

1697 TGF- β Intracellular Signaling is Required for Inhibition of MEE Proliferation. X.-M. CUI*, J. CHEN, T. YAMAMOTO, M. MOGASS, Y. CHAI, C.F. SHULER (University of Southern California School of Dentistry, California, USA).

TGF- β is a crucial factor participating in the regulation of the disappearance of the medial edge epithelium (MEE) during murine palatal fusion. It is believed that the MEE cells stop DNA synthesis prior to undergoing epithelial-mesenchymal transdifferentiation/migration. In general, TGF- β inhibits proliferation of epithelial cells through the ligands/receptors/SMADs signaling pathway. Previous studies localized TGF- β ligand, receptors and the downstream signaling effector SMAD2 in the MEE at the time of palatal fusion. We hypothesized that TGF- β inhibited MEE proliferation through its intracellular signaling pathway required for the onset of MEE disappearance. Timed pregnant TGF- β knockout mice were injected intraperitoneally with BrdU to examine cell proliferation of MEE. The fetal heads were processed for serial sections. Every fifth section when palatal shelves appeared contact in the midline were used for BrdU staining. The number of BrdU positive cells and MEE cells per section were counted. The rate of total BrdU positive cells over total number of MEE cells per head was calculated and compared between TGF- β wild-type and null mutant groups. 150 sections from 8 heads (4 from each group) were counted. The result showed that the rate of BrdU positive cells in MEE was significantly less in wild-type than in null mutant (t -test $p < 0.05$). The absence of TGF- β resulted in continued proliferation of MEE. Using immunohistochemistry with antibodies against phosphorylated SMAD2 on sections adjacent to those used for BrdU staining, we found intense nuclear staining exclusively localized in MEE in wild-type mice. The null mutant counterpart lacked a phosphorylated SMAD2 signal. These results suggest that a functional TGF- β intracellular signaling pathway with activated SMAD2 is required for the inhibition of MEE proliferation at the onset of disappearance of MEE during palatal fusion. Supported by NIDCR grant P01 DE-12941. xcui@hsc.usc.edu

1701 Interfacial characterization of resin-modified glass-ionomers bonded to dentin. B. VAN MEERBEEK*, Y. YOSHIDA, S. INOUE, R. FUKUDA, M. OKAZAKI, P. LAMBRECHTS, G. VANHERLE (Catholic Univ. of Leuven, Belgium; Hiroshima and Hokkaido Univ., Japan)

In order to better understand the mechanism of self-adhesiveness to dentin, the interaction between three resin-modified glass-ionomers (exp. Photac. Espe: FujiBond LC, GC; Vitrebond, 3M) and dentin was ultra-morphologically and chemically characterized using transmission electron microscopy (TEM), atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS). TEM and AFM disclosed that when dentin was pre-treated with a polyalkenoic acid conditioner, exp. Photac and FujiBond LC formed a submicron hybrid layer consisting of hydroxyapatite-coated collagen fibrils. On top of the hybrid layer, a 0.5-1 μ m amorphous phase was deposited. Compared to Fuji BOND LC, the amorphous phase presented with exp. Photac was more pronounced and was even formed when the conditioner was omitted. Vitrebond resulted in a close, but abrupt transition to dentin. No morphologic features indicating hybridization or the deposition of an amorphous phase could be detected. XPS of acrylic-maleic polycarboxylic acid copolymer (exp. Photac, FujiBond LC) and of Vitrebond co-polymer applied on synthetic hydroxyapatite revealed a significant shift of the COOH peak to a lower binding energy, suggesting the formation of an ionic bond with calcium of hydroxyapatite. XPS also disclosed that the amorphous phase deposited on top of the hybrid layer represents the formation of a calcium-polycarboxylate salt through reaction of the polyalkenoic acid with calcium extracted from the dentin surface. It is concluded that the self-adhesiveness of resin-modified glass-ionomers should be attributed to both micro-mechanical interlocking through hybridization and to ionic bond formation between the polyalkenoic acid copolymer and calcium of hydroxyapatite that remained attached to the partially exposed collagen fibrils. As result from an ion-exchange process, an amorphous polycarboxylate gel is deposited, especially when sufficient calcium is extracted from the dentin surface. bart.vanmeerbeek@med.kuleuven.ac.be

1698 Ultrastructural study of an all-in-one adhesive containing pre-reacted glass ionomer fillers. *Yiu CKY, Tay FR¹, Sano H², Tagami J³, Hashimoto M³, Pashley DH⁴ (Univ. of Hong Kong, ¹Hokkaido Univ., ²Tokyo Medical & Dental, ³Medical College of Georgia)

Reacmer Bond (Shofu Inc., Kyoto, Japan), is a novel fluoride releasing, tri-curable adhesive that utilizes Pre-Reacted Glass Ionomer (PRG) technology. It utilizes both unreacted, as well as fully-reacted glass ionomer particles (F-PRG) as fillers. This study examined the ultrastructure and elemental composition of resin-dentin interfaces that were treated with this single-step adhesive. Dentin disks prepared from human third molars were abraded with either 600- or 60-grit SiC paper to create smear layers of different thickness. They were bonded using Reacmer Bond and further laminated into disk-pairs. Two strips were prepared from each disk-pair, one of which was completely demineralized. Both undemineralized (U) and demineralized (D) specimens were processed for TEM and examined both stained and unstained. Unstained sections were further coated with carbon for STEM/EDX analysis. Results: Stained "D" sections revealed the presence of a 0.5-0.8 μ m thick hybrid layer in the 600-grit specimens. The hybrid layer was reduced in thickness and only partially present in the 60-grit specimens. The overlying resin layer exhibited completely different ultrastructural features in unstained "U" and "D" sections. In "U" sections, the conventional glass ionomer filler was characterized by an electron-dense glass core that was surrounded by a hydrogel layer. The F-PRG filler contained numerous spherical, electron-dense "seeds" within the pre-reacted hydrogel. The predominant elements present were Si, Al, La, F and Ca. In the 600-grit specimens, an inhibition zone could be seen along the partially demineralized dentin surface that contained increased levels of Ca, P and F. In "D" sections, numerous artificial, electron-dense dendritic deposits, rich in Ca, P and La, were evident within the resin matrix and inside both types of glass fillers. The resin matrix was also phase-separated into filler-free domains. The presence of a fluoride-containing inhibition zone, and the appearance of dendritic depositions after laboratory demineralization suggest that continuous ion exchange is possible within the polymerized resin matrix of Reacmer Bond. This probably accounts for its fluoride releasing and recharging potential.

1702 Adhesion/Decalcification mechanisms of acid interactions with human hard tissues. M. YOSHIOKA*, Y. YOSHIDA, B. VAN MEERBEEK, P. LAMBRECHTS, G. VANHERLE, M. OKAZAKI, H. SHINTANI (Hiroshima Univ., Japan; Catholic Univ. Leuven, Belgium)

In order to study adhesion/decalcification mechanisms of acid interactions with human hard tissues such as bone and tooth, the chemical interaction of 5 carboxylic (acetic, citric, lactic, maleic and oxalic acid) and 2 inorganic acids (hydrochloric and nitric acid) with enamel and 2 synthetic hydroxyapatite powders with respectively a high (HAp-H) and low crystallinity (HAp-L) was chemically analyzed using X-ray Photoelectron Spectroscopy (XPS), Atomic Absorption Spectrophotometry (AAS) and Spectrophotometry (S). X-ray Diffraction revealed that the crystallinity of HAp-H was considerably higher than that of enamel, while the crystallinity of HAp-L was similar to that of dentin and bone. XPS of acid-treated enamel suggested for all carboxylic acids ionic bonding to apatitic substrates. AAS and S showed for HAp-H and HAp-L that all carboxylic and inorganic acids except oxalic acid extracted Ca significantly more than P, leading to a Ca/P ratio close to that of HAp (2.16 w/w). Oxalic acid hardly extracted any Ca but substantially more P, leading to a significantly smaller Ca/P ratio than that of HAp. AAS showed that the salts of oxalic acid could hardly be dissolved, whereas the calcium salts of all other acids were very soluble in their respective acid solution. These results confirm the Adhesion/Decalcification Concept (AD-concept) previously advanced (Yoshida *et al.*, 2000 J Dent Res 79:418 Abstr. 2199): Depending on the dissolution rate of the respective calcium salts, acids either adhere to or decalcify apatitic substrates. It is concluded that the AD-concept originally detailing the interaction of carboxylic acids with human hard tissues can be extended to inorganic acids, such as hydrochloric and nitric acid. Furthermore, the HAp crystallinity was found not to affect the adhesion/decalcification behavior of acids when interacting with apatitic substrates, so that the AD-concept can be applied to all human hard tissues with varying HAp crystallinity.

1699 The glass ionomer phase in resin-based restorative materials. FR TAY¹, EL PASHLEY², C HUANG¹, M HASHIMOTO³, H SANO⁴, RJ SMALES⁵, DH PASHLEY¹ (Univ. of Hong Kong, ¹Medical College of GA, ²Hokkaido Univ., ³Univ. of Adelaide)

This TEM study examined the ultrastructural manifestation of the glass ionomer (GI) phase in a resin modified glass ionomer cement (RM-GIC; Fuji II LC, GC), a giomer (Reacmer Paste, Shofu), a compomer (Dyract AP, Dentsply) and a composite (Spectrum, Dentsply), before and after water uptake. Comparisons were made with the typical GIC reaction that is present in a conventional GIC (ChemFlex, Dentsply). 2-mm thick wafers were prepared for each of the restorative materials. They were stored at 100% relative humidity for 24 h before sectioning. Additional specimens of the RM-GIC and giomer were stored in de-ionized water at 37°C for 7 days, and those of the compomer and the composite for 84 days. Unstained, undemineralized sections of all specimens were coated with carbon for TEM examination. ChemFlex contained a polyalkenoate matrix with partially and fully-reacted glass particles that were surrounded with 250-300 nm thick siliceous hydrogel layers. Thinner hydrogel layers (80-100 nm) were found on the surface of glass fillers in Fuji II LC that became thicker upon water storage. Phase separation occurred in the resin matrix and became more distinct after water uptake. Reacmer Paste contained fully pre-reacted, dehydrated GIC silica gels, unreacted reactive glass fillers, silica particles and fumed silica in a homogenous resin matrix. There was no appreciable ultrastructural change after water storage. No glass ionomer phase was observed in Dyract AP and Spectrum after 24 h. A very thin siliceous hydrogel layer (< 200nm) formed on the surfaces of the glass particles in Dyract AP after water storage for 84 days. Dyract AP contained additional stannous fluoride crystals for fluoride-release. It is concluded that the variable extent to which the GI phase is manifested in this spectrum of reactive glass-containing hybrid restoratives is determined by the difference in their liquid/resin composition. (Supported by grant DE06427 from NIDCR).

1703 Submicron hiatus in acid-etched dentin are iatrogenic artifacts. KA AGEE¹, EL PASHLEY², A ITHAGARUN³, FR TAY⁴, DH PASHLEY¹ (Medical College of Georgia, Augusta, USA, University of Hong Kong, HKSAR, CHINA)

The submicron hiatus is a term that is referred to more and more frequently in the dentin bonding literature. It represents a potential space created at the base of the demineralized collagen network when dentin is acid-etched for bonding. These spaces were observed in SEM studies after acid-etched dentin were critical point dried or dehydrated in hexamethyldisiloxane. However, they have never been identified in TEM studies of dentin hybrid layers. This TEM study critically examined the cause of submicron hiatus formation using a silver staining technique. Two multi-step, total-etch adhesives (Single Bond, 3M; One-Step, Bisco) and two single-step, self-etching adhesives (Prompt L-Pop, ESPE; One-Up Bond F, Tokuyama) were examined. Flat dentin surfaces were bonded with these adhesives and a lining composite. For each adhesive, 0.8 mm thick slabs from the same tooth were coated with nail varnish applied 1 mm from the bonded interfaces. The varnish was either left to dry completely for 10 min before immersing in 50 wt% AgNO₃ for 24 h (group D), or painted on blotted tooth slabs that were dropped immediately into the AgNO₃ solution (group M). After developing, undemineralized, unstained, epoxy resin-embedded sections were prepared for transmission electron microscopy. Nanoleakage patterns were observed for all adhesives. Fine reticular silver deposits were also found in the undemineralized dentin. In group D, submicron hiatus were seen as tunnels of heavy silver deposits beneath hybrid layers. Specifically, a hiatus occurred between the undemineralized intertubular dentin and a cohesively fractured layer of the same that was still attached to the base of the hybrid layer. Hiatus were completely absent in group M. It is concluded that submicron hiatus are iatrogenic artifacts created by desiccation during specimen processing, and should be referred to as such in future studies of bonded dentin interfaces. (Supported by grant DE06427 from NIDCR).

1700 Ultrastructure and bonding of high strength GIC to dentin. *SHY Wei¹, FR Tay¹, H Ngo², RJ Smales³, DH Pashley¹ (The University of Hong Kong; ²The University of Adelaide, Australia; ³Medical College of Georgia, USA)

This study examined the microtensile bond strength (μ TBS) and ultrastructure of ChemFlex (Dentsply DeTrey, Konstanz, Germany), a highly viscous restorative glass ionomer cement (GIC), to sound dentin that was conditioned with various techniques. Mesial and distal enamel of extracted, human third molars were removed. Dentin surfaces were abraded with 180-grit SiC paper to create standardized smear layers for placement of the GIC. Three teeth were prepared for each conditioning protocol: [C] - no polyacrylic acid (PAA) treatment (control); [I] [P] - 10% PAA for 10s, no rinsing; [R] - 10% PAA for 10s, rinsed; [K] - 25% PAA for 25s, rinsed; and [H] - 10% phosphoric acid for 15s, rinsed. A 0.5mm layer of a less viscous GIC mixture was initially used to enable better adaptation to the moist, etched dentin. This was followed by GIC vertically using the recommended liquid-powder ratio. After being stored at 100% humidity for 24h, the teeth were vertically sectioned into 0.9 x 0.9mm beams for μ TBS evaluation, using the "non-trimming" technique. Beams stressed to failure were examined with SEM. Additional unstained beams from each group were prepared for TEM examination. Both demineralized and undemineralized specimens were examined. Results of μ TBS evaluation: [C] 7.2[±]1.7 MPa, [P] 14.0[±]3.7 MPa, [R] 14.0[±]3.4 MPa, [K] 15.0[±]2.4 MPa, [H] 15.3[±]2.2 MPa. Kruskal-Wallis ANOVA and Dunn's multiple comparison tests showed that [C] has a statistically lower μ TBS ($p < 0.05$). SEM fractographic analysis revealed exclusive adhesive failures along the surface of dentin in [C]. Apparent adhesive failures in the other groups were actually mixed failures. TEM examination revealed the presence of interaction layers (IL) in all groups. In [C], the IL was restricted to the smear layer. In the other groups, IL of varying thickness could be seen in the intertubular dentin. GIC particles could be seen within dentinal tubules in [K] and [P]. It is concluded that the low μ TBS observed in [C] reflects the weakness of the smear layer attachment to dentin. Similar μ TBS seen in the other groups suggests that such values represent more of the cohesive strength of GIC under tension, rather than true adhesive strength to dentin.

1704 Nanoleakage Patterns of Caries-affected Dentin of Three Adhesive Systems. S. KUBO¹, H. LI², M.F. BURROW³, M.J. TYAS³ (Nagasaki University, School of Dentistry, Nagasaki, Japan, ²The University of Melbourne, Melbourne, Australia)

The quality of the hybrid layer created in caries-affected dentin, which may have an influence on bonding durability, is still unclear. The purpose of this study was to investigate the nanoleakage patterns of caries-affected dentin after treatment with rotary instruments or Carisolv gel, and treated with various adhesive systems: PermaQuik (Ultradent), a conventional three-step system; Single Bond (3M), a single-bottle system; and One-Up Bond F (Tokuyama), a one-step self-etching-prime and bonding system. Flat occlusal dentin surfaces including carious lesions were prepared from extracted human molars and finished with wet 600-grit silicon carbide paper. Carious dentin was removed with CarisolvTM or steel round burs in conjunction with Caries Detector. The excavated dentin surfaces and flat occlusal surfaces were bonded with PermaQuik, Single Bond or One-Up Bond F. After 24 h storage in water at 37°C, bonded interfaces were finished, and the surrounding tooth surfaces were coated with nail varnish. Specimens were immersed in 50% (w/v) silver nitrate solution for 24 h, exposed to photodeveloping solution for 8 h, and sectioned longitudinally through the bonded excavated dentin or flat dentin surfaces. The sectioned surfaces were polished and carbon coated, and observed in a Field Emission-SEM. Silver deposition occurred along the base of the hybrid layer for all specimens. However, Single Bond showed a greater density of silver deposition in the caries-affected dentin compared with normal dentin. PermaQuik had a thicker hybrid layer in caries-affected dentin than normal dentin. One-Up Bond F exhibited a thin hybrid layer in normal dentin, which was often undetectable in caries-affected dentin. Caries-affected dentin was slightly stained with silver regardless of the adhesive system used. This study suggests that the hybrid layer created in caries-affected dentin is of poorer quality than that in normal dentin. This may affect the longevity of resin composite restorations.