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SEM Analysis of Hybrid Layer and Bonding Interface After Chlorhexidine Use

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Clinical Relevance

More studies suggest that the bond between composite and dentin degrades over time because of the action of matrix metalloproteinase on collagen fibrils left unprotected by acid etching. Clinical actions should be taken to prevent this from happening and thus the failure of what should be a very long-term restoration.

SUMMARY

Objective: **To evaluate the appearance of the hybrid layer of one total-etch and one self-etch bonding agent on human teeth with and without the application of 2% chlorhexidine after water storage.**

Materials and Methods: **Twelve human maxillary teeth (molars and premolars) had two Class II cavities (MO-OD) prepared. Teeth were separated in two groups (n=6) to receive either Adper Single Bond 2 or Adper SE Plus (3M ESPE). In one cavity, the dentin donding agent was applied following manufacturer's instructions. On the adjacent cavity, 2% chlorhexidine was applied for 30 seconds before the application of the bonding agent. Teeth were sectioned mesiodistally with a slow-speed diamond disk and stored in water at 37°C for four months. The teeth were prepared for scanning electron microscope observation. The appearance of the hybrid layer was observed and measured by two variables: clear image of hybrid layer and presence of resin tags in tubules. Data were analyzed with a Kruskal-Wallis test calculated at a 0.05 significance level.**

Results: **All groups treated with chlorhexidine had the clear presence of a hybrid layer, whereas only half the specimens without chlorhexidine had a clear hybrid layer. Chlorhexidine did not affect the presence of resin tags.**

Conclusions: **The use of 2% chlorhexidine before the application of a dentin-bonding agent**

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reduced the deterioration of the hybrid layer when exposed to water.

INTRODUCTION

When microtensile testing was introduced, a better study of the failure interface, especially under scanning electron microscopy (SEM) and transmission electron microscopy,[1,2] lead to the discovery that long-term storage in water changed the durability of the bonding interface.[3–6] The resin-dentin interface is a mixture of the adhesive resin with the exposed collagen fibers after the dentin has been demineralized with the use of an etchant. This interface was named the hybrid layer.[7,8] Many factors can affect the clinical longevity of this layer: occlusal forces, thermal changes produced by the different temperatures of foods, chemical agents in beverages, dentinal fluids, bacteria products, elution of resin monomers, and degradation of resin components.[9–20] The use of 0.2% chlorhexidine gluconate for 60 seconds was found to inhibit collagenolytic activity,[21] thus maintaining the resin-dentin interface. Pashley and others[21] recommended the use of chlorhexidine on acid-etched dentin before using total-etch adhesives. It did not affect the *in vitro* bond strength of aged specimens tested in microtensile testing, and there were less cohesive failures in dentin or in the hybrid layer when dentin was treated with chlorhexidine than without such application.[22] The purpose of this study is to evaluate the appearance of the hybrid layer of teeth treated with and without 2% chlorhexidine after aging for four months (125 days) in water.

MATERIALS AND METHODS

MO-OD Class II cavities were prepared on 12 maxillary teeth, six molars, and six premolars, using a 558 carbide bur, leaving a 1 mm-thick enamel wall to separate the mesial cavity from the distal cavity. Cavities were prepared to a depth of 1 mm below the dentin-enamel junction with no axial wall but elimination of the proximal enamel ridge on both sides (Figure 1). Teeth were then separated into two groups, one to receive Adper Single Bond 2 (3M ESPE, St. Paul, MN) and the other to receive Adper SE Plus (3M ESPE).

Adper Single Bond 2

The mesial cavity of each tooth was etched with 37% phosphoric acid for 15 seconds and rinsed thoroughly for 30 seconds. Excess water was eliminated. Two consecutive coats of Adper Single Bond 2 (SB2) were rubbed over the dentin surface for 10 seconds, air thinned, and light cured for 20 seconds (Elipar 2500, 3M ESPE). A third coat of adhesive was applied over the dentin surface, air thinned, and light cured for another 20 seconds. A resin composite (Filtek Supreme XT, shade A2, 3M ESPE) was applied in three increments. The first increment was applied in less than 0.5 mm in thickness to minimize the negative effect of polymerization shrinkage. All increments were light cured for 20 seconds.

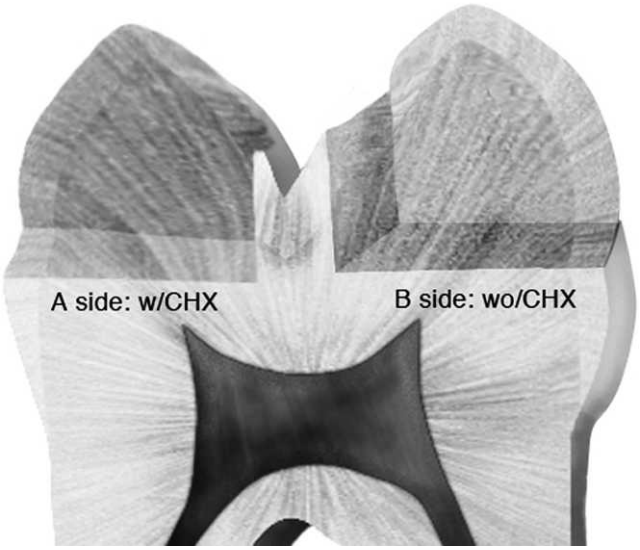

Figure 1. *Diagram for the cavity preparation. The distal cavity of all specimens was treated with chlorhexidine before the bonding agent was applied.*

The distal cavity of each tooth was etched with 37% phosphoric acid for 15 seconds and rinsed thoroughly for 30 seconds. Excess water was eliminated. Two percent chlorhexidine (Consepsis, Ultradent Prods. South Jordan, UT) was applied to the cavity for 30 seconds and the excess eliminated before SB2 and composite were applied as before.

Adper SE Plus

After finishing the preparation, the mesial cavity was rinsed and excess water eliminated. Adper SE Plus (SEP) Liquid A (3M ESPE) was applied on the dentin surface for 10 seconds until the dentin was completely covered. Liquid B was rubbed over the dentin surface for 10 seconds, air thinned, and light cured for 20 seconds. A second coat of Liquid B was applied on the dentin surface, air thinned, and light cured for another 20 seconds. Resin composite (Filtek Supreme XT, shade A2) was applied in three increments. The first increment was applied in less than 0.5 mm thickness. All increments were light cured for 20 seconds.

The distal cavity of each tooth was rinsed and excess water eliminated. Two percent chlorhexidine was applied to the cavity for 30 seconds. Excess chlorhexidine was eliminated before Adper SE Plus and composite were applied as before.

Specimen Preparation

All specimens were sectioned mesiodistally into two halves using a low-speed diamond saw. Specimens were placed under water at 37°C for 125 days. Then specimens were prepared for SEM (S-570, Hitachi, Tokyo, Japan) evaluation. All specimens were polished with silicon carbide paper under water, using sequentially 400, 600, 1000, and 2000 grit. The specimens were cleaned with 37% phosphoric acid for five seconds, rinsed in water for 30 seconds, and submerged in 3% NaOCl for five minutes. Then the specimens were placed in 70%, 80%, 90%, and 99% alcohol to eliminate all water present before being desiccated and prepared for SEM observation.

SEM Observation

All specimens were observed under 800× magnification, and an assessment of the dentin-bonding agent interface was made by two variables: clear image of hybrid layer on at least 75% of the length of the interface (yes=1, no=0) and presence of resin tags in tubules at least 75% of the length of the interface (present=1, not present=0). Scores were given for each parameter. Pictures of the most representative part of the interface image based on its score were taken for illustration purposes under 1000× magnification.

Nonparametric data were analyzed using a Kruskal-Wallis test calculated at a 0.05 significance level for each variable: quality of the hybrid layer and presence of resin tags.

RESULTS

The composite-dentin interface was given a score of 0 or 1 by two parameters, as shown in Table 1. Specimens treated with chlorhexidine before the application of the dentin-bonding agent (DBA) showed a higher presence of a hybrid layer in the interface when compared with the same bonding agent for the same tooth that was not treated with chlorhexidine, although no statistical difference was found in any of the variables in this study. Table 2 shows statistical analysis for variable: presence of hybrid layer.

SB2 produced the most uniform hybrid layer of the two bonding agents tested. The hybrid layer was present in the entire interface but varied in thickness in some areas of the specimen. SEP had two specimens in which, although the hybrid layer was identifiable, it was not as clear as other specimens treated with the same bonding agent or was not as clear as the one produced by SB2. For teeth not treated with chlorhexidine, both bonding agents produced a hybrid layer that was difficult to identify along most of the length of the interface for half the specimens evaluated (Figures 2–5).

Three specimens of SB2 without treatment with chlorhexidine showed no presence of resin tags or were very scarce. This observation was different from the one of SB2 when treated with chlorhexidine before it was applied, where all the specimens showed a very clear presence of resin tags in the

Table 1: *Specimen Scores on the Evaluation for the Two Variables*

	Treatment	Hybrid layer (yes answer)	Resin tags (yes answer)
SB2	Without chlorhexidine	3	3
	Chlorhexidine	6	6
SEP	Without chlorhexidine	3	3
	Chlorhexidine	6	4

Table 2: *Kruskal-Wallis Statistical Analysis for the Variable "Hybrid Layer Presence" With Single Bond 2*

	Sample size	Sum of ranks
SB2/− chlorhexidine	6	48
SB2/+ chlorhexidine	6	30
H	2.07692	
Degrees of freedom	1	
H (corrected)	3.6	
N	12	
p-level	0.14954	

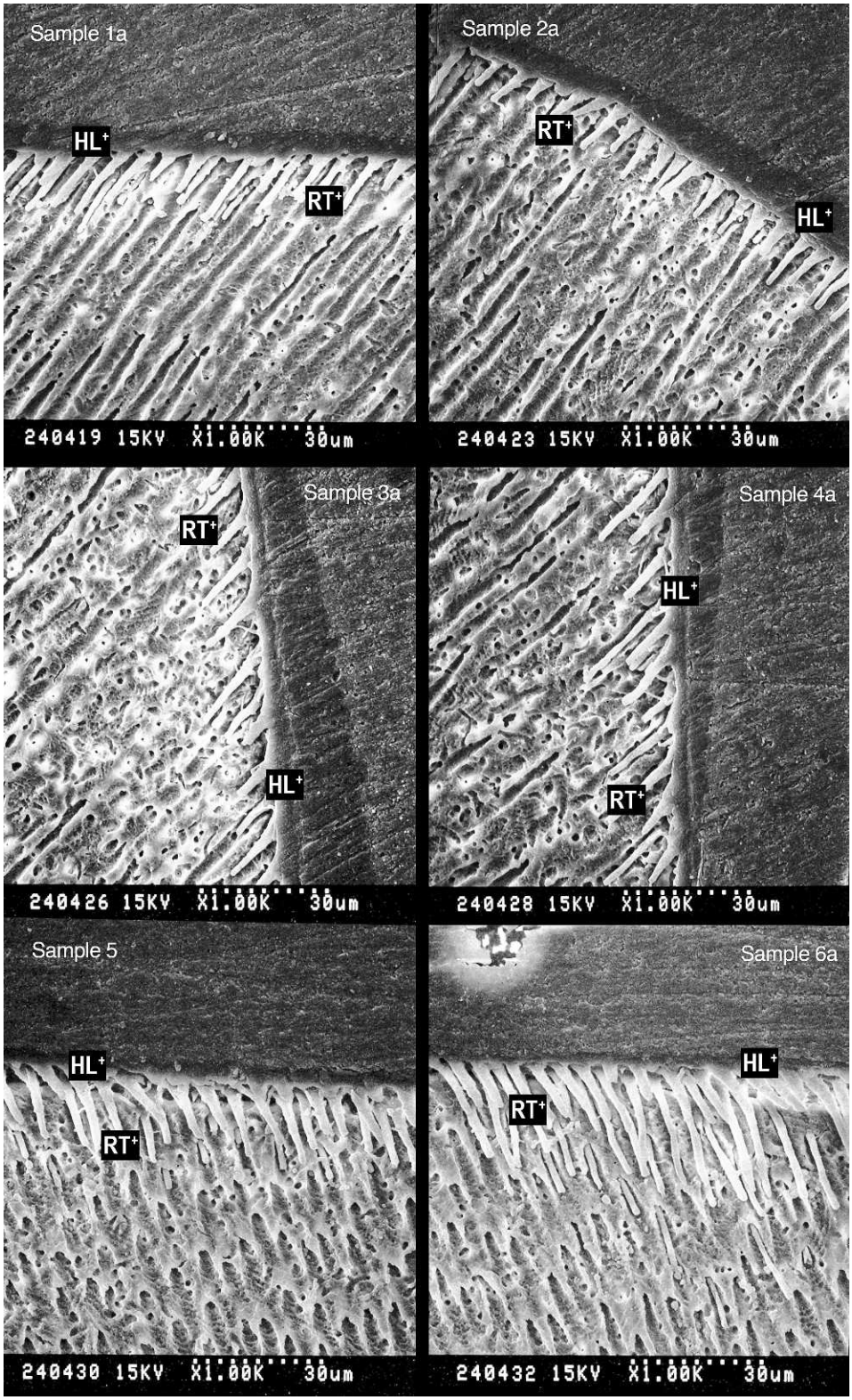

Figure 2. *Scanning Electron Microscopy images of composite-dentin interface with Single Bond 2 and 2% chlorhexidine after four months of water storage of all six specimens. HL+, HL– refers to a yes or no score on the presence of a hybrid layer; RT+, RT– refers to a yes or no score on the presence of resin tags.*

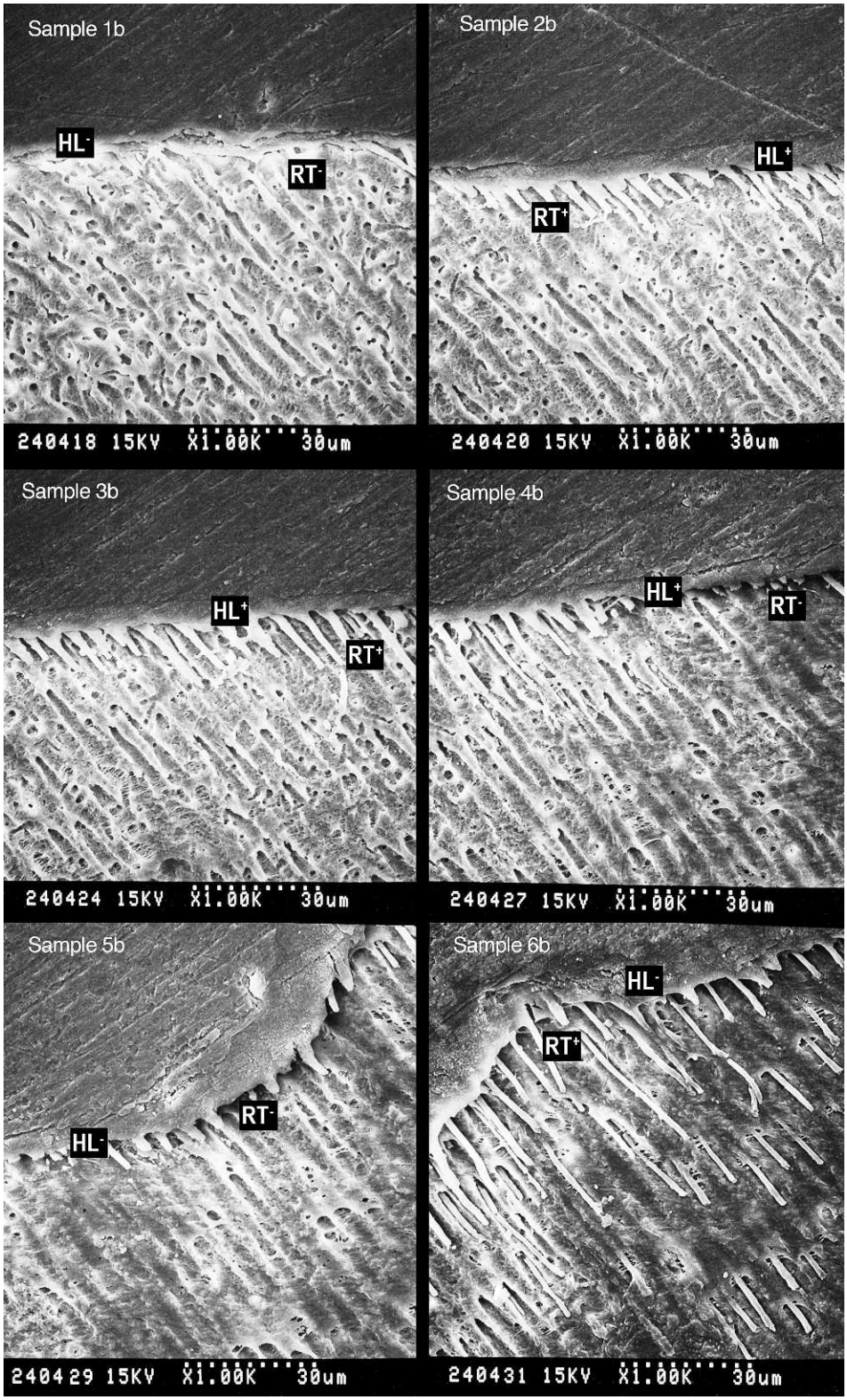

Figure 3. *Scanning Electron Microscopy images of composite-dentin interface with: Single Bond 2 without 2% chlorhexidine after four months of water storage of all six specimens in this study.*

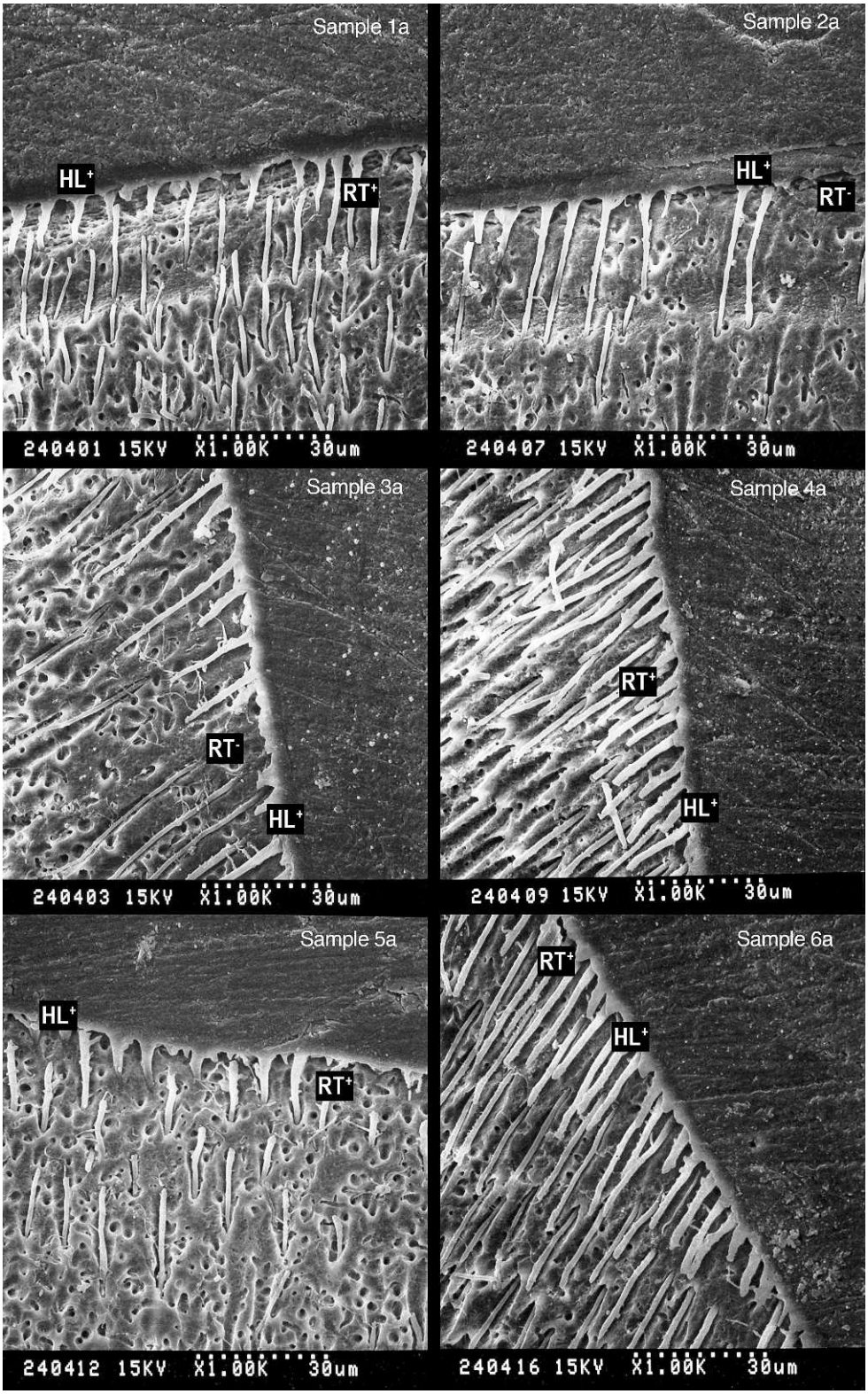

Figure 4. *Scanning Electron Microscopy images of composite-dentin interface with SE Plus and 2% chlorhexidine after four months of water storage of all six specimens in this study.*

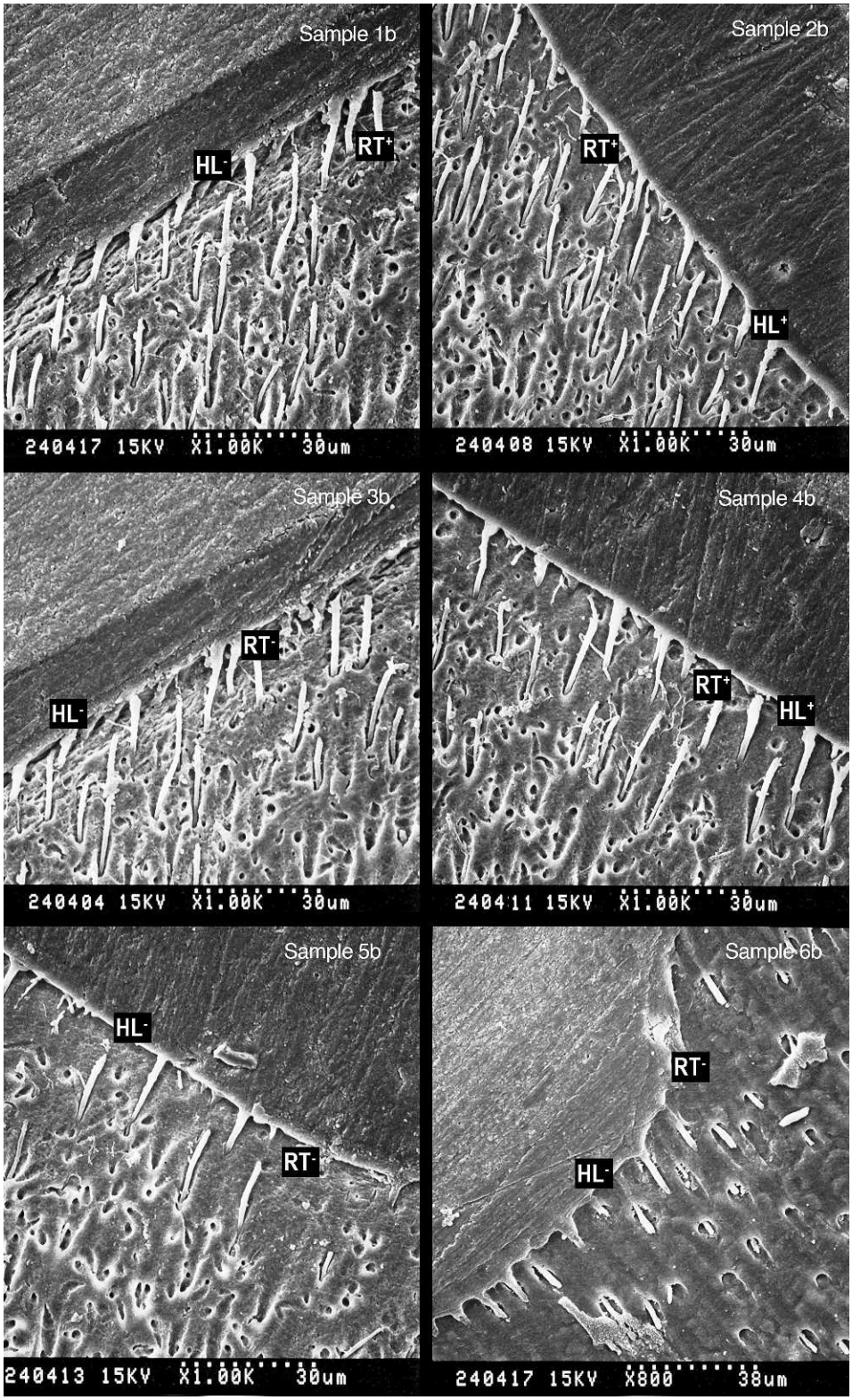

Figure 5. *Scanning Electron Microscopy images of composite-dentin interface with SE Plus without 2% chlorhexidine after four months of water storage of all six specimens in this study.*

dentin tubules. Regarding SEP, four of the specimens treated with chlorhexidine and three in the group not treated with chlorhexidine showed a clear presence of resin tags.

DISCUSSION

The deterioration of the hybrid layer after water storage is a concept well studied and accepted.[21–23] In this study, it was clear that the effect of water storage on the hybrid layer produced a reduction in thickness. The hybrid layer was difficult to isolate in half the samples that were not pretreated with chlorhexidine. Many authors have found that the use of chlorhexidine did not produce a negative effect on the bond strength of dentin adhesives when it was used before acid etching[24–27] as a cavity disinfectant. Many authors also have proposed that initial bond strength of chlorhexidine-treated specimens were comparable to the control groups,[23,27–29] and some authors have shown that the bond strength of dentin adhesives after water storage is preserved with the use of 2% chlorhexidine.[23,30]

The effect of protease inhibition by chlorhexidine suggests that the endogenous metalloproteinases cannot degrade the collagen fibrils left unprotected by acid etching.[31] This effect may explain why there is a more clearly defined hybrid layer along the entire interface in all the specimens treated in this study for both adhesives. Some specimens that were not treated with chlorhexidine showed a poorly defined hybrid layer.

The negative effect of water storage on the hybrid layer can be explained. First, hydrolysis of unstable polymeric hydrogels that are less concentrated occurs as they diffuse in the acid-etched dentin. Second, the unprotected collagen fibers get degraded by the matrix metalloproteinase.[31,32] This can also explain why the effect on resin tags is not as clear as in the hybrid layer. The concentration of polymers in the resin tags is higher, and therefore less hydrolysis can occur. Specimens not treated with chlorhexidine showed a lack of resin tags in some areas of the adhesive-dentin interface, but no statistical difference was found; therefore, one cannot conclude that there could be a positive effect in the use of the chlorhexidine in terms of the presence or the length of resin tags. Resin tag formation and tag length are more dependent on the application technique and the dentin adhesive itself.[33] If a high number of tags can be established on application, damaging effects by water storage can be minimized, and bond strength may not be affected.

This study used a small sample size, though using the same specimens to evaluate the treated and nontreated samples increased the power of the statistical analysis. Replication with a larger sample size is recommended.

CONCLUSION

The use of 2% chlorhexidine before the application of the DBA reduced the deterioration of the hybrid layer when exposed to water, but there was no statistical effect on its presence at the bonding agent-dentin interface or on the presence of resin tags in the tubules.

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