The purpose of the present study was to determine the resin-dentin interface conditions in Wet vs. Dry Dentin. Dentin disks were prepared from extracted human premolars. Sectioned dentin surfaces were used for SEM studies of wet vs. dried acid-etched dentin. These specimens were cut perpendicular to the surface into two equal halves. One-half of the sectioned specimen was observed by SEM in three treatment groups and the other half was observed for micro-morphological differences in the resin-dentin interface using Wavelength Dispersive X-ray Spectrometer (WDX). SEM photomicrographs of the dentin surface showed the collapse of collagen fibrils in the demineralized layer and enlargement of the tubule orifices. A collagen rich layer approximately 8-10 μm thick (WDX) was observed at the resin-dentin interface when treated with the conventional dry-bonding technique. Dentin surfaces treated by the wet-bonding technique (SB), as observed by SEM, showed an uncollapsed collagen layer, while the collagen-rich layer was approximately 1-2 μm thick (WDX). The present findings suggest that moist bonding is required for optimum infiltration of adhesive resin into the demineralized layer.

Key words: Dry bonding technique, Wet bonding technique, Adhesive resin

INTRODUCTION

The choice of an appropriate adhesive system together with bonding technique has always been an important concern for clinicians in dentistry or orthodontics. Many kinds of adhesive systems are available. Many of the current generation dentin adhesive systems involve removal of the smear layer to facilitate the infiltration of hydrophilic resin into acid-conditioned dentin. For instance, the use of the total-etch technique to enamel and dentin surfaces removes the inorganic mineralized material surrounding the collagen fibrils in dentin to a depth of approximately 4-5 μm. When using the dry bonding technique, adhesive resin infiltration appears to be limited to the top of the demineralized dentin zone. The absence of resin in the interfibrillar spaces of the bottom half of these areas suggests that these regions collapse when using the air-dry technique. Phosphoric acid etching of a dentin surface sometimes modifies the collagen in the demineralized dentin which collapses easily during the drying process. Therefore, the use of the dry-bonding technique is perhaps not the best choice for composite resin restorations.

The wet-bonding technique recommends the use of phosphoric acid (35%) (Single
Bond Dental Adhesive System - 3M) as an etching agent. The wet-bonding technique\textsuperscript{7} is considered as the more efficient bonding technique. In the wet-bonding technique, the dentin surface is maintained in a hydrated state prior to the application of the hydrophilic primer, leading to efficient infiltration of adhesive resin into the demineralized dentin layer.

Adhesive systems with hydrophilic primer dissolved in acetone, ethanol and Hydroxyethyl Methacrylate (HEMA)\textsuperscript{8-9} were found to increase the resin-dentin bond strength when the acid conditioned dentin surfaces were not air-dried but were left visibly moist prior to bonding\textsuperscript{10-12}. Air-drying of acid etched demineralized dentin has been shown to cause the demineralized unsupported collagen framework to shrink and collapse, thereby preventing the monomers of primer and adhesive resin to efficiently infiltrate the conditioned dentin surface\textsuperscript{13-14}. The collapse of the demineralized dentin layer may be avoided by addition of HEMA containing primers if maintained in a moist condition. The moist demineralized dentin layer may be crucial in preventing the collapse caused by dehydration and in keeping the collagenous network open for infiltration of hydrophilic monomers to form a hybrid layer zone\textsuperscript{15}. The presence of water is beneficial for optimizing bond strength and a polymeric seal with adhesive resin, but too much water may be detrimental\textsuperscript{16}. Recent studies have shown that 32\% phosphoric acid (All-Bond 2) and 10\% maleic acid (Scotchbond Multi-Purpose plus) in the presence of excess water will lead to lower resin bonding strength values\textsuperscript{17}.

A WDX type (Wavelength Dispersive X-ray Spectrometer) electron probe X-ray microanalyzer was used to observe the micromorphological changes of the resin-dentin interface\textsuperscript{17}. X-ray microanalyzers have two types of spectrometers, EDS (Energy Dispersive X-ray Spectrometer) and WDX. Analysis of light elements such as carbon, fluoride and nitrogen are best performed by the WDX type. Light element analysis is difficult for the EDS type spectrometer.

The purpose of the present study was to evaluate two types of bonding techniques: the dry-bonding technique (by air-drying) and the wet-bonding technique (by blot-drying) as described by Van Meerbeek et al.\textsuperscript{18} Observations of the micromorphology of the dentinal surfaces and resin-dentin interfaces were conducted by scanning electron microscopy (SEM) and using a WDX type of electron probe X-ray microanalyzer.

**MATERIALS AND METHODS**

The dentin specimens were prepared from premolars extracted for orthodontic reasons. They were stored at 4°C in isotonic saline for no longer than one week prior to use. Sectioned dentinal surfaces were obtained by cutting a circumferential groove 2.0 mm below the central groove of the occlusal surface. After cutting, the removed sectioned dentin disc was formed by means of a parallel section. The thickness of sectioned dentin was 0.1 mm and an area 4 × 4 mm was prepared with a low speed diamond saw (Micro cutter 201, Maruto Co. Ltd, Japan) under copious water supply (Fig. 1). For bonded specimens, a standardized smear layer was produced by wet-
sanding the dentin surface with 800 grit silicon carbide sandpaper. After bonding, the dentin disks were cross-sectioned perpendicular to the surface into two equal halves using a low speed diamond saw (Micro cutter 201, Maruto Co. Ltd, Japan).

The dentin specimens were divided into three treatment groups to investigate the two different kinds of conditioning modes (Table 1). Three treatment groups were conditioned as follows:

Group 1 (CPB-D): Conditioned with 37% phosphoric acid (Clearfil Photobond System, Kurary Co. Ltd, Osaka, Japan) for 30 seconds then thoroughly rinsed for 10 seconds with 3-way syringe water. The dentin surface was gently air-dried with a 3-way syringe for 10 seconds. Thus, all visible moisture was removed from the dentin surface, but excessive desiccation of the dentin surface was avoided (dry-bonding technique by air-drying).

Group 2 (SB): Conditioned with 35% phosphoric acid (Single Bond dental adhesive System, 3M, St. Paul, MN, USA) for 15 seconds then thoroughly rinsed with water. The excess moisture was removed with a dry cotton pellet to produce blot-dried dentin (wet bonding technique by blot-drying).

Group 3 (CPB-W): Conditioned with 37% phosphoric acid (Clearfil Photobond...
System, Kurary Co. Ltd, Osaka, Japan) for 30 seconds then thoroughly rinsed for 10 seconds with water. The excess moisture was removed with a dry cotton pellet to produce blot-dried dentin surface (wet bonding technique by blot-drying).

In both wet-bonding techniques, the visibly hydrated etched dentin surface was maintained. Dentin specimens were fixed simultaneously in 2% paraformaldehyde and 1.25% glutaradehyde (PA-GA) for 12 hours and dehydrated in ascending grades of ethanol (50%, 70%, 80%, 90%, 95% and 100%) for 10 min. with double application for each. All fragments were then transversally fractured before being critical point dried (Critical Point Dryer; HITACHI, Co. Ltd, Tokyo, Japan) and gold coated. These sections were observed using a scanning electron microscope (S-430, HITACHI, Co. Ltd, Tokyo, Japan).

The dentin specimens were subjected to the same treatment sequence as in group 1, 2 and 3, following further adhesive treatment with the respective primer and adhesive resin (CPB or SB). The dentin disks were cross-sectioned perpendicular to the resin-dentin interface for each using a low speed diamond saw (Maruto Co. Ltd, Japan). The specimens were finally analyzed with the WDX type electron probe X-ray microanalyzer (EPMA 8705, SHIMAZU, Co. Ltd, Japan) for elemental distribution of calcium (Ca) and nitrogen (N) on the resin-dentin interface after critical point drying and carbon coating. The two tested adhesive systems were applied by strict adherence to the instructions of their respective manufacturers.

RESULTS

Representative SEM photomicrographs (Fig. 2) illustrate the respective dentin surface produced by the etchants of the two adhesive systems following the three experimental post-conditioning procedures. They exhibited the typical appearance of etched dentin: open tubule orifices surrounded by cuffs of peritubular dentinal matrix. The SEM of the intertubular dentin revealed a rough mineralized surface with only occasional surface porosities.

Group 1 (Fig. 2, left), which represents the acid etched and air dried dentin for the CPB adhesive system, showed a collapse of the demineralized collagen matrix associated with enlarging of the tubule orifices to 4-5 μm in diameter due to the loss of peritubular dentin. The surface collagen fibers appears to form a condensed crust-like collapsed layer covered with aggregates of silica particles used to gel the etchant.

Group 2 (Fig. 2, central) represents the wet-bonding technique (blot-drying) used for the SB adhesive system showed an uncollapsed matrix with open collagen matrix. The tubule orifices were 3-4 μm in diameter, due to uncollapsed collagen matrix. The surface was covered with a thick layer of silica particles from the etchant.

Group 3 (Fig. 2, right) represents the wet bonding technique (blot-drying) used for the CPB adhesive system showed an uncollapsed matrix with a thick crust of silica. The tubule orifices were 3-4 μm in diameter, due to an uncollapsed collagen matrix.

The WDX images illustrated the elemental distribution of the collagen-rich layer
Fig. 2 SEM photomicrographs of a demineralized dentin surface with dry and wet-bonding techniques (Upper row: cross-section; Lower row: outer surface) (Scale bar: 5μm). Group 1: by air-drying (CPB-D); Group 2: by blot-dry (SB); Group 3: by blot-drying (CPB-W).

at the resin-dentin interface. Fig. 3 (a-c) illustrates the respective resin-dentin interface. The resin-dentin interface shows the presence of a collagen-rich layer about 8-10μm thick in air-dried specimens bonded with CPB-D (Fig. 3-a). This layer was only 1-2μm thick in the wet-bonding techniques used for both SB and CPB-W (Figs. 3b and 3c). The WDX image illustrating the collagen-rich layer was more prominent in the dry-bonding specimens (Fig. 3a).

DISCUSSION

Previously, for composite resin restorations, the popular bonding technique was complete air-drying of cavity walls (enamel or dentin surface) after acid etching and water rinsing. At that time, it was thought that the adhesive monomers could not diffuse into wet dentin. The complete diffusion of adhesive monomers into the air-dried, collapsed collagen layer in only 2-3 seconds was not possible. When using blot-drying after etching, the presence of some water on and in the demineralization layer keeps the collagen fibrils separated for adhesive monomer diffusion.

Pashley et al. (1993), examined the effect of phosphoric acid on dentin and reported that a collagen-rich layer developed as a result of demineralization, which could interfere with adhesive agent penetration. Dehydration of the acid-conditioned dentin surface through air-drying is thought to induce surface tension
Fig. 3: WDX analysis of micromorphological spectrum for resin-dentin interface on wet and dry dentin (SEI image scanning: Ca; calcium characteristic density; N; nitrogen characteristic density; WDX: type of electron probe X-ray microanalyzer analysis, the bar shows the calcium and nitrogen Kα density, scale bar: 10 μm).
stress, causing the exposed collagen network to collapse, shrink and form a compact layer that is impenetrable to resin monomer.

The wet-bonding technique can promote efficient resin monomer diffusion into the demineralized matrix only if excess water on the dentin surface is eliminated with the dry cotton after etching, water-rinsing, and then replaced by monomers during the subsequent priming step. In the currently available adhesive systems, hydrophilic primer monomers are dissolved in volatile solvents, such as acetone and ethanol, that aid displacement of the remaining water as well as carrying the polymerizable hydrophillic monomers into the opened dentin tubules and through the nano-spaces of the collagen web.10)

In the present study, there were large differences between specimens exposed to air-drying and wet-bonding techniques when viewed under an SEM. The dentin surface conditioners used by the CPB or SB systems are very similar in that they are both phosphoric acid solutions gelled with silica (CPB: 37%, SB: 35%). The moist or dry condition changes the morphology of the dentin surfaces. With the dry-bonding technique the dentin surface consists of a collapsed, shrunken collagen network with no apparent separation between the collagen fibrils. The dentin surface of demineralized collagen exposed to the wet bonding technique indicated the collagen fibrils were separated and expanded.

Some differences were seen in the resin-dentin interface bonded under dry vs. wet bonding techniques using WDX analysis. The resin-dentin interface showed an 8-10 μm thick collagen-rich layer in the dry-bonding group, while the same layer was only 1-2μm thick in the wet bonding group. The collagen-rich layer suggests the presence of a demineralized collagen rich layer that was not infiltrated by adhesive resin. The thickness of the collagen-rich layer may cause the collagen network to collapse and form a compact coagulate after air-drying that as an impenetrable demineralized layer for the adhesive resin monomer. However, the 1-2μm collagen-rich layer, present after blot-drying, did not affect the adhesive resin infiltration of the demineralized and the adhesive resin was able to infiltrate deeply into demineralized layer.

The collagen fiber network that was exposed following acid etching was affected by the adhesive resin which infiltrated into the collagen fiber layer, and penetrated into the nano-spaces (collagen web space) previously occupied by the apatite mineral to form a resin-collagen complex which was fully polymerized by the hybrid layer. In the current study, the absence of a smear layer may have facilitate the formation of this interface. In the wet bonding technique, the water, that was required for optimal adhesive resin inter-diffusion and higher shear bond strength generation, was available to maintain open interfibrillar spaces. In the dry-bonding technique, the presence of a collapsed thick demineralized collagen fibril layer, i.e. collagen smear layer, interfered with resin infiltration. This limits the adhesive resin infiltration to the surface of the demineralized layer. With the blot-drying technique, the expanded collagen fibril network allowed for deeper adhesive resin infiltration into the demineralized layer (Fig. 4). However, even when using the wet-bonding technique a
nitrogen rich layer was detected by WDX in the lower half of the resin inter-diffusion zone. This supports the recent findings of Spencer and Swafford (1999) who demonstrated an unprotected dentin matrix in the lower half of hybrid layers\textsuperscript{21}.

The moist or dry condition of the dentin surface was significant for adhesion. With the dry-bonding technique, the demineralized dentin was re-expanded during processing for SEM. Presumably, if this occurred in vivo, it would cause marginal microleakage and increase nanoleakage in the nanometer sized spaces within the uninfiltrated collagen layer. Using the wet-bonding technique, the dentin surface and the resin-infiltrated zone were expanded. The infiltration of adhesive resin into the expanded demineralized layer would prevent microleakage and nanoleakage\textsuperscript{22-23}.

**CONCLUSION**

The present findings indicate that moist bonding is required for optimum infiltration of adhesive resin into the demineralized layer.

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