17 S E M investigation of the dentin/adhesive interface.
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The aim of this study was by the use of SEM to observe a dentin/adhesive inter diffusion zone using the last generation of dentin bonding agents. Twenty-four Class I cavities were prepared in the occlusal surfaces of extracted intact premolars, with pulpal walls in the deep dentin. After simulation of all clinical procedures following manufacturers instructions for use, Superlux Prep (DMG), Scotchbond MP (3M), Optibond (Kerr) and All Bond 2 were applied. The cavities were thereafter restored with Superlux Universalhybrid (DMG). After 48 hours in a humidity chamber at 37°C, the teeth were split transversally by freeze-fracture, and acid treated (6mol/L HCl for 30 s.). Half part of the samples (24) were immersed in NaOCl for 10 min following acid etching. The specimens were dried in vacuum at 1 torr for 24 hours prior to gold-sputtering for SEM. The dentin/adhesive interface was examined with a JEOL 5300 microscope at different magnifications. SEM's of the dentin/adhesive interface showed an absence of microgaps, and a layer of resin-impregnated dentin (5-10 μm). The presence of resin tags, of various shapes and lengths were reported with Superlux Prep, Scotchbond MP and All Bond 2, and sporadically with Optibond. It appeared that the degree of resin infiltration (tissue sealing) into a complex structure of the dentin is considered to be the major factor in gap prevention and for strong and durable bond.

18 Phase separation of All-Bond 2 primers with excess surface moisture
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his *in vitro* optical micromorphological study examined the resin-dentin interface with All-Bond 2 Bisco) was applied to total etched dentin with various amounts of remaining surface moisture. Twenty-our 1 mm dentin discs were each total etched with 10% H_3PO_4 for 20 seconds and rinsed for 20 seconds. hey were divided into 3 groups based upon the status of remaining surface moisture: Group I - surface ently air dried for 3 seconds, Group II - surface blot dried (Kanca technique), Group III - 40 μL of stilled water spread thin on dentin surface after blot drying. Application of All Bond 2 (Bisco) primer as observed under a stereomicroscope prior to the application of bonding resin. Discs in each group ere further bonded together to form a disc pair. Similar procedures were repeated for 2 additional dentin scs, except one was primed with primer A alone and the other with primer B alone prior to the pplication of bonding resin. Bonded specimens were demineralized in EDTA, stained en bloc and post-xed together with 0.1% ruthenium red and 1% OsO_4, and embedded in epoxy resin. Semithin sections ere prepared, stained with toluidine blue, and observed under light microscopy. Excess surface moisture Group II ad III resulted in the entrapment of small (Gp II) and large (Gp III) meniscus-like spaces ithin the primer layer immediately above the interdiffusion zone (hybrid layer). These spaces were rtially filled with: a) toluidine blue stained globular structures that corresponded to the staining of imer B (BPDM), which were dispersed within b) a ruthenium red stained zone that corresponded to the aining of primer A (NTG-GMA). It was concluded that the present of surface moisture might have used the BPDM, which is immiscible with water, to separate out during the application of the primer ixture. (Supported by CRCG grant, University of Hong Kong).

19 Influence of Exposed Collagen Etched with Phosphoric Acid to Bonding
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The purpose of this study is to investigate the influence of exposed collagen with phosphoric acid (PA) to long - term durability of bonding to dentin. When adhesion was carried out to dentin etched with PA and dried, the bond strength was lower because the porosity of demineralized dentin was collapsed,which did not permit diffusion of monomers and hybrid layer was hardly prepared. Previous work demonstrated that the application of 4-META in acetone improved tensile bond strength to dentin etched with PA but kept wet, and the formation of hybrid layer was identified. The acetone solution could replace water in the pores and prevented the collapse, which encouraged monomer diffusion (J Jpn Dent Mater 13 (1) : 29-35.1994) . Prepared bovine dentin was etched with 65% PA , rinsed and followed by 5% 4-META in acetone and 4-META/TEGDMA-CQ_2 NPG photocure bonding resin was applied. The bonded specimens were immersed in 37°C water for various time intervals and tensile bond strength (TBS) was measured. TBS was decreased significantly ($p<0.05$) from 6.6 ± 1.0 to 3.4 ± 1.7 at 1 month , 3.9 ± 0.9 at 6 M and 2.0 ± 1.0MPa at 12 M after immersion in water. SEM views of fractured surfaces due to tensile load showed that cohesive failure part in collagenous layer which was not fully reinforced with the diffused resin increased with immersion periods. We could conclude that exposed collagen etched with PA was easily weakened by the hydrolysis and the long - term durability of bonding was rather poor in this bonding system. This study was supported by Ministry of Education, Japan. (0543213)

20 Quantitative contribution of the collagen network in dentin hybridization AJ GWINNETT,* FR TAY,[1] KM PANG,[1] SHY WEI[1] (*SUNY Stony Brook, NY, [1]Faculty of Dentistry, University of Hong Kong).

Absence of a zone of partial demineralization in dentin conditioned with certain acids led to the purpose of this study which was to determine the quantitative contribution of the resin infiltrated, demineralized collagenous network to interfacial dentin bond strength. Four groups each containing ten, caries-free molar teeth were established. Dentin, exposed in the transverse, mid-coronal plane of the tooth was finished with 320 grit, wet silicone carbide paper. Two groups served as controls bonded with All Bond 2 (10% H_3PO_4.20s) and Amalgambond (10-3 solution, 10s) according to manufacturer's instruction. Two additional groups were subjected to Type II collagenase digestion of the acid treated test site at 37°C for 6 hr. After washing, drying and bonding Bis Fil and Epic composite to these specimens with All Bond 2 and Amalgambond resins respectively, the completed assemblies, mounted in Watanabe test jigs, were stored in water at 37°C for 24 hr. Physical testing was conducted in a shear mode at a cross head speed of 5mm/min and monitored to assembly failure. Means and standard deviations in MPa units were subjected to ANOVA and student-test analysis. All Bond 2 and Amalgambond controls were 28.41 ± 3.9 and 19.04 ± 5.96 with collagen deficient groups scoring 26.43 ± 2.90 and 19.70 ± 4.25 respectively. No statistically significant difference existed between the control and experimental groups. It was concluded that the resin reinforced or hybridized, collagen network does not contribute any significant quantitative value per se to dentin bonding with the systems tested.

21 Bonding to Demineralized and Remineralized Dentin. M. NAGAMINE*, M. STANINEC and K. INOUE (Okayama Univ., Japan and Univ. of California, San Francisco, USA)

It is well-known that the presence of fluoride accelerates dentin remineralization. The aim of this study was to evaluate bonding strength to dentin de- and re-mineralized in the presence of fluoride. 216 fresh bovine dentin samples were prepared as follows: ID = intact control, DD = demineralized for 7 days in a solution of 3 mM Ca (Ca/P 1.67) titrated to pH 4.1 with lactic acid, RD = remineralized - first treated as DD, then 14 days in a solution of 6 mM Ca (Ca/P 1.67) and 0.12 mM NaF at pH 6.9. Bonding areas (ϕ 5 mm) were treated with 4 kinds of dentin bonding systems: S = Scotchbond MP (3M), K = KB200 (KURARAY), A = All Bond 2 (BISCO) and M = Mirage Wet-Bond (CHAMELEON) under 2 conditions (with and without H_3PO_4 etching). Composite (Z100; 3M) was bonded after each treatment and tested in tension as described by Staninec (AJD;5:187,1992) after 24h in 100% humidity at 37°C. Means (SD) of bond strengths (MPa) (N=9):

	ID-etch / no etch	DD-etch / no etch	RD-etch / no etch
S	8.52(3.70) / 1.89(0.78)	4.65(1.20) / 0.31(0.28)	7.10(1.49) / 4.68(1.37)
K	8.81(1.23) / 8.40(1.17)	3.85(0.73) / 3.89(1.61)	6.80(1.06) / 6.37(1.39)
A	8.55(1.58) / 4.68(0.85)	3.00(0.74) / 1.96(1.07)	4.61(1.13) / 3.86(1.21)
M	10.87(1.24) / 4.44(0.77)	3.73(0.74) / 1.44(0.44)	7.70(0.94) / 3.59(0.99)

RD etch was significantly different from ID etch or DD etch for all systems. Etch and non-etch means were different for S & M, but similar for K (ANOVA, $p<0.001$; t-test, $p<0.05$). All systems bonded best to intact dentin and better to remineralized dentin than to demineralized dentin. Only one bonded to unetched dentin as well as to etched dentin.

22 TEM Study on *In vivo* and *In vitro* Resin-Dentin Interfaces by Phenyl-P Based Primer.
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A commercially available dentin bonding system, Clearfil Liner Bond II®, is a two step system which comprises a self-etching primer containing phenyl-P, and a one liquid type light-cured bonding agent containing MDP and micro fillers. The tensile bond strengths to both enamel and dentin were reported to be over 20 MPa. In order to analyze the bonding mechanism of this system, the ultra-structure of the resin-dentin interfaces were observed under the TEM and SEM. Ultra thin sections of the interfaces with and without PTA staining obtained from *in vivo* and *in vitro* samples were observed under the TEM. The interfaces on polished section surfaces were observed under the SEM before and after Ar-ion beam etching. The hybrid layer at the interface was about 0.5 μm thick. The cross bands of the collagen fibers were clearly observed in the layer and hydroxyapatite crystals were preserved in the deep zone of the hybrid layer. The border between the hybrid layer and intact dentin below was unclear, therefore we propose that this hybrid layer should be called a "reaction layer". The *in vivo* reaction layer was much thinner than that of *in vitro* samples.

23 Structural changes in dentine surfaces demineralised with different acid primers.
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The effects of acid primers on dentine have mainly been studied following application of their associated resin bonding agents leading to inadequate fixation and questionable interpretation. Alterations in dentine structure produced by different acid primers were studied using SEM and TEM techniques without the addition of bonding resins to maximise tissue fixation. Thirty freshly extracted human molar teeth were sectioned coronally above the pulp chamber exposing dentine. Groups of six teeth were each treated with one of the following acids for the indicated time: orthophosphoric, 35% 15s; orthophosphoric, 10% 30s; nitric, 2.5% 60s; maleic, 10% 15s and oxalic, 4% 30s. Specimens were washed with normal saline and fixed in gluteraldehyde. Half the dentine surface in selected teeth was protected from acid with silicone rubber to provide a within sample control. SEM Specimens were air dried or CO^2 critical point dried (CPD). Mineralised TEM ultrathin sections were diamond cut. Nitric acid and 10% phosphoric acid produced the greatest depth of demineralisation (5-10μm). Air dried SEM samples showed a step drop in height between untreated and treated surfaces, whereas CPD samples and TEM sections showed no step. CPD and TEM samples showed considerable porosity with clear collagen banding in the demineralised layer. No structural differences were observed between collagen fibres treated with different acids, or between superficial and deeper fibres in the demineralised layer. Different acids have broadly similar effects on dentine surfaces, only appearing to vary in the resulting depth of demineralisation. The acids do not denature collagen contrary to other reports. "Smear layer removal" does not involve loss of a layer of dentine, but merely reorganisation of collagen fibres following surface demineralisation.

24 Computer Mapping of Interface Tracer Distribution With Serial Abrasion.
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Simple sectioning dentine microleakage tracer penetration tests are often confounded: dentine tubules are permeable to most tracers; unstandardized cavity size and shape affects the stresses causing gaps; water soluble tracers may be leached by wet sectioning; trapped air may prevent full tracer entry; tracer measurements are highly dependent on section location. The present method aimed to create a high resolution map of the stained interface whilst avoiding these confounding factors. Ten intact extracted upper adult central incisors were horizontally sectioned through the upper root, one third of the root length from the mid-buccal enamel limit. A dentine-bonded resin composite (Scotchbond MP, Z100, 3M) restoration was placed in a 2.1 mm diameter 2.0 mm deep cylindrical cavity milled centrally in the root face of the coronal portion of five teeth. Tubule orientation was approximately radial in the horizontal plane and angled at a mean value of 12.5 ± 4.8° cemento-coronally with respect to the root face in the radial plane. This ensured that tracer diffusing through tubules was lead away from the interface, and therefore was distinguishable from vertical interface penetration. Waterfast silver nitrate staining was then applied with initial vacuum at 30 mmHg. After initial polishing, serial precision grinding in approximately 100 μm increments with a precision grinding machine until no tracer remained, and computer image analysis of the 18 revealed surfaces, produced data for construction of detailed interface tracer maps. Control specimen tubule penetration was seen to be directed sufficiently radially outward to permit tubule penetration to be isolated from vertical interface penetration in test specimens, and ignored. Pure interface penetration ranged from 300 to 1600 μm in depth, and from 0.66 to 4.55 mm^2 in area. This method provided quantitative standardised high resolution mapping of interface tracer penetration, unconfounded by dentine penetration. None of the interfaces were fully sealed despite the present benign experimental conditions, demonstrating that this bonding is still inadequate.