**“Effect of Chlorhexidine on Nanoleakage and Bond Durability of Two Adhesives to Dentin”**

**“An In Vitro Study”**

**Introduction**

Nanoleakage (NL) refers to leaking through nanometer-sized channels inside the hybrid layer and/or the adhesive layer 1. NL could act as a path for water movement at the adhesive dentin interface. The water movement may extract unconverted monomers from adhesive resins over time, and so may affect bond durability 2.

Chlorhexidine is one of the most used agents for the inhibition of matrix metalloproteinases (MMPs) that are responsible for the breakdown of exposed collagen fibrils 3. Therefore, the aim of this study was to investigate the effect of chlorhexidine on nanoleakage and dentin bond durability of two universal adhesives after water storage.

The null hypothesis tested was that the use of chlorhexidine either as a therapeutic primer before the use of universal adhesives, or as being incorporated into these adhesives will not affect nanoleakage and bond durability of these adhesives to dentin.

**Materials and Methods**

**Tooth Selection and Preparation**

36 sound human lower molars were selected for this study. Teeth were extracted at Future University Dental Hospital, oral surgery department, after signing a consent. After teeth extraction, 0.5% chloramine was used to disinfect them, then they were stored in distilled water at 4°C in the refrigerator for 2-3 months before preparation 4.The enamel buccal surfaces of all teethwere removed to expose flat dentin surfaces.

**Grouping of the teeth and restorative procedures**

Materials’ name, chemical composition, manufacturer, lot number and company site are represented in **Table 1**.

The teeth were randomly assigned into three groups (n = 12) according to the bonding procedure performed (A). In the first group (A1) (SBU), All the dentin surfaces were etched with 35% phosphoric acid etchant for 15 seconds, rinsed with water for 10 seconds and dried for 2 seconds 5,6. Single Bond Universal Adhesive was applied and rubbed on dentin for 20 seconds 5,6, it was then air thinned and finally cured for 10 seconds according to the manufacturer’s instruction using LED light curing unit (Woodpecker, China) at intensity 1200 mW/Cm ².

In the second group (A2) (PUB), acid etch was also applied for 15 seconds onto dentin surfaces, then it was rinsed thoroughly for 5 seconds, and dried lightly using air/water syringe, after that a puddle coat of Peak Universal Bond was applied with a micro-brush and gently agitated for 10 seconds 7,8. It was air thinned for 10 seconds using ¼ to ½ air pressure 7,8 and light cured for 10 seconds.

In the third group (A3) (CHX+SBU), 2.0% Chlorhexidine Gluconate Disinfecting Solution was applied with a micro-brush after dentin etching and prior to adhesive application as a therapeutic primer 8 for 60 seconds under a slight rubbing motion, excess was removed with gentle air jet, leaving the dentin surface saturated with moisture 7. SBU was applied to dentin surfaces as mentioned in the first group (A1).

Resin Composite was built-up on the adhesive-applied dentin surfaces in three increments, each increment was 1.5 mm thick and constructed using a split teflon mold (7-mm internal length, 4-mm width and height of 4.5 mm). Buildups were light cured for 20 seconds.

Teeth for each bonding procedure were further subdivided into three subgroups (n=4), according to the period of storage in distilled water (S): either 24 hours (S1), three months (S2) or six months (S3) 9,10.

**Nanoleakage Evaluation**

After storage, specimens were vertically sectioned with a diamond saw under water coolant, across the adhesive/tooth substrate interface, into approximately 1-mm-thick slabs 2,1,11, 12. Three slabs, from each tooth at each storage period, were covered with nail varnish, leaving only 1 mm exposed at the bonded interface, and processed for nanoleakage evaluation (n=12).

These slabs were stored in 50 wt% ammoniacal silver nitrate in dark room for a day, followed by rinsing in distilled water. Under a fluorescent light, slabs were placed in photo-developing solution for 8 hours. Discs with different grits and 0.25 μm diamond paste were used for slabs polishing 5,6. Specimens were mounted on aluminum stubs and gold sputtered 4. The interfaces between resin and dentin were analyzed in a field-emission scanning electron microscope (FE-SEM) (The Quanta FEG-250 SEM, United States) operated in backscattered electron mode 5,6 at a magnification of 2000x. The NL in both adhesive and hybrid layers of each bonded slab was measured using energy-dispersive analytical X-ray (EDAX) 1,13,14,4. The nanoleakage was presented as a percentage of the evaluated area.

**Statistical analysis**

Data were presented as mean and standard deviation values, explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed parametric distribution.

One-way ANOVA was used to compare between the three groups in non-related samples followed by Tukey post hoc test. To compare between the three groups in related samples, repeated measure ANOVA was used. Paired sample t-test was used for the comparison between two groups in related samples.

Two-way ANOVA was used to study the interaction between the two variables.

The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

**Results**

Mean and standard deviation (SD) of silver ion uptake (wt. %) for each bonding procedure regardless of storage period are presented in **Table 2**.

CHX + SBU adhesive (A3) bonding procedure showed statistically the highest nanoleakage (2.09 ± 1.21), followed by Peak Universal Bond (A2) with nanoleakage value of (1.30 ± 0.55), then by Single Bond Universal Adhesive (A1) which showed the lowest nanoleakage value of (1.22 ± 0.67), where (*p*=0.034). There was no statistically significant difference between (A2 & A1) or between (A2 & A3), only a statistically significant difference was found between (A1)and (A3).

Mean and standard deviation (SD) of silver ion uptake (wt. %) for each storage period ( after 24 hours, three and six months) of the tested specimens regardless of the bonding procedure are presented in **Table 3.**

The highest nanoleakage was observed after six months of water storage (S3) (2.30 ± 0.84), intermediate values of nanoleakage were observed after three months of water storage (S2) (1.48 ± 0.81), and after 24 hours of water storage the lowest nanoleakage values were observed (S1) (0.82 ± 0.39), where (*p*<0.001). There was no statistically significant difference between (S1) and (S2), but a statistically significant difference was found between (S3) and each of (S1) and (S2).

The mean and standard deviation (SD) of silver ion uptake (wt. %) for each bonding procedure at each storage period are presented in **Table 4.**

After storage for 24 hours; PUB (A2) showed the highest nanoleakage (1.17± 0.48), followed by SBU (A1) with nanoleakage value of (0.69 ± 0.16), then by CHX + SBU which showed the lowest nanoleakage values (A3) (0.61± 0.22), where (*p*=0.070). There was no statistically significant difference between the three groups (A1), (A2) and (A3).

After storage for three and six months; CHX + SBU (A3) showed statistically the highest nanoleakage, followed by SBU (A1), then by PUB which showed the lowest nanoleakage values (A2). There was no statistically significant difference between (A1) and (A2), but a statistically significant difference occurred between both of them (A1 &A2) and (A3).

FE-SEM photomicrographs derived from backscattered electron mode at magnification of 2000 and EDAX histograms are shown in **Figures 1&2** respectively.

SBU nanoleakage pattern revealed almost no or very faint silver uptake at the interface after 24 hours. After storage, silver deposition increased and was found at the base of the hybrid layer. For PUB, a thick continuous linear silver accumulation through the entire thickness of the hybrid layer together with scattered silver deposits within the adhesive layer was found after 24 hours. After 3 months of storage, very faint silver uptake at the base of the hybrid layer was shown, but after 6 months of storage, dense deposits of silver in the form of water channels within the hybrid layer and almost a continuous linear silver uptake within the adhesive layer were observed. For CHX+ SBU, some reticular areas of silver uptake at the bottom of the hybrid layer were observed after one day of storage. After 3 months, silver-stained bands which were thick and continuous appeared. Water trees increased in number and height and became more prominent within the adhesive layer after longer storage in water (6 months).

**Discussion**

This study was carried out to investigate the effect of chlorhexidine on nanoleakage and dentin bond durability of two universal adhesives after different storage periods. The null hypothesis was partially accepted since CHX incorporated inside the adhesive revealed insignificant lower nanoleakage values after three and six months of water storage when compared to CHX free adhesive, on the other hand CHX as a therapeutic primer revealed the highest significant nanoleakage values after three and six months of water storage when compared with CHX containing and CHX free universal adhesives.

The nanoleakage evaluation was performed using a high-magnification field-emission scanning electron microscope in conjunction with EDAX which enabled distinct images to be captured together with sensitive and accurate analysis (2).

Water storage caused an increase in nanoleakage of SBU after six months, followed by three months, then by 24 hours with a statistically significant difference between six months and 24 hours. This might be attributed to the lack of a hydrophobic bonding resin in simplified Single Bond Universal Adhesive formulation. This reduced the bond stability over time, and the bonded interfaces behaved as semi-permeable membranes allowing the movement of water across them and expediting hydrolytic degradation.

These results were in agreement with **Marchesi et al** (15), who tested the performance of Scotchbond Universal over time using different bonding techniques on human coronal dentin. They found that long-term aging resulted in increased nanoleakage expression.

PUB produced an adhesive interface with non-significant higher deposition of NL after 24 hrs of water storage that decreased after three months but as said this decrease was non-significant. The low pH (pH = 2) of PUB might have caused an additional etching of the dentinal substrate, probably resulting in an increase in the demineralization and collagen exposure thereby increasing the NL at the beginning. These findings were in accordance with **Munoz et al** (6), who reported that performance of universal adhesives with water storage was shown to be material-dependent.

PUB showed its highest nanoleakage after six months of water storage. The presence of CHX in the primer or resin can sig­nificantly improve the resin- dentin bond stability, but chlorhexidine got released over time with gradual decrease in its concentration, therefore its inhibitory effect on MMPs decreased over time (16).

CHX + SBU adhesive showed an increase in nanoleakage after three and six months of water storage with a significant higher difference from the nanoleakage values after 24 hrs. This was because of the solubility of CHX and its large molecular size, it might have been leached out gradually at the resin-dentin interface, and so the remaining concentration became no longer appropriate to exert noticeable antiproteolytic effect (17).

Moreover, the combined application of CHX and MDP containing SBU increased the collagenolytic and gelatinolytic activities, as well as hydrolytic degradation of collagen fibrils, suggesting adverse effect on the inhibition of MMPs. Also CHX might have affected the formation of collagen protective MDP–Ca salts (11).

These findings were in accordance with, **Giacomini et al,** (18)reported that the proteolytic enzyme inhibition of CHX did not occur when it was used in association with a universal bonding system containing MDP, suggesting a negative interaction between CHX and 10-MDP.

On the other hand the results of this study were not in agreement with **Şişmanoğlu et al,** (19) whofound thatchlorhexidine treatment significantly improved adhesive performance of all tested universal adhesives containing MDP.

CHX had no prominent effect on bond durability and nanoleakage expression but still it is better to use it incorporated within the adhesive as recommended by the manufacturers.

**Conclusions**

Under the limitations of the present study the following conclusions were made:

1. The adhesive material itself still represents the corner stone of universal adhesive systems’ behavior.
2. Chlorhexidine containing universal adhesive suppressed the occurrence of nanoleakage and improved bond durability, while chlorhexidine as a separate therapeutic primer step had an adverse effect.
3. Prolonged time of water storage negatively affected bond durability of chlorhexidine containing and free universal adhesives by showing more nanoleakage at the interface.

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**Table 1:** Materials’name, chemical composition, manufacturer, lot number and company site.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Materials’ name** | **Chemical composition** | **Manufacturer** | **Lot number** | **Company site** |
| **Single Bond™ Universal Adhesive** | -MDP Phosphate Monomer  -Dimethacrylate resins  -Vitrebond™ Copolymer  -HEMA  - Filler  - Ethanol  -Water  - Initiators  - Silane  -PH=2.7 | 3M ESPE Deutschland GmbH  Germany  3M™ Single Bond Universal. | 00131A | multimedia.3m.com/. |
| **Peak™ Universal Bond.** | - ethyl alcohol  -2-hydroxyethyl Methacrylate (HEMA)  -Methacrylic Acid  -Chlorhexidine di(acetate) 0.2%  -PH=2.0 | Ultradent Products Inc.  505 W. Ultradent Drive (10200 S)  South Jordan, UT 84095,  USA | BJ1K7 | onlineordersupport@utradent.com |
| **Consepsis™ Chlorhexidine Gluconate Disinfecting Solution** | - A lightly flavored 2.0% chlorhexidine gluconate disinfecting solution.  - contains no surfactants or emollients. | Ultradent Products Inc.  505 W. Ultradent Drive (10200 S)  South Jordan, UT 84095 , USA | BHXL1 | onlineordersupport@ultradent.com |
| **Ultra-Etch™** | -35% phosphoric etchant solution  - contains no glycerin | Ultradent Products Inc.  505 W. Ultradent Drive (10200 S)  South Jordan, UT 84095, USA | BHXVJ | onlineordersupport@ultradent.com |
| **Filtek™ Z250 XT**  **Nano-Hybrid Universal**  **Resin composite** | -BIS-GMA  -UDMA  -BIS-EMA  -PEGDMA  -TEGDMA  -combination of surface modified zirconia/silica and 20 nm particles modified silica particles. | 3M ESPE  USA  Filtek™ Z250 XT - 3M | NA81959 | multimedia.3m.com/ |

Table 2: Mean and standard deviation of silver ion uptake (wt. %) of each bonding procedure regardless of storage period.

|  |  |  |
| --- | --- | --- |
| **Bonding Procedure** | **Silver Ion**  **Uptake (wt. %)** | |
|  | **Mean** | **SD** |
| **Single Bond Universal** | 1.22 | 0.67 |
| **Peak Bond Universal** | 1.3 | 0.55 |
| **CHX + Single Bond Universal** | 2.09 | 1.21 |
| **p-value** | **0.034\*** | |

\*; significant (p<0.05)

Table 3 Mean and standard deviation of silver ion uptake (wt. %) of each storage period regardless of the bonding procedure.

|  |  |  |
| --- | --- | --- |
| **Storage Period** | **Silver Ion Uptake**  **(wt. %)** | |
|  | **Mean** | **SD** |
| **24 hours** | 0.82 | 0.39 |
| **3 Months** | 1.48 | 0.81 |
| **6 Months** | 2.3 | 0.84 |
| **p-value** | **<0.001\*** | |

\*; significant (p<0.05)

Table 4: Mean and standard deviation of silver ion uptake (wt. %) at the three storage periods of the three bonding procedures.

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|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Silver Ion Uptake (wt. %)** | | | | | | |
|  | **Single Bond Universal** | | **Peak Universal Bond** | | **CHX+ Single Bond Universal** | | **P-value** |
|  | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |  |
| **24 hours** | 0.69 | 0.16 | 1.17 | 0.48 | 0.61 | 0.22 | **0.070ns** |
| **3 months** | 1.04 | 0.48 | 0.99 | 0.68 | 2.4 | 0.13 | **0.004\*** |
| **6 months** | 1.93 | 0.55 | 1.73 | 0.12 | 3.25 | 0.68 | **0.005\*** |
| **p-value** | **0.038\*** | | **0.092ns** | | **<0.001\*** | |  |

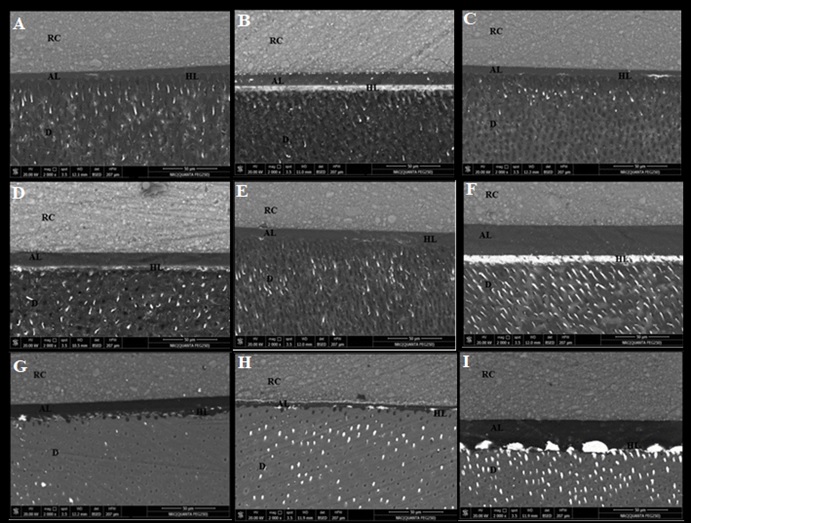
\*; significant (p<0.05) ns; non-significant (p>0.05)

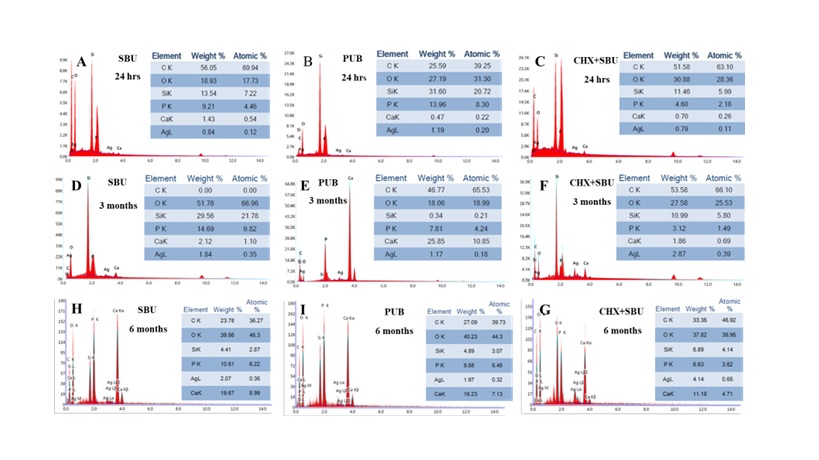
**6 months 3 months 24 hours**

**SBU PUB CHX+SBU**

**Figure 1.** Representative backscattered SEM images of the resin-dentin adhesive interfaces of each bonding procedure for 24 hours, three months and six months of water storage. RC: resin composite; D: dentin; HL: hybrid layer and AL: adhesive layer.

**A**





**Figure 2.** EDAX element analysis of the three bonding procedures at the three storage periods.