**Fluoride Release from Glass Ionomer with Nano Filled Coat and Varnish**

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**Introduction**

Fluoride is an important therapeutic and preventive agent in dental caries prevention and remineralization of partly demineralized dental tissues, when topically administered in the oral cavity (1). There are several mechanisms of anticariogenic fluoride action including inhibition of bacterial growth and metabolism, hindering demineralization and promoting remineralization (2). Fluoride release is, therefore, considered to be a valuable property of restorative dental materials, and was shown to be influenced by several factors. The material composition, storage conditions and curing method influence the degree of fluoride release (2).

Glass ionomer cements are widely used in contemporary dentistry (3). Their advantages over other restorative materials such as chemical adhesion, biocompatibility, protective and remineralizing action on dental tissues are well documented (4,5). Traditional GICs have unfavorable physical properties...
privremene materijale neprikladne za trajne ispune (6). Bojila fizikalna svojstva postiže se optimizacijom omjera kiseline i fluoroaluminosilikatnog stakla te veličine i distribucije čestica (4). Novi restorativni koncept na temelju tehnologije SIC-ova razvijen je 2007., a sastojao se od Fuji IX GP Extra SIC-a i coata (svjetlosno polimerizirajućeg premaza) punjenog nanopunilima, te je 2011. godine preimenovan u Equia Fil, Equia Forte (GC, Tokio, Japan) predstavljen 2015. kao novi materijal u sklopu stakleno-hibridne tehnologije. Ista knjuta je da sadržava visokoviskozni konvencionalni SIC u kombinaciji s coatom punjenim nanopunilima (Equia Forte Coat, GC, Tokio, Japan) (7). Equin pruža sastoji se od 95 % strontiju-fluoroaluminosilikatnog stakla, uključujući višokoreaktivne male čestice, a 5% čini poliakrilna kiselina. Tekuća komponenta sastoji se od 40 % vodene otropine i poliakrilne kiseline. Stroncij je odgovoran za povećanu radioaktivnost te nema neželjenih učinaka na izgled cementa (8). Ta zamjena kalcića stronicem povećala je otpuštanje fluora (9). Kako bi se fluor otpustio iz materijala, soli fluorida treba disociрати, a fluor difundirati kroz cement. Budući da je kalcić elektropozitivniji od stroncijca, CaF₂ je manje topljiv od SrF₂ (9). Equia Coat sastoji se od 50 % metil-metakrilata i 0,09 % kamfokornicina. Taj hidrofilni niskoviskozni površinski premaz brtvi površinu SIC-a, smanjuje abrazivno trošenje i povećava kompresivnu čvrstoću restauracije tijekom prvih mjeseča do postizanja potpune maturacije te poboljšava estetiku tvz. glaze-efektom (4, 10, 11). Nadalje, pokazalo se da klinička izvedba novoga restorativnog sustava zadovoljava (12, 13). Jedno od najvažnijih svojstava materijala temeljenih na SIC-u jest njihov protukarijesni potencijal. Odgođena demineralizacija susjednih zdravih tkiva i remineralizacija demineraliziranog podležećeg dentina, uvelike su posljedica otpuštanja fluora iz restorativnog materijala (14, 15).

Svrha ovog istraživanja in vitro bila je evaluirati i usporediti otpuštanje fluora iz Equia Forte Fil (GC, Tokio, Japan) premazanog dvama različitim površinskim premazima – Equia Forte Coatom (GC, Tokio, Japan) i zaštitnim premažom Fuji Varnish (GC, Tokio, Japan).

Materijali i postupci

Za izradu uzoraka korišteni su cilindrični aluminijski kalupi (promjera 8 mm i 2 mm visine). Promjer i visina mjereni su digitalnom pomičnom mjerkom. Equia Forte Fil pripremljen je prema uputama proizvođača i apliciran u kalupe. Gornja površina svakog uzorka prekrivena je celuloidnom premljenje prema uputama proizvođača i apliciran u kalupe. Equia Forte Fil je prepregzadene sa Fuji Varnish (GC, Tokio, Japan) (7). Equia's powder consists of 95% strontium fluoroaluminosilicate glass, including the newly added highly reactive small particles, and 5% polyacrylic acid. The liquid component consists of 40% aqueous polyacrylic acid. Strontium is responsible for increased radiopacity and it does not have any undesired effects on the appearance of the cement (8). This substitution of calcium with strontium has enhanced fluoride release (9). For the fluoride to be released, the salt needs to dissociate and diffuse through the bulk cement. Since calcium is more electropositive than strontium, CaF₂ is less soluble than SrF₂ (9). Equia's powder consists of 50% methyl methacrylate and 0,09% camphorquinone. This hydrophilic low viscosity nanofilled surface coating seals the GIC surface, reduces abrasive wear and the fracture strength of the restoration during the first months until complete maturation is achieved. Besides it improves esthetics by glaze effect (4,10,11). Furthermore, it was shown that the clinical performance of the newly developed restorative system is quite satisfying (12,13). One of the most important properties of GIC-based materials is their anti-cariogenic potential. Delayed demineralization of adjacent sound tissues and remineralization of demineralized underlying dentin are largely the result of fluoride release from the restorative material (14,15).

The aim of this in vitro study was to evaluate and compare the fluoride release from Equia Forte Fil (GC, Tokyo, Japan), coated with two different surface coating agents Equia Forte Coatom (GC, Tokyo, Japan) and Fuji Varnish protective coating (GC, Tokyo, Japan).

Materials and methods

Cylindrical aluminum molds (8 mm diameter and 2 mm depth) were used to prepare the samples. The diameter and depth were measured using an electronic digital caliper. Equia Forte Fil was prepared according to the manufacturer's instructions and packed into the molds. The top surface of each specimen was covered with a celluloid strip and a glass slide at room temperature and the specimen was allowed to set at room temperature for 10 min. Equia Forte Coatom was applied on six samples and light-cured for 20 s, six samples were coated with Fuji Varnish which was left to self-cure and six were left uncoated. Both agents were free of fluoride. Equia forte coating includes low viscosity monomer methyl methacrylate, phosphoric acid ester monomer and photoinitiator, whereas Fuji Varnish contains isopropyl acetate, acetone, cornmint oil and cinnamaldehyde. The specimens were subsequently removed from the molds by ap-
on 37 °C. Swaki uzorak uronjen je u 5 ml deionizirane vode u polietilenskim posudama i inkubiran 24 sata na 37 °C. Nakon 24 sata uzorci su izvadjeni iz posuda te je izmjerena koncentracija fluorida u destiliranoj vodi fluor selektivnom elektrodnom tipa 96 – 09 (Boston, Mass, SAD) i mikroprocesorom ORION EA 940 (Orion Res Inc, SAD). Prije mjerenja koncentracije fluorida, prema uputama proizvođača provjeren je točnost mjernog instrumenta i inžinjerka elektrode te je svakom uzorku dodano 0,5 ml TISAB-a III (Total Ionic Strength Adjustment Buffer; Merck KGaA, Darmstadt, Njemačka) kako bi se postigla konstantna ionska snaga i pH.

Nakon toga uzorci su isprani, osušeni, izvagani te ponovno uronjeni u novu posudu s 5 ml deionizirane vode. Mjerenje destilirane vode je mjerenje sadržaja fluorida u njoj učinjen je nakon 1, 4, 30 i 64 dana u triplikatima za svaki uzorak, a vrijednosti koncentracije izražene su u mgF/L (ppm F).

Podaci su obrađeni statističkim paketom SAS. ANOVA je korištena za usporedbu srednjih vrijednosti, Tukeyev test za multiple usporedbi te paired t-test s Bonferronijevom korekcijom za usporedbu srednjih vrijednosti u različitim točkama vremena. Razina značajnosti za sve testove bila je p < 0,05.

Uzorci koji nisu korišteni za određivanje razine otpuštanja fluorida, analizirani su s pomoću SEM-a. Uzorci su stavljeni u polimernu masu koja provodi struju, obrađeni brusnim papirom (P320, P500, P1000, P2400, P4000) na 300 rpm uz vođeno hlađenje, ispolirani na 150 rpm i primijenjenom silom od 30 N s pomoću dijamantnih pasta (3µm i 1 µm) i lubrikanta.

Rezultati

Rezultati otpuštanja iona fluorida nalaze se u tablici 1. Otpuštanje fluorida značajno se razlikovalo među skupinama (p < 0,0001) i u različitim točkama vremena (ANOVA, p < 0,0001). Najmanje otpuštenih iona fluorida zabilježeno je u skupini EQUIA + EC, zatim u skupini EQUIA + VC, a najveće otpuštanje fluorida bilo je u skupini s nepremazanim uzorcima. Tukeyev test pokazao je da je bilo slično otpuštanje fluorida nakon 24 sata iz uzoraka EQUIA + VC i EQUIA kont. Nakon 64 dana zabilježena je značajna razlika u otpuštanju fluorida između skupina EQUIA + EC i EQUIA kont. Rezultate kumulativnog otpuštanja iona fluorida vidi u tablici 2 i na slici 1.

Regresijska analiza pokazala je sljedeće relacione između kumulativnog otpuštanja fluorida (y) i vremena (t):

- EQUIA + EC: $y = 13,0 \cdot \ln t + 43,5$
- EQUIA + VC: $y = 27,6 \cdot \ln t + 75,3$
- EQUIA kont.: $y = 66,6 \cdot \ln t + 87,3$

Deskriptivna statistika za mase uzoraka prikazana je u tablici 3.

Razlike među skupinama nisu bile značajne (ANOVA test, p = 0,15), ali se masa značajno promijenila tijekom vremena (ANOVA test, p = 0,0001). Post hoc usporedba pokazala je da su se promjene dogodile u svim uzorcima.

Results

The results for fluoride ion release are given in Table 1. The fluoride release significantly differed between groups (p<0.0001) and at different time points (ANOVA, p<0.0001). The least fluoride release was noted in EQUIA+EC group, followed by EQUIA+VC group. The greatest fluoride release was in the group with uncoated samples. The Tukey’s test showed that after 24 hours the release of fluoride forms EQUIA+VC and EQUIA cont. samples were similar. After 64 days a significant difference in fluoride release was noted between EQUIA+EC and EQUIA cont. The results of cumulative fluoride ion release are given in Table 2 and shown in Fig. 1.

Regression analysis revealed the following relation between cumulative fluoride release (y) and time (t):

- EQUIA+EC: $y = 13,0 \cdot \ln t + 43,5$
- EQUIA+VC: $y = 27,6 \cdot \ln t + 75,3$
- EQUIA cont: $y = 66,6 \cdot \ln t + 87,3$

Descriptive statistics for sample weights are given in Table 3.

The differences between the groups were not significant (ANOVA test, p=0.15), but weight significantly changed over time (ANOVA test, p=0.0001). Post hoc comparison showed that changes occurred in all samples.
Tablica 1. Otpuštanje iona fluora – mg/l (srednja vrijednost i standardna devijacija – st. d.)

Table 1

|                | EQUIA+EC mean | EQUIA+EC st.d. | EQUIA+VC mean | EQUIA+VC st.d. | EQUIA cont mean | EQUIA cont st.d. |
|----------------|---------------|----------------|---------------|---------------|-----------------|-----------------|
| 24h            | 41.57 (21.3)  | 70.97 (11.2)   | 68.97 (12.6)  | 75.95 (17.9)  |                 |                 |
| 4 days         | 12.76 (9.2)   | 27.61 (14.1)   | 39.16 (11.5)  | 68.97 (12.6)  |                 |                 |
| 30 days        | 8.60 (4.2)    | 16.63 (10.5)   | (10.5)        | (11.5)        |                 |                 |
| 64 days        | 3.08 (3.4)    | 8.33 (6.6)     | 19.15 (14.6)  | 39.16 (11.5)  |                 |                 |

Tablica 2. Kumulativno otpuštanje iona fluora u mg/l (srednja vrijednost i standardna devijacija – st. d.)

Table 2

|                | EQUIA+EC mean | EQUIA+EC st.d. | EQUIA+VC mean | EQUIA+VC st.d. | EQUIA cont mean | EQUIA cont st.d. |
|----------------|---------------|----------------|---------------|---------------|-----------------|-----------------|
| 24h            | 41.57 (21.3)  | 70.97 (11.2)   | 68.97 (12.6)  | 75.95 (17.9)  |                 |                 |
| 4 days         | 54.33 (30.2)  | 98.58 (24.7)   | 144.92 (21.4) | 184.07 (27.5) |                 |                 |
| 30 days        | 62.93 (33.4)  | 115.21 (33.7)  | 184.07 (27.5) | 203.22 (34.6) |                 |                 |
| 64 days        | 66.01 (33.6)  | 123.54 (36.4)  | 203.22 (34.6) |                 |                 |                 |

Tablica 3. Mase uzoraka (srednja vrijednost i standardna devijacija – st. d.)

Table 3

|                | EQUIA+EC mean | EQUIA+EC st.d. | EQUIA+VC mean | EQUIA+VC st.d. | EQUIA cont mean | EQUIA cont st.d. |
|----------------|---------------|----------------|---------------|---------------|-----------------|-----------------|
| 24h            | 0.304 (0.04)  | 0.296 (0.02)   | 0.269 (0.02)  | 0.280 (0.02)  |                 |                 |
| 4 days         | 0.304 (0.04)  | 0.302 (0.02)   | 0.273 (0.02)  | 0.276 (0.02)  |                 |                 |
| 30 days        | 0.298 (0.04)  | 0.299 (0.02)   | 0.269 (0.02)  | 0.280 (0.02)  |                 |                 |
| 64 days        | 0.317 (0.04)  | 0.307 (0.02)   | 0.269 (0.02)  | 0.276 (0.02)  |                 |                 |

Slika 1. Otpuštanje fluora tijekom vremena za tri skupine uzoraka – EQUIA + EC, EQUIA+VC i EQUIA kont.

Figure 1 Fluoride release over time for the three groups of samples: EQUIA+EC, EQUIA+VC and EQUIA cont.

Slika 2. Reprezentativni uzorci Equia Forte s (A) Equia Forte Coatam (EQUIA + EC); (B) premazani Fuji Varnishem (EQUIA + VC); i (C) bez sredstava za premazivanje (EQUIA kont); SEM analiza otkrila je da Equia Forte Coat bolje adherira na staklenoionomerni materijal negoli Fuji Varnish.

Figure 2 Representative Equia Forte glass hybrid specimens with (A) Equia Forte Coat (EQUIA+EC); (B) covered with Fuji Varnish (EQUIA+VC), and (C) without coating agent (EQUIA cont). SEM analysis revealed that there was better adhesion of Equia Forte Coat than Fuji Varnish onto the underlying GIC material.
SEM analiza pokazala je da Equia Forte Coat bolje prilježe uz stakleni ionomer Equia Forte Fil negoli Fuji Varnish (slika 2.).

Rasprava

U načelu postoje dvije vrste premaza za zaštitu staklenih ionomera nakon postavljanja i početnog stvrdnjavanja, kao bi se sprječila kontaminacija vlagom i gubitak nevezane vode. To su jednostavne otopine polimera u otapalu i svjetlosnopolimerizirajući niskoviskozni monomeri. Pojedinca istraživanja pokazuju da svjetlosnopolimerizirajuće premazi (coats) učinkovitije štite SIC od isušivanja, negoli jednostavni premazi (varnish) te da poboljšavaju njegova fizikalna svojstva (16). U ovom istraživanju korišteni su Equia Forte Coat koji sadržava niskoviskozni polimer metil-metakrilat, monomer estera fosforne kiseline i fotoinicijator te Fiji Varnish koji sadržava izopropil acetat, aceton, kukuruzno ulje i cimnetriks. Rezultati pokazuju da je značajno više fluora otpušteno iz uzoraka Equia Forte Fil SIC-a premazanih Fuji Varnishem, sugerirajući da Equia Forte Coat bolje brtvi površinu SIC-a. SEM analiza pokazala je da je površina uzorka bila glatkija kada je bila premazana obama premazima, što može implicirati smanjenu tendenciju pričvršćivanja bakterija na površinu (17). Nadalje, SEM analiza također je pokazala da je adhezija Equia Forte Coata na podležće SIC bila bolja nego što je to bio slučaj s Fuji Varnishem. To je vjerojatno zbog tehnologije nanopunila na kojoj se temelji Equia Forte Coat, a koja omogućuje jednolicičnu disperziju čestica punila (17). Fuji Varnish, pak sadržava molekule polimera oltpjene u organomatskom otapalu. Nakon što se ispun SIC-a premaže varnishem, otapalo hlap i ostavljaju molekule kao tanki sloj ili film. Te molekule veće su od nanočestica iz Equia Forte Coata i to vjerojatno utječe na debljinska film na površini (slika 2.).

Prije je utvrđeno da način stvrdnjavanja – svjetlosnopolimerizirajući ili kemijski – utječe na razinu otpuštanja fluora iz smolom modificiranih SIC-ova i dvostruko polimerizirajućih čestica smolastih cementa. Pokazalo se da polimerizacija inicira na svjetlom povećava gustoću polimernih mreža, što rezultira smanjenjem propuštanjem fluoride ionsa za ione fluorida (18, 19). No naši rezultati, koji pokazuju veće otpuštanje fluora u uzorcima premazanih Fuji Varnishem koji nije polimeriziran svjetlom, ne mogu se pripisati povećanoj gustoći veza unutar SIC-a nakon polimerizacije svjetlom pri premaživanju coatom, jer je Equia Forte Fil materijal koji se stvrdjava samo kemijski.

U ovom istraživanju ioni fluora otpušteni su u logaritmskoj tijekom rhmskoj vremenskoj ovisnosti u svim trima skupinama uzoraka (slika 1.), na koji je bila premazana obama premazima (slika 2.). Kako je već spomenuto, ovaj početni burst-efekt poželjan je u smislu protutubišnjevog uskraćivanja slično kao u prijašnjim studijama (20, 21). Kako je već spomenuto, ovaj početni burst-efekt poželjan je u smislu protutubišnjevog uskraćivanja slično kao u prijašnjim studijama (20, 21).

The SEM analysis showed that Equia Forte Coat adhered better to Equia Forte Fil glass ionomer than Fuji Varnish (Figure 2).

Discussion

Generally, there are two types of coatings used for the protection of GICs after placement and initial hardening to avoid contamination by moisture and loss of unbound water: simple solutions of polymer in solvent and light-curable low viscosity monomers. There are experiments revealing that light-curable coats protect GICs more effectively from drying out than simple varnish, and that they improve the physical properties of GICs (16). Coating agents used in our study were Equia Forte Coat containing a low viscosity monomer methyl methacrylate, phosphoric acid ester monomer and photoinitiator, and Fuji Varnish containing isopropyl acetate, acetone, cornmint oil and cinnamaldehyde. Our results show that there was significantly more fluoride released from Equia Forte Fil specimens when they were coated with Fuji Varnish indicating that Equia Coat seals the GIC surface more effectively. The SEM analysis of the specimens showed that the surface was smoother when covered with both coating agents, which could imply a reduced tendency of bacteria to adhere to the surface. (17). Furthermore, the SEM analysis also showed that Equia Forte Coat adhered better than Fuji Varnish to the underlying GIC. This is probably due to the nanofiller technology used in Equia Forte Coat enabling uniform dispersion of the filler particles (17). Fuji Varnish on the other hand, contains polymer molecules dissolved in organic solvent. After the GIC filling is covered with varnish, the solvent evaporates, leaving the solute as a thin layer or film. The solute molecules are larger than the nano-particles of the Equia Forte Coat and this probably influences the film thickness of both coatings (Figure 2).

It was previously shown that curing method, either light or chemical curing, influences fluoride release from resin modified glass ionomers and dual-cured resin cements, and it was shown that the photoinitiator polymerization enhancement cross-linking density resulting in the reduced resin matrix permeability for fluoride ions (18,19). However, our results of increased fluoride release in the samples coated with Fuji Varnish that was not light cured can hardly be explained with the enhanced cross-linking upon light curing, since Equia Forte Fil is a material that sets by chemical curing alone.

In our study, fluoride was released in a logarithmic time dependence in all three groups (Figure 1), similarly as in previous studies (20, 21). As it was already mentioned, this initial burst effect is desirable in the context of anticariogenic action because it stimulates remineralization of enamel and dentin and has an antibacterial effect (14, 15, 22, 17). In the case of samples coated with Equia Forte Coat, the period of fairly constant fluoride release rate was reached after 4 days, similarly as in uncoated and varnished samples, but the initial fluoride release was significantly smaller in the group coated with Equia Forte Coat. This is in concordance with other studies where 60-76% of reduction in fluoride release from the coated GICs was reported (23, 24). This probably occurred be-
puštanje fluora bilo smanjeno za 60 do 76 % kada su SIC-ovi bili premazani (23, 24). Pretpostavlja se da je to zato što je površinski sloj nematuriranog SIC-a topljiviji i skloniji erozi-ji ako nije zaštićen premazom (25). Općenito, rezultati ovog istraživanja u skladu su s rezultatima ranjih istraživanja ko-ja pokazuju da je najveće otpuštanje fluora u prvih 24 do 48 sati, a varira od 5 do 155 ppm za različite SIC-ove (26, 27).

Nakon početnog bursta, ioni fluora i dalje se otpuštaju jer ne reagiraju kemijskij tijekom reakcije svrđnjavanja te mogu difundirati niz koncentracijski gradijent i biti otpušteni u usnu šupljinu ili preuzeti u stakleni ionomer ako je izložen otopinama s visokom koncentracijom fluora (15, 20). U našem istraživanju je već sada počeo proces spezijski: u prijašnjim istraživanjima, u konvencionalnim i smolom modificiranim SIC-ovima (15, 26, 27, 26). Na te-melju zabilješki o otpuštanju fluora u ovisnosti o vremenu, može se pretpostaviti dinamika otpuštanja fluora u budućnosti i trenutak u budućnosti kada će otpuštanje fluora prestati. U istraživanju Arbabzadeh-Zavareh i suradnika (28) količina fluora u uzorcima na datu datu se izrađene su može na 24 do 48 sati, a varira od 5 do 155 ppm za različite SIC-ove (26, 27).

Sukob interesa

Autori nisu bili u sukobu interesa.

Zahvala

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Conflict of Interest

None declared

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Abstract

Objective: This in vitro study compares the fluoride release from microlaminated glass ionomer based on glass hybrid technology coated with two different surface coating agents. Materials and Methods: A total of 18 samples were divided into three groups of six samples each: (1) glass ionomer Equia Forte Fil coated with Equia Forte Coat (Equia+EC), (2) glass ionomer Equia Forte Fil coated with GC Fuji Varnish (Equia+VC) and (3) uncoated glass ionomer Equia Forte (EQUIA cont). Fluoride release was measured using an ion-selective electrode (ORION EA 940) after 24 hours, 4 days, 30 days and 64 days. Repeated measures ANOVA, multiple comparisons, Tukey’s test and paired t-test were used to test the differences between the groups. Results: The differences between the groups and four time points were statistically significant (ANOVA, p<0.0001). Cumulative fluoride ion release after 64 days was 66.01 mg/l, 123.54 mg/l and 203.22 mg/l for EQUIA+EC, EQUIA+VC and EQUIA cont, respectively. All the differences were statistically significant except the difference between EQUIA+VC and EQUIA cont after 24 hours. Conclusions: The amount of released fluoride was significantly lower in the samples coated with nanofilled surface coating agent compared to the samples coated with varnish and uncoated samples.

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