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EVALUATION OF MICROTENSIL BOND STRENGTH OF COMPOSITE UNDER THREE AGING CONDITIONS: A COMPARATIVE *IN-VITRO* STUDY

Fadi Hammoud[1] | LouisHardan[2] | Maryse Nassif[3] | Tala Ghoul (El)[4] | Georges Najjar[5] | Cynthia Kassis[6]

Objectives: To investigate the effects of different in-vitro artificial aging protocols on the micro tensile bond strength (µTBS) of composite restorations applied to dentin.

Methods: A total of 88 teeth were prepared on dentin and divided into three distinct aging conditions and a control group : G1) Control group with no aging (n=22); G2) Thermal Cycling (Thermocycling) involving 10,000 cycles between 5°C and 55°C, with a dwell time of 20 seconds (n=22); G3) Mechanical Loading (Cyclic loading) with a 50 N load, a frequency of 1.5 Hz, and 10,000 cycles (n=22); G4) Combined Thermo-mechanical cyclic loading (TMCL) using the parameters of both Thermocycling and Cyclic loading (n=22). Following the preparation of specimens on dentin using standardized procedures, they were subjected to their respective aging conditions and subsequently underwent micro tensile bond strength µTBS testing. The resulting failure modes were classified into adhesive, mixed, and cohesive failures. Statistical analyses were conducted with $\alpha = 0.05$ to determine significant differences between groups.

Results: All aged groups showed lower µTBS compared to the control group. The TMCL group exhibited a significantly lower µTBS compared to both the Control and cyclic loading groups. However, no significant differences were observed between the Control, Thermocycling, and cyclic loading groups. Adhesive failure emerged as the predominant mode of failure across all aging groups.

Conclusions: The combined aging condition of TMCL significantly influenced the µTBS of composite restorations, particularly at the adhesive layer located at the dentin/composite interface. Thermal cycling induced a lower µTBS compared to cyclic loading. These findings underscore the importance of standardized aging protocols for reliable comparison of adhesive performance.

Clinical significance: The study establishes that the combined Thermo-mechanical cyclic loading (TMCL) condition, evaluated using the micro tensile bond strength (µTBS) test, emerges as the most suitable in-vitro aging protocol for accurately assessing adhesive performance. This finding offers valuable guidance for researchers and clinicians in selecting a standardized aging approach that closely mimics real-world conditions.

Keywords: Adhesives, Aging, Composite resin, In-vitro tests, Mechanical test, Tensile strength.

Corresponding author:
Fadi Hammoud, e-mail: fadi.hammoud@net.usj.edu.lb, Phone: +961 71 490 001

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The authors declare no conflicts of interest.

1. DDS, MSc, Department of Restorative and Esthetic Dentistry, Faculty of Dental Medicine, Saint-Joseph University of Beirut, Lebanon. E-mail: fadi.hammoud@net.usj.edu.lb
2. DDS, CEA, DEA, PhD, Department of Restorative Dentistry, Faculty of Dental Medicine, Saint-Joseph University of Beirut, Lebanon. E-mail: louis.hardan@usj.edu.lb
3. DDS, MSc, Department of Prosthodontics and Occlusion, and Department of Digital Dentistry, Faculty of Dental Medicine, Saint-Joseph University of Beirut, Lebanon. E-mail: maryse.nassif@net.usj.edu.lb
4. DDS, MPH, Center for Public Health, Department of Epidemiology, Medical University of Vienna, Vienna, Austria. E-mail: tala.elghoul@meduniwien.ac.at
5. DS, MSc, Cranio-Facial Research Laboratory and Department of Removable prostho, Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. Email: georges.el.najjar@gmail.com
6. DDS, MSc, DEA, PhD, Department of Restorative and Esthetic Dentistry, Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. Email: cynthia.kassis@usj.edu.lb

ÉVALUATION DE LA RÉSISTANCE À LA MICROTRACTION D'UN COMPOSITE SOUS TROIS CONDITIONS DE VIEILLISSEMENT: UNE ÉTUDE COMPARATIVE IN VITRO

Objectifs: Étudier les effets de différents protocoles de vieillissement artificiel in vitro sur la résistance à la microtraction (μTBS) des restaurations en composite appliquées sur la dentine.

Méthodes: Un total de 88 dents ont été préparées sur la dentine et réparties en trois conditions de vieillissement distinctes ainsi qu'un groupe témoin : G1) Groupe témoin sans vieillissement (n=22) ; G2) Thermocyclage (10 000 cycles entre 5°C et 55°C, temps de maintien de 20 secondes, n=22) ; G3) Chargement mécanique cyclique (force de 50 N, fréquence de 1,5 Hz, 10 000 cycles, n=22) ; G4) Chargement thermo-mécanique combiné (TMCL) associant les paramètres du thermocyclage et du chargement mécanique cyclique (n=22). Après la préparation des échantillons selon des protocoles standardisés, ceux-ci ont été soumis aux conditions de vieillissement respectives, puis été évalués par un test de résistance à la microtraction (μTBS). Les modes de rupture ont été catégorisés en ruptures adhésives, mixtes et cohésives. Des analyses statistiques ont été effectuées avec un seuil de significativité α = 0,05 afin d'identifier les différences significatives entre les groupes.

Résultats: Tous les groupes subis à un vieillissement ont montré une μTBS inférieure à celle du groupe témoin. Le groupe TMCL a montré une μTBS significativement plus faible que les groupes témoin et chargement cyclique. Aucune différence significative n'a été observée entre les groupes témoin, thermocyclage et chargement cyclique. La rupture adhésive a été le mode de défaillance prédominant dans tous les groupes soumis à un vieillissement.

Conclusions: La condition de vieillissement combinée TMCL a significativement influencé la μTBS des restaurations en composite, notamment au niveau de l'interface dentine/composite. Le thermocyclage a induit une μTBS plus faible que le chargement cyclique. Ces résultats soulignent l'importance de l'utilisation de protocoles de vieillissement standardisés pour une évaluation fiable des performances adhésives.

Signification clinique: Cette étude démontre que le chargement thermo-mécanique combiné (TMCL), évalué par le test de μTBS, constitue le protocole de vieillissement in vitro le plus pertinent pour évaluer les performances adhésives. Ces conclusions fournissent des indications précieuses pour la sélection de méthodes de vieillissement standardisées, reflétant les conditions cliniques réelles.

Mots clés: Adhésifs, Vieillissement, Résine composite, Tests in vitro, Test mécanique, Résistance à la traction.

Introduction

Dental composites are increasingly being used in restorative dentistry due to their aesthetic and functional properties. However, the long-term success of composite restorations is dependent on their durability and resistance to degradation through their mechanical, chemical, and biological properties [1]. The longevity of dental composite restorations can be influenced by external factors as well, including patient-related habits and clinical considerations such as restoration size, location, and occlusal forces [2]. One of the most important factors affecting the durability of composite restorations is the aging oral environment characterized by exposure to moisture, temperature changes, and mechanical forces. These factors can lead to degradation of the composite resin, resulting in weakening of the bond between the resin and the tooth structure [3].

While clinical trials offer accurate insights into biomaterial behavior, their complexity, expense, and variability challenge standardization [4]. In response, in-vitro studies have emerged as a valuable tool to assess dental biomaterials under simulated clinical conditions, providing valuable insights. To replicate the aging environment, various in-vitro techniques have been employed to mimic oral degradation processes. Despite the extensive volume of in-vitro studies, standardized protocols for artificial aging does not exist [5]. Nevertheless, thermocycling remains widely recognized as an essential method for mimicking the aging process in dental materials [6, 7]. In thermal cycling, specimens are subjected to cyclic temperature changes, simulating the thermal stress that occurs in the oral cavity during eating and drinking [8]. On the other hand, mechanical loading is a widely used protocol as well that involves subjecting the specimens to cyclic or static mechanical stresses, simulating the mechanical stresses that occur in the oral cavity during mastication and bruxism [9]. While each of these techniques has its advantages and limitations, some studies have used a combination of different artificial aging procedures to better simulate intra-oral conditions [10–13].

On the other hand, the importance of bond strength tests is crucial in evaluating new composites and understanding how the aging oral environment impacts the outcomes [14, 15]. Thus, many bond strength tests have been used such as macro bond strength tests (Macrotensile, Macroshear and Push-out) and micro bond strength tests (Microtensile and Microshear) [16, 17]. Sano et al., introduced the Microtensile bond strength methodology employing smaller specimens with bond surfaces measuring less than 1 mm^2 recognized as the most practical bond strength test, with a better discriminative power when compared to other tests [18, 19].

Significant knowledge gaps persist despite extensive research on the aging behavior of dental composites. These gaps concern both the impact of various aging protocols on the microtensile bond strength of composite resin and the comparative analysis of these different techniques [4, 10, 20].

In their recent systematic review and meta-analysis, da Rosa et al. found that there is no clear consensus on the effect of different aging protocols on the microtensile bond strength of composite resin [21]. Conversely, some studies have reported that thermal cycling and cyclic loading can significantly reduce the microtensile bond strength of composite resin [5, 22]. Therefore, this study aimed to assess the micro-tensile bond strength of composite resin at the dentin-composite interface when exposed to different aging conditions, including Cyclic Loading, Thermal Cycling, a combined aging technique (TMCL), and a Control group. Simultaneously, this study analyzed the failure mode of the composite resin across these different aging conditions. The null hypothesis is that the aging conditions did not have a significant effect on the micro-tensile bond strength of the composite resin at the dentin-composite interface, and there was no statistically significant difference between the micro-tensile bond strength values obtained from different aging protocols.

Materials and Methods

Sample preparation and distribution

After the approval of the Ethical Committee of Saint-Joseph University (Beirut, Lebanon; ref.USJ-2022-88), freshly extracted non-carious human third molars were collected and stored in distilled water then 0.1% thymol solution for one week before utilization (N=88) [23]. Given the inherent variability in human third molars, teeth with comparable shape and size were preselected, with an average mesiodistal (MD) dimension of 11 mm and a buccolingual (VP) dimension of 10 mm. Subsequently, the selected teeth were randomly divided into four equal groups (n=22 within each group) for further experimentation. The sample size was determined using SigmaPlot 14.0 software (Systat Software, Inc., Chicago, IL, USA).

Group 1 (G1) served as the Control group and did not undergo any artificial aging condition. For Group 2 (G2), the teeth were subjected to Thermocycling using the THE-1200 Thermocycler (SD Mechatronik, Germany) for 10,000 cycles, ranging from 5°C to 55°C, with a 20-second dwell time at each temperature and a 12-second interval between temperature shifts. In Group 3 (G3), Cyclic loading was applied using the YL01-Cyclic Dental Tester (YLE GmbH, Germany). The teeth were exposed to 10,000 mastication cycles at a dynamic loading of 50 N and a frequency of 1.5 Hz. Lastly, Group 4 (G4) experienced both Thermocycling and Cyclic loading (TMCL). The specimens were first subjected to 10,000 mastication cycles at a dynamic loading of 50 N and a frequency of 1.5 Hz, followed by thermocycling for 10,000 cycles ranging from 5°C to 55°C, with a 20-second dwell time at each temperature and a 12-second interval between temperature shifts. Group distribution is described in table 6.

Once divided into their respective groups, all tooth samples underwent standardized preparation by a single operator using identical equipment. The preparation involved using a high-speed water-cooled Exakt microtome (EXAKT Technologies, USA) equipped with a 200 μm thick diamond band saw. This process removed the occlusal enamel of each tooth, creating a flat coronal dentin surface while keeping the peripheral axial enamel intact. Following the enamel removal, the samples were subjected to an etching step using DeTrey Conditioner 36 (Dentsply Sirona) for 15 seconds on Dentin . After etching, they were thoroughly rinsed for 20 seconds and dried before undergoing a bonding procedure using Prime and Bond Universal (Dentsply Sirona). The bonding material was then photo-activated for 20 seconds using a 3rd generation LED light curing unit (Eighteeth curing pen, Changzhou, China) with a power intensity of 1000 mW/cm^2 [14]. Subsequently, the teeth were restored with Neo Spectra ST composite (Dentsply Sirona) in two increments of 2 mm each. Each increment was photo-polymerized for 20 seconds with the same light-cure unit. The total composite height was measured using a periodontal probe, and it amounted to 4 mm. After this standardized preparation and restoration process, the specimens in each group were exposed to their respective artificial aging protocols as described earlier. This was done following a 24-hour storage period in distilled water at 37 °C.

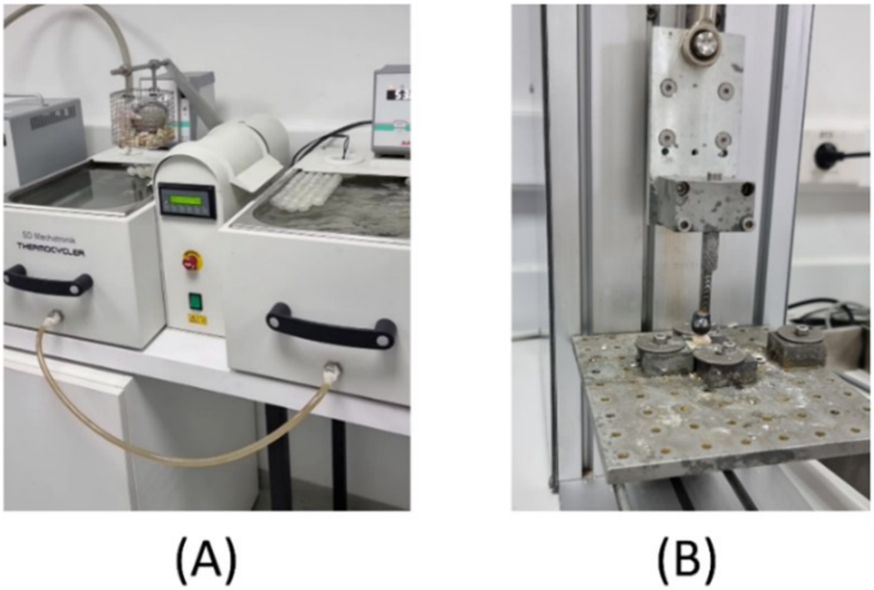

Figure 1. Photography of the Utilized Artificial Aging Machines. (A) The Thermal Cycler, THE-1200 Thermocycler (SD Mechatronik, Germany), programmed for 10,000 cycles, ranging from 5°C to 55°C, with a 30-second dwell time at each temperature and a 12-second interval between temperature transitions. (B) The Mechanical Loading Apparatus, YL01-Cyclic Dental Tester (YLE GmbH, Germany), configured for 10,000 mastication cycles at a dynamic loading of 50 N and a frequency of 1.5 Hz.

Table 1. Experimental Groups and Protocols used for Micro Tensile Bond Strength Evaluation (n=22)

Group	Protocol
Group 1: Control group (n=22)	- No artificial aging procedure
Group 2: Thermocycling group (n=22)	- Temperature range: 5°C to 55°C - Number of cycles: up to 10,000 cycles - Dwell time per temperature: 20 seconds
Group 3: Cyclic loading group (n=22)	- Number of cycles: 10,000 cycles - Force: 50 N - Frequency: 1.5 Hz
Group 4: Thermo-mechanical cyclic loading group (TMCL) (n=22)	**Thermocycling** - Temperature range: 5°C to 55°C - Number of cycles: up to 10,000 cycles - Dwell time per temperature: 20 seconds **Cyclic loading** - Number of cycles: 10,000 cycles - Force: 50 N - Frequency: 1.5 Hz

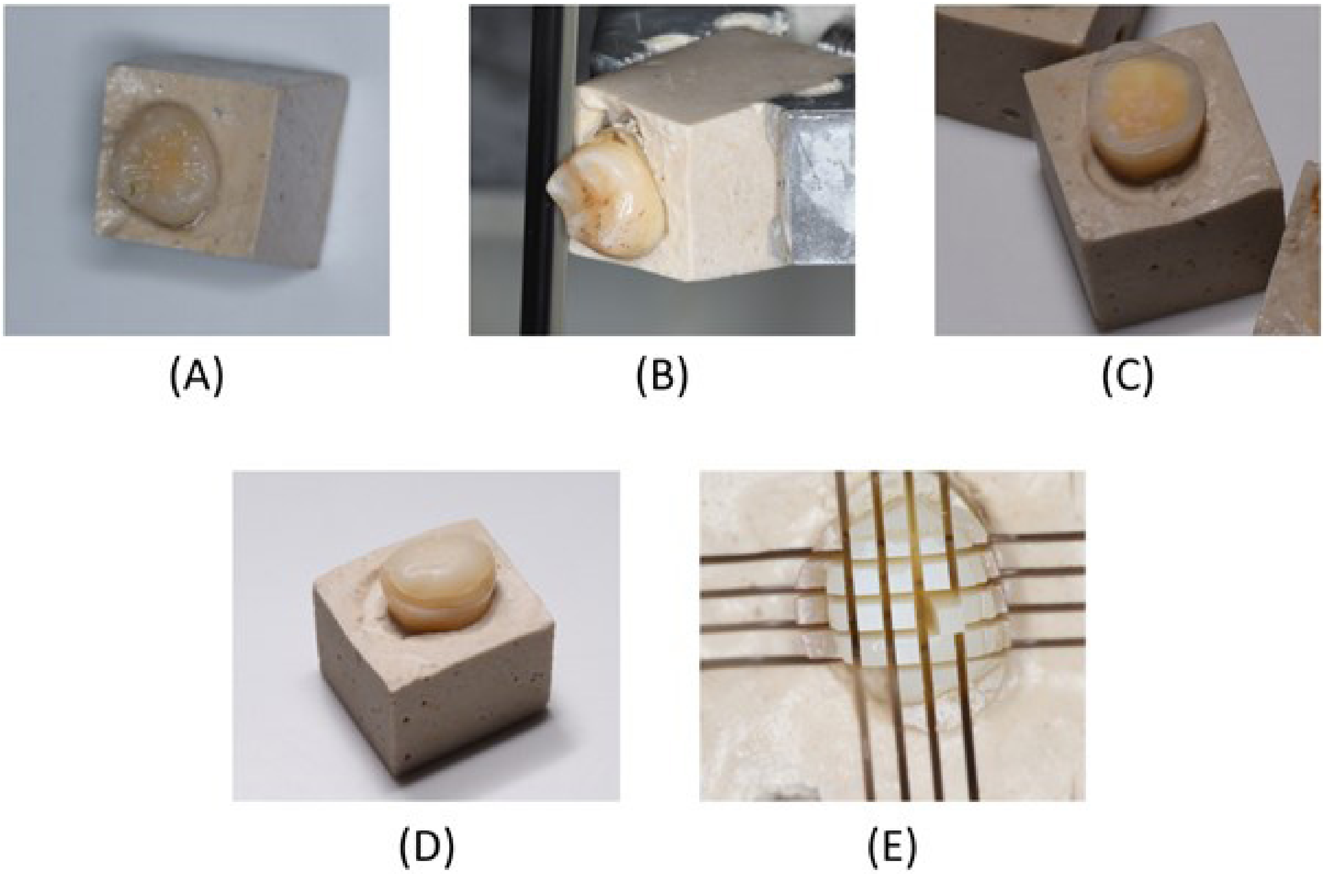

Figure 2. Photography representation of the steps to prepare a specimen for the Microtensile bond test. After pouring a specimen into a solid material (A), the specimens are mounted on a microtome (B) and are subjected to a flat coronal preparation (C). The specimens are then etched, bonded, and restored with composite (D). After being subjected to their respective artificial aging protocols, they are prepared into a stick-shaped specimens using the same Microtome (E).

Testing parameters

The micro-tensile bond strength (μTBS) was evaluated using a Universal testing machine YL-01 (YLE GmbH Waldstraße 1/1a, 64732 Bad König, Germany) available in the Laboratory of Histology of hard tissues – USJ. After undergoing specific artificial aging conditions, specimen slices (n=22 per group) with an approximate surface area of 1 mm^2 thickness were prepared perpendicular to the bonded interface. This was accomplished using a saw equipped with a water jet (Exact Technologies Inc., Norderstedt, Germany) to create stick-shaped specimens measuring 8 mm in length (4 mm of dentin and 4 mm of composite) as illustrated in figure 2. The thickness of 1 mm^2 was determined using a digital caliper (Mitutoyo, Tokyo, Japan) to calculate the bonded area and slices with extra-thin (0.7 mm) and extra-thick (1.4 mm) dimensions were excluded from the analysis. Approximately ten dentin beams were obtained, from which six central beams were carefully selected, and comprised 4 mm of resin composite and 4 mm of dentin structure [24]. These prepared slices were then affixed to a gripping device (Geraldeli's jig) using flow composite (Neo Spectra ST Flow, Dentsply Sirona) and securely placed in the Universal testing machine for the micro-tensile bond strength test.

The micro-tensile bond strength test was then performed at a consistent crosshead speed of 1 mm/min, utilizing a 50 N load cell until bond failure. To calculate the μTBS value, the force applied during debonding (in Newtons) was divided by the bonded surface area of the specimen (in mm^2), and the result was expressed in units of megapascals (MPa).

Following the micro-tensile bond strength test, the fractured specimens were carefully examined to determine the failure mode. The failure mode was classified into four categories:

- Type I: Adhesive failure, where the bond fails at the interface between the adhesive and the dentin.
- Type II: Mixed failure, characterized by a combination of adhesive fracture and partial cohesive fracture within the composite restoration or dentin.
- Type III: Cohesive failure within the composite layer.
- Type IV: Cohesive failure within the dentin.

Observation of the failure modes was carried out using a stereomicroscope (Olympus BX60) at a magnification of x40. All observations and analyses were conducted by a single operator to ensure consistency and accuracy in the results.

Statistical tests

The statistical analysis of this study was performed using a statistical software (STATA version 15.0, StataCorp LP, College Station, TX, USA). The level of significance was set at 0.05. Initially, means and standard deviations were calculated across the four groups of different artificial aging techniques. To evaluate the normal distribution of continuous variables of the data within these categories, both the Shapiro-Wilk and Kolmogorov-Smirnov tests were employed. One-way analysis of variance (ANOVA) test was used to compare the means of the micro tensile bond strength followed by multiple comparisons using the Bonferroni correction tests. Additionally, the association between the type of fracture and the corresponding artificial aging condition was assessed using Fisher's exact test.

Results

Micro tensile bond strength (μTBS)

The distribution of Micro Tensile Bond Strength values (in MPa) within the four distinct groups is illustrated in Figure 4, and the corresponding means and standard deviations are presented in Table 2. One-way ANOVA test revealed a significant difference (p-value<0.01) of the micro tensile bond strengths across the various artificial aging techniques employed on adhesive interface. The Control group (G1) exhibited the highest mean value of micro tensile bond strength at 22.14 ± 2.45 MPa. Following closely was Cyclic loading (G3) at 21.18 ± 2.10 MPa, succeeded by the Thermal cycling group (G2) at 20.19 ± 1.54 MPa while the Thermo-mechanical Cyclic Loading group (TMCL) (G4) displayed the lowest mean value at 17.06 ± 2.10 MPa. Multiple comparisons using the Bonferroni correction tests revealed notable differences among the artificial aging techniques (table 3). Specifically, a significant difference was found between the Control and TMCL groups, highlighting a micro tensile bond strength advantage in favor of the Control group of 5.08 MPa (p-value=0.002). Furthermore, a significant difference in mean micro tensile bond strength was observed between the Cycling Loading and TMCL groups, with the Cycling Loading group showing a higher bond strength of 4.12 MPa (p-value=0.02). While the micro tensile bond strength (μTBS) values of all artificially aged groups exhibited a reduction in comparison to the control group (G1), statistical analysis revealed on the other hand that no statistically significant differences were observed between the bond strengths of G1 (Control) and G2 (Thermal cycling), G1 and G3 (Cycling Loading), as well as G2 and G3 (p > 0.05).

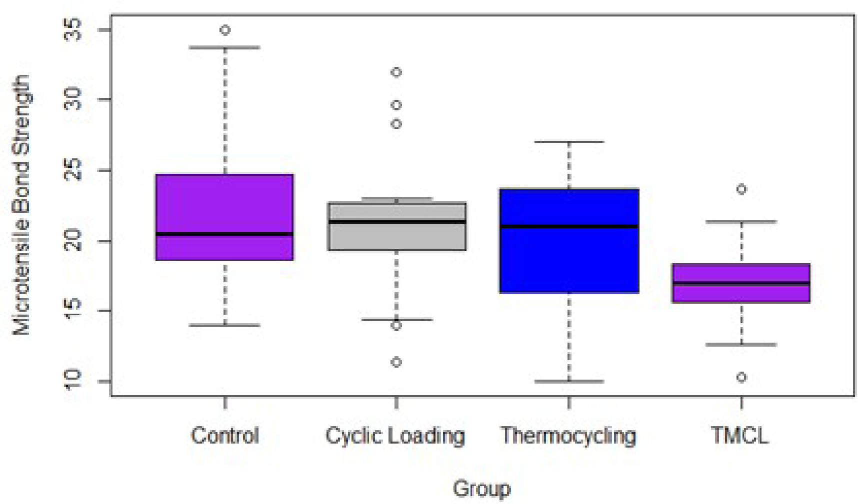

Figure 3. Box Plot Distribution of Microtensile Bond Strength by Group.

Table 2. Results of One-Way ANOVA Test Comparing the Mean Micro Tensile Bond Strength Values Under Various Artificial Aging Conditions

Groups (n=22)	Micro tensile Bond strength		P-value
	Mean (MPa)	± SD	
Control Group	22.14	± 2.45	0.002*
Thermocycling	20.19	± 1.54	
Cyclic Loading	21.18	± 2.10	
TMCL	17.06	± 2.10	

**Significant if p<0.05*

Table 3. Mean difference of Micro Tensile Bond strength between G4/G1 and G4/G3 which showed a significant difference

Groups	Micro tensile Bond strength		P-value
	Mean Difference (MPa)	CI (95%)	
TMCL (G4) and Control (G1)	-5.08	(-8.76; -1.39)	0.002*
TMCL (G4) and Cyclic loading (G3)	-4.12	(-7.8; -0.44)	0.02*

**Significant if p<0.05*

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Failure mode analysis

The frequencies of observed failure modes are depicted in figure 5 and table 4. Adhesive, mixed, and cohesive failure modes were categorized with adhesive failure (Type I) as the most common failure mode detected in all the tested groups (G1: 84.80%, G2: 77.27%, G3:66.67%, G4:75.77%). In contrast, cohesive failures within dentin (Type IV) represented only a small percentage in all four groups. Overall, the Fisher Exact test, which was employed to assess the association between failure mode and the various aging groups, yielded a p-value of 0.210, indicating no significant difference (p-value=0.210>0.05) between the failure modes and the aging condition.

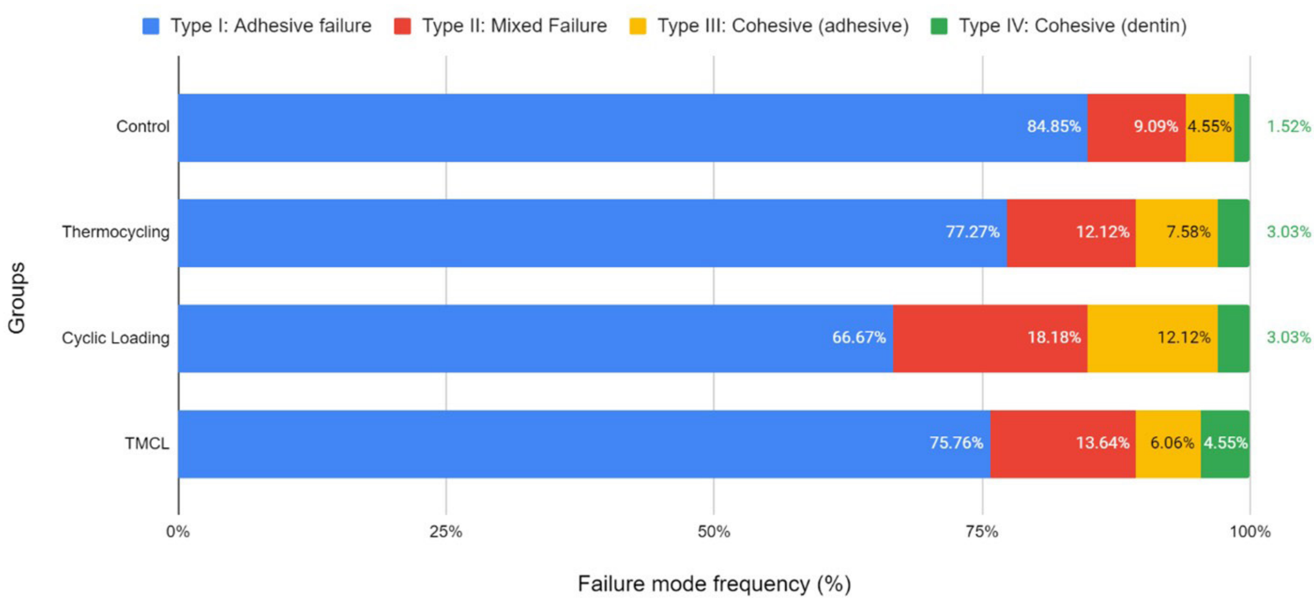

Figure 4. Frequency (%) of failure mode observed among the four groups.

Table 4. Table representing the failure mode distribution. Total number of sticks are given in parentheses (total number per group: 66)

Groups	Failure mode				P-value
	Type I: Adhesive failure	**Type II: Mixed Failure**	**Type III: Cohesive (composite)**	**Type IV: Cohesive (dentin)**	
Control	84.85% (56)	9.09% (6)	4.55% (3)	1.52% (1)	0.210
Thermocycling	77.27% (51)	12.12% (8)	7.58% (5)	3.03% (2)	
Cyclic Loading	66.67% (44)	18.18% (12)	12.12% (8)	3.03% (2)	
TMCL	75.76% (50)	13.64% (9)	6.06% (4)	4.55% (3)	

**Significant if p<0.05*

Discussion

The evaluation of new bonding systems often relies on lengthy long-term clinical trials, which, despite their potential, are challenging by being time consuming, with high costs, and variations in study parameters and investigator expertise [4]. Under clinical conditions, adhesive restorations face a range of challenges from exposure to water, saliva, thermal stresses and masticatory forces. These factors, whether acting independently or collectively have the potential to induce fatigue within the adhesive dentin bond, deteriorating the composite resin, and weakening the collagen structures [3, 25]. To address these complexities, the present in-vitro investigation aimed to simulate in-vivo aging conditions and systematically assess their impact on the micro tensile bond strengths of composite materials. This study employed three of the most widely used aging protocols and a control group [4,9,10] in order to assess the micro tensile bond strength on the dentin/composite interface. As mentioned before, the selection of the μTBS test was based on its unique advantages, including the ability to acquire multiple specimens from a single tooth offering enhanced precision and discriminatory power compared to alternative methods like the microshear bond strength test. Consequently, it is widely recognized as the most precise method for assessing the authentic strength of the bond between an adhesive and its substrate [16, 19].

The results of the present study showed that the in-vitro aging conditions evidently decreased the Micro Tensile Bond Strength of the adhesive compared to the control group, the first null hypothesis was consequently rejected. These results may be explained by the fact that fatigue decreases the bond strength of the adhesive, and these findings align with many research such as Amaral et al. [26], Daneshkazemi et al [12], Abdalla et al. [27], Vivanco et al. [11], Bedran-de-Castro et al. [28] and many others. Other studies showed contradictory results when using thermocycling alone such as the one of Aguilar et al. [29], Ulker et al. [30] and cyclic loading alone such as the one of Nikaido et al. [31] and this can be explained by the lack of standard artificial aging methods which can make it difficult to compare outcomes from different studies in the literature [5, 10].

Thermal cycling is a widely accepted laboratory method used to replicate the effects of temperature variations in the oral cavity on dental restorative materials [7]marginal gap and bond strength laboratory tests. Temperature changes used have rarely been substantiated with temperature measurements made in vivo and vary considerably between reports. Justification and standardization of regimen are required. Data, sources and study selection: An assessment of reports describing temperature changes of teeth in vivo is followed by an analysis of 130 studies of laboratory thermal cycling of teeth by 99 first authors selected from 25 journals. A clinically relevant thermal cycling regimen was derived from the in vivo information, and is suggested as a benchmark standard. Conclusions: Variation of regimens used was large, making comparison of reports difficult. Reports of testing the effects of thermal cycling were often contradictory, but generally leakage increased with thermal stress, although it has never been demonstrated that cyclic testing is relevant to clinical failures. However, should this be done, the standard cyclic regimen defined is: 35°C (28s, inducing repetitive stresses at the tooth-material interface. This results in harming the adhesive by crack propagation due to the difference of coefficient of thermal expansion between the substrates and the adhesive [26]. On the other hand, the hydrolysis effects during thermocycling (20) inducing a hydrolytic degradation by the breakdown of the covalent bonds between polymers, negatively influenced the bond strength, which was observed in this study. It is also considered the prime reason for hybrid layer degradation [32]. Moreover, during thermal cycling, the collagen fibrils situated at the base of the hybrid layer deteriorates. This deterioration disrupts the bonding between these collagen fibrils and the adhesive layer, ultimately leading to the collapse of the adhesive-dentine interface [20].

According to the ISO standard (ISO TR 11450), the thermal cycling protocol suggests 500 cycles within the temperature range of 5°C to 55°C, with a dwell time of over 20 seconds. However, research has demonstrated that this recommended number of cycles (of 500) doesn't accurately simulate the prolonged challenge of bond durability and bond strength of adhesives systems thus studies using the ISO procedures are not considerably impacted [4]. Szczesio-Wlodarczyk et al.[5] demonstrated that 7500 cycles were effective enough for simulating the oral environment on the strength properties of resin composite. As a result, the present study adopted a more rigorous approach, utilizing 10,000 cycles. This extended cycle count, as supported by Gale and Darvell et al., effectively represents the equivalent of one year's worth of clinical functionality [7]. By subjecting specimens to 10,000 cycles at temperatures ranging from 5°C to 55°C (simulating most temperature changes in actual intraoral conditions), and a dwell time lasting 20 seconds, our study aimed to establish a standardized protocol and recommend these conditions to other researchers planning to study bond strength.

Mechanical loading, often referred to as cyclic loading, is a crucial component in studies assessing the bond strength of adhesives, as it has a direct impact on the adhesive interface. When subjected to mechanical loading, adhesives experience fluctuating stresses along their interface, leading to fatigue degradation characterized by the initiation and propagation of microcracks through the matrix [33]. These microcracks can compromise the integrity of the adhesive bond [34]. Additionally, the presence of vacancies within the material structure can significantly accelerate crack propagation under mechanical stress. This combination of fluctuating stresses and the presence of voids within the adhesive matrix greatly enhances the risk of initiating and propagating microcracks [35]. According to Montagner et al [36], there's an absence of universally accepted standards and consensus regarding the optimal parameters for the mechanical loading protocol. Nevertheless, Lima et al. [10] demonstrated that parameters such as a 50 N load, a frequency of 1.5-1.7 Hz, and cycle counts ranging from 10,000 to 100,000 cycles are being the most frequently used. In our study, a 50 N load was used given that loads within the range of 50-90 N are commonly employed in similar investigations. Notably, 70 N was identified as representing a clinically normative bite force [37]. Additionally, a frequency of 1.5 Hz was chosen, closely mirroring the natural chewing cycles observed in vivo [38]. For the mechanical loading, a total of 10,000 cycles were deemed suitable, aligning with the established cycle range for this aging technique.

In the present study, no significant difference was observed between the thermal cycling and control group, as well as the cyclic loading and control group. However, a significant difference was shown between the TMCL and the control, as well as the TMCL and the cyclic loading group. This can be explained by the fact that TMCL is a more severe form of loading that combines both thermal and mechanical stresses, reflective of the complex oral environment which can cause more damage to the adhesive interface resulting in a significant decrease in bond strength compared to the control and cyclic loading groups [10]. Nonetheless, the lack of a significant difference between the thermal cycling group and cyclic loading group compared to the control group, can be attributed to the specific loading conditions applied to these groups. Notably among the various factors, the number of cycles usually considered to be the most influential, which could be increased in further studies to notice a significant change in the micro tensile bond strength [26]. In support of this observation, Teixeira et al. demonstrated that thermocycling proved to be an impactful method leading to pronounced degradation of the bond interface and notably lower bond strength, particularly evident at higher cycle numbers such as 20,000 or 30,000 cycles [39]. An absence of an additional mechanical force to the thermocycling group may have limited the extent of damage to the adhesive interface, resulting in a relatively subtle impact on bond strength.

Similarly, in the cyclic loading group, the specimens were subjected to mechanical loading cycles with the absence of concurrent thermal stresses as seen in the thermo-mechanical cyclic loading group (TMCL), leading to a lesser effect on the bond strength of the adhesive interface. Increasing the number of cycles as well as the loading force and the frequency could negatively influence the bond strength [22, 37]. In our study, the observed significant reduction in micro tensile bond strength within the TMCL (Thermal-Mechanical Cycling) group can be attributed to the synergistic effect of thermal and mechanical stresses [11]. This deterioration in bond strength can be attributed to factors such as increased microleakage and water penetration, which can have adverse effects on the integrity of the adhesive interface. Moreover, unprotected collagen fibrils within the adhesive interface are known to be more susceptible to proteolytic degradation, hydrolysis, and damage from both functional and thermal stress [32]. This phenomenon aligns with the findings reported by Da Silva et al. [40]. Bedran De Castro et al. [28] actually demonstrated that the simultaneous application of thermal and mechanical load cycling led to a noteworthy reduction in microtensile bond strength between a total-etch adhesive and dentin. This decline was more pronounced when compared to specimens subjected to either thermocycling or mechanical loading [30]. Thus, the second null hypothesis is partially rejected with the TMCL group only showing a statistically significant difference compared to the control group and the cyclic loading group.

In the current investigation, the thermocycling group exhibited a lower bond strength value compared the cyclic loading group with the TMCL showing a significant difference with the cyclic loading group. This shows that the thermocycling group negatively influenced the bond strength compared to the cyclic loading group and could be explained by the low number of cycles used for the cyclic loading group. According to Lima et al [10], 1.2 million is the most used number of cycle in studies while our study only used 10,000 cycles. This low number of cycles may not be enough to exhibit a significant decrease in the bond strength as compared to higher numbers of cycles [22].

Following the Microtensile Bond Strength (μTBS) testing, the dentin-facing sides of the failed specimens were analyzed using a stereomicroscope (Olympus BX60) at 40x magnifications. These examinations aimed to classify the failure modes into distinct categories: Type I (Adhesive failure), Type II (Mixed failure), Type III (cohesive failure within the composite), and Type IV (cohesive failure within the dentin). The task of defining categories for classifying failure modes is challenging, and the differentiation between these modes was subjective within the limitation of this study. Observations from Figure 5 and Table 4 showed that adhesive failure emerged as the predominant fracture mode across all four groups. This was followed by mixed failure, then lastly cohesive failure. These findings align with those of numerous other studies. For instance, Braga et al. synthesized the results of 37 studies, concluding that adhesive failure was the prevailing mode [17]. Eren et al. emphasized the significance of the microtensile bond strength technique in understanding the adhesive interface. They associated this technique with a higher occurrence of adhesive failures and fewer cohesive failures [41]. This is explained by the great precision and discriminative capability in assessing the adhesive performance of the Microtensile bond strength test [19].

Notably, there was an increase in mixed and cohesive failure modes in the aged groups compared to the control group. Similar findings were reported by Dieckmann et al., who linked the rise in cohesive failure among aged specimens to composite substrate degradation and compromised mechanical properties due to aging [42]. The application of cyclic loading conditions contributed to an increase of mixed and cohesive failures. This occurrence can be explained by the cumulative impact of mechanical stress, leading to the emergence of microcracks or defects within the material [34]. Importantly, cohesive failures might not be as prominent under static conditions or during thermocycling, as these conditions lack the repetitive and fluctuating stress patterns characteristic of cyclic loading. In addition, cohesive failures might occur due to issues during composite application (presence of voids or air bubbles) or inherent weaknesses in the dentin, resulting in fractures within either the composite or dentin structure [43]. Certain studies have showed a higher number of cohesive failure and this could be due to misalignment of specimen's position, small cracks formation during slicing or positioning which could be mistakenly interpreted as cohesive failures [12].

The variability in results observed across different studies, including the current investigation, points to a lack of standardized aging protocols and storage environments [10]. Variation in the number of cycles, loading forces, temperature ranges, storage environments and dwell times significantly contributes to the discrepancies in results. This inconsistency makes it challenging to directly compare findings among in-vitro studies, limiting our understanding of how adhesives perform [4]. To address this, it's crucial to establish standardized aging protocols that mimic real-world conditions. A consensus on parameters will facilitate meaningful comparisons between studies, leading to a more comprehensive understanding of adhesive performance. However, regarding the limitations, this investigation focused solely on micro tensile bond strength, and the impact of aging was assessed in-vitro, which may not fully replicate the dynamic oral environment. Moreover, the study employed three specific sets of aging protocols with a low number of cycles specifically for the cyclic loading protocol. Although we aimed to simulate clinical conditions, further research with broader aging protocols incorporating mechanical, thermal, and chemical factors is needed to better explore the various factors affecting adhesive bond strength in a closer oral condition.

Conclusion

Within the limitations of the present study, the following conclusions can be drawn:

1. The combined aging effects (TMCL) of Thermocycling and Cyclic loading negatively impacted the μTBS of the adhesive to a greater extent than when these aging conditions were applied individually.
2. Thermocycling exerted a more pronounced influence on the μTBS of the adhesive compared to cyclic loading.

Author Contributions Statement

Fadi Hammoud: Study conception, design, manuscript preparation, Louis Hardan: Data review, manuscript refinement, Maryse Nassif: Literature review, laboratory research, Tala Ghoul (El): Statistical analysis, data interpretation, Georges Najjar: Laboratory research, data evaluation and Cynthia Kassis: Study design, methodological input.

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