

# Fluoride release for restorative materials and its effect on biofilm formation in natural saliva

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**Abstract** This study investigated the influence of natural saliva of varying pH on surface biofilm formation of restorative materials and how this influenced fluoride release. Columnar specimens of glass ionomer cement (GIC), resin modified glass ionomer cement (RMGIC), compomer, giomer and composite, were prepared, matured for 24 h at 37 °C and 100% humidity, lapped and then placed in natural stimulated saliva with a pH of 3.8 or 7.1. Fluoride release was determined daily using an ion-selective electrode. The surfaces of selected specimens were observed using Confocal Laser Scanning Microscopy in conjunction with a fluorescent dye. The surface biofilm formation and bacterial growth was most dominant under neutral conditions and on the surfaces of GICs compared with other materials. GICs released significantly higher amounts of fluoride than other materials. The results suggest that the increased fluoride release of GICs did not reduce the amount of bacterial growth and biofilm formation on the surfaces of these materials when stored in natural saliva.

## Introduction

The beneficial effects of fluoride in dentistry are well established [1] and the potential benefits of fluoride releasing restorative materials such as glass ionomer cements are well documented [2]. It has been reported that fluoride has an ability to modify the structure of dental hard tissues to increase resistance to acids [3] and to impart antibacterial properties, preventing the growth of harmful bacteria in dental plaque, which is considered as a type of natural biofilm deposited on the surfaces of both teeth and dental restorations [4].

Glass ionomer cements have two advantageous characteristics. They adhere to dental hard tissues [5] and release fluoride [6]. However, glass ionomer cements also have some well characterised disadvantages including a greater brittleness and solubility than resin matrix materials [7]. For this reason manufacturers have attempted to blend the properties of the parent groups (glass ionomer cement and resin-matrix composites) to produce new products such as resin-modified glass ionomers and compomers, which can be considered as hybrids of the parent groups [8].

Plaque adhesive properties may be an index with which to predict the anticariogenicity of materials [9]. Since biofilm formation may be influenced by the different components of dental restorative materials [10], it is of interest to investigate the effect of fluoride release on biofilm formation. Low concentrations of fluoride are not found to eliminate the bacterial populations from the oral cavity, but can modify the bacterial metabolism with a concomitant decrease in acid production [11]. In the laboratory it has been shown that GIs demonstrate an inhibitory effect on the growth and adherence of oral bacteria which has been related to the acid released (pH depression) from the cement while setting [12] and to the short-term release of fluoride [13].

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Little corresponding information exists so far regarding the fluoride release of different dental materials in natural saliva *in vitro*. Therefore the purpose of the present work was to determine the extent to which fluoride release into natural saliva occurs in the glass ionomer cements and hybrid products and, more significantly, the extent to which biofilms containing bacteria can be grown on the surfaces of materials and the relationship, if any, between changing the pH of saliva, fluoride release and biofilm formation. The hypothesis to be tested was that fluoride release under the different storage conditions would inhibit biofilm formation.

## Materials and methods

### Test specimens

Plastic moulds (2 × 2 × 24 mm) were used to make 12 columnar specimens from each of the five restorative materials shown in Table 1. The materials were chosen to represent products with expected high, medium and low fluoride release. All materials were mixed according to the manufacturers instructions and were placed in the moulds which were mounted on a polymethylmethacrylate plate covered with a Melinex film (Toray, Tokyo, Japan), covered with a second film and then compressed using a second plate. Light activated materials were activated for 40 s of overlapping surface exposure using a Visilux<sup>TM</sup>2 Visible Light Curing Unit (3 M ESPE dental products, Germany). The specimens were stored in their moulds for 24 h at 37 °C and 100% humidity. All the specimens were then removed from their moulds, dry-hand-lapped using 1200-grit silicon carbide paper, weighed and then placed in appropriate storage media.

### Saliva collection and fluoride measurements

Three volunteers donated a total of 120 ml of natural stimulated saliva on each day of the four weeks of the study. The saliva was collected (between 9:30 and 10:30 a.m.), mixed and used at two pH levels: Neutral pH 7.1 and acidified pH 3.8 (by adding lactic acid pH 1.8 to the collected saliva). The static low pH of 3.8 was used to simulate how the materials would perform or tolerate such extreme pH levels for a much longer period of time than the 4 weeks measured in this study.

Three millilitres of mixed saliva and two columnar specimens (to increase the surface area for fluoride release) were placed in each test tube to represent one test sample. Long thin test tubes were used which allowed the specimens to stand fully immersed in the 3 ml of natural saliva and ensured minimal contact with the test tube. After 24 h storage at 37 °C, all specimens were rinsed with de-ionised distilled water, dried with soft tissue paper and then placed in freshly collected saliva. Additional natural saliva which was used at weekends was collected from the three volunteers on the last working day (Friday), and stored in a freezer until use. Daily fluoride release in saliva was measured using an Ion Selective Electrode (ISE). A mixture of 3 ml of saliva and 0.3 ml of a buffer solution (TISAB III, Orion ionplus, Thermo Electron Corporation, Beverly, MA, USA) was used and the fluoride concentrations were measured using a calibrated fluoride ion specific electrode (96-09BN), used in conjunction with an ion meter (model 720A, Orion Research Inc. Beverly, MA, USA) to an accuracy of 0.1 ppm.

### Biofilm formation

At the end of the experiment the biofilm formation on the surfaces of randomly selected specimens was investigated

**Table 1** Materials used in this study

Type of material	Product	Composition	Manufacturer
Composite	UniFil-S	Fluoro-alumino-silicate glass, silica filler, UDMA, dimethacrylate, catalyst	GC, Japan
Giomer	Reactmer	F-PRG, glass filler, UDMA, HEMA, catalyst	Shofu, Japan
Compomer	Dyract AP	Sr–Al–Na–F–Si–glass, Strontium fluoride, UDMA, TCB resin, highly cross linking methacrylate-monomer	Densply, Germany
Resin modified GIC	Fuji II LC	Fluoro-alumino-silicate glass, polyacrylic acid, HEMA, TEGDMA, UDMA, water, catalyst	GC, Japan
Conventional GIC	Ketac-Fil plus aplicap	Calcium-alumino-fluoro-silicate glass, copolymer of acrylic and maleic acid, tartaric acid, water	3 M ESPE, Germany

UDMA = urethane dimethacrylate

F-PRG = full reaction type pre-reacted glass-ionomer

HEMA = 2-hydroxyethyl methacrylate

TEGDMA = triethylene glycol dimethacrylate

Sr–Al–Na–F–S–glass = Strontium–Aluminium–Sodium–Fluoro–Silicate

using Confocal Laser Scanning Microscopy. The specimens were carefully removed from the test tube and exposed to a freshly prepared solution of DAPI dye (33  $\mu\text{g}/\text{ml}$  of 4,6-Diamidino-2-Phenylindole Dihydrochloride dye diluted 100 times with sterile distilled water). The dye was allowed to remain on the specimens for two minutes, before immersing the specimens in a small petri dish half filled with distilled water. The water dipping lens (HCX APO, 40 $\times$ , NA 0.80) of a Confocal Laser Scanning Microscope (Leica TCS SP2 UV, Leica Microsystems, Heidelberg, Germany), at 40 $\times$  magnification, was used to investigate the central part of each specimen for the presence of biofilm formation and bacterial growth. The DAPI dye was excited at 360 nm wavelength while the fluorescence was determined between 400–580 nm. The method used gave only qualitative or at best semi-quantitative results. The change in weight of all specimens was determined after 4 weeks. The specimens were removed from their containers, dried with soft tissue paper, waved in the air for 15 s and then weighed.

### Statistical analysis

One way analysis of variance and Tukey's pairwise comparisons at a significance level of 0.05 were used to examine differences in fluoride release and percentage weight changes between materials.

## Results

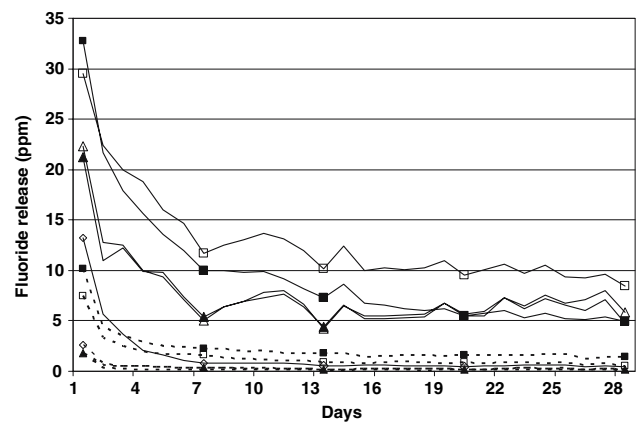
### Fluoride release

The daily fluoride release (ppm) in saliva of all materials under both acidified and neutral conditions is shown in Fig. 1. Under acidified conditions the glass ionomer cement (GIC) dominantly showed the highest release, while the Giomer showed the lowest. During the first two and a half weeks, the resin modified glass ionomer cement (RMGIC) released greater amounts of fluoride than both Composite and Compomer, but the difference reduced with time.

Acidified conditions produced a greater difference in the amount of cumulative fluoride release between the materials (Table 2). Under neutral conditions the RMGIC released significantly the highest amount of fluoride followed by GIC. Both GIC and RMGIC released significantly more fluoride ( $P < 0.001$ ) than the other three materials with the Composite giving the lowest release.

### Biofilm formation

Compared to the images of the control (baseline) surfaces of each material, the surface biofilm formation of all



**Fig. 1** The daily fluoride release (ppm) of: GIC  $\square$ , RMGIC  $\blacksquare$ , Compomer  $\triangle$ , Giomer  $\diamond$ , and Composite  $\blacktriangle$ , under acidified (full lines) and neutral (dotted lines) stimulated natural saliva storage conditions, by combining the daily means of fluoride readings of three samples for each group over 28 days. Although the measurements were carried out on a daily basis, measurements on day 1, 7, 13, 21, and 28 are highlighted in this figure to simplify the differentiation between the various groups

materials was greatest under neutral conditions (Fig. 2). Greater salivary precipitations appeared to cover the surfaces and obscured the clear vision of bacteria in the different layers of the biofilm. The biofilm thickness was determined by counting the number of laser scanning sections required to produce the three dimensional images. The biofilm formation appeared to be thicker on the surfaces of both GIC and RMGIC than on other materials. The maximum thickness for each of the materials was: 10.5  $\mu\text{m}$  for both GIC and RMGIC, 5.25  $\mu\text{m}$  for composite, 6.65  $\mu\text{m}$  for Giomer, and 8.4  $\mu\text{m}$  for Compomer. On the other hand, no distinctive differences were found on the surface images of materials under acidified conditions, with only a few scattered colonies of bacteria covering the surfaces. Special viewing glasses are required to properly appreciate the three dimensional surface images of each material, so to avoid any misunderstanding and/or confusion surface images of RMGIC are given as an example (Fig. 2). These images were chosen since the single images are representative of the overall three dimensional view for this material.

### Weight changes

Table 2 gives the mean (SD) percentage weight changes of materials under acidic and neutral conditions. Under neutral conditions, all materials increased in weight. GIC and Composite showed the smallest increase and were not significantly different ( $P > 0.05$ ). Under acidified conditions, the GIC and Composite showed a decrease in weight. The Compomer showed no significant change in weight,

**Table 2** The mean (SD) cumulative fluoride release (ppm) and % change in weight of restorative materials placed for 28 days in acidified and neutral stimulated natural saliva

	Materials	Composite	Giomer	Compomer	RMGIC	GIC
Acidified Saliva	Fluoride release (ppm)	210.7 (7.9) <sup>c</sup>	40.9 (0.8) <sup>d</sup>	211.0 (15.5) <sup>c</sup>	267.3 (9.0) <sup>b</sup>	358.9 (9.5) <sup>a</sup>
	% change in weight	− 0.45 (0.1) <sup>d</sup>	5.45 (0.2) <sup>a</sup>	0.01 (0.02) <sup>c</sup>	1.70 (0.08) <sup>b</sup>	− 1.10 (0.1) <sup>c</sup>
Neutral Saliva	Fluoride release (ppm)	5.9 (0.2) <sup>c</sup>	10.9 (0.4) <sup>c</sup>	10.1 (0.5) <sup>c</sup>	63.7 (4.1) <sup>a</sup>	39.6 (1.2) <sup>b</sup>
	% change in weight	0.56 (0.04) <sup>d</sup>	4.84 (0.29) <sup>a</sup>	1.22 (0.03) <sup>c</sup>	3.47 (0.17) <sup>b</sup>	0.63 (0.08) <sup>d</sup>

Negative value indicates weight loss

Same superscript letter within a row indicates no significant difference ( $p > 0.05$ )

whilst the RMGIC and particularly the Giomer showed a significant increase in weight even in acidified conditions.

## Discussion

The way in which fluoride release patterns altered with changing conditions in this work suggested that a number of competing factors contributed to the final outcome. Furthermore, attempting to relate changes in the fluoride release profile to the changing weight of the specimens was confounded by a number of factors. For example, fluoride release from a cement or resin may occur by diffusion from within the material or dissolution at the surface [14]. Both mechanisms would be expected to produce a weight loss. However, this weight loss may be partially or fully compensated by water absorption [15]. Changing the pH of the environment may shift the balance of competing processes. Under acidic conditions, the dissolution process is expected to be more dominant and this probably explains the greater fluoride release for each material under these conditions [16]. The surface biofilms formed in natural saliva are likely to have an inhibitory effect on both dissolution and diffusion and the fact that biofilms formed readily in neutral saliva but not in acidified saliva may explain the changes in material behaviour seen when conditions were altered. For example, in acidified conditions fluoride release from the glass ionomer cement (GIC) was significantly greater than from the resin modified glass ionomer cement (RMGIC), whilst in neutral conditions the reverse was true (Table 2). This indicated that diffusion dominated in the RMGIC but that dissolution was a more significant factor for the GIC.

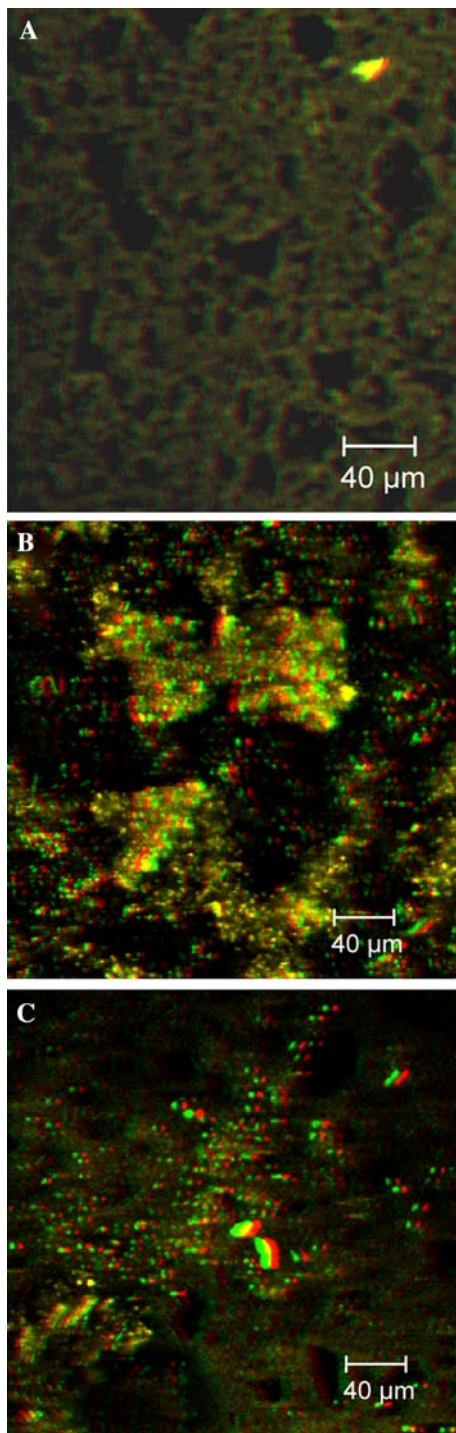
Under acidified conditions, the GIC released greatest amount of fluoride. This was thought to be due to the presence of a relatively soluble fluoride compound [17] surrounded by a labile glass ionomer matrix phase. This, in addition to the low water sorption may explain the decrease in weight of this material and to a lesser extent of the Composite, with time [18, 19]. The Compomer and Composite showed significantly lower fluoride release than the

GICs. This may partially be explained by the low solubility of the fluoride containing salts [20] and little or no acid base reaction and salt matrix formation [19]. The Giomer on the other hand released significantly the lowest amount of fluoride under acidified conditions. This may be explained by the presence of pre-reacted glass fillers that are fully consumed and converted to a hydrogel matrix which is more resistant to acid attack and suppressed the surface reaction in acidic solutions [21]. Both Giomer and RMGIC showed a significant increase in weight on storage, with the former material being significantly higher, under both storage conditions. The presence of hydrophilic poly HEMA chains in these materials plus a significantly thicker hydrogel layer surrounding the pre-reacted glass filler in the Giomer may be responsible for this observation [22].

Under neutral conditions, the RMGIC released significantly higher amounts of fluoride than the GIC. This is contrary to previously published findings using water as the storage medium [23, 24]. The formation of a surface biofilm on the GIC and RMGIC materials seems to shift the balance in favour of diffusion rather than dissolution as the dominant mechanism for fluoride release. The biofilm appears not to inhibit diffusion as much as dissolution and hence the RMGIC, in which water diffusion is encouraged by the presence of a poly HEMA hydrogel [25], was observed to release significantly more fluoride in saliva than the GIC, for which the surface dissolution responsible for some fluoride release, is inhibited by the biofilm.

The presence of fully reacted GIC type filler particles in the Giomer, in addition to HEMA hydrophilic monomer, may contribute to an increased water absorption and diffusion [26]. However, the fluoride release of the Giomer remained significantly lower than both GIC and RMGIC, and this suggested that in Giomer products, such as Reactmer, a significant amount of the original fluoride in the glass is lost during the manufacturing process. The water sorption characteristics of Compomers resembles more closely the composites than RMGIC, due to the copolymerisation of hydrophilic acidic monomers with comparatively more hydrophobic monomers such as (UDMA) in the resin network [25]. Both compomer and composite





**Fig. 2** The surface images of the RMGIC specimens examined under the Confocal Laser Scanning microscope in conjunction with DAPI dye, magnified  $\times 40$  times. **(A)** Control surfaces that were not exposed to saliva; **(B)** Surface images after being exposed to neutral saliva for 28 days; **(C)** Surface images after being exposed to acidified saliva for 28 days. Notice the bacterial colonies appearing as bright spots within regions of lower brightness of biofilm formation

materials released significantly lower amounts of fluoride than both GIC and RMGIC during the first two and a half weeks. The diversity of methods and experimental protocols used, prevent the direct comparison of the results of different studies. However, the results of other studies where similar materials were placed in aqueous media [27–30] also showed compomer to release significantly less fluoride than the RMGIC.

The investigation of the surface of each material revealed that the biofilm formed under neutral conditions was thicker and covered a greater proportion of the surface than under acidified conditions. Although it is reported that the GICs have an inhibitory, anti-bacterial and anti-adherent effect [31], the results of the present study did not support the assumption that bacteria would not adhere to the surface of GICs [32]. The decreased fluoride release after the first few days [33] and the increased surface roughness [34] could explain these findings. Hence, the antibacterial properties of GICs may only be significant during the initial fluoride burst.

## Conclusion

The difference in behaviour of the various materials may be related to the ratio of resin to salt in the material matrix. Hence, materials which are predominantly salt matrix (GIC and RMGIC) are more sensitive to changes in pH than products which have predominantly a resin matrix. Under both acidified and neutral storage conditions, the GICs released significantly greater amounts of fluoride than the Composite, Giomer and Compomer. However, this did not reduce the amount of bacterial growth and biofilm formation on the surfaces in neutral saliva suggesting that either fluoride is not a dominant factor in controlling biofilm formation or that its concentration is so low as to be ineffective.

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