Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 6 Issue 11 November 2022

Research Article

Comparative Evaluation of *Streptococcus mutans* biofilm collected from Excessive Adhesive Flash (EAF) in patients undergoing fixed orthodontic appliance therapy bonded with two different adhesives

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DOI: 10.31080/ASDS.2022.06.1497

Received: October 03, 2022

Published: October 18, 2022

Abstract

Objective: To assess accumulation of *Streptococcus Mutans* (SM), in biofilm collected from Excessive Adhesive Flash (EAF) produced from two different orthodontic adhesive resins, in patients undertaking fixed appliance therapy.

Materials and Methods: A prospective interventional split mouth design was followed. 20 patients were segregated based on the resin used: Group 1: Right maxillary lateral incisor bonded with non- tooth coloured resin [adh 1: Transbond Plus]; Group 2: LeR maxillary lateral incisor bonded with tooth coloured resin [adh 2: Transbond XT]. Plaque was procured from EAF at these sites at 4 inter- vals: T0- before bonding; T1-1-month aRer bonding; T2-2 months aRer bonding; T3-3 months aRer bonding. Colonization of SM was assessed by colony count.

Results: There was a statistically significant surge in SM count, at T1 in both groups. The colony counts of SM were greater in Group 2. A subsequent decrease was seen in colony counts of both groups at T2 and T3, as mouth rinse was prescribed aRer T1.

Conclusions: The EAF formed from both adhesives, showed increased colonisation of SM at T1. The amount of SM on the EAF generated from Transbond XT was greater in comparison to Transbond Plus.

Keywords: Excessive Adhesive Flash; *Streptococcus Mutans*; Orthodontic

Introduction

Multibracket orthodontic appliances have an increased affinity for dental plaque retention, thereby creating difficulty in maintenance of oral hygiene for such patients [1]. Patients receiving treatment with fixed orthodontic appliances are at a substantial risk of enamel demineralization, as there islack of accessibility for cleansing the minute regions of these appliances [2]. Orthodontic treatment causes oral ecological variations, bringing about a surge in *Streptococcus Mutans* (SM) in saliva, and plaque accumulation [3]. Formation of dental plaque is the preliminary step in dental caries initiation, and SM is considered as one of the chief colonizers in the multi-species dental biofilm [4]. During fixed orthodontic appliance treatment, up to 5-fold increment in the number of SM has been observed [5]. Additionally, orthodontic bonding materials and tooth attachments may retain plaque and facilitate initiation of! white spot lesions" (WSL) and dental caries [5].

While performing bracket bonding procedures, an unspecified quantity of adhesive gets leR on the tooth surface, invariably along the periphery between the appliance and enamel interface. This is termed as! Excessive Adhesive Flash" (EAF) [2]. If this EAF is not cautiously eliminated during the process of bonding, it may recurrently contribute as a mechanically noxious stimuli leading to irritation of the gingiva, specifically on teeth, where the height from bracket pad to marginal gingiva is less [6]. Secondarily, bacteria will easily occupy the rough surface and speed up the incidence of WSLs and dental caries [2]. The order of incidence of WSLs aRer orthodontic treatment has been recorded as lateral incisor (34%), canine (31%), premolar (28%), and central incisor (17%), for the maxillary teeth [7]. Thereby, EAF acts as a harbour that anchors varieties of pathogenic bacteria, especially SM [2].

With the advent of light-cured composite resin materials in dentistry, orthodontists are able to re- move EAF with no trouble, in a soR state, before it sets [6]. These adhesives may be tooth-coloured or non-tooth coloured (with a colour transition feature) [6]. The usage of non-tooth-coloured composite resins has been advocated to aid in improved visualization of the adhesive, thereby reducing the amount of EAF generated during bracket bonding procedures [2].

Few studies have been conducted *in vivo*, with respect to EAF and its role in orthodontic treatment. Hence, this investigation was planned, to assess the accumulation of SM, in biofilm collected from EAF produced from two different types of orthodontic adhesive resins, in patients undertaking fixed appliance therapy.

Materials and Methods

A prospective interventional study design was planned and followed. ARer screening, 20 patients who volunteered and seemed motivated to be a part of the study, were recruited based on the following:

Inclusion criteria

- Patients undertaking fixed orthodontic appliance therapy, immaterial of the type of malocclusion with pre-adjusted edgewise brackets.
- Patients between 15-25 years of age.
- Patients with maxillary lateral incisors bereR of caries, hypoplasia or other developmental anomalies.
- Patients bereR of any systemic diseases, periodontal diseases or on antibiotic therapy having oral manifestations.
- Patients with no history of mouth rinse usage in the past 3 months.
- Patients with no significant history of hypersensitivity to the materials being used.

Exclusion criteria

- Patients with fixed or removable lateral incisor prostheses.
- Patients with blocked out, missing, peg shaped, deformed or developmentally abnormal lateral incisors.
- Patients with chronic systemic diseases, dentofacial abnormalities or craniofacial syndromes
- Patients with history of smoking, alcoholism or addiction to other deleterious substances.

For each of these 20 patients, a split mouth design was followed uniformly where the patients‡ lateral incisors were segregated into two groups: Group 1: Maxillary lateral incisor of the right side bonded with non-tooth coloured orthodontic adhesive resin [adh 1: Transbond Plus]; Group 2: Maxillary lateral incisor of the leR side bonded with tooth coloured orthodontic adhesive resin [adh 2: Transbond XT]. Informed consent was acquired from these patients, and the procedure was explained at length. Before the treatment, and during every follow-up visit, patients were motivated to follow proper oral hygiene instructions. Verbal reinforcements and physical demonstrations were provided on performing effective

oral hygiene maintenance, particularly, proximal to the brackets and ligatures.

Patients were asked to refrain from consuming food or beverages or implementing any oral hygiene procedures, for two hours prior to collection of plaque samples. Designated portion of the anterior dentition for plaque collection was isolated with cotton rolls, promptly dried, disclosing agent (Plaque-D by MAARC) was administered for visualisation of biofilm, and plaque was collected from the labial surface of maxillary right lateral incisor using a sterile curette, prior to bonding, to deter- mine the carriage of SM by the patient, which was termed as T0. The same procedure was repeated for the maxillary leR lateral incisor. Appliance bonding procedures were then carried out using non- tooth coloured orthodontic adhesive resin for the maxillary right quadrant, and tooth coloured orthodontic adhesive resin for the maxillary leR quadrant, while maintaining proper isolation. The placement of appliances was done by a single operator for all patients to avoid inter-operator bias. ARer 1 month, plaque samples were obtained from EAF on lateral incisors from both sides, and this reading was termed as T1. Such an interval had been chosen assuming that there will be sufficient colonization of bacteria around the surface of the orthodontic adhesive. These readings were com- pared with T0 to understand the difference in colonization of SM, before and aRer placement of fixed appliances. T1 readings obtained by collecting plaque samples from lateral incisors of both right and leR side, respectively were also compared with each other to evaluate which orthodontic adhesive resin attracts more colony forming SM. Similarly, samples were collected at T2-2 months and T3-3 months aRer bonding.

Methods applied for quantification of SM

Plaque samples were added to a test tube containing 2ml of transport fluid (brain-heart infusion broth) and taken to microbiology laboratory within 2 hours for processing. Samples were then plated on Mitis Salivarius Bacitracin agar for identification of SM. A single agar plate was divided into two sections, where one half was used to streak the sample collected from leR side, and the other half was used for right side. Streaking was done using sterile nichrome loops of 4mm diameter. Agar plates were incubated at 37˚C in a candle jar or in an anaerobic jar with gas pack for 48 hours. Total colony count for quantification of SM was obtained from the agar plate. The colonies obtained were then sub-cultured on new agar plates and incubated for 48 hours. The sub-culture colonies were used for: Gram staining, Catalase test.

The data obtained was subjected to analysis using SPSS 22.0 version and Graph Pad Prism 5.0 version. $P < 0.05$ was considered as level of significance. The statistical tests implemented for scrutiny of the results were: Students' paired and unpaired t tests.

Results

Comparison of colony of SM in adh 1-Transbond Plus with baseline: (Table 1, Figure 1)

Time Interval	Mean	N	Std. Deviation	Std. Error Mean	Mean Difference	T-value	P-value
T0	25.75	20	9.82	2.19	-	-	-
T1	76.35	20	16.43	3.67	50.60 ± 9.18	24.63	0.0001, S
T2	64.25	20	15.62	3.49	38.50 ± 8.43	20.41	0.0001, S
T3	62.05	20	16.74	3.74	36.30 ± 9.93	16.34	0.0001, S

Table 1: Comparison of colony of streptococcus mutans in adh 1 Transbond Plus with baseline.

Student's Paired t test.

Mean colony formation at time T0 was 25.75%9.82, at time T1 it was 76.35%16.43 (t = 24.63, p = 0.0001), at time T2 it was 64.25%15.62 (t = 20.41, p = 0.0001) and at time T3 it was 62.05%16.74 (t = 16.34, p = 0.0001).

Comparison of colony of SM in adh 2-Transbond XT with baseline: (Table 2, Figure 2)

Mean colony formation at time T0 was 25.85%9.81, at time T1 it was 88.40%20.31 (t = 18.64, p = 0.0001), at time T2 it was 76.85%17.42 (t = 17.28, p = 0.0001) and at time T3 it was 75.85%16.82 (t = 17.73, p = 0.0001).

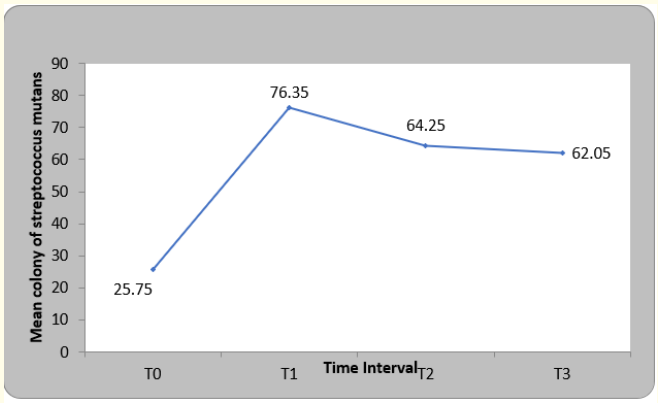

Figure 1: Graph showing Comparison of colony of *streptococcus mutans* in adh 1 -Transbond Plus with baseline.

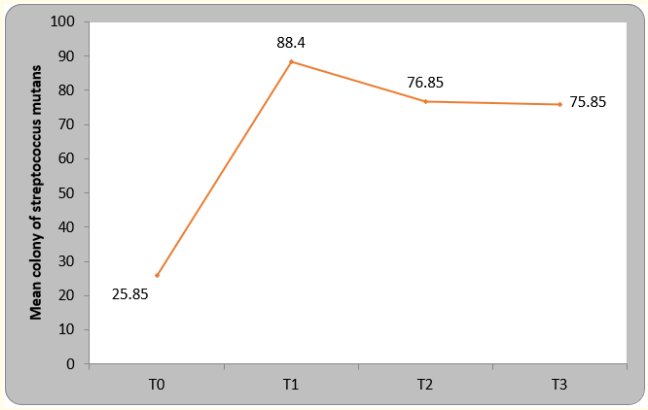

Figure 2: Graph showing Comparison of colony of *streptococcus mutans* in adh 2 Transbond XT with baseline.

Time Interval	Mean	N	Std. Deviation	Std. Error Mean	Mean Difference	T-value	P-value
T0	25.85	20	9.81	2.19	-	-	-
T1	88.40	20	20.31	4.54	62.55±15	18.64	0.0001, S
T2	76.85	20	17.42	3.89	51±13.19	17.28	0.0001, S
T3	75.85	20	16.82	3.76	50±12.61	17.73	0.0001, S

Table 2: Comparison of colony of streptococcus mutans in adh 2-Transbond XT with baseline.

Student's Paired t test.

Comparison of colony of SM in adh 1-Transbond Plus and adh 2-Transbond XT: (Table 3, Figure 3)

- **Mean colony formation in adhesive 1:** Transbond Plus group was 25.75% 9.82, and in adhesive 2: Transbond XT group, it was 25.85% 9.81 at time T0 (t = 0.03, p = 0.97).
- **Mean colony formation in adhesive 1:** Transbond Plus group was 76.35% 16.43, and in adhesive 2: Transbond XT group, it was 88.40% 20.31 at time T1 (t = 2.06, p = 0.046).
- **Mean colony formation in adhesive 1:** Transbond Plus group was 64.25% 15.62, and in adhesive 2: Transbond XT group, it was 76.85% 17.42 at time T2 (t = 2.40, p = 0.021).
- **Mean colony formation in adhesive 1:** Transbond Plus group was 62.05% 16.74, and in adhesive 2: Transbond XT group, it was 75.85% 16.82 at time T3 (t = 2.60, p = 0.013).

Time	Group	N	Mean	Std. Deviation	Std. Error Mean	T-value	P-value
T0	adh 1-Transbond Plus	20	25.75	9.82	2.19	0.03	0.97, NS
	adh2-Transbond XT	20	25.85	9.81	2.19		
T1	adh 1-Transbond Plus	20	76.35	16.43	3.67	2.06	0.046, S
	adh2-Transbond XT	20	88.40	20.31	4.54		
T2	adh 1-Transbond Plus	20	64.25	15.62	3.49	2.40	0.021, S
	adh2-Transbond XT	20	76.85	17.42	3.89		
T3	adh 1-Transbond Plus	20	62.05	16.74	3.74	2.60	0.013, S
	adh2-Transbond XT	20	75.85	16.82	3.76		

Table 3: Comparison of colony of streptococcus mutans in adh 1Transbond Plus and adh 2-Transbond XT.

Student's unpaired t test.

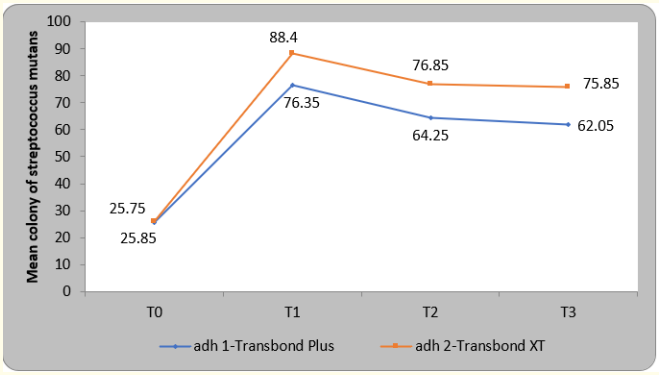

Figure 3: Graph showing Comparison of colony of *streptococcus mutans* in adh 1-Transbond Plus and adh 2-Transbond XT.

Discussion

Following the introduction of the edgewise principle, the bracket bonding technique utilising phosphoric acid etching and composite adhesive resin, is considered to be one of the most significant advancements in the field of orthodontics [8]. However, the design and surface features of orthodontic attachments, and composite adhesive resin affect plaque retention [9]. Roughness of these surfaces influences rapid adhesion and cultivation of oral microflora [10,11]. In routine practice, some quantity of EAF remains inadvertently in conjunction with, the periphery between the bracket base and the tooth enamel upon appliance bonding [6].

The application of orthodontic composite adhesive resin with a colour transition feature has been recommended to facilitate improved visualization of the material, thus simplifying the prompt removal of EAF during appliance bonding procedures [6]. However, very few studies comparing the sur- face characteristics of conventional adhesives to those with a colour change feature have been documented in existing literature.

Likewise, not many studies had been conducted to assess which of the above mentioned two composites will accumulate more acidogenic bacteria. Also, most studies comparing orthodontic adhesives have been carried out *in-vitro*.

Therefore, this study was planned where two different orthodontic adhesives, one being convention- al and the other with a colour change feature were compared for the amount of accumulation of SM.

According to Ahn [1]., *et al*, the prevalence of SM was observed to be greater on maxillary incisor brackets (50%), compared to mandibular incisor brackets (33.8%). Hence, the maxillary incisor region was targeted for this study. According to existing literature, facial surfaces of maxillary lateral incisors are the most affected by orthodontic treatment, and show maximum incidence of demineralization, which is why this site was chosen for plaque collection [13-15].

Precautionary efforts in susceptible populations have been fixated on immediate inhibition of the cariogenic oral micro-organisms by chemotherapeutic agents as an aide-de-camp to enhanced oral hygiene practices [3,16]. Therefore, aRer taking samples for T1, patients were educated to use mouth rinse for maintenance of oral hygiene.

T0, T1, T2 and T3 readings were compared with each other, by appropriate statistical methods and the difference obtained in the colonization of SM at each interval was noted. The following observations were noted.

Alteration in the oral microflora one month aVer bonding

There was a statistically significant surge in the SM count, one-month aRer bonding the appliance in both the groups, irrespective of the orthodontic adhesive being used.

This observation is in agreement with the observations of Naranjo [17]., *et al,* where a notable increase was observed in the biofilm bacterial levels aRer appliance bonding. The findings of our study were also similar to the findings of Jung [18]., *et al* and Arab [19]., *et al.*

However, according to the research of Carrillo [20]., *et al* on 34 orthodontic patients, even though SM levels rise in both plaque and saliva, one-month aRer appliance bonding, the increment was not statistically significant.

Also, in contrast to our study, Mota [21]., *et al* and Koga [22]., *et al*, found no significant increase in the salivary SM levels, aRer appliance bonding in young adults.

The amount of colony forming units decreased gradually in both groups at T2 and T3, aRer mouth rinses were prescribed. However,

they were still greater in number compared to baseline (T0), with a statistically significant difference between them. (Table 1, Table 2).

Comparison between orthodontic adhesives

The number of colonies of SM were greater in the Group 1, in comparison to Group 2. The difference in the amount of colonisation persists even aRer prescription of mouth rinses, with Transbond XT group exhibiting a significantly greater quantity in comparison to Transbond Plus group.

Lee [23]., *et al.* found that orthodontic adhesives had greater surface free energy characteristics and low- er surface roughness than bracket materials, owing to which they showed a higher capacity to retain SM than bracket materials. Practically, common regions for decalcification are at the orthodontic adhesive and enamel interface, encircling the bracket base where it unites with the tooth structure [10,23]. Filler particles in the resin matrix are continually exposed, due to sequential wear, thereby contributing to a persistently roughened surface [24]. Literature is abounding with investigations proving that acidogenic micro-organisms will readily inhabit such surfaces and cause an upsurge in the incidence of WSLs [25].

The results of our study are in a similar vein with those of the research published by Ho [2]., *et al* (2017), where Transbond XT (3M) and Grengloo (Ormco) were compared with each other for adherence and biofilm formation of SM. On evaluating through scanning electron microscopy, Transbond XT presented greater EAF on the tooth surface than Grengloo, which may have resulted in considerably higher amounts of biofilm formation, and subsequently increased SM adhesion on the brackets bonded with Transbond XT. It was also noted that the Transbond XT surface exhibited more hydrophilicity than the Grengloo surface, suggesting that bacterial adhesion may be influenced by greater hydrophilicity and increased surface energy of bonding adhesives.

However, according to the research work of Armstrong [6]., *et al*, no significant reduction could be appreciated in the quantity of EAF generated around orthodontic brackets even with the additional colour transition feature incorporated in the bonding material.

However, both these studies were conducted in-vitro, whereas our study was performed *in vivo*. Even though we assessed the growth in the number of SM, specific strains of SM responsible for WSLs in these patients were not isolated. Variations on the basis of malocclusion and sex were also not taken into account.

Conclusion

The colony counts of SM increased in the oral cavity within one-month aRer bonding procedures. The EAF formed from both Transbond XT and Transbond Plus, showed increased colonisation of SM. The colony counts of SM were greater on EAF generated from the tooth coloured adhesive, i.e., Trans- bond XT in comparison to the non-tooth coloured adhesive, i.e., Transbond Plus.

Bibliography

1. Jurišić S., *et al*. "Assessment of efficacy of two chlorhexidine mouth rinses on oral hygiene and gingival health in adolescents wearing two types of orthodontic brackets". *International Journal of Dental Hygiene* 16.2 (2018): e52-e57.

2. Ho CSF., *et al*. "Streptococcus mutans forms xylitol-resistant biofilm on excess adhesive flash in novel ex-vivo orthodontic bracket model". *American Journal of Orthodontics and Dentofacial Orthopedics* 151.4 (2017): 669-677.

3. Fard BK., *et al*. "Effectiveness of Mouth Washes on Streptococci in Plaque around Orthodontic Appliances". *ISRN Dentistry* 2011 (2011): 1-4.

4. Saffari F., *et al*. "The effects of chlorhexidine and persica mouthwashes on colonization of Streptococcus mutans on fixed orthodontics O-rings". *Journal of Dentistry* 16.1 (2015): 54.

5. Artun J and Brobakken BO. "Prevalence of carious white spots aRer orthodontic treatment with multibonded appliances". *European Journal of Orthodontics* 8.4 (1986): 229-234.

6. Armstrong D., *et al*. "Excess Adhesive Flash Upon Bracket Placement: A Typodont Study Comparing APC PLUS and Transbond XT". *The Angle Orthodontist* 77.6 (2007): 1101-1108.

7. Chapman JA., *et al*. "Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances". *American Journal of Orthodontics and Dentofacial Orthopedics* 138.2 (2010): 188-194.

8. Ahn S-J., *et al*. "Roles of Salivary Proteins in the Adherence of Oral Streptococci to Various Orthodontic Brackets". *Journal of Dental Research* 81.6 (2002): 411-415.

9. Lundström F and Krasse BO. "Caries incidence in orthodontic patients with high levels of Strep- tococcus mutans". *European Journal of Orthodontics* 9.1 (1987): 117-121.

10. Gwinnett AJ and Ceen RF. "Plaque distribution on bonded brackets: A scanning microscope study". *American Journal of Orthodontics* 75.6 (1979): 667-677.

11. Weitman RT and Eames WB. "Plaque accumulation on composite surfaces aRer various finishing procedures". *The Journal of the American Dental Association* 91.1 (1975): 101-106.

12. Ahn S-J., *et al*. "Prevalence of cariogenic streptococci on incisor brackets detected by polymerase chain reaction". *American Journal of Orthodontics and Dentofacial Orthopedics* 131.6 (2007): 736-741.

13. Bishara SE and Ostby AW. "White Spot Lesions: Formation, Prevention, and Treatment". *Seminars in Orthodontics* 14.3 (2008): 174-182.

14. Richter AE., *et al*. "Incidence of caries lesions among patients treated with comprehensive orthodontics". *American Journal of Orthodontics and Dentofacial Orthopedics* 139.5 (2011): 657-664.

15. Julien KC., *et al*. "Prevalence of white spot lesion formation during orthodontic treatment". *The Angle Orthodontist* 83.4 (2013): 641-647.

16. Raina R. "A Comparison of Antibacterial Efficacy of 0.5% Sodium Fluoride Impregnated Miswak and Plain Miswak Sticks on *Streprococcus mutans* - A Randomized Controlled Trial". *Journal of Clinical and Diagnostic Research* 11.2 (2017): ZC01.

17. Naranjo AA., *et al*. "Changes in the subgingival microbiota and periodontal parameters before and 3 months aRer bracket placement". *American Journal of Orthodontics and Dentofacial Orthopedics* 130.3 (2006): 275.e17-275.e22.

18. Jung W-S., *et al*. "Quantitative analysis of changes in salivary mutans streptococci aRer orthodontic treatment". *American Journal of Orthodontics and Dentofacial Orthopedics* 145.5 (2014): 603-609.

19. Arab S., *et al*. "Effect of fixed orthodontic treatment on salivary flow, pH and microbial count". *Journal of Dentistry (Tehran, Iran)* 13.1 (2016): 18.

20. Lara-Carrillo E., *et al*. "Effect of orthodontic treatment on saliva, plaque and the levels of Streptococcus mutans and Lactobacillus". *Medicina Oral, Patologia Oral, Cirugia Bucal* (2010): e924-e929.

21. Mota SM., *et al*. "*Streptococcus mutans* counts in plaque adjacent to orthodontic brackets bonded with resin-modified glass ionomer cement or resin- based composite". *Brazilian Oral Research* 22.1 (2008): 55-60.

22. Koga-Ito CY., *et al*. "Correlation among mutans streptococci counts, dental caries, and IgA to Streptococcus mutans in saliva". *Brazilian Oral Research* 18.4 (2004): 350-355.

23. Lee S-P., *et al*. "Surface Characteristics of Orthodontic Materials and Their Effects on Adhesion of Mutans streptococci". *The Angle Orthodontist* 79.2 (2009): 353-360.

24. Sukontapatipark W. "Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study". *European Journal of Orthodontics* 23.5 (2001): 475-484.

25. Zachrisson BU. "A posttreatment evaluation of direct bonding in orthodontics". *American Journal of Orthodontics* 71.2 (1977): 173-189.