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SEM-Based Micromorphological Analysis of the Hybrid Layer Formed by Two Contemporary Adhesive Systems

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Abstract: ***This study employed scanning electron microscopy (SEM) to compare the micromorphological characteristics of hybrid layers formed by two adhesive systems-Excite (etch-and-rinse) and Adhese Universal Pen (self-etch). Thirty-six dentine specimens were prepared from extracted human molars and evaluated for hybrid layer thickness, uniformity, and resin tag morphology. Results demonstrated that the total-etch system produced significantly thicker and more uniform hybrid layers with longer resin tags, whereas the self-etch system generated thinner, irregular layers. Statistical analysis confirmed the superiority of the etch-and-rinse system in achieving deeper resin infiltration. These findings suggest that etching strategy markedly influences the quality of dentine bonding, with practical implications for the longevity of adhesive restorations.***

Keywords: adhesive systems, dentine bonding, hybrid layer, resin tags, scanning electron microscopy (*SEM*)

1. Introduction

Adhesive dentistry has revolutionised restorative techniques by enabling minimally invasive cavity preparation and strong micromechanical bonding between resin composites and dental hard tissues [1][2]. The quality and durability of this bond rely largely on the formation of a stable hybrid layer. Despite continuous advancements in adhesive technology, each generation of bonding systems presents specific challenges in achieving uniform resin infiltration and complete polymerisation within demineralised dentine. Inadequate hybridisation or incomplete sealing may compromise bond strength, leading to marginal leakage and long-term degradation [3][4][5].

The hybrid and adhesive layers represent key interfaces in the micromechanical bonding between composite resins and dental hard tissues. Their morphology, continuity, and infiltration quality are essential for the long-term success of adhesive restorations [1]. However, the thickness of these layers alone is not directly correlated with bond strength; rather, the quality of resin infiltration and the degree of polymerisation determine the durability of adhesion [7].

The hybrid layer (*HL*) is a resin–infiltrated zone of demineralised dentine, whose thickness depends on the etching protocol and the aggressiveness of the adhesive system [8]. In etch-and-rinse systems, HL thickness typically ranges from 3–5 µm, while in self-etch adhesives, it is significantly thinner—approximately 0.4–1.5 µm [6,8]. Over-etching may result in a highly demineralized dentine zone that resists full resin infiltration, creating weak sublayers that reduce bond strength [7].

Studies have demonstrated that the mean HL thickness in mild self-etch adhesives may be approximately 2.2 ± 0.3 µm, whereas prolonged etching (e.g., 15 s with phosphoric acid) can result in hybrid layers up to 8–9 µm [8,10]. Nevertheless, thicker layers are not necessarily advantageous, as incomplete infiltration and inhomogeneous polymerisation may compromise the integrity of the interface and increase susceptibility to nanoleakage [7,9].

The adhesive layer acts as an elastic intermediary between the rigid composite and the dentine substrate. Optimal adhesive film thickness generally falls between 10 µm and 17 µm, depending on the adhesive system and solvent composition [8,10]. An excessively thick adhesive layer, often resulting from insufficient solvent evaporation or multiple unthinned applications, may reduce microtensile bond strength [8]. Conversely, a uniformly thin layer with good polymerisation provides effective stress distribution and reduced microleakage [10,11]. In some configurations, a slightly thicker layer within internal line angles may act as a stress-relieving cushion, though this effect is highly technique-sensitive [11].

Resin tags are adhesive micro-extensions that penetrate the dentinal tubules, contributing to micromechanical interlocking [1]. Their length and morphology vary with the conditioning method, dentine depth, and pulpal pressure. In etch-and-rinse systems, typical tag lengths range from 50 µm to 130 µm, with numerous and well-defined projections [8,12]. Self-etch adhesives, particularly those with mild or ultra-mild acidity, form shorter and less frequent tags of about 12–20 µm [3]. The use of 37% phosphoric acid on enamel results in tag penetration depths of approximately 50–60 µm, whereas self-etch primers yield around 40 µm [10,12]. Longer or more numerous resin tags do not necessarily correlate with stronger adhesion, as over-etching or inadequate resin infiltration can result in voids and nanoleakage [1,3].

Several variables affect hybrid layer and tag formation:
- **Etching protocol and acidity:** Etch-and-rinse systems create thicker hybrid layers and longer tags; mild self-etch systems form thinner, more controlled interfaces.
- **Dentine condition and moisture:** Overly wet or deep dentine and positive pulpal pressure can reduce tag formation quality.

- **Adhesive composition and technique:** Active application, thorough solvent evaporation, and controlled light curing are crucial. Double application or an additional hydrophobic coat may enhance long-term stability in certain systems.

2. Materials and Methods

2.1 Sample selection

Six extracted human molars with intact crowns and without carious lesions, restorations, or visible cracks were selected for this study. Teeth were obtained following extraction for orthodontic or periodontal reasons, with informed consent and ethical approval. After extraction, the teeth were cleaned of soft tissue residues and stored in 0.9% saline solution with thymol at 4°C until specimen preparation. Prior to cavity preparation, all teeth were examined under x40 magnification using a stereomicroscope (*Leica, Leica Microsystem, Wetzlar, Germany*) to confirm the absence of structural defects, such as demineralised areas, initial caries lesions, cracks, fractures, or surface irregularities.

2.2 Sample preparation

The teeth were sectioned in a mesiodistal direction under water cooling to obtain representative areas of the dentine–adhesive–composite interface. The sectioned surfaces were polished using abrasive discs of progressively decreasing grit size. Four specimens were obtained from each tooth and distributed into two groups according to the adhesive protocol applied. The specimens were divided into groups as follows:

- Group I ($n = 12$): specimens treated using the total-etch technique with a 5th generation adhesive system – Excite (*Ivoclar Vivadent, Liechtenstein*).
- Group II ($n = 12$): specimens treated with a self-etch adhesive system, 8th generation – Adhese Universal Pen (*Ivoclar Vivadent, Liechtenstein*), applied according to the manufacturer's instructions.

Following completion of the adhesive procedures, all cavities were directly restored with a nanohybrid composite resin (brand and shade to be specified, if applicable). The specimens were stored in a saline solution with thumol at 4°C until further analysis.

2.3 SEM analysis

Specimens were polished sequentially using discs of decreasing grit size, followed by dehydration in ascending concentrations of ethanol (*50%, 70%, 90%, and 100%*). Subsequently, the samples were mounted on aluminium stubs and sputter-coated with a thin layer of gold to ensure surface conductivity and prevent charging during examination.

Observations were carried out using a scanning electron microscope (*TESCAN LYRA, TESCAN ORSAY HOLDING, Brno, Czech Republic*) operated at an accelerating voltage of 10 kV. The specimens were examined at magnifications of x1000 and x3000. The hybrid layer was analysed for thickness, uniformity, and micromorphological features, including the presence and morphology of resin tags at the dentine–adhesive interface.

2.4 Statistical analysis

The obtained data were statistically analysed using descriptive statistics, including mean values, standard deviation, and range. Data normality was verified using the Shapiro–Wilk test. Intergroup comparisons between the two adhesive systems were performed using either the independent-samples t-test or the Mann–Whitney U test, depending on the data distribution. Correlations between hybrid layer thickness and resin tag depth were assessed using Pearson's or Spearman's correlation coefficients. A significance level of $p < 0.05$ was considered statistically significant.

3. Results

In the Group I specimens, treated with the total-etch adhesive system (*Excite*), the hybrid layer showed a relatively uniform morphology, with an average thickness of approximately 3.5-5.2 µm. Minor variations were observed among different slices, reflecting local differences in dentine permeability and resin infiltration. Overall, the hybrid layer exhibited a continuous interface with well-defined boundaries, indicating effective resin infiltration as a result of phosphoric acid conditioning (Table 1, Fig.1).

Table 1: Hybrid layer thickness measurements (**Group I** – Excite, etch-and-rinse system)

**M	*S1 (µm)	S2 (µm)	S3 (µm)	S4 (µm)	S5 (µm)	S6 (µm)	S7 (µm)	S8 (µm)	S9 (µm)	S10 (µm)	S11 (µm)	S12 (µm)
1	3.51	3.94	3.65	4.12	4.25	3.71	4.86	4.58	3.99	4.45	4.33	3.12
2	3.22	3.76	3.56	4.16	3.69	5.21	5.11	4.92	3.93	4.73	3.64	4.15
3	4.22	3.72	4.81	4.51	3.95	4.44	4.36	3.16	3.21	3.75	3.52	4.15
4	3.61	5.25	5.1	4.96	3.94	4.72	3.67	4.14	4.28	4.12	3.67	5.20
5	5.1	4.94	3.92	4.71	3.66	4.19	4.24	3.76	4.8	4.54	3.92	4.42
6	4.32	3.24	3.73	3.5	4.12	3.62	5.23	5.14	4.91	3.98	4.73	3.18

Each slice* (S) represents one specimen section; six measurements **(M) were taken per slice.

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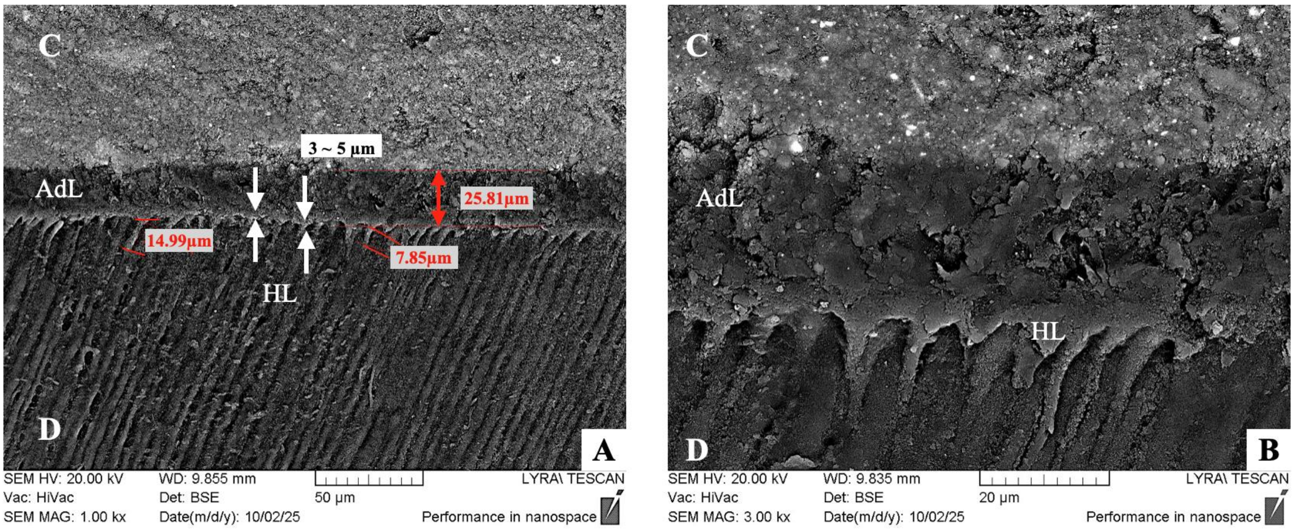

Figure 1: Representative SEM micrographs of the hybrid layer morphology in specimens treated with the total-etch adhesive system (*Excite*). **A**/ and **B**/ show the adhesive–dentine interface at ×1000 and ×3000 magnification, respectively. A well-defined hybrid layer (*HL*) with a thickness of approximately 3–5 µm is visible, along with clearly distinguishable resin tags penetrating into the dentinal tubules (*small red arrows*). The adhesive layer (*AdL)* is uniform and continuous, indicating effective resin infiltration and polymerisation after phosphoric-acid etching (*double-headed red arrow*). The micromorphology reveals good adaptation between the hybrid and adhesive layers, with no evident gaps or voids along the interface. This is visualized in more detail at **B**/ magnification ×3000.

In Group II specimens, where the self-etch adhesive system (*Adhese Universal Pen*) was applied, the hybrid layer appeared thinner and more variable, with thicknesses ranging from 0.7 µm to 2.9 µm. The layer was more homogeneous in the superficial dentine regions, but in some slices, discontinuities and less distinct transitions between resin and dentine were observed. These findings are consistent with the milder etching potential of self-etch systems and their limited demineralisation depth. The reduced thickness and less prominent resin tags may influence the micromechanical retention and long-term stability of the adhesive interface (Table 2, Fig.2*)*.

Table 2: Hybrid layer thickness measurements (**Group II** – *Adhese Universal Pen*, self-etch system)

**M	*S1 (µm)	S2 (µm)	S3 (µm)	S4 (µm)	S5 (µm)	S6 (µm)	S7 (µm)	S8 (µm)	S9 (µm)	S10 (µm)	S11 (µm)	S12 (µm)
1	1.51	1.94	2.65	1.12	1.25	0.71	1.86	1.58	1.99	2.45	3.33	1.12
2	1.22	0.96	1.56	1.16	1.69	1.72	0.11	0.92	0.93	1.73	2.64	2.15
3	2.22	1.72	1.81	1.51	2.95	3.14	3.36	2.16	1.21	2.75	2.52	2.15
4	1.61	1.25	1.1	0.96	1.94	1.7	1.67	1.14	1.28	1.12	1.67	1.20
5	1.1	0.92	0.91	1.71	1.02	1.09	1.04	1.16	1.08	1.52	1.92	1.04
6	1.32	1.18	1.24	0.73	1.05	1.01	1.62	1.02	1.01	0.91	0.98	0.79

Each slice* (S) represents one specimen section; six measurements **(M) were taken per slice.

Descriptive statistics revealed that the mean hybrid layer thickness for Group I (*Excite*) was 4.23 ± 0.54 µm (range 3.16–5.25 µm), whereas for Group II (*Adhese Universal Pen*) it was 1.47 ± 0.63 µm (range 0.71–2.95 µm). The data in both groups followed a normal distribution, as indicated by the Shapiro–Wilk test ($p > 0.05$). An independent-samples t-test showed a statistically significant difference between the two adhesive systems ($p < 0.001$), indicating that the hybrid layer formed with the total-etch system was significantly thicker than that formed with the self-etch system.

The total-etch adhesive system (*Excite)* produced a significantly thicker, more uniform hybrid layer than the self-etch system (*Adhese Universal Pen*), confirming the influence of the etching strategy on hybrid layer morphology (Table 3).

Mean hybrid layer thickness (µm) for the total-etch adhesive (*Excite*) and self-etch adhesive (*Adhese Universal Pen*). The total-etch system produced a significantly thicker hybrid layer ($p < 0.001$). To provide a clearer visual comparison between the two adhesive protocols, the statistical data were also illustrated graphically. The bar chart highlights the considerable difference in mean hybrid layer thickness, demonstrating the more pronounced demineralisation and resin infiltration achieved with the total-etch system compared with the milder self-etch approach.

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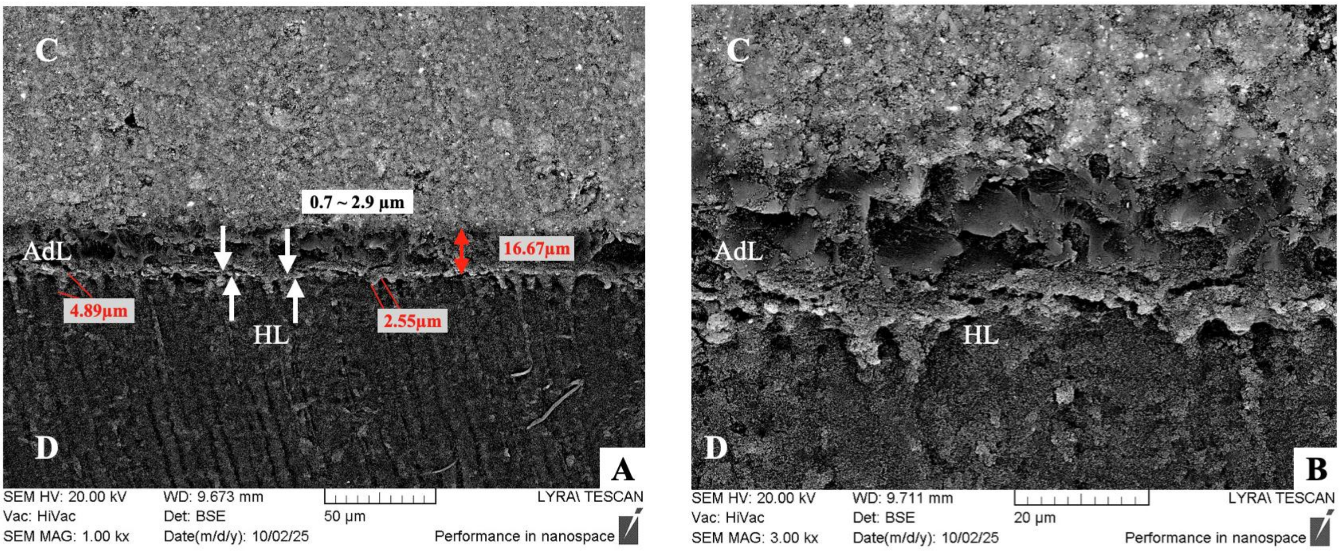

Figure 2: Representative SEM micrographs of the hybrid layer morphology in specimens treated with the self-etch adhesive system (*Adhese Universal Pen*). **A/** and **B/** show the adhesive–dentine interface at ×1000 and ×3000 magnification, respectively. A thinner and more irregular hybrid layer (*HL*), approximately 0.7–2.9 µm in thickness, is observed (*double-headed white arrows*). The adhesive layer (*AdL*) appears continuous but less uniform compared with the total-etch system, with occasional discontinuities and areas of partial resin infiltration. Shorter, less defined resin tags can be seen penetrating the dentinal tubules (*small red arrows*). These morphological features are consistent with the mild etching potential of self-etch adhesives, which results in limited demineralisation and resin penetration depth. The interface characteristics are shown in greater detail at ×3000 magnification in image **B/**.

Table 3: Descriptive and comparative statistics of hybrid layer thickness for the two adhesive systems

Parameter	Group I – Excite (etch-and-rinse)	Group II – Adhese Universal Pen (self-etch)	p-value
Mean ± SD (µm)	4.23 ± 0.54	1.47 ± 0.63	< 0.001
Range (µm)	3.16 – 5.25	0.71 – 2.95	—
Normality (Shapiro–Wilk)	p = 0.132 (ns)	p = 0.087 (ns)	—
Statistical test	—	—	Independent samples t-test

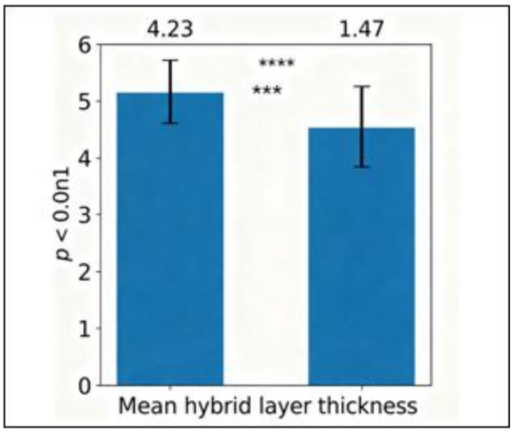

Figure 3: Mean hybrid layer thickness (µm) in two adhesive systems

The effect size (*Cohen's d = 5.31*) indicated a very strong practical significance of the difference between the two groups. Furthermore, the 95% confidence intervals for the mean values (*Excite:* 4.12–4.34 µm; *Adhese Universal Pen:* 1.32–1.62 µm) did not overlap, confirming the robustness of the statistical result. These findings emphasise the marked impact of the etching strategy on hybrid layer formation and are consistent with the micromorphological observations from SEM analysis. The considerably greater hybrid layer thickness observed in the total-etch group may be attributed to the complete removal of the smear layer and the deeper demineralisation achieved by phosphoric acid, which allows for enhanced resin monomer penetration into the dentine substrate. In contrast, the self-etch adhesive, characterised by a milder acidic pH, results in superficial demineralisation and partial infiltration of the smear layer, leading to a thinner and more irregular hybrid layer. Statistically and morphologically, these results confirm that etching aggressiveness remains a key determinant of the hybrid layer's integrity and thickness.

4. Discussion

The current study clearly demonstrates that the total-etch adhesive system (*Excite*) produced a significantly thicker hybrid layer compared to the self-etch system (*Adhese Universal Pen*). Beyond hybrid layer thickness alone, our SEM observations revealed deeper, more abundant resin tags in the etch-and-rinse group, whereas the self-etch group exhibited shallower, fewer tags. Such morphological differences underscore the importance of tag length and penetration depth as supplementary factors to hybrid layer thickness in establishing a robust resin–dentine interface [13,14]. In particular, long resin tags formed under phosphoric acid etching conditions likely reflect enhanced micromechanical interlocking and deeper monomer infiltration [8]. Conversely, milder self-etch adhesives, while easier to apply and less technique-sensitive, result in shallow demineralisation and limited tubule penetration, which may compromise long-term stability [15].

It is noteworthy that despite thinner hybrid layers and shorter resin tags in self-etch adhesives, some studies have reported comparable initial bond strengths, attributed to chemical bonding mechanisms [16]. However, the limited penetration depth could render the interface more prone to nanoleakage, enzymatic degradation, and stress concentration over time [10]. The significantly greater effect size in our comparison (*Cohen's d = 5.31*) further emphasises that not only thickness, but also the quality and depth of resin infiltration, play critical roles. Indeed, recent literature suggests that the smear layer integrity and substrate condition may influence tag formation and hybrid layer continuity more than nominal thickness [1,11]. Clinically, these findings suggest that in scenarios where ideal dentine preparation and moisture control can be achieved, the etch-and-rinse strategy may deliver superior micromechanical retention. In contrast, simplified self-etch systems might be preferable in less favourable conditions but may require careful adjunctive protocols (e.g., active agitation, extended dwell time) to compensate for reduced tag penetration [17]. Ultimately, long-term clinical trials are necessary to determine whether the deeper tag networks and thicker hybrid layers translate into enhanced restoration longevity. In summary, our results corroborate that etching aggressiveness and consequent tubule penetration remain determinants of hybrid layer integrity and performance [18].

Clinical Implications

Clinically, optimal adhesion is not achieved by maximising the thickness of the bonding layers, but by ensuring uniform resin infiltration and complete polymerisation throughout the interface. Over-etching of dentine should be avoided, as it may create a demineralised zone that the adhesive resin cannot fully penetrate. Active application and careful air-thinning of the adhesive are essential to produce a continuous, bubble-free film with optimal substrate adhesion. Although microstructural features such as hybrid layer thickness and resin tag length provide valuable morphological evidence of bonding, it is the integrity and long-term stability of the hybridised zone that ultimately determine clinical performance and restoration longevity [14] [19] [20] [21] [22] [23] [24].

5. Conclusion

Within the limitations of this in vitro study, the findings demonstrated that the etching strategy plays a decisive role in hybrid layer morphology and resin infiltration. The total-etch adhesive system (*Excite*) produced a significantly thicker, more uniform hybrid layer, accompanied by deeper, more distinct resin tags that penetrated the dentinal tubules. In contrast, the self-etch adhesive (*Adhese Universal Pen)* produced a thinner, more superficial hybrid layer, with shorter, less defined tags. These micromorphological differences were statistically significant and reflect the influence of etching aggressiveness on the formation of a stable resin–dentine interface.

Clinically, these results highlight that successful adhesion depends not merely on layer thickness, but on homogeneous infiltration, adequate polymerisation, and the long-term integrity of the hybridised zone. Further studies, including long-term ageing and nanoleakage evaluation, are recommended to assess how these structural differences impact the durability and clinical performance of adhesive restorations.

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