Original Article

An *in vitro* evaluation of the effect of dentin deproteinization on coronal microleakage in endodontically treated teeth

Vikram Shetty K, Kapil Jhajharia, Vishwajit Rampratap Chaurasia[1], Ashu Jhamb[2], Varsha Rohra[3], Akanksha Manmohan Sharma[4]

Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, Melaka Manipal Medical College, Melaka, Malaysia, [1]Department of Conservative Dentistry and Endodontics and [4]BDS Student, KLE's Dental College and Hospital, Belgaum, Karnataka, [2]Department of Conservative Dentistry and Endodontics, Yamuna Institute of Dental Sciences and Research, Gadholi, Yamunanagar, Haryana, [3]Department of Conservative Dentistry and Endodontics, RKDF Dental College and Research Centre, Bhopal, Madhya Pradesh, India

Corresponding author (email: <kvikramshetty@gmail.com>)
Dr. Vikram Shetty K, Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, Melaka Manipal Medical College, Melaka, Malaysia.

Abstract

Background: The most common cause of failure of endodontic therapy is inadequate apical and coronal seal. Proper coronal seal reduces the risk of endodontic failure. Hence, the present study was done to test the role of self-etching primers in reducing microleakage through coronal seal. **Materials and Methods:** Following root canal preparation and obturation, 46 specimen teeth were subjected to one of the test methods as follows: Group I – deproteinization with 3% sodium hypochlorite and etching with 37% phosphoric acid; Group II – deproteinization with 3% sodium hypochlorite and chelation with 15% ethylenediaminetetraacetic acid (EDTA) (Glyde) without etching. Group I and Group II were further divided into two subgroups with 10 specimens in each: In subgroup A, Clearfil Liner Bond 2V was used and in subgroup B, Excite was used. Group III (obturated without access restorative material) had six specimens. **Results:** Spectrophotometric analysis was done to quantitatively analyze the amount of dye leakage. Microleakage values obtained in Group I and Group II were comparable. In Group I, marginally better values were obtained with the Clearfil Liner Bond 2V in comparison with Excite. In Group II, microleakage values obtained with Clearfil Liner Bond 2V and Excite were similar and statistically not significant. In Group III (control) where no access restoratives were placed, maximum leakage was observed. **Conclusions:** Maximum leakage values were observed in Group III, when obturated without access restorative and when exposed to artificial saliva. Clearfil Liner Bond 2V as a self-etching primer showed better values in preventing microleakage. Deproteinization may be important to reduce microleakage when using the fifth-generation bonding system (Excite) and sixth-generation bonding system (Clearfil Liner Bond 2V).

Key words: *Coronal microleakage, dentin, deproteinization, endodontically treated teeth, sodium hypochlorite*

INTRODUCTION

The most common cause of failure of endodontic therapy is inadequate apical seal. Inadequate apical seal results in passage of bacteria and periapical fluid into the root canal and failure of endodontic therapy.[1] Coronal seal is as important as apical seal for the success of endodontic treatment. The apical seal may be adversely affected by loss of or defective coronal seal. Inadequate coronal seal may occur due to fracture of tooth structure, missing of temporary filling materials, marginal leakage of the final restoration, and recurrent caries.[2-6] The three-dimensional filling of root canal system prevents the penetration of microorganisms and toxins from the oral cavity through the root canal into the periradicular tissues. Despite apical leakage still

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being considered an important factor for endodontic failures, in the last few years, more attention has been given on the procedures performed to achieve an effective coronal sealing soon after the completion of root canal therapy.[6,7]

Good coronal restoration of endodontically treated teeth helps in successful treatment and healing of periradicular inflammation.[8] Various restorative materials have been tried to restore the access cavity, including glass ionomer cement (GIC), composite, intermediate restorative material (IRM), and amalgam.[5,9] Composite resins are the most commonly used materials to restore access cavities. They are esthetic, and they strengthen the existing coronal tooth structure by bonding to the tooth structure. Conventional GIC restorations are useful for bulk filling of access cavities.

Hence, the present study was done to test the hypothesis that the deproteinized dentin in endodontic access preparations is likely to bring in changed microleakage values and to determine whether the self-etching primers would play a role in significantly reducing microleakage through coronal pathway.

MATERIALS AND METHODS

Forty-six freshly extracted intact human maxillary incisors free from caries or cracks were collected, stored, and disinfected. The teeth were then placed in 0.9% physiologic saline solution for 10 days prior to access cavity preparation. The samples were divided into three experimental groups. Group I and Group II were further divided into two subgroups with 10 specimens in each and Group III had six specimens. Standard access cavity preparations, biomechanical preparation with step-back flare technique, and obturation were done using AH plus as a sealer and gutta-percha as standard obturating material.

In Group I, deproteinization with 3% sodium hypochlorite followed by etching with 37% phosphoric acid for 15 s was done. The specimens were then washed with a water spray for 30 s and gentle air drying was done for a few seconds. After this, the respective bonding systems were applied to the two subgroup samples and bonded to the composite resin. In Group II, deproteinization with 3% sodium hypochlorite and 15% ethylenediaminetetraacetic acid (EDTA) (Glyde) was done without etching. After this, the respective bonding systems were applied to the two subgroup samples and bonded to the composite resin. In subgroup A, the adhesive system used was Clearfil Liner Bond 2V. In subgroup B, the adhesive system used was Excite. All the subgroups were restored using Tetric Ceram composite resin. In Group III, the specimens were obturated without access restorative material.

All specimens were placed in artificial saliva for 24 h. The entire surface of the restored teeth was covered with nail varnish except for the restoration and a 2 mm margin around it, and the teeth were then placed in 2% methylene blue dye for 72 h. Following this, the specimens were washed with distilled water, the nail varnish removed, and then they were placed in 35% nitric acid for 72 h. Standard solutions of 1%, 0.5%, 0.005%, 0.002%, 0.001%, and 0.0005% of methylene blue in 35% nitric acid were prepared and stored for 72 h. At the end of this time period, the liquid was centrifuged for 1 min (200 rpm) and the supernatant was subjected to spectrophotometric analysis at 670 nm to analyze the amount of leakage.

RESULTS

Spectrophotometric analysis was done to quantitatively analyze the amount of dye leakage in dentin deproteinization with 3% sodium hypochlorite with etching and deproteinization without etching but chelating with 15% EDTA.

Spectrophotometric analysis was done to quantitatively analyze the amount of dye leakage. Microleakage values obtained in Group I and Group II were comparable. In Group I, marginally better values were obtained with Clearfil Liner Bond 2V in comparison with Excite and the values were statistically not significant. In Group II, the microleakage values obtained with Clearfil Liner Bond 2V and Excite were similar and statistically not significant. In Group III (control) where no access restoratives were placed, maximum leakage was observed [Tables 1-3].

DISCUSSION

Endodontic therapy has become a routine procedure for treating and retaining non-vital teeth. Several researchers have observed that coronal microleakage is a major factor in the etiology of endodontic treatment failure.[10,11] Basaran *et al*. stated that the survival of endodontically treated teeth depends more on the coronal sealing provided by the coronal restoration than the apical sealing provided by endodontic therapy.[12] A coronal restoration after endodontic therapy could

prevent the movement of bacteria and their products, hence long-term prognosis of endodontically treated teeth depends on the quality of the final restoration.[9] Successful coronal restoration of endodontically treated teeth can be assessed by the presence or absence of periapical findings in the *in vivo* method, whereas in *in vitro* method, microleakage can be assessed by checking the amount of dye penetrating from the coronal restoration area. Similar *in vitro* procedure was done in the present study to assess the quality of coronal restoration.

Restoration of endodontically treated teeth using resin composites has been increasingly done recently due to the development of better dentin bonding systems. A resin composite restoration enables an endodontically treated tooth to be restored by replacing only the missing tooth structure because a dentin bonding system can reinforce the remaining tooth structure. The development of single bottle adhesives (self-etching primers) naturally reduced the number of clinical steps when using composite resins as access restoratives.[6,13] Certain factors associated with restoration of endodontic access cavities need special attention. Chemical irrigants such as sodium hypochlorite and their combination with EDTA as dentin softening agents or as a final flush have all been shown to adversely affect the bond strength of resin composites to dentin.[6]

To simulate the natural oral conditions, all the samples were kept submerged in artificial saliva for 24 h before they were surfaced and subjected to dye leakage studies. Our present investigation involved two experimental groups with two subgroups in each. In Group I, we attempted deproteinization and etching and in Group II, we did not etch the substrate and deproteinization was followed by chelation with 15% EDTA. Group II (experimental group) was included for the main reason that deproteinization as it is occurs due to the utilization of sodium hypochlorite as a universal irrigant and also, most endodontists globally utilize 15% EDTA as a routine adjunct with sodium hypochlorite. Group III served as control in which the six specimens were devoid of any access restorative. Particularly, we have utilized Clearfil Liner Bond 2V as a representative of sixth-generation bonding system and Excite as a representative of fifth-generation bonding system for comparative evaluation of microleakage values.

Microleakage is the diffusion of a substance into a fluid-filled gap or a defect between the filling material and the tooth substrate. In the present investigation, we have utilized Zakariasons dye recovery method, which is a more dependable method in quantifying the microleakage, to quantitatively measure the amount of dye leakage. Quantitative microleakage evaluation method was developed by Douglas and Zakariason.[14] Mandras *et al*. reported that this methodology eliminates the subjective operator evaluation that is used in qualitative evaluations and measures the infiltrated dye.[10]

The use of organic dyes as tracers is one of the oldest and most common methods of detecting leakage *in vitro*. In this ongoing investigation, 2% methylene blue dye was utilized. The specimens were left immersed in the dye for 72 h.

In the present investigation, microleakage values obtained in Group I and Group II were comparable. In Group I, marginally better values were obtained with Clearfil Liner Bond 2V in comparison with Excite and the values were statistically not significant. In Group II, the microleakage values obtained with Clearfil Liner Bond 2V and Excite were similar and statistically not significant. However, in Group III (control), where no access restoratives were placed, maximum leakage was observed as expected [Tables 1,2,4]. Maximum transmission amounts to minimum leakage. In their *in vitro* study, Agrawal *et al.* obtained the highest mean microleakage score for Xeno V after saline irrigation (Group 3: 2.90 ± 0.3162) which was followed by Xeno V with Chlorhexidine (CHX) irrigation (Group 1: 2.80 ± 0.4216) and Xeno V with EDTA + NaOCl irrigation (Group 2: 2.30 ± 0.8233) and the least mean microleakage score was obtained for Adper Easy One after EDTA + NaOCl irrigation.[6] Bayram *et al.* observed in their *in vitro* study that using CoroSeal (CS) material as an intraorrifice barrier material reduced the amount of microleakage, as compared with fissure sealant (FS), flowable composite (FC), and polycarboxylate cement (PC).[9]

Hommez *et al.* observed by clinical and radiographic evaluation that 31.1 and 36.8% of teeth with good and poor coronal restorations scored clinically, respectively,

Table 1: Volumetric dye penetration values obtained in different groups with respect to transmission of light

Groups	Subgroups	Transmission of light (%)
I	A	80, 87, 75, 95, 95, 97, 83, 95, 96, 83
	B	90, 77, 87, 82, 78, 90, 86, 90, 82, 82
II	A	85, 90, 86, 88, 93, 88, 75, 94, 90, 90
	B	92, 90, 88, 90, 92, 90, 88, 84, 86, 75
III		75, 77, 80, 82, 75, 83

had apical periodontitis and this difference was not statistically significant.[15] Mannocci *et al.* in a 3-year follow-up clinical study observed that the clinical success rates of endodontically treated premolars restored with fiber posts and direct composite restorations after 3 years of service were equivalent to a similar treatment of full coverage with metal-ceramic crowns.[16]

In the present investigation, we have also attempted to test the reliability of dentin bonding when EDTA was utilized without etching. The relevance of creating an endodontic access restorative substrate when treated with 15% EDTA was that most endodontists use EDTA as an adjunct with sodium hypochlorite as a dentin softener in enlarging the pulp space. Belli *et al*. observed that on re-treatment of pulp chamber with sodium hypochlorite, the pre-dentin matrix was removed leaving a smoother, mineralized matrix for bonding.[17]

Self-etching primers are the new generation adhesives with no long-term clinical studies available on them. In the present study, even though the results were not statistically significant, Clearfil Liner Bond 2V as a self-etching primer showed better values in preventing microleakage [Tables 3,5,6]. It is hoped that the adhesive resin systems and bonding techniques, especially with the newer self-etching primers, can be adapted for use within the pulp chamber to provide a second layer of defense against microleakage. The self-etching primers are simple, convenient to use, and single-step etchants and adhesives with a good track record, even though it is for short term. It should be possible to develop simple adhesive systems that can be conveniently applied but are translucent and soft

Table 2: Multiple comparisons of the subgroups with reference to transmission of light

Subgroups	Groups	Mean	Standard deviation	*t*	*P*	Comments
A	I	88.6000	7.9750	0.2310	0.82	Not significant
	II	87.9000	5.3219			
B	I	84.4000	4.9035	1.3910	0.181	Not significant
	II	87.5000	5.0607			
Comparison between Group I and Group III						
A	I	88.6000	7.9750	2.8590	0.013	Significant
	III	78.6667	3.5024			
Comparison between Group II and Group III						
A	II	87.9000	5.3219	3.7620	0.000	Very highly significant
	III	78.6667	3.5024			

Table 3: Multiple comparisons with respect to optical density

Subgroups	Groups	Mean	Standard deviation	*t*	*P*	Comments
Comparison between Group I and Group III						
A	I	0.064	0.03	3.1930	0.007	Highly significant
	III	0.115	0.04			
Comparison between Group II and Group III						
A	II	0.0580	0.0253	3.6630	0.003	Highly significant
	III	0.1150	0.0373			

Table 4: Mean and standard deviation of volumetric dye penetration in different groups with reference to transmission of light

Groups	Subgroups	Number of specimens	Mean	Standard deviation	*t*	*P*	Comments
I	A	10	88.6000	7.9750	1.4190	0.173	Not significant
	B	10	84.4000	4.9035			
II	A	10	87.9000	5.3219	0.17200	0.865	Not significant
	B	10	87.5000	5.0607			
III		6	78.6667	3.5024	1.4298		

enough to permit re-treatment when necessary. Van Meerbreek *et al.* observed least leakage with self-etching adhesives, which is in agreement with our study. The bonding mechanism of etch and rinse adhesives to dentin is primarily diffusion based, depending on the hybridization or infiltration of resin within the exposed collagen fibril scaffold.[18]

Yavari *et al*. observed in their *in vitro* study that Calcium enriched mixture (CEM) cement and Mineral trioxide aggregate cement (MT A) as intraorifice sealing biomaterials, are more effective than amalgam and composite resin in preventing salivary leakage in endodontically treated teeth.[5]

From previous clinical studies, a number of conclusions concerning the importance of a coronal seal relative to prognosis have been drawn. Ray and Trope stated that the technical quality of coronal restoration may be significantly more important than the technical quality of endodontic treatment for optimal periodontal health.[19] Swanson and Madison found all teeth obturated using gutta-percha and sealer in the absence of temporary restoration showed leakage ranging from 79 to 85% of root length within 56 days when exposed to saliva.[1] This is in agreement with the results of the present study wherein we had included a control group with no access restorative.

Table 5: Volumetric dye penetration values in the different groups with respect to optical density

Groups	Subgroups	Optical density (value between 0-2)
I	A	0.09, 0.06, 0.12, 0.05, 0.05, 0.03, 0.07, 0.05, 0.04, 0.08
	B	0.05, 0.11, 0.06, 0.08, 0.12, 0.05, 0.06, 0.04, 0.08, 0.08
II	A	0.07, 0.05, 0.06, 0.06, 0.03, 0.06, 0.12, 0.03, 0.05, 0.05
	B	0.04, 0.05, 0.06, 0.05, 0.04, 0.05, 0.06, 0.08, 0.06, 0.12
III		0.12, 0.11, 0.18, 0.09, 0.12, 0.07

Using full-coverage restorations for endodontically treated anterior teeth is controversial. Most endodontically treated anterior teeth do not discolor if basic tenets in endodontic therapy have been observed. The basic tenets include mainly the removal of the pulp in entirety, including the pulp horns, and using a good irrigant like sodium hypochlorite which has tissue-dissolving capabilities, as well as a second-line bleaching agent. Hence, an ideal endodontic access restorative may be the only post-endodontic restoration required for endodontically treated anterior tooth.

Therefore, an ideal endodontic access restorative should bond to not only non-vital dentin but also to a substrate which is both sodium hypochlorite treated and chelated. Resins have long been used as access restoratives, even though it involves a number of steps for completing the procedure. With the introduction of self-etching primers, the procedure has become simple, with a single step, less time consuming, and showing better bonding against dentin substrate. However, further clinical and *in vitro* studies are required to promote composite resins with self-etching primers as the top priority access restorative materials.

Limitation of the study

Experimental studies cannot exactly reproduce the clinical condition. The relationship of *in vitro* leakage to *in vivo* situation has not yet been established.

CONCLUSION

Maximum leakage values were observed in Group III, when obturated without access restorative and when exposed to artificial saliva. Clearfil Liner Bond 2V as a self-etching primer showed better values in preventing microleakage. Deproteinization may be important to reduce microleakage, when using the fifth-generation bonding system (Excite) and sixth-generation bonding system (Clearfil Liner Bond 2V).

Table 6: Mean and standard deviation of volumetric dye penetration in different groups with reference to optical density

Groups	Subgroups	Mean	Standard deviation	*t*	*P*	Comments
I	A	0.0640	0.0267	0.7590	0.458	Not significant
	B	0.0730	0.0263			
II	A	0.0580	0.0253	0.2730	0.788	Not significant
	B	0.0610	0.0238			
III		0.1150	0.0373			

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