Antibacterial Effects of Several Current Orthodontic Materials against *Streptococcus mutans*

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ABSTRACT

The aim of this study was to examine the antibacterial effect of several current orthodontic materials against a certain oral bacterium. The antibacterial activities of six orthodontic composite resins (Transbond LR, Light Cure Retainer (LCR), Light Bond, System 1+, Kurasper F, Transbond XT adhesive), two orthodontic bonding materials (Transbond XT primer and System 1+ activator) and two glass ionomer cements (GIC) [Multicure Glass Ionomer and Ketac Cem GIC] were evaluated against Streptococcus mutans. *The hard materials were put into the Teflon mould. The liquid materials were put on a paper disc. All materials were handled under aseptic conditions and placed on agar culture plates. All plates were incubated at 5% CO_2 and 37 °C for 48 hours. The bacterial growth inhibition zones including the diameter of the sample were measured in millimetres. As a result of this study, the multicure GIC showed the highest antibacterial effectiveness, but no inhibition zones were noted for ketac cem GIC. The light bond adhesive of the Reliance orthodontic bonding system produced high antibacterial effect against* S mutans, *while the Reliance composite (LCR) did not show any antibacterial effect (*$p < 0.05$*). Both composite and primer of the transbond XT system demonstrated significant antibacterial effect against the test bacterium when compared to transbond LR (*$p < 0.05$*). Among the materials tested, kurasper F, Ormco system 1+ and system 1+ activator showed slight or no inhibitory effect against the test bacterium in this study. In patients who have relatively high salivary levels of* Streptococci mutans *before treatment, the multicure GIC, the Reliance light bond adhesive, and transbond XT system which had high level antibacterial properties could be applied.*

Keywords: Antibacterial effect, orthodontic materials, *Streptococcus mutans*

Efectos Antibacterianos de Varios Materiales Ortodóncicos Usuales frente al *Streptococcus mutans*

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RESUMEN

El objetivo de este estudio fue examinar el efecto antibacteriano de varios materiales ortodóncicos actuales sobre cierta bacteria oral. Se evaluaron las actividades antibacterianas frente al Streptococcus mutans, *de seis resinas compuestas (composites) ortodóncicas (Transbond LR, Light Cure Retainer (LCR), Light Bond, System 1+, Kurasper F, Transbond XT), dos adhesivos ortodóncicos (Transbond XT y Sistema 1+ activador) y dos cementos de ionómeros de vidrio (GIC) [ionómero de vidrio Multi-cure y Ketac Cem GIC]. Los materiales duros fueron puestos en el molde de Teflón. Los materiales líquidos fueron puestos en un disco del papel. De todos los materiales fueron manipulados bajo condiciones asépticas y pusieron en el agar cultive los platos. Todos las placas fueron manipuladas en condiciones asépticas, y colocados en placas de cultivo agar. Todas las placas fueron incubadas a 5% CO_2 y 37 °C durante 48 horas. Las zonas de inhibición del crecimiento bacteriano, incluido el diámetro de la*

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muestra, fueron medidas en milímetros. Un aspecto del resultado de este estudio, fue que el Multi-Cure GIC mostró la efectividad antibacteriana más alta, en cambio no se observó ninguna zona de inhibición para el ketac cem GIC. El adhesivo Light Bond del sistema Reliance para la adhesión ortodóncica, produjo altos efectos antibacterianos frente al S mutans, *mientras que el composite de Reliance (LCR) no mostró efecto antibacteriano alguno (p < 0.05).*

*Tanto el composite como el iniciador (primer) del sistema XY transbond probaron poseer un efecto antibacteriano significativo frente a la bacteria de la prueba, cuando se les comparó con el transbond LR (*p < *0.05). Entre los materiales probados, kurasper F, Ormco Sistema 1+ y sistema 1+ activador no mostraron efecto inhibitorio alguno, o sólo ligeramente, frente a las bacterias de la prueba en este estudio. En pacientes con niveles salivales relativamente altos de* Streptococci *mutans antes del tratamiento, podrían aplicarse el Multi-Cure GIC, el adhesivo Reliance Light Bond, y el sistema transbond XT – los cuales tuvieron un alto nivel de propiedades antibacterianas.*

Palabras claves: efecto antibacteriano, materiales ortodóncicos, *Streptococcus mutans*

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INTRODUCTION

Fixed orthodontic appliances are attached to the teeth by various dental cements, adhesive resins, and hybrid cement-resin combinations that offer improved physical properties and clinical benefits (1). Due to the high frequency of white spot lesions after fixed orthodontic appliance therapy (2, 3), the therapeutic effects of orthodontic bonding materials have become a matter of primary importance. In these materials, remineralization of enamel by release of fluoride is expected (4−7). Since the antibacterial effect is another important property because inactivation of bacteria means a direct strategy to eradicate the cause of dental demineralization, some studies have examined the antibacterial activity of commercial orthodontic bondings and their constituents (8−11). Many different microorganisms may be associated with caries. Oral *Streptococci*, in particular those of the mutans group, as well as some lactobacillus and actinomyces species often play a part in the onset of smooth surface caries (12, 13).

Patients who undergo orthodontic therapy have changes in their oral area, such as a low-pH environment, increased retentive sites for *Streptococcus mutans* (*S mutans*) and increased retention of food particles, which may lead to increased proportions and absolute numbers of salivary *S mutans* (14–16).

Sandham *et al* (17) treated 26 children with chlorzoin at four time points during the month before initiating orthodontic treatment. Treatment resulted in a decrease in salivary mutans *Streptococci* counts after one week (23 subjects had nondetectable *S mutans* levels) and one month post-treatment (22 subjects still had nondetectable *S mutans* levels), gradually decreasing toward the end of the study, with 11 subjects free of detectable *S mutans* six months later.

To investigate the antibacterial property of dental materials after being cured, agar disc-diffusion was employed. Cured materials were placed on agar plates and inoculated with oral bacteria such as *Streptococci* (10, 18−20).

The aim of this study was to investigate the *in vitro* antibacterial activity of several current orthodontic materials against *S mutans* which is most heavily implicated in dental caries.

SUBJECTS AND METHODS

The orthodontic materials tested in this study are shown in Table 1. The antibacterial activities of each material were evaluated against *Streptococcus mutans* (NCTC 10449).

Table 1: Brand names and types of the test agents

Test Agents	Type	Manufacturers and lot numbers
Light bond	Primer light cure	Reliance, USA, 104160
Transbond XT primer	Primer light cure	3M Unitek, USA, JT/4BP
System 1+ activator	Primer chemical cure	Ormco USA, 02A63
System 1+	Composite chemical cure	Ormco USA, 02A63
Transbond LR	Composite light cure	3M Unitek, USA, BE/4BP
Light cure retainer (LCR)	Composite light cure	Reliance Itasca, USA, 122323003
Kurasper F	Composite light cure	Kuraray, Japan, 41156
Transbond XT adhesive	Composite light cure	3M Unitek, USA, JT/4BP
Ketac cem radiopaque	Glass ionomer cement chemical cure	3M ESPE, Germany, 158218
Unitek multicure glass ionomer	Glass ionomer cement multicure	3M Unitek, USA, 4DP/4EL
Sterile saline	Negative control	
Cefadroxil 30 mg	Positive control	Oxoid, England

The bacteria were obtained from culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, Selcuk University, Turkey. The bacteria were microaerophylically grown in trypticase soy broth (Oxoid) at 37 °C for 48 hours. The bacterial concentration was adjusted to 10^6 cells/mL. The agar was evenly distributed in the plates of 10 mm-in-diameter. Approximately 100 µl of suspensions of bacteria were swabbed over the surface of the agar.

The antibacterial activities of six orthodontic composite resin [Transbond LR (3M Unitek, USA), Light Cure Retainer (Reliance Itasca, Illinois, USA), Light Bond (Reliance, USA), System 1+ (Ormco, USA), Kurasper F (Kuraray, Japan), Transbond XT adhesive (3M Unitek, USA)], two orthodontic bonding materials [Transbond XT primer (3M Unitek, USA) and System 1+ activator (Ormco, USA)] and two glass ionomer cements (GIC) [Unitek Multicure Glass Ionomer (3M Unitek, USA) and Ketac Cem Radiopaque (3M ESPE, Germany)] were tested. Negative and positive con-trols were also used as sterile saline and cefadroxil (30 mg) on paper discs, respectively.

The moulds (inner diameter: 6 mm, height: 2 mm) which were formed with glass slides and Teflon tubes were sterilized with ethylene oxide gas. Ketac cem radiopaque, Unitek multicure glass ionomer and system 1+ were mixed, respectively according to manufacturers' instructions and put into the Teflon moulds between two glasses. Unitek multicure glass ionomer was also cured for 30 seconds with the light activation unit (Optilux 501). Transbond LR, light cure retainer, kurasper F and transbond XT adhesive were put into the Teflon moulds between two glasses and cured for 20 seconds with the light activation unit (Optilux 501). Twenty microlitres of light bond, transbond XT primer and system 1+ activator were put on a paper disc of 6 mm in diameter.

All materials were handled under aseptic conditions and put on an agar surface. All plates were incubated at 5% CO_2 and 37 °C for 48 hours. Zones of bacterial growth inhibition including the diameter of the sample were measured in millimetres. Antimicrobial tests were repeated six times, and the mean diameter of the inhibition zone values for each material was determined.

Descriptive statistics, including the arithmetic means and standard deviation (SD) were calculated for each group. Kolmogorov-Smirnov test was used to evaluate the homogeneity of data distribution. Additionally, Kruskal-Wallis and Mann Whitney U tests were performed on results of antibacterial activity; *p*-values less than or equal to 0.05 were evaluated as statistically significant. All of the statistical analyses were performed with the SPSS software package (SPSS version 12.0, Chicago, IL, USA).

RESULTS

Tables 2 and 3 show the mean values of the inhibition zones produced by each material tested and differences between dental materials, respectively. The Unitek multicure GIC showed the highest antibacterial effectiveness against *S mutans* (Mean: 29.67). Among the GIC materials tested, no inhibition zone was noted for ketac cem radiopaque. The light bond adhesive of the Reliance orthodontic bonding system produced the second highest antibacterial effectiveness against *S mutans* (Mean: 21.33), while the Reliance light cure retainer did not show any antibacterial effect. Both composite and primer of the transbond XT system demonstrated significant antibacterial effectiveness against the test bacteria when compared to transbond LR ($p < 0.05$). Among the materials tested, kurasper F showed a slight inhibitory effect against the test bacteria in the present study (mean: 8.67). Ormco system 1+ did not show any inhibition but system 1+ activator produced slight inhibitory effect (Mean: 9.67). This relation between them was statistically significant ($p < 0.05$).

Table 2: Diameters of antibacterial inhibition zones

Materials	n	Mean (mm)	SD	*p*
Light bond	6	21.33	0.82	
Transbond XT primer	6	18.83	1.33	
System 1+ activator	6	9.67	0.52	
System 1+	6	0.00	0.00	
Transbond LR	6	0.00	0.00	
Light cure retainer (LCR)	6	0.00	0.00	0.000*
Kurasper F	6	8.67	0.82	
Transbond XT adhesive	6	12.83	0.75	
Ketac cem radiopaque	6	0.00	0.00	
Unitek multicure glass ionomer	6	29.67	0.52	
Negative control (sterile saline)	6	0.00	0.00	
Positive control (cefadroxil)	6	27.17	0.41	

Kruskal-Wallis test

DISCUSSION

In this study, the lack of antibacterial properties of cured composites (Ormco system 1+, transbond LR and light cure retainer) and the glass ionomer (ketac cem radiopaque) means that there is no inhibitory effect against plaque accumulation on the surface, and bacteria such as *Streptococci mutans* can easily grow on the composites (21).

The frequent occurrences of gingivitis when composites were placed at the subgingival area have also been reported (22). One of the reasons for these unfavourable characteristics is suggested to be the lack of inhibitory effects against bacteria on the cured surface of the composites (23). In the current study, the bondings showed higher antibacterial effects against the *S mutans* than their respective resin composites.

The antibacterial activity measured in this study does not correlate entirely with the type of the cements. Transbond XT and transbond LR are composite resin-based cements from the same manufacturer (3M Unitek). However, transbond LR did not exhibit any antibacterial effect, similar to light cure retainer and Ormco system 1+ composite resin

Table 3: Differences between the dental materials

	Light bond	Transbond XT primer	System 1+ activator	System 1+	Transbond LR	Light cure retainer (LCR)	Kurasper F	Transbond XT adhesive	Ketac cem radiopaque	Unitek multicure glass ionomer	Negative control (sterile saline)	Positive control (cefadroxil)
Light bond	_											
Transbond XT primer	0.007*	_										
System1+ activator	0.003*	0.003*	_									
System1+	0.002*	0.002*	0.002*	_								
Transbond LR	0.002*	0.002*	0.002*	1.000	_							
Light cure retainer (LCR)	0.002*	0.002*	0.002*	1.000	1.000	_						
Kurasper F	0.003*	0.003*	0.041*	0.002*	0.002*	0.002*	_					
Transbond XT adhesive	0.003*	0.003*	0.003*	0.002*	0.002*	0.002*	0.003*	_				
Ketac cem radiopaque	0.002*	0.002*	0.002*	1.000	1.000	1.000	0.002*	0.002*	_			
Unitek multicure glass ionomer	0.003*	0.003*	0.003*	0.002*	0.002*	0.002*	0.003*	0.003*	0.002*	_		
Negative control (sterile saline)	0.002*	0.002*	0.002*	1.000	1.000	1.000	0.002*	0.002*	1.000	0.002*	_	
Positive control (cefadroxil)	0.003*	0.003*	0.002*	0.001*	0.001*	0.001*	0.003*	0.003*	0.001*	0.002*	0.001*	_

*Mann-Whitney U test

of materials tested in this study. This might be attributed to components added to the material by the manufacturers. Similarly, one of the GIC did not exhibit an antibacterial property, indicating that components might be involved in the measurable effect.

Incorporation of fluorides into dental materials, as well as into orthodontic cements, is based on the notion that fluoride will be released gradually from the set material *in vivo*, thus providing continuous long-acting anticariogenic effect (24). Fluoride ions might have a bacteriostatic effect on *S mutans* (5, 6, 25−27). Orthodontic cements based on glass ionomer and reinforced glass ionomer have been shown to release fluoride (28). Badawi *et al* (29) suggested that the use of fluoride-releasing bonding materials may support the growth of supragingival plaque, which does not contain *S mutans*. Ortendahl *et al* (13) recommended that in patients who have relatively high salivary levels of *Streptococci mutans* before treatment and especially in those who harbour *S sobrinus*, the use of GIC for bonding may prevent incipient caries formation during orthodontic treatment. However, the fluoride concentration in a specific dental material's composition does not reflect its rate of release. Thus, the antibacterial properties due to fluoride concentration are expected to vary from one material to another. As shown in this study, the GIC materials have different levels of antibacterial effects. The Unitek multicure GIC showed an effective antibacterial capability relative to ketac cem radiopaque. Therefore, in patients who have relatively high salivary levels of *Streptococci mutans,* the use of the GIC could be a good preference.

In most studies, it was found that cured composite did not release any antibacterial components, producing no inhibition halo. Therefore, no elution of adequate amounts of antibacterial components from cured composites to inhibit bacterial growth has been demonstrated (23). The addition of soluble antimicrobials into the resin matrix is a simple way to aim at the release of the agent from the materials in a wet environment. For this objective, chlorhexidine is most frequently used (30). It has been demonstrated that the clear inhibition of bacteria including oral *Streptococci* was observed around the composites containing 1% or greater of chlorhexidine by the agar disc-diffusion tests. However, the resins containing soluble antimicrobials show the release profile in which leaching of large amounts of the agent occurred within a few days followed by a dramatic decrease in the concentration (31). Another problem for the incorporation of antimicrobials into the monomer phase is an adverse influence on mechanical properties. Jedrychowski *et al* (32) reported that the addition of 1% chlorhexidine gluconate resulted in the reduction of tensile and compressive strengths (23). As such, addition of soluble antimicrobials into the resin matrix is not the exact way to prevent caries formation during fixed orthodontic treatment. The antimicrobial agents must be slowly diffused long term from the orthodontic material to the oral area, and the resin matrix should be recharged with antimicrobial gargles for regular diffusion of antimicrobial agents to the oral area.

CONCLUSIONS

The orthodontic composites on the market have been designed to produce a superior clinical performance, but these commercial products do not have substantially reliable antibacterial properties, and even for those materials which have been demonstrated to exhibit some antibacterial effects, their clinical significance may not be adequate. As shown in this study, the clear antibacterial activity of several experimental antibacterial composites was confirmed by *in vitro* tests. In patients who have relatively high salivary levels of *Streptococci mutans* before treatment, the Unitek multicure GIC, the Reliance light bond adhesive, and transbond XT system which have high level antibacterial properties could be applied.

Further experiments simulating clinical situations will clarify whether the bonding or adhesive materials are effective in inhibiting bacterial growth or bacterial attachment under *in vivo* conditions, and their clinical benefits may be better clarified.

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