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Effect of S. mutans biofilm on the hybrid ceramic-resin cement bond strength assessed by different methods

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Aim: The purpose of this study was to investigate the biofilm effect on the hybrid ceramic-resin cement bond strength (BS) by comparing two methods. Methods: Teeth were distributed into groups (n=5), according to the resin cement (Maxcem Elite-(MC) or NX3 Nexus-(NX)) and degradation method (24h or 7 days in distilled water; 7 or 30 days incubated with biofilm and 30 days in sterile media). Treated surfaces of Vita Enamic blocks (5x6x7mm) were luted to treated or no treated dentin surfaces and light-cured. After 24h, beams were obtained (1x1x10mm) and stored accordingly. The flexural bond strength (FBS) was assessed by four-point bending test. Additional beams were obtained from new teeth (n=5), stored for 24h or 7 days in distilled water, and submitted to a microtensile bond strength (μTBS) assay. Failure modes were determined by scanning electron microscopy (100X). The flexure strength of the cements (n=10) was assessed by a four-point bending test. Data were analyzed by 1 and 2-ways ANOVA, and Tukey's test (α=0.05). **Results:** There was no significant difference between the degradation methods for the FBS groups. For the μTBS, the significant difference was as follows: NX 7days > NX 24h > MC 7days = MC 24h. Failure mode was mainly adhesive and mixed, but with an increase of cohesive within cement and pre-failures for the MC groups assessed by μTBS. NX had better performance than MC, regardless of the method. **Conclusions:** The biofilm had no effect on the materials BS and FBS test was a useful method to evaluate BS of materials with poor performance.

Keywords: Biofilms. Resin cements. Dental bonding. Tensile strength.

Introduction

Success of indirect restorations depends on a combination of several factors, such as aesthetics, occlusal balance and long-term bond stability between substrate, adhesive layer and restorative material[1]. In the challenging oral environment, dental materials are subjected to biodegradation, which is caused by the deleterious effect of oral biofilm on their structure and properties. Bacterial acids can promote an increase in surface roughness, matrix and interfacial softening, decrease in surface hardness and chemical degradation of the hybrid layer, directly affecting the bond strength of indirect restorations and promoting the loss of cervical sealing[2-5]. To date, few authors[4] have evaluated the effect of actual biofilm growth on the bond strength of restorative materials to dentin.

Vita Enamic (Vita Zahnfabrik, BS, Germany) is a hybrid CAD-CAM material, composed by feldsphatic ceramic (75 wt%) and a dimethacrylate polymer network (25wt%)[6-9]. CAD-CAM materials are preferably adhesively cemented in order to promote better bond stability, and conventional resin cements with dental adhesives are typically used. However, in an attempt to diminish the technique sensitivity of the process, self-adhesive luting agents can be used to eliminate the need for treating the surface of the teeth with an adhesive before applying the cement[10,11]. While the resin cements and ceramics have different resistance to biodegradation, the bond strength of hybrid materials to conventional and self-adhesive resin cements when subjected to the action of a growing biofilm is yet to be determined.

Bond strength can be assessed by different methods, with microtensile bond strength (μTBS) being the most popular test used in the literature[3,12,13]. If performed correctly, it produces a uniform interfacial stress distribution, resulting in reliable outcomes[13]. Despite its popularity, it is a time-consuming and highly technique-sensitive assay[12]. The mounting of specimens to the proper jig can lead to premature stress, resulting in many pre-test failures and data with high standard deviation, especially for materials with low bond strength values[10,13]. Flexure bond strength assessed by a four-point bending test (FBS) has been shown to be a promising method to evaluate the bonding performance of materials[13-15]. Four-point bending geometry concentrates the maximum tensile stress on the convex surface (bottom), removing the stress concentration at the surface of the interface, which is claimed to be more clinically relevant than the direct tension test[13-15]. Also, the easy placement of samples on the four-point bending device leads to a less technique sensitive assay. However, there is still a lack of evidence regarding the reliability of such results, raising the importance of studies comparing FBS to other well-stablished bonding methods, such as the microtensile test.

Therefore, the aims of this study were to evaluate the effect of a growing *S. mutans* biofilm on the bond strength of a hybrid CAD-CAM material to two different luting agents and to compare the bonding performance by two assays: flexure and microtensile bond strength. The hypotheses of this research were: (1) the biofilm will negatively affect the bond strength, and (2) both bond strength methods will provide similar outcomes.

Methods and Materials

Specimen preparation

Fifty caries-free human third molars were stored in an aqueous solution of 0.5% chloramine-T for 7 days and stored in distilled water until use. Before extraction, patients had been informed about the use of the teeth for research purposes, and verbal consent had been obtained. Deep dentin was exposed by removing the occlusal enamel with a low speed, water-cooled diamond saw (IsoMet 1000 Precision Saw, Buehler, IL, USA). Dentin surfaces were abraded on #600 silicon carbide paper for 30s to create a standardized smear layer, and then ultrasonically cleaned in water for 5 min. Teeth were then randomly distributed into ten groups (n=5), according to the luting agent (Maxcem Elite or NX3 Nexus, Kerr, CA, USA) and the storage condition (Table 1).

Table 1. Group distribution (n=5).

Storage Condition/ Resin cement	NX3 Nexus (NX)	Maxcem Elite (MC)
24hrs in distilled water	NXc	MCc
7 days in distilled water	NX7w	MC7w
7 days with biofilm	NX7b	MC7b
30 days in sterile media	NX30m	MC30m
30 days with biofilm	NX30b	NX30b

Dentin surfaces were treated according to the resin cement group. For the self-adhesive cement (MC), no further surface preparation was performed. For the conventional luting agent (NX), surfaces were etched for 15s with a 37.5% phosphoric acid gel (Gel Etchant, Kerr, CA, USA) and then rinsed abundantly with water. After removing excess moisture with an absorbent paper, leaving a glistening surface, an etch-and-rinse adhesive system (Optibond S, Kerr, CA, USA) was actively applied by means of a microbrush for 15 s, gently air-dried for 3 s at a standardized distance of 5 cm and light-cured for 20s, using a curing unit (Elipar S10, 3M ESPE, MN, USA), with an output of at 900 mW/cm^2, monitored with a radiometer (SDS KERR Model 100, Optilux Radiometer, Kerr, CA, USA). The light-curing unit tip (9.8mm of diameter) was at standardized distance of 5 mm from the dentin surface.

Rectangle-shaped hybrid ceramic (Vita Enamic, Vita Zahnfabrik, BS, Germany) specimens (5 x 6 x 7 mm) were prepared cut from standard blocks with a diamond saw. The bonding surface of each specimen was then etched with a 5% hydrofluoric acid (Vita Ceramics Etch, Vita Zahnfabrik, BS, Germany) for 60 s and rinsed ultrasonically with distilled water for 5 min. The surfaces were then air-dried, and one layer of a silane primer (RelyX Ceramic Primer, 3M ESPE, MN, USA) was applied for 20 s and then allowed to dry for 30 s.

Each luting agent was prepared by aid of auto-mixing dispenser provided by the manufacturer and applied on the treated hybrid ceramic surface by the same mixing tips.

The rectangular specimen was then placed on the dentin surface with a constant pressure of 100g to standardize the thickness of the cement layer (~130 μm). After gently removing the excess cement with a microbrush, the complex was light-cured for 10 s at 900 mW/cm^2 from two opposite sides at a 90º angle with the edge of the light guide resting on the dentin surface. The specimens were then stored in distilled water at 37ºC for 24hrs. The specimens were then cut into beams (1 X 1 X 10 mm) with the bonded interface in the middle using a low-speed, water-cooled diamond saw at 300 rpm. The cross-sectional area of each stick was measured for subsequent calculation of the bond strength.

Degradation methods

The bonded sticks originating from the same teeth were then assigned to each group, according to the degradation method: 1 or 7 days in distilled water at 37ºC; 30 days storage in sterile Todd-Hewitt (TH) media (Thermo Fisher Scientific, MA, USA), changed each 4 days, and 7 or 30 days co-incubated with an inoculate of IDH-RenG luciferase reporter strain *Streptococcus mutans* grown as a biofilm[16].

For the samples tested with living biofilm, an overnight culture of S. mutans was added in fresh sterile TH media, and the optical density was set at 0.8 at 600 nm (OD600). This particular strain of *S. mutans* is genetically modified to result in a bioluminescent phenotype, able to provide quantitate data regarding cell viability under light emission conditions[16].

After the beams were sterilized by storage in 70% ethanol for 5 min and rinsed with autoclaved water, they were placed in a sterile 24-well plate along with 0.5 ml of the bacterial suspension. To encourage biofilm formation, 1% of a 40% sucrose solution was also added. The specimens were then incubated at 37ºC in 5% CO_2 for 7 or 30 days. Bacterial growth medium was refreshed every day without disturbing the formed biofilm.

After the incubation period, a luciferase assay was performed to assess the viability of the bacteria in the biofilm, essentially as previously described[16]. Briefly, samples were moved carefully with the aid of sterile tweezers and placed into a luminescence 24-well plate and incubated with fresh media for 1 hr at 37ºC in 5% CO2. After, light emission from growing bacteria cells was measured by adding 5 μl of a substrate solution (1 mM d-luciferin in 0.1 M sodium citrate buffer [pH 5.0]) to each well. The plate was immediately placed in an optical plate reader (Glomax Discover Multimode Microplate Reader, Promega, Madison, WI, USA) and light emission recorded, representing the quantity of viable *S. mutans* cells.

Four-point bending assay

To determine the flexural bond strength (FBS) after the various degradation methods, beams were washed in tap water for 5 min, carefully dried and subjected to a four-point bending test. The 10 mm beams were fixed between the four supports with the bonded interface centered within the inner rollers and loaded until fracture using a universal testing machine (Q-test, MTS, Eden Prairie, WI, USA) at 1 mm/min crosshead speed[17,18] (Figure 1).

Figure1. Example of a beam accordingly positioned between the supports for the flexural bond strength assay.

The FBS (MPa) was calculated using the following equation:

$$FBS = \frac{9 \times F \times L}{8 \times W \times T^2}$$

where F (*N*) was the load at fracture, L the support span (8.48mm), W and T the specimen width and thickness, respectively.

Microtensile bond strength

The enamel crown of an additional twenty caries-free human third molars was removed to expose dentin by cutting with the diamond saw. These teeth were randomly distributed into four groups (n=3), according to the storage period (24 hrs or 7 days at 37ºC) and luting agent (Maxcem Elite and NX3 Nexus). Specimen preparation was performed in the same way as previously described for the FBS. There were many pretest failures for Maxcem specimens (more than 50% of the sticks for the 24 h specimens and about 40% for the 7 days specimens), but essentially no pre-test failures for Nexus. Though more teeth were prepared, only teeth in which at least three sticks could be tested were included in the analysis, leaving n=3 for all four groups[19].

After the respective storage periods, each ceramic-dentin stick was removed from the solution and gently dried. They were attached to a microtensile testing device (Odeme Dental Research, Luzerna, SC, Brazil) using cyanoacrylate adhesive and subjected to a tensile force in the universal testing machine at 1 mm/min cross-head speed.

Failure mode

Bond test samples were mounted on metallic stubs, coated with 60% gold:palladium in a sputter coater (Anatech,Hayward, CA, USA) and observed under a scanning elec-

tron microscope (SEM) (Quanta 200 SEM, FEI company, OR, USA), at magnification x100, in order determine the failure modes.

Flexural strength of the cements

The flexural strength of the luting agents was assessed using the four-point bending test. To prepare these specimens, 1 X 1 X 10 mm polyvinylsiloxane molds were filled with each cement (n=10), sandwiched between glass slides and light-cured at 900 mW/cm^2 for 10s on each side (2 exposures of 5 s each to cover the entire surface). After polishing the samples to remove any excess, they were stored in distilled water at 37ºC for 24h. The specimens were gently dried and mounted in the four-point bending device to measure the flexural strength, using the same conditions previously described for the FBS test.

Statistical analysis

The data from the FBS test and the μTBS were analyzed by 2-way ANOVA, followed by Tukey's multiple comparison test (α = 0.05). Regression analysis was used to correlate the results from both tests. Comparison of the flexure strength of the two cements was test done with a student's t-test (α = 0.05).

Results

Flexural bond strength

Mean and standard deviation (SD) values for the FBS are presented in Figure 2.

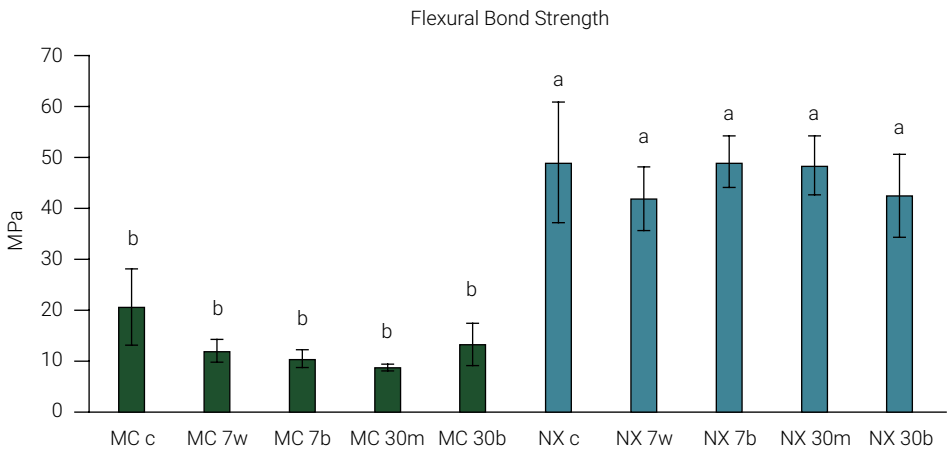

Figure 2. Flexural bond strength (MPa) of the different groups after the degradation methods (mean ± sd). Bars with dissimilar letters indicate values that are significantly different from each other (p <0.05).

Analysis of variance showed a significant difference among the cements, with the FBS of NX being higher for all conditions than MC (p<0.001). Because there was no significant difference between the degradation methods and no interaction effect.,

individual one-way ANOVAs were run for the two cements. No significant differences between the aging conditions was shown for either cement.

The failure modes were classified as adhesive, cohesive within cement, cohesive within dentin and mixed (Figure 3). The failure modes of the different groups are presented in Figure 4.

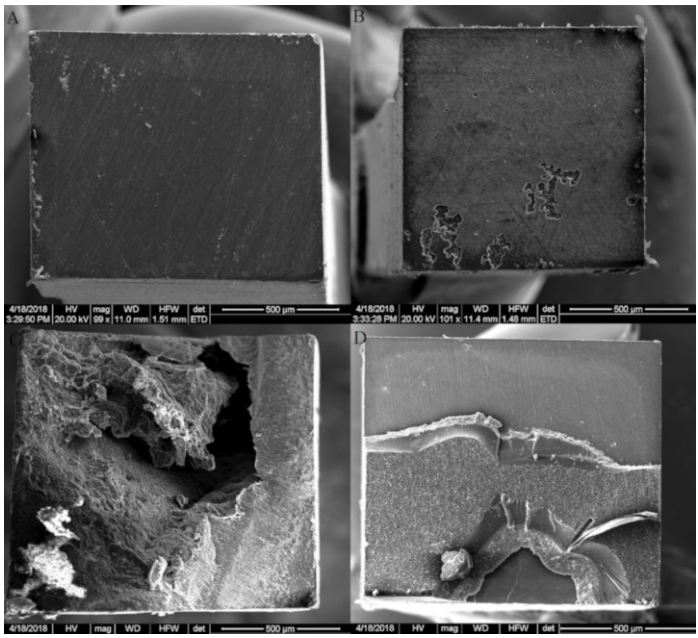

Figure 3. Examples of the failure modes assessed by SEM (x100): (A) Adhesive; (B) Cohesive within cement; (C) Cohesive within dentin and (D) Mixed.

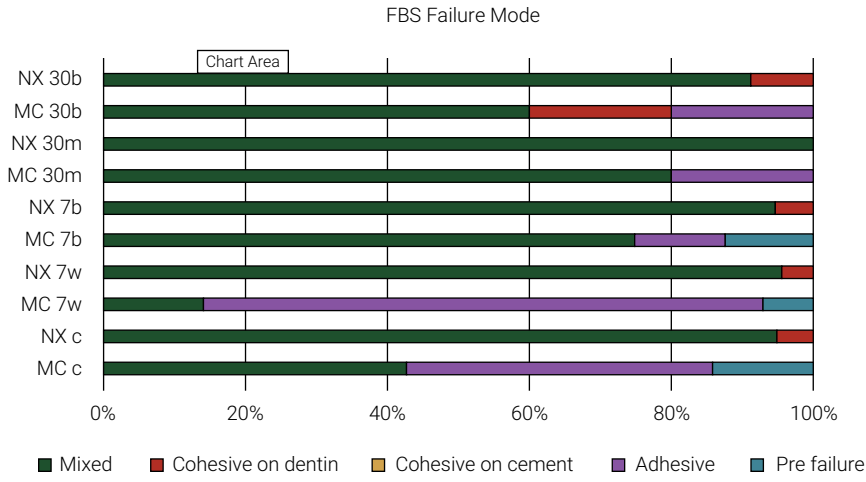

Figure 4. Failure mode assessed by SEM (x100) after four-point bending assay.

For all the groups, there was a predominance of mixed and adhesive failures. Pre-test failures only occurred for the MC cement stored in water for 24 h and 7 days and co-incubated with bacteria for 7 days. The viability of biofilm of the samples co-incubated with *S.mutans* were assessed by a luciferase assay. The biofilm was considered viable, without significant difference between the groups ($p > 0.05$).

Microtensile bond strength

Mean and standard deviation (SD) values for the microtensile bond strength (MPa) of the different groups are presented in Figure 5.

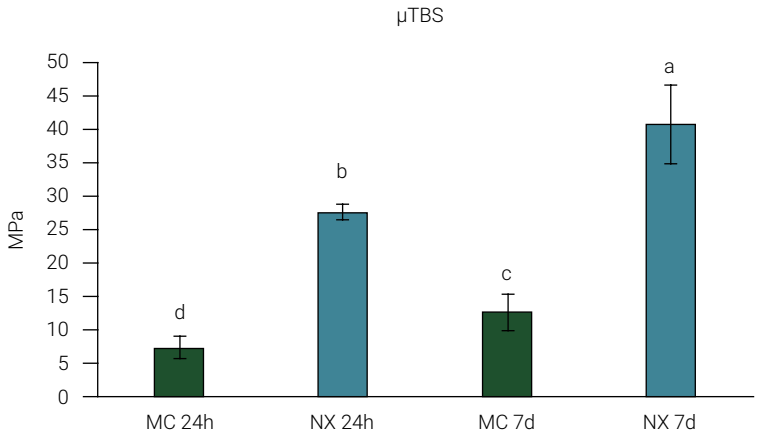

Figure 5. Microtensile bond strength (MPa) of the different groups after the degradation methods (mean ± sd). Bars with dissimilar letters indicate values that are significantly different from each other ($p < 0.05$)

Regardless of the storage period, NX presented higher values than MC. Also, the values at 7 days were greater than those at 24 hours for both cements. The failure mode of the different groups is presented in Figure 6.

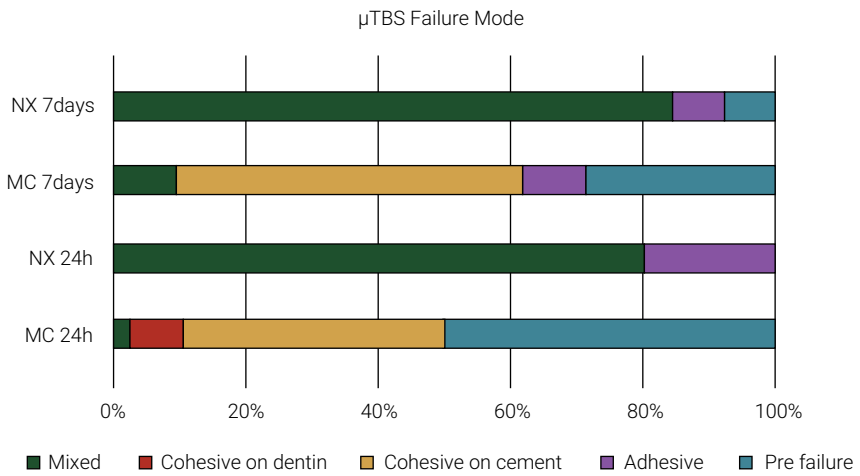

Figure 6. Failure mode assessed by SEM (x100) after µTBS assay.

For the NX groups, there was a predominance of mixed and adhesive failures. For the MC groups, more cohesive within cement and pre-test failures were observed.

Flexural strength of the cements

Mean and standard deviation (SD) values for flexural strength of both cements (MPa) after 24 h in distilled water at 37ºC are presented in Figure 7.

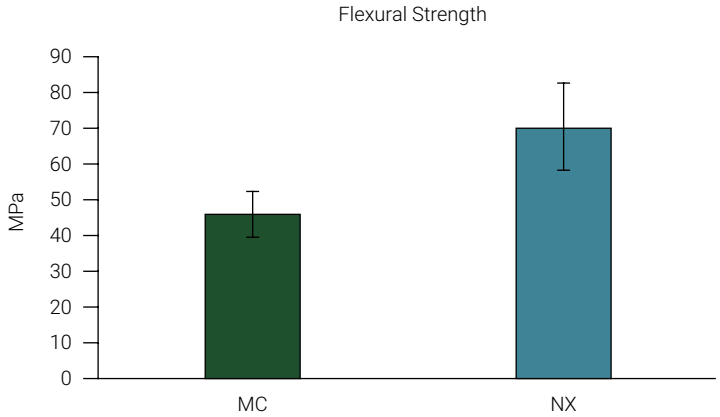

Figure 7. Flexure strength of cements (MPa) after 24 h in water (mean ± sd; n=9). The FS for NX was significantly higher than for MC ($p < 0.001$).

Comparison between bond strength methods

A regression plot between the FBS and µTBS results for both cements at 24 h and 7 days in distilled water at 37ºC is represented in Figure 8.

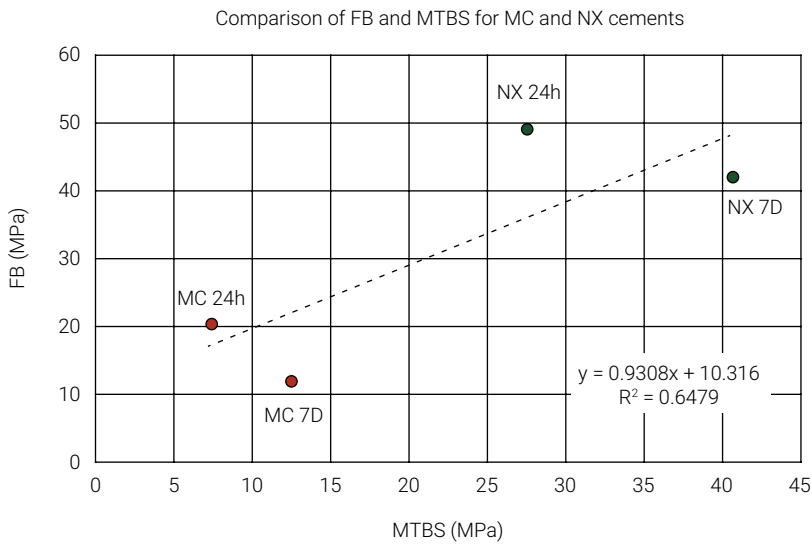

Figure 8. Correlation between FBS and μTBS assays (R^2= 0.664).

For the 1-week period, both test methods gave similar values. At 24h, FBS values were higher (~50%) than μTBS values for both cements.

Discussion

The bond strength stability of the restorative material-cement-dentin interface is a key factor in the success of indirect restorations. The aim of this study was to investigate the effect of different degradation methods on the hybrid ceramic-resin cement flexure bond strength, and to compare the results from the flexure bond strength to the more common microtensile bond strength test for the two cements.

Viable oral biofilms produce significant concentration of acids, mainly propionic, acetic and lactic[3,20]. The hydroxyl and carboxyl functional groups of these acids can establish a high level of hydrogen bonds with the polar sites of the methacrylate monomers present in the adhesive and cement, increasing the acid uptake by the polymeric phase of the hybrid layer. Synergistically, these entrapped acid molecules can reduce the local pH, favoring the hydrolysis of ester groups and leading to the degradation of the hybrid layer that results in a reduction in the interfacial bond strength[2,3,21,22].

Amaral et al.[2] (2015) showed evidence for decreased bond strength values for resin composites bonded to bovine teeth after storage in lactic and propionic acids. Similar results were found by Reis et al.[3] (2015), showing approximately a 33% decrease of the resin composite-human dentin bond strength after storage in acetic acid for 1 week and propionic acid for 1 month. In contrast, the present study showed no significant difference between the degradation methods when samples were assessed by a four-point bending test (Fig. 2), rejecting the first hypothesis. Actual biofilms may produce cariogenic acids at a slow rate and these may have accumulated at a lower concentration than those utilized in the studies that tested the direct effect

of the acids on the bonded interface, thus explaining the different outcomes. Within the limitations of in vitro studies, incubation of restorative materials with cariogenic bacteria may be considered more clinically relevant in comparison with chemical degradation alone (e.g. storage in cariogenic solution), since more variables, such as bacterial metabolism and biofilm structure, are simulated.

Another hypothesis for the lack of difference between the degradation methods, especially for those stored for 30 days, may be related to the time frame. One of the major causes for decrease of bond strength is the degradation produced by water sorption[3,13,14]. In an aqueous environment, the plasticization of the resin matrix[23] and the hydrolysis of the unprotected collagen fibrils by host-derived proteases[6,9] can promote the collapse of the hybrid layer. This hydrolytic degradation, however, is time-dependent. Several studies showed that hydrolytic bonding degradation occurs only after 6 months of water storage[1,3,11,24,25]. Therefore, the period of time chosen for this study may have not been sufficient to produce significant degradation of the adhesive interface.

For the μTBS, samples stored for 7 days had higher bond strength results than those tested after 24h (Fig. 4). Both luting agents used in this study were dual-cured, achieving an adequate degree of cure by the synergistic effect of light exposure and a redox initiator for the free radical formation. While the immediate photo-activation will guarantee an initial mechanical stability, enhanced properties will be obtained after the chemical curing reaction occurs[26]. Although authors claimed that most of the curing occurs within 24 h[27], a residual setting may still occur after this period, explaining the better bonding performance after 7 days. Also, in the short-term, the presence of an aqueous environment can increase the bond strength by forming hydrogen bonds between the polar components in the resin with water[13,28].

Regardless of the bond strength testing method, the NX cement presented better bonding performance as compared with the MC (Figs. 2 and 4). Others have shown that self-adhesive luting agents have lower bond strengths to dentin than conventional resin cements that are used in conjunction with a dentin adhesive[10,11,29-31]. Characteristics such as low etching potential of the functional acid monomers of the self-adhesive cements and their high viscosity promote only partial or no modification of the smear layer, resulting in a weaker hybrid layer in comparison with conventional resin cements when used with their associated primer and etchants[24,32,33].

Maxcem also presented poorer mechanical properties than Nexus, as shown by the comparison of their flexural strengths (Fig.4). The lower values for MC are consistent with the literature. Fuirichi et al.[34] (2016) showed that MC, when compared with other self-adhesive and conventional resin cements, had presented the lowest flexural strength. Although the mechanical properties of self-adhesive cements are material-dependent, it has been shown that some self-adhesive luting agents tend to present poorer mechanical behavior due to specific factors: incompatibility between the acidic functional monomers and the others resinous components[31], reduced degree of conversion[34,35], resin matrix hydrophilicity and unprotected surfaces on filler particles[36], these latter may be responsible for a higher susceptibility to wear. The poorer mechanical properties of MC can be also observed in this study in the failure mode analysis after the μTBS test (Fig.5). For both storage periods, MC presented a higher

percentage of 'cohesive within the cement' failure, indicating that the weaker cement failed before the interface failed.

A direct comparison between μTBS and FBS is somewhat dubious considering the different types of forces and dynamics acting in each test. During the assay, μTBS specimens are subjected exclusively to tensile forces distributed over a well-defined bonding area. In contrast, during the four-point bending test, a mixture of tensile (bottom) and compression (top) forces are produced in the area within the supports spans[13-15]. Another difference that may complicate the comparison concerns sample preparation. For most FBS studies, beams of each of the substrates are produced separately and are then bonded to each other with the cement materials, which likely incorporates more variables and less sample standardization[13-15]. In the present study, specimen preparation was performed identically for both test methods, thus eliminating variables such as irregular beam cutting and luting procedures, making the comparison between methods potentially more accurate.

The major difference between both tests pertains to the sample mounting before the actual test. For the μTBS, the beams must be glued to a jig prior to the test, which, besides being time-consuming, may lead to premature stress on the beams[13]. This is especially critical for materials with poor bonding performance, such as aged specimens and the self-adhesive cement tested in this study. In contrast, FBS samples must only be aligned horizontally within the supports, reducing any excessive manipulation of the beams[13-15], as shown in the analysis of the failure mode assessed after each experiment (Figs. 3 and 5). The μTBS specimens presented more pre-test failures than the FBS specimens, many of which occurred while mounting the specimen to the testing jig. This was especially critical for the MC 24h group. This corroborates the results presented by this material on the assays performed, demonstrating that its poorer mechanical and adhesion properties can influence its performance during the microtensile bond strength test.

For the samples tested after 7 days, the number of pre-test failures decreased, which correlated with the increase of the bond strength that likely occurred due to the completion of cure and maturation of the bond.

The sensitivity of the μTBS method can also be observed on the correlation plot between the assays (Fig.7). When tested at 24 hours, specimens in the FBS test produced higher values than those from the μTBS test. However, at 7 days, the two methods gave essentially identical results. As mentioned, at 24h, the polymerization of the cements may not have been completed, resulting in specimens that were more sensitive to the application of manipulation stresses, e.g. to possible shear forces induced during μTBS assembling. Nevertheless, it was possible to observe that both assays presented a similar trend between the different materials and storage periods, validating the second hypothesis.

Considering a restoration loaded/cemented interface in tension, the microtensile bond strength provides a closer representation of what is occurring at the adhesive interface than the four-point bending[13]. Additionally, few data are available regarding this test in comparison with the abundant evidence related to μTBS, the latter being considered the gold-standard test method. On the other hand, the ease of performing

the FBS, the lower sensitivity of the FBS technique, and the ability to determine the different trends between the materials, as shown by this study, makes the FBS a useful method to determine bond strengths of dental interfaces.

Regarding the effect of the biofilm on the bond strength of the interface of the materials tested, the limitations of this study were related to the limited verosimilarity conditions that an *in vitro* design promotes. Further studies, including *in situ* analysis should be performed, in order to assess the alternations of pH, bacterial flora, temperature, salivary flow, etc, that occur on the oral cavity.

Furthermore, additional analyses including a wider range of resin cements and hybrid/ceramic materials may provide more consistent information regarding the comparison of different bond strength methods.

In conclusion, within the limitations of this study, it can be concluded that biofilm exposure did not affect the hybrid ceramic-resin cement flexural bond strength. Both bond strength methods provided similar outcomes, stating that NX presented higher bond strength than MC for both storage periods. Therefore, FBS was a useful method to compare different materials, especially for those with low mechanical properties which are more sensitive to pre-test manipulation.

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Data availability

Datasets related to this article will be available upon request to the corresponding author.

Conflicts of interest

None.

Author Contribution

Amanda Mahammad Mushashe: conception, design, experiment performance analysis and interpretation of data, paper writing.

Sarah Aquino de Almeida: conception, design, experiment performance analysis and interpretation of data.

Jack Libório Ferracane: conception, design, literature review, analysis and interpretation of data.

Justin Merritt: design, analysis, and interpretation of data.

Carla Castiglia Gonzaga: literature review and critical review of the manuscript.

Gisele Maria Correr: literature review and critical review of the manuscript.

All authors actively participated in the manuscript's findings and revised and approved the final version of the manuscript.

References

1. de Oliveira Lino LF, Machado CM, de Paula VG, Vidotti HA, Coelho PG, Benalcázar Jalkh EB et al. Effect of aging and testing method on bond strength of CAD/CAM fiber-reinforced composite to dentin. Dent Mater. 2018;34(11):1690-701.doi: 10.1016/j.dental.2018.08.302
2. Amaral CD, Correa DS, Miragaya LM, Silva EM. Influence of organic acids from the oral biofilm on the bond strength of self-etch adhesives to dentin. Braz Dent J. 2015;26(5):497-502. doi: 10.1590/0103-6440201300260.
3. Reis A, Martins GC, de Paula EA, Sanchez AD, Loguercio AD. Alternative aging solutions to accelerate resin-dentin bond degradation. J Adhes Dent. 2015 Aug;17(4):321-8. doi: 10.3290/j.jad.a34591.
4. Follak AC, Miotti LL, Lenzi TL, Rocha RO, Soares FZ. Degradation of multimode adhesive system bond strength to artificial caries-affected dentin due to water storage. Oper Dent. 2018;43(2)E92-E101. doi: 10.2341/17-129-L.
5. Jain A, Armstrong SR, Banas JA, Qian F, Maia RR, Teixeira EC. Dental adhesive microtensile bond strength following a biofilm-based in vitro aged model. J Appl Oral Sci. 2020;28 e20190737. doi: 10.1590/1678-7757-2019-0737.
6. Sen N, Us YO. Mechanical and optical properties of monolithic CAD-CAM restorative materials. J Prosthet Dent. 2018;119(4):593-9. doi: 10.1016/j.prosdent.2017.06.012.
7. Cruz MEM, Simões R, Martins SB, Trindade FZ, Dovigo LN, Fonseca RG. Influence of simulated gastric juice on surface characteristics of CAD-CAM monolithic materials. J Prosthet Dent. 2020;123(3):483-90.doi: 10.1016/j.prosdent.2019.04.018.
8. Elmougy A, Schiemann AM, Wood D, Pollington S, Martin N. Characterization of machinable structural polymers in restorative dentistry. Dent Mater. 2018;34(10):1509-17. doi: 10.1016/j.dental.2018.06.007.
9. Beyabanaki E, Eftekhar Ashtiani R, Feyzi M, Zandinejad A. Evaluation of microshear bond strength of four different CAD-CAM polymer-infiltrated ceramic materials after thermocycling. J Prosthodont. 2022 Aug;31(7):623-628. doi: 10.1111/jopr.13469.
10. Velo MMAC, Nascimento TRL, Scotti CK, Bombonatti JFS, Furuse AY, Silva VD et al. Improved mechanical performance of self-adhesive resin cement filled with hybrid nanofibers-embedded with niobium pentoxide. Dent Mater. 2019;35(11):e272-e85. doi: 10.1016/j.dental.2019.08.102.
11. Mushashe AM, Gonzaga CC, Cunha LF, Furuse AY, Moro A, Correr GM. Effect of enamel and dentin surface treatment on the self-adhesive resin cement bond strength. Braz Dent J. 2016;27(5):537-42. doi: 10.1590/0103-6440201600445.
12. Campos RE, Santos Filho PCF, de Oliveira Júnior OB, Ambrosano GMB, Pereira CA. Comparative evaluation of 3 microbond strength tests using 4 adhesive systems: mechanical, finite element, and failure analysis. J Prosthet Dent. 2018;119(1):166-74. doi: 10.1016/j.prosdent.2017.02.024.
13. Wong ACH, Tian T, Tsoi JHK, Burrow M, Matinlinna JP. Aspects of adhesion tests on resin-glass ceramic bonding. Dent Mater. 2017;33(9):1045-55. doi: 10.1016/j.dental.2017.06.013.
14. Albuquerque PPAC, Duarte MFB, Moreno MBP, Schneider LFJ, Moraes RR, Cesar PF et al. Physicochemical properties and microshear bond strength of experimental self-adhesive resin cements to dentin or yttria-stabilized tetragonal zirconia polycrystal. J Adhes Dent. 2019;21(2):133-41. doi: 10.3290/j.jad.a42363.

15. Sanli S, Comlekoglu M, Comlekoglu E, Sonugelen M, Pamir T, Darvell BW. Influence of surface treatment on the resin-bonding of zirconia. Dent Mater. 2015;31(6):657–68. doi: 10.1016/j.dental.2015.03.004.

16. Merritt J, Senpuku H, Kreth J. Let there be bioluminescence: development of a biophotonic imaging platform for in situ analyses of oral biofilms in animal models. Environ Microbiol. 2016;18(1):174-90. doi: 10.1111/1462-2920.12953.

17. Shin Y, Kim Y, Cho BH. Flexural test as an alternative to tensile test for bond strength of resin cement to zirconia. J Mech Behav Biomed Mater. 2021 Jul;119:104525. doi: 10.1016/j.jmbbm.2021.104525.

18. Öznurhan F, Ünal M, Kapdan A, Öztürk C. Flexural and microtensile bond strength of bulk fill materials. J Clin Pediatr Dent. 2015;39(3):241-6. doi: 10.17796/1053-4628-39.3.241.

19. Rai S, Nikhil V, Jha P. Does eugenol affect the microtensile bond strength of self-adhering composite? - An in vitro study. J Conserv Dent. 2021;24(4):354-58. doi: 10.4103/jcd.jcd_60_21.

20. Al-Dulaijan YA, Cheng L, Weir MD, Melo MAS, Liu H, Oates TW, Wang L, Xu HHK. Novel rechargeable calcium phosphate nanocomposite with antibacterial activity to suppress biofilm acids and dental caries. J Dent. 2018;72(1):44-52. doi: 10.1016/j.jdent.2018.03.003.

21. Fukuoka A, Koshiro K, Inoue S, Yoshida Y, Tanaka T, Ikeda T Suzuki K et al. Hydrolytic stability of one-step self-etching adhesives bonded to dentin. J Adhes Dent. 2011;13(3):243-8. doi: 10.3290/j.jad.a19226.

22. Chen C, Chen Y, Lu Z, Qian M, Xie H, Tay FR. The effects of water on degradation of the zirconia-resin bond. J Dent. 2017;64(1):23-9. doi: 10.1016/j.jdent.2017.04.004.

23. Malysa A, Wezgowiec J, Grzebieluch W, Danel DP, Wieckiewicz M. Effect of thermocycling on the bond strength of self-adhesive resin cements used for luting CAD/CAM ceramics to human. Int J Mol Sci. 2022;23(2):745. doi: 10.3390/ijms23020745.

24. Santos MJ, Bapoo H, Rizkalla AS, Santos GC. Effect of dentin-cleaning techniques on the shear bond strength of self-adhesive resin luting cement to dentin. Oper Dent. 2011;36(5):512-20. doi: 10.2341/10-392-L.

25. Sundfeld D, Palialol ARM, Fugolin APP, Ambrosano GMB, Correr-Sobrinho L, Martins LRM, et al. The effect of hydrofluoric acid and resin cement formulation on the bond strength to lithium disilicate ceramic. Braz Oral Res. 2018;32:e43. doi: 10.1590/1807-3107bor-2018.vol32.0043.

26. Kanamori Y, Takahashi R, Nikaido T, Bamidis EP, Burrow MF, Tagami J. The effect of curing mode of a high-power LED unit on bond strengths of dualcure resin cements to dentin and CAD/CAM resin blocks. Dent Mater J. 2019;38(6):947-54. doi: 10.4012/dmj.2018-344.

27. Huang B, Cvitkovitch DG, Santerre JP, Finer Y. Biodegradation of resin-dentin interfaces is dependent on the restorative material, mode of adhesion, esterase or MMP inhibition. Dent Mater. 2018;34(9):1253-62. doi: 10.1016/j.dental.2018.05.008.

28. Kim HJ, Bagheri R, Kim YK, Son JS & Kwon TW. Influence of curing mode on the surface energy and sorption/solubility of dental self-adhesive resin cements. Materials (Basel). 2017;10(2):129. doi: 10.3390/ma10020129.

29. Higashi M, Matsumoto M, Kawaguchi A, Miura J, Minamino T, Kabetani T et al. Bonding effectiveness of self-adhesive and conventional-type adhesive resin cements to CAD/CAM resin blocks Part 1: Effects of sandblasting and silanization. Dent Mater J. 2016;35(1):21-8. doi: 10.4012/dmj.2015-234

30. Kim AJ, Shin SJ, Yu SH, Oh S, Bae JM. Shear bond strengths of various resin cements between three types of adherends and bovine teeth with and without thermocycling. Dent Mater J. 2022;41(2):323-32. doi: 10.4012/dmj.2021-259.

31. Mazzoni A, Maravić T, Tezvergil-Mutluay A, Tjäderhane L, Scaffa PMC, Seseogullari-Dirihan R et al. Biochemical and immunohistochemical identification of MMP-7 in human dentin. J Dent. 2018;79:90-5. doi: 10.1016/j.jdent.2018.10.008.

32. Cantoro A, Goracci G, Vichi A, Mazzoni A, Fadda GM, Ferrari M. Retentive strength and sealing ability of new self-adhesive resin cements in fiber post luting. Dent Mater. 2011;27(10):e197-204. doi: 10.1016/j.dental.2011.07.003.

33. Pisani-Proença J, Erhardt MSG, Amaral R, Valandro LF, Bottino MA, Castillo-Salmerón RD. Influence of different surface conditioning protocols on microtensile bond strength of self-adhesive resin cements to dentin. J Prosthet Dent. 2011;105(4):227-35. doi: 10.1016/S0022-3913(11)60037-1.

34. Fuirichi T, Takamizawa T, Tsujimoto A, Miyasaki M, Barkmeier WW, Latta MA. Mechanical properties and sliding-impact wear resistance of self-adhesive resin cements. Oper Dent.2016;41(3):E83-92. doi: 10.2341/15-033-L.

35. Almeida CM, Meereis CTW, Leal FB, Ogliari AO, Piva E, Ogliari FA. Evaluation of long-term bond strength and selected properties of self-adhesive resin cements. Braz Oral Res. 2018;32:e15. doi: 10.1590/1807-3107bor-2018.vol32.0015.

36. Ferracane JL, Stansbury JW, Burke FJ. Self-adhesive resin cements - chemistry, properties and clinical considerations. J Oral Rehabil. 2011;38(4):295-314. doi: 10.1111/j.1365-2842.2010.02148.x.