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Orthodontic materials with fluoride have an antimicrobial ability for the prevention of white spot lesions

Ortodontski materijali s fluoridom imaju antimikrobnu sposobnost za prevenciju karijesnih lezija

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Abstract. ***Aim:*** Orthodontic brackets during their wear cause demineralization of enamel, which is the initial step in caries development. The prevention of such spots is crucial to ensure healthy teeth, but patients' compliance is not the most optimal way. The aim of this study was to compare the antimicrobial properties of fluoride-containing orthodontic materials to the materials without additional fluoride. ***Materials and Methods:*** Antibacterial effectiveness of orthodontic materials with fluoride – Transbond Plus SEP Bonding agent, Transpond Plus Adhesive agent, Fuji I Band cement, Fuji Ortho LC Adhesive agent, Ortho Solo Bonding agent, and without antimicrobial substances – Transpond XT Bonding agent, Transbond XT Primer were tested with the inhibition on most common causes *L. achidophilus* (ATCC 4356) and *S. mutans* (ATCC 10449) and compared to negative control. Antimicrobial effectiveness of each material was measured with the agar diffusion method and expressed with the diameters of inhibition zones around the disk. ***Results:*** Materials containing fluoride showed more antimicrobial effectiveness compared to materials without fluoride or negative control ($p<0.001$), respectively. Materials from the group with no antibacterial substances were not statistically different compared to the negative control ($P>0.05$). ***Conclusion:*** Materials containing fluoride showed more significant antimicrobial effectiveness when compared to the materials without antimicrobial substance and thus might have the potential of antimicrobial properties *in vivo*.

Key words: antimicrobial agents; fluoride; orthodontic adhesives; white spot lesions

Sažetak. ***Cilj:*** Ortodontski breketi tijekom nošenja uzrokuju demineralizaciju cakline, što je početni korak u razvoju karijesa. Prevencija takvih mrlja presudna je za osiguravanje zdravih zuba, ali suradljivost pacijenata nije uvijek optimalna. Cilj ovog istraživanja bio je usporediti antimikrobna svojstva ortodontskih materijala koji sadrže fluorid s materijalima bez dodatnog fluorida. ***Materijal i metode:*** Antibakterijsku učinkovitost ortodontskih materijala s fluoridom – Transbond Plus SEP Bonding agent, Transpond Plus ljepilo, Fuji I Band cement, Fuji Ortho LC ljepilo, Orto Solo Bonding agent, i učinkovitost ortodontskih materijala bez antimikrobnih tvari – Transpond XT Bonding agent, Transbond XT Primer, testirali smo s inhibicijom na najčešće uzročne bakterije *L. achidophilus* (ATCC 4356) i *S. mutans* (ATCC 10449) i usporedili učinkovitost s negativnom kontrolom. Antimikrobna učinkovitost svakog materijala izmjerena je metodom difuzije agara i izražena promjerom zona inhibicije oko diska. ***Rezultati:*** Materijali koji sadrže fluorid pokazali su veću antimikrobnu učinkovitost u usporedbi s materijalima bez fluorida ili u usporedbi s negativnom kontrolom ($p < 0,001$). Materijali koji ne sadrže antibakterijske tvari nisu se statistički razlikovali u usporedbi s negativnom kontrolom ($p > 0,05$). ***Zaključak:*** Materijali koji sadrže fluorid pokazali su značajniju antimikrobnu učinkovitost u usporedbi s materijalima bez antimikrobne tvari i stoga mogu imati potencijal antimikrobnih svojstava u uvjetima *in vivo*.

Ključne riječi: antimikrobna sredstva; bijele mrlje; fluorid; ortodontski adhezivi

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INTRODUCTION

Orthodontic brackets frequently impair the cleanliness of teeth during their wear[1–3]. It is physically difficult to clean and brush the teeth around the brackets, therefore cariogenic bacteria, such as *Lactobacillus acidophilus* (*L. acidophilus*) or *Streptococcus mutans* (*S. mutans*), can form biofilm which leads to tooth decay or demineralization around orthodontic brackets[4-6].

Orthodontic brackets during their wear cause demineralization of enamel, which is the initial step in caries development. The prevention of such spots is crucial to ensure healthy teeth. Fluoride-containing materials showed greater antimicrobial effectiveness but require frequent application of more fluoride to enhance their antimicrobial ability.

Demineralization or development of white spot lesions (WSLs) might disappear through natural remineralization without additional substances once the orthodontic brackets are removed and oral hygiene is restored[7]. However, the removal of the appliances is not always enough, as demineralization spots do not disappear and can last for years[8,9].

Prevention of WSLs encompasses various strategies. Oral hygiene and dietary control are one thing[10]. but application of fluoride agents as a remineralizing agent, can help remineralize the lesions[11]. Additionally, to the fluoride itself, antimicrobial fluoride-obtaining adhesives have arrived at the market for orthodontic bonding, and these were proven as useful in remineralization[12,13]. Fluoride adsorbs minerals of the enamel and enhances protective mechanisms against acid dissolution, counteracts with bacterial enzymes, which leads to bacterial plaque inactivity, and speeds up the remineralization process by attracting calcium ions in carious lesions[14-18]. As fluoride comes in different forms, the option which rely on patient's compliance and cooperation however is often not reliable[19-21]. Therefore, the solution of incorporation of antimicrobial agents into the orthodontic adhesives (e.g. fluoride-releasing orthodontic adhesives) have shown to be effective in reversing WSLs after debonding[15].

The aim of our study was to evaluate the antimicrobial effectiveness of fluoride in fluoride-containing orthodontic materials and compare their antimicrobial ability to the materials without additional fluoride.

MATERIALS AND METHODS

Antimicrobial effect of orthodontic materials containing fluoride were analyzed *in vitro*. Materials were classified into two different groups; cases with orthodontic materials that contained fluoride and controls with materials that contained to antimicrobial substance. Negative controls were performed with each batch to ensure there was no contamination. The following materials were tested for 10-times; Group 1 containing fluoride – Transbond Plus SEP Bonding agent, Transpond Plus Adhesive agent, Fuji I Band cement, Fuji Ortho LC Adhesive agent, Ortho Solo Bonding agent); Group 2 with no antimicrobial substances – Transpond XT Bonding agent, Transbond XT Primer.

Bacterial samples

Performance of additional fluoride was tested on two most commonly causes of WSLs *L. achidophilus* (ATCC 4356) and *S. mutans* (ATCC 10449) as a bacterial sample. The frozen microbial strains were dissolved and inoculated onto the blood agar plate. The plate was incubated for 48 hours in anaerobic atmosphere at 37 °C and examined after two days. The bacterial growth was evaluated and confirmed with morphological characteristics (colony shape, colour, thickness, haemolysis on agar plate) and gram staining.

Orthodontic samples

The standardized quantity of each orthodontic material was created with the plastic mold disk using 10 mg of a respective material. When molded, disks were then placed on freshly inoculated BHI agar plates. Each plate also contained a negative control with a blank paper disk.

Bacterial culture

Suspensions of *L. acidophilus* and *S. mutans* culture were levelled to 0.5 McFarlands ($1.5x10^9$ cells/mL) and prepared in the thioglycolate broth.

200 µL of suspension was mixed with 3.5 mL of soft agar and poured over the BHI agar plate.

Antimicrobial testing

In-house disk diffusion test was performed. Disk diffusion method of antimicrobial testing was used. Orthodontic disks were placed onto the hardened BHI agar. The plates containing *S. mutans* strain were cultivated at 37 °C under anaerobic conditions for 48 hours. Meanwhile the plates with *L. acidophilus* were cultivated at 37 °C under microaerophilic conditions also for 48 hours. Antimicrobial ability of orthodontic materials was evaluated with the measurement of inhibition zones around each disk. Diameter of the zones were measured in millimetres (mm).

Statistical analysis

Data were analyzed using SPSS 21 (IBM, New York, USA). Two-Way ANOVA with pairwise comparisons was used to assess differences between the effectiveness on different bacterial strains and for the comparisons of orthodontic materials with their mean diameters of inhibition zones. In case of abnormal data distribution Kruskal-Wallis test was used. Statistical significance was set at $P<0.05$ for all comparisons.

RESULTS

Overall, 160 specimens were tested. The effectiveness with inhibition zones is presented in Table 1. There were no differences in terms of significant effectiveness between the two bacterial strains. *L. acidophilus* on average showed slightly larger zones of inhibition with agents containing fluoride (9.1±2.0 mm) compared to *S. mutans* (9.0±3.2 mm), but none of the tested agents showed any significance. Similarly, was observed with the agents without fluoride (1.2±1.2 mm and 0.8±0.9 mm, respectively). Moreover, the ANOVA showed no statistically significant difference (F-value=20.121; $P=0.100$). However, the ANOVA test showed statistically significant differences when comparing orthodontic materials among each other (F-value=55.081; $P<0.001$). Pairwise comparisons with Kruskal-Wallis test showed that all fluoride-containing materials were statistically different to the negative controls ($P<0.001$), respectively (Table 2). However, materials from Group 2 with no antibacterial substance were not statistically significant when compared to the negative controls ($P>0.05$).

Among all orthodontic materials Transbond Plus agents showed the largest zones of inhibition of above 10 mm in diameter, which was different to other materials. Furthermore, fluoride-containing orthodontic materials showed statistically better antimicrobial effectiveness when compared to the materials from Group 2, without additional antimicrobial substance. On average diameter was 9.1±2.6 mm versus 0.9±1.0 mm. These showed significantly less antimicrobial ef-

Table 1. Antimicrobial effectiveness on L. acidophilus and S. mutans with the zones of inhibition for a respective group of orthodontic materials

Antimicrobial agent	Orthodontic material	TOTAL [mm] N=160	Zone of inhibition		*p*-value
			L. acidophilus [mm] (n=80)	*S. mutans* [mm] (n=80)	
Fluoride	Transbond Plus SEP bonding agent (n=20)	10.4±3.8	9.3±1.9	11.2±5.1	0.108
	Transbond Plus adhesive agent (n=20)	10.3±2.8	10.6±2.6	9.8±3.1	0.587
	Fuji I band cement (n=20)	8.2±2.5	8.5±2.6	8.0±2.5	0.489
	Fuji Ortho LC adhesive agent (n=20)	7.8±1.4	8.2±1.5	7.4±1.3	0.357
	Ortho solo bonding agent (n=20)	8.5±1.6	8.6±1.2	8.4±2.0	0.734
	Total	9.1±2.6	9.1±2.0	9.0±3.2	0.086
None	Transbond XT bonding agent (n=20)	1.1±1.0	1.3±1.1	0.9±1.0	0.765*
	Transbond XT primer (n=20)	0.9±1.0	1.1±1.1	0.8±0.8	0.924*
	Total	0.9±1.0	1.2±1.2	0.8±0.9	0.603*
	Negative control (n=20)	0.0±0.0	0.0±0.0	0.0±0.0	1.000

* Kruskal-Wallis test was used for comparisons between the effectiveness on different bacterial strains

Table 2. Pairwise comparisons between the orthodontic materials for combined effectiveness data on *L. acidophilus* and *S. mutans*

	Transbond Plus SEP bonding agent	Transbond Plus adhesive agent	Fuji I band cement	Fuji Ortho LC adhesive agent	Ortho solo bonding agent	Transbond XT bonding agent	Transbond XT primer	Negative control
Transbond Plus SEP bonding agent	1	0.185	0.007	<0.001	0.014	<0.001	<0.001	<0.001
Transbond Plus adhesive agent	0.185	1	0.091	0.039	0.259	<0.001	<0.001	<0.001
Fuji I band cement	0.007	0.091	1	0.560	0.684	<0.001	<0.001	<0.001
Fuji Ortho LC adhesive agent	<0.001	0.039	0.631	1	0.329	<0.001	<0.001	<0.001
Ortho solo bonding agent	0.014	0.259	0.734	0.329	1	<0.001	<0.001	<0.001
Transbond XT bonding agent	<0.001	<0.001	<0.001	<0.001	<0.001	1	0.934	0.411
Transbond XT primer	<0.001	<0.001	<0.001	<0.001	<0.001	0.934	1	0.472
Negative control	<0.001	<0.001	<0.001	<0.001	<0.001	0.411	0.472	1

* Pairwise comparisons between the orthodontic materials were tested with series of ANOVA tests or Kruskal-Wallis test

fectiveness and where not statistically different compared to negative controls (Table 2).

DISCUSSION

Enamel demineralization of WSLs are frequent problem after removal of orthodontic brackets. Different treatment and prevention measures have been proposed, which are fully dependable on patients' compliance and physician choice[22]. Preventing WSLs is important in order to obtain more aesthetic results after debonding of brackets. There is the increase in the number of substances that can be used to prevent these lesions, and application of fluoride in different forms has showed great effectiveness in the prevention of not only bacterial growth and caries, but also in the prevention of such lesions[23,24]. The aim of the current study was to determine whether fluoride-containing orthodontic materials have an advantage in the prevention of WSLs than compared to the materials without antimicrobial substances. Fluoride-containing adhesives to prevent enamel demineralization were used in previous studies and showed encouraging results[25,26].

Table 1 summarizes the zones of inhibition for orthodontic material according against *L. acidophilus* and *S. mutans*. The fluoride-containing orthodontic materials inhibited the growth of *L. acidophilus* and *S. mutans*, measured with the inhibition zones with disk diffusion method and showed antimicrobial effect against both. Past studies proved that fluoride has the antimicrobial ability against oral microorganisms[7,16,17,27,28]. In our study also the materials with no incorporated antimicrobial substances from Group 2 showed some antimicrobial efficacy, implying that orthodontic adhesives on their own might have some antimicrobial activity, but these were not statistically significant when compared to the negative controls.

Fluoride-containing orthodontic materials showed significantly larger zone of inhibition than the materials without fluoride. Materials with no antimicrobial substance showed no or extremely poor antimicrobial effectiveness. These results coincide with the findings by Kelly[29], who reported that orthodontic materials with no additional incorporation of antimicrobial substances do not inhibit the growth of bacteria and do not form an inhibition zone. A poor inhibition was observed in our analysis. As the material itself cannot diffuse into the agar, therefore cannot have an antimicrobial ability, but small inhibition zone might occur due to material physical restrictions for bacterial growth.

The results of our study showed that Transbond Plus SEP bonding agent containing fluoride pro-

ved to have the greatest antimicrobial effectiveness compared to all other tested materials. The comparisons were statistically significantly different to all tested materials, except when compared to Transbond Plus adhesive agent (P=0.185). There results suggest that an amount of incorporated fluoride is the main reason for the difference of the results in the matter or inhibition zones for a given type of the material. In our study it was proven that all fluoride-containing materials had shown antimicrobial effectiveness, as they were statistically significant when compared to negative controls or even to materials without incorporated fluoride. Other studies are in line with the results of our current study and reported antibacterial effectiveness for Fuji I, Fuji Ortho LC, Ortho solo, and Transbond Plus, and no effectiveness for Transbond XT bonding agent and Transbond XT primer with the same dish diffusion method and bacterial growth inhibition zones[29]. Therefore, fluoride confirmed that it has antibacterial effectiveness.

The incorporation of fluoride into the orthodontic material itself showed several advantages over the materials without fluoride. Thus, providing higher effectiveness also *in vivo* models. The effectiveness of fluoride against *L. acidophilus* and *S. mutans*, as the most frequent causes of development of WSLs, has therefore the potential to prevent growth of this cariogenic bacteria and prevent the development of caries and WSLs. As mentioned, the positive effectiveness of fluoride proved in the current study coincides with the findings of previous reports, still suggesting fluoride is the antimicrobial agent of choice when establishing health oral cavity[30]. Although fluoride provides a significant benefit, the only disadvantage of fluoride in various forms is that it requires often applications in order to ensure the continuous effectiveness[22,25,31]. Over time, the effectiveness of orthodontic materials containing fluoride require application of more fluoride to enhance their antimicrobial ability.

Orthodontic materials with incorporated antimicrobial substances, such as fluoride, can act as modern and powerful assets for orthodontists to maintain the growth of cariogenic bacteria, and thus prevent the WSLs as an initial steps of caries development. The results obtained from this study clearly show that fluoride might have the potential to increase the preventive measures of WSLs development *in vivo*. However, this study had limitations. The study was small, investigating only 7 respective orthodontic materials and include only the materials with and without fluoride. Nowadays, orthodontists use materials that have incorporation of various antimicrobial substances, but these were not used in this study. It would be interesting to analyze the antimicrobial effectiveness of all more agents. Our study also did not simulate the full oral cavity conditions, as for instance artificial saliva was not used. Therefore, limitations of *in vitro* tests include the lack of application into *in vivo* conditions.

In vitro study does not simulate the full oral cavity conditions of in vivo conditions. As there is not a lot of studies on the maintaining effect of incorporated fluoride it is unclear antimicrobial effectiveness are maintained during the wearing of orthodontic brackets. Therefore, fluoride-containing materials need to be studied more often also with other adhesive materials for the prevention of WSLs during orthodontic therapy.

CONCLUSIONS

Disk diffusion method showed that orthodontic materials containing fluoride have antimicrobial ability. Furthermore, as there is not a lot of studies on the maintaining effect of incorporated fluoride it is unclear antimicrobial effectiveness are maintained during the wearing of orthodontic brackets. Fluoride-containing materials showed greater antimicrobial effectiveness but require frequent application of more fluoride to enhance their antimicrobial ability. However, fluoride-containing materials have shown the potential to prevent the development of WSLs during orthodontic therapy.

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