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Article

Effect of Lesion Depth on the Efficacy of Resin Infiltration in Orthodontic White Spot Lesions

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Abstract

Background and objectives: White spot lesions (WSLs) represent a common enamel demineralization complication associated with fixed orthodontic treatment. Resin infiltration is widely used as a minimally invasive approach to arrest lesion progression and improve esthetics; however, the influence of lesion severity on treatment effectiveness remains insufficiently understood. This in vitro study aimed to evaluate the impact of WSL severity on resin infiltration performance under conditions simulating orthodontic demineralization. **Material and Methods:** Ninety extracted human premolars were subjected to controlled acidic exposure to produce mild, moderate, and severe lesions. All specimens were treated using a standardized resin infiltration protocol. Lesion depth, resin penetration, optical masking effect (ΔE), and surface microhardness were evaluated using confocal microscopy, spectrophotometry, and Vickers hardness testing. **Results:** Lesion depth increased significantly with demineralization duration ($p < 0.001$). Resin penetration showed a strong positive correlation with lesion depth ($r = 0.81$), while infiltration efficiency was highest in moderate lesions. Optical masking effectiveness decreased significantly with increasing lesion severity ($p < 0.01$). Surface microhardness improved significantly after infiltration in all groups, with the greatest recovery observed in moderate lesions. **Conclusions:** Lesion severity significantly influences the clinical performance of resin infiltration. Early and moderately developed WSLs respond more favorably to infiltration treatment, emphasizing the importance of timely intervention during orthodontic therapy.

Keywords: white spot lesions (WSLs); resin infiltration; enamel demineralization; lesion depth; optical masking effect; surface microhardness

1. Introduction

White spot lesions (WSLs) are among the most frequent and clinically relevant adverse effects associated with fixed orthodontic treatment; they represent the earliest clinically detectable manifestation of enamel demineralization associated with fixed orthodontic therapy. [1]. They are subsurface enamel demineralization areas that clinically appear as opaque, chalky-white regions due to changes in enamel's refractive index [1,2]. These lesions develop as a result of an imbalance between demineralization and remineralization processes at the tooth surface, strongly associated

with prolonged plaque accumulation and increased cariogenic bacterial activity around orthodontic appliances. The structural characteristics of WSLs vary considerably depending on lesion severity and duration of acidic challenge, which may influence the effectiveness of minimally invasive therapeutic approaches such as resin infiltration [1-4].

Despite significant advances in orthodontic materials and techniques, fixed appliances continue to create multiple plaque-retentive areas, particularly around brackets, ligatures, and archwires [3]. These retentive zones favor the accumulation of biofilm rich in acidogenic microorganisms, leading to a rapid decrease in local pH and subsequent mineral loss from enamel [4]. Recent studies published after 2021 consistently report a high incidence and prevalence of WSLs in patients undergoing fixed orthodontic therapy, emphasizing that these lesions may develop within weeks of appliance placement, especially in the presence of inadequate oral hygiene [1,2,5].

From a clinical perspective, WSLs are not merely an aesthetic concern. Although initially non-cavitated, these lesions reflect a structurally weakened enamel surface with increased porosity, which predisposes the tooth to further progression toward cavitated carious lesions if left untreated [2,6,7]. Moreover, the aesthetic impact of WSLs often becomes more evident after appliance removal, when patients expect an improvement in dental appearance following orthodontic treatment [7,8]. As a result, WSLs can compromise patient satisfaction and overall treatment outcomes [9].

Early diagnosis and risk assessment are therefore essential components of modern orthodontic care [1,10]. Contemporary diagnostic approaches, including standardized digital photography and quantitative light-induced fluorescence (QLF), allow for the detection and monitoring of early enamel demineralization before cavitation occurs [11,12]. Recent literature highlights that patients with pre-existing enamel defects or initial WSLs before orthodontic treatment are at increased risk for the development of additional lesions during therapy [1].

Preventive strategies remain the cornerstone of WSL management and include rigorous oral hygiene protocols, dietary counseling, fluoride-based products, and emerging biomimetic remineralization systems [13]. However, evidence published in the last few years suggests that preventive measures alone may not be sufficient to eliminate the risk of WSL formation, particularly in high-risk orthodontic patients [14]. Consequently, minimally invasive therapeutic approaches aimed at arresting lesion progression and improving aesthetics have gained increasing attention [1,7,15].

Resin infiltration therapy has emerged as a micro-invasive treatment modality for non-cavitated enamel lesions [15,16]. By penetrating the porous structure of demineralized enamel, low-viscosity infiltrating resins occlude diffusion pathways for acids, stabilize the lesion, and modify the optical properties of enamel, thereby reducing the visible contrast between sound and affected tissues [16-18]. Recent studies have confirmed the clinical effectiveness of resin infiltration in arresting WSL progression and achieving favorable aesthetic outcomes [1,7,8,13,14,15,17].

In parallel with commercially available infiltrants, ongoing research has focused on the development of experimental resin systems with improved mechanical properties, reduced water sorption, and enhanced biological performance [18,19]. Modifications of the resin matrix and the incorporation of functional fillers capable of fluoride release represent promising strategies to improve the long-term stability and preventive potential of infiltrating materials [20,21].

The present study aimed to evaluate the influence of lesion severity on the performance of resin infiltration in orthodontically induced white spot lesions. Specifically, the study assessed the relationship between demineralization depth and the following parameters: resin penetration depth, optical masking effect, and surface microhardness recovery [22,23].

A secondary objective was to determine whether resin infiltration exhibits differential effectiveness across early, moderate, and advanced stages of enamel demineralization under controlled laboratory conditions simulating the cariogenic environment associated with fixed orthodontic appliances.

2. Materials and Methods

Study Design

This in vitro laboratory study was designed to evaluate the influence of white spot lesion (WSL) severity on resin infiltration performance under conditions simulating orthodontic demineralization. The primary outcomes assessed included resin penetration depth, optical masking effectiveness, and surface microhardness recovery. Ethical approval was obtained from the University George Emil Palade Ethic Committee (Approval No. 2709/27.12.2024). Specimen collection and preparation. Specimen collection and preparation were performed between January and February 2025. The artificial demineralization procedures and resin infiltration treatments were conducted from March to April 2025. Laboratory measurements, including confocal microscopy, spectrophotometric analysis, and microhardness testing, were completed between May and June 2025. Statistical analysis and data interpretation were carried out in July 2025.

A priori sample size calculation was performed using G*Power software (Version 3.1, Heinrich-Heine University, Düsseldorf, Germany). Based on data from previous in vitro studies evaluating resin infiltration penetration depth and microhardness recovery in artificial white spot lesions, a large effect size ($f = 0.40$) was assumed.

With a significance level of $\alpha = 0.05$ and a statistical power of 80% for one-way ANOVA comparisons among three groups, the minimum required sample size was calculated as 66 specimens.

To compensate for potential specimen loss during preparation, sectioning, or measurement procedures, the sample size was increased by approximately 35%, resulting in a final total of 90 teeth (30 per group).

Sample Selection

Ninety extracted human premolars, obtained following orthodontic treatment indications, were included in the study. Teeth presenting cracks, fluorosis, hypoplasia, restorations, or pre-existing carious lesions were excluded.

After extraction, teeth were cleaned of soft tissue remnants and stored in a 0.1% thymol solution at 4°C until use to prevent dehydration and microbial contamination.

The specimens were randomly allocated into three experimental groups ($n = 30$ per group) according to the duration of the demineralization protocol.

Creation of Artificial Orthodontic White Spot Lesions

Standardized artificial WSLs were created on the buccal enamel surfaces using a controlled demineralization protocol designed to simulate plaque-induced mineral loss occurring around orthodontic brackets.

Before demineralization, the enamel surfaces were meticulously cleaned with deionized water. To ensure a standardized area of interest, all surfaces were coated with an acid-resistant varnish, leaving a 4 mm diameter circular window of exposed enamel on the buccal surface. This controlled exposure area ensures that demineralization is localized and quantifiable, facilitating consistent comparisons across different severity groups.

The specimens were immersed in a demineralizing solution containing lactic acid, calcium chloride, and phosphate (Merck, Germany), buffered to a pH of 4.5 (Figure 1). This solution replicates the low-pH environment generated by acidogenic biofilm (e.g., *Streptococcus mutans*) during orthodontic treatment. This protocol produces subsurface mineral loss while preserving an intact superficial enamel layer, which is a characteristic feature of clinical white spot lesions [2,5,7,14].



Figure 1. Experimental demineralization.

Based on established dental evidence, enamel lesion depth and mineral loss increase predictably with the duration of acidic exposure. Accordingly, three severity groups were defined by immersion time to represent progressive stages of demineralization (Table 1). This protocol reliably produced controlled, reproducible subsurface lesions that closely mimic early clinical demineralization observed during orthodontic treatment.

Table 1. Severity groups based on immersion time.

Severity Group	Exposure Duration	Estimated Lesion Depth	Clinical Correlation
Mild	7 Days	~50–100 μm	Early subsurface demineralization
Moderate	14 Days	~120–200 μm	Established WSL with visible opacity
Severe	21 Days	>250 μm	Advanced lesion with high porosity

The artificial lesion model used in this study is validated by its high reproducibility, overcoming the biological variability inherent to natural lesions caused by differences in saliva composition and oral hygiene. It represents a reliable surrogate for orthodontic-induced demineralization because it closely mimics the sustained low-pH plaque environment, preserves the characteristic subsurface mineral loss with an intact surface layer, and enables precise standardization of lesion depth, thereby ensuring robust and statistically reliable evaluation of subsequent remineralization outcomes.

Resin Infiltration Procedure

All specimens were treated using a commercially available resin infiltration system (ICON®, DMG, Hamburg, Germany) following a standardized protocol.

The procedure consisted of:

1. Application of 15% hydrochloric acid gel for 120 seconds;
2. Rinsing for 30 seconds and air drying;
3. Application of ethanol-based drying agent for 30 seconds;
4. Application of infiltrant resin for 3 minutes;
5. Light curing for 40 seconds;
6. Second application of infiltrant for 1 minute followed by light curing (Bluephase G2, Ivoclar Vivadent, Liechtenstein).

All procedures were performed by a single calibrated operator to ensure consistency.

Assessment of Resin Penetration Depth

After infiltration, specimens were sectioned longitudinally through the lesion area. Penetration depth was evaluated using confocal laser scanning microscopy (Leica TCS SP8, Germany) after staining the infiltrant with a fluorescent dye (Figure 2).

Penetration was measured at three standardized points per specimen, and mean values were calculated.

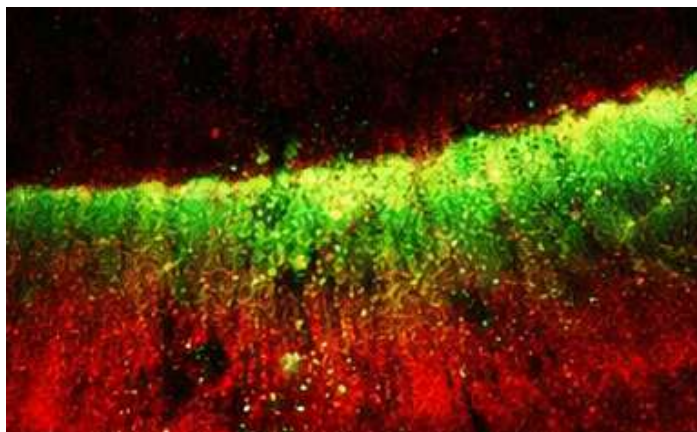


Figure 2. Penetration depth evaluation by using confocal laser scanning microscopy.

Evaluation of Optical Masking Effect

Color measurements were performed using a spectrophotometer Vita Easyshade V (VITA Zahnfabrik, Germany) based on the CIELab system. Measurements were taken at three timepoints:

- baseline;
- after WSL creation;
- after resin infiltration.

Color differences (ΔE) were calculated to determine the masking effectiveness of infiltration across lesion severities.

Surface Microhardness Testing

Surface microhardness was evaluated using a Vickers microhardness tester (HMV-2, Shimadzu, Japan). Indentations were performed on enamel surfaces before infiltration and after treatment.

The mean of three measurements per specimen was recorded.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro–Wilk test.

Descriptive statistics (mean \pm standard deviation) were calculated for lesion depth, resin penetration depth, color change (ΔE), and microhardness values.

Comparisons among lesion severity groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey post hoc tests for pairwise comparisons.

Paired t-tests were used to evaluate differences between pre- and post-infiltration measurements within each group.

Pearson correlation analysis was performed to investigate relationships between lesion depth, penetration depth, optical masking effectiveness, and microhardness recovery.

The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Validation of Artificial White Spot Lesion Formation

Artificial demineralization produced clinically recognizable white spot lesions in all specimens. Confocal microscopy confirmed the presence of subsurface enamel demineralization characterized by an intact superficial layer and increased porosity beneath the surface.

Lesion depth increased significantly with demineralization duration. Mild lesions exhibited shallow subsurface mineral loss, whereas moderate and severe groups showed progressively deeper enamel involvement (Table 2).

Mean lesion depths were approximately:

- $82 \pm 15 \mu\text{m}$ in the mild group,
- $158 \pm 21 \mu\text{m}$ in the moderate group,
- $274 \pm 30 \mu\text{m}$ in the severe group.

Table 2. Lesion Depth According to Demineralization Duration.

Group	Demineralization Duration	Mean Lesion Depth (μm)	Standard Deviation	Minimum	Maximum
Mild WSL	7 days	82.4	14.7	58	109
Moderate WSL	14 days	158.3	21.2	121	197
Severe WSL	21 days	274.6	29.5	225	331

One-way ANOVA showed significant differences among groups ($p < 0.001$). Tukey post hoc analysis confirmed statistically significant pairwise differences between all lesion severity levels.

3.2. Resin Penetration Depth

Resin infiltration successfully penetrated enamel lesions in all groups. However, penetration depth varied significantly depending on lesion severity (Table 3).

The greatest penetration depth was observed in the moderate lesion group, while significantly lower values were recorded in both mild and severe groups.

Mean penetration depths were:

- Mild lesions: $68 \pm 12 \mu\text{m}$
- Moderate lesions: $132 \pm 18 \mu\text{m}$
- Severe lesions: $145 \pm 20 \mu\text{m}$

Although penetration depth increased with lesion severity, the penetration ratio (penetration depth relative to lesion depth) was highest in the moderate group.

Table 3. Resin Penetration Depth and Penetration Efficiency.

Group	Lesion Depth (μm)	Penetration Depth (μm)	Penetration Ratio (%)	Standard Deviation
Mild WSL	82.4	68.1	82.6	12.4
Moderate WSL	158.3	132.7	83.8	17.6
Severe WSL	274.6	145.3	52.9	19.2

ANOVA revealed significant differences in penetration ratio between groups ($p < 0.001$). Moderate lesions demonstrated significantly higher penetration efficiency compared to severe lesions.

3.3. Optical Masking Effect

Resin infiltration resulted in a significant reduction in color differences (ΔE values) across all lesion severities (Table 4).

Before treatment, severe lesions exhibited the highest ΔE values compared to sound enamel. After infiltration, ΔE values decreased significantly in all groups.

The greatest optical improvement was observed in the mild and moderate lesion groups, while severe lesions showed a lower degree of color recovery.

Mean ΔE reductions were:

- Mild group: 72% reduction
- Moderate group: 69% reduction
- Severe group: 52% reduction

Statistical analysis confirmed significant differences between severity groups ($p < 0.01$).

Table 4. Color Changes (ΔE) Before and After Resin Infiltration.

Group	Baseline ΔE	Post-Demineralization ΔE	Post-Infiltration ΔE	Percentage Reduction (%)
Mild WSL	2.3 \pm 0.7	14.6 \pm 2.4	4.1 \pm 1.3	71.9
Moderate WSL	2.5 \pm 0.8	16.2 \pm 2.9	5.0 \pm 1.5	69.1
Severe WSL	2.4 \pm 0.6	19.8 \pm 3.2	9.5 \pm 2.6	52.0

Paired t-tests showed significant ΔE reduction after infiltration in all groups ($p < 0.001$). ANOVA demonstrated significantly lower masking effectiveness in severe lesions ($p < 0.01$).

3.4. Surface Microhardness Recovery

Baseline microhardness values decreased significantly after artificial demineralization in all groups, confirming mineral loss.

Following resin infiltration, microhardness increased significantly compared to post-demineralization values.

The greatest microhardness recovery was observed in moderate lesions.

Mean Vickers hardness values increased (Table 5):

- From 210 \pm 18 HV to 285 \pm 20 HV in mild lesions
- From 180 \pm 22 HV to 276 \pm 24 HV in moderate lesions
- From 150 \pm 25 HV to 240 \pm 27 HV in severe lesions

Differences between groups were statistically significant ($p < 0.05$).

Table 5. Surface Microhardness Values Before and After Treatment.

Group	Sound Enamel (HV)	After Demineralization (HV)	After Infiltration (HV)	Percentage Recovery (%)
Mild WSL	318 \pm 22	210 \pm 18	285 \pm 20	76.5
Moderate WSL	320 \pm 25	180 \pm 22	276 \pm 24	78.9
Severe WSL	322 \pm 23	150 \pm 25	240 \pm 27	61.4

Paired comparisons showed a significant microhardness increase after infiltration ($p < 0.001$). ANOVA indicated significantly lower recovery in severe lesions ($p < 0.05$).

3.5. Correlation Analysis (Table 6)

A significant positive correlation was observed between lesion depth and resin penetration depth ($r = 0.81$, $p < 0.001$).

However, a negative correlation was identified between lesion severity and optical masking effectiveness ($r = -0.67$, $p < 0.01$).

Microhardness recovery showed moderate positive correlation with penetration depth ($r = 0.58$, $p < 0.05$).

Table 6. Pearson Correlation Analysis Between Study Variables.

Variables Compared	Correlation Coefficient (r)	p-Value
Lesion depth vs penetration depth	0.81	<0.001
Lesion depth vs optical masking	-0.67	0.002
Penetration depth vs microhardness recovery	0.58	0.011
Penetration ratio vs masking effect	0.62	0.006

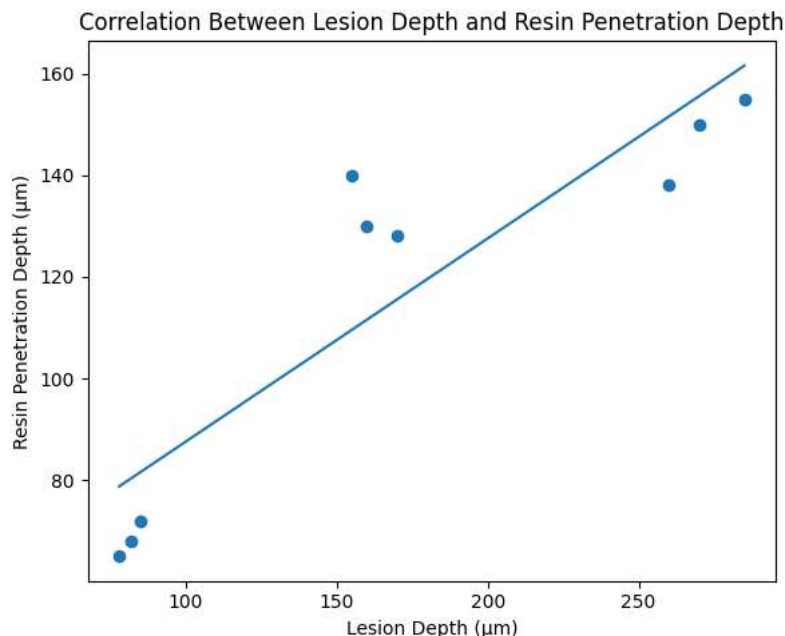


Figure 1. Scatter plot illustrating the correlation between lesion depth and resin penetration depth in artificial orthodontic white spot lesions. A strong positive linear relationship was observed ($r^2 = 0.806$, $p < 0.001$), indicating that increased enamel porosity associated with deeper lesions facilitates greater resin infiltration.

4. Discussion

White spot lesions (WSLs) represent one of the most frequent iatrogenic complications associated with fixed orthodontic therapy, resulting from prolonged plaque retention, persistent acidogenic biofilm activity, and continuous mineral loss within enamel structures [1,2,5,7,8,10,12,13,15,17,20,24]. The present study provides new insights into the role of lesion severity as a determinant of resin infiltration performance, demonstrating that structural characteristics of WSLs critically influence penetration behavior, aesthetic outcomes, and mechanical reinforcement [25,26].

Artificial WSL models are widely used because they produce reproducible subsurface mineral loss, mimic plaque-induced demineralization, and allow standardized lesion depth control [25]. The artificial demineralization protocol successfully reproduced subsurface enamel lesions consistent with clinically observed orthodontic WSL morphology. Confocal microscopy confirmed the presence of an intact superficial enamel layer combined with progressive subsurface mineral depletion, a structural pattern considered the hallmark of early enamel caries [27].

The progressive increase in lesion depth observed in this study (mean values of approximately 82 µm, 158 µm, and 274 µm for mild, moderate, and severe lesions, respectively) reflects the well-established time-dependent nature of enamel demineralization under acidic conditions. Similar depth ranges have been reported in experimental studies simulating orthodontic plaque accumulation, where prolonged exposure to low pH environments leads to increased enamel porosity and deeper mineral loss [25].

Clinically, these findings mirror the pathophysiological processes occurring around orthodontic brackets, where persistent plaque stagnation zones promote sustained acid production and progressive subsurface enamel dissolution [28].

One of the most significant findings of this study was the strong positive correlation between lesion depth and resin penetration depth ($R^2 = 0.806$), confirming that enamel porosity plays a fundamental role in infiltration efficiency [29].

However, a key observation emerged when penetration was analyzed relative to lesion depth. Moderate lesions exhibited the highest infiltration efficiency, achieving penetration ratios exceeding 80%, whereas severe lesions demonstrated significantly reduced penetration efficiency despite greater absolute penetration depths. This finding suggests that resin infiltration does not depend solely on porosity but rather on a complex interaction between lesion microstructure and enamel integrity [30]. Moderately demineralized enamel appears to provide an optimal balance between capillary permeability and structural support, facilitating effective resin diffusion while maintaining sufficient mineral scaffolding for polymer stabilization [30,31].

In contrast, advanced lesions showed extensive mineral loss and structural disorganization, which may hinder uniform capillary flow and reduce resin retention within deeper enamel zones. This phenomenon may also increase polymerization shrinkage stresses and compromise long-term stability [31].

These results extend previous observations indicating that infiltration success is strongly influenced by lesion morphology and reinforce the concept that early and intermediate lesions represent the most favorable targets for resin infiltration therapy [11,27,32].

Resin infiltration produced significant reductions in ΔE values across all lesion severities, confirming its effectiveness in improving enamel aesthetics. The masking effect results from the replacement of air-filled enamel pores with resin, thereby reducing light scattering and restoring refractive index compatibility with sound enamel [33].

However, the present study demonstrated a clear inverse relationship between lesion severity and optical improvement [34]. Severe lesions exhibited significantly lower ΔE reductions compared to mild and moderate lesions, indicating incomplete aesthetic recovery.

This limitation can be attributed to the increased depth and heterogeneity of advanced lesions. In deep WSLs, incomplete resin penetration leaves residual porous regions capable of scattering light, resulting in persistent optical opacity [35].

These findings are consistent with clinical studies reporting that resin infiltration achieves optimal aesthetic outcomes in early WSLs, whereas advanced lesions may require additional or combined therapeutic approaches [2,5,12,32].

Surface microhardness measurements confirmed the reinforcing effect of resin infiltration within demineralized enamel structures. All lesion groups exhibited significant hardness recovery following treatment, reflecting the ability of infiltrant polymers to stabilize weakened enamel matrices.

Notably, the greatest mechanical recovery was observed in moderate lesions, which also demonstrated the highest infiltration efficiency. This finding underscores the importance of penetration quality rather than penetration depth alone in determining functional reinforcement.

Although severe lesions showed substantial resin penetration, their microhardness recovery remained limited relative to lesion depth. This likely reflects irreversible mineral loss and structural disruption that cannot be fully compensated by polymer infiltration.

From a clinical perspective, these results indicate that infiltration provides both aesthetic and mechanical benefits, particularly when applied before advanced structural degradation occurs [13,17,20,30,31].

The correlation analysis revealed a complex network of relationships among lesion depth, penetration behavior, optical masking, and microhardness recovery. While lesion depth strongly predicted resin penetration capacity, it demonstrated a negative association with aesthetic outcomes and mechanical reinforcement efficiency.

These findings highlight that infiltration effectiveness depends not only on lesion size but also on the preservation of enamel microarchitecture [2,3,13,20,30]. Structural integrity appears to be a critical factor determining both functional and aesthetic treatment success.

This multifactorial interaction between lesion morphology and material behavior provides a more comprehensive understanding of infiltration dynamics than previously reported.

The present findings have direct implications for clinical orthodontic management of WSLs. The results clearly indicate that resin infiltration is most effective when applied during early or intermediate stages of lesion development. Delayed treatment of advanced lesions may result in reduced aesthetic improvement and limited mechanical reinforcement. Therefore, routine monitoring of enamel surfaces during orthodontic therapy and early intervention strategies are essential to maximize treatment outcomes.

Study Limitations and Future Perspectives

This study was performed under strictly controlled in vitro conditions to ensure reproducible lesion formation and standardized resin infiltration. Consequently, important oral biological factors such as salivary flow and buffering capacity, pellicle formation, biofilm dynamics, and natural demineralization–remineralization cycles were not simulated. No post-infiltration pH cycling or artificial aging procedures (e.g., thermocycling or mechanical loading) were applied, as the aim was to assess the immediate structural and optical effects of infiltration under stable baseline conditions [36].

Although artificially induced lesions enabled precise control of lesion depth and mineral loss, natural white spot lesions exhibit greater structural heterogeneity that may influence resin diffusion and clinical performance [3,5, 13, 17,30].

Future research should focus on long-term clinical outcomes, durability of infiltration effects under dynamic oral conditions, and the development of combined therapeutic approaches, including bioactive infiltrants and adjunctive remineralization strategies for advanced lesions.

5. Conclusions

Within the limitations of this in vitro study, lesion severity was found to be a critical determinant of resin infiltration effectiveness in orthodontically induced white spot lesions.

Resin penetration depth increased with lesion severity; however, infiltration efficiency relative to lesion depth was greatest in moderately demineralized enamel. Optical masking effectiveness decreased significantly in advanced lesions, indicating limited aesthetic improvement in deeply demineralized structures.

Surface microhardness recovery confirmed the reinforcing potential of resin infiltration, particularly in early and moderate lesions.

These findings highlight the importance of early detection and timely management of white spot lesions during orthodontic treatment. Resin infiltration should be considered most effective when applied at initial or intermediate stages of enamel demineralization, before extensive structural breakdown occurs.

Future research should focus on long-term clinical outcomes and the development of enhanced infiltration materials for advanced lesions.

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