Evaluation of the Effect of Atmospheric Pressure Variations on the Marginal Seal of Class I Composite Resin Restorations Bonded with a Three-Step Etch-and-Rinse Adhesive: An In Vitro Study

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ABSTRACT

Background: The durability of composite resin restorations under pressure variations (e.g., diving, air travel, hyperbaric oxygen therapy) remains a concern due to potential microleakage at the tooth–composite interface.

Objective: To assess whether a single exposure to hyperbaric or hypobaric pressure affects the marginal sealing of Class I cavities restored using a three-step etch-and-rinse adhesive system (Optibond FL).

Methods: Forty extracted human molars were randomly assigned to eight groups (n = 5) and restored with Ceram.x Spectra ST HV composite resin following standard adhesive protocols. The groups were exposed to simulated hyperbaric (up to 3.5×10^3 hPa) or hypobaric (down to 0.75×10^3 hPa) conditions for varying durations. One group served as a control (no pressure change). Specimens were immersed in 1% methylene blue dye for 24 hours, sectioned, and analyzed using a VR-H4J profilometer to measure dye penetration at the tooth–composite interface. Data were analyzed using the Shapiro–Wilk test and Kruskal–Wallis test ($\alpha = 0.05$).

Results: No statistically significant differences in microleakage were observed between the control group and pressure-exposed groups ($p > 0.05$). In total, 87.5% of the samples showed no detectable microleakage. Only 5 out of 40 teeth showed minimal dye penetration.

Conclusion: Within the limits of this in vitro study, a single exposure to hyperbaric or hypobaric conditions did not affect the marginal integrity of Class I restorations bonded with the MR3 adhesive system. Pressure variations of the magnitude and duration tested appear unlikely to compromise the seal when proper adhesive protocols are followed.

Keywords: Microleakage, Composite resin, Atmospheric pressure variation, Adhesive system

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INTRODUCTION

Human evolution has consistently been driven by the desire to transcend natural environmental limitations. Among the most notable milestones in this quest are mastering air travel and exploring underwater environments, achievements that have expanded our physical, scientific, and medical frontiers. However, these advancements also introduce new physiological challenges, particularly due to exposure to fluctuating atmospheric pressures. Such pressure variations can have profound effects on the human body, including the oral cavity, where they may result in conditions such as dental barotrauma, defined as physical damage to a tooth or dental restoration caused by changes in ambient pressure and barodontalgia, which is a pain in the teeth triggered by these pressure variations.[1-4]

These conditions are not limited to extreme environments. They are frequently reported among scuba divers, aviators, and even commercial airline passengers despite cabin pressurization systems. Furthermore, patients undergoing hyperbaric oxygen therapy (HBOT), increasingly used in clinical settings to promote healing in various medical conditions, are also subject to elevated pressure levels that may impact dental structures.[5] Such exposures are becoming more commonplace, making it increasingly important to understand their implications for oral health.

Atmospheric pressure variations can be broadly categorized into two types: hypobaric conditions-where pressure is lower than at sea level, as experienced at high altitudes-and hyperbaric conditions, characterized by pressures above atmospheric levels, such as those encountered during deep-sea diving or in hyperbaric chambers. Even within commercial aircraft cabins, pressurization typically simulates altitudes of 1,800-2,400 meters, which still subjects passengers to significant hypobaric stress.[5] These shifts can influence both biological tissues and dental biomaterials, potentially affecting the integrity of dental restorations and leading to discomfort or failure.

Despite the growing prevalence of such exposure, the behavior of dental materials, particularly adhesive systems and resin composites, under varying pressure conditions remains inadequately explored.[4,6] As hyperbaric medicine expands and advanced dental materials continue to evolve[7], understanding how these materials perform in non-normobaric environments is becoming increasingly relevant. The need for this knowledge is particularly urgent, given lifestyle trends that involve frequent air travel, recreational diving, and novel medical therapies that utilize pressure modulation.

Composite resins have become the material of choice for dental restorations due to their favorable aesthetic qualities, mechanical durability, and ability to adhere to tooth structures through chemical and micromechanical bonding. These properties are enabled mainly by dental adhesive systems, which allow for a more conservative restorative approach compared to traditional amalgam fillings.[8-10] Among these, etch-and-rinse systems, particularly the three-step (ER3) protocol, are known for their high bond strength and reliability when applied correctly.

However, composite materials' principal limitations lie in their inherent polymerization shrinkage during light curing. This shrinkage can create gaps at the tooth-restoration interface, leading to marginal microleakage, characterized by the infiltration of bacteria, fluids, and ions. Over time, such leakage can compromise the longevity and effectiveness of the restoration, increasing the risk of secondary caries and postoperative sensitivity.[11,12]

Given these concerns, this study aims to evaluate the impact of atmospheric pressure changes on the marginal seal between composite resin and dental tissue when using a three-step etch-and-rinse adhesive system (ER3). By investigating how this adhesive system performs under varying pressure conditions, we aim to generate data that can inform clinical protocols, especially for patients regularly exposed to hypobaric or hyperbaric environments. These insights may contribute to more resilient dental restorations and support adapting dental care strategies to evolving medical practices and lifestyle patterns.

The null hypothesis of this study is that there is no significant difference in the marginal sealing performance of composite restorations bonded with the ER3 adhesive system under different atmospheric pressure conditions.

MATERIALS & METHODS

Ethics: The study was carried out in compliance with ethical standards and received prior authorization in accordance with institutional requirements.

Ethical Approval and Informed Consent: The study was approved by the Ethics Committee of the Queen Fabiola University Children's Hospital, Université libre de Bruxelles (CEH 51/14), and conducted by the principles of the Declaration of Helsinki. Informed consent was obtained from all adult participants or, in the case of minors (adolescents), from their parents or legal guardians.

Sample Selection: This study was conducted on 40 extracted human permanent maxillary and mandibular molars. The extracted teeth consisted primarily of permanent molars removed for periodontal reasons, as well as non-carious impacted third molars. Only teeth free of carious lesions, cracks, restorations, or structural defects were included. Selection was performed using a stereomicroscope to ensure the structural integrity of the dental tissues. The teeth were subsequently cleaned and stored in a physiological saline solution until use.

The sample size was determined based on similar in vitro microleakage studies evaluating dental restorations, in which group sizes ranging from 5 to 10 specimens are commonly reported (13,14). Given the exploratory nature of the present study and the technical constraints associated with pressure chamber experiments, a total of 40 teeth (n = 5 per group) was considered appropriate to allow preliminary comparisons while maintaining experimental feasibility.

- **Atmospheric Pressure:** At sea level, all bodies are subjected to atmospheric pressure, which results from the weight of the atmosphere exerting a constant force on the Earth's surface. Its standard value is approximately 1,013.25 hPa.

- **Hyperbaric Pressure:** During scuba diving, the human body is exposed to an absolute pressure corresponding to the sum of atmospheric and hydrostatic pressure resulting from the weight of the water column above. This hydrostatic pressure increases by approximately 1,000 hPa (or 1 atm) for every 10 meters of depth in seawater (15).

For this study, an absolute pressure of 2.8×10^3 hPa was selected, corresponding to the pressure observed at a depth of 18 meters below sea level. We chose this specific value because it is commonly used in hyperbaric oxygen therapy, which delivers oxygen at similar maximum pressures. This therapeutic context further supported our choice.

Additionally, a pressure of 5×10^3 hPa, equivalent to the pressure found at a depth of 40 meters below sea level, was also included in the study. This depth represents the upper limit of recreational diving, beyond which technical diving begins. Selecting this pressure allowed us to explore physiological responses at the threshold between recreational and technical diving conditions.

- **Hypobaric Pressure:** As altitude increases, atmospheric pressure decreases and air density drops, resulting in reduced oxygen availability. On average, atmospheric pressure decreases by approximately 10% for every 1,000 meters of elevation.[16,17]

At typical cruising altitudes of commercial aircraft between 10,000- and 12,000-meters atmospheric pressure falls to around **250 hPa (0.25×10^3 hPa)**. This level is too low to sustain human life for extended periods, particularly in individuals with underlying health conditions.

To counteract this, aircraft cabins are pressurized to simulate an altitude where the pressure corresponds to **at least 75% of sea-level atmospheric pressure**, regardless of the actual flight altitude. For this study, a cabin pressure of **750 hPa (0.75×10^3 hPa)** was selected as the reference value.

Specimen Preparation: Standard Class I cavities were prepared on all teeth with the following approximate dimensions: 2 mm in width and 2.5 mm in depth, using a one mm-thick cylindrical diamond-tipped bur under continuous water irrigation. We preserved the marginal ridges by maintaining a thickness of 1.5 mm. Each tooth was then sectioned horizontally at the cemento-enamel junction using an extra-long cylindrical diamond bur, leaving only the crown portion for the subsequent procedures.

Bonding Procedure: We used the Optibond™ FL system (Kerr, Orange, CA, USA) for resin bonding. The teeth were first etched with 37% phosphoric acid for 20 seconds, then thoroughly rinsed with running water. A primer was then applied using a micro-brush and vigorously rubbed into the surface, followed by gentle air-drying for 20 seconds. We applied an even layer of adhesive, dispersed it using a gentle air stream, and light-cured it for 20 seconds.

Restorative Procedure: The cavities were restored using a light-curing composite resin (Ceram.x Spectra® ST HV, Dentsply Sirona, Charlotte, NC, USA), applied in increments of no more than 2 mm. Each layer was light-cured for 20 seconds. Final finishing and polishing were performed using a red-ring diamond olive-shaped bur, followed by polishing with a cup mounted on a contra-angle handpiece. All cavity preparations, adhesive procedures, and restorative procedures were performed by a single operator to ensure procedural standardization and minimize operator-related variability.

The experiments were conducted at the CREST laboratory of the Université Libre de Bruxelles. To replicate varying pressure conditions, two types of pressure chambers were employed. To simulate a hyperbaric environment, we used a pressure chamber (E3000 Series Critical Point Drying Apparatus) capable of gradually increasing and maintaining pressure levels up to 200 × 10^3 hPa. The chamber was connected to an air compressor (Compressor Powerplus POWX1727S, 550W, 6L–10 ACC) to supply pressurized air and raise the internal pressure. A manual manometer was also attached to monitor and measure the pressure inside the chamber in real time. To simulate a hypobaric pressure environment, a vacuum-sealed cold trap chamber (Goldleaflab Stainless Trap for Mechanical Cold Trap, KF-25) was used as a low-pressure chamber. This device is designed to maintain low pressure reliably over extended periods of time. The chamber was connected to a dual-stage vacuum pump (Vacuumchambers.eu Vacuum Pump VP280, 10 CFM) to decrease the internal atmospheric pressure gradually. To monitor pressure levels during the experiment's different phases, an electronic vacuum gauge (Thyracont VD81 Compact Vacuum Meter) was connected to the system, enabling precise measurement of the negative pressure inside the chamber.

Sample distribution: Following composite resin restoration, the 40 teeth were randomly divided into eight groups of five teeth each (n = 5). Each group was subjected to a distinct pressure condition (Table 1).

Atmospheric Pressure (Control Group)

- **Group 1:** The teeth were immersed in physiological saline and maintained at ambient atmospheric pressure throughout the experiment, without any pressure variation. This group served as the control.

Hyperbaric Pressure Conditions

- **Group 2:** The teeth were exposed to a pressure of 2.8 × 10^3 hPa, reached within approximately 3 minutes, maintained for 30 minutes, followed by a gradual return to atmospheric pressure over 3 minutes.
- **Group 3:** The teeth were exposed to the same pressure of 2.8 × 10^3 hPa, also reached in 3 minutes, maintained for 60 minutes, with a gradual decompression over 3 minutes.
- **Group 4:** The teeth were exposed to a pressure of 5 × 10^3 hPa, reached in 5 minutes, maintained for 5 minutes, then returned to atmospheric pressure over 10 minutes.
- **Group 5:** The same pressure of 5 × 10^3 hPa was applied, reached in 5 minutes, maintained for 60 minutes, and followed by decompression over 5 minutes.

Hypobaric Pressure Conditions

- **Group 6:** The teeth were exposed to a reduced pressure of 0.75 × 10^3 hPa, reached over 15 minutes, maintained for 60 minutes, and then gradually returned to atmospheric pressure in 15 minutes.
- **Group 7:** The pressure of 0.75 × 10^3 hPa was maintained for 5 hours and 30 minutes, following the same 15-minute compression and decompression phases.
- **Group 8:** The teeth were exposed to 0.75 × 10^3 hPa for11 hours and 30 minutes, with pressure changes also occurring over 15 minutes.

Table 1: Distribution of samples according to pressure type, duration, and sample size

Group	Pressure condition	Pressure (hPa)	Exposure duration	N
1	Atmospheric (control)	1,013	No pressure variation (control condition)	5
2	Hyperbaric	2.8 x 10^3	30 min	5
3	Hyperbaric	2.8 x 10^3	60 min	5
4	Hyperbaric	5.0 x 10^3	30 min	5
5	Hyperbaric	5.0 x 10^3	60 min	5
6	Hypobaric	0.75 x 10^3	60 min	5
7	Hypobaric	0.75 x 10^3	5 h 30 min	5
8	Hypobaric	0.75 x 10^3	11 h 30 min	5

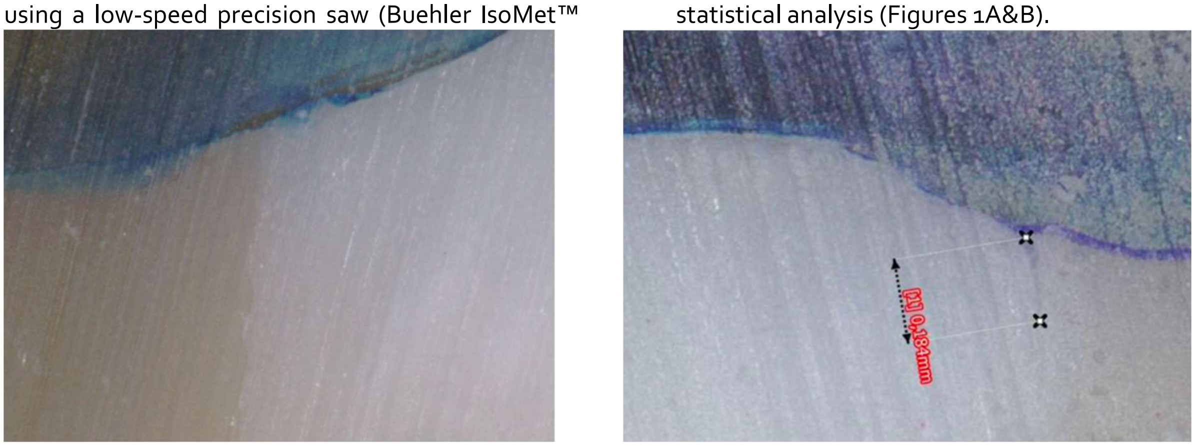
Microleakage Assessment at the Tooth–Composite Interface

To evaluate microleakage at the tooth–composite resin interface, a methylene blue dye penetration test was performed. After exposure to hyperbaric or hypobaric conditions, all samples were fully immersed in a 1% methylene blue solution for 24 hours at room temperature.

Following immersion, the teeth were thoroughly rinsed with distilled water to remove any surface dye residue. Each sample was then embedded in epoxy resin molds, using stabilization screws to ensure consistent positioning during sectioning.

The teeth were sectioned into one mm-thick slices using a low-speed precision saw (Buehler IsoMet™ Low Speed Saw) following a buccolingual cutting plane.

Microleakage analysis was performed using a 3D profilometer (Keyence, VR-6000, Osaka, Japan), which enabled micrometer-scale observation with magnification of up to 160×Microleakage was assessed by measuring the depth of methylene blue dye penetration at the interface between the tooth and the composite resin restoration. After sectioning the samples, measurements were performed using the VR-H4J image analysis software (IRIS Development, Tours, France). The dye penetration was recorded in millimeters (mm), and the maximum depth of infiltration along the interface was used for statistical analysis (Figures 1A&B).



A **B**

Figure 1: Profilometric evaluation of microleakage in a tooth cross-section. **A)** Tooth section showing no microleakage under ×160 magnification. **B)** Tooth section with visible microleakage measured using a profilometer at ×160 magnification

Statistical Analysis

The collected data were transferred into an Excel spreadsheet and analyzed using descriptive statistics to calculate the mean dye penetration depth for each experimental group. The primary outcome variable was the maximum depth of dye penetration (mm) measured at the tooth-composite interface for each specimen. Failure was defined as the presence of any detectable dye penetration (> 0 mm). Descriptive statistics were calculated for each experimental group.

The Shapiro-Wilk test was used to assess data normality within each group. Several groups showed zero variance, with all observations equal to zero, thereby precluding assessment of normality. Consequently, non-parametric statistical methods were selected.

Overall, intergroup comparisons were performed using the Kruskal-Wallis test to evaluate whether atmospheric pressure variations (hyperbaric or hypobaric) had a significant effect on microleakage when compared with the control group.

Given the small sample size per group ($n = 5$) and the high proportion of zero values, post-hoc pairwise comparisons were not performed, as they would not provide additional statistical power or reliable interpretation. Instead, failure rates (presence/absence of dye penetration) were reported descriptively for each group, and comparisons between the control group and pressure-exposed groups were interpreted cautiously.

All statistical analyses were conducted using GraphPad Prism version 10.5.0 (GraphPad Software, Inc., La Jolla, CA, USA). The level of statistical significance was set at $p < 0.05$.

RESULTS

Microleakage results under different pressure conditions were obtained by measuring the depth of

methylene blue dye penetration at the tooth–composite resin interface using the VR-H4J software. Measurements are expressed in millimeters (mm).
The Shapiro-Wilk test was used to assess the normality of data distribution within each group (n = 5). For groups 2, 3, and 8, all observed values were identical (zero), resulting in zero variance and rendering the normality test inapplicable (Table 2).

Table 2: Normality test results for the experimental groups under different pressure conditions (n = 5 per group).

	Atmospheric Pressure	**Hyperbaric Pressure**				**Hypobaric Pressure**		
Groups	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Minimum	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Maximum	0,2390	0,000	0,000	0,08400	0,1830	0,2820	0,1580	0,000
Mean	0,04780	0,000	0,000	0,01680	0,03660	0,05640	0,03160	0,000
±SD	0,1069	0,000	0,000	0,03757	0,08184	0,1261	0,07066	0,000
SEM	0,04780	0,000	0,000	0,01680	0,03660	0,05640	0,03160	0,000
P value	0,0001	n.e	n.e	0,0001	0,0001	0,0001	0,0001	n.e

The Shapiro-Wilk test was used to assess normality. Groups showing identical values were marked as not evaluable (n.e.), due to zero variance, which invalidates the test.
Statistical analysis using the Kruskal-Wallis test revealed no significant differences between the control group and the groups exposed to hyperbaric or hypobaric pressure conditions (Figure 3). Failure, defined as the presence of dye penetration at the tooth–composite interface, was reported descriptively for each experimental group. Group-wise comparisons between the control group and each pressure-exposed group did not reveal any statistically significant differences ($p > 0.05$). Under hyperbaric conditions, no statistically significant differences were observed between groups exposed to the same pressure for different durations. When the exposure duration was kept constant, no significant differences were observed between groups exposed to varying pressure levels.

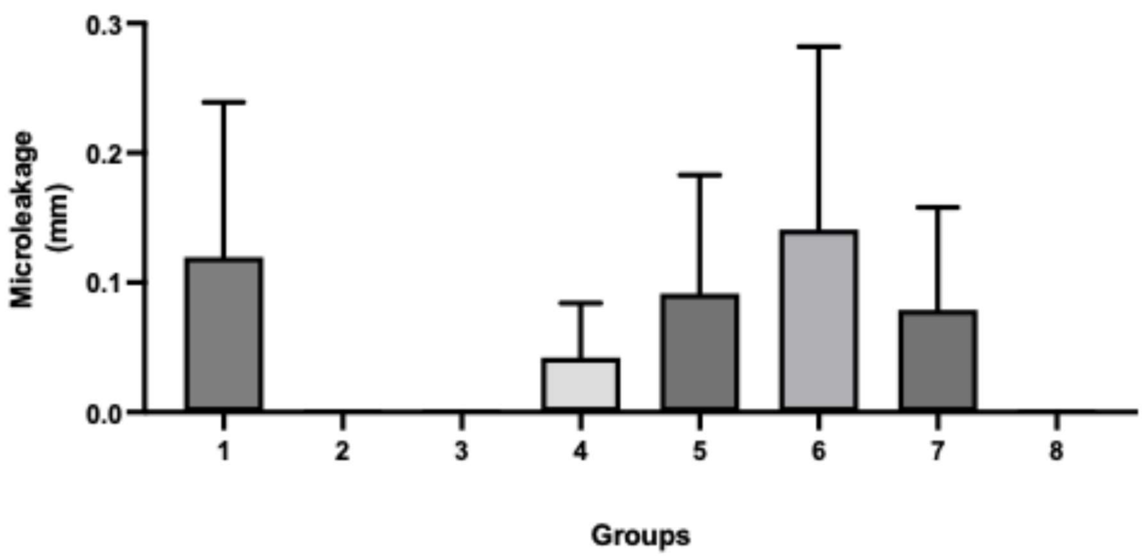


Figure 3: The mean and range of microleakage values within the different groups.

Under hypobaric pressure conditions, no statistically significant differences were observed among groups exposed to 0.75×10^3 hPa for varying durations. Throughout all eight experimental groups, the depth of microleakage remained very low. In each group, at least four out of five teeth showed no detectable dye penetration under the profilometer (value = 0 mm), indicating that a minimum of 80% of the samples were completely sealed. These findings demonstrate the high sealing capability of the ER3 adhesive system, regardless of the pressure conditions to which the specimens were exposed.

No microleakage was observed in 87.5% of samples across all groups. Only 5 out of 40 teeth showed visible microleakage, resulting in a 12.5% rate (Figure 4).

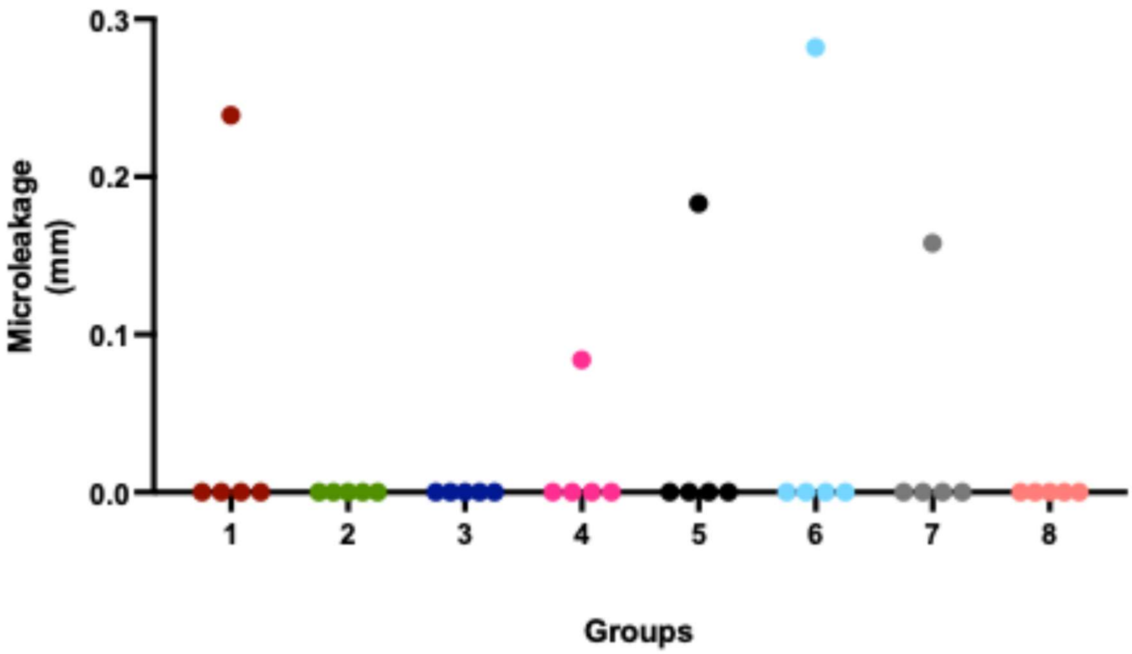


Figure 4: Incidence of microleakage across the eight experimental groups.

DISCUSSION

The objective of this study was to evaluate the influence of atmospheric pressure changes on the seal integrity between dental tissues and composite resin using an ER3 adhesive system. The results suggest that a single exposure to pressure variation does not significantly affect marginal seal integrity within the experimental conditions of this study.

The absence of significant microleakage observed in this study is consistent with the well-documented performance of three-step etch-and-rinse adhesive systems. These are widely regarded as the gold standard for composite bonding.[18,19] When properly applied, such systems provide reliable, durable adhesion to enamel, ensuring excellent marginal sealing. Therefore, the favorable marginal integrity in this study was expected and should be seen in light of the established efficacy of etch-and-rinse adhesives, not as an unexpected outcome.

The findings highlight that recreational diving up to a depth of 40 meters for one hour, with gradual descent and ascent phases at a rate of 8 meters per minute, does not cause leakage at the tooth-composite interface. Similarly, a 12-hour commercial flight showed no impact on marginal sealing.

Moreover, a hyperbaric oxygen therapy session, during which oxygen is administered at a pressure of 2.8×10^3 hPa, also did not induce microleakage at the dental tissue-composite interface. Across all groups, restorations maintained remarkable sealing even under significant pressure variations. Indeed, 90% of the samples showed no microleakage, and the few observed infiltrations were minimal (<0.3 mm) and confined to enamel.

The quality of adhesion to enamel partly explains this. The highly mineralized enamel provides a favorable surface for micromechanical bonding, primarily achieved through the selective dissolution of hydroxyapatite crystals via acid etching, which creates microporosities that allow resin infiltration and the formation of resin tags, ensuring the effective and durable retention of composite to enamel.[20,21]

These results can also be attributed to the absence of trapped air bubbles at the composite-dental tissue interface, particularly in the enamel. According to Boyle's law ($PV = K$, where P is pressure, V is volume, and K is a constant), at constant temperature, the volume of a gas is inversely proportional to the pressure applied.[22] If air bubbles were present at the junction, pressure variations would change their volume, generating stresses that could compromise marginal sealing.

During exposure to high pressure, air bubbles compress, which generally does not affect the marginal seal or cause structural defects. However, the risk arises during pressure decrease, particularly when returning to normal pressure after hyperbaric exposure or during hypobaric exposure. To minimize this risk, strict adherence to operative protocols and good compaction of composite resin are essential.

Some in vitro studies have explored microleakage under pressure variations, notably Shafigh et al.[23] who demonstrated a similar microleakage rate after pressure changes in MOD composite restorations bonded with an ER2 system, supporting our findings. These studies also show that applying a thin layer of flowable composite can reduce microleakage[7], while dentin porosities tend to increase it.

Conversely, other studies have reported a significant increase in microleakage at the dental tissue-composite interface following repeated pressure variations.[24,25]. These studies also confirm that applying a thin layer of flowable composite reduces microleakage[24], whereas dentin porosities contribute to its increase.[22]

According to the literature, the primary challenge of adhesive systems is their inability to ensure optimal dentin adhesion.[26]This lack of retention risks leaving gaps and air bubbles at the interface, which can potentially cause dental complications under pressure variations. This risk is exceptionally high with improper etchant use, such as over- or under-drying the acid.[27] Careful application of phosphoric acid and controlled dentin drying are therefore critical.

The clinical performance of the ER3 system, particularly Optibond FL used in our study, has been confirmed by studies reporting clinical retention rates between 86% and 98%, with 100% achieved after 5 years of follow-up.[28-30] This study suggests that this system offers superior bonding efficacy compared to other adhesive systems.

Another study comparing various restorative materials highlighted the low polymerization shrinkage of the resin used in this work (Dentsply Sirona Ceram.x Spectra ST HV) relative to other materials tested.[11]. This supports the absence of microleakage in most samples, likely due to its high inorganic filler content.

Limitations and Perspectives

This study is an in vitro investigation, where the analyzed teeth were kept isolated from saliva, occlusal forces, and pulpal responses, which can influence marginal sealing. Although this experimental model allows some control over variables, it does not perfectly replicate the biological conditions of the oral cavity. Furthermore, the limited sample size per group reduces the statistical power and, consequently, the generalizability of the conclusions. A larger-scale study would strengthen the validity of these observations.

The method used to evaluate microleakage involved immersion in 1% methylene blue dye, a commonly used dye for its penetration ability and visual contrast[31], making it a simple and effective tool for detecting sealing defects at the dental tissue-composite interface. Nevertheless, this technique has some limitations. Despite sectioning the teeth at various levels, the thickness of the slices could lead to missing microleakage that does not occur precisely at the cut.

Observations were performed using a profilometer with magnification up to ×160, allowing relatively precise readings of dye penetration levels. However, scanning electron microscopy (SEM) could have provided more detailed information on the adhesive interface morphology and represents a promising avenue for future research.

Finally, this study focused exclusively on the effects of a single pressure variation exposure in a Class I cavity restored with an ER3 adhesive system. Further research should investigate the potential impact of multiple successive exposures under similar conditions and establish whether a recommended waiting time between exposures is necessary.

Given the large number of people engaging in scuba diving or air travel, it would be relevant to adapt dental anamnesis by incorporating targeted questions about these activities. Inquiring about patients' flying or diving habits, or their recent exposure to flights, dives, or hyperbaric oxygen therapy following dental treatment, could help tailor care according to available recommendations.[6,32]

CONCLUSION

Within the limits of this in vitro study and under optimal bonding conditions using the ER3 system, a single exposure to either hyperbaric or hypobaric pressure variations did not affect the marginal seal of teeth with Class I cavities, regardless of the pressure level or duration of exposure.

Conflict of intertest: The authors declare that they have no conflict of interest related to this study.

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