# Effect of Qmix and Other Irrigants on Dentin Adhesives in Pulp Chambers of Primary Teeth: SEM Study

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***Objective:*** *To compare the effect of different endodontic irrigants on microleakage of adhesives used within pulp chamber of primary molars.* ***Study Design:*** *72 Primary molars were divided into 6 groups, according to 3 irrigants and 2 adhesives used. After de-roofing the pulp chamber, pulp was extirpated. In 36 samples, pulp chambers were bonded with Xeno V⁺ after irrigation with either QMix (Group1); 17%EDTA+5%NaOCl (Group2) or normal saline (Group3) and in other half samples, pulp chambers were bonded with SinglebondUniversal after irrigation with either QMix (Group 4); 17%EDTA+5% NaOCl (Group5) or normal saline (Group 6). All the samples were restored with Filtek Z350. Ten teeth from each group were assessed for dye penetration. Two samples in each group were viewed under scanning electron microscope. Data was statistically analyzed using Mann–Whitney and Kruskal Wallis tests at a significance level of $P < 0.05$.* ***Results:*** *Mean microleakage scores were: Group 1-1.5±0.70, Group 2-1.6±.51, Group 3-2.4±0.96, Group 4-1.2±0.42, Group 5-1.2±0.42, Group 6-1.1±0.32.* ***Conclusions:*** *Irrigation with QMix significantly reduced the microleakage of XenoV⁺ but had no significant effect on microleakage of SinglebondUniversal. Irrigation with EDTA/NaOCl or QMix had no detrimental effect on the sealing ability of either of the adhesive tested.*

*Key words:–Qmix, Endodontic irrigants, Primary molars, Pulp chamber dentin, SEM.*

## INTRODUCTION

Preserving the integrity of primary dentition is the most important aspect of preventive dentistry. It is vital to retain the primary dentition until its normal exfoliation, as it is essential for the growth of facial-skeletal complex. One of the common reasons for the premature loss of primary teeth is dental caries. An alternative to avoid such loss would be an endodontic treatment, in which the main goal is to preserve the teeth in the same position. A clean root canal system along with a three-dimensional seal is the clinician's path to success. It is a simple procedure, by which the loose, necrotic, contaminated materials are flushed away.[1]

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The success of endodontic treatment depends on the eradication of microorganisms from the root canal system and prevention of reinfection.[2] Mechanical instrumentation alone does not result in a bacteria free root canal system, when the complex anatomy is considered. Thus, irrigants are essential to ensure bacterial minimization and elimination of organic tissue remnants. An ideal root canal irrigant must have maximum tissue dissolving and antibacterial effect, and must induce mild or no inflammatory response in the tissues.[3]

Bonding to pulp chamber dentin is differently affected by different endodontic chemical irrigants.[4] These irrigants are used during shaping and cleaning of the root canal space. Sodium hypochlorite has been extensively used in endodontic therapy to provide gross debridement, disinfection, lubrication, and dissolution of tissues.[5]

EDTA is an effective chelating agent, which is widely used in endodontic preparation. It effectively removes smear layer by chelating the inorganic components of the dentine. Therefore, by facilitating cleaning and removal of infected tissue, EDTA contributes to the elimination of bacteria in the root canal. It has also been shown that removal of the smear layer by EDTA improves the antibacterial effect of locally used disinfecting agents in deeper layers of dentin.[6] Niu et al studied the ultrastructure on canal walls after EDTA and combined EDTA +NaOCl irrigation by scanning electron microscopy and found that more debris was removed by irrigation with EDTA followed by NaOCl than with EDTA alone.[7]

Q-Mix is a newer irrigant developed by Dr. Markus Haapasalo et al, UBC, Canada. This irrigant eradicates bacteria, removes smear layer and persists in bio films. It contains ethylenediaminetetraacetic acid (EDTA), Chlorhexidine and cetrimide (N-Cetyl N,N,N-TriM-tehylAmmoniumBromide) mixed in distilled water with acceptable additional salt and its pH is slightly above neutral.[8] Stojicic et al. investigated the effectiveness of smear layer removal by Qmix using scanning electron microscopy. QMix removed the smear layer equally as well as they concluded that the ability to remove the smear layer by Qmix was comparable to that of EDTA.[9]

The immediate sealing of endodontically treated teeth using restorative materials is a powerful tool in preventing early coronal leakage. Among non-temporary restorative materials, dentin adhesives have been advocated for use within the pulp chamber in an attempt to work as a durable barrier against microleakage hampering apical and coronal microleakage.[10,11]

Various studies regarding use of irrigants in alteration of coronal dentin are present but very few depict effect in sealing ability of self-etch adhesives to pulpal dentin in primary teeth. Therefore, this in vitro study aims to evaluate and compare the effect of different endodontic irrigants on the microleakage of two self-etch adhesives placed within the pulp chamber of primary teeth and scanning electron microscope is done to see the resin-dentine interface.

## MATERIALS AND METHOD

This study was approved by the Ethics Committee for Research of the institution.

Seventy two freshly extracted human primary molars that were extracted due to serial extraction, trauma and orthodontic reasons with more than two-thirds of the root length remaining were collected and teeth were stored in saline at room temperature before the experiment. Roof of the pulp chamber was exposed using a carborundum disc (Bego, Germany) horizontally 1.5 mm coronal to the cementoenemel junction and roots were sectioned 2 mm apical to the bifurcation with water coolant. Pulp tissue was removed carefully with the help of excavator and broaches (Spirocolorinox, Dentsply Maillefer, Switzerland). Canal orifices were widened with Gates Glidden (Gates drills, Mani, Inc., Tochigi, Japan) drill no. 2-3. Care was taken to replace broach after preparation of every four samples to avoid compromising the efficient biomechanical preparation. Cavities were prepared at end of roots by straight fissure bur to a depth of 2mm and were sealed with interim restorative material.

Teeth were randomly divided into two groups of 36 teeth each according to two adhesives used: Xeno $V^+$ (Dentsply De Trey, Konstanz, Germany) and Single bond universal SBU ( 3M ESPE, St Paul, USA). In SBU group, etchant (Adper Scotch Bond- 3M ESPE) was used prior to application of adhesive. Therefore SBU act as total-etch.

Each group was further sub-divided into three groups of 12 teeth each, according to the irrigation solution used: Qmix 2 in 1(Dentsply Tulsa Dental Specialties, Tulsa, USA); 17% EDTA ( SmearClear, Kerr, SybronEndo, Glendora, USA) and 5% NaOCl or saline.

- Group 1: (Xeno$V^+$ with QMix) Pulp chambers were continuously irrigated with 1 ml of QMix for 60 s.
- Group 2: (Xeno$V^+$ with EDTA followed by NaOCl) Pulp chambers were continuously irrigated with 1 ml of EDTA for 60 seconds followed with 3 ml of 5% NaOCl for 60 s.
- Group 3: (Xeno$V^+$ with normal saline as control) Pulp chambers were continuously irrigated with normal saline solution for 60 s.
- Group 4: (SBU with QMix) Pulp chambers were continuously irrigated with 1 ml of QMix for 60 s.
- Group 5: (SBU with EDTA followed by NaOCl ) Pulp chambers were continuously irrigated with 1 ml of EDTA for 60 s followed with 3 ml of NaOCl for 60 s.
- Group 6: (SBU with normal saline as control) Pulp chambers were continuously irrigated with normal saline solution for 60 s.

Distilled water was used in all the above samples for 60 s as final flush and was blot dried.

Pulp chambers in groups 1, 2, and 3 were bonded with Xeno $V^+$ and groups 4, 5, and 6 were bonded with Single Bond Universal adhesive according to manufacturer's directions. All the samples were restored with composite resin Filtek 350 (3M, ESPE, St. Paul, USA) in one to two increments and condensed with condenser and were light cured at 500 mw/ $cm^2$ by Spectrum 800 (Dentsply, Caulk, Milford, USA) for 20 s. Ten teeth from each group were used for dye penetration and two specimens were subjected to scanning electron microscopic analysis.

### Preparation of samples for microleakage

Sixty teeth were used for microleakage testing by stereomicroscope (10 samples per group). Specimens were coated with two layers of nail polish leaving 1 mm window around restoration margin and were immersed in 2% methylene blue for 2 days. They were washed under running water and air dried at room temperature for 24 h. All teeth were than sectioned for evaluation under stereomicroscope and were scored [Table 1].

**Table 1: Dye leakage scoring criteria**

| Dye Leakage Score | Criteria For Scoring |
|---|---|
| 0 | NO LEAKAGE |
| 1 | LEAKAGE EXTENDING INTO PULP CHAMBER |
| 2 | LEAKAGE INVOLVING PULP FLOOR |
| 3 | LEAKAGE INVOLVING ROOT CANAL |

### Preparation of samples for analysis of surface morphology using SEM

12 primary molars (2 samples per group) were used for SEM analysis. After being restored with composite, the sectioned surface of molars were ground on wet 210 grit SiC paper to a flat enamel surface. Acid base treatment was done and then samples were dehydrated in ascending ethanol concentration (50%, 75% and 95% for 20 minutes each and 100% for 1 hour), then transferred to a critical point dryer for 30 minutes.

The specimens were then gold sputter coated and the surfaces were examined in a SEM at 1000X – 5000X magnification.

### Statistical analysis

The data were statistically analyzed by Kruskal–Wallis and Mann–Whitney U-tests using SPSS Base 15.0 software at significance level of $P < 0.05$.

## RESULTS

### Dye leakage study

Dye penetration scores and statistical analysis results are depicted in Table 2.

None of the groups completely prevented dye leakage.

- With saline as an irrigant, microleakage of single bond universal was significantly less than Xeno V$^+$ (Group 3 and 6; P=.003).
- EDTA and NaOCl irrigants had no significant effect on the microleakage of single bond universal (Group 5 & 6; P=.542) but significantly reduced the microleakage of Xeno V$^+$ as compared to the control group (Group 3 and 4; .037)
- Similarly irrigation with Qmix had no significant effect on the microleakage of single bond universal (Group 4 & 6; P= .542) but significantly reduced the microleakage of Xeno V$^+$ (Group 1 & 3; P=.036).

**Table 2: Mean Microleakage scores and standard deviation observed in different groups**

| S. No. | Group | No. of samples | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|---|
| 1. | Group 1 | 10 | 1.5 | .7071 | 1 | 3 |
| 2. | Group 2 | 10 | 1.6 | .5164 | 1 | 2 |
| 3. | Group 3 | 10 | 2.4 | .9661 | 1 | 3 |
| 4. | Group 4 | 10 | 1.2 | .4216 | 1 | 2 |
| 5. | Group 5 | 10 | 1.2 | .4216 | 1 | 2 |
| 6. | Group 6 | 10 | 1.1 | .3162 | 1 | 2 |

### Scanning Electron Microscopy

Scanning Electron Microscopic micrographs of dentin treated with different irrigants and its interface with adhesives are shown in figures 1-6.

In general, better interfaced adaptation was observed for single bond universal, regardless of the irrigation regimen used. (Fig 4-6)

Samples with Xeno V$^+$ depicted generalized gap at the resin-dentine interface. (Fig 1-3) Worst interfacial adaption was observed for Xeno V$^+$ in the control group. (Fig 3)

**Table 3: Intergroup comparison of Mean Microleakage scores**

| S.No. | Comparison | z | "p" |
|---|---|---|---|
| 1 | Group 1 vs Group 2 | -0.597 | 0.551 |
| 2 | Group 1 vs Group 3 | -2.099 | 0.036 |
| 3 | Group 1 vs Group 4 | -1.037 | 0.3 |
| 4 | Group 1 vs Group 5 | -1.037 | 0.3 |
| 5 | Group 1 vs Group 6 | -1.550 | 0.121 |
| 6 | Group 2 vs Group 3 | -2.084 | 0.037 |
| 7 | Group 2 vs Group 4 | -1.780 | 0.075 |
| 8 | Group 2 vs Group 5 | -1.780 | 0.075 |
| 9 | Group 2 vs Group 6 | -2.285 | 0.022 |
| 10 | Group 3 vs Group 4 | -2.719 | 0.007 |
| 11 | Group 3 vs Group 5 | -2.719 | 0.007 |
| 12 | Group 3 vs Group 6 | -2.938 | 0.003 |
| 13 | Group 4 vs Group 5 | 0 | 1 |
| 14 | Group 4 vs Group 6 | -0.610 | 0.542 |
| 15 | Group 5 vs Group 6 | -0.610 | 0.542 |

**Figure 1: SEM micrograph of the dentin composite interface after Q MIX treatment and bonded with Xeno V $^+$ showing the presence of generalized interfacial gap.**

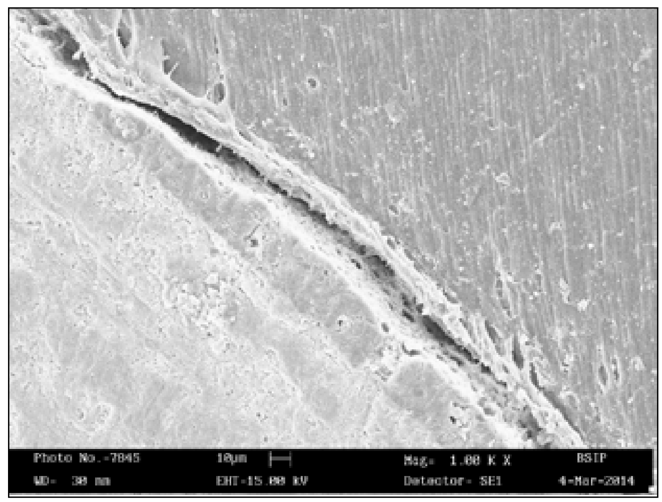


**Figure 2: SEM micrograph after 17% EDTA and 5% NaOCl treatment and bonded with Xeno V $^+$ showing a slightly reduced interfacial gap.**

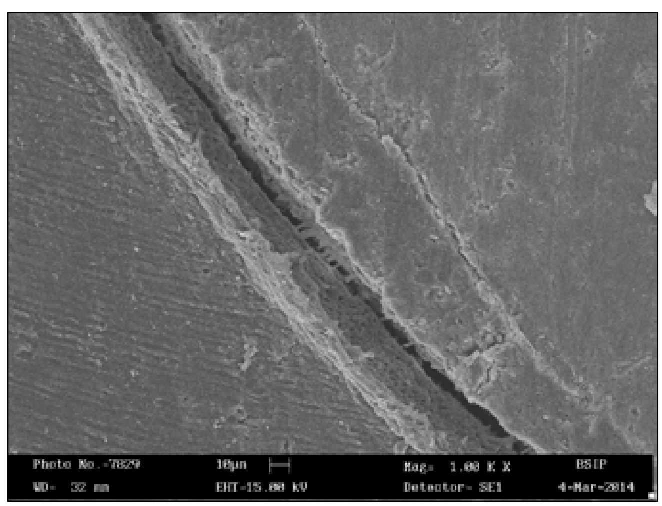


**Figure 3: SEM micrograph of the dentin composite interface after normal saline treatment and bonded with XenoV$^+$ showing maximum interfacial gap.**

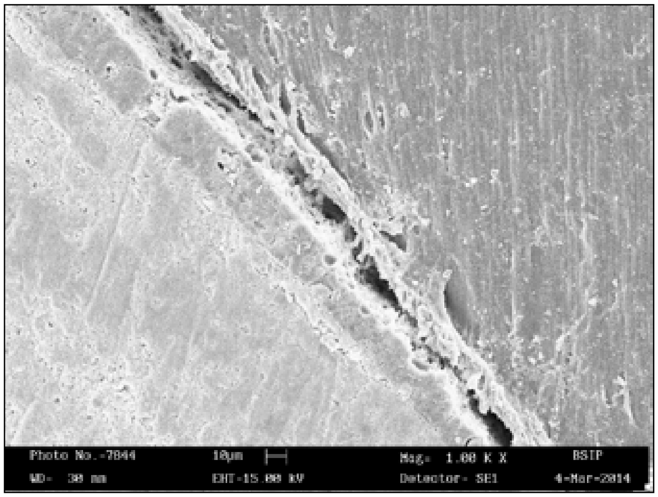



**Figure 4: SEM micrograph of the dentin composite interface after Q MIX treatment and bonded with Single bond Universal showing less interfacial gap.**

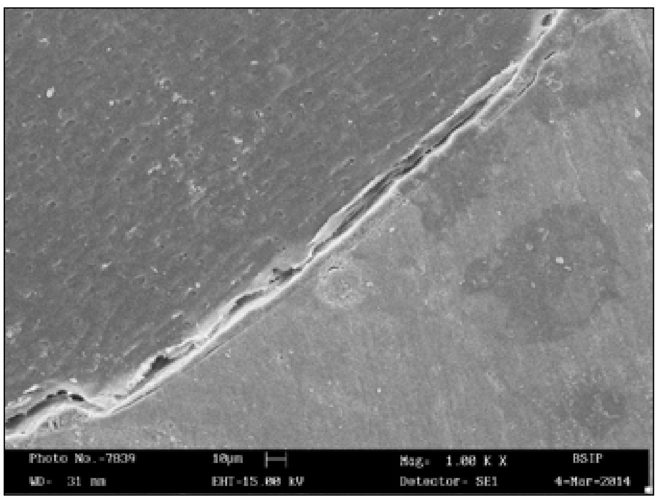



**Figure 5: SEM micrograph after 17% EDTA and 5% NaOCl treatment and bonded with Single bond Universal with minimum interfacial gap.**

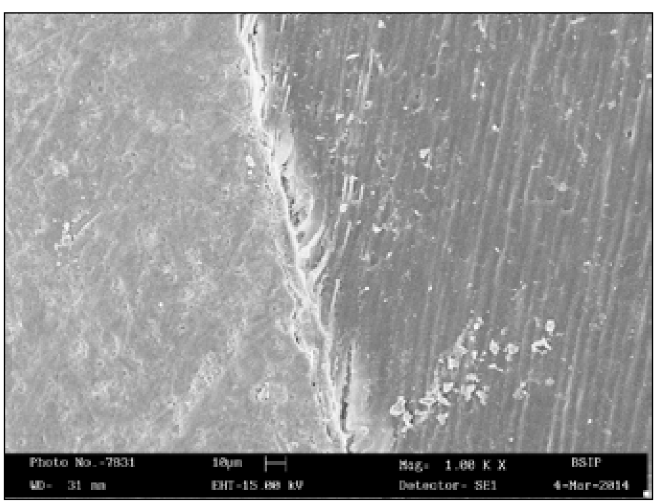
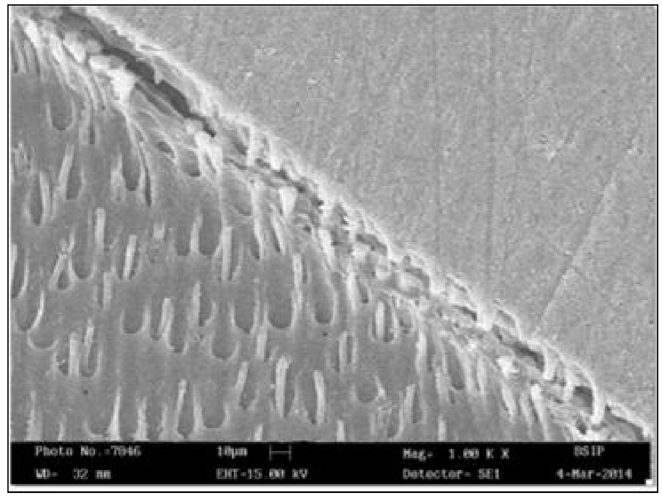



**Figure 6: SEM micrograph of the dentin composite interface after normal saline treatment and bonded with Single bond Universal showing good adaptation with resin tag formation.**




## DISCUSSION

Coronal microleakage has been identified as an important cause of endodontic failure. The quality of coronal restoration is of prime importance to create a bacteria impervious seal after root canal treatment.

Microleakage was observed with both Xeno V+ and single bond universal control group in this study which could be attributed to different characteristic of pulp chamber dentine. Bonding to pulp chamber dentine is more difficult to achieve. The structure of the pulp chamber wall is complicated, including collagen rich predentin, sclerotic dentine and regular and irregular secondary dentine.[12] The pulp chamber walls have a high density of tubules ($65000mm^2$) with large tubule diameters, small amount of intertubular dentine and many accessory canals. In addition, because there have been no cavity preparation, a smear layer typically has not been formed. Consequently, bonding of the adhesive material to pulp chamber walls is different from bonding to other dentinal surface covered with smear layer. Presence of high C factor and polymerization shrinkage of composite resins might also be responsible for the extensive microleakage reported in this study.[13] C factor or configuration factor is ratio of bonded to unbounded surfaces. The greater the percentage of unbounded surfaces, the less stress is placed on bonded surface from polymerization contraction. The unbounded surface allows plastic deformation overflow within resin mass during polymerization. When resin polymerize, the volumetric shrinkage can also result in gap formation leading to increase in microleakage.

The dentine hybridization technique initially described by Nakabayashi et al lead to significant improvement in quality of adhesion substantively increasing bond strength value and diminishing microleakage.[14] Hybridization involves molecular level infiltration of resin polymer into demineralized dentin which was achieved previously by cumbersome, technique sensitive steps of etching, priming and bonding. With evolution, these steps have been combined to form dentin bonding agent commonly known as "self-etch adhesive". In self-etch adhesive, two steps of etching and priming are done simultaneously thus risk of discrepancy between these two processes is low or no inexistent.[15]

The present study was designed to compare the effect of endodontic irrigants on the sealing ability of two self-etch adhesives namely Xeno V+ and single bond universal respectively in the pulp chamber of primary teeth- in vitro.

When single bond universal and Xeno V+ were compared, SBU revealed significantly (P<.05) better performance in comparison to Xeno V+.

Single bond Universal incorporates the functional monomer methacryloyl-oxydecyldihydrogen phosphate (MDP). MDP is known for its primary chemical interaction with hydroxyapatite, which occurs within a clinically relevant time span of 20 seconds. This kind of chemical interaction did not increase the immediate microtensile bond strength, but investigations evaluating the biodegradation resistance of adhesive interfaces have shown that it could enhance long-term stability. Single bond Universal contains Vitrebond Copolymer (3M ESPE), which creates an additional bond to hydroxyapatite, and furthermore, SBU results in better bonding of the filler particles inside the adhesive.[16] Moreover, Single bond Universal also remained stable over time if applied in accordance with manufacturer's instructions. This is probably due to its improved curing capability, which allows a high degree of polymer cross-linking, even in the thin adhesive layer created by the single-application technique.[17] So perhaps the filled adhesives form a sufficiently thick adhesive layer in one application step, resulting in its being less prone to oxygen inhibition.

Interaction of self-etch adhesive with dentine may be limited by many factors from both substrate and adhesive itself.[18] Various irrigants used during root canal instrumentation alter the chemical composition of the dentine surface and affect its interaction with material used for coronal sealing.

The present study indicate that EDTA followed by NaOCl irrigants had no significant effect on the microleakage of single bond universal (Group 5 & 6; P=.542) but significantly reduced the microleakage of Xeno V+ as compared to the control group (Group 2 and 3; P= .037)

EDTA acts as a mild chelating agent at a neutral pH that produces different effects on dentin, depending on its concentration and time of exposure. EDTA contains carboxylic acid groups, giving it the ability to remove hydroxyapatite selectively. Because most of the intrafibrillar minerals remain, the structural support by the minerals is preserved, and resin infiltration is facilitated. Shafiei et al. reported that EDTA conditioning significantly decreases microleakage of self-etch adhesives.[19]

NaOCl is a non-specific deproteinizing agent, that in aqueous solution from superoxide radical, $O_2^-$ and induces oxidation that fragments long peptide chain of proteins. It also causes chlorination of amino terminal group and hypochlorous acid formation which increase proteolytic susceptibility of collagen.[20]

NaOCl may facilitate further exposure of the inorganic material through removal of the organic matrix, and thus increase the demineralization effect. Deproteinization by NaOCl transforms demineralized, collegen-rich dentine into porous hydrophilic structure with multiple irregularities in peri- and intertubular dentine with good retention of adhesive resin into the modified dentin substrate. Wakasbayashi[21] and Campos et al[22] observed improvement of marginal sealing after deproteinization while Pioch et al[23] reported complete elimination of nanoleakage. Tubule surface area of deproteinized substrate is increased thereby increasing wettability of collagen depleted substrate and facilitate inter and inter tubular resin infilteration.[24] According to Talendano et al NaOCl treatment leads to increase in capillary effect, in addition to increase in surface energy thereby increasing wettability.[24]

The association of EDTA and NaOCl solutions has proved effective in removing smear layer formed during endodontic instrumentation. EDTA acts upon the inorganic components of the smear layer, causes the decalcification of peri- and intertubular dentine, and leaves the collagen exposed. Subsequently, the use of NaOCl dissolves the collagen, leaving the entrances to the dentinal tubules more open and exposed.[25]

The sequences of these irrigating materials can also affect bonding. When EDTA is used before hypochlorite, EDTA remove the smear layer, and prepare the dentinal walls for better adhesion of filling materials. For effective removal of both the organic and inorganic components of the smear layer, irrigating root canals with 10 ml of 17% EDTA, followed by 10 ml of 5% NaOCl is recommended. Baumgartner and Mader reported that the combination of EDTA and NaOCl caused a progressive dissolution of dentin at the expense of peritubular and intertubular areas, and they suggested that this effect may have resulted from the alternating action of NaOCl, which dissolved the organic component of the dentin, and EDTA, which demineralized the inorganic component.[26]

In current study, irrigation with QMix had no significant effect on the microleakage of single bond universal (Group 4 and Group 6; P= .542) but significantly reduced the microleakage of Xeno V+ (Group 1 & Group 3; P=.036).

QMix (Dentsply Tulsa Dental,Tulsa, OK, USA), a novel irrigant for smear layer removal with added antimicrobial agents. QMix is composed of EDTA, CHX and a surfactant, which consequently enhanced the demineralization of radicular dentine due to the chelating effect of EDTA, whilst disinfectant the same time (Stojicic et al.2012, Dai et al. 2011).[9,27] The underlying principle of adding a surface active agent in QMix is to lower the surface tension of solution and increase its wettability and consequently enhance the flow of the irrigating solution into the root canal and its contact with the smear layer.

Elnaghy AM investigated the effect of QMix irrigant compared with several other irrigating solutions on the bond strength of glass fiber posts to root dentine and on smear layer removal after post space preparation and they concluded that QMix is an effective irrigant that can remove smear layer, open dentinal tubules and simplify the irrigation protocol, without compromising the bonding strength of glass fiber posts cemented with a self-adhesive resin cement to root dentine.[28] Dai et al. examined the ability of QMix to remove canal wall smear layers and concluded the QMix was as effective as 17% EDTA in removing canal wall smear layers after the use of 5.25% NaOCl as the initial rinse. An insignificant difference in Ca loss and micro hardness was seen between 5% NaOCl-EDTA and 5% NaOCl-QMix groups. As QMix contains EDTA in its composition along with chlorhexidine and a detergent, the effect of QMix on root dentin could have been almost similar to EDTA.[27]

Future studies should aim to investigate the effect of other endodontic irrigants on the adhesion of contemporary simplified adhesive systems to pulp chamber dentin, as the effect varies with the specific adhesive system used.

## CONCLUSIONS

1. Both SBU and Xeno $V^+$ adhesives depicted microleakage when bonded to pulp chamber dentin. However SBU which is total etch adhesive depicted significantly reduced microleakage after compared to Xeno $V^+$ self-etch adhesives.
2. EDTA and NaOCl irrigation area of pulp chamber significantly reduced the microleakage scores of Xeno$V^+$ generation but did not affect the microleakage of SBU.
3. Irrigation with QMix also significantly reduced the microleakage of Xeno$V^+$ but had no significant effect on the microleakage of SBU.
4. Irrigation with EDTA/NaOCl or QMix had no determined effect on the sealing ability of either of the adhesive tested.
5. In general total etch adhesive depicted good sealing ability regardless of the irrigation regimen used.

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