Effect of aging on bond strength between orthodontic metallic brackets and enamel

2022 Research Aid Awards (RAA)

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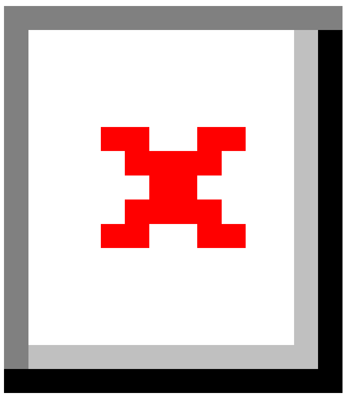
FollowUp Form

Award Information



In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*
Effect of aging on bond strength between orthodontic metallic brackets and enamel

Award Type
Research Aid Award (RAA)

Period of AAOF Support
July 1, 2022 through June 30, 2023

Institution
University of Toronto

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
Dr. Grace De Souza / Dr. Laura Tam

Amount of Funding
$5,000.00

Abstract
(add specific directions for each type here)

Introduction: The effects of the oral cavity on orthodontic brackets need to be considered in the evaluation of the performance of these appliances. An in vitro protocol that simulates thermal aging and mechanical cyclic loading processes for the duration of the orthodontic treatment, approximately 2 years, is proposed.
Objectives: The aims of this study are to determine the optimal artificial aging protocol to test the performance of brackets for future in vitro studies; to evaluate the effect of artificial aging methods on the bond strength between metallic bracket and enamel, on the amount of enamel loss following bracket debonding, and on the mode of bond failure.
Hypothesis: The main question is to determine the most appropriate artificial aging method to simulate the oral environment. We believe that the combination of thermocycling followed by mechanical cyclic loading allows to closely simulate the influence of the oral cavity on brackets. Also, this project will determine the effect of different artificial aging methods on the bond strength between metallic bracket and enamel, on the amount of enamel loss, and on the mode of bond failure at debonding. We believe that a decrease in bond strength between enamel and adhesive will be observed after aging in comparison with the control group, and the greatest decrease will be found in the group of thermo-mechanical cyclic loading. Thermocycling will affect bond strength between enamel and adhesive more severely than mechanical loading. The site of bond failure after artificial aging will be more frequently located at the enamel-adhesive interface.
Methods: 60 extracted maxillary premolars will be selected for bonding with metallic brackets (American Orthodontics Master Series) and with a 2-step etch-and-bond technique (Transbond XT, 3M). The teeth will be equally divided into 4 groups according to the artificial aging method. The control group (CO) will be stored in distilled water at room temperature for 21 days. The thermocycling group (TC) will undergo 20,000 cycles (5°C and 55°C) in a thermocycler (Mechatronik). The mechanical cyclic loading group (MC) will be tested in a chewing simulator (Mechatronik) for 10,000 cycles at a constant load of 10N and at a speed of 60mm/min. The thermo-mechanical cyclic loading group (TMC) will be tested under the same conditions than TC and MC groups respectively. The shear bond strength will be measured by using the Instron universal machine. The teeth will be visualized under stereomicroscope to assess the percentage of adhesive remnant. The debonded brackets will be examined with scanning electron microscopy with energy dispersive x-ray spectrometry to evaluate the percentage of calcium and phosphorus, components of enamel, on the bracket base. One way analysis of variance (ANOVA) and Tukey's test will be used for shear bond strength ($p<0.05$). Kruskal-Wallis test will be applied on the percentage of adhesive remnant, of calcium and of phosphorus ($p<0.05$).

Clinical implication: With an optimal in vitro aging protocol to evaluate the performance of orthodontic brackets, researchers will be able to test new fixed appliances available on the market before using them on patients.

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".
OR
Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

AAOF Jia Lin Liu.pdf
Please refer to the document attached for the results of the study.

Were the original, specific aims of the proposal realized?*

Most of the aims of the proposal were realized and some of them had to be modified throughout the project.

Aim 1: To determine the optimal artificial aging protocol to test the performance of brackets for future in vitro studies.
This aim was not included in the final report of this study, because it was impossible to determine the specific criteria that would qualify an artificial aging protocol as optimal. In order to realize this objective, an in vivo part of the project needs to be developed.

Aim 2: To evaluate the effect of artificial aging methods on the bond strength between metallic bracket and enamel.
This aim was realized. There is a difference in SBS of metallic brackets bonded to human enamel following artificial aging. TC and TMC aging resulted in significantly less SBS when compared with the controls.

Aim 3: To evaluate the effect of artificial aging methods on the amount of enamel loss following bracket debonding.
This aim was realized. There was no difference in enamel loss following artificial aging. However, due to the small number of samples, only a qualitative analysis was conducted.

Aim 4: To evaluate the effect of artificial aging methods on the mode of bond failure.
This aim was realized. For all the four methods, ARI score of 1 was most prevalent, which implies that the mode of failure was mostly a combination of adhesive and cohesive failures.

Were the results published?*

No

Have the results of this proposal been presented?*
Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*
I used the AAOF funding in order to complete my master thesis project required to become an orthodontist. Through this project, I was able to develop my critical thinking as a researcher and my ability to apply the results from in vitro studies to clinical application. The AAOF funding also allowed me to consider a career in academics in the future.

Accounting: Were there any leftover funds?
$0.00

Not Published

Are there plans to publish? If not, why not?*
There are plans to publish the study in a near future in Journal of Dental Research or Clinical and Experimental Dental Research. The manuscript for publication is in work at the moment.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*
1.Effect of Artificial Aging Protocols on Bond Strength Between Orthodontic Metallic Brackets and Human Enamel
Dr. Jia Lin Liu; Dr. Grace De Souza; Dr. Laura Tam
Latest Advancements in Canadian Orthodontic Research Symposium at the annual Canadian Association of Orthodontists Scientific Session; 2022; Saskatoon (Canada)

2.Effect of Artificial Aging Protocols on Bond Strength Between Orthodontic Metallic Brackets and Human Enamel
Dr. Jia Lin Liu; Dr. Grace De Souza; Dr. Laura Tam
E-Poster presentation at American Association of Orthodontists Annual Session; 2022; Miami (United States)

Was AAOF support acknowledged?

If so, please describe:

Yes, the support of AAOF was acknowledged for both presentations and it was indicated in the poster.

Internal Review

Reviewer comments

Reviewer Status*

File Attachment Summary

Applicant File Uploads

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LIST OF ABBREVIATIONS

SBS: shear bond strength

ARI: adhesive remnant index

N: Newton

MPA: Mega Pascal

CO: control

TC: thermocycling

MC: mechanical cyclic loading

TMC: thermocycling + mechanical cyclic loading

SD: standard deviation

ANOVA: one-way analysis of variance

RESULTS

Shear Bond Strength

The mean SBS values and the correspondent standard deviations (SD) for the four groups are presented in Table 1. One-way analysis of variance (ANOVA) showed that there was a significant difference among the four groups ($p < 0.001$; F = 7.959). Further post-hoc analysis with Tukey's test revealed that CO group was statistically different from TC and TMC. The SBS values of TC ($p < 0.001$) and TMC ($p = 0.002$) groups were significantly lower than CO group, and there was no difference between TC and TMC ($p > 0.05$). MC group was the only one that did not show any difference compared with CO group.

Table 1: Mean SBS and SD values of the experimental groups.

Artificial aging protocol (Mean ± SD MPa)			
Group CO	Group TC	Group MC	Group TMC
16.29 ± 5.48^{A}	8.52 ± 5.20^{B}	12.34 ± 3.77^{AB}	9.86 ± 4.04^{B}
Post-hoc Tukey's test; α=0.05 Different upper case letter indicates significant difference between two means according to post-hoc Tukey's test			

Adhesive Remnant on Enamel

Values of ARI based on the images of the samples taken under the stereo microscope are shown in Table 2. The different samples representing each possible score, except for score 0, are presented in Figure 1.

Table 2: ARI scores [frequency (percentage)] for the experimental groups.

ARI Score	CO	MC	TC	TMC
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1	10 (67%)	7 (47%)	11 (73%)	12 (80%)
2	3 (20%)	6 (40%)	4 (27%)	3 (20%)
3	2 (13%)	2 (13%)	0 (0%)	0 (0%)
Score 0: no adhesive remnant on enamel surface Score 1: less than ½ adhesive remnant on enamel surface Score 2: more than ½ adhesive remnant on enamel surface Score 3: all adhesive remnant on enamel surface (distinct impression of bracket mesh)				

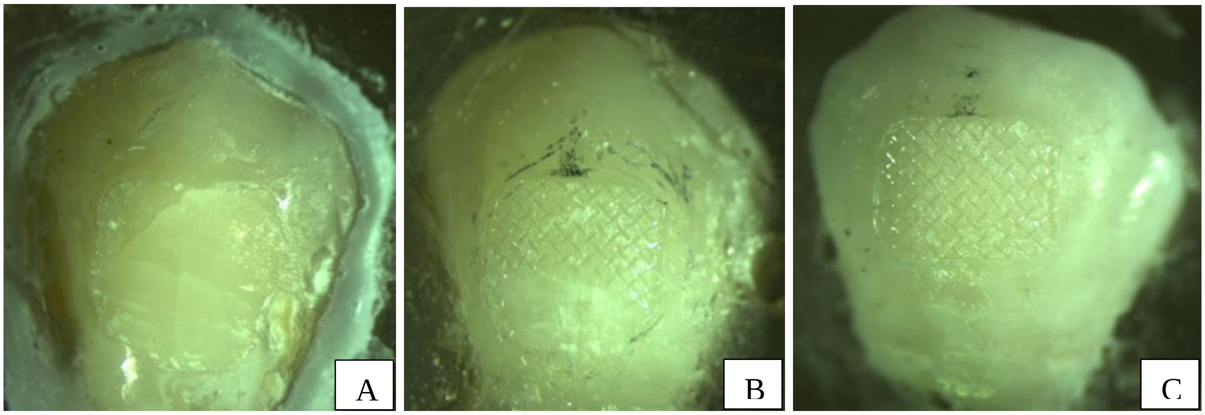


Figure 1: Stereomicroscope images representing different ARI scores (10x magnification). No sample demonstrated ARI score 0. A shows ARI score 1. B shows ARI score 2. C shows ARI score 3.

For all the four groups, ARI score of 1 was most prevalent, which implies that the mode of failure was mostly a combination of adhesive and cohesive failures. No group displayed an ARI score of 0. Table 3 shows the mean percentage of adhesive remnant on enamel and the standard deviation associated for all the four groups. Kruskal-Wallis test by ranks demonstrated no significant differences among the four groups for the mean percentage of adhesive remnant on enamel (p = 0.139).

Table 3: Mean and standard deviation for area percentage of adhesive remnant on tooth for the experimental groups.

Artificial aging protocol (Mean ± SD percentage)			
Group CO	Group TC	Group MC	Group TMC
43.82 ± 32.03	32.94 ± 20.37	59.70 ± 34.17	33.81 ± 26.18
Kruskal-Wallis test by ranks; α=0.05 No significant differences between means according to Kruskal-Wallis test			

Scanning Electron Microscopy

A qualitative evaluation of enamel loss was done using the microscope images of the 20 specimens scanned with SEM. Figure 2 represents an image of a debonded bracket bonding base. At the gingival portion of the bonding base, which represents the top portion of the picture, a piece of adhesive attached to the bracket can be observed. Some enamel fragments are still bonded on the adhesive. Also, adhesive remnant is still trapped in the mesh pad. Through the elemental maps of calcium and phosphorus generated by energy dispersive x-ray spectrometry in Figures 3 and 4, the fragments of enamel bonded to the adhesive remnant in the gingival side of the bracket are more visible.

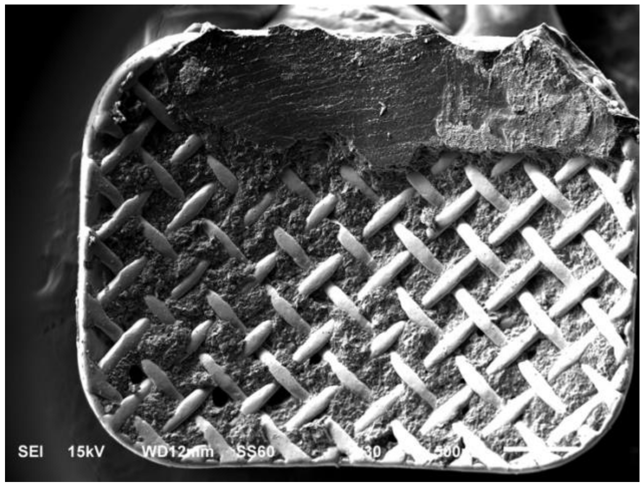
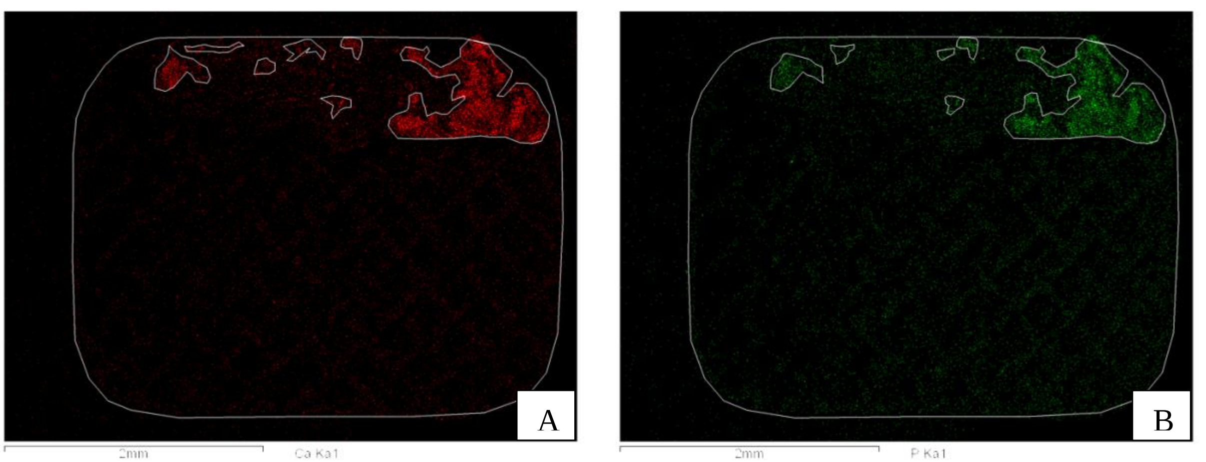


Figure 2: Photomicrography of bracket bonding pad (27x magnification) indicating a piece of adhesive resin with fragments of enamel.



Figures 3 and 4: Elemental maps of calcium (A) and phosphorus (B) generated through energy dispersive x-ray spectrometry with bracket base outlined (27x magnification).

In order to compare the samples scanned with SEM, Table 4 shows the area percentage of enamel present on the bracket base. Most of the 20 samples analyzed had between more than 0%

but less than 10% of bracket base covered with enamel. Only two brackets showed more than 10% of enamel. Table 5 shows an approximate location of the enamel present on the bonding base. Most of the samples from all the four groups had the enamel in the gingival portion of the bonding base. However, for TC and TMC groups that underwent thermocycling, some samples demonstrated an occlusal location for the enamel present on the bonding base.

Table 4: Enamel present on bracket base (frequency and percentage) for the experimental groups.

Area percentage of bracket base covered with enamel	Group CO	Group TC	Group MC	Group TMC
0%	0 (0%)	0 (0%)	0 (0%)	0 (0%)
>0%-<5%	4 (80%)	4 (80%)	2 (40%)	1 (20%)
5%-<10%	0 (0%)	1 (20%)	3 (60%)	3 (60%)
≥10%	1 (20%)	0 (0%)	0 (0%)	1 (20%)

Table 5: Location of enamel present on bracket base (frequency and percentage) for the experimental groups.

Location of enamel on bonding pad	Group CO	Group TC	Group MC	Group TMC
Occlusal	0 (0%)	3 (60%)	0 (0%)	1 (20%)
Gingival	5 (100%)	2 (40%)	5 (100%)	4 (80%)

DISCUSSION

This *in vitro* experiment demonstrated that there is a difference in SBS of metallic brackets bonded to human enamel following artificial aging. TC and TMC aging resulted in significantly less SBS when compared with the controls. All the SBS mean values from the four groups analyzed achieved the critical SBS necessary for clinical practice according to Reynolds, which is between 6 and 8 MPa.[1] However, the bond strength cannot be more than 14 MPa to decrease the risks of enamel fracture[2], but only CO group exceeded this value.

TC and TMC groups both involved thermocycling for 20,000 cycles as part of the artificial aging protocol. For TC group, the SBS values are in agreement with the results from the study by Sokucu et al.[3] Indeed, the authors demonstrated a significant decrease in SBS values of metallic brackets bonded to premolars after thermocycling using Transbond XT Light Cure Adhesive (3M). However, instead of choosing 20,000 cycles corresponding to two years, Sokucu et al. selected only 10,000 cycles based on one year under humidity and temperature changes in the oral cavity.[3] As for TMC group, the SBS values are consistent with those from Ibrahim et al.[4] Both experiments reported a significant decrease in SBS after a combination of thermocycling and mechanical cyclic loading. However, the settings for the protocol of Ibrahim et al. are different in comparison with the current experiment, since the authors evaluated sapphire brackets instead of metallic brackets and selected 5,000 cycles for thermocycling and for mechanical cyclic loading with the staircase method.[4] In contrast, other studies that included a combination of both artificial aging methods did not find a significant decrease in SBS, but the protocols of these experiments vary greatly.[5-7] The reason behind the reduction in SBS due to thermocycling procedure involves the differences in coefficients of thermal expansion of enamel, metallic bracket, and adhesive resin, sorted in ascending order of coefficients of thermal expansion.[8] Indeed, the two extreme temperatures used in thermocycling, along with the cyclical stress, affect weak areas in the bonding interface and cause them to expand.[8] Also, hygroscopic expansion and chemical degradation of the materials can be caused by thermocycling due to the absorption and the solubility of the adhesive resin.[8] Indeed, Yap et al. reported that composite resins absorbed more water under thermocycling conditions.[9]

There is a lack of studies comparing different artificial aging methods. In this project, the post-hoc Tukey's test did not find any significant difference between TC and TMC groups, and the only difference between them is the mechanical cyclic loading portion of the protocol. The principle of mechanical cyclic loading is based on the initiation and the propagation of microcracks that weaken the enamel-adhesive interface.[10] In the present study, instead of using the staircase method, a constant load of 0-10 N for 10,000 cycles was applied in a chewing simulator as the mechanical aging in MC and TMC groups. Even though there are in average 1,800 occlusal contacts per day that occur during chewing and swallowing[11], not all of these contacts affect the brackets in the oral cavity. For the staircase method, the findings in the literature vary between a significant decrease[12, 13] or no difference[14] in SBS values after mechanical cyclic loading. Some studies selected the constant load method, but the load amount and the number of cycles were higher than this experiment, up to 0-50 N and 1,200,000 cycles.[7] The protocol from Imani et al. was similar to the current project, but with a high load of 0-40 N and the same 10,000 cycles.[15] The amount of 40 N was selected, because it is considered as the lowest amount of force applied to brackets during mastication.[16] According to Imani et al., 10,000 cycles represented the highest number of cycles used for fatigue testing.[17, 18] However, the mechanical cyclic loading procedure from these studies did not affect SBS significantly.[7, 15]

In the current study, the mechanical cyclic loading procedure did not significantly affect the results as well. SBS for MC group was similar to CO group and SBS for TC group was similar to TMC group. The findings for MC group might have been different if higher settings were chosen, since 0-10 N and 10,000 cycles are too low to represent two years of clinical service in the oral cavity. The protocol established by Ok et al. to evaluate the microleakage under brackets after laser-aided enamel conditioning can improve the current protocol for MC and TMC.[19] The authors considered 240,000 cycles at a load of 0-50 N as equal to one year in the oral environment[20] and 500,000 cycles as equal to two years.[19] This number of cycles is based on a study evaluating the wear of a posterior composite against a maxillary palatal cusp in an artificial mouth laboratory set-up, but no bond strength was evaluated.[21]

In order to overcome the weaknesses of the ARI scoring system, the method developed by O'Brien et al.[22] was applied and it assesses the percentage of adhesive remaining on enamel. For this experiment, there was no difference in the percentage of adhesive remaining on tooth after artificial aging according to Kruskal-Wallis test. According to Al-Salehi et al., there is a correlation between bond strength and bond failure mode, since higher values of bond strength are correlated to a combination of cohesive and adhesive fractures.[23] Although similar mean percentages of resin remnant were observed for MC and CO groups, high mean values of SBS were also observed. The majority of the studies in the literature on artificial aging have not reported the percentage of adhesive but the ARI scoring system instead.

According to the ARI scores, most of the samples from the four groups fall into score 1, which represents less than half of the adhesive remnant on tooth. In TC and TMC groups, which had low mean SBS values, most of the samples showed less than half of the adhesive. For TMC group, 80% of the premolars were associated with a score of 1, while other studies using a similar scoring system, and a combination of thermocycling and mechanical cyclic loading categorized most of the samples as score 0.[5-7] However, there were no samples with score 0 in the present study. As for TC group, 73% of the premolars had a score of 1 and these results are consistent with the ARI values obtained by Sokucu et al.[3] Most of the samples from MC and CO groups, which had high mean SBS values, were classified as score 1 (47%) and score 2 (40%) respectively. These scores are similar to the ARI scores found in the study by Algera et al.[14] where a high frequency of score 2 was reported, and in the study by Imani et al.[15] where most of the samples had a score that corresponds to 50-75% of adhesive remnant on enamel surface. Furthermore, the exposure time to curing light can affect SBS values and ARI scores. In the study by Gronberg et al.[24], an exposure time below 10s for metallic brackets produced low SBS values that are still clinically acceptable according to Reynolds.[1] However, there was an incomplete polymerization under the bracket base for this exposure time. Indeed, the high frequency of ARI score 3 from their experiment indicated that all the adhesive resin was left on the surface of the tooth due to the lack of cured adhesive resin in the metallic mesh of the bracket bonding pad.[24] In the current study, an exposure time of 12s to curing light was selected. The mean SBS values obtained were all clinically acceptable and the frequency of ARI score 3 was low among all the groups, which indicates the proper polymerization under the bracket base.

A quantitative evaluation of adhesive remnant by percentage of tooth surface covered complements well the qualitative evaluation with ARI scoring system. According to Cerheli et al., the qualitative method of ARI to evaluate adhesive remnant was able to generate similar results to the quantitative image analysis method.[25] However, in the present study, ARI was not able to identify small differences among the groups, since it classifies most of the failures in score 1, which is less than 50% of adhesive remnant on tooth, and this represents a large range of possible values. The percentage of adhesive remnant would allow researchers to find the minor differences between the samples that fall into the same score.

According to the SEM findings, there was no difference in enamel loss following artificial aging. However, due to the small number of samples, only a qualitative analysis was conducted. Enamel damage occurs when the mode of failure is at the enamel-adhesive interface during debonding, and a high amount of stress is placed on the enamel. Based on the categories of percentage of enamel found on the bracket bonding pad, most of the groups had more than 0% and less than 10% of enamel. The only two samples that showed more than 10% of enamel loss had a visible enamel fracture associated. Even though the number of samples is low, all the samples demonstrated some enamel loss in contrast with the *ex vivo* study by Cochrane et al. where the authors found that 13.3% of the metallic brackets exhibited enamel on the bonding pad, and the majority of these brackets (8.7%) had between more than 0% and less than 1% of enamel present.[26] Another *ex vivo* study by Pont et al. showed that the bonding pads displayed in average 12.6 ± 7.8% of calcium, which is higher than most of the samples of this current *in vitro* study.[27] In addition, there is a positive correlation between the amount of adhesive remnant on the bracket base and the amount of calcium found on the bracket base.[27, 28] Due to the *ex vivo* nature of these two studies, the authors were not able to assess the bond strength during the debonding process and the debonding technique used *in vivo* is different. Furthermore, dehydrated enamel *in vitro* has decreased resistance to fracture.[29] From these observations, bond failure at the bracket-adhesive interface seems to be preferable to avoid transferring the stress to the enamel and causing enamel damage.

Based on the location of enamel on the bonding pads, most of the enamel was found in the gingival portion of the bonding pads for the four groups. However, some specimens from TC and

TMC groups displayed an occlusal location of the enamel. The occlusal side of the bracket is where the chisel applies the shear force during SBS test. It is possible that the different coefficients of thermal expansion between enamel and adhesive resin created micro-fractures under the entire bracket.[30, 31] Then, enamel fracture was induced on the occlusal side that received first the force applied by the chisel to simulate the debonding process for these specific specimens. Based on the number of samples analyzed, it was not possible to determine a correlation between one specific artificial aging protocol and enamel loss.

For clinicians, it is suggested to investigate the bond strength of new brackets and adhesive systems in an *in vitro* setting before using these products on patients. The protocols for thermocycling and thermo-mechanical cycling loading from the current study can simulate artificial aging for a duration equivalent to two years in the oral cavity. These methods are useful to determine the SBS applicable at the time of debonding process.

In terms of limitations of this project, the extrapolation of the results from an *in vitro* experiment to clinical application remains difficult. Even though the samples went through different artificial aging methods estimated to be equivalent to two years in the mouth, the oral cavity is a complex and dynamic environment that processes food and beverages and experiences constant changes in temperature and pH. In addition, due to circumstances related to the COVID-19 pandemic that was ongoing during this project, the collection of premolars proved to be a challenging task. Indeed, some of the teeth used for the experiment were stored for more than 12 months in distilled water. According to Aydın et al., the storage of teeth in distilled water for more than a year significantly decreases the microhardness of enamel and dentin.[32] Also, since the premolars were extracted with the use of forceps, it is possible that some of the teeth possessed craze lines that could not be detected under stereo microscope, and it may also have affected the findings. As for the application of shear force with a shear blade for the debonding of the brackets, it does not represent the clinical reality of the debonding procedure, where a debonding plier is used *in vivo* to grip and compress the bracket wings on the bracket from both sides.[33] In addition, the analysis of enamel loss with elemental maps was limited to a qualitative evaluation due to the low number of samples randomly selected. An increased number of specimens for this section of the experiment could have provided more information on the amount of enamel loss and the location of enamel found on the bonding pads.

CONCLUSION

- Artificial aging involving thermocycling or a combination of thermocycling and mechanical cyclic loading caused a decrease in bond strength.

- All the artificial aging methods from this project met the gold standard for clinical bond strength established by Reynolds.[1]

- For the percentage of adhesive remnant on tooth surface, there was no difference among the groups. ARI scores demonstrated mostly a combination of cohesive and adhesive fractures for all the groups.

- The evaluation of enamel loss after debonding revealed that there was no difference between the protocols, but all the samples tested showed a small quantity of enamel on the bracket bonding pad.

- The protocol for mechanical cyclic loading of this project needs to be modified to simulate closely the conditions of the oral cavity by selecting a higher load and a higher frequency of cycles. Also, the number of samples tested under SEM needs to be increased.

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