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Title	Does gradual dehydration affect the mechanical properties and bonding outcome of adhesives to dentin? [an abstract of dissertation and a summary of dissertation review]
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学位論文内容の要旨

博士の専攻分野の名称 博士(歯学) 氏名 チョウドリー アブ ファエム モハマッド アルマス

学位論文題名

Does gradual dehydration affect the mechanical properties and bonding outcome of adhesives to dentin?

(乾燥処理時間は象牙質の物性と接着性能に影響を与えるか?)

This study evaluated the effects of gradual dehydration on the Hardness (H) and Elastic Modulus (E) of adhesives and dentin, and on the Microtensile Bond Strength (μ TBS) of adhesives to dentin.

The study was approved by the Hokkaido University Ethical Committee (# 2013-7). All teeth were collected after the patient's informed consent. Twenty five sound human third molars were stored in an aqueous solution of 0.5% Chloramine-T at 4° C and used within 6 months of extraction. For µTBS test, flat, occlusal dentin surfaces of twenty third molars were exposed and subsequently polished with 600-grit SiC paper (Sankyo-Rikagaku Co., Saitama, Japan) under running water for 60 s. They were then randomly divided into four groups (n =5): Clearfil Mega Bond Wet (MBW), Clearfil Mega Bond Dry (MBD), Clearfil SE Bond 2 Wet (SE2W) and Clearfil SE Bond 2 Dry (SE2D). Adhesives were applied according to the manufacturer's instruction and light cured (Optilux 401, Demetron/Kerr, Orange, CA, USA) at ≥550 mW/cm². Following composite resin (Clearfil AP-X, Kuraray Co, Ltd, Osaka, Japan) build-up, the specimens were stored in distilled water at 37° C for 24 h. Resin/dentin beams (cross-sectional area: 1 mm²) were prepared by a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA) and a total of 15 beams per group were randomly selected and tested. A pilot study established that it takes approximately 3 min to remove each bonded beam from the storage medium, wipe off water, measure the cross-sectional area and attach to the Ciucchi's jig with a cyanoacrylate adhesive (Model Repair II Pink, Dentsply-Sankin, Tokyo, Japan). In the wet groups (MBW and SE2W), each beam was tested 2 min after fixing to the grips of the testing device to allow adequate setting of the cyanoacrylate adhesive to prevent glue failure. During this period, a small piece of wet paper (Kimwipe S-200, Nippon paper Crecia Co., Tokyo, Japan) was used to cover the beams. In the dry groups (MBD and SE2D), each beam was kept on the bench for 7 min after fixing to the jig without wet paper covering. Therefore, the wet group was tested at 5 min after removal from the storage medium and the beams were kept wet until tested; and the dry group was tested at 10 min after removal from the distilled water and underwent free dehydration during this period. All tests were conducted at room conditions (23° C and 30% RH). The µTBS test was carried out at a crosshead speed of 1 mm/min (EZ-S, Shimadzu Co., Kyoto, Japan) until failure occurred. The data were analyzed by two-way ANOVA to demonstrate the effects of adhesive and condition (dry vs. wet), followed by Tukey's test at 5% level of significance. The fractured specimens were coated with Pt-Pd (E-1030, Hitachi, Tokyo, Japan), and were observed using a field emission scanning electron microscope (FE-SEM; S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10kV. Failure modes were categorized as: A, Adhesive failure; CD, Cohesive failure in dentin; CC, Cohesive failure in composite; M, Mixed failure.

Five dentin slabs (1 per tooth; approximately 11 mm x 1.5 mm) were prepared (IsoMet 1000, Buehler, Lake Bluff, IL, USA) and then sequentially finished with no. 1000, 1200, and 2000-

grit waterproof SiC paper (Sankyo-Rikagaku Co., Saitama, Japan) under running water; and polished with 6, 3, and 1 µm particle size diamond pastes (DP-Paste, Struers, Denmark) for a period of 1 min each. The specimens were cleaned (Fine ultrasonic cleaner, Gao Hui Mechanical and Electrical International Trade Co. Ltd., Nanjing, China; model FU-2H) with phosphate buffered saline solution (PBS; Wako Pure Chemical Ind., Ltd., Osaka, Japan) for 3 min after every finishing and polishing step. The slabs were then further cut to prepare 1 mm^2 beams and preserved in PBS until used. Adhesive disks (n = 5/adhesive) measuring 2.0 ± 0.3 mm in thickness and 10 ± 0.2 mm in diameter were also prepared from plastic ring molds and then stored in distilled water at 37° C for 24 h before testing. For H and E test, dentin beams (n = 15; 3/tooth) were removed from PBS, blotted of excess water (Kimwipe S-200, Nippon paper Crecia Co., Tokyo, Japan), fixed on glass slides and tested with a dynamic ultra micro hardness tester (DUH-211, Shimadzu, Japan). The intertubular dentin at the centre of each beam was targeted. If any part of the indentation mark occurred on a dentinal tubule, the data was discarded and retaken. Indentations were performed at 5 min, 10 min, 15 min, 20 min, 1 h and 24 h after removal from the PBS, with maximum loads of 5.04 mN, at a constant speed of 0.2926 mN/s, with a 45 s holding at peak load. Adhesive discs (n = 5/adhesive) were also tested at similar time intervals and loading configurations except the holding at the peak load was 10 s. H and E values were obtained from the default software of the testing device. All the samples were tested in the range of ambient temperatures 22° C -24° C with a maximum humidity of 30%. At least a 10 µm distance between adjacent indentations was maintained and the Poisson's ratio assumed was 0.3.

For weight-loss test, polished dentin beams (n = 5, 1/tooth) were removed from PBS, quickly blotted dry and the weight loss of the specimens was recorded (METTLER TOLEDO, AB204-S Analytical Balance) after 5 min, 10 min, 15 min, 20 min, 1 h and 24 h of free, ambient dehydration. Adhesive discs (n = 5/group) were also weighed in the same sequence as dentin. H, E and weight loss data of dentin and adhesives were subjected to one-way repeated measures ANOVA, followed by Bonferroni's test at a 5% level of significance.

Two-way ANOVA indicated significant differences in bond strength for adhesives (F= 8.97; p < 0.05) and for conditions (dry vs. wet; F= 15.63; p < 0.001). The interaction between the factors was not significant (F= 2.004; p > 0.05). Contrary to SE2 (p > 0.05), dehydration significantly increased the µTBS of MB (p < 0.05). However, SE2 showed significantly higher µTBS than MB (SE2W vs. MBW; p < 0.05) without dehydration. SEM observations revealed a predominance of cohesive dentin failure in all the groups except for MBW, where the mixed failure prevailed over other failure patterns.

Gradual dehydration caused significant differences between MB's mean H (F= 68.685; p < 0.001) and E (F= 4.635; p < 0.05) values obtained at the tested time points. While H became significantly different at 20 min (p < 0.05) from the baseline (5 min), for E, the difference became significant at 24 h (p < 0.05). Similar trend was observed for SE2's mean H (F= 34.856; p < 0.001) and E (F= 6.004; p < 0.05) values, where H became significantly different at 1 h (p < 0.05). However, for E, the difference became significant at 24 h (p < 0.05). In case of dentin, gradual dehydration only caused significant differences between mean H (F= 11.260; p < 0.001) values which became significantly different at 24 h (p < 0.05).

Gradual dehydration caused significant differences between mean weight values of dentin beams (F= 15.807; p < 0.001), MB discs (F= 160.977; p < 0.001) and SE2 discs (F= 193.627; p < 0.001). For dentin, the difference became significant at 10 min (p < 0.001) from the baseline (5 min), for MB discs at 20 min (p < 0.05) and for SE2 discs at 10 min (p < 0.05).

Gradual dehydration of μTBS testing specimens can cause significant changes in the test outcomes and should be avoided as a significant source of test variation.