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In vitro fluoride uptake by bovine enamel from aesthetic restorative materials

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Objectives. The purposes of this *in vitro* study were to determine whether different types of fluoride-containing restoratives produce differing levels of fluoride uptake by bovine enamel, and to determine the effect of time on this uptake.

Methods. Seven aesthetic restorative materials were evaluated. Forty bovine enamel slabs were prepared for each tested material, five of which were used to determine baseline fluoride concentrations. Each slab was attached to a disc of the tested material and suspended in synthetic saliva for up to 64 days. After removal, the specimens were acid etched with perchloric acid, and the dissolved enamel was analysed for fluoride and calcium. Fluoride was determined by direct potensiometric analysis, whereas the amount of calcium was evaluated by means of atomic absorption spectrophotometry.

Results. Higher values of fluoride uptake, not significantly different, were recorded in the first two groups. A statistically significant difference was found in fluoride uptake between Fuji II LC and the three compomers in all test intervals. No significant differences were found in the amounts of fluoride uptake between the three compomers. The highest fluoride uptake from all compomers was recorded by F2000.

Conclusion. Enamel acquired significant amounts of fluoride from all materials with variations during the test intervals.

Introduction

Fluoride is generally well accepted as an anticariogenic agent, and it seems that it may be able to reduce recurrent caries[1]. Many mechanisms are involved in the anticariogenic effects of fluoride, including the formation of fluoroapatite with solubility lower than the original carbonated apatite; thus, the enamel resistance to subsequent acid attack is increased[2], remineralization is enhanced, and carbohydrate metabolism in dental plaque is inhibited[3].

The interest in the clinical use of conventional glass ionomer cements (GIC) arises mainly from their behaviour as adhesive bioactive materials with therapeutic action[4]. The latter originates from their ability to release fluoride. Resin-modified glass ionomer cements (RMGIC) and polyacid-modified composite resins (compomers) have been developed in an attempt to incorporate the advantageous properties of composites and GICs into one material. These materials have different setting mechanisms. The RMGICs are set by an acid–base reaction and free radical polymerization mechanisms[5]. The compomers set by free radical polymerization only with a limited acid–base reaction occurring later as the material absorbs water from the oral environment[6]. Fluoride release from RMGICs is known to be similar to that of conventional glass ionomers, whereas compomers produce a low and relatively constant fluoride release[7].

In view of the complex chemistry and physicochemistry of RMGICs and compomers, an investigation of the fluoride release and subsequently fluoride uptake by dental tissues is not an easy task and can only be performed adequately by a systematic approach[8]. This becomes clear when one realizes that the elution of fluoride and uptake by dental tissues can be affected quantitatively by several intrinsic (related to the chemical and physical

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Table 1. Materials, composition, and manufacturers of the examined materials.

Brand (type)	Composition	Manufacturer (batch number)
Fuji IX GP fast (GIC)	Liquid: PAA, tartaric acid, water Fillers: Ca–Al–F–Si glass (SiO_2, AlO, SrC, NaF, CaC_2, CaP_2), 74% wt Mean filler size: 4.4 μm	GC Corporation, Tokyo, Japan (800160)
Ionofil Molar AC (GIC)	Liquid: PAA, tartaric acid, water Fillers: Ca–Al–F–Si glass, 50% wt Mean filler size: 6 μm	VOCO, Cuxhaven, Germany (97012)
Vitremer (RMGIC)	Liquid: Poly (acrylic–itaconic acid) with methacrylate acid, water, tartaric acid, HEMA Fillers: Fluoroaluminosilicate glass, microencapsulated redox catalysts, 71% wt Mean filler size: 3 μm	3M ESPE, Dental Products, St Paul, MN, USA (20010820)
Fuji II LC (RMGIC)	Liquid: PAA, HEMA, 2-hydroxy-1, 3-dimethacryloxypropane, tartaric acid, camphorquinone, water Fillers: Ca–Al–F–Si glass (SiO_2, AlO, SrC, NaF, CaC_2, CaP_2), 76% wt Mean filler size: 1.8 μm	GC Corporation (0105107)
F2000 (compomer)	Liquid: CDMA oligomer, GDMA Fillers: Al–F–Si glass, 84% wt Mean filler size: 3 μm	3M ESPE (19971014)
Compoglass F (compomer)	Liquid: TEGDMA, DCDMA, UDMA Fillers: Ba–Al–F silicate glass, YbF_3, mixed oxides microencapsulated redox catalysts, 77% wt, 56% vol. Mean filler size: 0.2–1.6 μm	Vivadent Ets., Schaan, Liechtenstein (G-21950)
Dyract AP (compomer)	Liquid: TCB, UDMA Fillers: Sr–Al–Na–F–P silicate glass, SrF_2 75% wt, 46% vol. Mean filler size: 0.8 μm	Dentsply DeTrey, Konstanz, Germany (0401002296)

CDMA, dimethacrylate functional oligomer of citric acid; DCDMA, cycloaliphatic dicarboxylic acid dimethacrylate; GDMA, glyceryl dimethacrylate; HEMA, 2-hydroxyl ethyl methacrylate; PAA, polyacrylic acid; TCB, a reaction product of butane tetracarboxylic acid and hydroxyl methyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

formulations) as well as experimental variables. The latter can cause noticeable differences in the amounts of fluoride released by a given glass ionomer.

The purposes of this *in vitro* study were to determine whether different types of fluoride-containing restoratives produce differing levels of fluoride uptake by bovine enamel, and to determine the effect of time on this uptake. The fluoride uptake by bovine enamel from two GICs, two RMGICs, and three compomers was evaluated at time intervals of 1, 2, 4, 8, 16, 32, and 64 days.

Materials and methods

The materials used in this study and their respective compositions as provided by the manufacturers are listed in Table 1. The three compomers were in modules, and the two GICs and Fuji II LC (GC Corporation, Tokyo, Japan), which were encapsulated, required mechanical mixing by means of an Ultramat 2 (Southern Dental Industries, Victoria, Australia) for 10 s. Vitremer was supplied in a powder: liquid ratio (3M ESPE, USA), thus requiring hand mixing and adjustable consistency, and it

was mixed and handled according to the manufacturer's instruction.

Thirty-five cylindrical discs (2 mm in diameter and 1 mm in thickness) were prepared from each examined material. The cement paste was injected into a Teflon split mould. After the mould was filled, a strip was placed over. A slide glass was placed over the strip and held in place with pressure to exude excess material. The materials were photopolymerized with a visible light unit (XL 3000, 3M ESPE Dental Products, St. Paul, MN, USA) emitting 600–800 mW/cm^2 for the time suggested by the manufacturer. The light was tested for light output using the radiometer included in the XL 3000.

Bovine incisor teeth from the permanent dentitions of 45- to 48-month-old cattle were obtained from a meat-packing plant and stored frozen until use. All cattle were born and lived in the same area. Each tooth was left at room temperature for about 3 h before specimen preparation. Then, the teeth were rinsed thoroughly with water, and the buccal surfaces were lightly cleaned with a rubber cup and flour of pumice, washed, dried, and swabbed with a cotton pellet soaked in acetone to remove residual organic debris. Only specimens without carious lesions or other defects on the buccal surface were used in this study.

A total of 280 enamel tooth slabs (4 × 5 mm) were cut with a diamond saw (IsoMet, Buehler, Lake Bluff, IL, USA) under water spray from the buccal surfaces of the bovine teeth, and were divided into seven distinct groups, one for each tested material. Each of these groups contained 40 enamel slabs that belonged to one bovine jaw in order to ensure the same initial fluoride enamel content. Five untreated enamel specimens of each group were used as controls for the determination of the baseline concentrations of fluoride in bovine enamel. The remaining 35 slabs were divided into seven subgroups (five specimens each) and tested for fluoride uptake at seven time intervals (1, 2, 4, 8, 16, 32, and 64 days).

The slabs were attached with sticky wax to a plastic post with the enamel surfaces facing upwards. To limit the area for enamel uptake, the enamel surface was covered with a sticky Teflon paper (0.08 mm thick, UNIGASKET, Sarnico, Italy) with a circular hole in the middle with standard dimensions (1.5 mm diameter). The remaining slab surfaces were covered with nail varnish.

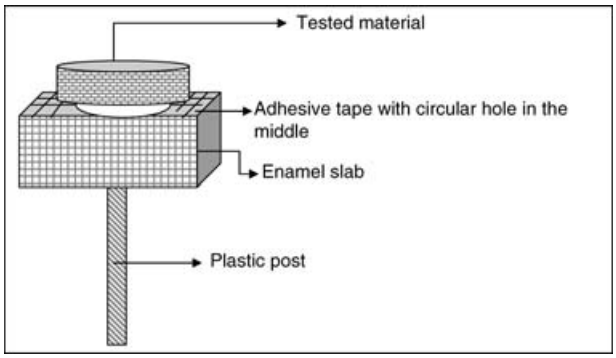

Fig. 1. Relationship of the tested material disc and the enamel slab.

For each slab, a disc of the examined material was transferred on the Teflon paper and attached with sticky wax without sealing off the whole circumference (Fig. 1). The tooth slabs with the attached disc materials were then suspended in polystyrene tubes of 10 mL (300900, EUROTUBO DELTALAB, Barcelona, Spain) containing 2 mL of synthetic saliva [3 m*I* calcium, 1.8 m*I* phosphorus, 150 m*I* sodium chloride, and 1% carboxymethylcellulose (CM-cellulose)] adjusted to pH 7.0 with sodium hydroxide. The tubes were covered with laboratory film (Parafilm M, American National Can, Chicago, IL, USA). The solutions were kept unstirred, but were renewed every 2 days. During the incubation period, there were no recorded pH changes. Because the sides of the enamel were exposed to synthetic saliva, the tissue hydration influenced the fluoride transfer from the materials to the enamel, as it happens *in vivo*.

At the tested time intervals, the specimens were removed from the synthetic saliva and dried with compressed air. The specimens were only used once to avoid contamination. The disc of the material was removed, and the integrity of the seal was evaluated by depositing 0.4 μL of distilled water on the demarcated enamel with a 1-μL microsyringe (Hamilton, Basel, Switzerland). The disappearance of the drop of water from the demarcated biopsy site indicated a defective marginal seal, and in that

case, the specimen was discharged. For that reason, a total of 12 specimens were discharged which were replaced.

The acid etch biopsy method was used to obtain enamel samples[9] to determine the enamel fluoride uptake. The enamel slabs were then suspended in a new tube containing 150 μL of 1 M $HClO_4$ and remained there for 1 min. Immediately after etching, the solution was buffered by pipeting 600 μL of 1 M $Na_3C_6H_5O_7$ directly onto the tooth surface and into the beaker. Following that, the etched area was washed three times with *bis*-distilled water 100, 100, and 50 μL each. By this means the total volume of the solution was 1 mL. To determine the pH of the solution, a combination pH electrode was used (Glass–Ag, AgCl, 405-7/120, Mettler Toledo, Greifensee, Switzerland), connected with a pH reading device (Crison Microph 2002, Crison Instruments, Barcelona, Spain).

The amount of fluoride in the samples was determined by direct potensiometric analysis with the use of a combination fluoride–ion selective electrode (Orion combination fluoride ionalyser 96-09-00, Orion Research, Cambridge, MA, USA) connected to an ion analyser (Crison Microph 2002). Fluoride standards were added to a solution of 150 μL of 1 M $HClO_4$ and 600 μL of 1 M $Na_3C_6H_5O_7$, pH 5.7. The standard curve was plotted from readings of solutions containing 0.02, 0.05, 0.1, 0.5, 1, and 2 p.p.m. fluoride. Standard room temperature conditions (21–25 °C) were kept by means of a heat–cool unit.

After the evaluation of fluoride concentration, an aliquot of 0.5 mL of the solution was transferred in a new tube. One millilitre of $SrCl_2$ and 8.5 mL of *bis*-distilled water were also added, so the final volume of the new solution was 10 mL. All the reactants used in the study were from Merck (Darmstadt, Germany). The amount of enamel which diluted with the acid etch method was evaluated by determining the calcium concentration of the respective solution. The amount of calcium was determined by means of atomic absorption spectrophotometry (model 403, PerkinElmer, Norwalk, CT, USA)[10], on the basis of a calcium content of 40% (±0.24) in sound bovine enamel[11].

Statistical analysis

Statistical comparisons were made among each time interval for all seven materials. The amount of fluoride uptake was calculated by subtracting the baseline fluoride concentrations from the concentration measured at 1, 2, 4, 8, 16, 32, and 64 days. The normality of the studied parameters was checked using the Kolmogorov–Smirnov test, and because all followed normal distribution, statistical analyses were based on parametric tests. The values of the studied parameters in each group were compared by analysis of variance (ANOVA), and the homogeneity of variance was checked with Levene's test. According to the results of Levene's test, *post hoc* comparisons were performed using Scheffe's and Dunnett's T3. Differences were deemed statistically significant at $P < 0.05$. Statistical analysis was carried out with SPSS 10.1 computer program.

Results

The recorded mean values and standard deviations for each test interval of fluoride uptake are presented in Table 2. Measurable amounts of fluoride uptake were found for all examined materials throughout the test period. Among the three groups of materials, the higher values of fluoride uptake were recorded from the GICs and RMGICs. No significant differences were found among these materials. In contrast, the ANOVA test showed significant differences ($P < 0.05$) in fluoride uptake between Fuji II LC and the three compomers in all test intervals. With the exception of the first day, significant differences were also found among Fuji IX and the three compomers.

We also examined the relationship between fluoride uptake by bovine enamel associated with each examined material and the square root of time (in days). Figure 2 demonstrates that this relationship is almost linear. We therefore used the equation $[F] = mt^{1/2} + C$, where $[F]$ represents the fluoride uptake (in p.p.m.), m is the slope of the curve, t is the time (in days), and C is the constant of the equation (Table 3). The square of the correlation coefficient (R^2) between fluoride uptake and square root of time ranges between 0.985

Table 2. Mean values and standard deviations, in parentheses, of the amounts of fluoride uptake from the examined materials during the test intervals.

Day	Fuji IX	Ionofil Molar	Fuji II LC	Vitremer	F2000	Compoglass F	Dyract AP
1	141 (44)	123 (73)	208* (59)	113 (20)	55 (45)	73 (31)	56 (29)
2	233† (46)	200 (59)	284* (55)	193 (44)	95 (35)	117 (43)	90 (22)
4	312‡ (37)	291 (86)	369* (49)	254 (28)	160 (41)	168 (41)	150 (52)
8	394§ (26)	408 (84)	464¶ (43)	307 (27)	230 (36)	225 (38)	215 (58)
16	521* (81)	512* (62)	579¶ (66)	429 (62)	315 (51)	284 (44)	275 (45)
32	702** (128)	684** (129)	741* (91)	628* (21)	414 (85)	381 (66)	362 (51)
64	1039* (178)	940†† (158)	1014* (213)	937 (99)	596 (45)	623 (115)	575 (64)

Fluoride in bovine enamel is given in p.p.m.
*Significantly different compared with all compomers ($P < 0.05$).
†Significantly different compared only with F2000 ($P < 0.05$).
‡Significantly different compared only with F2000 and Compoglass ($P < 0.05$).
§Significantly different compared with Vitremer, F2000 and Compoglass ($P < 0.05$).
¶Significantly different compared with all compomers and Vitremer ($P < 0.05$).
**Significantly different compared only with Compoglass ($P < 0.05$).
††Significantly different compared only with Dyract AP ($P < 0.05$).

Table 3. Relationship between fluoride uptake and time.

	Fuji IX	Ionofil Molar	Fuji II LC	Vitremer	F2000	Compoglass F	Dyract AP
R^2	0.997	0.996	0.996	0.993	0.994	0.985	0.990
C	44	49	128	6	–2	5	–5
m	122	113	111	113	75	74	70

C, the constant of the equation $[F] = mt^{1/2} + C$; m, the slope of the curve; R^2, the square of the correlation coefficient between fluoride uptake and square root of time.

and 0.997 for all examined materials, pointing to a strong correlation between fluoride uptake and time. C reflects the rapid short-term release of fluoride; among RMGICs, this 'burst effect' was observed with Fuji II LC, but not with Vitremer. The GICs also exerted a 'burst effect', similar between them but lower than Fuji II LC; in contrast, none of the compomers had any 'burst effect'. Finally, the m values, which represent the degree of fluoride uptake by bovine enamel associated with each material, were similar in GIC and RMGIC, but substantially lower in compomers. In addition, no differences were observed in m values among materials of the same class (i.e., Fuji IX GP fast versus Ionofil molar AC, Vitremer versus Fuji II LC and F2000 versus Compoglass F versus Dyract AP).

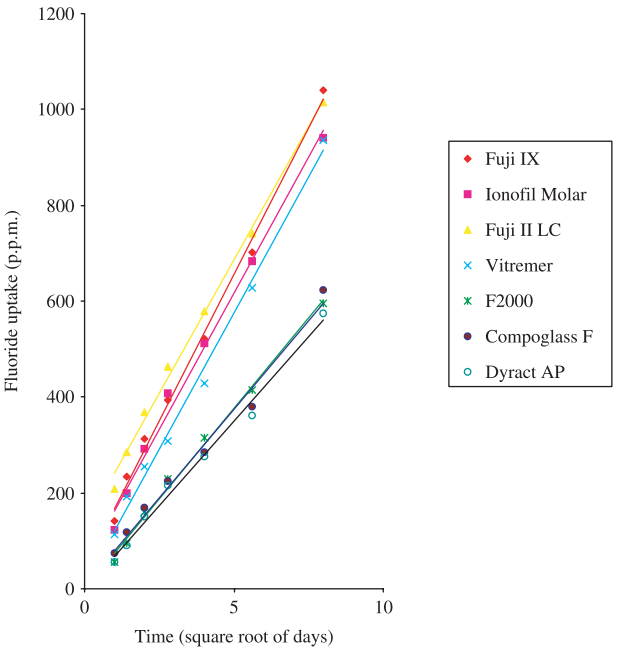

Fig. 2. Fluoride uptake associated with the examined materials during the test intervals.

Discussion

In this study, an acid etch method was used to determine the enamel fluoride uptake from

seven fluoride-containing materials. The method used has been described previously[12] and is in good agreement with the abrasion technique[13]. An underestimate of the results by acid etching could occur if the fluoride in the fluid phase is transferred into deeper enamel layers, as a consequence of the acid penetrating between the enamel prisms faster than the prisms themselves get dissolved[14]. Selective dissolution at prism boundaries, however, is more likely to occur during the etching process.

Bovine enamel is more porous than human enamel, and this might lead to greater fluoride uptake from the former compared with the latter; this possibly represents a limitation of our study. We, however, chose bovine enamel to have a relatively large group of samples all from one jaw and reassure the baseline of fluoride content. Bovine teeth provide large, flat test surfaces with low fluoride content and comparable anatomy and calcification[15]. In contrast, human teeth provide small surfaces with differences in fluoride content among different groups of teeth. In this study, no attempt was made to evaluate fluoride concentration in different enamel layers.

It has been suggested that the favourable condition for fluoroapatite formation in sound enamel is a constant, low level of fluoride ions for at least 24 h[16], and that only 1 p.p.m. fluoride is necessary for the promotion of remineralization[17]. The changes in the fluoride concentration of the enamel, relative to the time of exposure to the tested materials, indicate that fluoride ions were released from the materials continuously over the 64-day investigation period. Undoubtedly, some of the fluoride ions form the fluoroapatite that resists dissolution during the subsequent acid attack, while allowing the acid to diffuse between the resistant crystals to the enamel minerals that were not affected sufficiently by fluoride. Tam *et al.*[18] examined the ability of the conventional versus the RMGIC restorations to resist decay by developing an initial anticariogenicity profile (from fluoride release to fluoride uptake, to resistance to artificial caries challenge). They reported that the amount of fluoride released from conventional and RMGIC is proportional to the amount of fluoride uptake. In another study, it has been shown that fluoride uptake from an RMGIC can prevent demineralization of enamel[19]. Protection of enamel and dentine around freshly placed glass ionomer restorations has been shown *in vitro* and *in situ*. Clinical studies support these *in vitro* data[20]. Under intraoral conditions, fluoride will also leach from restoratives into saliva and may subsequently precipitate on the tooth surfaces adjacent to the cavity. Donly *et al.*[21] demonstrated that RMGIC restorations can enhance the prevention of enamel demineralization on adjacent teeth.

Besides the aqueous environment of the oral cavity, numerous factors, like ionic composition and ionic strength of the saliva, are important parameters, which may influence the quantity of substances released from a restorative material. In this study, the storage medium of the samples was synthetic saliva. This storage medium was previously used in similar experimental studies[22]. There was no protein present, which would attach to the enamel surface, and the only organic component was CM-cellulose. It has been demonstrated that in the presence of enzymes such as porcine liver esterase, fluoride release from compomers is increased compared to neutral buffers[23]. Thus, although quantitatively, our results are not directly applicable for a specific human cavity wall, they could offer a model for the study of interaction between these materials and reference enamel. The use of synthetic saliva instead of a storage medium more similar to human saliva, however, represents a limitation of our study.

The development of an ideal restorative material that would provide a permanent seal with tooth structure has not yet been achieved. Problems associated with interfacial defects, like marginal discoloration and secondary caries, are the most often reported cause for clinical failure of tooth coloured restorations. In the design of this study, we took for granted that a gap exists between the restorative material and the enamel although the size of the gap in the experimental design would not exactly reflect the clinical situation.

In contrast to the situation that occurs in an actual restoration, the *in vitro* experiments can be expected to give lower values of fluoride uptake, because the materials are simply

placed after being set close to enamel, thus having the disadvantage of the fluoride being diffused prior to its contact with the enamel. A more desirable procedure for demonstrating fluoride transfer from a restoration to adjacent enamel would have been to place the material in contact with the enamel during setting time, similar to an *in vivo* restoration. The method described in our study was chosen because it would have been impossible to ensure complete removal of the material prior to the analysis of the enamel – a problem encountered by Forsten *et al.*[24] in a similar work.

The considerable amount of fluoride uptake evaluated from RMGIC is in agreement with the results of previous investigations, which also revealed significant amounts of fluoride when this kind of material was applied to enamel[18]. The degree of fluoride uptake by bovine enamel was similar with GIC and RMGIC, but substantially lower with compomers. Fluoride uptake was initially high from Fuji II LC, but then it declined rapidly, after the first 24 h diminishing to a significantly lower level within the next days. This pattern of fluoride uptake is in accordance with the fluoride release process of these materials and suggests that fluoride release occurs as two different processes, one short term and rapid and the other more gradual and prolonged[25]. Interestingly, among the RMGIC evaluated, only Fuji II LC showed this 'burst effect', whereas Vitremer did not. These two RMGICs, however, did not differ in terms of overall fluoride release. A 'burst effect' was also observed with both GICs, and there were no differences between Fuji IX and Ionofil Molar; however, this effect was of a lower magnitude compared with Fuji II LC. In contrast, no 'burst effect' was noted at the fluoride uptake from the compomers. The absence of this phenomenon is also revealed in surveys concerning fluoride release of these materials[26]. The pattern of uptake was similar for all three compomers with a gradual decrease in the amount of fluoride over time. Although there were no significant differences among compomers in all time intervals, the fluoride uptake from the compomers decreases accordingly from Compoglass F > F2000 > Dyract AP. Geurtsen *et al.*[23] measured the fluoride release by the same compomer products, after storage in solutions with various syntheses. They reported that fluoride release decreases in the same rank. Vercruysse *et al.*[27] also reported that Compoglass F released greater amounts of fluoride than Dyract AP.

The filler composition and the particle size influence significantly the fluoride release. Reducing the filler particle size can increase fluoride release because smaller particles have larger surface areas. This is why manufacturers have developed Dyract AP with a mean particle size of 0.8 μm, while predecessor Dyract has a mean particle size of 2.4 μm. As a result, Dyract AP has higher fluoride release than Dyract[28]. Fluoroaluminosilicate glass is the major component of the filler and the main source of fluoride in all of the materials in this study. Calcium is the essential part of the glass filler particles in Fuji II LC. It initiates the reaction with the acids or polyacids to form a cross-linked gel network. The Ca–Al–F silicate glass fillers are more soluble and weaker, and thus release more fluoride, than those fillers used in compomers that do not contain calcium[28]. In compomers, the initially light-polymerized material takes up water with time, and the carboxylic groups of the acidic monomer undergo an acid–base reaction with metal ions of the glass filler. This in turn leads to the formation of carboxylic salts and to the release of fluoride. This reaction is weak and results in lower fluoride release[27]. In Compoglass F and Dyract AP, barium and strontium are added, respectively, in the filler glass to increase radiopacity. Some other components such as ytterbium trifluoride (YbF_3) and strontium fluoride (SrF_2) are added, respectively, in these materials with the purpose to also increase the fluoride release. The YbF_3 glass particles could be dissolved by water by diffusing into and out of the material. A recent *in vitro* study, however, reported that only negligible amounts of fluoride were released from resin composites containing YbF_3[29].

In an effort to simulate the oral environment, bovine enamel slabs were suspended in a neutral environment. It has been demonstrated that fluoride release from fluoride-containing materials is increased when lowering the pH

of the storage medium[23,30]. The increased fluoride release at low pH values may be predominantly because of an enhanced hydrolytic degradation occurring at the matrix–filler interface. Intraorally, this could be the case especially with an established plaque-induced acidogenic challenge[31]. It would be of great interest to investigate if during an acidic attack, the increased release of fluoride leads to an increased incorporation into the enamel as well. An additional control could be the placement of the tooth surface in a solution of fluoride without any material being present in order to confirm the fluoride uptake from aqueous media. The purpose of this study, however, was limited to comparing the fluoride uptake from different materials, all of which were submerged in identical conditions of aqueous media; therefore, such an experiment was not conducted.

This study evaluated the amount of fluoride incorporated into the enamel, and no attempt was made to relate this to the theoretical anticariogenicity of the tissue. The question still remains: how much fluoride uptake by the enamel is enough to inhibit recurrent or secondary caries? While important clinically, this question has not yet had a definite answer[32].

Until the ideal fluoride concentration is determined, the use of dental materials with the greatest long-term fluoride release is preferable, especially in patients with moderate-to-high caries activity.

What this paper adds

- This paper adds knowledge about fluoride uptake by enamel from restorative materials.

Why this paper is important to paediatric dentists

- It is important for the clinician to know the variation in fluoride uptake from different aesthetic restorative materials to select the most suitable in every case.

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