Clin Oral Invest (2005) 9: 1–7
DOI 10.1007/s00784-004-0273-4

ORIGINAL ARTICLE

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Permeability of marginal hybrid layers in composite restorations

Received: 2 February 2004 / Accepted: 11 May 2004 / Published online: 21 January 2005

Abstract The goal of adhesive dentistry is to restore the peripheral seal of dentin lost from removal of enamel. Unfortunately, the hybrid layer (HL) that is used to create that seal is permeable to small ions or molecules, even in the absence of detectable, interfacial gap formation via nanoleakage. This nanoleakage results from several mechanisms including incomplete infiltration of adhesive monomers into demineralized collagen matrix, presence of hydrophilic monomers, and insufficient removal of solvent or water that remains trapped inside the HL. These mechanisms lead to a porous interface with nanometer-sized channels that increase the permeability of the HL. The null hypothesis tested in this study was that water and acidic solution storage are able to alter in vitro the resin-dentin interface, further increasing the marginal hybrid layer (MHL) permeability. Class II cavities were made in vitro. The specimens were stored in water for 1 week and in lactic acid solution for 3 days. Polyvinyl siloxane impressions of restoration margins were taken before and after storage in water and lactic acid solution. Polyether replicas were obtained using the silicon impressions as molds. Replicas and original samples were observed under scanning electron microscopy. Lines of water droplets were detected on MHLs and overlying adhesive only after storage. Replicas obtained after acidic solution storage showed great numbers of irregularities such as gaps, voids, and degradation of the dentin-restoration surface margin, but also a great number of droplets. Dentin-restoration resin interfaces absorb water and are damaged by storage in dilute lactic acid. The presence of water droplets probably indicates water that flows out of the interface during the setting time of the impression and thus represents an index of marginal HL water permeability.

Keywords Bonding agents · Dentin permeability · Hybrid layer · Replica · Restoration

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Introduction

Conventional thought is that a perfect seal along the resin-dentin interface can be established within demineralized collagen matrix when it is completely infiltrated by adhesive resins in permanent and primary teeth [8, 9, 16]. This notion is based on the assumption that the polymerized resins used for bonding are nonporous and impermeable to fluids. However, small ions or molecules can permeate the hybrid layer (HL) even in the absence of detectable interfacial gap formation. This phenomenon is called “nanoleakage” and results from several mecha-

nisms that include the incomplete infiltration of adhesive monomers into a hydroxyapatite-depleted collagen network [24, 25] and retention of residual solvent or water that remains trapped inside the HL, creating porosities or nanochannels that increase the HL's permeability [28]. The functional groups of many adhesive monomers may have only weak chemical affinity for demineralized collagen, leaving much water bound to collagen [31].

Adhesive resins contain hydrophilic monomers that, after polymerization, may behave as a hydrogel, creating a three-dimensional copolymer network that can attract water and swell, similar to a sponge [1, 2, 17, 18, 30]. Water and other oral plaque constituents (lactic acids, salivary esterases, proteolytic enzymes, etc.) may cross the HL, even in the presence of optimally hybridized dentin, and contribute to the leaching of adhesives or to the degradation of collagen fibrils and their bond with resins [10].

The importance of water uptake in the long-term durability of restorations is still under question, but it may be related to water tree formation as described by Tay and Pashley [28]. A recent study [5] reported that water storage leads to degradation of HLs if they are directly exposed to water for 4 years. Dentinal nanoleakage phenomena increased when bonded specimens were stored in water but not in oil. This morphological alteration may be a consequence of the hydrolytic degradation of polymers and exposed collagen network over time [15].

For these reasons, the HLs formed in most peripheral dentin sites that have no enamel or cementum are potentially highly vulnerable to nano- and microleakage and deterioration [5, 10, 15]. The HLs located at these sites are called marginal hybrid layers (MHLs), since they are commonly found in cavosurface margins ending in dentin [21].

MHL exposed to the oral environment has several features that render it less effective than internal HL in preserving marginal sealing. For instance, MHL exposed to the oral environment is very thin [21]. This may be due to a higher acid resistance of dentin in this zone than deeper dentin areas and to the unfavorable orientation of tubules that limits the penetration of monomers [26].

Recently, a replica technique was used to demonstrate water uptake and release from dentin HL. During the setting of impression material, absorbed water flowed back to the outer surface of the HL and was trapped by the setting impression, producing a line of blisters that represented a replica of water droplets [2, 14]. These investigations support the hypothesis that there can be bidirectional water movement within the adhesive-HL complex [2, 3, 4]. In the present study, the morphology of MHL along the external margins of restorations was evaluated with the same technique, after storage in water and in a lactic acid solution, to simulate the effects of exposure to the oral environment. The hypotheses tested by this study were: (1) water droplets are detected along the marginal HL after storage in water of restoration samples and (2) lactic acid storage increases the number of droplets along marginal HL.

Materials and methods

Sample preparation

Thirty erupted third molars obtained from young patients (age range 25–40 years, mean 28.2), stored at 4°C in saline solution for no more than 1 month, were selected for the study. Nonretentive, standardized, class II cavities (3.5 mm width and 3.0 mm depth) were prepared with the proximal box extended to just below the cementum-enamel junction on the distal surface. This permitted examination of resin-dentin bonds was made under clinically relevant conditions. Medium- and fine-grit diamond burs were used with a high-speed, water-cooled handpiece (Castellini, Bologna, Italy). Different restorative systems were used: (1) Quadrant UniBond/Quadrant Universal LC (Cavex, Haarlem, The Netherlands), (2) Clearfil SE Bond/Clearfil APX (Kuraray, Osaka, Japan), (3) iBond + Venus (Haereus Kulzer, USA), (4) Quadrant Uni-1-bond + Universal LC (Cavex), and (5) Scotchbond 1+Z250 (3M-ESPE, St. Paul, Minn., USA).

Dentin pretreatment procedures

The specimens were randomly divided into the five groups of six teeth each: the dentin surface of groups 1, 4, and 5 was etched with 37% H_3PO_4 (Scotchbond etchant) (3M-ESPE) for 15 s. The specimens in each group were then rinsed with water for 20 s.

Bonding procedures

Resin composites were applied using a stainless spatula with 1-mm-thick increments and light-cured for 40 s. All bonding agents were used following the manufacturers' directions.

1. Quadrant UniBond (total etch with bonding technique): after dentin pretreatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread with an air syringe to remove excess adhesive and evaporate the solvent. The adhesive was light-cured for 20 s at 400 mW/cm^{2}with a previously tested unit (Visilux Command 2) (3M).
2. Clearfil SE Bond (self-etching technique): the bond primer was applied with a microbrush and gently air-dried for 6–8 s to evaporate the solvent. A layer of Clearfil SE Bond adhesive was applied with a microbrush, spread with air, and light-cured for 20 s.
3. iBond (self-etching technique): adhesive was applied with a microbrush for 30 s and gently spread with air for 6 s to evaporate the solvent. The adhesive layer was light-cured for 20 s.
4. Quadrant Uni-1-Bond (total etch with bonding technique): after dentin pretreatment procedures, the specimens were gently air-dried for 2 s. Adhesive was

applied with a microbrush and gently spread with an air syringe to remove excess adhesive and evaporate the solvent. The adhesive was light-cured for 20 s.

5. Scotchbond 1 (total etch with bonding technique): after dentin pretreatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread with an air syringe to remove excess adhesive and evaporate the solvent. The adhesive was light-cured for 20 s.

Replica preparation

After finishing with the Sof-Lex PopOn system (3M), each restoration was immediately polished along the margins with wet silicon carbide abrasive papers (nos. 600, 800, 1000, 1200, 2400, and 4000). The specimens were dried with gentle air blast for 10 s, and the first replica of each restoration was immediately made using a polyvinyl-siloxane impression material (President Jet light) (Coltene, Alstatten, Switzerland), applying the material to the restoration surface without any pressure. After 6 min, the impression material was separated from the tooth surface to obtain a negative replica of each sample. Positive replicas were then made using a polyether impression material (Permadyne Garant) (3M) using the silicon negative replica as a mold. Each restored tooth was stored in deionized water (pH 6.4) at 37°C for 1 week. Then each sample was removed from the water and gently dried with an air syringe for 10 s, and a second impression was taken as previously described.

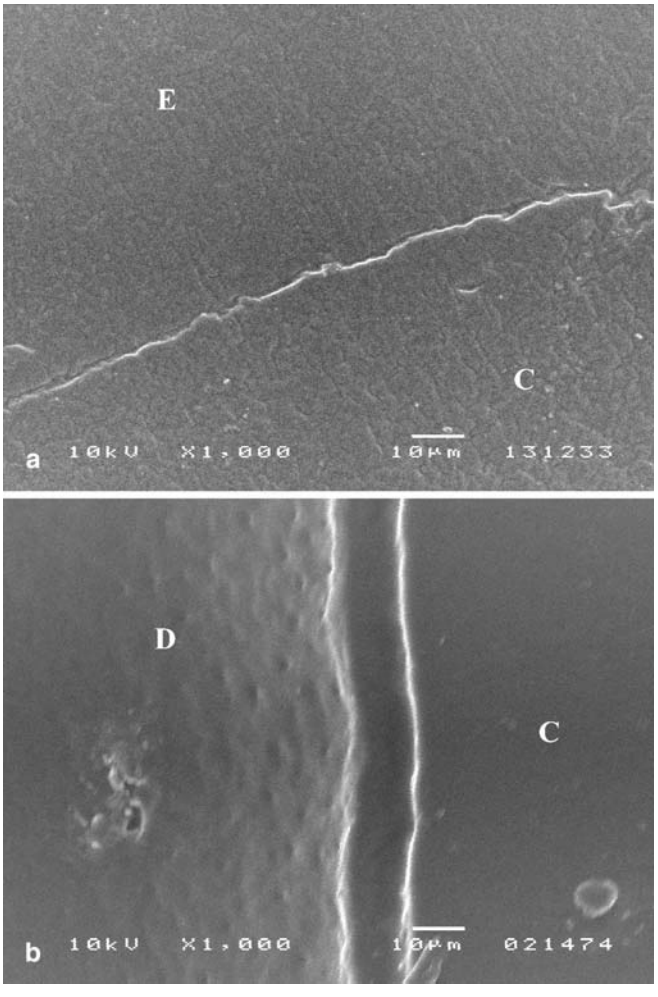

Fig. 1a, b Scanning electron micrographic images of dentin-restoration interface (×1000). **a** Margins of a resin composite restoration immediately after polishing and finishing procedures. Image shows the replica of a specimen obtained before its immersion in water. The visible composite (*C*) and the dentin surface (*D*) are easily identified. *E* enamel. **b** Image of a marginal gap obtained immediately after polishing and finishing and before water immersion. No water droplets are visible

Demineralization procedures

All restored teeth that had been stored in water for 1 week were then immersed in a demineralizing lactic acid solution (pH 4.4, 0.1 M, 37°C) for 3 days. The solution was changed every 8 h. After storage in this solution, each restored tooth was removed, washed under tap water for 2 min, and gently air-dried for 10 s, and a third replica was obtained.

Scanning electron microscope examination

Replicas (before and after water storage and after storage in lactic acid solution) were gold-coated and prepared for scanning electron microscope (SEM) observation (JEOL, Tokyo, Japan).

The restored teeth were then fixed in 4% glutaraldehyde (pH 7.4) in 0.2 M cacodylate buffer for 24 h at room temperature, rinsed in cacodylate buffer solution, dried, gold-coated, and analyzed by SEM in order to evaluate directly the HL morphology and exclude any preparation artefacts during the impression procedures.

The length of marginal gaps was measured as percentage of margin alteration respect to the total length of the margin. Positions of voids, droplets and blister-like structures, gaps, and fractures were recorded and inspected under SEM at ×1000 magnification.

Results

The dentin-restoration interface was easily observed on SEM images of the replicas. Interfacial gaps 1–5 μm wide (Fig. 1a, b) were occasionally seen in restorations of all groups of materials (Table 1, Table 2). Replication of samples obtained before water immersion resulted completely free of water droplet replicas (Fig. 1a, b). No droplets were observed on dentin smear layer surfaces or enamel surface replicas.

Scanning electron microscope examination of the specimen replicas stored in water revealed small, scattered droplets (0.5–3 μm in diameter) only along the dentin margins of composite restorations associated with the MHL.

Table 1 Composition of the adhesives used in this study. *HEMA* hydroxyethyl methacrylate, *META* methacryl oxyethyl trimethyllic anhydride

Adhesive	Components	Composition
Quadrant Unibond	Primer; bond	HEMA, purified water, ethanol, triethyleneglycol dimethacrylate, maleic acid ester, methacrylated polycarboxylic acids, maleic acid, and CQ; bisGMA,triethylene glycol dimethacrylate, maleic acid ester, Ba-Al-F-B-silicate glass, silicon dioxide, and CQ
Clearfil SE Bond	Self-etching primer; adhesive	HEMA, hydrophilic dimethacrylate, 10-MDP, MDPB, N,N-diethanol p-toluidine, CQ, and water; silanated silica, BisGMA, HEMA, hydrophilic dimethacrylate, 10-MDP, toluidine, and CQ
iBond	Liquid	Acetone, 4-META, glutaraldehyde
Uni-1-Bond	Liquid	UMA, HEMA, ethanol, CQ, butoxyethyl dimethyl aminobenzoate, 4-META, methacrylated polycarbonic acid, water, maleic acid
Scotchbond 1	Liquid	BisGMA, HEMA, hydrophilic dimethacrylate, polyalchenoic acid copolymer, ethanol, water

All materials showed droplets, as illustrated in Fig. 2a–c. No droplets were observed on dentin smear layer surfaces. Twenty droplets were detected for approximately every 100 μm of MHL. Composite and enamel surface replication also resulted free of droplets.

Lines of bigger water droplet replicas (0.5–3.5 μm) were seen along the entire length of the MHL in replicas (Fig. 3a, b) after storage in lactic acid solution. Open dentinal tubules were well observed on the dentin surface, suggesting that the acid solution was able to remove the smear layer produced during the polishing procedure. The diameter of tubules ranged from 0.5 μm (partially opened) to 2.0 μm (completely opened) (Fig. 4). No water droplets were detected on the tops of open dentinal tubules or on dentin surfaces.

Enamel surfaces appeared altered after storage in lactic acid. In many specimens, it was possible to observe an etching pattern, with loss of intraprismatic structure or roughening of the interprismatic enamel (Fig. 5a). The etching of enamel was most clearly visible close to the restoration margins, while the enamel surfaces distant from margins was relatively acid-resistant. All resin-enamel margins appeared free of the droplets seen on resin-dentin margins (Fig. 5b).

Obviously, the original samples resulted free of water droplets and blister-like structures. The morphology of original dentin surfaces and marginal gaps was similar to that observed in the replicas. Several gaps and fractures were observed but were free of droplets.

Discussion

The replica method for detecting margin alterations has been widely used in the past [7, 14]. In this study, such a technique was used to analyze evidence for water released from the MHL before and after immersion of teeth samples in water and lactic acid solution.

Interestingly, in this study, all replicas of restored teeth stored in water showed lines of droplets located only along or close to the resin-dentin margin. As revealed by the SEM images, this line was evident approximately 1 μm above the interface between resin and dentin. Since the droplets were visible only on the replicas of samples stored in water and not on the original specimens that were dehydrated prior to SEM observation, we believe that they were formed by water flowing out of the adhesive-MHL region during setting of the impression material [3, 4, 29]. Droplets were not present on sound dentin, resin composite, or marginal resin-enamel interfaces but only along the HL and at the bottom part of adhesives, suggesting that, during water storage, these structures took up and released more water than the other structures and tissues. We believe that these droplets are not artefacts produced by moisture condensation during impression taking, as they were absent when specimens were not preincubated in water and were never seen in replicas of resin-enamel margins. All materials showed the formation of droplet lines.

Table 2 Ranges of marginal gaps calculated as percentage of margin affected by gap with respect to total length of restoration margin. Presence of replica water droplets is reported as frequent (more than 20–25 per 100 μm), rare (less than 15–20 per 100 μm), or absent

Bonding agent	Before water immersion		After water immersion		After acid solution immersion	
	Percent of margin affected by gap	Water droplets	Percent of margin affected by gap	Water droplets	Percent of margin affected by gap	Water droplets
Quadrant Unibond + Universal LC	10–15	Absent	10–20	Frequent	15–25	Frequent
Clearfil SE Bond + APX	5–10	Absent	5–10	Rare	10–12	Frequent
iBond + Venus	15–20	Absent	15–25	Frequent	20–30	Frequent
Quadrant Uni-1-bond + Universal LC	15–25	Absent	15–25	Frequent	20–30	Frequent
Scotchbond 1 + Z250	10–15	Absent	10–20	Frequent	15–25	Frequent

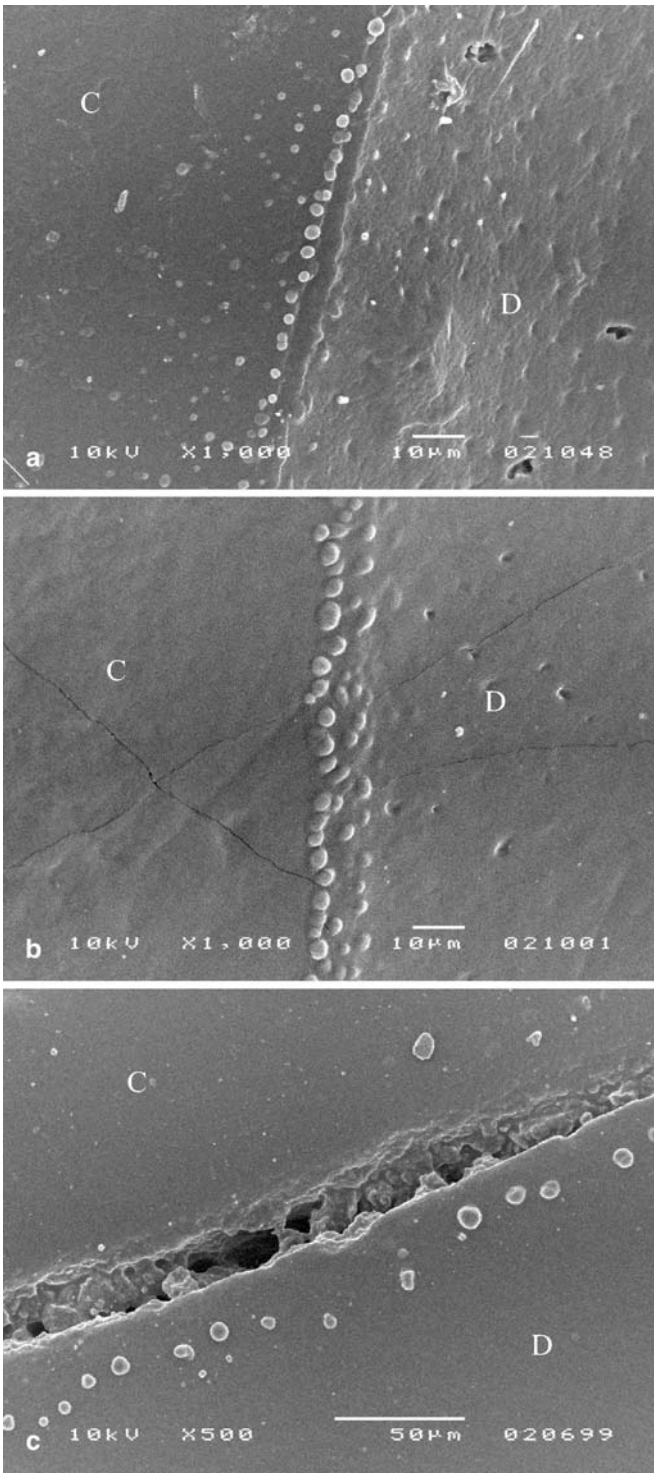

Fig. 2a–c Replicas of restoration specimens after immersion in water. *C* composite, *D* dentin. **a** Scattered droplets (0.5–2 µm in diameter) were detected at margins of restoration associated with the MHL and overlying adhesive (×1000). **b** Dentin-composite interface after immersion of the sample in water. Dentin is well visible. Water droplets are detected only along the hybrid layer and in its proximity (×1000). **c** Replica of specimen stored in water. Scattered droplets are visible along the MHL. A gap is well visible (×500)

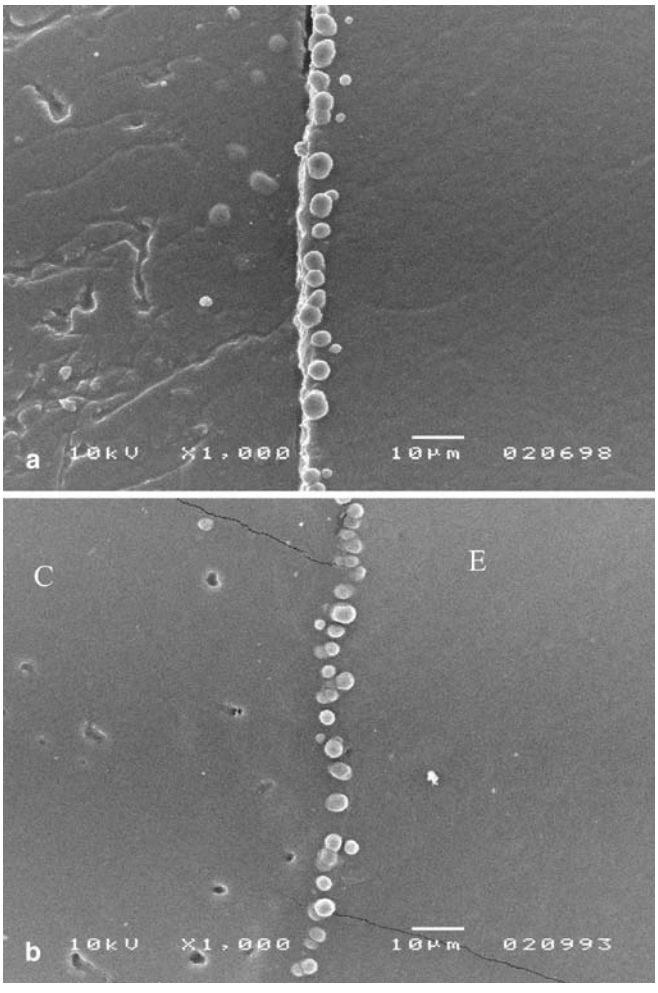

Fig. 3a, b Replicas of specimens stored in water and lactic acid solution (×1000). **a** A line of small and large droplets (0.5–5 µm in diameter) is observed along the MHL and the adhesive layer. **b** Another specimen. *C* composite, *E* enamel

Moisture transfer in organic coatings on porous materials has been studied extensively in nondental fields [11, 13, 20, 27]. It may occur via different mechanisms. While the demonstration of interconnecting water channels (water trees) in dentin adhesives [28] is an example of capillary fluid movement, in the absence of water-conducting pathways, water movement across polymer coatings may still occur via nanopores in the polymer that are created by the segmental mobility of the polymer chains, according to the free volume theory of Cohen and Turnbull [11]. Water molecules and small ions can move through these nanopores via a hopping mechanism that occurs in the range of picoseconds during the beta relaxation of these polymer chains [11]. Water diffusivity in polymer networks is probably enhanced by the incorporation of hydrophilic groups in the adhesive copolymers [27].

Permeability of water through these adhesives is caused not only by a loss of integrity between the adhesive and dentin but by the nanoporosity of the HL-adhesive complex. Most adhesive formulations include hydroxyethyl

methacrylate and acidic monomers [6, 31, 32] that may cause swelling pressures commonly seen in hydrogels [18, 33]. We speculate that hydrophilic resins in restorations absorb water during water storage, causing minute swelling in these structures that stretches the cross-linked polymer network [33]. When the specimens are removed from water, briefly dried, and then covered with impression material, the absorbed water is squeezed out by recoil of the polymer network at the free surface.

Interestingly, we found increased numbers and dimensions of droplets after acidic storage. We speculate that this was due either to a degradation effect of the acidic solution on the resin or that the low pH caused more water sorption [18, 21]. The presence of a wide marginal gap may increase the uptake and release of water.

Sound dentin is also a porous tissue. It contains approximately 10%/weight of aqueous fluids, most of which is in dentinal tubules. When dentin was removed from water and gently dried for 5–7 s, no water droplets were seen in the replicated dentin surface. Presumably, the mineralized dentin matrix is too stiff to stretch at a molecular level during water sorption, so the matrix cannot recoil when it is removed from water.

The results of the present study also revealed that there is no obvious direct relation between marginal integrity and number/dimensions of droplets on resin-bond dentin. Thus, we speculate that water absorbed by the HL was "stored" in small voids, porosities, or channels within the hybrid and adhesive layers and subsequently released, independently of any marginal breakdown [28]. This work indicates that both HLs and at least some contemporary adhesives absorb water. This water can come out of free surfaces within 3–6 min. Whether a similar bidirectional movement of water can occur under occlusal loading remains to be determined.

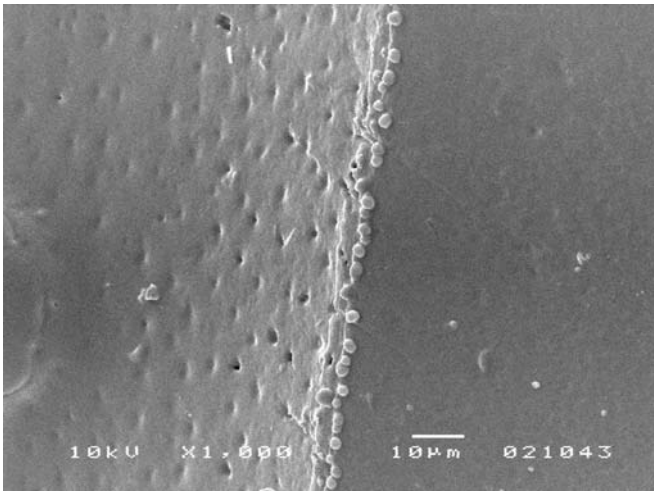

Fig. 4 Marginal dentin at the cervical portion of a cavity restoration (×1000). Dentinal tubules appear open and free of a residual smear layer. The tubule diameter ranges from 0.5 µm to 2 µm. Interesting is that the presence of open dentinal tubules is not responsible for the formation of water droplets in the replica, suggesting that dentinal fluids from tubules are not able to create any droplet-like structures. Droplets are visible only along the MHL

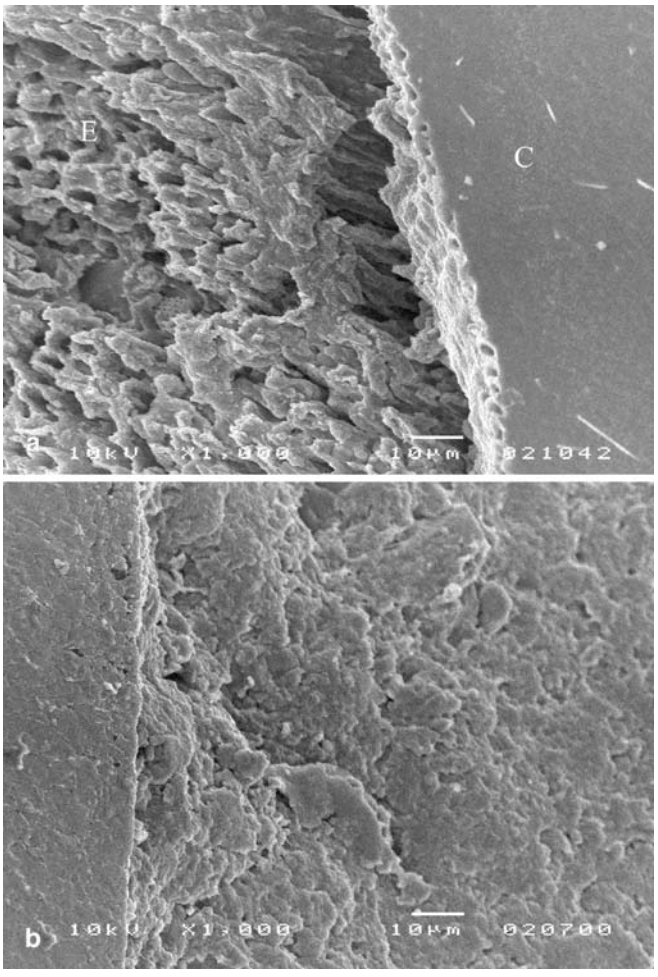

Fig. 5a, b Images by SEM of the enamel-composite margin (×1000). **a** Deterioration of an enamel surface after lactic acid immersion. The erosion of enamel is most clearly visible close to the margin of the restoration. *E* enamel, *C* composite. **b** Typical enamel alterations along the margin of the restoration

The development of marginal defects and secondary caries around restoration margins may be considered one of the major reasons for restoration failures and replacements [5, 12]. Little information is available on the mechanisms of marginal deterioration in the gap-free interfaces. Enamel surface resulted rich in porosities and alterations, especially close to the margin of restoration [22] but free of water droplets. The permeability characteristics of polymers have important implications in polymer degradation and have been extensively investigated in other fields [23, 34]. In MHL, we may suppose that the relatively high permeability of this area vulnerable to water may be responsible for the degradation of resin-dentin bond strength [19] and for the rapid, long-term hydrolytic deterioration of structural properties of the interface [10].

Acknowledgements This work was supported, in part, by the grant Progetto Pluriennale Biofilm Dipartimento Scienze Odontostomatologiche e COFIN ex 60% 2001–2002 UNIBO.

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