Long-term antibacterial properties of fluoride-releasing orthodontic bonding adhesives

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ABSTRACT

Objectives: The aim of this study was to compare antibacterial properties of 3 different fluoride-releasing orthodontic adhesives during three months by using the direct contact test (DCT).

Materials and Methods: The materials tested in the present study were Transbond Plus (3M Unitek, Monrovia, USA), Light Bond (Reliance Ortho Prod. Inc. Itasca, IL, USA), and Kurasper F (Kuraray Medical Inc., Okayama, Japan). Eight specimens of each material type were prepared for estimation. For the DCT, wells of 96-microtitre plates were coated with each of three bonding adhesives. Eight uncoated wells, using identical inocula size, served as a positive control (bacteria with medium). A *Streptococcus mutans* suspension was placed on the surface of each specimen that was held for 1 hour at 37°C. Bacterial growth was monitored for 16 hours with a temperature-controlled microplate spectrophotometer. The kinetics of the growth in each well was recorded continuously at 650 nm in every 30 minutes. Three additional measurements were performed on these tested materials after aging for 1 week, 1 month and 3 months. One-way ANOVA and Tamhane's T2 multiple comparison tests were applied to the data. The level of significance was set as $p<0.05$.

Results: The DCT showed that there were significant differences found in freshly mixed samples between four groups ($p<0.001$, F=20.901). Freshly mixed samples of Transbond Plus showed antibacterial activity ($p<0.001$). Kurasper F and Light Bond did not differ from positive control. In the measurements of aged specimens, all materials showed an increase in the logarithmic growth rate of *Streptococcus mutans*.

Conclusion: Only Transbond Plus showed antibacterial properties in freshly mixed specimens. However, none of the tested orthodontics composites showed antibacterial properties after aging.

Key Words: Antibacterial agents, direct contact test, fluorides, orthodontic adhesives, *streptococcus mutans*.

INTRODUCTION

Orthodontic bands and brackets are highly susceptible to biofilm formation that threatens the integrity of the teeth and the soft tissues by means of decalcification and periodontal disease. Professional tooth cleaning, local application of fluorides and use of antimicrobial mouth rinses are basic strategies to prevent the clinical side-effects of the fixed orthodontic treatment.[1,2] Enamel decalcification can

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also be reduced using sealants.[3] *Streptococcus mutans* is considered to be the primary organisms responsible for enamel demineralization. There is a significant increase in the salivary and plaque levels of these acidogenic, aciduric bacteria in patients undergoing fixed appliance treatment.[4-8] Studies have shown that decalcification is a significant risk in orthodontic patients with rates reported from 15% to 75%.[9]

Because none of the preventive strategies have the potential to inhibit bacterial adhesion on the bracket surfaces, incipient carious lesions have been demonstrated in vivo around the brackets after only 4 weeks.[10-12] The results of decalcification vary from no evident

change to white spots on the enamel of teeth, or even cavitation. They appear as unsightly lesions on previously sound teeth at the end of orthodontic treatment.

Fluoride is the most anticariogenic agent known to date.[12] A variety of mechanisms are involved in the anticariogenic effects of fluoride, including the inhibition of demineralization, the enhancement of remineralization, the prevention of pellicle and plaque formation, and the inhibition of microbial growth and metabolism.[13]

Researches have recommended that topical fluorides are likely to decrease decalcification during active orthodontic phase.[14,15] One method of fluoride application is to incorporate it into the adhesive.[14] Fluoride-releasing bonding materials not only decrease decalcification, but also stimulate the development of a calcium fluoride layer on enamel surfaces adjacent to the brackets. This layer has a potential reserve to release fluoride ions slowly during the demineralization and remineralization processes. It also serves as a barrier against acid challenge.[16,17]

Resin composites may contain fluoride in a variety of forms, such as inorganic salts, leachable glasses or organic fluoride. Thereby, not only the amount of fluoride, but also the type and particle size of the fluoridated filler, the type of resin, silane treatment and porosity might be important factors contributing to fluoride release.[18,19] The direct contact test (DCT) quantitatively measures the effect of direct and close contact between the test microorganism and the tested materials, regardless of the solubility and diffusibility of their components.[20]

Some studies[21-24] have presented the antibacterial properties of different resin materials. There have been no reports that investigated the long-term antibacterial effects of fluoride releasing orthodontic composites. The aim of this in vitro study was to investigate the antibacterial properties of 3 different fluoride-releasing orthodontic adhesives by using the DCT during 3-month aging period.

MATERIALS AND METHODS

The fluoride-releasing adhesives used in this study are shown in Table 1. *Streptococcus mutans* (Refik Saydam National Public Health, 676, Ankara,Turkey) was grown aerobically to late logarithmic or early stationary phase from frozen stock cultures in brain hearth infusion (BHI) broth containing 0.5% bacitracin for 48 hours at 37ºC before applying it to the specimens according to the experimental design.

Direct Contact Test (DCT)

The DCT[20] is based on the turbidometric determination of bacterial growth in 96-well microtitre plates (96-well, flat-bottom Nunclon; Nunc, Copenhagen, Denmark). The kinetics of the outgrowth in each well was recorded continuously at 650 nm in every 30 minutes, using a temperature-controlled spectrophotometer (µquant, Bio-Tek Instruments Inc., Winooski VT, USA).

In all wells, the sidewall was coated with evenly measured amount of tested materials [Transbond Plus (3M Unitek, Monrovia, USA), Light Bond (Reliance Ortho Prod. Inc. Itasca, IL, USA) and Kurasper F (Kuraray Medical Inc., Okayama, Japan)] while the plate was held vertically. Light-cured primers were brushed on and applied to the sidewalls of the wells. A thin film thickness was provided with care. To ensure a steady surface area, a thin coat adhesive was applied with a micro-brush. The test samples were light polymerized due to the manufacturers' recommendations. Special care was taken to avoid the material flow to the bottom of the well, which would interfere with the light path through the microplate well and would result in a false reading.

A 10µL (10^6 CFU) bacterial suspension was placed on each sample and incubated while the plate remained in a vertical

position. After incubation for 1 hour in a humid atmosphere at 37°C, evaporation of the liquid was evident and direct contact between bacteria and the tested material surfaces ensured. Then, 220 µL of BHI broth was added to each of the wells and the plate was placed in the spectrophotometer. The bacterial outgrowth was estimated after direct contact with the tested material on the basis of the changes in the readings of optical density at 650 nm, which were recorded automatically by the spectrophotometer every 30 minutes for 16 hours.

Table 1. Orthodontic composites used in this study.

Brand	Components	Chemical Composition	Manufacturer	Lot number
Light Bond	Light bond	Bisphenol A Diglycidylmethacrylate. Urethane Dimethacrylate, Triethyleneglycol Dimethacrylate, Hydrofluoride Methacrylate	Reliance Ortho Prod. Inc., Itasca, IL, USA	0911076
	Paste	Bisphenol A Diglycidylmethacrylate, Silica-crystalline, Silica, Fused Amorphous Silica, Sodium Fluoride		09066775
Kurasper F	F bond	Methacryloyl Fluoride-MMA copolymer, 2-Hydroxyethyl-Methacrylate, Silanated Colloidal Silica, Sodium Fluoride, Dimethacrylates	Kuraray Medical Inc., Okayama, Japan	00041C
	Paste	Dimethacrylates, Silanated Barium Glass filler, Silica filler		00052B
Transbond Plus	Transbond plus Self-Etching Primer	Water, Methacryloyl Phosphate Monomer, Phosphine Oxide, Fluoride Complex	3M Unitek, Monrovia, USA	361980
	Transbond Plus Paste	Silane-treated Quartz, Glass reacted with Hydrolyzed Silane, Polyethylene Glycol Dimethacrylate, Citric Acid Dimethacrylate Oligomer, Silane-Treated Silica, Bisphenol A Diglycidyl Ether Dimethacrylate		N104121

Eight uncoated wells, using identical inocula size, served as a positive control (bacteria with medium) so that bacterial outgrowth could be monitored in the absence of the tested material. To show no microbial growth was derived from BHI broth, the negative control group consisted of a set of wells containing 220 µL of BHI broth.

Serial measurements were performed after the tested materials were aged for 1 week, 1 month and 3 months. Aging was arranged with 250 µL of phosphate-buffered saline (PBS), which was replaced every 48 hours.

Statistical Analyses

Bacterial growth curves were evaluated for each well. Using the equation y=ax+b, a regression line on the ascending linear portion of the curve was estimated. This equation provided the value of the slope corresponding to the growth rate. Descriptive statistics, including the mean, standard deviation, minimum and maximum values were calculated for each of the three groups. Shapiro-Wilks normality and Levene's variance homogeneity tests were applied to the data. The data did not show normal distribution and there were not homogeneity of variances among the groups. One-way ANOVA and Tamhane's T2 multiple comparison tests were applied to the data.

The level of significance was set as p<0.05. All statistical analyses were performed using the Statistical Package for Social Sciences, version 13.0 for Windows (SPSS for Windows 13.0; SPSS, Chicago, Illinois, USA).

RESULTS

Figure 1-4 present the growth of *Streptococcus mutans* in a 96-well microtitre plate. Each point on the growth curve is the average of the optic density measured in eight wells at any given time. Each curve includes 32 measurements taken within 16 hours. There is no significant microbial growth observed in the negative control group in all measurements ($p=0.007$, fresh sample; $p<0.001$, 1 week; $p=0.001$, 1 month; $p<0.001$, 3 months).

There are significant differences in freshly mixed samples between four groups ($p<0.001$, F=20.901). Freshly mixed samples of Transbond Plus showed antibacterial activity ($p<0.001$). Kurasper F ($p=0.878$) and Light Bond ($p=0.747$) did not differ from positive control (Figure 1).

In the serial experiments with 1 week, 1 month and 3 month- aged specimens, all materials showed an increase in the logarithmic growth rate of *Streptococcus mutans* (Figure 2-4).
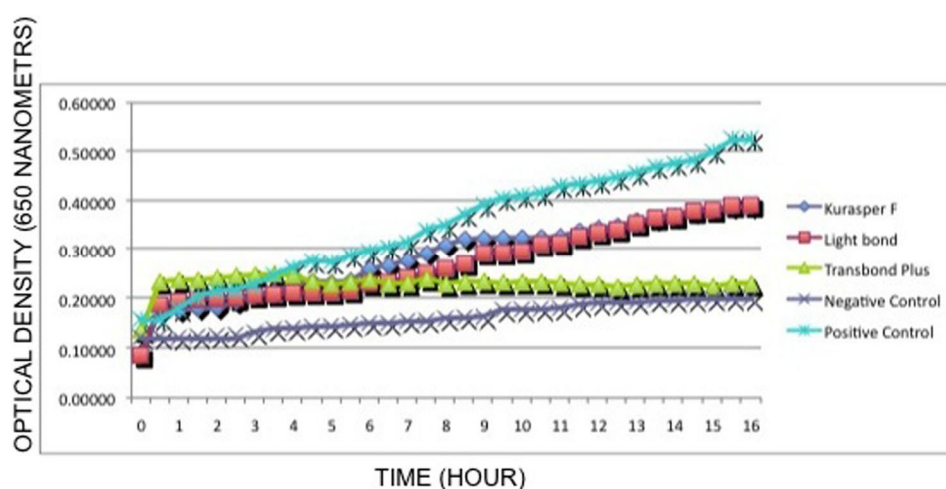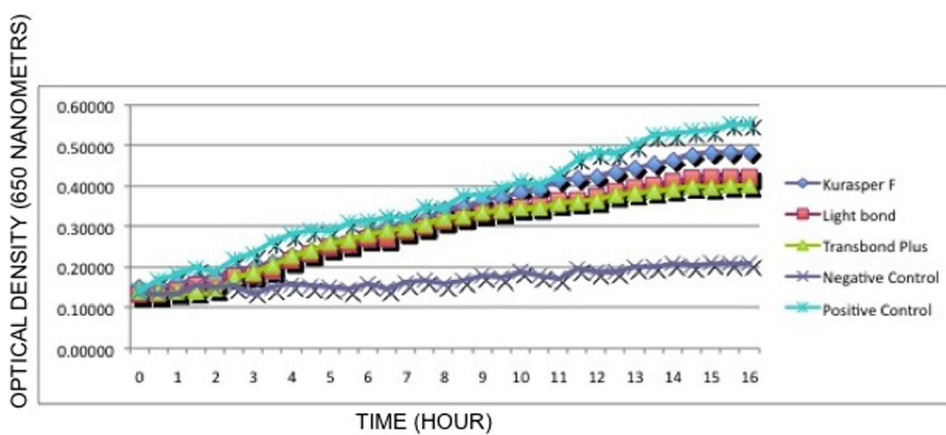

Figure 1. Bacterial growth after direct contact with fresh material. Each point on the growth curve represents the average optical density measured at 650 nm in eight wells.
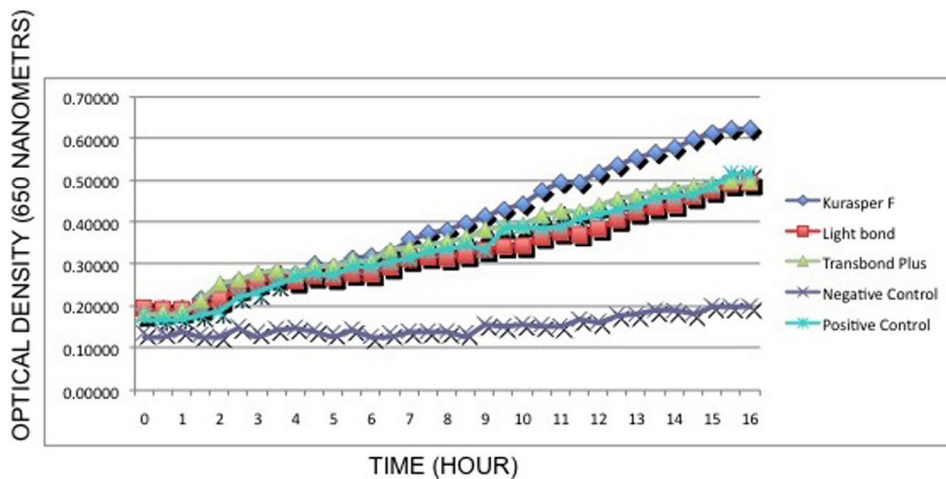
Figure 2. Bacterial growth after direct contact with 1 week aged material. Each point on the growth curve represents the average optical density measured at 650 nm in eight wells.
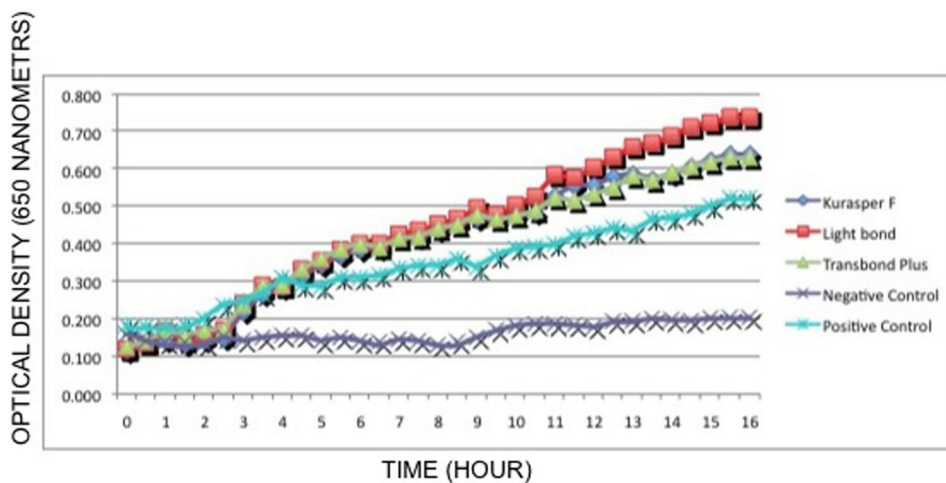
Figure 3. Bacterial growth after direct contact with 1 month aged material. Each point on the growth curve represents the average optical density measured at 650 nm in eight wells.

Figure 4. Bacterial growth after direct contact with 3 month aged material. Each point on the growth curve represents the average optical density measured at 650 nm in eight wells.

DISCUSSION

The oral situations of orthodontic patients experiences some changes, such as pH reduction, increased accumulation of food particles available for *Streptococcus mutans* collection.[5] Such changes may contribute to the development of the decalcification lesions frequently observed at the end of orthodontic treatments.[5,25] The high prevalence of post-orthodontic treatment decalcifications is caused by the increment of *Streptococcus mutans* adjacent to the orthodontic appliances.[4,26] *Streptococcus mutans* has been widely used for testing the antimicrobial activity of restorative materials in dentistry.[20,27,28] Growth of the *Streptococcus mutans* was investigated in the present study using the DCT. Both the agar diffusion test (ADT) and the DCT has been used to analyze the antibacterial properties of dental materials.[6,23,27] Lewinstein et al.[21] found that the DCT was more effective than ADT in order to detect the antibacterial properties. With a temperature-controlled spectrophotometer and the appropriate software, the DCT allows the researchers to evaluate the number of viable bacteria at the end of the direct contact incubation period using calibration growth curves.

Dental plaque is a microbial biofilm formed by organisms tightly bound to a solid substrate and to each other by means of an exopolymer matrix. Bacteria exhibit different properties when contained within a biofilm.[7,8] Such biofilms are characterized by several cell layers, with bacteria stratification arranged by metabolism and aerotolerance. When the orthodontic bands and brackets are applied, new attachment sites for microorganisms would be created, and the appliances may lead to opportunities for non-oral microorganisms to be maintained longer or even to colonize in the mouth.[29] All strategies must be focused on reducing or removing oral biofilm and aimed at strengthening the resistance of teeth against bacterial metabolic waste products, i.e. bacterial acids.[1]

While the amount of fluoride, type and particle size of the fluoridated filler, the type of resin, silane treatment, and porosity might be important factors contributing to fluoride release[18,19] and these may differ from brand to brand, in the present study we aimed to compare the effectiveness of three different fluoride-releasing composites. The analyses of our measurements revealed that Transbond Plus had antibacterial activity in only fresh material. The antibacterial characteristics of orthodontic adhesives were compared in only one study in the scientific literature. Matalon et al.[22] examined different types of orthodontic materials (cement-composite and those that release fluoride and not) and evaluated the antibacterial properties of conventional glass ionomer cement (CX-Plus), resin forced glass ionomer cement (GC Fuji Ortho LC), composite resin (Transbond XT) and fluoride-releasing composite resin (Transbond Plus) using the DCT. The researchers stated that while GC Fuji Ortho LC and Transbond Plus showed the antibacterial properties in fresh material, Transbond Plus did not show the antibacterial properties after aging. Our findings are similar to the result of Matalon et al.[22]

To obtain the antibacterial effects, on tooth structures, several methods such as bonding systems containing antimicrobial agents,[30,31] fluoridated toothpastes, mouth rinses and gels have been proposed. However, toothpastes, mouth rinses and gels greatly depend on patient compliance and provide only intermittent protection against demineralization.[32] Systematic usage of fluoride varnish seems to reduce lesion formation in orthodontic patients.[33] Besides, fluoride releasing cements, elastomeric modules and chains could be administered during fixed appliance treatment.[34]

The number of fluoride-containing composites for the orthodontic purposes increases each year, and both fluoride containing glass ionomer and composite

adhesives have been shown to reduce incipient carious lesions in patients wearing fixed appliances.[14,35] Fluoride has some important effects, including the inhibition of demineralization, the enhancement of remineralization, and the inhibition of microbial growth and metabolism.[13] Remineralization by releasing fluoride is important, but the antibacterial property of fluoride is a direct strategy for eliminating the cause of dental caries.[36] The antibacterial properties of Durelon, Ketac-cem, and Harvard cements were evaluated by using the DCT.[21] The authors found that Durelon and Harvard cement had significant antibacterial effects. The antibacterial activity of several glass ionomer cements, dentin bonding systems and luting agents were investigated using the ADT. Marked antibacterial activity was revealed with the glass ionomer cement, whereas amalgams, composites, luting agents, and bonding systems did not affect the bacterial growth.[37] Feuerstein et al.[24] indicated that four different tested self-etching adhesive systems (AdheSe, Adper Prompt L-Pop, Clearfil Protect Bond and Xeno III) had a bactericidal effect on *Streptococcus mutans* within 16 hours by using the DCT.

In the present study, bacterial growth in fresh material was evaluated for 16 hours. For the aged material three measurements (one week, one month and three months later) were performed. No bacterial growth inhibition was determined during aging periods. The levels of fluoride release at a constant rate are required for effectiveness. The fluoride release was high on the first day, fell rapidly over the next day, then gradually decreased to a nearly constant level by the end of the third day.[17] Because orthodontic patients have routine examining appointments, elastomeric ligature ties impregnated with fluoride would be a solution to provide a long-term low-dose fluoride release.[38] This method of fluoride delivery would eliminate any need for patient compliance and would replace fluoride at each orthodontic visit.

Fluoride-releasing materials may act as a fluoride reservoir and may increase the fluoride level in saliva and plaque. The results of the studies, which compared the rates of fluoride release over time from the orthodontic materials, showed that the mean fluoride releasing rate declined with time.[39,40]

The fluoride-releasing rate of resin materials can increase after the topical fluoride is applied. Resin materials can recharge the fluoride and release it again into the environment. In general, materials with a higher initial rate of fluoride release have a higher recharge capability.[19,41] However, fluoride release from aged and re-fluoridated specimens did not reach the initial rate of fluoride release.[41]

CONCLUSIONS

1. Freshly mixed samples of Transbond Plus showed the antibacterial activity.

2. None of the materials showed the antibacterial activity after aging protocol.

ACKNOWLEDGEMENT

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