Dentin bonding: SEM comparison of the dentin surface in primary and permanent teeth

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Abstract

The literature suggest differences between primary and permanent teeth regarding the composition and morphology of the dentin. The purpose of this study was to compare the effect of two dentin conditioners on the micromorphology of the dentin surface of primary and permanent teeth. Human extracted and noncarious molars were divided into four groups and conditioned with either 10% phosphoric acid (All-Bond 2™) or 10% maleic acid (Scotchbond Multi-Purpose™) for different time periods. SEM photomicrographs (1500x) were taken from the conditioned dentin and evaluated blindly by three calibrated examiners. The results indicate that the smear layer was removed more easily from primary teeth than from permanent teeth (P = 0.0001), which suggests greater reactivity to acidic dentin conditioners. We also found that the longer the time of application of dentin conditioner the more smear layer is removed (P = 0.0094). In comparing primary and permanent dentin, the results of this study indicate that less time is required for appropriate acid conditioning of primary dentin surfaces. Such a differentiated protocol for bonding to primary tooth dentin results in surface morphological characteristics similar to those found in conditioned permanent teeth. (Pediatr Dent 19:246–52, 1997)

Clinical indications for tooth-colored restorations have increased with the evolution of adhesive and composite resin systems. More conservative cavity preparations, as well as increased public concern about esthetics, are also strong motivations for using composite resins. However, failures of these restorations in primary teeth are still a common problem for pediatric dentists. The bond strength of composite resins to the dentin surface is lower in primary teeth than in permanent teeth,[1,2] leading eventually to poorer performance of this material when used for primary dentition restorations. Two studies with similar methodologies can be used to illustrate differences in adhesion to both dentin types. When Amalgambond™ was used in permanent teeth, it presented bond strengths of 23.3 ± 5.7 MPa and All-Bond™ 19.3 ± 5.6 Mpa[3], while in primary teeth Amalgambond had bond strengths of 12.6 ± 7.5 MPa and All-Bond 11.6 ± 6.6 Mpa.[4]

Comparison of the composition and morphology of dentin in primary and permanent teeth indicates some differences. Neutron activation analysis was used to measure the mineral content of dentin, and lower concentrations of calcium and phosphorus were measured for primary teeth than for permanent teeth, but this difference was not statistically significant.[5] When energy dispersive spectroscopy was used, the concentrations of calcium and phosphorus were shown to be decreased in both peritubular and intertubular dentin of primary teeth, compared with permanent teeth.[6] In a microhardness study, the dentin from the central area of the crowns of permanent teeth was shown to be harder than dentin from the same area of primary teeth.[7] This finding suggested that permanent tooth dentin is more mineralized than primary tooth dentin. The concentration is higher and the diameter of dentin tubules is larger close to the pulpal surface (0.4–0.5 mm) in permanent teeth than in primary teeth, leading to decreased dentinal permeability in primary teeth.[8] Previous work from this laboratory[9] has shown differences in resin-dentin interdiffusion zone (hybrid layer) width between primary and permanent tooth dentin after treatment with two different dentin bonding systems. It was shown that the same protocol for dentin bonding produces a hybrid layer in primary teeth that is comparatively thicker than in permanent teeth. We believe that this finding might be due to differences in reactivity of primary tooth dentin to the acidic solutions used for conditioning the surface prior to the application of primers and adhesive resins (as recommended by the manufacturer).

The effects of dentin surface treatment and consequent characteristics of the dentinal substrate used for bonding affect the performance of composite resin restorations.[10] However, all the parameters established for preparation of an adequate dentin substrate for bonding have been studied in permanent teeth, and the results merely extrapolated for primary teeth without taking into consideration compositional and morphological differences that may exist between the two dentitions. The purpose of this SEM study, therefore, was to evaluate the impact of dentin surface treatments on

the micromorphology of the conditioned dentin of primary and permanent teeth.

Methods and materials

Specimen preparation

Ten primary and 10 permanent noncarious, previously erupted molars were selected for this study. The extracted teeth were stored in a solution of 0.2% sodium azide in distilled water at 4°C. All teeth were used within 6 months of extraction. Primary teeth in final stage of rhizolysis (old primary teeth) were paired with old permanent teeth, and primary teeth that still presented complete root structure (young primary teeth) were paired with recently erupted permanent teeth. This procedure was done to avoid eventual biases caused by age changes normally manifested through the biological cycle of the dentin in both primary and permanent teeth.

The crowns were divided from the roots using a high-speed diamond bur just apical to the cementoenamel junction, and the pulp tissue was removed using a stainless steel hand instrument. Labial surfaces were used in order to have a homogeneous depth of the dentin in relation to the pulp chamber. The preparation was done with a conical carbide bur # 7664 in high speed with copious water spray, parallel to the long axis of the tooth, exposing an area of superficial dentin within 1 mm of the dentinoenamel junction.[11] The dentin was then divided into four distinct zones in order to obtain a separate area for each dentin conditioning time protocol (Fig 1).

Dentin conditioning

Hydrostatic intrapulpal pressure was used in all samples to simulate the average tissue pressure of healthy pulps (about 25 mmHg).[12] Each crown was fixed to an acrylic platform penetrated in its center by a tube to connect the pulpal chamber to a pressure apparatus. Physiological intrapulpal pressure was reproduced by filling the pulp chambers with distilled water and connecting the mounted teeth to a 34-cm-high and 1-cm-diameter column.[13] All teeth were kept under positive hydrostatic intrapulpal pressure for 12 hr prior to dentin conditioning in order to establish an equilibrium between external (dentin surface) and internal (pulp chamber) pressures and also to achieve a homogeneous baseline pressure for all samples. Up to nine teeth were placed at each time on the intrapulpal pressure apparatus. These teeth were mounted in such a way that primary and permanent teeth were alternated on the table, resulting in an equal distribution through the platform to avoid biases caused by minor pressure differences that might have occurred in different locations on the table.

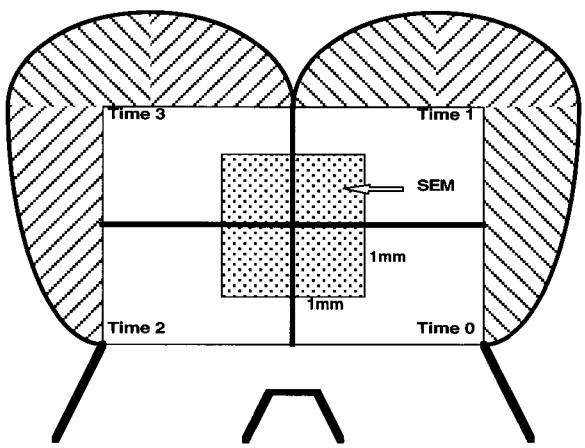

Fig 1. Schematic representation explaining the methodology used for evaluating the dentin surface. All photomicrographs were taken from the 1-mm² shaded areas at the center of the labial surface.

Intrapulpal pressure then was reduced to zero in order to simulate effects of application of an anesthetic with vasoconstrictor (commonly used in clinical situations). The dentin was conditioned after 15 min with either 10% phosphoric acid gel (All-Bond 2™, Bisco Dental Products, Itasca, IL) or 10% maleic acid gel (Scotchbond Multi-Purpose™, 3M Co, St Paul, MN) for four different time periods: 0 sec (control), 7 sec (half of the manufacturer's recommended conditioning time), 15 sec (recommended time), and 30 sec (twice the recommended time). These four areas in the labial surface were assigned randomly to the different etching times so that each tooth presented a different configuration in the relation of etching time to quadrant at the labial surface.

The conditioning of the experimental areas was done in a standardized sequence: the first area to be etched was the one that received the longest etching time (30 sec). When the first area had 15 sec of etching, acid was applied to the second area (the one to receive 15 sec total of acid etch). Finally, after 23 sec of etching time in the first area (and consequently, 8 sec in the second), the third area was conditioned for 7 sec. Then all samples were rinsed with 180 cc of distilled water for 30 sec with a hypodermic syringe. By doing this sequence, all etching procedures were done at once, and consequently, all areas received the same amount of water irrigation, including the control sites (no acid etch). After irrigation, all teeth were air dried for 5 sec and then stored in a desiccation cabinet (The Chemical Rubber Co, Cleveland, OH) for 24 hr.

Microscopic evaluation

All samples were mounted in metal stubs and coated with gold in a Sputter Coater™, Model S 150B (Edwards Co, West Sussex, England), during two cycles of 45 sec each, as a preparation for SEM. Each sample was then analyzed in a scanning electron microscope (Model 1000B, Amray, Bedford, MA) with an accelerating voltage of 8.0 Kv. All photomicrographs were

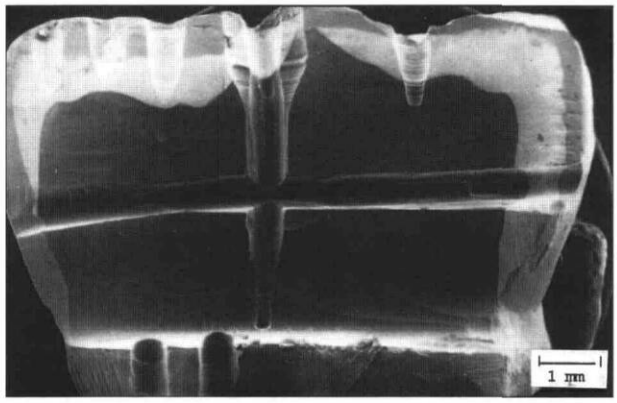

Fig 2. Photomicrograph illustrating a primary tooth prepared for evaluation of the dentin surface (11x). Each quadrant received a different time for dentin conditioning (0, 7, 15, or 30 sec).

taken from a zone of approximately 1.0 mm², at the central portion of the labial surface (Figs 1 and 2). A thorough scan of the area was performed to evaluate the general morphological characteristics of the dentin and to allow the operator to select the most representative fields (within the 1.0-mm² zone described above) for taking the photomicrographs.

The descriptive analysis of the surface characteristics of conditioned dentin was performed by three calibrated examiners. Each examiner received a complete set of photomicrographs (1500x magnification) and evaluated an area corresponding to approximately 60x40µ. A blind evaluation was performed, that is, the labels of the photomicrographs were covered so the examiners did not know the type of tooth (primary or permanent), the time of acid etch employed (0, 7, 15 or 30 sec) or the acid utilized (10% phosphoric acid or 10% maleic acid) for each sample. The examiners were asked to record the number of dentin tubules that remained partially or completely obliterated by smear layer, the condition of the peritubular dentin at the aperture of the dentin tubules (removed or intact), and the topography of the intertubular dentin (rough/smooth).

Statistical analyses

Multivariate analysis of variance (MANOVA) was used to evaluate the relationship of time for dentin conditioning with tooth type (primary or permanent teeth) and dentin conditioner (10% phosphoric acid or 10% maleic acid). The hypothesis that time had no effect on removal of the smear layer was rejected ($P = 0.0094$). Once the significance of time had been established, repeated measures ANOVA was performed to test the hypothesis of main group effects (tooth type, dentin conditioner, and the interaction tooth type/adhesive system) on removal of smear layer from dentin tubules.

Results

The results of the smear layer removal evaluation are described as percentages of dentin tubules that remained partially or totally obliterated by this layer after application of 10% phosphoric acid and 10% maleic acid (Table). The data are presented in two groups (according to the dentin conditioner used) for clearer appreciation of the differences between primary and permanent teeth and the differences among selected times for dentin conditioning.

The unetched dentin (0 sec of dentin conditioning) presented smear layers with identical micromorphology in primary and permanent teeth, at the magnifications used in this study (up to 8000x). The smear layer

TABLE. PRESENCE OF SMEAR LAYER IN DENTIN TUBULES (MEAN ± SD) IN PRIMARY ($N = 5$) AND PERMANENT TEETH ($N = 5$)

	10% Phosphoric Acid (All-Bond 2)		*10% Maleic Acid (Scotchbond Multi-Purpose)*	
	Primary	*Permanent*	*Primary*	*Permanent*
0 sec	100.00 ± 0	100.00 ± 0	100.00 ± 0	100.00 ± 0
7 sec	33.22 ± 23.91	89.96 ± 3.43	27.82 ± 24.00	84.43 ± 17.19
15 sec	8.49 ± 4.73	61.57 ± 25.41	12.41 ± 11.54	36.99 ± 34.84
30 sec	5.03 ± 1.34	7.55 ± 2.89	—	—

*Data is described in percentage

obtained with our protocol for cavity preparation (high-speed carbide burs under copious water spray) is about 1–2 µm thick, both in primary and permanent teeth. However, when a dentin conditioner was used, the smear layer was removed more readily from the dentin surface and dentin tubules of primary teeth than of permanent teeth ($P = 0.0001$). Seven seconds of dentin conditioning allowed for maintenance of more smear layer in dentin tubules than did 15 sec ($P = 0.0094$), and the two dentin conditioners tested produced similar removal of smear layer ($P = 0.9466$). But the effect of tooth type (primary or permanent) on removal of the smear layer was not dependent on the dentin conditioner used ($P = 0.3659$). The application of dentin conditioners for 30 sec (twice the time recommended by the manufacturers) caused a drastic removal of smear layer in both primary and permanent teeth. The results indicated that nearly 100% of the dentin tubules were opened completely and without any trace of smear layer, as a result of intense action of the dentin conditioner. As described in the discussion section, there are several reasons to believe that it is not desirable to over-etch dentin in preparation for bonding procedures. Therefore, this time for dentin conditioning was not included in the statistical analysis of this study.

The effect of dentin conditioning with 10% phosphoric acid and 10% maleic acid on peritubular dentin of pri-

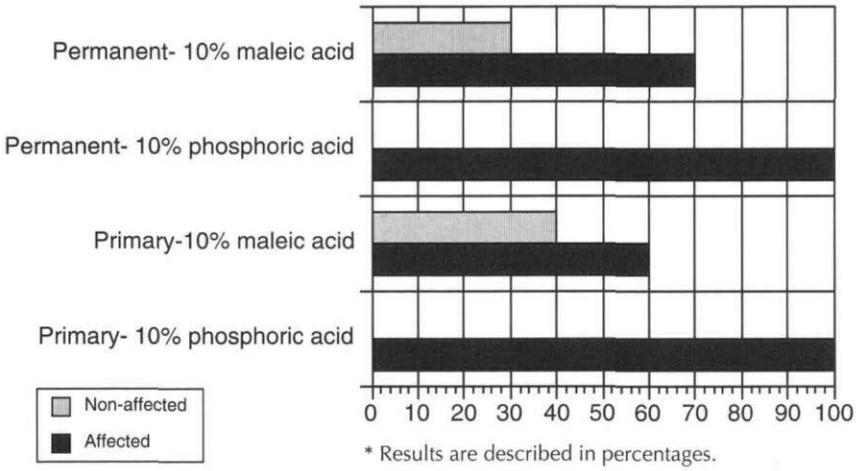

Fig 3. Effect of the dentin conditioners on the peritubular dentin of primary ($N = 10$) and permanent teeth ($N = 10$).

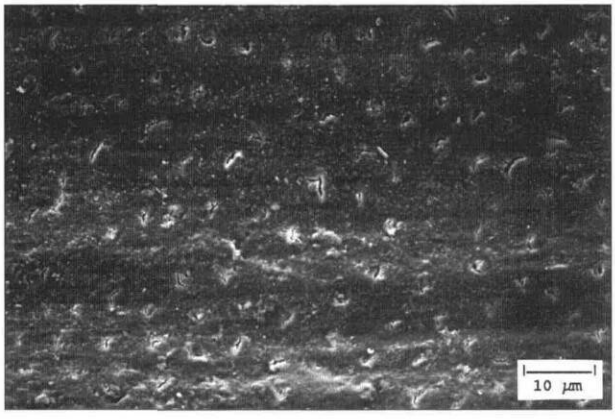

Fig 4. Photomicrograph showing an illustrative area of intact smear layer (no dentin conditioning) in a primary molar (1500x).

mary and permanent teeth was evaluated based on examination of dentin tubule borders. The examiners were asked to report removal of peritubular dentin when these borders were rounded, and nonremoval when the borders were defined sharply. The results of this evaluation (Fig 3) indicated that the action of 10% phosphoric acid on peritubular dentin seems to be more intense and more readily noticeable in both primary or permanent teeth, compared with 10% maleic acid.

The effect of the two dentin conditioners on intertubular dentin of primary and permanent teeth was determined based on an evaluation of its surface topography. The examiners were asked to report the intertubular dentin as rough or smooth. Data obtained from this evaluation were scattered and distributed randomly among different groups, not allowing establishment of any relationship between intertubular dentin topography and the other variables examined (tooth type, time for dentin conditioning, and acid solution). Due to the lack of information in the literature related to this feature and the fact that no pattern could be established, no further analysis was performed regarding intertubular dentin topography.

Discussion

This study was designed to evaluate the morphological characteristics of the conditioned dentin used as a substrate for bonding composite resin restorations to primary and permanent teeth. The three aspects evaluated were the responsiveness of the smear layer to the use of acidic solutions for dentin conditioning, the morphology of peritubular dentin, and the topography of the intertubular dentin after acidic conditioning. Significant differences were found regarding the effect of the acidic dentin conditioners on smear layer removal, suggesting that the substrate produced when primary tooth dentin is conditioned does not reproduce the substrate found in permanent teeth.

Care was taken to closely mimic the in vivo situation. A carbide bur was used for cavity preparation. Different instruments produce different amounts of smear layer and dentin surface topography.[14, 15] Con-

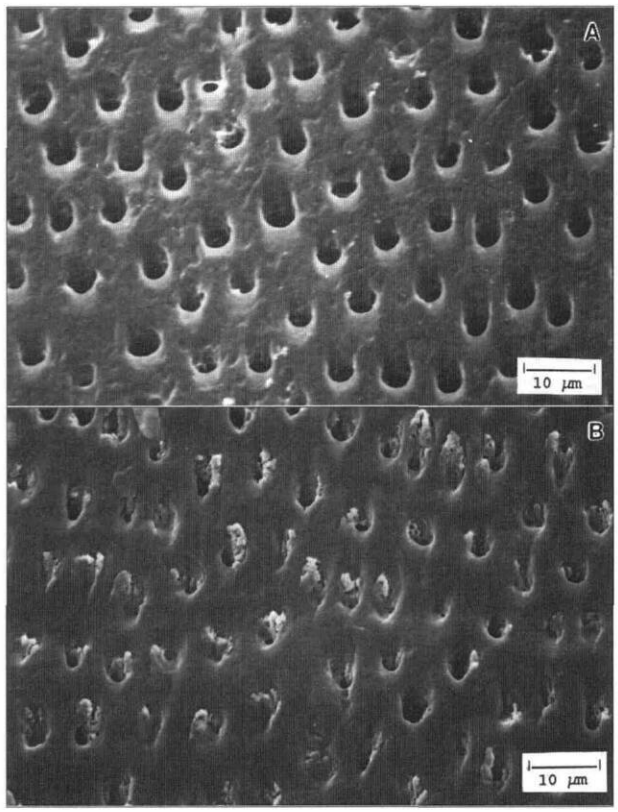

Fig 5. Photomicrographs showing an illustrative area of the dentin surface of (A) a primary molar (1500x) and (B) a permanent molar (1500x). The dentin of both specimens was conditioned for 7 sec with 10% maleic acid.

sequently, attention should be given to this step in order to use an instrument for cavity preparation that has clinical relevance, as it is in the case of carbide burs.

Another attempt to simulate the in vivo condition was the use of hydrostatic intrapulpal pressure.[16,17] The presence of fluids under physiological pressure inside the dentin tubules alters the pattern of demineralization caused by dentin conditioners by diluting the acids and, consequently, decreases their action on removal of peritubular dentin. It may also influence the extension of the dentin that is etched on the lateral walls of the dentin tubules and, consequently, the area available for primer/adhesive resin diffusion among the collagen fibers resulting in the formation of a hybrid layer. So, the use of physiological intrapulpal pressure allows for a more reliable system to evaluate, in vitro, the action of dentin conditioners.

Every time tooth structure is cut, a smear layer is created (Fig 4).[15] This layer of debris has important implications for restorative procedures. It has the protective effect of reducing the diffusion of elements from the restorative material to the pulp[18] and of limiting bacterial invasion.[19] It also decreases the fluid flow within the dentin tubules after restorative procedures, a fact that may have a positive impact on reducing postoperative sensitivity.[20]

On the other hand, the smear layer must be removed from the intertubular dentin and the opening of the dentin tubules to allow for the establishment of a hybrid layer, which ultimately is responsible for a strong and stable adhesion to dentin.[21–24] The extent to which smear layer is ideally removed from permanent teeth is already established. Each manufacturer indicates a time for dentin conditioning that is determined specifically for its adhesive system, but the protocol for removing smear layer from primary teeth is yet to be established.

The results obtained in this study reject the hypothesis that primary tooth dentin reactivity is identical to permanent dentin. The acids used to condition the dentin surface removed smear layer more rapidly from primary than from permanent teeth (Figs 5 and 6). The literature contains no direct explanations for these findings, but some speculations can be made. The composition of smear layer is related directly to the composition of the underlying dentin.[18,25] Based on this fact, a reasonable explanation for the differences found between primary and permanent teeth is that they may have different chemical compositions or chemical reactivity (James K. Avery, personal communication). Consequently, the smear layer from primary teeth becomes more responsive to the acids present in dentin conditioners.

Another possible reason for differences in smear layer removal may be related to the number of dentin

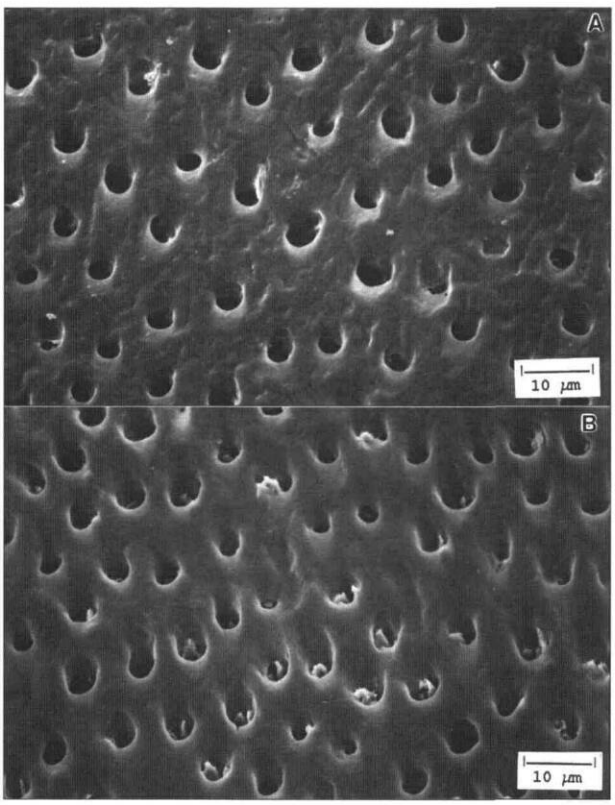

Fig 6. Photomicrographs showing an illustrative area of the dentin surface of (A) a primary molar (1500x) and (B) a permanent molar (1500x). The dentin of both specimens was conditioned for 15 sec with 10% maleic acid.

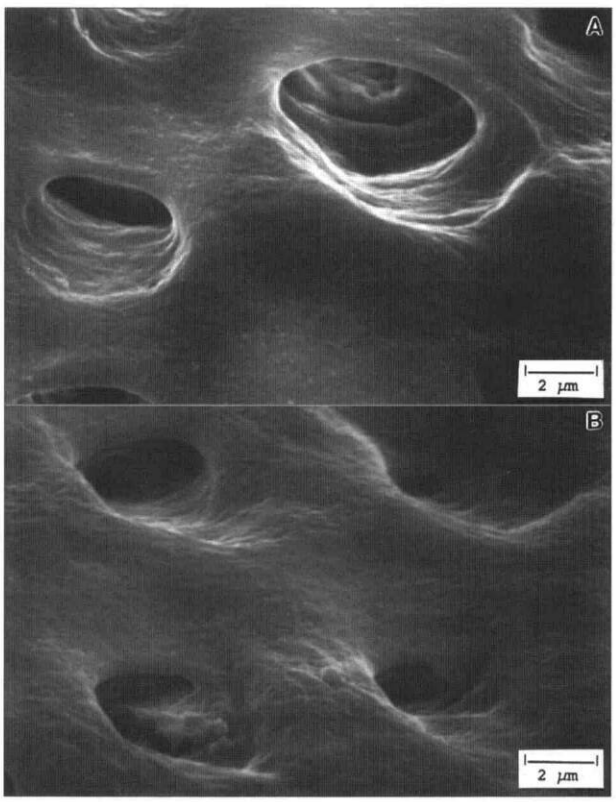

Fig 7. Photomicrographs showing an illustrative area of the dentin surface of (A) a primary molar (8000x) and (B) a permanent molar (8000x). The dentin of both specimens was conditioned for 15 sec with 10% phosphoric acid. The peritubular dentin is partially removed and the dentin tubule openings present a funnel shape.

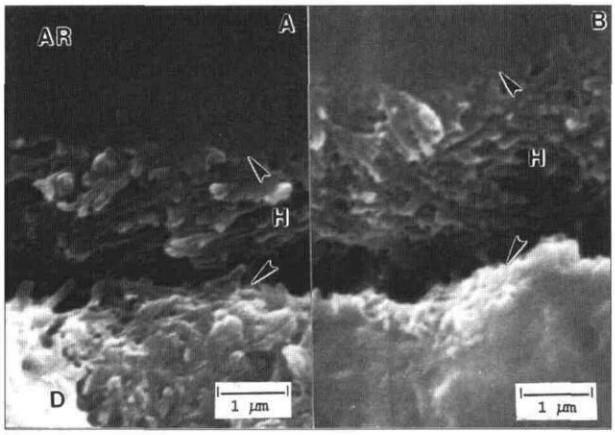

Fig 8. Photomicrographs showing the depth of demineralization of the dentin and hybrid layer thickness in (A) *permanent tooth* (13000x) and in (B) *primary tooth* (13000x). The dentin was conditioned for 15 sec with 10% maleic acid, and the Scotchbond Multi-Purpose/ Z100 system™ was used in both teeth. AR = adhesive resin; H = hybrid layer; D = dentin.

tubules present. The decreased dentin permeability of primary teeth is caused by smaller tubule concentration and diameter.[8] Thus, it can be hypothesized that primary teeth present less moisture on the dentin surface, thereby altering the effectiveness of the dentin conditioners on smear layer removal. Well-controlled studies of the consequences of a differentiated concentration and diameter of dentin tubules and a comparative analysis of the dentin and smear layer constituents in both dentitions may provide the necessary information for better understanding the mechanisms involved in smear layer removal and the reasons for the significant differences found in this research.

When the removal of smear layer was analyzed under the perspective of the dentin conditioner used, both 10% phosphoric acid and 10% maleic acid presented very similar results. The results also indicated that both primary and permanent teeth had their peritubular dentin affected by the use of these two dentin conditioners, even in short periods of time (7 sec). This action ultimately results in funnel-shaped openings of the dentin tubules at the surface level (Fig 7). The most noticeable feature regarding peritubular dentin removal observed in our study was the fact that 10% phosphoric acid seemed to have a more intense action on peritubular dentin than 10% maleic acid.

Another perspective of peritubular dentin removal was observed by the authors during a previously reported evaluation of the resin-dentin interface in primary and permanent teeth.[9] The cross-sections obtained for that SEM study showed clear enlargement of the opening ends of the dentin tubules of both primary and permanent teeth, compared with deeper areas in the dentin (not affected by the acid). The extent of this effect seems to be related to the duration of the dentin conditioning step (i.e., the longer the acid is applied to the dentin, the more significant is the removal of peritubular dentin).

It is clear that even short periods of dentin conditioning can potentially promote significant alterations in the structure of the dentin. Acidic dentin conditioning should be evaluated seriously since there are indications that dentin conditioning should be effective but not excessive. Conditioning the dentin is fundamental to remove the smear layer, partially demineralize the intertubular dentin, and expose the collagen fibers to allow the establishment of a hybrid layer.[26] However, when the depth of demineralization of the intertubular dentin is excessive, the collagen fibers collapse and form a dense layer that may not be fully impregnated by the primer and adhesive resin.[17] In these circumstances, the mineral matrix removed is not replaced fully by the primer, leaving a weaker area at the bottom of the hybrid layer, which potentially becomes a pathway for microleakage[27,28] or a site for bonding failure.[10]

Removal of smear layer is related directly to the concentration of acid and time of contact.[29] Based on the findings of this study, either shorter times for application of dentin conditioner or use of weaker concentrations should be considered for primary teeth. This study supports the concept that it is possible to control the amount of smear layer removed from the dentin surface by controlling the application time of the dentin conditioner. The clinical application of the knowledge generated by this work is that a shorter time for conditioning primary tooth dentin is indicated to promote a removal of smear layer and surface morphology similar to that observed in conditioned permanent tooth dentin.

The authors have found that the hybrid layer created at the resin-dentin interface of primary teeth is thicker than the one observed in permanent teeth, as demonstrated in cross-sectional views of dentin used as substrate for bonding (Fig 8).[9] We have also shown that a porosity can be seen at the bottom of the hybrid layer in primary teeth that were conditioned for 15 sec.[9] These results, combined with the observations of our study, offer a possible explanation for the problematic paradigm of bonding to primary tooth dentin. The use of the same protocol for bonding to primary and permanent teeth may be the reason why primary teeth consistently exhibited lower values in shear bond strength tests with dentin bonding systems.[1,2] Establishing a differentiated protocol for dentin conditioning primary teeth creates a dentinal substrate that resembles more closely the one found in permanent teeth. The findings of this work show that time for conditioning primary teeth dentin should be approximately 50% less than the time recommended for permanent tooth. However, well-designed clinical trials and reliable shear bond strength studies using the criteria established by Pashley[16] for in vitro simulation of in vivo conditions should be performed before this technique can be considered clinically acceptable.

Conclusions

The conclusions of this study are:

1. Application of dentin conditioners (10% phosphoric acid or 10% maleic acid) produces a different substrate for bonding to the dentin of primary teeth compared with permanent teeth.
2. The dentin conditioners used in this study removed more effectively the smear layer present at the dentin surface of primary teeth than of permanent teeth.
3. Application time of the dentin conditioner determines the amount of smear layer and peritubular dentin removed from the dentinal surface.
4. The effectiveness of the dentin conditioners (10% phosphoric acid or 10% maleic acid) on smear layer removal was very similar.
5. To produce a conditioned dentin surface in primary teeth with morphological features similar to that of permanent teeth, the time for conditioning the dentin of primary teeth should be approximately 50% less than the time recommended for permanent teeth.

Dr. Nör is a PhD candidate in the Oral Health Sciences Program and adjunct lecturer of pediatric dentistry; Dr. Feigal is program director and professor of pediatric dentistry; Dr. Dennison is program director and professor of operative dentistry; Chris Edwards is the manager of the Dental Microanalysis Facility, School of Dentistry, University of Michigan, Ann Arbor. This research was done in partial fulfillment of Dr. Nör's requirements for a Master of Science degree in pediatric dentistry at the University of Michigan.

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