Title	Is moist bonding necessary following acid conditioning of dentin in-vivo?
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Grafting of MMA onto collagen using NPG with and without ferric compounds
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Efforts have shown that the bonding systems containing N-phenylglycine(NPG) or its derivatives, NPG-GMA,NTG-GMA yields strong adhesive bonding of composite resins to both dentin and enamel This study was to measure graft polymerization of methyl methacrylate (MMA) onto collagen using NPG with and without ammonium ferric sulfate (AmmFeSul) Typical composition is collagen Type I (Sigma chemical Co.) 0.1 g, purified MMA 1.0 ml, NPG10^{-4} mol$\sim 10^{-3}$ mol and distilled water 5.0 ml The grafting was stopped by pouring the mixture into 150 ml ethanol. The precipitations were filtered by glass filter ,washed with ethanol and dried at 23℃ in vacuo to constant weight The dried precipitations were extracted with acetone in a Soxhlet apparatus for 10 hr to remove homopolymer of MMA Total conversion,grafting and grafting efficiency were calculated as follows

Conversion (%) =(wts of grafted polymer and homopolymer)/(wt of MMA) × 100
Grafting (%) = (wt of grafted polymer)/(wt of collagen) × 100
Grafting Efficiency (%) = (wt of grafted polymer)/ (wts of grafted polymer and homopolymer) × 100

Code		47	48	49	50	51	52
NPG	mol	1×10^{-4}	5×10^{-4}	1×10^{-3}	1×10^{-4}	5×10^{-4}	1×10^{-3}
AmmFeSul	mol	0	0	0	1×10^{-4}	1×10^{-4}	1×10^{-4}
Conversion	%	87.08	88.98	80.30	65.89	87.39	78.39
Grafting	%	11.0	15.0	20.0	70.0	41.0	50.0
Efficiency	%	1.34	1.76	3.10	11.25	4.97	6.76

For a given reaction temperature and time(37℃,5 hr), the grafting and grafting efficiency obtained in the presence of AmmFeSul are significantly higher than in its absence.

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An ATR-FTIR Study on the Interaction of Adhesive with Dentin,
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The aim of this study was to investigate the chemical interaction of 4-acryloxyethyltrimellitic acid (4-AET) with the dentin apatite and collagen, using scanning electron microscopy (SEM) and fourier transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR). 4-AET and its calcium salt (4-AET·Ca) were synthesized for identification. An experimental primer containing 4-AET/HEMA (40/60) was prepared. A block of bovine dentin (8 x 5 x 3, mm) was treated with NaOCl (for Apatite group) or 1N-HCl (for Collagen group), and also treated with the primer. ATR-FTIR measurements of the primer and the surface of the dentin treated with or without the primer were carried out. The SEM micrographs revealed that apatite crystal was exposed (Apatite group) or the apatite was selectively removed (Collagen group) from the surface of dentin. The ATR results and the difference spectra of the Apatite group indicated that the absorvance of phosphoric ion (1020 cm^{-1}, -PO_4^{3-}) of hydroxyapatite was significantly reduced and Ca-carboxylate (1557 and 1413 cm^{-1}) was formed. The ATR results and the difference spectra of the Collagen group indicated that the amide I band was shifted and a new peak (1670 cm^{-1}) was grown. It was suggested that an ionized di-valence carboxylic group ($R\text{-}(\text{-}COO^-)_2$) in the 4-AET molecule could combine with the Ca^{2+} cation of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, to form a chelate bond (or a Ca salt), or an inter hydrogen bond between carboxyl group of 4-AET and the amide bond of dentin collagen was formed at the interface between the 4-AET and ground dentin.

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Field Emission Scanning Microscopy of Etched Dentin Under Hydrated Conditions.
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To date, scanning electron microscopic investigations of the etched dentin surface have generally involved specimen preparatory procedures that may alter the native structure. For example, dentin samples are usually desiccated and coated with metal prior to observation under high vacuum. Using a field emission environmental SEM (FE-ESEM, Philips XL30), the purpose of this study was to compare the morphologic structure of the smeared dentin surface treated with 3 different acids. In contrast to previous studies, the samples were hydrated throughout the observation period. The occlusal one-third of the crown was removed from 6 extracted unerupted human third molars. An uniform smear layer was created by abrading the exposed dentin with 600 grit SiC under water. The prepared dentin was treated for 15 seconds with: liquid or gel 35% phosphoric acid (H_3PO_4); 10% citric acid, 3% ferric chloride (10:3); or 2.5% nitric acid (HNO_3). Following acid etching the samples were rinsed thoroughly; in contrast to previous investigations the samples were wet throughout the preparation. The samples were fractured to permit cross-sectional viewing and stored in normal saline until transfer into the FE-ESEM. The sample chamber was maintained at 99% humidity; the samples were imaged at 20kV with a gaseous secondary electron detector. In this first field emission environmental SEM investigation of hydrated etched dentin surfaces, structure characteristic of collagen fibrils was noted lining the tubule walls of samples treated with H_3PO_4 or 10:3. This structure was obliterated in samples treated with HNO_3 and the walls of the tubules were pitted. The gel consistency of the H_3PO_4 acid produced an inconsistent etch pattern across the dentin surface. Supported in part by USPHS Research Grant DE12252, DE09696.

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Effects of Acids on the Susceptibility of Dentin to Denaturation.
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Native collagen is not very susceptible to enzymatic attack by trypsin, unlike denatured collagen. Addition of 3% ferric chloride to 10% citric acid was reported to decrease the denaturing effect of citric acid (Mizunuma, 1986). The purpose of this study was to evaluate the effects of various additives to acidic solutions that are commonly used to etch dentin. The null hypothesis was that additives have no protective effect on the denaturing action of acid conditioners. Disks of human dentin (1 mm thick) were dipped in acid conditioners with or without additives for 60 sec, rinsed and incubated in trypsin 24 h. Treatments included H_20, 37% phosphoric acid (PA), 10% citric acid (CA), 3 or 6% ferric chloride (3 or 6 FC) 50% HEMA, or 0.5M EDTA. The supernatant was hydrolyzed in 6 N HCl at 110° for 20 hrs, neutralized and the amount of hydroxyproline (HOP) released (from solubilized collagen) was quantified spectrophotometrically. Values are μg HOP released/cm^2 of dentin surface, mean ± S.D., N=3; pHs listed below S.Ds. * = $p < 0.05$ between groups.

H_20	PA	PA+HEMA	CA	CA+3FC	CA+6FC	CA+HEMA	EDTA	3FC	6FC
2.80 *	11.83 *	7.68	5.34 *	11.62	14.83 *	2.99 *	4.83	6.67	7.91
±0.77	2.04	±1.04	± 1.66	± 1.39	± 2.83	± 0.12	± 0.19	± 0.72	± 1.80
6.4	0.9	7.0	1.9	1.2	1.0	2.0	7.4	1.8	1.6

Addition of 50% HEMA reduced the denaturing action of 37% phosphoric acid and 10% citric acid, while addition of 3 or 6% ferric chloride to 10% citric acid increased the denaturing action of citric acid by lowering the pH. Thus, the null hypothesis is rejected for HEMA but supported for ferric chloride. Supported, in part, by grant DE06427 from the NIDR and by the MCG Biocompatibility Group.

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Effects of Polycarboxylic Acids on Enamel and Dentin.
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The aim of this study was to characterize the effects of 4 polycarboxylic acids on enamel and dentin. The acids studied were 50wt% solutions of PAA(polyacrylic acid,MW=5,000,Aldrich),GC-PA_1(polyalkenoic acid,MW=8,000,GC),GC-PA_2(polyalkenoic acid,MW=15,000,GC), and of V-MPA (methacrylate modified polyacrylic acid,MW>50,000, Vivadent). GC- PA, and GC-PA_2 were made up of 90wt% acrylic acid and 10wt% maleic acid. Inductively Coupled Plasma (ICP) was used to measure the decalcification rate of hydroxyapatite (HA) by the acids. In addition, hardness (H) and elastic modulus (E) of enamel and dentin surfaces exposed to the acids were measured using a nano-indenter. XPS of treated enamel and dentin confirmed that only an ultra-thin layer of the acids was deposited on the surface, so that its thickness did not influence H and E. ICP showed that decalcification of HA by the acids decreased in the following order: V-MPA>GC-PA_2>GC-PA_1>PAA. Regarding the effect of the polyacids on H and E of enamel, the acids were ranked in an opposite order(PAA> GC-PA_1>GC-PA_2>V-MPA) with PAA-treated enamel having the highest values(H=1426MPa; E=74.6GPa) and V-MPA-treated enamel the lowest(H=94MPa;E=10.7GPa). Regarding the effect on H and E of dentin, the acids were ranked as follows:PAA>V-MPA> GC-PA_2>GC-PA_1. PAA-treated dentin was measured to have the highest values (H=238MPa;E=15.2GPa) and GC-PA_1 -treated dentin the lowest(H= 51MPa;E=2.3GPa). These data showed that irrespective of MW the more the polyacid decalcified enamel, the lower H and E of enamel became. However, the decalcification rate of dentin by the polyacids appeared to depend also on MW of polyacid : the larger MW, the less dentin was demineralized. This may be due to a filtering effect of collagen fibrils that once exposed at the dentin surface may prevent the polyacid to penetrate and thus demineralized deeper. Despite its low MW, PAA decalcified dentin the least of all acids and thus kept H and E of dentin the highest. The decalcification rate of PAA may have been so low that it hardly penetrated into the surface and thus its MW could not have limited decalcification.

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The role of the collagen network on the dentin adhesion in primary teeth
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The aim of this research was to evaluate the influence of hybrid layer formation on the adhesion in primary teeth. For this study were used 20 deciduous first molars caries free from the Teeth Bank of The School of Dentistry - SP, that were included in epoxic resin, grounded with carbide papers creating a flat surface in dentin, without pulp exposure's. The teeth were randomly divided in two groups. The dentin bonding system used was Single Bond (3M). Group 1 (Control group): the dentin was etched for 15 sec., washed, dried using an absorvent paper leaving a wet surface, then were applied two layers of the adhesive, which one was photocured for 20 sec. Group 2 (Experimental): the dentin was etched for 15 sec., washed, dried using an absorvent paper leaving a wet surface, then the surface was treated with sodium hypoclorite (10%) for 60 sec., washed, dried using an absorvent paper leaving a wet surface and were applied two layers of the adhesive, which one was photocured for 20 sec. After the adhesive was applied a resin cone was created (Z100 – 3M) on the dentin surface using a teflon matrix. The teeth were stored in distilled water for 24 hours at 37° C. The tensile bond strength test was performed in a Mini- Instrom machine, with a cross head speed of 0.5 mm/sec. The results were obtained at the moment of the sample fracture. The results in MPa were: Group 1 – 12.90 +/- 4.78; and Group 2 – 10.52 +/- 1.90. Statistical analysis Mann-Whitney showed no statistical differences between groups. Based on the methodology *and the results we can concluded that the collagen network did not have a principal role on the adhesion to primary teeth dentin.*

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Is Moist Bonding Necessary Following Acid Conditioning Of Dentin *In-Vivo*?
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In-vitro studies showed that keeping acid-conditioned dentin moist is crucial for optimal infiltration of water-free, acetone-based adhesive systems. As dentinal fluid flow in vital teeth increases following removal of the smear layer, moist bonding may be superfluous *in-vivo*. On the other hand, intrapulpal pressure is reduced under the influence of anesthetics containing a vasoconstrictor. The objective of this study was to compare, with the use of a resin replica technique, surface features of deep, acid-conditioned dentin from vital human molars that were anesthetized with: **Group I**: a local anesthetic without a vasoconstrictor (Mepivacaine 3%), and **Group II**: one containing a vasoconstrictor (Lidocaine 2% with 1:80,000 epinephrine). Ten Class I cavities with deep dentinal caries were included in each group. Following complete caries removal, a total-etch technique was performed with 32% phosphoric acid (Uni-Etch, Bisco) for 15 s. After rinsing, each cavity was air-dried for 1 s, then a slow setting vinyl polysiloxane (President, Coltene) impression was taken. As a control, impressions were taken from three additional cavities that were anesthetized with Mepivacaine 3% but not acid-etched. A TEM-grade epoxy resin (TAAB 812, TAAB Laboratories) was used to prepare replicas from the impressions. Polymerized replicas were coated with gold and examined with a scanning electron microscope. In Group I, exudates were visible from tubular orifices in seven of the replicas. The rest were covered with a smooth, amorphous film that was different from the granular appearance of the smear layer in the control cavities. In Group II, patent tubular orifices without exudate were observed in all specimens. Odontoblast process-like structures were seen from some dentinal tubules. Following the use of a local anesthetic containing a vasoconstrictor, it is concluded that reduction of intrapulpal pressure and/or reversal of pulpal flow direction results in minimal fluid exudation from dentinal tubules when vital, deep dentin is acid-conditioned, making moist bonding necessary. (Supported by RGC grant 10201901, HKU)

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Effect of Calcium Phosphate Dissolved in Etchant on Dentin Bonding.
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The purpose of this study was to show the restoration mechanism of collapsed demineralized-dentin with a primer to create diffusion pathway for monomers which is essential for the hybridization with dentin and makes resin bonding to dentin possible. It was hypothesized that the collapsed demineralized-dentin could be more easily restored if amount of dissolved dentinal peptides such as phosphopro-teins could be minimized by dissolving multi-cations in etchants. Bovine dentin prepared with 600 grit paper was etched with 10% phosphoric acid dissolved 0, 3 or 5% of calcium hydrogen phosphate (Ca salt) for 10 s, rinsed and dried. This etched dentin was primed with 1% 2-methacryloyloxyethyl phenyl phosphoric acid (Phenyl-P) dissolved in 30% aqueous 2-hydroxyethyl methacrylate (HEMA) (1P-30H) for 30 s. The excess primer was removed by an air-blast. The primed demineralized dentin was applied with 100% HEMA for 30 s, and then a light-cured TEGDMA was placed for 1 min and irradi-ated for 1 min. A resin composite was polymerized on the bonding agent. Mini-dumbbell test speci-mens were trimmed from the bonded samples. The tensile bond strength (TBS) was measured. Frac-tured surfaces of specimens and hybridized dentin were analyzed by SEM. TBSs (MPa) were 9.9±2.8 (0% Ca salt), 14.2±2.9 (3% Ca salt), 15.4±5.0 (5% Ca salt), and TBS increased significantly by dissolving Ca salt ($p<0.05$, student t-test).But there was no significant difference between 3 and 5 % group ($p>0.01$). It can be concluded that Ca salt could decrease the amount of dissolved peptides by immobilization of dissolved dentinal peptides and decreasing the hydrogen bonding between collagen and the dissolved peptides. The bonding primers are required to the split hydrogen bonding for mono-mer diffusion. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (09557151, 08457503 and 09229220).