Effects of Toothbrush Abrasion on Biofilm Retention of Thermoplastic Orthodontic Appliances

Major Duy Q. Nguyen, DDS, Orthodontic Resident, United States Air Force Postgraduate Dental School and Uniformed University of Health Sciences Postgraduate Dental College

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AIR FORCE POSTGRADUATE DENTAL SCHOOL
SAN ANTONIO, TEXAS

USU
Uniformed Services University

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Background and Purpose

- Removable thermoplastic orthodontic appliances (RTAs) are increasingly utilized in both active treatment and retention. Although RTAs offer several advantages, it also may act as a reservoir for biofilms that may increase discoloration and risk for demineralization. Levrini et al demonstrated that mechanical debridement was required to remove bacterial biofilms from RTA. Additionally, multiple studies reported that brushing with toothpaste is a more effective means of removing biofilm when compared to brushing with distilled water alone. However, many providers anecdotally instruct patients to avoid doing so due to concern of abrasion on the appliances. The objective of this study is to conclusively determine if brushing with toothpaste cause abrasion to the removable thermoplastic appliances? If so, is that abrasion significant enough to increase biofilm retention of the appliance?

Materials and Methods

TABLE 1. Toothbrush abrasion protocol by grouping.

Experimental Group	Toothbrush	RDA value	Clensing Agent / manufacturer
1	Oral-B indicator 35 Soft	0	Dawn Dish Soap (Palmolive, NYC, NY)
2	Oral-B indicator 35 Soft	70	Colgate Total (Palmolive, NYC, NY)
3	Oral-B indicator 35 Soft	189	Crest Pro-Health (Proctor and Gamble, OH)

45 standardized 0.030 inch thick Polyester thermoplastic samples (Essix Ace) were fabricated from a 3D printed template using a positive pressure forming machine (Biostar). The samples were divided into three groups, which were subjected to simulated brushing to two weeks and six months. Each group utilized a cleansing agent with increasing abrasiveness based on the relative detin abrasivity (RDA) values: Group 1- Dawn Dish Soap (non-abrasive), Group 2 – Colgate Total, and Group 3- Crest Pro-Health .

Materials and Methods

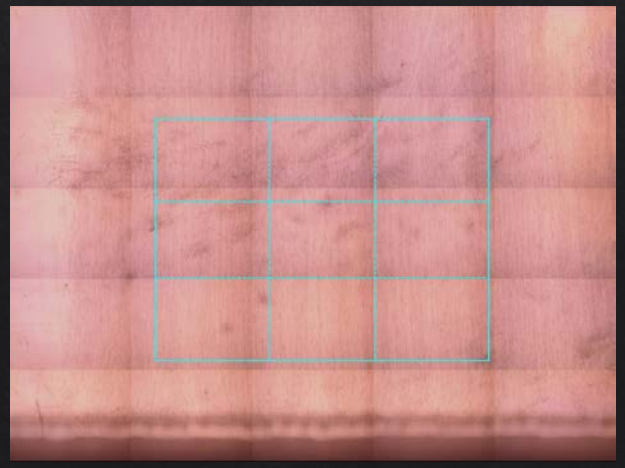
To investigate the effects of toothbrush abrasion, bacterial retention and surface roughness of the samples were evaluated at baseline, 2 weeks, and 6 months of simulated brushing.

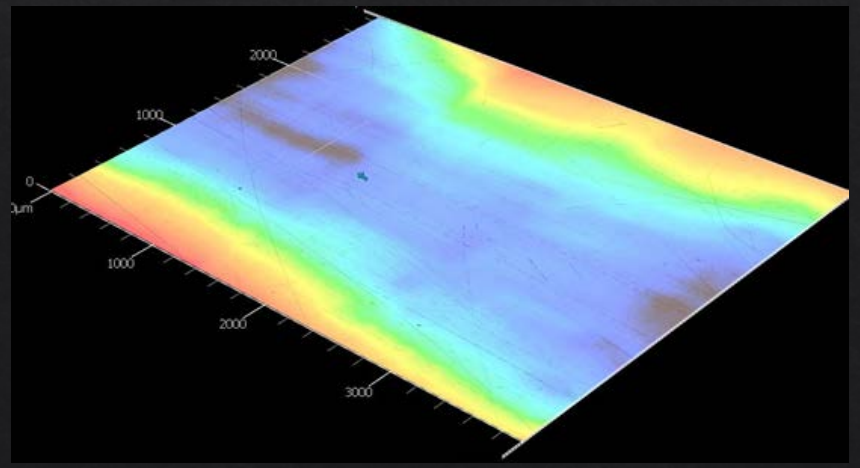
Biofilm retention was evaluated via an immersion of the samples in a 5ml bacterial suspension of *Streptococcus Mutans (10^6 CFU/ml)* in artificial saliva for 12 hours. Once inoculated, the samples were rinsed with deionized water and vortexed in sterile saline to remove biofilm from the sample. The saline mixture was then serially diluted and plated on Trypticase Soy Agar with 5% Sheep Blood (TSA II) and incubated at 37 +/-2°C in ambient air for 48 hours. The CFU's were counted and CFU/ml will be calculated.

Materials and Methods

Surface roughness analysis was completed on 5 samples per group utilizing scans from a non-contact 3D laser scanning confocal microscope (VK-X250, Keyence, Itasca, IL). Samples were placed on the 3D template, which was mounted onto an index to standardize scanning area. Using the proprietary software, each scan was normalized to remove artifacts and the arithmetical mean height was utilized to measure the surface roughness of the samples in μm.

Figures left to right: 1. sample mounted with index in VK-X250 scanner. 2. Screenshot of scanning area, note abraded area (center) vs unabraded surface (left). 3. 3D rendering of scanned baseline sample.





Materials and Methods

An automated toothbrushing machine (Sarbi Dental Enterprises) was utilized to standardize abrasion onto the samples. Samples were placed on the 3D printed templates which were mounted onto the machine. Samples were brushed with sectioned toothbrush heads (Oral B Indicator 35 Soft, Proctor and Gamble) and brushed at 60 strokes/min under 2N of force. The samples were brushed in a slurry mixture (250 ml distilled H_2O / 96 g) of toothpaste). The samples were brushed at an equivalent of 138 strokes/week for a total of 276 strokes at 2 weeks, and 3312 strokes at 6 months.

Statistics

The sample size was estimated based on the medium effect size in bacterial buildups, with a significance level α of 0.05. A total of 36 samples or 12 per group will achieve 80% power (a total of 45 samples were used). Paired t-tests and one-way analysis of variance (ANOVA) were utilized to compare the effects of toothbrush abrasion on surface roughness and bacterial retention of the samples within the three groups. Tukey's post hoc test for comparison of the means were calculated at 0.05 level of significance. Statistical analyses were performed using SAS version 9.4 (Statistical Analysis Software, Cary, NC).

Results: Bacterial Retention

- Paired t-tests demonstrated that Group 3 (Crest Pro-Health) had significant increase in biofilm retention from baseline - 2 weeks as well as 6 months.
- Interestingly, Group 2 (Colgate Total) had decrease in biofilm retention (baseline - 2 wks).
- A one-way ANOVA yielded significant group difference at 2 weeks ($p < 0.0001$) and 6 months ($p < 0.0044$).
- Finally Tukey's post hoc test Group 3 changes were significantly greater than groups 1 & 2. While differences between groups 1 & 2 were not statistically significant.

Table 2. Bacterial Growth (mean, st dev) (CFU/mL))

Group	Baseline	2 weeks	Change Baseline – 2 wks	6 months	Change Baseline – 6 months
1 - (Dawn Dish Soap)	4086 (1397)	4478 (1501)	392 (1307)	14940 (6437)	10854 (5934)
2 - (Colgate Total Toothpaste)	4833 (1132)	4107 (1259)	-727 (1118)	15100 (6489)	10267 (5872)
3 - (Crest Pro-Health Toothpaste)	5220 (2423)	10927 (4117)	5707 (4108)	22147 (5027)	16927 (5411)

Results: Surface Roughness

2 weeks & 6 months:

- Group 3 had significant increase in surface roughness
- ANOVA analysis – no significant group difference ($p = 0.56$). This may be due to low power with a small sample size.

TABLE 3. Surface Roughness (mean, st dev) (μm)

Group	Baseline	2 weeks	Change Baseline – 2 wks	6 months	Change Baseline – 6 months
1 - (Dawn Dish Soap)	2.18 (0.58)	2.34 (0.22)	0.16 (0.45)	2.35 (0.25)	0.16 (0.42)
2 - (Colgate Total Toothpaste)	2.84 (1.10)	3.20 (0.85)	0.36 (0.26)	3.22 (0.73)	0.38 (0.46)
3 - (Crest Pro-Health Toothpaste)	2.80 (0.60)	3.13 (0.64)	0.33 (0.05)	3.16 (0.75)	0.36 (0.16)

Discussion

The results show that the brushing with a more abrasive agent did increase the surface roughness, as well as the bacterial retention of the Essix material. At both 2 weeks and 6 months, group 3 demonstrated the most significant increase in both surface roughness as well as in bacterial CFUs. From these results we can reject the null hypothesis that toothbrush abrasion will not increase biofilm retention of the appliance.

Chang et al demonstrated that brushing with toothpaste (Colgate Total) was more effective in removing 99.9% of bacteria vs 92.8% using only distilled water. This study also demonstrated that there was no statistical difference between group 1 and 2 with regards to biofilm retention and surface roughness, emphasizing that the abrasiveness of the dentifrice is critical factor. The results would support that instructing a patient to brush with Colgate Total is no more abrasive than brushing with the a toothbrush alone.

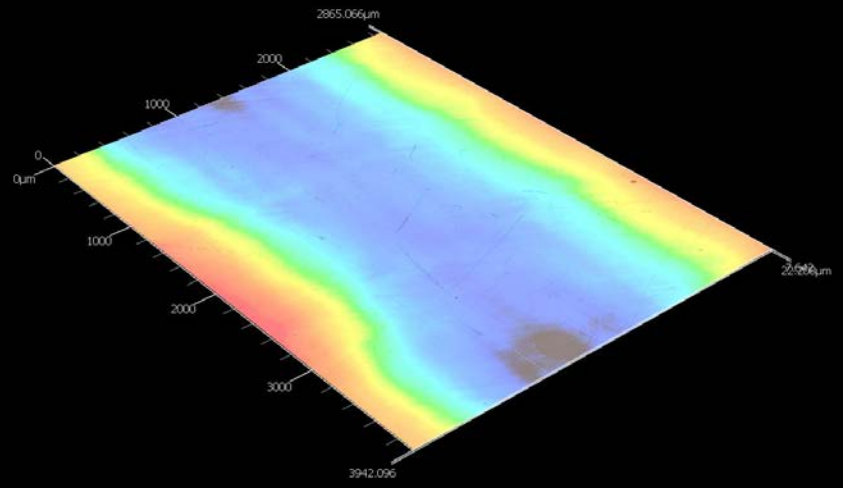
Discussion

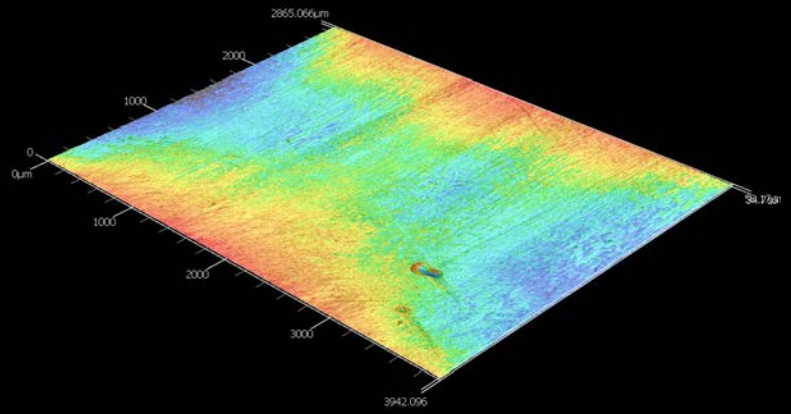
The limitation of this study is that flat surface samples were utilized compared to the normal tray a patient may utilize. However, this was done to mitigate the interference contributed by variation in surface anatomy of the dentition so we can focus solely on the material properties. Future studies could potentially attempt the evaluation with a greater variation in RDA values or potentially utilize an en-vivo split mouth study.

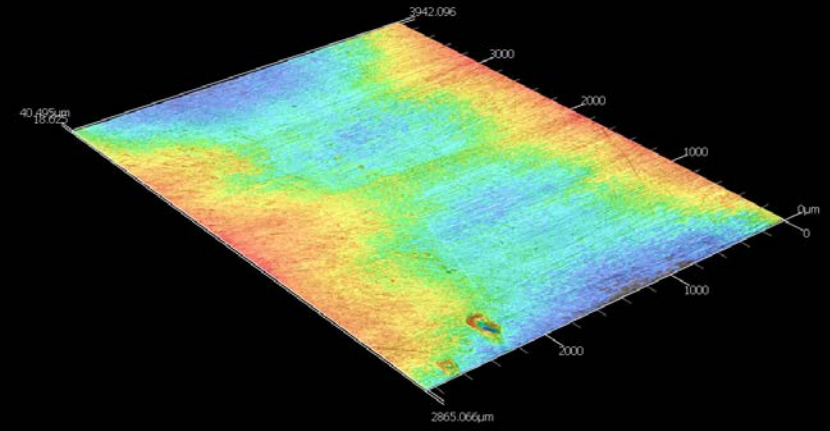
Discussion

Another limitation of this study was the small sample size evaluated in the surface roughness study. The decision to add a limited number of samples to evaluate surface roughness was for improved information for discussion and 3D imaging. Though the results were consistent, there was a lack of power. Future studies could utilize a greater sample size or possibly examine the changes to light transmittance with varying RDA values of toothpaste (or other cleansing agents).

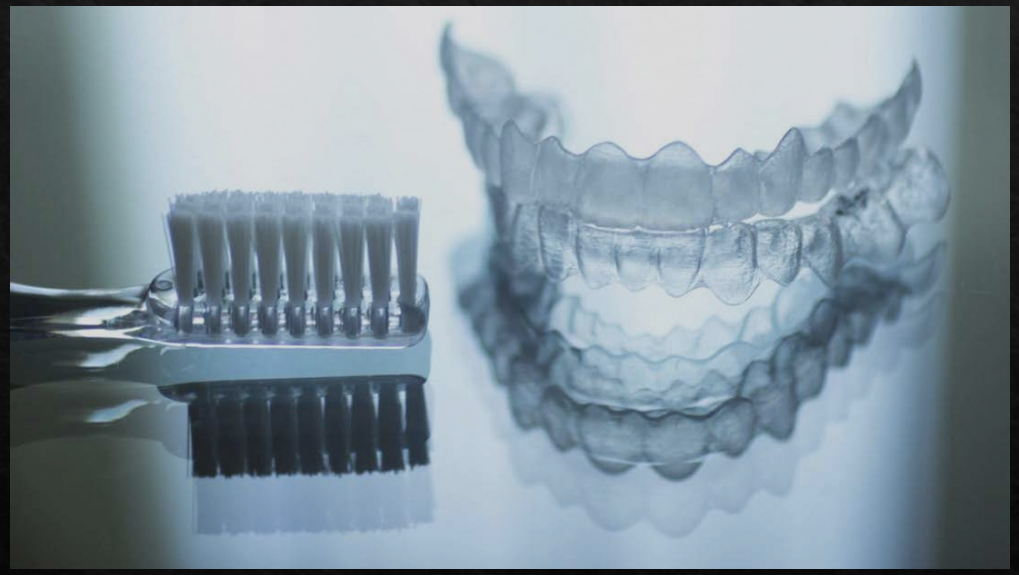
Images left to right: 3D rendering of the same sample using scans at baseline, 2 weeks, and 6 months.







Conclusion



Based on the data, practitioners should recommend that patients brush their removable thermoplastic orthodontic appliances with toothpaste, if the toothpaste has a lower RDA value.

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