ions with strontium ions within the composition of the powder which slightly enhances fluoride release rate (Hassan et al., 2012; Thuy et al., 2008). As SrF2 is more readily dissociated in less acidic environment than CaF2 resulting in a higher fluoride release. In order to release fluoride content of a salt, it needs to be dissociated and diffused through the bulk cement. Since calcium is more electropositive than strontium, CaF2 is less soluble than SrF2 (Brzović-Rajić et al., 2018; Moreau & Xu, 2010). High fluoride release can also be related to the ultrafine fillers, which may lead to increase the surface area for acid-base reaction to take place leading to enhanced fluoride release (Dasgupta et al., 2018). It was also suggested that the hydrogel phase formed during the acid-base reaction of the glass hybrid cements is thicker than for the conventional type. Low fluoride release in Fuji IX could be related to glass filler content with fewer monovalent ions. In addition, cross linking the polymer chains holding them close together which led to less water transport and, consequently less fluoride release (Dijkman et al., 1993).

CONCLUSION

Based on the results and within the limitations of the current study, our results demonstrated that flexural strength after 24 hours of EQUIA Forte Fil (hybrid glass) was higher than of Fuji IX (conventional) without significant differences. EQUIA Forte Fil showed better bond to dentin structure, and more fluoride release than that of Fuji IX.

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DECLARATION OF CONFLICT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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