



Title	An ultrastructural study of bonding of a self-etching primer to sclerotic dentin
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1537 Effect of light emission on the flexural strength of 5 composites
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Recently 2 new polymerization modes were proposed involving either high and low intensity emission. The purpose of this *in vitro* study was to evaluate the influence of 3 different curing units on flexural strength of 5 current composites (Z100 P60 / 3M, Prodigy Condensable / Kerr, Aniston Vivadent Solitaire, Heraeus Kulzer). We have compared a plasma unit (Apollo 95E DMD) used during 3 sec (intensity of 1400 mW / cm²) a low intensity lamp (Elipar Highlight ESPE) used with the 2 step mode (10 sec at 100 mW / cm² and 30 sec at 600 mW / cm²) and a conventional halogen lamp (Optilux 400 Demetron) used during 40 sec (intensity of 600 mW / cm²) taken as a reference. In order to check the overall mechanical performances, 50 parallelepipedic samples (24 x 2 x 2 mm) divided in 5 groups were tested with a three point bend test at a cross head speed of 1 mm / min. Data were statistically analyzed using 2 ways ANOVA and PLSD test. Results: Table 1 presents the flexural strengths in MPa (same letters) to compare the influence of curing units and vertical lines to compare the influence of the composite represent data with no significant difference.

Table 1	Optilux	Apollo	Elipar Highlight
Z100	137 (21) a	134 (22) a	137 (20) a
P60	154 (19) b	137 (17) b	134 (26) b
Prodigy Condensable	110 (17) c	75 (10) c	106 (22) c
Aniston	101 (16) d	80 (11) d	89 (25) d
Solitaire	72 (6)	0	55 (9)

For 4 composites, there was no statistical difference between the conventional and the low intensity lamp. With the plasma unit, the values significantly decreased for Aniston and Prodigy Condensable. It was also impossible to remove the samples of Solitaire from the mold because of a lack of polymerization. This study was supported by ESPE.

1538 An ultrastructural study of bonding of a self-etching primer to sclerotic dentin
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This study examined the ultrastructural features of the resin sclerotic dentin interface following the application of Clearfil Liner Bond II (Kuraray Co.) to natural cervical wedge shaped lesions. Twenty deep cervical natural lesions were cleaned gently with a slurry of pumice and chlorhexidine and then bonded using the self etching primer. Micromorphology of the bonded interface at different locations within the lesions were examined using SEM. Both demineralized and undemineralized specimens were examined with TEM either a) unstained, b) stained with uranyl acetate and lead citrate or c) stained with phosphotungstic acid and uranyl acetate. Undemineralized unstained sections were also examined with STEM/EDS. Ultrastructural features were compared with the use of the same self etching primer on artificial lesions created in sound cervical dentin. A hypermineralized surface layer devoid of intact banded collagen was invariably present on the surface of the natural lesions. Depending upon its thickness at different locations of the lesions, the action of the self etching primer may be limited to this surface layer alone producing a hybridized hypermineralized surface layer. Penetration of the self etching primer into the underlying sclerotic dentin produced a hybridized complex containing a hybridized hypermineralized surface layer as well as a subsurface layer of hybridized intertubular dentin. Bacterial colonization of the lesion surface resulted in the formation of an additional zone of hybridized intermicrobial matrix over the surface of the lesions. It is concluded that there are four factors that may have resulted in the reported decrease in bond strength in natural cervical sclerotic lesions: (a) the presence of a hybridized intermicrobial matrix together with entrapped bacteria may have weakened the bonds, (b) inability of a self etching primer to etch through a thick surface hypermineralized layer, (c) presence of a layer of poorly mineralized, denatured collagen at the base of the surface hypermineralized layer, and (d) retention of acid resistant sclerotic casts that obliterate the tubular lumina and prevent effective resin tag formation. (Supported by RGC Grants 10202534 HKU and DE06427 NIDCR)

1539 Three Dimensional Microscopic Investigation of Resin/Cavity Wall Integrity
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The aim of this study was to evaluate three dimensional resin/cavity wall integrity of six current dentin bonding systems by using a microscopic method which is derived from a technique used for the microscopic examinations of carbonate rocks and fossils. A total of 12 non carious extracted human premolar teeth were randomly distributed into 6 groups of 2 teeth each. The groups were solid bond (SB), Clearfil Liner Bond2V (CLB), Clearfil SE Bond (CSE), Prime&bond 2.1 (PB), Optibond (OB), Fuji Bond (FB), Mesial and distal standard class II slot cavities were prepared on the teeth. The bonding systems and composite resin (Clearfil APX) applied to the teeth according to the manufacturers instructions. While one tooth from each group was embedded horizontally in acrylic blocks the other one is embedded vertically. Flat occlusal and proximal surfaces were prepared on the teeth by polishing. After acid etching of the surfaces with H₃(PO₄) (30%) for 1 minute an acetate peel section produced by regrounding the surfaces after each peel 9 horizontally and 18 vertically serial peels were obtained from each tooth. The horizontal and vertical microscopic evaluations of the peels showed gap free resin/cavity wall integrity in all groups. But when the Resin Infiltrated Layers (RIL) were evaluated separately both in enamel and dentin FB, OB, CSE and PB produced very clear and thick RIL along enamel margins. In the dentinal area, the most clear RIL and resin tags were observed in the OB group. The technique used here is an easy, cheap and rapid way of taking serial ultra thin sections of teeth without decalcification. It can be used many purposes in histologic examinations. One of them is investigations of resin/cavity wall integrity as used here.

1540 Micromechanics Morphology and Chemistry Direct at the Dentin/Adhesive Interface
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The purpose of this study was to determine the moduli of elasticity directly at the dentin/adhesive (d/a) interface and to correlate the moduli with the chemistry and morphology. The occlusal one third of the crown was removed from extracted unerupted human third molars. A uniform smear layer was created with 600 grit SiC under water. The prepared dentin surfaces were treated with Single Bond (SB 3M Dental Products) adhesive according to manufacturer's instructions. Samples from each tooth were analyzed using all 3 techniques. 3 µm thick sections of the d/a interface were cut and stained with Goldner's trichrome stain for light microscopy. 10 x 2 x 2 mm companion slabs were cut for analysis with micro Raman spectroscopy and scanning acoustic microscopy. Micro Raman spectra were acquired at 1 µm intervals across the d/a interface. This data was compared to a series of reference spectra including those acquired from model compounds of type I collagen and SB. Based on the light microscopy the total depth of dentin demineralization was ~ 6.6 µm. The micro Raman spectroscopic results suggest that the contribution from SB is <50% throughout half of the demineralized layer. When the adhesive concentration drops to ~25% the demineralized dentin, which is primarily type I collagen, is available for reaction with the Goldner's trichrome stain. The results indicate a zone of exposed protein ~2 to 3 µm wide at the dentin/adhesive interface. The same specimens were then imaged with an Olympus UH3 Scanning Acoustic Microscope (SAM) using a 400 MHz burst mode lens. At this frequency the lateral resolution in the SAM micrograph is 2.5 µm. Based on an internal calibration method, the elastic moduli are: dentin, 28 GPa, demineralized dentin, 1.3 GPa, adhesive, 5 GPa, protein interface less than 2 GPa. Supported in part USPHS DE12487.

1541 Permeability of Demineralized Dentin to HEMA
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The purpose of this work was to test the hypothesis that HEMA uptake by demineralized dentin depends upon the degree of expansion of the matrix. Dentin cubes (2x2x2 mm) were prepared from mid coronal dentin in extracted human teeth. They were incubated in 100% HEMA for up to 1000 min and then removed, blotted free of excess adherent HEMA, and then extracted with water for 1 hr to recover all HEMA taken up by the cubes, which was quantitated spectrophotometrically. The dentin cubes were then demineralized in 0.5 M EDTA (pH 7) for 10 days and the HEMA uptake remeasured at 1, 10, 100 and 1000 min. The cubes were then air dried and the HEMA uptake remeasured. Scanning electron microscopy was done on specimens that were expanded vs collapsed. The results were expressed as mean (SD) x 10⁷ moles mm⁻³ N=8.

Dentin condition	Time (min)	HEMA uptake (x10 ⁷ moles mm ⁻³)
Mineralized dentin	1000 min	4.6 (0.9)*
Deminerlized dentin (moist)	1 min	4.1 (0.4)*
Deminerlized dentin (moist)	10 min	27.3 (0.9)*
Deminerlized dentin (moist)	100 min	44.4 (2.2)*
Deminerlized dentin (moist)	1000 min	52.7 (1.7)*
Collapsed dentin	1000 min	2.9 (0.4)*

SEM examination of dried, collapsed dentin revealed an absence of interfibrillar spaces. The results support the hypothesis that HEMA uptake is directly proportional to the degree of expansion of interfibrillar spaces. Collapsed dry dentin took up little HEMA, while wet dentin allowed time-dependent uptake. Supported in part by DE06427 from the NIDCR.

1542 Penetrability of Dentin Cavity Walls Following Application of Different Adhesives
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The aim of this study was to investigate the penetrability of dentinal tubules in cavity walls lined with different dentin bonding systems. Occlusal Class I cavities were prepared in 93 intact premolars. The cavities in the control group had intact smear layer without a lining, while those in the experimental group were lined with Gluma CPS Scotchbond Multipurpose Plus or One Step. The penetrability of the dentinal tubules was tested with either a dye (basic fuchsin) or bacteria (*S. faecalis*) immediately after adhesive lining and after one month storage in water at 37°C. Some of the lined samples were sectioned and examined under the SEM. In some of the samples in the experimental group the dye penetrated to the pulp and the bacteria for up to 125 µm into the dentinal tubules immediately after lining. Kruskal Wallis ANOVA and Tukey test showed that the depths of dye and bacterial penetration were significantly less in teeth lined with the bonding systems than those in the control group (p < 0.05). However, after storage in water there was no statistically significant difference between the control and experimental groups (p > 0.05). SEM examination showed that the hybrid layer and resin tags were present in cavity walls immediately after lining but absent after storage in water. Under the experimental conditions, therefore, the adhesive linings were ineffective in preventing dye or bacterial penetration of the dentinal tubules. This project was registered at CDRC, King Saud University.

1543 Ultramorphology of the Hybrid Layer: a TEM study of Non decalcified Interfaces
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Adhesion to dentin is believed to depend upon dentin hybridization. The objective of this *in vitro* study was to characterize the bonding mechanism at the interface between dentin treated with a self etching primer (SEP) and unaffected dentin. Fifteen 800 µm thick dentin disks were obtained from middle dentin and assigned to five groups: (1) Clearfil SE Bond, Kuraray Co (SE) applied as per manufacturer's instructions; (2) Clearfil SE Bond primer rinsed off for 30 sec (SE/rinse); (3) Dentin etched with 35% H₃PO₄ for 15 sec + SE (SE/Etch); (4) Dentin decalcified with 0.5M EDTA for 2 min + SE (SE/EDTA); (5) PQ Ultradent (PQ) as the negative control. Four sticks with a cross sectional area of 1 mm² were taken from each bonded specimen. The sticks were not decalcified nor stained. The specimens were fixed, dehydrated, embedded in epoxy resin, sectioned with an ultra microtome (85 nm thick) and mounted on Ni grids to observe under the TEM. Some sections were immersed in phosphotungstic acid for 5 min to highlight the collagen fibers and re observed. SE resulted in a 0.6 µm thick hybrid layer (HL) with an intense concentration of hydroxyapatite crystals and granular deposits. The bottom of the HL displayed an electron dense zone that may limit the ingress of the SEP. Rinsing off the primer (SE/rinse) did not remove the hydroxyapatite crystals from the HL, but removed part of the granular deposits. The use of H₃PO₄ or EDTA prior to applying SE resulted in a deeper HL with total dissolution of hydroxyapatite crystals. The HL formed with PQ was free of any mineral deposits for all the sections but displayed a mesh of collagen fibers throughout the HL down to the transition to unaffected dentin. SEPs are less aggressive to dentin than H₃PO₄, leaving hydroxyapatite crystals retained in the HL. The bonding mechanism of SEPs may depend upon interlocking with hydroxyapatite crystals.

1544 High resolution Micro Raman Laser Spectroscopy of the Dentin Hybrid Layer
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Adhesion of resin bond agents to dentin is currently believed to result from impregnation of adhesive resin into superficially decalcified dentin. The purpose of this study was to investigate the chemical composition of the resin impregnated dentin (hybrid) layer using high resolution micro Raman spectroscopy (System 2000 Renishaw). Two step bonding systems: Mac Bond II (MB Tokuyama), Clearfil Mega Bond (CB Kuraray) and Single Bond (SB 3M) were employed. Resin composites were bonded to bovine dentin with the bonding systems and specimens were sectioned parallel to dentinal tubules. These surfaces were then polished down to 0.1 µm diamond pastes. Raman spectra were successively recorded along a line perpendicular to the dentin adhesive interface with a 0.6 µm focal size He Ne laser. The sample stage was moved by steps of 0.2 µm on a computer controlled X-Y table. Additional spectra from regions of only dentin and only adhesive resin were recorded for control. The relative amounts of hydroxyapatite (960cm⁻¹ P-O) adhesive resin (637cm⁻¹ aromatic ring) and organic substrate (1450cm⁻¹ C-H) in the dentin adhesive bonding area were calculated. From the Raman spectroscopy results resin impregnation into superficially decalcified dentin was estimated to extend 1-2 µm for MB and CB and 4-5 µm for SB. Furthermore, the hybrid layer represents a gradual transition in the relative amount of adhesive from the resin side to dentin side. Evidence of poor saturation of the adhesive resin in the demineralized dentin with SB was detected. From the results of this study, inhomogeneity of the hybrid layer composition was detected, and the degree of resin impregnation was found to be different among the bonding systems tested.