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Antimicrobial Effect of Orthodontic Materials on Cariogenic Bacteria *Streptococcus mutans* and *Lactobacillus acidophilus*

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BE 1 **Sokol Krasniqi**
A 1 **Milaim Sejdini**
CE 2 **David Stubljar**
CD 3 **Tomislav Jukic**
AD 4 **Alojz Ihan**
F 1 **Kaltrina Aliu**
AE 5 **Xhevdet Aliu**

1 Faculty of Medicine, University of Pristina, Pristina, Kosovo
2 Department of Research and Development, In-Medico, Metlika, Slovenia
3 Department of Internal Medicine, History of Medicine and Medical Ethics, Faculty of Medicine, Osijek, Croatia
4 Medical Faculty, Institute of Microbiology and Immunology, Ljubljana, Slovenia
5 Faculty of Dentistry, University for Business and Technology, Pristina, Kosovo

Corresponding Author: Xhevdet Aliu, e-mail: xhevdetaliu99@gmail.com
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Background: White spot lesions (WSLs) are a common complication after orthodontic treatment. The aim of this study was to characterize and compare the antimicrobial properties of selenium-containing *vs.* fluoride-containing orthodontic materials.

Material/Methods: Antibacterial efficacy of orthodontic materials (SeLECT Defense bonding agent, Adhesive agent, Band Cement, 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, and Transbond XT primer) was tested with the inhibition of 2 bacterial strains: *S. mutans* (ATCC 10449) and *L. acidophilus* (ATCC 4356). The antimicrobial efficacy of the materials was measured by agar diffusion test. The diameters of inhibition zones around each disk were measured in millimeters (mm).

Results: Materials containing selenium and fluoride showed significant differences from the negative control (both $p<0.001$). Orthodontic materials containing fluoride as a potential antimicrobial agent showed larger zones of inhibition in total (9.1±2.6 mm), the selenium group was the second-most effective (4.7±4.9 mm), and the group without any potential antimicrobial agent showed the least antimicrobial effect (0.9±1.0 mm). Materials from the group with no antibacterial agent were not significantly different from the negative control group ($p>0.05$).

Conclusions: Materials containing selenium carried the most significance when comparing microorganisms with the agent, since they were the only ones showing difference between the 2 microorganisms. They showed statistically significant difference in efficacy against *S. mutans*, and poor antimicrobial effect against *L. acidophilus*. These data suggest that orthodontic materials containing selenium might have the potential to prevent WSLs due to their antimicrobial properties.

MeSH Keywords: **Anti-Infective Agents • Fluorides • Organoselenium Compounds • Orthodontic Appliances • Orthodontic Brackets**

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Background

Enamel demineralization, known as white spot lesions (WSLs), is the worst adverse effect that impairs the aesthetics of fixed orthodontic treatments. The development of WSLs occurs due to prolonged accumulation of bacterial plaques, and is associated with high production of acid by acidogenic bacteria [1]. Orthodontic appliances can influence the ability to clean teeth, alter the oral microflora, and increase the levels of acidogenic plaque bacteria, which lead to the development of dental biofilm while wearing the appliance [2–6]. Cariogenic biofilm can lead to dental decay [7] or demineralization around orthodontic brackets. Within such a biofilm, *Streptococcus mutans* (*S. mutans*) is the main etiological factor responsible for initiation and progression of tooth decay [8,9]. Despite many attempts at prophylaxis and prevention, the prevalence of WSLs remains as high as 61% when debonding orthodontic appliances [10]. It is generally believed that these lesions will recover through natural remineralization with saliva once the orthodontic appliances have been removed and oral hygiene is restored [11]. However, the removal of bacterial plaque alone is not enough to achieve complete repair of WSLs, and some spots can last for more than 10 years [12,13].

Various strategies have been suggested to reduce demineralization and white spot formation during treatment, but they mainly rely on patient compliance. The main approaches are still mechanical biofilm removal and the use of potentially antimicrobial substances, such as fluoride or other antimicrobial agents [14,15]. The use of fluoride in various forms inhibits the metabolism of bacteria that cause caries and increase the resistance of enamel and dentine [16–19]. However, their effectiveness depends on patient compliance, which is challenging and not reliable, especially among children [20–22].

Although WSLs are recognized as a major problem of orthodontic treatments, orthodontists are still using orthodontic materials with limited or no preventive measures. The most commonly used preventive measures against plaque accumulation are intensive oral hygiene [1,23] fluoridated rinses [23], and fluoridated toothpastes [1,10,24–27]. Some orthodontists use fluoride varnishes [24] or fluoride-containing adhesives/primers [10, 24] and fluoride-releasing sealants or antimicrobial varnishes [10,28]. Despite the efficacy of these applications, they remain inefficient because they need frequent reapplications or recharges of fluoride throughout the treatment duration [1,10,29].

Nevertheless, studies have found that specific orthodontic materials, for instance those containing selenium, prevent the formation of WSLs. Tran et al. [29–31] reported the antibacterial properties of selenium, but there have been few studies comparing commonly used fluoridated orthodontic materials with potentially novel antimicrobial agents, such as selenium. Thus, in the present study our objective was to characterize the antimicrobial properties of orthodontic materials containing potential antimicrobial agents (selenium, fluoride, or no agent) and to compare them, so that in the future they might be used for the prevention of WSLs in people who are wearing fixed orthodontic appliances. The aims of this investigation were to evaluate and compare the *in vitro* effectiveness of selenium materials and fluoride materials on growth of *S. mutans* and *Lactobacillus acidophilus* (*L. acidophilus*) and therefore translate the results into practice in preventing demineralization of the enamel surrounding orthodontic brackets.

Material and Methods

This study was designed as an *in vitro* analysis of the antimicrobial effect of different orthodontic materials on cariogenic bacterial strains. Three different types of orthodontic materials were assessed (Table 1): those containing selenium, fluoride, and materials without any of the potential antimicrobial substances. Blank paper disks impregnated with physiological solution were used as a negative control.

Preparation of samples

Samples were equally divided into 2 testing groups according to the inoculated bacterial strains: *S. mutans* or *L. acidophilus*. The 2 bacterial testing groups were further divided into 4 subgroups according to the material: Group 1 contained selenium (SeLECT Defense bonding agent, SeLECT Defense Adhesive agent, SeLECT Defense Band Cement), Group 2 contained fluoride (Transbond Plus SEP bonding agent, Transbond Plus Adhesive agent, Fuji I Band cement, Fuji Ortho LC Adhesive agent, and Ortho Solo Bonding agent), Group 3 contained no antimicrobial substances (Transbond XT bonding agent and Transbond XT primer), and Group 4 was negative controls.

Preparation of bacterial strains

The antimicrobial efficacy of orthodontic materials was tested with the inhibition of growth for 2 bacterial strains: *S. mutans* (ATCC 10449) and *L. acidophilus* (ATCC 4356). The frozen ATCC microbial strains were dissolved and then inoculated onto blood agar plates with *S. mutans*, or chocolate agar plates with *L. acidophilus*. The plates inoculated with *S. mutans* were incubated for 48 h in an anaerobic atmosphere at 37°C. The plates inoculated with *L. acidophilus* were incubated for 48 hours under microaerophilic conditions at 37°C. Atmospheres were created using the Anoxomat System™ (MART Microbiology BV, Netherlands). Plates were examined after 2 days. When there was no detection of bacterial growth, the incubation period was extended to 1 week.

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Table 1. Orthodontic materials which were tested with agar diffusion assay.

Material	Type	Manufacturer	Potential antibacterial agent
Group 1			**Selenium**
SeLECT Defense	Bonding agent	Element 34	Selenium
SeLECT Defense	Adhesive agent	Element 34	Selenium
SeLECT Defense	Band cement	Element 34	Selenium
Group 2			**Fluoride**
Transbond Plus SEP	Bonding agent	3M	Fluoride
Transbond Plus	Adhesive agent	3M	Fluoride
Fuji I	Band cement	GC America	Fluoride
Fuji Ortho LC	Adhesive agent	GC America	Fluoride
Ortho Solo	Bonding agent	Ormco	Fluoride
Group 3			**None**
Transbond XT	Bonding agent	3M	None
Transbond XT	Primer	3M	None
Control group			
Blank paper disk*	Negative control	BD	None

* Blank paper disk served as a control in the agar diffusion assay only.

The growth of bacterial colonies on plates was evaluated and confirmed according to the morphological characteristics (shape of colonies, colour of colonies, thickness of colonies, smell, and hemolysis on agar plate) and gram staining.

Preparation of orthodontic disks and disk diffusion assay

The mold disk was created to ensure the standardized quantity of each tested material when applied onto testing plates. The disks of each orthodontic material were prepared using approximately 10 mg of product. Disks were formed using a plastic mold. For each material, a new mold was used to prevent cross-contamination between materials. Expressed disks were immediately placed on freshly inoculated plates with BHI agar using aseptic techniques. Each plate contained an empty paper disk in the middle of the agar plate as a negative control.

Suspensions of each bacterial culture were standardized to 0.5 McFarland's (1.5×10^9 cells/mL) and prepared in the thioglycolate broth. Afterwards, 200 µL of suspension of *S. mutans* or *L. acidophilus* were pipetted and mixed with 3.5 mL of soft agar and poured evenly over the BHI agar plate. Material disks were applied directly onto the hardened layer of BHI agar. *S. mutans* plates were cultivated at 37°C under anaerobic conditions for the next 48 h and *L. acidophilus* plates were cultivated at 37°C under microaerophilic conditions for the next 48 h.

The antimicrobial effect of orthodontic materials was evaluated after plate incubations as the diameters of the inhibition zones around each disk. Zones were measured in millimeters (mm) with digital calipers. All specimens included a negative control to exclude the possibility of false-positive findings.

Statistical analysis

Statistical analysis was performed using SPSS 21 (IBM, New York, USA). Two-Way ANOVA with pairwise comparisons was used to assess differences between orthodontic materials in mean diameters of inhibition zones. In case of abnormal data distribution, the Kruskal-Wallis test was used. Statistical significance was set at $p<0.05$.

Results

A total of 154 testing samples were analyzed with two-way ANOVA for identification of potential correlations between the variables. Mean inhibition zones, determined as an arithmetic average of diameters of inhibition zones in plates for respective material, are shown in Table 2.

At this point, the differences were statistically assessed between the 2 bacterial strains. *L. acidophilus* showed smaller zones of inhibition compared to *S. mutans*. In total, average zones of inhibition were 6.7±4.6 mm for *S. mutans* and 4.4±4.6 mm for *L. acidophilus*. Thus, these 2 microorganisms showed statistically significant differences (F-value=55.121; $p<0.001$). Statistically significant differences were also observed when

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Table 2. Mean zones of inhibition (diameter in mm)±standard deviation (SD) for orthodontic materials according to disk diffusion method.

Antimicrobial agent	Orthodontic material	Inhibition zone [mm]
Selenium	SeLECT Defense bonding agent	5.2±5.4
	SeLECT Defense adhesive agent	4.8±5.2
	SeLECT Defense band cement	4.0±4.1
	Total	4.7±4.9
Fluoride	Transbond Plus SEP bonding agent	10.4±3.8
	Transbond Plus adhesive agent	10.1±2.7
	Fuji I band cement	8.4±2.4
	Fuji Ortho LC adhesive agent	7.9±1.4
	Ortho solo bonding agent	8.6±1.5
	Total	9.1±2.6
None	Transbond XT bonding agent	1.0±1.0
	Transbond XT primer	0.8±1.1
	Total	0.9±1.0
	Negative control	0.0±0.0

Table 3. Mean zones of inhibition (diameter in mm)±standard deviation (SD) of orthodontic materials according to antimicrobial agent against *S. mutans* and *L. acidophilus* by disk diffusion method.

Antimicrobial agent	Orthodontic material	*S. mutans**	*L. acidophilus*	p-Value
Selenium	SeLECT Defense bonding agent[c,f,g,i,j]	10.1±2.7	0.3±0.4	<0.001
	SeLECT Defense adhesive agent[g,i,j]	9.5±2.7	0.1±0.1	<0.001
	SeLECT Defense band cement[a,d,i,j]	8.0±0.7	0.0±0.1	<0.001
	Total	9.2±2.3	0.1±0.2	<0.001
Fluoride	Transbond Plus SEP bonding agent[c,f,g,h,i,j]	11.2±5.1	9.5±1.6	0.108
	Transbond Plus adhesive agent[g,i,j]	9.8±3.1	10.4±2.4	0.587
	Fuji I band cement[a,d,i,j]	8.0±2.5	8.7±2.4	0.489
	Fuji Ortho LC adhesive agent[a,b,d,e,i,j]	7.4±1.3	8.4±1.5	0.357
	Ortho solo bonding agent[d,i,j]	8.4±2.0	8.8±1.0	0.734
	Total	9.0±3.2	9.2±1.9	0.086
None	Transbond XT bonding agent[a,b,c,d,e,f,g,h]	0.9±1.0	1.2±1.1	0.765
	Transbond XT primer[a,b,c,d,e,f,g,h]	0.8±0.8	0.9±1.3	0.924
	Total	0.8±0.9	1.0±1.2	0.603
	Negative control	0.0±0.0	0.0±0.0	1.000

* Different index letters denote different statistical difference determined by post hoc test (Tukey test) for *S. mutans*. [a] SeLECT Defense bonding agent; [b] SeLECT Defense adhesive agent; [c] SeLECT Defense band cement; [d] Transbond Plus SEP bonding agent; [e] Transbond Plus adhesive agent; [f] Fuji I band cement; [g] Fuji Ortho LC adhesive agent; [h] Ortho solo bonding agent; [i] Transbond XT bonding agent; [j] Transbond XT primer.

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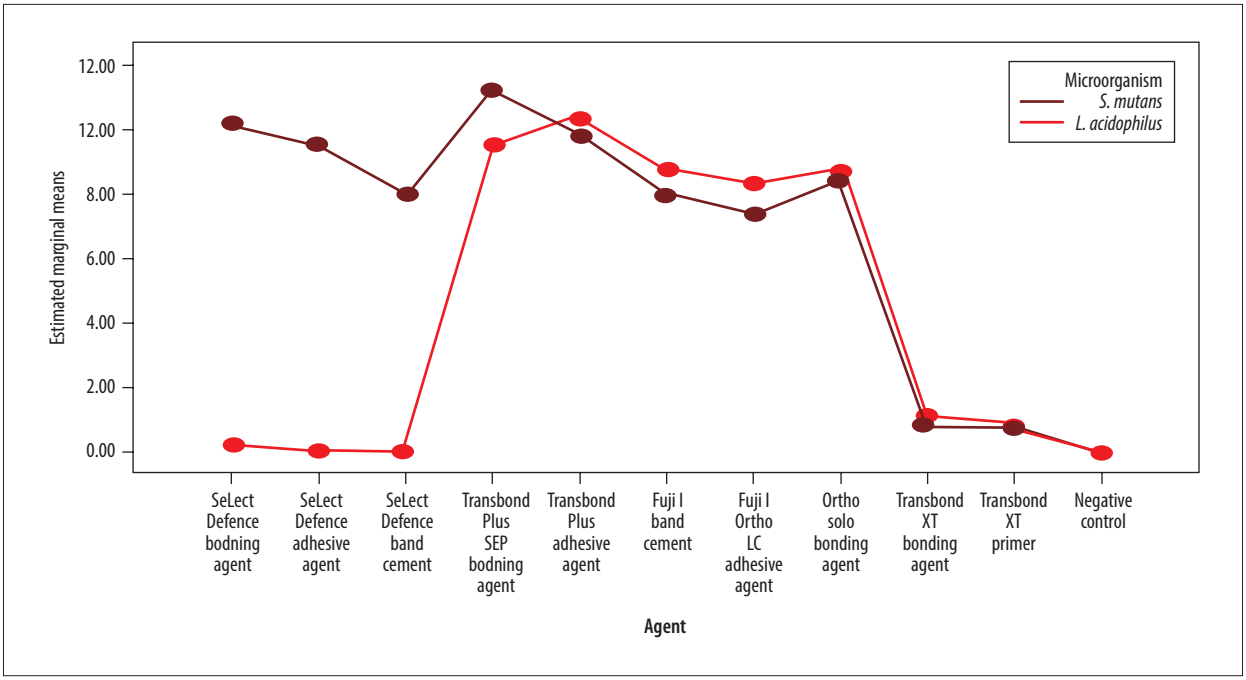

Figure 1. Estimated marginal means of inhibition zones for respective orthodontic materials compared between the 2 tested microorganisms.

comparing different orthodontic materials among each other (F-value=52.081; p<0.001). Materials containing the antibacterial agents selenium and fluoride were significantly different when compared to negative control (p<0.001). However, materials with no antibacterial agent were not significantly different compared to the negative control (p>0.05). Transbond Plus SEP bonding agent showed the highest antimicrobial effect overall and was significantly different from all other materials except compared to Transbond Plus adhesive agent (p=0.708). A similar performance was observed for Transbond Plus adhesive agent. Other materials containing fluoride showed relatively comparable results when compared to the selenium group.

The mean zones of inhibition according to antimicrobial agent and against *S. mutans* and *L. acidophilus* are summarized in Table 3. SeLECT Defense materials containing selenium had the greatest effect when comparing microorganisms with agent, since they were the only materials showing difference in performance between the 2 microorganisms (p<0.001) (Figure 1). These materials showed significant efficacy against *S. mutans*, but no antimicrobial effect against *L. acidophilus*, which influenced the overall antimicrobial efficacy against both strains. All fluoride-containing materials showed no difference between the microorganisms and showed antimicrobial effect against both. At this point, selenium-containing bonding materials showed significant differences from fluoride-containing materials and similar efficacy compared to the most efficacious Transbond Plus SEP bonding agent (p=0.285) and Transbond Plus adhesive agent (p=0.786). Furthermore, orthodontic materials from Group 3, without any antimicrobial agents, showed poor antimicrobial effect and where not significantly different from negative controls. No zones of inhibitions were detected in any of the negative controls.

Discussion

This study was conducted to assess the role of potential antimicrobial agents incorporated into orthodontic materials to prevent the development of WSLs or tooth caries *in vivo* in people who are wearing fixed orthodontic brackets. Biofilm plaque formation promotes the growth of *S. mutans*, which is a caries-causing bacterium and is a major cause of pH reduction [3,32,33]. Organic acids produced by bacteria decrease the pH and WSLs are formed [33,34]. Derks et al. [28] discovered that although orthodontists know about the various demineralization therapies available, few implement any of the strategies in their clinical practice. Thus, in our study, we aimed to characterize the antimicrobial properties of different orthodontic materials containing different antimicrobial agents (selenium, fluoride, or no agent).

The in-house agar diffusion assay was used for evaluation of antimicrobial efficacy of orthodontic products, evidenced by a measurement of inhibition zones on agar plates around the applied material. The current study is one of the few to characterize the antimicrobial properties of SeLECT Defense materials containing selenium and to compare them to other commonly

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

used products containing conventional fluoride. The study was designed as an *in vitro* analysis to determine antibacterial activity against growth of *S. mutans* and *L. acidophilus* strains.

The incorporation of fluoride or selenium into orthodontic material inhibited the growth of *S. mutans* or *L. acidophilus*, measured as inhibition zones in mm after agar plate incubation. Previous studies have reported that fluoride has shown broad antimicrobial activity against microorganisms in oral biofilms [10,17,25,35,36]. Research by Tran et al. has shown that selenium is an excellent antimicrobial agent [30–32]. In the present study, Group 3 materials with no additional antimicrobial substances showed lower antimicrobial efficacy. *L. acidophilus* showed smaller zones of inhibition compared to *S. mutans* (F-value=55.121; p<0.001). Statistically significant differences were also observed when comparing different orthodontic materials among each other (F-value=52.081; p<0.001). Materials have different effects on a particular microbial strain, which was statistically confirmed with the correlation analysis of type of microorganism *vs.* antimicrobial agent (F-value=17.483; p<0.001).

Orthodontic materials containing fluoride as an antimicrobial agent showed larger zones of inhibition for both bacteria (9.1±2.6 mm), the selenium group was the second-most effective (4.7±4.9 mm), and Group 3, despite the lack of fluoride release, showed some antimicrobial effect (0.9±1.0 mm). However, differences were not statistically significant compared to the negative control (p>0.05). As shown in the present study, the inhibition resulting from direct contact demonstrated that the materials with no antimicrobial agent had no or extremely poor antibacterial effect in its pure state. All findings are consistent with the result from the study by Kelly [37], who reported that agents with no additional substances in orthodontic materials do not form an inhibitory zone on bacterial agar plates. They also suggested that as the material itself does not diffuse into agar, it therefore might not have an antimicrobial effect, but a small inhibition zone might occur due to material physical restrictions of bacterial growth.

Combined results with *S. mutans* and *L. acidophilus* showed that Transbond Plus SEP bonding agent had the strongest antimicrobial effect overall and was significantly different from all other materials, except for Transbond Plus adhesive agent (p=0.708). Thus, similar performance was observed for Transbond Plus adhesive agent, which was significantly more efficacious compared to other materials in inhibiting both bacterial strains. Such findings suggest that the amount of fluoride released is the main determinant of results in a zone of inhibition for a given bacterial species or material. All assays demonstrated that all tested materials have antimicrobial properties. Previous studies agree with the results of our study and have also reported positive findings of antibacterial properties for Fuji I, Fuji Ortho LC, Ortho solo, and Transbond Plus, and negative findings for Transbond XT bonding agent and Transbond XT primer in agar diffusion and growth inhibition assays [37]. Therefore, as with our results, fluoride was confirmed to have antibacterial, antifungal, antiviral, antitumor, and anti-inflammatory properties. The inhibition zones against *S. mutans* and *L. acidophilus* were also determined. The zones were larger in the selenium group with *S. mutans*. These results are explained by the ineffectiveness of selenium against *L. acidophilus*. The agar diffusion assay results suggested that *L. acidophilus* is not sensitive to any of the SeLECT Defense materials. The selenium group showed larger zones of inhibition with *S. mutans,* with an average of 9.2±2.3 mm, whereas those used with *L. acidophilus* were only 0.1±0.2 mm. The fluoride group showed average zones of inhibition of 9.0±3.2 mm with *S. mutans* and a much more significant 9.2±1.9 mm with *L. acidophilus*.

SeLECT Defense materials containing selenium showed the most significant differences when comparing microorganisms with agent, since they were the only materials showing differences in performance between the 2 microorganisms. They showed statistically significant differences in efficacy against *S. mutans* no antimicrobial effect against *L. acidophilus*. This could be because, in general, the strict anaerobes are less susceptible than the facultative microorganisms, but this was not confirmed with the fluoride. *S. mutans* is responsible for tooth decay by initiating biofilm formation, colonizing the tooth surface by being able to synthesize extracellular polysaccharides from sucrose [38]. Because of the further accumulation of biofilm, the number of capnophilic and obligatory anaerobic bacteria increase, and change the antimicrobial biofilm composition from streptococcus-dominated to *Lactobacillus* spp.-dominated [38], which are involved in root caries and periodontal disease, respectively [39,40]. *L. acidophilus* colonizes the periodontal pockets, where they co-exist with other microorganisms [40]. *L. acidophilus* plays a role in the pathogenesis of periodontal disease, gingivitis, and some odontogenic infections [40]. *S. mutans* and *L. acidophilus* are not equally sensitive to the same orthodontic materials, indicating that these species of bacteria may have different defense mechanisms. These findings agree with the study by Kelly [37]. Interesting, materials with selenium only produced zones of inhibition on agar plates inoculated with *S. mutans*. It is important to consider that *L. acidophilus* is most commonly implicated in deep carious lesions, while *S. mutans* may be present in incipient caries, such as WSLs [17]. *S. mutans* has a variety of virulence factors that contribute to its ability to initiate the caries process to produce WSLs, while *L. acidophilus* utilizes its ability to thrive in the low pH environment of existing carious lesions. Many authors suggest that without the initiation of WSL by *S. mutans*, *L. acidophilus* will not be present [17]. Therefore, it is of primary importance that antimicrobial orthodontic materials,

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such as SeLECT Defense, are effective against *S. mutans*, and secondarily effective against *L. acidophilus*.

As mentioned, despite not being effective against *L. acidophilus*, the addition of selenium into the material showed several advantages over that of fluoride, such as providing potential anti-*S. mutans* agents with higher effectiveness, which is important for further growth of *L. acidophilus* in *in vivo* conditions. The effectiveness of selenium against *S. mutans* has been reported as being more efficacious than fluoride. The mechanism of action for selenium totally differs from that of fluoride. The antimicrobial mechanism of action of selenium is its ability to catalyze the formation of superoxide radicals, which are bactericidal in nature [2]. During phases of bacterial growth, radical ions can irreversibly damage DNA as it is replicated. A material that produces radical ions which kill bacterial cells in this way would be considered bactericidal. There also exist radical-mediated pathways which inhibit metabolic enzyme function to prevent bacterial growth, and these are considered bacteriostatic. Superoxide radicals are, by definition, short-lived due to their highly reactive nature [41]. They are limited to the selenium-coated surfaces of SeLECT Defense products, and do not leach out into the oral environment beyond 35 nanometers [41]. Thus, selenium has 2 actions: bactericidal and bacteriostatic. The agar diffusion assay used in our study could not differentiate between bactericidal *vs.* bacteriostatic effects, and was also not sensitive enough to detect potential zones of inhibition of small magnitude with *L. acidophilus,* although SeLECT Defense products resulted in measurable zones of inhibition with *S. mutans*. However, clinically, bacteriostatic and bactericidal materials equally prevent WSLs, because WSLs formation is dependent on the growth of bacterial plaque and subsequent production of lactic acid, not simply the presence of cariogenic bacteria. These results thus suggest that SeLECT Defense products have antimicrobial properties, but the assay cannot determine if the antibacterial agent is a selenium-catalyzed superoxide radical.

Results of a study by Hammad [42] confirmed that selenium inhibits bacterial plaque formation on human teeth and the stability of the antibacterial effect over 6 months. The positive effects of fluoride noted in the present study coincide with the findings of previous reports [23]. So, although fluoride provides a significant benefit to patients at high risk of caries, a drawback is that it often requires multiple applications [1,25,29,43]. As a result, over time, the efficiency of these dental materials would be compromised, which requires the re-application of more fluoride to enhance their antimicrobial activity. In contrast, because of the covalent attachment of selenium to the polymer of the material, only very small amounts of unpolymerized selenium are released from the material. The significant difference between bacterial attachment and biofilm formation on fluoride *vs.* selenium is likely to be due to the O_2, catalytically produced by the selenium, which causes oxidative stress that damages the bacterial cell walls and DNA. Superoxide radicals are toxic to microorganisms but not to humans, even in large amounts. Furthermore, i*n vivo* and *in vitro* studies have proven that SeLECT Defense products are effective as antimicrobial agents and as prophylactic products against demineralization, while simultaneously displaying adequate shear bond strength and durability when used with different adhesives [42]. Therefore, the results of our study suggest that SeLECT Defense products are promising new antimicrobial sealants if used early enough as a preventive measure. This is supported by the findings of previous studies such as Bishara et al. [24] and Ogaard et al. [10], who found that selenium products prevent the formation of WSLs.

Modified orthodontic materials that have antibacterial effects can act as powerful antimicrobial agents that maintain control of the bacterial biofilm, preventing initial colonization of cariogenic bacteria. The results obtained in the present study are difficult to compare with those in previous studies because few authors have standardized the methodology and different bacterial strains were used. The present study demonstrated that fluoride and selenium might have the potential to augment measures to preventive WSLs development *in vivo*. Other studies agree with our findings, since selenium compounds covalently attached to different biomaterials have shown inhibition of bacterial biofilms [29,42]. A study by Tran et al. [29] found that selenium polymerized into dental sealant is effective in inhibiting bacterial attachment and biofilm formation of the 2 main oral pathogens, *S. mutans* and *S. salivarius*. The same was also observed in a comparable study of an organo-selenium-containing pit and fissure sealant with that of a selenium-free sealant for clinical retention and prevention of plaque and caries development by Amaechi et al. [44].

Our study has some limitations. The study was small, investigating only 7 specimens of respective orthodontic material and did not totally simulate the full oral cavity with orthodontic treatment environment (e.g., the use of artificial saliva). It is extremely difficult to recreate the oral environment outside of the mouth. Therefore, limitations of *in vitro* tests include the lack of application to *in vivo* conditions. Additionally, this *in vitro* study used only limited types of orthodontic materials. We encourage future investigators to expand the scope of their research and to test more products.

Conclusions

While this study demonstrated that orthodontic products containing selenium possess antimicrobial properties, it is important to emphasize that this was an *in vitro* study testing bacterial species in isolation. Furthermore, as have been few *in*

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vivo clinical trials, and it is unclear whether these antimicrobial properties are maintained throughout the course of orthodontic treatment. However, our results do suggest that orthodontic materials, including those containing selenium, might have the potential to prevent WSLs due to their antimicrobial properties. Materials containing fluoride showed greater antimicrobial effectiveness due to their efficacy against *L. acidophilus*. The addition of selenium or fluoride showed antibacterial activity when in contact with *S. mutans*. However, a much larger randomized clinical trial is needed to determine if the incidence of WSLs during orthodontic therapy can be decreased by using antimicrobial orthodontic products containing selenium.

Conflict of interest

None.

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