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Title	Is the double-sided microtensile bond strength test appropriate to assess the performance of adhesives to dentin? [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(歯学)
Dissertation Number	甲第13483号
Issue Date	2019-03-25
Doc URL	https://hdl.handle.net/2115/74022
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/4.0/
Type	doctoral thesis
File Information	Ahmed_Zubaer_abstract.pdf, 論文内容の要旨



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 アハメッド ズバエル

学位論文題名

Is the double-sided microtensile bond strength test appropriate to assess the performance of adhesives to dentin?

(両側微小引張試験は象牙質歯質接着性を評価する際に妥当か?)

This study was to compare the conventional μ TBS test with an alternative double-sided μ TBS test using self-etch adhesives.

Sixty non-carious human molars were used for this study. The teeth were collected under a protocol reviewed and approved by the University Ethics Committee (2014-1). After extraction they were stored in an aqueous solution of 0.5% chloramine-T at 4°C. Four current self-etch adhesives were used: Clearfil™ SE Bond2 ((SE, Kuraray Noritake Dental Inc., Kurashiki, Japan), Clearfil™ Universal Bond (CU, Kuraray Noritake Dental Inc., Kurashiki, Japan), Scotchbond™ Universal (SB, 3M ESPE, St Paul, MN, USA) and G-Premio Bond (GP, GC Corporation, Tokyo, Japan).

This study was designed into three experiments. In Experiment-1, to evaluate the bond strength of different self-etch adhesive systems was assessed by means of conventional microtensile bond strength test (n=4). Sixteen human molars were randomly divided into four adhesive groups: SE, CU, SB, GP. Teeth were sectioned on the mid portion of dentin using a diamond saw mounted in low-speed cutting machine (IsoMet 1000, Buehler, Lake Bluff, IL, USA) under copious water. Exposed dentin surfaces were polished with 600-grit SiC paper (Sankyo-Rikagaku Co., Saitama, Japan) for 60 s. The adhesives were applied on dentin surfaces according to manufacturer's instructions. Composite resin were built-up (Clearfil AP-X, shade A2, Kuraray Noritake Dental Inc., Kurashiki, Japan.). Prepared teeth were stored in distilled water at 37 °C for 24 h.

In Experiment-2, to determine the appropriate dentin disc thickness for double-sided μ TBS test. The bond strength values of SE and CU were assessed using the double-sided test. The effect of dentin thickness on the bond strength was verified (n=8). Thirty-two human molars were randomly divided into four dentin thickness groups: 0.5, 1.0, 1.5 and 2.0 mm. The dentin discs were prepared by removing the dentin from the pulp side, that is, 0.5 mm of dentin refers to the superficial dentin. Both adhesives were bonded simultaneously on the same dentin disc. They were used alternately, that is, when SE as applied to the coronal side of the dentin disc, CU was applied to the pulpal side and vice-versa. Teeth were prepared and stored as described above in Experiment-1.

EXP-3 was employed to compare simultaneously different adhesives using same dentin beam. The double-sided test was used to assess the bond strength of CU, SB and GP to dentin. The effect of dentin thickness on the bond strength was verified (n=4). 1-mm thick

dentin discs were used, and both surfaces (coronal and pulpal) were simultaneously and alternately bonded with the combination of pairs of adhesives. In total, there were six combinations of adhesives systems. Teeth were prepared and stored as described in Experiment-1.

After storage, each sample was section in both, “x” and “y” directions, to obtain beams (cross sectional area 1 mm²), using an Isomet diamond saw (Isomet 1000, Buehler, Lake Bluff, Illinois, USA). The beams were fixed to a Ciucchi’s jig with cyanoacrylate glue (Model repair 2 Blue, Dentsply-Sankin, Otahara, Japan) and subjected to a tensile force at a crosshead speed of 1 mm/min in a desktop testing apparatus (EZ test, Shimadzu, Kyoto, Japan).

μTBS results were expressed in MPa. Data were tested for normality and homoscedasticity ($p < 0.05$). In case of Experiment-1 and Experiment-3, data was statistically analyzed using One-way ANOVA followed by Tukey’s *post hoc* test. For Experiment-2 Two-way ANOVA followed by Tukey’s *post hoc* test was used. Statistical analyses were performed with SPSS software (SPSS statistics version 20.0, SPSS, Chicago, IL, USA) ($p < 0.05$). Failure modes of the specimens were observed using the stereoscope (20x; Asone, Osaka, Japan). Further, the fractured specimens were mounted on an aluminum stub, then coated with Pt-Pd for 120 s (Ion sputter, E-1030, Hitachi, Tokyo, Japan). Coated beams were observed using a scanning electron microscope (SEM, S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV. Fracture and specific features on dentin surfaces were examined at lower magnification (80×) to categorize the mode of fractured.

There were no pre-test failures in this study. In case of Experiment-1, One-way ANOVA analysis revealed significant difference in μTBS means of tested adhesives to dentin ($p < 0.05$). The pairwise comparison using Tukey’s *post hoc* test showed that SE presented the highest bond strength values followed by SB>CU> GP. In Experiment-2, the Two-way ANOVA analysis revealed that dentin disc thickness significantly affected the μTBS of adhesives ($p < 0.05$). The Tukey’s *post hoc* analysis showed that using 1.5 mm and 2.0 mm dentin thickness produced the lowest bond strength results. 1.0 mm dentin disc thickness showed to be most appropriate substrate for double-sided μTBS test. In Experiment-3, the One-way ANOVA analysis showed that differences on bond strength were found for adhesives ($p < 0.05$). According to the *post hoc* pairwise comparisons, there was no significant difference between mean microtensile bond strength values from paired-adhesives ($p < 0.05$). SB-CU and CU-SB groups showed the highest bond strength values and there was no significant difference between these values (respectively when SB was at coronal and pulpal side).

In general, the failure modes were mainly categorized as adhesive failure, cohesive failure and mixed failure. SEM observation confirmed that most failures were mixed. In Experiment-2, The bonded dentin beams always failed on CU side. In Experiment-3, most failure occurred on GP side followed by SB and CU sides.

From the results of this study, it might be concluded that Conventional μTBS testing was still considered a good technique to assess the bond strength of adhesives to dentin. Within the limitations of this study, double-sided μTBS test is not always applicable to assess the performance of adhesives to dentin. Further investigations should be conducted to better explore the double-sided μTBS test.