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BASIC RESEARCH:

In Vitro Analysis of Marginal Sealing Using Light Curing Techniques on Aged and Unaged Composite Resins

Análisis *in vitro* del sellado marginal mediante técnicas de fotopolimerización en resinas compuestas envejecidas y no envejecidas

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ABSTRACT: To evaluate the marginal sealing of resin composite restorations subjected to various light-curing protocols, both with and without artificial aging through thermocycling. A comparative, longitudinal *in vitro* experimental study was performed with 120 bovine teeth distributed across 8 groups. The teeth were treated with different light intensities (650-1200 mW/cm², 800 mW/cm², 1200 mW/cm², 2500-2800 mW/cm²) along with different curing times (15", 20", 10", 3"), as described in the ISO 11405-2015 standard. Thermocycling comprised of 10,000 cycles to replicate one year of intraoral conditions, and microleakage was measured with dye penetration and the Khera and Chan scale (0-3). The majority of samples (41.7%) displayed dye penetration in the middle third of the interface (grade 2). Significant differences were detected among samples with and without thermocycling in the specific groups (p=0.027 and p=0.013), confirming a possible effect of artificial aging. For the highest light intensities (2500-2800 mW/cm²), no significant differences were present (p=0.527), indicating a possible lower effect of thermocycling at these intensities. The results demonstrate that marginal sealing varies by curing protocols and aging processes. Thermocycling affected microleakage with moderate light intensity, while higher intensities and shorter curing times had a reduced effect. These results point to the need for optimizing the curing protocols to provide better long-term durability for the restoration. Further work should examine other variables that may alter marginal sealing.

KEYWORDS: Marginal sealing; Light curing; Thermocycling.

RESUMEN: Evaluar el sellado marginal de restauraciones de composite resinoso sometidas a diversos protocolos de fotopolimerización, con y sin envejecimiento artificial mediante termociclado. Se realizó un estudio experimental *in vitro* longitudinal comparativo con 120 dientes bovinos distribuidos en 8 grupos. Los dientes fueron tratados con diferentes intensidades de luz (650-1200 mW/cm², 800 mW/cm², 1200 mW/cm², 2500-2800 mW/cm²) junto con diferentes tiempos de polimerización (15«, 20», 10«, 3»), tal y como se describe en la norma ISO 11405-2015. El termociclado consistió en 10.000 ciclos para replicar un año de condiciones intraorales, y la microfiltración se midió con penetración de colorante y la escala de Khera y Chan (0-3). La mayoría de las muestras (41,7%) presentaban penetración de colorante en el tercio medio de la interfase (grado 2). Se detectaron diferencias significativas entre las muestras con y sin termociclado en los grupos específicos (p=0,027 y p=0,013), lo que confirma un posible efecto del envejecimiento artificial. Para las intensidades de luz más altas (2500-2800 mW/cm²), no se presentaron diferencias significativas (p=0,527), lo que indica un posible menor efecto del termociclado a estas intensidades. Los resultados demuestran que el sellado marginal varía según los protocolos de curado y los procesos de envejecimiento. El termociclado afectó a la microfiltración con una intensidad de luz moderada, mientras que las intensidades más altas y los tiempos de curado más cortos tuvieron un efecto reducido. Estos resultados apuntan a la necesidad de optimizar los protocolos de polimerización para proporcionar una mejor durabilidad a largo plazo de la restauración. En futuros trabajos deberán examinarse otras variables que puedan alterar el sellado marginal.

PALABRAS CLAVE: Sellado marginal; Fotopolimerización; Termociclado.

INTRODUCTION

Restoration of the affected teeth is essential to maintain the integrity of the primary dentition until they exfoliate and the permanent teeth emerge. Resins are the treatment of choice for many clinicians, mainly due to their excellent esthetics and ease of repair in case of crown fracture (1). However, the correct shade matching of the composite resin to the surrounding tooth structure remains a challenge (2). The resilience of composite resins as restorative materials is well-established, as demonstrated by numerous systematic reviews (3-7). However, some researchers emphasize that maintaining a clean operative field is critical because contamination can significantly compromise the bond strength of the composite resins.

Contamination of the surgical site can adversely affect the bond strength and longevity of the restorations, according to *in vitro* studies and systematic reviews (8). Isolation options in dentistry that control moisture include the use of cotton rolls, saliva ejectors, and rubber dams. These options not only improve visibility and access during procedures, but they also prevent patients from swallowing or inhaling dental tools or materials. Additionally, isolation options protect against contamination of the operative field and will lead to better clinical outcomes (9).

Laboratory studies have also shown the negative effect of salivary contamination on the bond strength of composite resin restorations to enamel and dentin (7). Composite resins designed for esthetic restorations vary in filler particle type, size, and amount, as well as the composition of their resin matrix. The organic matrix structure and properties of the inorganic fillers also contribute to staining properties and surface smoothness of the restoration (10).

With progress in nanotechnologies and an increasing availability of nanocomposites, a new class of composite resins is emerging that has been designated as bulk-fill restorative materials (11). These materials are gaining popularity because they are easy to work with. In general, bulk-fill resins are designed to be applied in layers of 2 mm or greater in thickness, unlike traditional composites. Thus, they simplify restorative procedures.

Therefore, the aim of this study was to evaluate the marginal seal of a composite resin using different light-curing techniques, both under aging and non-aging conditions.

MATERIALS AND METHODS

STUDY DESIGN

This is an experimental study of an *in vitro* nature, organized in a comparative and longitudinal design. The marginal sealing of the restorations constructed with the composite resin was evaluated by light-curing protocols in both initial conditions and following artificial aging by thermocycling. This was all performed following the required standards of ISO 11405-2015, which formally guarantees the reproducibility and reliability of any results acquired. The CRIS Guidelines (Checklist for Reporting In-vitro Studies) were used to report the findings.

SAMPLE SIZE

The sample size was obtained through the mean comparison formula with an alpha of 0.5 and a beta of 0.8. An N=120 was obtained and divided into 8 groups (n=15 specimens). The specimens were obtained from dental pieces treated with lamps with different types of light intensity in polymerization, Tetric N ceram™ composite resin photo cured with times of 15“, 20”, 10“, 3” and Single Bond Universal™ 3M adhesive.

ALLOCATION

- Group 1: Tetric N ceram™ light cured at 650-1200mW/cm² light intensity at 15” (Polywave®).
- Group 2: Tetric N ceram™ light cured at 800mW/cm² light intensity at 20” (Monowave®).
- Group 3: Tetric N ceram™ light cured at 1200 mW/cm² light intensity at 10” (Polywave®).
- Group 4: Tetric N ceram™ light cured at 2500-2800 mW/cm² light intensity at 3” (Wood Pecker®).

SELECTION CRITERIA

The study included bovine premolar or incisor teeth in good health, with intact crowns and an extraction age of no more than 6 months, which had also been treated with resin. Conversely, those teeth with caries, fractured crowns, restorations with lack of compactness, enamel alterations or visible pigmentation were excluded.

OBTAINING THE SPECIMENS

Lower teeth from the bovine species were obtained from ranking, regarding criteria, of the animal farm Yerbateros, Lima-Peru. Once they were extracted, the teeth were washed with physiological saline solution to remove any remains of the periodontal tissue. After that, the teeth underwent a deep cleaning process using a scaler, fine pumice stone and water; all tissues were removed from the teeth, and a soft cup was used to protect the dental surfaces during the process. Finally, the pieces were again stored in a jar of deionized water; one German WLO Vorff Not ZScker®

oven at 37°C was used to replicate the oral conditions to maintain hydration until the completion of the experimental protocol. This provided the best quality preservation of the dental pieces.

CAVITY PREPARATIONS

A controlled environment of 23±2°C, which is most optimal for resinous materials, was maintained for the cavity preparations. Class V cavities were prepared on the mesial and distal surfaces of each tooth and restored with direct composite resin. For cavity preparation and restoration, a 330-Bur and a high-speed handpiece (Extra Torque 605 C Kavo®) with an aqueous cooling device were used. The dimensions were standardized by a template and chamfered with a diamond bur. Every five preparations, new burs were used to standardize the preparation. A total of 120 cavities were restored with Bulk fill™ nanohybrid resin with polymerization using state-of-the-art LED technology.

TOOTH RESTORATION

The approach of restoration began by using absolute acid etching with 37% phosphoric acid (15 seconds on enamel and 5 seconds on dentin). It was washed with water for 30 s and dried with gauze without drying the dentin. Single Bond Universal™ adhesive (3M ESPE) was then applied with Monitex microbrush™, rubbing on dentin to optimize penetration and gently on enamel. The adhesive should have a uniform gloss of 2 mm prior to light curing (10 seconds). The cavity was restored with composite resin using an incremental technique and light curing according to the experimental protocols.

SEALING

After the restoration, the apical foramen of each tooth was sealed with Vitaloy acrylic. The specimens, except for the occlusal/incisal side, were coated with nail polish to prevent seepage during thermocycling, performed at alternating temperatures of 5 °C, 37 °C and 55 °C, following ISO-TS 11405-2015. Each bath lasted 20 seconds with 5-10 seconds of transfer, completing 10,000 cycles, equivalent to one year of exposure in the oral cavity. The samples were then immersed in 2% methylene blue for 4 h to evaluate the penetration and marginal sealing of the restorations.

MICROFILTRATION

The filtration of the dye in the samples was assessed using a stereoscope with 25X magnification. The external surfaces were comprehensively cleaned with both water and acetone to remove contaminants before the samples were stabilized in blocks of clear acrylic resin. The samples were then cut longitudinally along the major axis in the mesiodistal direction with a low-speed, water-cooled bioactive disk to obtain a hemisection for analysis. To minimize bias, this was also completed the same day. Filteration was evaluated by viewing the samples based on a table developed for this purpose, and by using Khera and Chan's ordinal scale (0 to 3), which confers the penetration of dye by depth.

DATA ANALYSIS

The collected data were recorded in a Microsoft Excel 2019 spreadsheet and subsequently analyzed using SPSS Version 27 statistical software. Descriptive analysis was performed to explore the absolute and relative frequencies. For inferential analysis, no normality test was performed due to the characteristics of the study and the variables evaluated. Instead, Fisher's exact test was used directly since it is suitable for comparing proportions and analyzing categorical variables in small sample size contexts.

RESULTS

Overall when evaluating Tetric N ceram™ marginal sealant light cured at 650-1200mW/cm² light intensity at 15", 10.1% of the samples did not show any level of microleakage (grade 0). The majority of the samples (41.7%) showed penetration in the middle third of the interface (grade 2), regardless of the performance of the thermocycling. Likewise, 26.6% showed penetration limited to the occlusal third (grade 1), while 21.6% showed penetration to the apical third (grade 3). Statistical analysis indicated that there was no significant difference between the samples with and without thermocycling (p=0.324). These results highlight that the intensity of light curing, within this range, may not be the only determining factor in the marginal seal (Table 1).

Assessment of the marginal seal of Tetric N ceram™ light cured with an intensity of 800mW/cm² for 20" indicated differing levels of microleakage based on the thermocycling condition. Of the samples tested only 1.7% demonstrated no dye penetration (grade 0), while the most samples (55.0%) had limited penetration in the middle third of the interface (grade 2). A total of 18.3% of the samples demonstrated microleakage limited to the occlusal third (grade 1) with 25.0% of samples demonstrating marked penetration that reached the apical third (grade 3) of the interface. Statistically significant differences were observed between samples with and without thermocycling (p=0.027) indicating the factorial influence of the artificial aging condition on the marginal seal (Table 2).

Assessing the marginal seal of Tetric N ceram™ at a light intensity of 1200 mW/cm² cured for 10", light curing intensity had a meaningful effect of marginal seal, in the samples that were thermocycled 15.0% had no dye penetration (grade 0) while only 1.7% did not demonstrate any dye penetration in the samples that were not thermocycled. The largest amount of samples (41.7%) demonstrated some dye penetration into the middle third of the interphase (grade 2). 26.6% of the samples showed dye penetration into the apical third of the interphase (grade 3). The difference between thermocycling and non-thermocyling samples was statistically significant (p=0.013) and implies a change of quality of marginal seal due to artificial aging (Table 3).

The outcomes indicated that with a light intensity of 2500-2800 mW/cm² and light curing time of 3 s, most samples (58.3%) demonstrated penetration in the middle third of the interface (grade 2). A total of 6.7% exhibited no microleakage (grade 0), 8.3% exhibited microleakage confined to the occlusal third (grade 1), and 26.7% exhibited penetration to the apical third (grade 3). No statistically significant differences existed between samples with and without thermocycling (p=0.527), which suggested that under these conditions, the artificial aging process did not affect the marginal seal (Table 4).

Table 1. Marginal seal of Tetric N ceram™ light cured at 650-1200mW/cm² light intensity at 15”.

Grade	Thermocycling				Total		
	No		Yes				
	n	%	n	%	n	%	p
0	4	6.7	2	3.4	6	10.1	
1	5	8.3	11	18.3	16	26.6	0.324
2	13	21.7	12	20.0	25	41.7	
3	8	13.3	5	8.3	13	21.6	
Total	30	50.0	30	50.0	60	100.0	

0: No penetration of the stain. 1: Penetration in the occlusal third of the sealer enamel interface. 2: Penetration extending into the middle third of the interface. 3: Penetration to the apical third of the interface.

Table 2. Marginal seal of Tetric N ceram™ light cured at 800mW/cm² light intensity at 20”.

Grade	Thermocycling				Total		
	No		Yes				
	n	%	n	%	n	%	p
0	1	1.7	0	0.0	1	1.7	
1	2	3.3	9	15.0	11	18.3	0.027
2	21	35.0	12	20.0	33	55.0	
3	6	10.0	9	15.0	15	25.0	
Total	30	50.0	30	50.0	60	100.0	

0: No penetration of the stain. 1: Penetration in the occlusal third of the sealer enamel interface. 2: Penetration extending into the middle third of the interface. 3: Penetration to the apical third of the interface.

Table 3. Marginal seal of Tetric N ceram™ light cured at 1200 mW/cm² light intensity at 10”.

Grade	Thermocycling				Total		
	No		Yes				
	n	%	n	%	n	%	p
0	1	1.7	9	15.0	10	16.7	
1	3	5.0	6	10.0	9	15.0	0.013
2	16	26.7	9	15.0	25	41.7	
3	10	16.6	6	10.0	16	26.6	
Total	30	50.0	30	50.0	60	100.0	

0: No penetration of the stain. 1: Penetration in the occlusal third of the sealer enamel interface. 2: Penetration extending into the middle third of the interface. 3: Penetration to the apical third of the interface.

Table 4. Marginal seal of Tetric N ceram™ light cured at 2500-2800 mW/cm² light intensity at 3".

Grade	Thermocycling				Total		
	No		Yes				
	n	%	n	%	n	%	p
0	3	5.0	1	1.7	4	6.7	
1	3	5.0	2	3.3	5	8.3	0.527
2	18	30.0	17	28.3	35	58.3	
3	6	10.0	10	16.7	16	26.7	
Total	30	50.0	30	50.0	60	100.0	

0: No penetration of the stain. 1: Penetration in the occlusal third of the sealer enamel interface. 2: Penetration extending into the middle third of the interface. 3: Penetration to the apical third of the interface.

DISCUSSION

The use of light-curing composite resin (CR) restorations has gained popularity due to their improved mechanical properties, satisfactory esthetic results, and increasing interest from the general population (12-14). The procedures for the application of this material were relatively standardized. First, the cavity was prepared following well-established conservative principles, then the adhesive system was applied, followed by composite placement and then light curing. This last process was of vital importance, as it directly influenced the final properties of the composite. Insufficient curing could result in decreased surface hardness, increased water absorption, and reduced wear resistance of the restorative material. In contrast, adequate light-curing ensured durable restoration with optimal mechanical and esthetic properties (15,16).

In the study by Ding L. *et al.*, they analyzed 48 tooth surfaces experimentally by *in vitro* analysis. The results showed that the application of the infiltrating composite reduced the enamel porosity and significantly increased the surface microhardness. Furthermore, in their study with 163 tooth surfaces, they observed that different treatments applied on surfaces with initial values of $-10 < \Delta F < -6$ and $-1000 < \Delta Q < -20$ obtained satisfactory results (17).

Jablonski-Momeni A. *et al*. (18) conducted a study comparing in situ and in vitro approaches to investigate the remineralization of enamel lesions. Two dentifrices were tested: one containing 1450 ppm sodium fluoride (Group A) and a placebo (Group B). Differences in remineralization potential were significant in the *in vitro* studies ($p<0.001$) and in situ after 21 days ($p=0.034$), but not at 10 days ($p=0.4$). µCT results for *in vitro* and in situ were consistent, indicating that in vitro studies using pH cycling could replace in situ protocols.

In one study, by Greenlaw R. *et al.*, they conducted an *in vitro* investigation with 69 premolars to evaluate a visible light-curing resin system intended for orthodontic bonding. The bond strength of the light-curing resin system at 30 h was found to be approximately half the strength observed for the chemically activated resin systems. The initial bond strength at 1 h was only 26% of the bond strength at 30 h. During recementation and cleanup, the enamel loss with the light-curing resin was approximately half the loss observed with the chemically polymerized, heavily filled resin. In addition, cleaning the remaining resin from the visible light-curing resin system only required the use of hand scrapers (19).

Mavropoulos A. *et al.* investigated the minimum polymerization time required for bonding stainless steel brackets using intensive LED polymerization units. A total of 75 bovine incisors were divided into five groups, and brackets were fixed using Transbond XT adhesive. Two groups used the Ortholux LED lamp for 5 and 10 s, and two others used the Ultra-Lume LED 5 for the same durations. The control group employed a halogen lamp (Optilux 501) for 40 s. Significant differences in the SBS values (P<0.001) were observed. Adequate SBS was achieved with 10 s of intensive LED polymerization, comparable to the control, while 5 s showed lower SBS. It was concluded that intensive LED lamps offer an effective and economical alternative to halogen lamps for orthodontic bonding (20).

Uusitalo E. *et al*. examined how light transmits through the dental enamel and dentin, focusing on the effects of exposed dentinal tubules. They assessed light attenuation in enamel and dentin samples of varying thicknesses (1-4 mm) under wet and dry conditions (n=5). Using a light-curing unit with a maximum power of 1869 mW/cm², irradiance was measured before and after treating the samples with EDTA. For 1 mm wet discs, the transmitted light was 500 mW/cm² for enamel and 398 mW/cm² for dentin (p<0.05). Transmission decreased with greater thickness, and the wet specimens attenuated less light than the dry ones. Post-EDTA treatment, dentin transmission increased to 439 mW/cm². Intact premolars and incisors exhibited irradiances of 6.2 mW/cm² (8.2 mm thickness) and 37.6 mW/cm² (5.6 mm thickness), respectively (21).

This study has some limitations that should be considered when interpreting the results (22,23). First, this is an *in vitro* model, which implies that the experimental conditions, although controlled, do not completely replicate the dynamic environment of the oral cavity in a living being. In addition, the use of bovine teeth, although representative, could differ in certain aspects from the characteristics of human dental tissue. Another relevant aspect is the relatively limited sample size per group, which could restrict the generalizability of the findings. In addition, the focus on specific light-curing times and intensities does not allow us to evaluate how other variables might influence marginal sealing. Finally, the thermocycling process, while simulating aging, does not fully reflect the mechanical and chemical challenges that restorations are subjected to in a real clinical setting. These limitations highlight the need for further research to corroborate and complement these results.

CONCLUSION

The study showed that the marginal seal of the composite restorations varied according to the thermocycling conditions and the light intensity used in light curing. In general, most of the samples showed microleakage in the middle third of the interface (grade 2). It was observed that thermocycling increased microleakage in certain groups, while in others, with high intensities and short times, there was no significant difference. This suggests that although light-curing intensity and duration are key factors, other elements could influence marginal seal performance. These findings reinforce the importance of optimizing protocols to improve the quality and durability of dental restorations.

CONFLICT OF INTEREST

No conflict of interest.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: J.M., L.Q.T., H.O.A., and F.M.T.
Literature review: F.E.C., W.M and F.M.T.
Methodology and validation: F.M.T .and F.E.C.
Formal analysis: J.M., F.M.T., and F.E.C
Investigation and data collection: J.M., H.O.A. and L.Q.T.
Resources: F.E.C. and W.M.
Data analysis and interpretation: F.M.T., and F.E.C.
Writing-review and editing: J.M. L.Q.T., and F.M.T
Supervision: F.M.T.
Project administration: J.M., and L.Q.T.

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