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EVALUATION OF THE AMOUNT OF FLUORIDE RELEASE AND MICRO HARDNESS OF ENAMEL ADJACENT TO DIFFERENT FLUORIDE RELEASING RESTORATIVE MATERIALS

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ABSTRACT: The aim of this *in vitro* study was to evaluate the remineralizing effects of three fluoride releasing restorative materials by determining their fluoride release and the micro hardness of enamel adjacent to these restorative materials. Standard cavities (3×1.5×1.5 mm size) were prepared on buccal surfaces of extracted human molar teeth. Half of the cavities were filled with dental wax and served as control and the other halves were restored with a conventional restorative glass ionomer/Fuji Bulk (FB), a glass hybrid/Equia Forte (EF), or a giomer/Beautifil Bulk (BB) (n=8). Teeth were subjected to a de-/remineralization cycle for 5 days. Fluoride release was measured by a fluoride ion selective electrode and micro hardness of adjacent enamel was evaluated with a universal hardness tester before and after pH cycling. The data were analyzed statistically (α=0.05). The highest fluoride release was observed in EF and the lowest was seen in BB (p<0.0001). The lowest decrease in micro hardness was observed in FB and the highest was seen in BB. Fluoride releasing restorative materials stimulated the remineralization of adjacent dental hard tissue *in-vitro.*

Keywords: Bioactive restorative materials; Fluoride release; Micro hardness; pH cycle.

INTRODUCTION

Today, with the increased popularity of minimally invasive dentistry which aims to preserve as much healthy dental tissue as possible, bioactive restoration materials that allow the formation of special biological responses and repair of tissues with remineralization potential, have become more preferred.[1,2] Replacing dental tissues using restorative materials was considered as a passive process before bioactive materials were developed. However, after analyzing the interaction between biologically active materials and dental tissues, it was understood that special biological responses developed in these tissues.[1,3,4]

Studies examining bioactive restorative materials indicated that their fluoride (F) releasing properties have an important role to inhibit the demineralization and to enhance the remineralization process.[1,3,5] In this way, it has been shown that dental hard tissues maintain their mineral density and physico-mechanical properties.[6]

Glass ionomers (GIs) are designed as a F reservoir which maintain a steady flow of F ions into the neighboring tooth hard tissues, thus improving the resistance to secondary caries.[7-10] However, inadequacy of conventional GIs to be used in stress-bearing areas have led to the launching of newly developed F releasing restoratives with better physical properties. Recent developments, aimed to eliminate these disadvantages, resulted with the introduction of new materials called glass hybrids, reinforced with smaller and more reactive silicate particles and acrylic acid molecules with higher molecular weight.[8,11] Equia Forte (GC, Tokyo, Japan) is a glass hybrid restorative system that has increased flexural strength and fracture toughness, which are mandatory along with greater abrasion resistance and F release.[8,12]

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Recently, another new conventional GI system (Fuji Bulk; GC, Tokyo Japan) was introduced. The manufacturer of this new GI claims that owing high F release capacity, a purpose-designed glass filler and a new higher-molecular-weight polyacrylic acid enable this GI to have increased resistance to acidic oral environment.[13]

One of the recent developments among the F releasing dental materials are giomers, which combine the esthetics by means of the possibility to have a finished surface and better mechanical resistance. Giomers were produced with the aim of incorporating the favorable features of composite resins and GIs: protection against carious lesion, good mechanical resistance and esthetics and they have a conventional bis-GMA matrix and bioactive glass fillers.[14]

Until now, there have been no studies that evaluated the amount of F release and microhardness of enamel adjacent to these restorative materials. Therefore, the aim of this *in vitro* study was to analyse the effects of three different F releasing restoratives; a conventional restorative GI [Fuji Bulk (FB)], a glass hybrid restorative system [Equia Forte (EF)] and a giomer [Beautifil Bulk (BB)] on adjacent enamel tissue by examining the amount of F release and the micro hardness The tested null hypotheses were; there would be no differences among the restorative materials: (1) in F release and (2) in micro hardness after de-/remineralization cycle.

MATERIALS AND METHODS

The study protocol was validated by the Non-Interventional Clinical Researches Ethics Board of the university with the project number of GO 19/225, 2019/14-25. Informed written consents were obtained from the donors according to the settled protocol of Institutional Review Board.

Sample size calculation:

Sample size calculation parameters were: 0.05% significance level a power of 0.80, resulting in 8 specimens per group (G*Power 3.1.9.2).

Tooth selection and specimen preparation:

For the evaluation of F release and change in enamel microhardness, extracted human first molars without visible defects were used. The teeth were subjected to disinfection protocol for 24 hr in the 0.1% thymol solution and then thoroughly cleaned. The roots of the teeth were removed 1 mm away from the cemento-enamel junctions with diamond discs under water cooling and the crowns were embedded in chemically cured acrylic resin (Integra, Ankara, Turkey). After the acrylic resins were cured, the crowns were taken out of the molds and the buccal surfaces were flattened and polished under water-coolant using 600, 800, 1000, 1200, and 2000 grit silicon carbide papers (English Abrasives, London, United Kingdom) via a polishing machine (Mecapol p230, Madrid, Spain), respectively. Then, standardized buccal cavities (3×1.5×1.5 mm size) were prepared in the middle third of the crowns and divided into 3 groups (n=8), randomly. Half of each cavity was filled with dental wax (Polywax 774, Bilkimya, Izmir, Turkey) and the other half received one of the restorative materials as shown in Table 1 according to manufacturers' instructions as follows:

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Table 1. Manufacturer and chemical composition of materials tested

Material	Manufacturer	Chemical Composition
Fuji BULK	GC Corp., Tokyo, Japan	Powder: Ultra fine high reactive flluoroaluminosilicate glass (92-97% w), poliacrylic acid (3-8% w) Liquid: High molecular weight polyacrylic acid (35-40% w), distilled water (45-55% w), polybasic carboxylic acid (5-10% w)
Equia Forte	GC Corp., Tokyo, Japan	Powder: Fluoroaluminosilicate glass (92-97%w), polyacrylic acid (3-8% w), iron (III) oxide (<0.5 % w) Liquid: Polyacrylic acid (35-40% w), distilled water (45-55% w), polybasic carboxylic acid (5-10% w)
Equia Forte Coat	GC Corp., Tokyo, Japan	Urethane methacrylate, methyl methacrylate, camphoquinone, colloidal silica, phosphoric ester monomer
Single Bond Universal	3M ESPE, Seefeld, Germany	10-MDP, phosphoric ester monomer, HEMA, silane, dimethacrylate, Vitrebond copolimer, filler, ethanol, water, initiator
Beautifil Bulk	Shofu Inc., Kyoto, Japan	Matrix: Bis-GMA, UDMA, Bis-MPEPP, TEGDMA Filler (74.5% w): S-PRG filler (F-B-Al-Si glass)

Group 1 – FB (n=8): To aerate the powder inside the capsule, the encapsulated restorative material was tumbled for 5 sec and automatically mixed for 10 sec (DB-338, Foshan Medical Inst Co., Foshan, China). The activated material was injected into the cavity and covered by a transparent Mylar strip and a glass slab. A firm finger press was applied over the glass slab to spread the material equally in the cavity. After waiting the setting time of material recommended by the manufacturer, Mylar strip and glass slab were removed and the restoration was subjected to finishing and wet polishing by high-speed fine diamonds (Diatech, Swiss Dental, Heerbrugg, Switzerland) and silicones (HiLusterPLUS, Kerr Corp, Orange, CA, USA). Finally, the specimens were coated with a light-cured resin coating (Equia Forte Coat, GC, Corp., Tokyo, Japan) and irradiated for 20 sec by a photocuring light (Starlight s, Mectron spa, Carasco, Italy, 1400 mW/cm^2).

Group 2 – EF (n=8): EF capsule was mixed and injected into the cavity as outlined in Group 1. After waiting the setting time of GI recommended by the manufacturer, the finishing, polishing and coating procedures were done in the same way as outlined in Group 1.

Group 3 – BB (n=8): A universal adhesive (Single Bond Universal, 3M, Seefeld, Germany) was applied in self-etching mode. The adhesive was applied both on enamel and dentin by a single-use brush with a scrubbing motion for 20 sec, air dried for 5 sec and irradiated for 10 sec. Then, BB was applied into the cavity and irradiated for 20 sec. The finishing and polishing of the restoration were done by ultrafine diamonds and silicone instruments.

After the restorations were completed, specimens were stored in 37ºC, 100% humidity environment for 24 hr.

Demineralization/remineralization cycling:

All specimens were submitted to pH cycling procedure according to Vieira et al.[15] to mimic dynamic oral conditions. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid adjusted to pH 4.8. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl adjusted to pH 7.0. Each specimen was stored in 10 mL demineralizing solution for 6 hr and in remineralizing solution for 18 hr for 5 days. The solutions were renewed daily and collected in separate containers to measure the amount of released F ions.[16]

Fluoride release:

F release measurement was conducted by a F ion selective electrode (Seven Compact pH Meter S210, Mettler, Toledo, Ohio, USA). The instrument was calibrated according to manufacturer's instruction using four standard F solutions containing 0.01, 0.05, 0.1, and 0.5 ppm F, respectively.[17] Before measurements, 1 mL of Total Ionic Strength Adjustment Buffer III (TISAB III) was added to each solution to provide constant background ionic strength, decomplex F and adjust pH.[18] F release data was recorded in parts per million (ppm).

Micro hardness test:

Vickers micro hardness test was carried on enamel adjacent to restorations and dental wax before and after de-/remineralization cycle. A microhardness tester (HMV-2, Shimadzu, Kyoto, Japan) with a Vickers diamond indenter and a static load of 10 g was applied to the enamel for 15 sec.[19] Five indentations were done at distances of 100, 200, and 300 µm from the interface of the restoration and dental wax on enamel.[20] All micro hardness values measured before and after the pH cycle were recorded in order to calculate the micro hardness change using the following equation:

$$\text{Micro hardness change} = VHN_{after} - VHN_{before}$$

Statistical analysis:

Statistical analyzes were done by SPSS software package (SPSS 20.0 for Windows, IBM Corp., Armonk, NY, USA) in the $\alpha = 0.05$ confidence interval. The data obtained from F release and microhardness were analyzed using multivariate analysis of variance (ANOVA) and Tukey's post hoc test ($\alpha = 0.05$).

RESULTS

Fluoride release:

The mean ± standard deviations (± SD) of F release from each material during pH cycle are shown in Table 2 and the Figure.

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Table 2. The mean ± standard deviation (±SD) of F release from each material during pH cycle (ppm)

	Day 1	Day 2	Day 3	Day 4	Day 5
Fuji Bulk	1.469[a,b] (±0.047)	1.161[a,b] (±0.038)	0,587[a,b] (±0.031)	0,438[a,b] (±0.013)	0,356[a,b] (±0.026)
Equia Forte	1,924[a,b] (±0.058)	1,593[a,b] (±0.048)	0,724[a,b] (±0.041)	0,592[a,b] (±0.041)	0,457[a,b] (±0.027)
Beautifil Bulk	1,065[a,b] (±0.051)	0,724[a,b] (±0.039)	0,372[a,b] (±0.037)	0,298[a,b] (±0.024)	0,230[a,b] (±0.022)

F release differences among the materials according to repeated measures ANOVA ([a]): (p<0.0001).
Daily F release differences without material effect according to repeated measures ANOVA ([b]): (p=0.002).

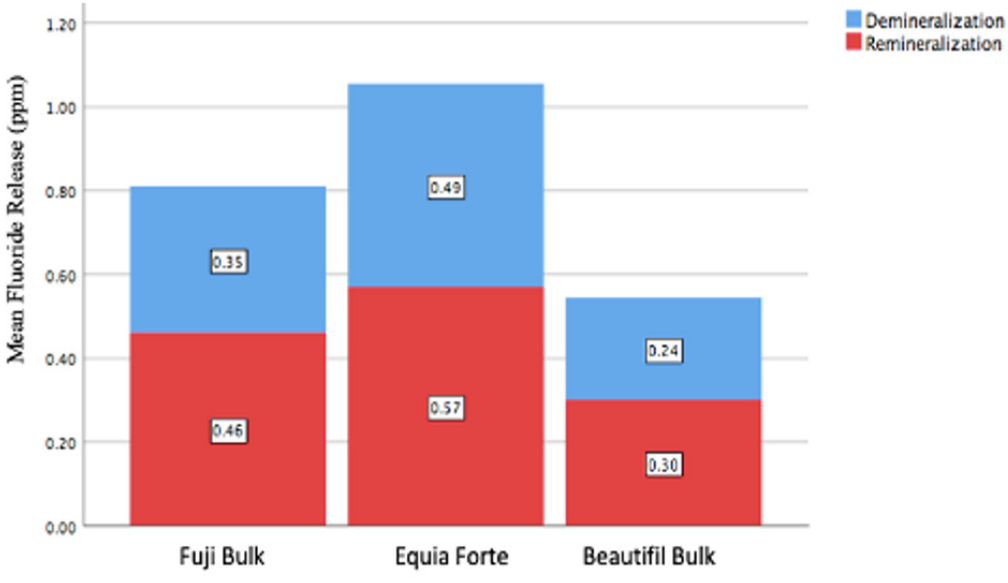

Figure. Cumulative F release from restorative materials tested.

Time (p<0.0001), restorative material (p<0.0001), and pH cycle phase (p<0.0001) had an effect on the F release. For all tested materials, maximum F was released during the first 24 hr and decreased with time (p=0.002). Significant differences were observed among the materials in the following order: EF>FB>BB at all evaluation periods (p<0.0001). Regardless of the material tested, pH cycle (the solutions in which the samples kept) was found to be significantly effective on cumulative F

release ($p<0.0001$). The amount of F release in the remineralization solution is higher than the amount in the demineralization solution.

Micro hardness:

Micro hardness significantly decreased in control groups ($p<0.0001$) (Table 3).

Table 3. Micro hardness (VHN) changes in enamel next to the restorative materials at 3 different distances

	100 µm	200 µm	300 µm
Fuji Bulk	31.5	–4	–13
Fuji Bulk / Control	–18.5	–41.5	–41. 5
Equia Forte	–99	–106	–135
Equia Forte / Control	–150	–126.5	–152
Beautifil Bulk	–98	–133.5	–145.5
Beautifil Bulk / Control	–136.5	–151.5	–148.5

An increase in micro hardness was observed at 100-µm distance from FB. However, a decrease in micro hardness was observed in EF and BB groups. At 200 and 300 µm distances, none of the restorative materials inhibit decrease in micro hardness. When the average micro hardness change at all distances were examined, lowest decrease in micro hardness was observed in FB, whereas the highest decrease was observed in BB. Differences in the average micro hardness change values were significant for FB-EF and FB-BB ($p<0.0001$) but no significant difference was detected between EF and BB ($p=0.491$).

DISCUSSION

In vitro studies play an important role in evaluating the effectiveness of materials developed to be used in dental practice.[21] When compared to *in vivo* studies, *in vitro* studies are faster, easier to execute, and more economical. However, they show limitations to mimic variable clinical conditions.[22] By standardization of variables, significant results can be obtained about the evaluated parameters in *vitro* studies.[21,23] The present study was conducted under *in vitro* conditions and permanent human teeth were used as they reflect the results more easily to clinical conditions. Depending on diet habits, saliva amount, and buffering capacity of individuals, dynamic mouth environment with pH fluctuating constantly, is a very challenging feature for both dental hard tissues and existing restorations.[24] Investigation of the effects of bioactive restorative materials on dental tissues under static conditions may be insufficient due to the fact that the data obtained may not be able to reflect the clinical conditions.[25] So, in the present study, the restored teeth were subjected to the de-/remineralization cycle in order to understand the behavior of the restorative materials under the dynamic conditions of the mouth and to obtain more meaningful findings that will lead to further clinical studies in which these materials will be examined.[26,27]

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In previous studies, cylindrical cavities with different diameters and heights were often prepared to measure the F release of bioactive materials.[17,28,29] However, the total surface area and volume of specimens with various diameters and heights prepared in these studies were beyond the amount of material used in routine restorative procedures. It is known that the amount of F release is related to the surface area and total volume of the surfaces of the restoration opening to the oral environment.[5,28,30,31] In this study, cavities were prepared based on Class V restorations frequently applied in the dental routine.

There were significant differences among F release of the three restorative materials tested. So, the first null hypothesis is rejected. F releasing capacity was listed as EF > FB > BB. These results are compatible with previous studies that reported the F release of different restorative materials.[28,29] The difference in the amount of hydrogel matrix formed during the hardening process of restorative materials may be the reason for the differences among the F release of the materials in the current study. EF consists of ultrafine and highly reactive glass fillers dispersed within a conventional GI structure. Therefore, as a result of the acid-base reaction, the hydrogel matrix phase occurs in a very thick layer. BB, in the structure of giomer, does not show any acid-base reaction as a result of polymerization and contact with water. Accordingly, the hydrogel matrix phase either does not occur at all or occurs in a very small amount.[29] Another reason for EF to release higher F may be the Sr^{++} ions added to the content instead of Ca^{++} ions. SrF_2 is divided into easier components than CaF_2. So, the amount of F ions in the environment increases more.[32,33]

F release of all tested materials in the present study varied depending on time. At the end of the first two days, F release of FB and EF showed a sharp decrease. During the pH cycle, they revealed more than half of the total F release in the first two days. This feature, called as “burst effect”, has also been demonstrated in other studies evaluating the F release properties of bioactive materials.[18,28,32,34] In this study, F release of BB decreased over time, but this graph was not in the form of a significant decrease at the end of the first two days similar to the other restorative materials; it was closer to a regular decline graph. This data was consistent with the study of Garoushi et al.[18]

The surface of both FB and EF were coated according the to manufacturer’s recommendations after finishing and polishing. This coating could affect F release by covering the surface of the restorative material, reducing the surface area that may show diffusion and preventing the contact of the pores where the ion exchange takes place with water and coating the superficial layer that shows more dissolution potential.[32] Studies investigating the effects of coating agents on F release indicated that coating the surface of GIs reduces F release by 60–70%.[34,35] Brzovic Rajic et al.[32] measured the F release of EF with different surface treatments and stated that coating reduced the F release. The researchers reported that nano-sized fillers of coating agent showed a much more homogeneous distribution and covered the GI surface more effectively. It was thought that F release of FB and EF would have been higher if their surfaces had not been coated. However, it should be taken into consideration that these restorative materials were coated in accordance with the manufacturer's instructions, therefore they may show a similar F release pattern in clinical conditions.

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Forsten[36] and Vieira and Modesto[37] stated that F release was higher in environments with low pH than environments with neutral pH. The authors reported that this may be due to faster and more dissolving of the surfaces of the restorative material at acidic pH. [36,37] In the present study, F release showed a significant difference for both solutions. F release in the remineralization solution was higher than the demineralization solution regardless of restorative material. This result is not compatible with studies investigating the effect of different solutions on F release.[36,37] However, it should be in mind that the storage time of the specimens in solutions was different. Specimens were kept in the demineralization solution for a third of the remineralization solution time due to the determined pH cycle, which may have affected the data obtained.

Although it varied depending on the restorative material, all restorative materials in this study protected the adjacent enamel from the demineralizing effect of the pH cycle. These findings are compatible with most studies[20,38,39] investigating the effects of F releasing restorative materials on adjacent dental tissues but are not compatible with the results of Ayres et al.[40]

When FB was compared with EF and BB, the decrease in micro hardness of adjacent enamel was found to be lower and the difference between EF and BB was not significant. FB was claimed by the manufacturer as the most acid resistant bioactive material available on the market. In the present study, FB was the most resistant material to acid attacks. This may be due to the addition of specially designed glass fillers and higher molecular weight polyacrylic acid. In addition, effects of the materials on adjacent enamel micro hardness were found to be dependent to the distance from the restoration interface. As the distance to restoration-tooth interface increased, the protective effect of the restorative material on the adjacent enamel decreased.

While these results are consistent with the study of Alkattan et al.[20], which reported that the protective effect of the restoration decreases due to distance; they are not compatible with the study of Borges et al.,[38] that argued that distance does not have an effect. Significant differences were seen among the micro hardness changes in control and experimental groups for all three restorative materials. The effect of the three restorative materials tested on the micro hardness change of enamel was different. Therefore, the second null hypothesis, which predicted that there would be no difference among the tested materials, is also rejected.

The present study was conducted *in vitro* and it did not simulate clinical conditions by the absence of the influence of saliva and dietary habits. So, *in vivo* studies are needed to achieve more precise results and to confirm the capability of these materials.

CONCLUSION

In this *in vitro* study, although their effects differ, restorative materials with F releasing properties seemed to protect enamel from demineralization and provided remineralization.

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