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Original Article

Comparison of microbial adhesion and biofilm formation on orthodontic wax materials; an in vitro study

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Abstract *Background/purpose:* Orthodontic wax materials are available on the dental market and are given by orthodontists due to pain, sores and irritation caused by treatment. The aim of the study was to compare biofilm formation and microbial adhesion at different time points on different protective materials used against orthodontic wounds in vitro.
Materials and methods: Microbial adhesion and biofilm formation were evaluated against *Streptococcus mutans* ATCC 25175 and *Lactobacillus acidophilus* ATCC 4356 standard strains on orthodontic wax materials at the 0, 24th, 48th, 72nd, 96th and 120th hour. The Kruskal Wallis test and Bonferroni test were used for statistical evaluations. Statistical significance was set at $p < 0.05$.
Results: It was observed that *S. mutans* formed statistically significantly more biofilm on OrthoDots®CLEAR (OrVance) than Ora-Aid (TBM Corporation) at the 48th hour ($p < 0.05$). Furthermore, *L. acidophilus* formed statistically significantly more biofilm on OrthoDots®-CLEAR (OrVance) than Brace Gard®(Infa-Lab Inc.) at the 72nd, 96th and 120th hours ($p < 0.05$).
Conclusion: Significant differences were noted among the different orthodontic wax materials and both *S. mutans* and *L. acidophilus* created biofilm on all waxes at different time points in vitro. To prevent biofilm formation, these waxes need to be refreshed and should not be used for more than 24 h. According to our study, biofilm production performances of pathogens on Brace Gard®(Infa-Lab Inc.) are minimal and therefore it may be a better option to use in clinics. However, to our knowledge, this is the first study investigating biofilm formation on waxes and more studies are needed in this field.

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Introduction

Orthodontic treatment is a long-lasting form of treatment that eliminates both aesthetic and functional concerns of the patient. Before starting orthodontic treatment, patients should pay attention to their oral hygiene and all dental or periodontal problems need to be treated.[1] Orthodontic appliances can prevent patients from having good oral hygiene, and the components of the appliances can cause changes in the oral microflora by reducing pH and increasing the retention areas for microorganisms, which increases an orthodontic patient's risk of developing white spot lesion and inflammatory reactions in gingival tissue.[2,3]

The oral microbiota serve as the habitat for millions of microorganisms. Some of them are known to be bacterial aetiological agents on the basis of periodontal diseases and demineralisation. The presence of *Streptococcus mutans* and *Lactobacillus acidophilus* can increase these risks.[1,3] Moreover, many researchers have reported that the amount of *S. mutans* and *L. acidophilus* increases after orthodontic treatment.[4,5] *S. mutans* is the bacteria with the best capacity for adhesion and biofilm formation on orthodontic materials in enamel demineralisation and caries formation.[5] It also increases salivary concentrations of *L. acidophilus*, which are considered to be the source of the acid that demineralises the enamel.[3]

Pain and discomfort are some of the common complications in orthodontic treatment. They are caused by irritation of the oral mucosa resulting from trauma and increased friction between tissues and brackets, wires and tubes.[6,7] With the formation of the wound, it may take 7—14 days to complete the epithelial healing. Therefore, preventing bacterial invasion during this period prevents secondary infection and supports the healing process.[8] Wounds caused by orthodontic irritation may get worse due to food intake or tongue irritation. It has been recognised that there is a need for barrier materials to protect wounds from being irritated by these stimuli, to prevent secondary infection and to reduce the patient's discomfort.[7,9,10] Orthodontic materials such as wax are available on the dental market and are given by orthodontists due to pain, sores and irritation caused by orthodontic treatment. In the US alone, it is estimated that 11 million packs of dental wax are dispensed or purchased by consumers annually.[11—14]

Many types of barrier materials used to prevent irritation caused by fixed orthodontic treatment are commercially available. One material is the adhesive periodontal wound dressing Ora-Aid (TBM Corporation, Gwangju, Korea) material. This product is a self-adhesive oral dressing material to protect mouth sores. It acts as a buffer between orthodontic appliances and wound, protecting the wound from secondary infections. It supports wound healing by isolating the wound. It is a disposable material and it can stay in the mouth for a long time due to its adhesive feature.[11]

Another material is the special food grade silicone wax Brace Gard® (Infa-Lab Inc., Rockaway, NJ, USA) material. The manufacturer states that silicone material is smoother and more comfortable than normal wax, is unaffected by mouth chemistry and does not crumble off the brackets. Unlike normal wax, silicone lasts longer and can be removed and then reapplied. Moreover, silicone is clear, providing a better appearance.[12]

Another disposable conventional orthodontic wound barrier material is Ormco wax (Ormco, Glendora, CA, USA) made from thermoplastic resin silicone. This product is a water-insoluble solid material that has no odour and taste.[13]

OrthoDots®CLEAR (OrVance, Caledonia, MI, USA). is a dispensed healthcare product that features hygienic unit-of-use packaging, tamper-evident packaging, labelling with product traceability, and ingredient disclosure. OrthoDots®CLEAR (OrVance) is made from two high-quality ingredients: medical grade silicone and polyvinylpyrrolidone (PVP). PVP has a long history of use in many oral care products, nutritional supplements and pharmaceuticals with limited risks to patient safety. It is claimed that OrthoDots®CLEAR (OrVance) is different from other dental wax with these features. It can stay on the surface for a minimum of 48 h.[14]

Clinically, the area where demineralisation and white spot lesion are most commonly located is the tooth surface around the bracket. The use of orthodontic wax in mouth sores caused by irritation of the brackets is also mostly in this region.[15—17] Although some researchers have investigated the adhesion of microorganisms and biofilm formation on bands,[3] brackets,[1,18] polyurethane elastomeric rings[19] and acrylic components,[20,21] no study has evaluated orthodontic wound protecting materials that are used in almost every fixed orthodontic treatment. The aim of this study was to experimentally analyse the microbial adhesion and biofilm formation ability of *S. mutans* and *L. acidophilus* individually and together on orthodontic wax materials' surfaces at different time points and to compare orthodontic wax materials.

Materials and methods

The study was deemed exempt by the Faculty of Dentistry Research Ethics Committee. Four orthodontic wax materials were selected: Ora-Aid (TBM Corporation), Brace Gard® (Infa-Lab Inc.), Ormco wax (Ormco), and OrthoDots®CLEAR (OrVance). The intraoral orthodontic wax materials used in this study were all commercially available and also they were preferred in the study because they are the most used wax materials. Disk-shaped specimens 6 mm in diameter and 1.2 mm in thickness were prepared. These specimens were placed in wells of the 96-well microwell plate in the laboratory environment. A coding system was used for blind evaluation and all laboratory analyses were performed with this.

S. mutans ATCC 25175 and *L. acidophilus* ATCC 4356 standard strains were used in vitro to investigate the amount of biofilm formation over time after their interaction with orthodontic wax materials. Bacterial suspensions were prepared using the triptic soy broth (TSB) (Oxoid, Basingstoke, UK) medium and optical density was prepared to be .5 McFarland turbidity standard (1.5×10^8 CFU/mL) at 600 nm 10 μL bacterial suspension and 90 μL TSB medium (Oxoid, UK) were added to the wells that contained wax materials. Subsequently, the microwell plates were incubated at 37 °C in the CO_2 incubator to calculated different

time points. All plates were incubated up to 120 h and the data for biofilm amount was recorded at 0th,24th,48th,72nd, 96th and 120th hours spectrophotometrically. The crystal violet staining method was used to detect biofilm formation of *S. mutans* ATCC 25175 and *L. acidophilus* 4356 standard strains individually and together on orthodontic wax material surfaces (22). After the incubations, the liquid in the wells was drained and 100 μL of .4% crystal violet was added and then incubated for 15 min. The wells were washed twice and 95% alcohol was added to the wells to dissolve the biofilm formed after bacterial interactions. Then, 96-well plates were scanned at 540 nm wavelength (OD540) using an Epoch spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA), and turbidity was measured in the wells and compared to the 0 h results.[22,23] All tests were performed in triplicate.

Statistical analysis

All statistical evaluations were performed in the SPSS software program (IBM, SPSS Statistics) [22]. Statistical significance was set at $p < 0.05$. Intraclass correlation coefficients (ICCs) were calculated to determine intrarater and interrater reliability. The Kruskal Wallis test was used to examine the difference between the mean of the amount of biofilm formation in vitro over time against *S. mutans* ATCC 25175 and *L. acidophilus* 4356 individually and together on intraoral orthodontic wax materials. The Bonferroni test was used to identify the group or groups that created the difference.

Results

ICCs were calculated as weighted kappa score was .89 (range: .91—.88). When comparing the *S. mutans* biofilm formation on different types of orthodontic wax materials during the 0—120 h observation period, it was found that the bacteria formed statistically significantly more biofilm on OrthoDots®CLEAR (OrVance) than Ora-Aid (TBM Corporation) at the 48th hour ($p < 0.05$). The effects of orthodontic wax materials on *S.mutans* biofilm formation are shown in Table 1.

When the L. *acidophilus* biofilm formation on different orthodontic wax materials was compared over the 0—120 h observation period, it was found that the bacteria formed statistically significantly more biofilm on OrthoDots®CLEAR (OrVance) than Brace Gard® (Infa-Lab Inc.) at the 72nd, 96th and 120th hours ($p < 0.05$). The effects of orthodontic wax materials on *L. acidophilus* biofilm formation are shown in Table 2.

Considering the ability of biofilm formation of *S. mutans* and *L. acidophilus* together on different orthodontic wax materials, it was found that more biofilm was formed at the 48th hour on OrthoDots®CLEAR (OrVance) compared to Brace Gard® (Infa-Lab Inc.). This significant difference continued to be observed at the 72nd, 96th and 120th hours ($p < 0.05$). The effects of all orthodontic wax materials on the biofilm formation of *S.mutans* and *L.acidophilus* together are shown in Table 3.

When the biofilm formation rates of *S. mutans* and *L. acidophilus* together were compared over 0—120 h on the same orthodontic wax, the amount of biofilm formation found on Brace Gard® (Infa-Lab Inc.) at the 96th and 120th hours was found to be significantly larger than the amount observed at the 72 nd h ($p < 0.05$). Furthermore, it was observed that a significantly larger amount of biofilm was formed on Ormco wax (Ormco) at the 96th hour compared to the time the experiment was started ($p < 0.05$). Biofilm formation rates of *S. mutans* and *L. acidophilus* together at 120 h on different orthodontic wax materials are shown in Table 4.

Table 1 Comparison amount of biofilm formed after interaction of *S. mutans* and intraoral orthodontic wax materials in-vitro environment over time.

Time	Material	Min	Max	$\overline{X}$	SS	X^2	p
0th hour	Ora-Aid	2.14	12.03	6.26	5.14	5.66	.12
	Brace Gard	2.78	2.92	2.86	.07		
	Ormco Wax	.64	15.48	5.59	8.55		
	OrthoDots Clear	15.17	19.55	17.42	2.19		
24th hour	Ora-Aid	2.15	11.11	6.28	4.52	4.84	.18
	Brace Gard	1.88	2.31	2.12	.22		
	Ormco Wax	14.39	14.60	14.51	.10		
	OrthoDots Clear	1.14	19.12	8.67	9.33		
48th hour	Ora-Aid	2.91	5.57	3.93	1.43	9.46	.02*
	Brace Gard	4.07	4.18	4.11	.06		
	Ormco Wax	12.93	14.34	13.86	.81		
	OrthoDots Clear	19.92	20.28	20.14	.19		
72nd hour	Ora-Aid	6.87	12.40	9.36	2.80	4.12	.24
	Brace Gard	6.52	11.40	8.22	2.75		
	Ormco Wax	14.55	17.54	15.55	1.71		
	OrthoDots Clear	6.29	19.04	14.76	7.33		
96th hour	Ora-Aid	15.39	16.66	15.84	.71	5.66	.12
	Brace Gard	3.84	8.32	6.59	2.41		
	Ormco Wax	14.74	15.21	14.96	.23		
	OrthoDots Clear	6.49	20.30	15.13	7.53		
120th hour	Ora-Aid	16.66	18.68	17.99	1.15	7.61	.05
	Brace Gard	4.51	5.31	4.87	.40		
	Ormco Wax	11.08	11.66	11.33	.29		
	OrthoDots Clear	3.36	15.36	7.44	6.85		

*$p < 0.05$.

Discussion

Orthodontic treatment, which is a branch of dentistry, is a long-lasting discipline where side effects can occur. The most common complications are root resorption, pain, pulpal changes, periodontal irritation (wound) and disease, decalcification, and temporomandibular dysfunction.[24] Experiencing local tissue damage during orthodontic treatment is a very common condition that negatively affects both treatment duration and patient motivation. In a study conducted by Kvam et al., the presence of small

Table 2 Comparison amount of biofilm formed after interaction of *L. acidophilus* and intraoral orthodontic wax materials in-vitro environment over time.

Time	Material	Min	Max	$\overline{X}$	SS	X^2	p
0th hour	Ora-Aid	.51	9.34	4.01	4.68	6.59	.08
	Brace Gard	4.22	5.00	4.70	.42		
	Ormco Wax	.64	15.48	5.59	8.55		
	OrthoDots Clear	20.54	21.27	20.80	.40		
24th hour	Ora-Aid	1.90	9.88	5.41	4.07	9.35	.02*
	Brace Gard	2.41	6.07	3.68	2.06		
	Ormco Wax	14.39	14.60	14.51	.10		
	OrthoDots Clear	15.95	18.14	17.20	1.12		
48th hour	Ora-Aid	5.95	10.90	8.04	2.56	8.53	.03*
	Brace Gard	6.25	6.49	6.34	.12		
	Ormco Wax	12.93	14.34	13.86	.81		
	OrthoDots Clear	12.72	20.79	17.80	4.42		
72nd hour	Ora-Aid	3.80	6.71	4.85	1.61	10.38	.01*
	Brace Gard	3.45	3.75	3.62	.15		
	Ormco Wax	14.55	17.54	15.55	1.71		
	OrthoDots Clear	18.14	20.31	19.52	1.19		
96th hour	Ora-Aid	10.57	18.14	15.57	4.33	8.43	.03*
	Brace Gard	2.80	3.94	3.45	.58		
	Ormco Wax	14.74	15.21	14.96	.23		
	OrthoDots Clear	17.68	24.51	22.20	3.91		
120th hour	Ora-Aid	16.21	19.82	18.58	2.05	9.66	.02*
	Brace Gard	3.37	7.01	5.43	1.86		
	Ormco Wax	11.08	11.66	11.33	.29		
	OrthoDots Clear	18.09	20.21	19.50	1.22		

*$p < 0.05$.

Table 3 Comparison amount of biofilm formed after interaction with *S. mutans* and *L. acidophilus* strains with intraoral orthodontic wax materials in vitro environment over time.

Time	Material	Min	Max	$\overline{X}$	SS	X^2	p
0th hour	Ora-Aid	2.14	11.61	7.37	4.81	9.46	.02*
	Brace Gard	3.55	4.00	3.84	.25		
	Ormco Wax	1.06	1.43	1.20	.19		
	OrthoDots Clear	20.49	22.51	21.38	1.03		
24th hour	Ora-Aid	2.04	8.06	5.02	3.01	7.64	.05
	Brace Gard	3.47	10.62	5.85	4.12		
	Ormco Wax	1.25	4.37	2.45	1.67		
	OrthoDots Clear	14.68	17.83	16.69	1.74		
48th hour	Ora-Aid	6.65	11.82	8.48	2.89	10.38	.01*
	Brace Gard	2.56	2.87	2.75	.17		
	Ormco Wax	12.85	16.01	14.28	1.60		
	OrthoDots Clear	19.58	20.02	19.74	.24		
72nd hour	Ora-Aid	2.97	3.66	3.41	.38	9.52	.02*
	Brace Gard	2.01	2.32	2.11	.17		
	Ormco Wax	12.44	13.97	13.46	.88		
	OrthoDots Clear	4.40	20.46	13.88	8.41		
96th hour	Ora-Aid	10.26	17.84	15.31	4.37	8.12	.04*
	Brace Gard	5.16	12.04	9.69	3.92		
	Ormco Wax	14.06	16.56	15.67	1.39		
	OrthoDots Clear	18.62	23.06	20.96	2.22		
120th hour	Ora-Aid	6.16	17.39	13.57	6.41	7.61	.05
	Brace Gard	8.79	12.27	10.02	1.94		
	Ormco Wax	15.20	15.55	15.42	.19		
	OrthoDots Clear	17.69	18.83	18.35	.59		

*$p < 0.05$.

lesions was found in 75.8% of patients and large lesions were found in 2.5% of patients.[25] Ulcerations, pain, and discomfort caused by irritation are most frequently encountered. Mucosal trauma lesions may occur due to ulceration caused by brackets and tubes, irritation caused by the arc wire in the molar region or excessive use of the appliances in the vestibule or palatinal side.[7,9,10,25]

Orthodontists have options to prevent or relieve mucosal irritation by using fixed orthodontic appliances. The simplest solution in traumatic injuries is to eliminate the cause factor. However, since it is not possible to remove fixed orthodontic appliances from the mouth during the treatment, the use of a barrier material will contribute to the healing by acting as a buffer between the irritation zone and orthodontic appliances. Products containing fluoride, chlorhexidine gluconate, hyaluranic acid or antibiotics, which are in the form of mouthwash or gel, can be used to prevent bacterial adhesion and reduce the lesions that occur during orthodontic treatment or to heal the wound; however, they have insufficient adherence to the mucosa and orthodontic appliances.[6,26,27] These agents, which may be beneficial in wound healing, are insufficient in preventing the development of secondary infections and microbial adhesion.[6] The preferred properties of topically applied agents are the ability to afford a smooth surface, prevent irritation and be flexible, as well as adhesive and dimensional stability. Therefore, in this study, orthodontic wax material was preferred as the protective material for investigetion. Orthodontists can give the patient wax to cover the brackets, wires or tubes. The oral mucosa is keratinised very quickly and the patient gets used to the newly installed orthodontic appliances. Wax material given to the patient minimises the initial trauma and discomfort of the patient.[6,27,28] The orthodontic wax products used in this study are strips, which are easy to apply and can reduce the patient's discomfort because of very low foreign body feeling.

As orthodontic devices remain in the mouth for a long time and are very widely used, there is a need to conduct research in microbiologically.[1,3] Orthodontic wax materials are the most frequently used materials against wounds that occur during treatment. The orthodontic material is in contact with the wound until it heals, and this can provide an attachment surface for the bacteria. The more the adhesion surface of the orthodontic material increases, the

Table 4 Comparison amount of biofilm formed by *S. mutans* and *L. acidophilus* strains coexistence in-vitro after interaction with intraoral orthodontic wax materials according to time.

Material	Time	Min	Max	$\overline{X}$	SS	X^2	p
Ora-Aid	0th hour	2.14	11.61	7.37	4.81	9.93	.07
	24th hour	2.04	8.06	5.02	3.01		
	48th hour	6.65	11.82	8.48	2.89		
	72nd hour	2.97	3.66	3.41	.38		
	96th hour	10.26	17.84	15.31	4.37		
	120th hour	6.16	17.39	13.57	6.41		
Brace Gard	0th hour	3.55	4.00	3.84	.25	14.50	.01*
	24th hour	3.47	10.62	5.85	4.12		
	48th hour	2.56	2.87	2.75	.17		
	72nd hour	2.01	2.32	2.11	.17		
	96th hour	5.16	12.04	9.69	3.92		
	120th hour	8.79	12.27	10.02	1.94		
Ormco Wax	0th hour	1.06	1.43	1.20	.19	14.58	.01*
	24th hour	1.25	4.37	2.45	1.67		
	48th hour	12.85	16.01	14.28	1.60		
	72nd hour	12.44	13.97	13.46	.88		
	96th hour	14.06	16.56	15.67	1.39		
	120th hour	15.20	15.55	15.42	.19		
OrthoDots Clear	0th hour	20.49	22.51	21.38	1.03	11.33	.04*
	24th hour	14.68	17.83	16.69	1.74		
	48th hour	19.58	20.02	19.74	.24		
	72nd hour	4.40	20.46	13.88	8.41		
	96th hour	18.62	23.06	20.96	2.22		
	120th hour	17.69	18.83	18.35	.59		

*$p < 0.05$.

greater the adhesion and accumulation of bacteria. In the studies carried out previously, band and acrylic resin created more adhesion area than brackets, and it was found that there was more *S. mutans* adhesion.[1,5]

Biofilm formation is a microbially complex process and a cosmopolitan structure, as often more than one microorganism is involved in this process. In addition, biofilm formation causes enamel demineralisation, white spot lesion and dental caries in the following period. *S. mutans* and *L. acidophilus* are the main bacterial pathogens in the formation of caries, and it is important to prepare the environments where two pathogens coexist in vitro and to evaluate their biofilm formation abilities together.[3,4,29] In our study, the ability of *S. mutans* and *L. acidophilus* to form biofilm on different orthodontic wax materials in the environment of co-inoculum was compared. Starting from the 48th hour, intense biofilm formation was observed on OrthoDots®CLEAR (OrVance) and a significant difference was found between the amount of biofilm on OrthoDots®-CLEAR (OrVance) and Brace Gard® (Infa-Lab Inc.). However, when the biofilm formation properties of the two pathogens together on Brace Gard® (Infa-Lab Inc.) over 120 h were examined, the development of biofilm was observed at 96 h. However, it is noteworthy that Brace Gard® (Infa-Lab Inc.) had a smaller amount of biofilm on it than the other orthodontic waxes used in the study. It was observed that OrthoDots®CLEAR (OrVance) created a more suitable surface for *S. mutans* to form biofilm compared to Ora-Aid (TBM Corporation). Moreover, it was concluded that during orthodontic treatment, may contribute to enamel demineralisation and white spot lesion formation. When the biofilm formation capacity of *L. acidophilus* on different orthodontic wax surfaces was examined, it was observed that OrthoDots®CLEAR (OrVance) had created a more suitable surface for *L.acidophilus* to form biofilm than Brace Gard® (Infa-Lab Inc.), especially after 72 h, and it was concluded that during the orthodontic treatment process, may provoke the development of dental caries on the surface of the tooth.

It was concluded that the effect of surface properties on *S. mutans* adhesion was not significantly affected by saliva coating and there was a significant correlation between surface roughness, surface free energy characteristics and *S. mutans* adhesion.[30] In this study, saliva was not used for coating the samples, as previous studies[31,32] had shown that the adhesion patterns of *S. mutans* and *L. acidophilus* were not significantly affected by saliva coating.[33]

There is no chemical adhesion between the orthodontic appliances and wax materials. If the wax comes off, the patient has to reapply it. Although it seems to be a single-use material, manufacturers have suggested that it can hold up to 96 h or more for some materials and remain in place. For this reason, in our study, evaluation of biofilm formation and microbial adhesion over 120 h was performed.

Biofilm formation assays were used to assess the colonisation capacity of selected bacteria on wax material. Moreover, information was provided on whether to support dental plaque formation next to fixed orthodontic devices that are clinically responsible for the formation of white spot lesion.[34] In this in vitro study that was based on pure cultures of selected bacterial strains, the effects of the interaction of a large number of bacterial species, saliva and bacteria in the mouth were not taken into account. This is a limitation of the study.

All orthodontic materials can play a role in enamel demineralisation as they create regions that can cause bacterial adhesion in the mouth environment. Analysis of adhesion and biofilm formation of cariogenic *S. mutans* and *L. acidophilus* will reinforce understanding of the factors causing enamel demineralisation.[35–37] This study was conducted to determine the level of *S. mutans* and *L. acidophilus*, which were adhered to, cultured and tested on orthodontic wax material, as both microorganisms play an important role in enamel demineralisation and white spot lesion formation. Thus, the current study addresses the need for further investigations of biofilm formation on different types of orthodontic wound protective materials. The findings could be helpful in the development of materials with minimal or anti-biofilm-forming surface chemistries.

Orthodontic treatment is a long-term treatment and it should be ensured that the materials used in the treatment are not suitable for biofilm formation. Although there are studies on biofilm formation on various orthodontic materials in this area, there are no studies that use orthodontic waxes. Since there is no previous study on bacterial adhesion and biofilm formation related to these wax materials, it is not possible to compare the data directly. Our study is the first in this field and it is important in terms of showing

that microorganisms can also form biofilm on orthodontic wax materials. The fact that different amounts of biofilm are formed on different types of wax materials will guide orthodontists in the selection of orthodontic wax in treatment. According to our results, Brace Gard® (Infa-Lab Inc.) is a better option than other wax materials as less biofilm formation is observed on it than on the other three orthodontic wax materials. Nevertheless, more studies are needed in this area. Further research is needed to potentially reduce plaque adhesion and increase the protective properties of protective and barrier materials such as orthodontic wax used during treatment with fixed devices.

This study was conducted to analyse the level of bacterial adhesion and biofilm formation on orthodontic wax materials. These materials serve as extra space for biofilm formation. In this in vitro study, significant differences were noted between the different types of orthodontic wax materials. The type of orthodontic wax material may influence the bacterial adhesion. According to our study, biofilm production performances of pathogens on Brace Gard® (Infa-Lab Inc.) are minimal and therefore it may be a better wax option to use in clinics. However, as this is the first study investigating biofilm formation on waxes, more studies are needed in this field. This research provides valuable information for identifying the orthodontic wax materials with a minor risk for development of white spot lesions and formation of caries.

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article.

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