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SCIENTIFIC SECTION

Effect of fluoride exposure on cariostatic potential of orthodontic bonding agents: an *in vitro* evaluation

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Abstract

Aims: The aims of this *in vitro* study were to compare the cariostatic potential of a resin modified glass ionomer cement (Fuji Ortho LC) to that of a resin control (Transbond) for bracket bonding and to compare the effect of extrinsic fluoride application on the cariostatic potential of each material.

Setting: *Ex vivo* study.

Materials and methods: Orthodontic brackets were bonded to 40 extracted premolars, 20 with Fuji Ortho LC and 20 with Transbond. The teeth were subjected to pH cycling, pH 4.55, and pH 6.8, over a 30-day period. Ten teeth bonded with each material were immersed in a 1000 ppm fluoride solution for 2 minutes each day. Fluoride release was measured throughout the study from all teeth. After 30 days, the teeth were assessed visually for signs of enamel decalcification.

Results: Significant differences in decalcification existed macroscopically between all four groups of teeth, with the exception of those bonded with Fuji Ortho LC alone compared with Transbond alone (P = 0.22), and Fuji Ortho LC alone compared with Transbond with added fluoride (P = 0.3). Fluoride release from Fuji Ortho LC alone fell to minimal values, but with the addition of extrinsic fluoride the levels fell initially and then followed an upward trend. There was minimal fluoride release, from Transbond alone, but with daily addition of extrinsic fluoride, subsequent fluoride release was increased. Significant differences existed in the amount of fluoride released between all groups, except comparing Fuji Ortho LC alone and Transbond with added fluoride.

Conclusions: The results of this study have indicated that with an *in vitro* tooth-bracket model, the creation of white spot inhibition could best be achieved by the use of a resin-modified glass ionomer cement, supplemented with fluoride exposure. The least protection was afforded by the composite control. The resin-modified glass ionomer cement alone and the composite with added fluoride demonstrated equivalent protection.

Index words:
Composite resin, fluoride release, resin-modified glass ionomer cement.

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Introduction

Enamel demineralization has a recorded prevalence of up to 96per cent in patients undergoing fixed appliance therapy.[1] Although mineral loss has been recorded after only 4 weeks,[2] the earliest clinical evidence of enamel demineralization is an opaque white spot. This lesion can be insidious and may lead to cavitation.[3]

The benefits of fluoride in the inhibition of carious lesion development and enhancement of lesion remineralization are well-documented.[2,3] Fluoride-releasing ability is, therefore, perceived as a desirable property of an orthodontic bonding agent, but laboratory studies[4,5] and clinical studies of fluoride releasing composites,[1] as means of preventing demineralization have proved equivocal.

Conventional glass ionomer cements, which release and absorb fluoride have been explored as possible means of bracket bonding. Although their potential to prevent demineralization has been identified in labora-

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tory studies,[5] clinical trials have produced conflicting findings.[6,7] In addition, these cements have unreliable and inferior adhesive properties compared to composite resins and, therefore, are not recommended for routine orthodontic bonding.[8]

Modified composites (compomers) and resin-modified glass ionomer cements have been developed, which have improved bond strength compared to conventional glass ionomer cements, while retaining the ability to release and uptake fluoride. Both of these newer cements have demonstrated favourable performance clinically for bracket bonding and prevention of enamel demineralization,[9,10] although a greater bracket failure rate and similar rates of demineralization have been recorded with a resin-modified glass ionomer in comparison to a composite resin.[11]

The magnitude of fluoride release from resin-modified glass ionomers, although product dependent, appears similar to that from conventional glass ionomers.[12,13] With each cement type, fluoride uptake and release has been demonstrated following exposure to fluoride toothpaste,[14] fluoride solution,[15,16] fluoride gel,[13] and fluoride mouthrinse.[14,17] Composite resin has also been shown to imbibe and release fluoride *in vitro* following exposure to a fluoride mouthrinse, but the level of fluoride release was considerably lower than that of a resin-modified glass ionomer cement, which had not been exposed to the fluoride solution.[18]

Irrespective of whether brackets are bonded with resin-modified glass ionomer or composite resin, it would appear, therefore, that daily exposure to a fluoride source offers the possibility of a sustained level of fluoride release from these bonding agents. Moreover, sustained low level fluoride release has been inferred to be more cariostatic than single high dose applications.[19]

To date, it appears that only one study has assessed demineralization in association with a resin-modified glass ionomer cement compared to a composite resin using a tooth-bracket model.[20] Specimens were pH cycled between synthetic saliva and an artificial caries solution, and brushed twice daily with a fluoridated dentrifice. The resin-modified glass ionomer, with or without exposure to a fluoridated dentifrice, demonstrated significantly greater protection against demineralization than the composite. No attempt, however, was made in that study to assess the actual levels of fluoride released during the different experimental protocols or to correlate the fluoride levels with cariostatic performance for each material.

The aims of this *in vitro* study were, first, to compare the cariostatic potential and levels of fluoride released by a resin-modified glass ionomer cement to that of a composite resin control for bracket bonding, and secondly, to compare the effect of extrinsic fluoride application on the cariostatic potential of each bonding agent. The null hypothesis tested was that there was no significant difference in the cariostatic potential and level of fluoride release of either bonding agent, with or without exposure to a fluoride solution.

Materials and methods

Tooth preparation, allocation and bonding procedure

Twenty pairs of premolars, extracted for orthodontic purposes, were obtained from patients aged between 12 and 16 years of age resident in a non-fluoridated area from birth. The teeth were cleaned thoroughly with a water and pumice slurry, and stored in 0.12 per cent thymol until required. The paired teeth always came from the same individual to standardize for caries experience/susceptibility and previous fluoride exposure. All teeth were examined macroscopically to ensure that the buccal surfaces were intact and caries-free.

An orthodontic bracket (0.022-inch pre-adjusted edgewise premolar bracket, 3M Unitek, Monrovia, CA, USA) was bonded to the mid-buccal aspect of each tooth. One tooth from each pair was bonded randomly with either a resin-modified glass ionomer cement (Fuji Ortho LC. G.C. America Inc., Chicago, Ill., Lot 210377) or a composite resin (Transbond, 3M Unitek, Monrovia, California, USA, Lot 090897 427). Teeth were subsequently painted with an acid-resistant nail varnish (Max Factor, Procter and Gamble, Surrey, UK) apart from 1 mm around the bracket periphery and were then allowed to dry at room temperature for 24 hours.

Demineralization, remineralization, and fluoride cycling

Each tooth was immersed individually in a plastic vial with 2 ml of demineralizing solution (2 mM $CaCl_2$, 2 mM NaH_2PO_4, addition of 50 mM CH_3COOH to pH 4.55) for 4 hours. After rinsing with deionized water and gentle drying to avoid cross-over contamination, the teeth were then placed in 2 ml remineralizing solution (2 mM $CaCl_2$, 2 mM NaH_2PO_4, addition of 0.1 M NaOH to pH 6.8) for 20 hours as described by Creanor *et al.* [21] Ten teeth bonded with each cement were exposed additionally on a daily basis to 1000 ppm fluoride for 2 min to simulate exposure to a fluoridated dentifrice. This resulted in four

experimental groups: Fuji Ortho LC with and without fluoride exposure, and Transbond with and without fluoride exposure. After each episode of fluoride exposure, teeth were rinsed thoroughly in deionized water and air dried before being returned to the remineralization solution. This procedure was repeated daily for 30 days.

Throughout the experimental period, 1 ml of the de- and remineralizing solutions for each bonding agent, with and without added fluoride was removed, using Oxford micropipettes, and stored in a plastic Eppendorf tube at –20°C until fluoride analysis was carried out. After 30 days the teeth were washed in deionized water, the nail varnish removed with acetone and the teeth were then stored individually in 0.12 per cent thymol until a visual assessment of decalcification was undertaken.

Measurement of fluoride release

The fluoride concentrations were measured on a daily basis, from days 1 to 30. All solutions were analysed for ionic fluoride concentration using an Orion combination fluoride ion-selective electrode (Orion Research Electrode No. 9609BN) attached to an ion analyser (Orion Research Expandable Ion Analyser EA940, Boston, Massachusetts, USA). One millilitre of the solution to be tested was added to 1 ml of low-level TISAB (total ionic strength adjustment buffer) in a microsample dish and the electrode and dish were then covered with 'cling film' to minimize evaporation. The solution was stirred during the measuring procedure on a non-heating magnetic stirrer and the electrode was allowed to stabilize for 5 min before recording the reading in millivolts. Between measurements, the electrode membrane was rinsed gently with deionized water.

Using standard solutions of fluoride, of various ionic concentrations, a calibration curve was generated with the aid of a computer software programme prior to each measuring session. Using this curve, fluoride measurements (in millivolts) were converted to corresponding fluoride concentrations in parts per million (ppm).

Assessment of decalcification

Teeth were debonded, using debonding pliers (3M Unitek, Monrovia, CA, USA), with care taken to ensure there was no damage to the enamel surface. Any bonding adhesive remaining on the buccal enamel was left in place. Assessment of decalcification was undertaken directly by examination under ×4 magnification by the same examiner using a modification of the caries index described by Geiger *et al.*,[22] which was adopted by Marcusson *et al.*:[6] 0 = no white spot formation; 1 = slight white spot formation; 2 = severe white spot formation; 3 = excessive white spot formation (cavitation). The examiner was blind as to which teeth had been bonded with each material, and whether or not fluoride had been added. To assess intra-examiner reliability scoring was repeated after 2 weeks, with the order of teeth changed on the second occasion.

Statistical analysis

For the decalcification scores, intra-examiner reliability for assessment of decalcification was made using Kappa statistics. Mann–Whitney tests were performed to assess if significant differences existed in the distribution of decalcification scores between the four groups. Follow-up multiple comparisons were made using the Bonferroni correction method. One-way ANOVA followed by Tukey comparisons tests were performed to assess if differences existed in the amount of fluoride released between the four experimental groups.

Results

Decalcification scores

On day 6, all teeth bonded with Transbond, without added fluoride, were withdrawn from the pH cycling regime, as cavitation was imminent. All teeth bonded with Fuji Ortho LC, without added fluoride, were removed at the same time to allow equivalent time comparisons. These teeth were scored for degree of decalcification and, subsequently, returned to the pH cycling regime for the remainder of the experimental period. After removal of the brackets and the acid-resistant nail varnish, the teeth were assessed for decalcification. The Kappa score for the assessment of decalcification was 0.85. This shows intra-examiner reliability to be very good.

On day 6, 60 per cent of teeth bonded with Fuji Ortho LC showed no signs of decalcification, with the remaining 40 per cent having a score of 1. All teeth bonded with Transbond had areas of decalcification that scored 2. All of the teeth bonded with Fuji Ortho LC exhibited white spots, of scores 1 or 2. The majority of teeth bonded with Transbond with added fluoride had white spots, score 1. Eighty per cent of those teeth bonded with Fuji Ortho LC with added fluoride remained intact macroscopically, with the remaining teeth showing slight white spot

formation. Comparisons of the distribution of decalcification scores are given in Table 1. All comparisons were statistically significant, except for Fuji Ortho LC versus Transbond (P = 1.0) and Fuji Ortho LC versus Transbond with added fluoride (P = 0.3).

Fluoride release (Table 2)

Transbond (Figure 1). Total fluoride release was virtually negligible, with on average 0.08 ppm fluoride released on day 1 and 0.06 ppm released on day 6. There was a slight reduction in the fluoride released over the 6-day period. Very little differences existed in the amount of fluoride released during the de- and remineralization periods.

Transbond and extrinsic fluoride (Figure 2). The total amount of fluoride released on day 1 was 0.19 ppm (SD = 0.000) and the levels followed an upward trend towards day 7, with a concentration of 0.41 ppm (SD = 0.089) ppm fluoride. The amount of fluoride then fell rapidly towards day 10 (0.18 ppm, SD = 0.11) followed by a more gradual fall towards day 15 (0.1 ppm, SD = 0.003). This was followed by an increase in the fluoride levels recorded, with a gradual increase towards day 30, with a final average fluoride release of 0.35 ppm (SD = 0.12). The fluoride released during the remineralization period followed the same general trend as that of the cumulative fluoride release. During the demineralization period, however, the fluoride released remained relatively constant throughout the 30-day trial period, with no more than 0.1 ppm fluoride released at any stage.

Table 1 Distribution of decalcification scores for each of the four groups

	Decalcification score				
	0	1	2	3	Total
Fuji Ortho LC (day 6)	6	4	0	0	10
Fuji Ortho LC (day 30)	0	4	6	0	10
Fuji Ortho LC + fluoride	8	2	0	0	10
Transbond (day 6)	0	0	10	0	10
Transbond + fluoride	2	7	1	0	10

Statistical comparison between each protocol.

Materials for comparison	*P* values
Fuji Ortho LC *v.* Transbond	1.00
Fuji Ortho LC *v.* Transbond & fluoride	0.30
Fuji Ortho LC *v.* Fuji Ortho LC & fluoride	0.012
Fuji Ortho LC and fluoride *v.* Transbond	0.0006
Fuji Ortho LC and fluoride *v.* Transbond and fluoride	0.05
Transbond *v.* Transbond and fluoride	0.042
Transbond *v.* Fuji Ortho LC [day 6]	0.006

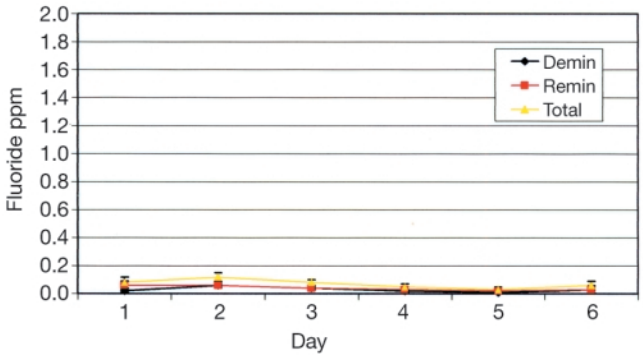

Fig. 1 Fluoride release–Transbond (days 1–6).

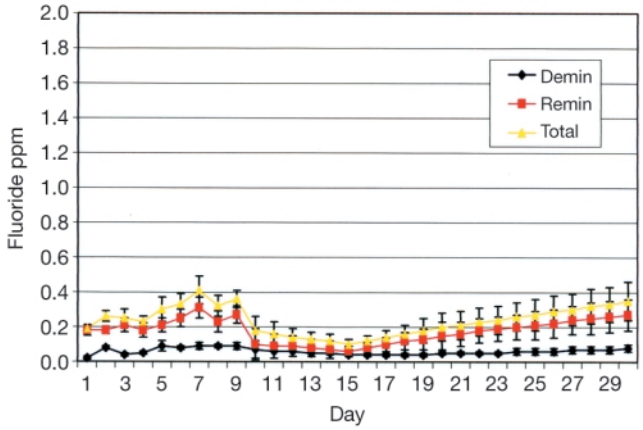

Fig. 2 Fluoride release–Transbond and extrinsic fluoride (days 1–30).

Table 2 Mean cumulative fluoride release (ppm, SD) for each experimental group during demineralization and remineralization periods

	Transbond (6 days)	Fuji Ortho LC (30 days)	Transbond and fluoride (30 days)	Fuji Ortho LC and fluoride (30 days)
Demineralization	0.22 (0.045)	2.55 (0.68)	1.79 (0.11)	5.14 (0.34)
Remineralization	0.23 (0.058)	3.37 (1.25)	5.26 (0.36)	10.62 (0.40)
Total	0.45 (0.051)	5.92 (0.98)	7.06 (0.45)	15.76 (0.88)

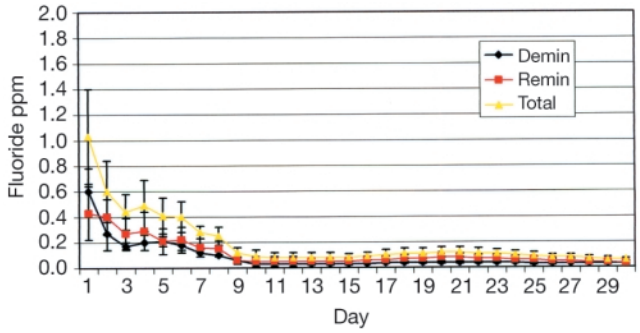

Fig. 3 Fuji Ortho LC–no extrinsic fluoride (days 1–30).

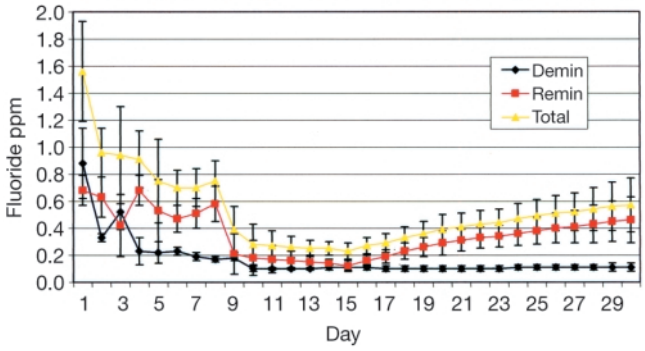

Fig. 4 Fluoride release–Fuji Ortho LC and extrinsic fluoride (days 1–30).

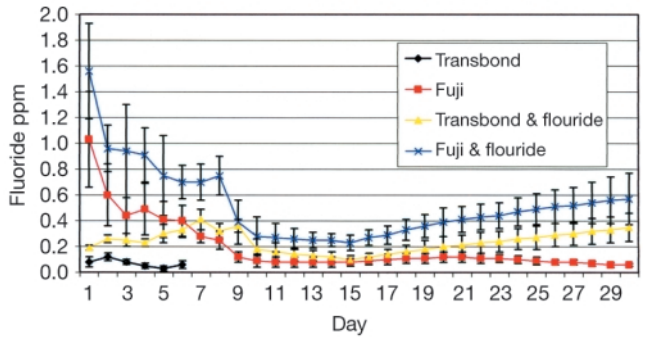

Fig. 5 Summary of the total fluoride released from Fuji Ortho LC and Transbond, with and without extrinsic fluoride (days 1–30).

Fuji Ortho LC (Figure 3). Total fluoride release from Fuji Ortho LC followed a downward trend, with a rapid fall from 1.03 ppm (SD = 0.37) fluoride on day 1, to 0.4 ppm (SD = 0.19) on day 3, and a more gradual reduction towards day 10 with an average fluoride release of 0.09 ppm (SD = 0.06). Thereafter, the fluoride release remained relatively constant. This graph shows the fluoride released during both the de- and remineralization periods and the cumulative fluoride release over the 30-day trial period. There was slightly more fluoride released during the remineralization period, particularly in the first few days, except on day 1.

Fuji Ortho LC and extrinsic fluoride (Figure 4)
Fuji Ortho LC with added fluoride released the most fluoride of all 4 groups, with an average of 1.56 ppm fluoride (SD = 0.39) released on day 1. After a sharp fall to 0.96 ppm (SD = 0.18) on day 2, the fluoride levels fell more gradually towards day 8 (0.75 ppm, SD = 0.17) and then fell sharply again until day 10 (0.28 ppm, SD = 0.15). During the remineralization period, fluoride release remained relatively constant until day 15 and then increased gradually towards day 30, with an average of 0.57 ppm (SD = 0.21) fluoride released on that day. The pattern of fluoride release during the demineralization period did not show the same gradual reduction over the first 8 days. Instead, the levels of fluoride release initially fell rapidly, and after day 4 remained at a relatively constant level.

Combined fluoride release graph (Figure 5). A graph showing the total fluoride released from all four sub-groups allows direct comparisons to be made about the average fluoride release patterns of the test and control materials. It is evident that the addition of fluoride changes the fluoride release profiles of both Fuji Ortho LC and Transbond.

Statistical analysis of total fluoride release

Using ANOVA, significant differences existed in the average fluoride released from the two materials, with and without added fluoride ($P < 0.001$; Table 2). A follow-up Tukey multiple comparisons test indicated significant differences between all groups, except for fluoride release from Fuji Ortho LC alone versus Transbond with extrinsic fluoride.

Fluoride release and decalcification scores (Table 3)

In combining the results of average total fluoride release with the average decalcification score, it is apparent that the greater the amount of fluoride released, the lower the mean decalcification score recorded (Table 3; note the results for Transbond are after 6 days, all other results are after 30 days).

When comparing the individual results of fluoride released from each tooth and its decalcification score, no definite relationship existed between the two variables,

Table 3 Total fluoride released (ppm, SD) versus the mean decalcification scores

	Mean cumulative fluoride release (ppm) (SD)	Mean decalcification score
Transbond	0.45 (0.051)	2
Fuji Ortho LC	5.92 (0.98)	1.6
Transbond and fluoride	7.06 (0.45)	0.8
Fuji Ortho LC and fluoride	15.76 (0.88)	0.2

except in the case of Fuji Ortho LC with added fluoride. This was the only group in which a lower fluoride release value was associated with a higher decalcification score. In all other groups, the amount of fluoride released did not appear to correlate with the decalcification score.

Discussion

The results of this study have indicated that with an *in vitro* tooth-bracket model, the creation of white spot inhibition could best be achieved by the use of a resin-modified glass ionomer cement, supplemented with fluoride exposure. The least protection was afforded by the composite control. The resin-modified glass ionomer cement alone and the composite with added fluoride demonstrated equivalent protection.

Although other studies have used a tooth-bracket model,[4,23–26] it appears that few studies have subjected specimens to recurrent fluoride exposure.[5,20] The latter study employed a tooth-bracket model along with a pH cycling regime to assess the potential caries inhibition of a resin-modified glass ionomer cement compared with a composite control. In that study, like the one reported here, the potential benefit of additional fluoride exposure to the cariostatic properties of the intrinsic fluoride of these materials was assessed.

No previous study has assessed the levels of fluoride that have been released into both the de- and remineralizing media. This is an important consideration, as it is now well documented that fluoride enhances remineralization, as well as reducing the demineralizing potential of any acidic challenge. For Fuji Ortho LC without added fluoride, the fluoride release pattern into both the de- and remineralizing media was consistent with other studies, which have reported fluoride release into either water or an artificial saliva from a similar product.[13,27,28] An initial burst effect was witnessed followed by a fall to baseline around 5–10 days. Transbond released only negligible amounts of fluoride at all time points.

With the addition of fluoride, the pattern of release for both cements into the demineralization solution remained similar to the protocol without the addition of fluoride. The pattern of fluoride release into the remineralizing solution, however, deserves further comment. Up to day 15 the pattern of fluoride release is similar to the release into the demineralizing solution, but thereafter it shows a steady increase up to day 30 for both materials. Why this occurs is not readily apparent. Possible sources of this increased level of fluoride are three-fold. First, fluoride from the additional fluoride exposure may have been taken up by the porous demineralized enamel and then released. Secondly, fluoride may have been released from the outer enamel surface. Thirdly, the cement itself may be the source of fluoride.

When the mean cumulative fluoride release from each of the four groups is compared with the mean decalcification score, it is evident that in this protocol the level of fluoride released from Fuji Ortho LC alone was inadequate to combat the severity of the demineralizing process. With the addition of fluoride, the mean decalcification scores for both materials were reduced considerably. The effect, however, was greatest with Fuji Ortho LC. Interestingly, for Transbond, which is a composite resin, the addition of fluoride appeared to halve the mean decalcification score. This result supports the clinical advice that patients with fixed orthodontic appliances adhere to regular toothbrushing with a fluoridated dentifrice and/or fluoride mouthrinsing. It is important to bear in mind that even low fluoride concentrations, similar to those observed here, could have a retarding effect on the demineralization process and are therefore of potential clinical significance.

Although the increase in fluoride concentration adjacent to an orthodontic bonding agent is important, the clinical relevance remains unclear as the ideal level of fluoride in enamel required to confer protection from demineralization is unknown.[29]

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