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THE EFFECT OF VARIOUS ORGANIC SOLVENTS ON MICROHARDNESS OF DENTIN AND THE PRELIMINARY STUDY ON THE ANTIBACTERIAL FUNCTION.

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Abstract

The purpose of this study was to assess the efficacy of desocclusol, eucalyptol and d-limonene on removal of gutta-percha, their effect on microhardness of dentin, and the antibacterial properties against Enterococcus faecalis (E. faecalis) during root canal retreatment.

Forty single-canal roots, obturated with gutta-percha, were prepared and randomly divided into 4 groups (n=10). Group that free of any solvent was control group. Three of experimental groups were exposed to desocclusol, eucalyptol and d-limonene respectively during endodontic retreatment. Efficacy of each solvent was assessed by comparation of time duration, amount of apical extrusion and residual debris in the root canals. Vickers microhardness of each sample was recorded. Antibacterial activity on E. faecalis was also observed. The data were subjected to repeated statistical analysis.

All three organic solvents were found to be effective in the removal of root canal filling material compared with control group ($p<0.05$). D-limonene was more effective in significantly increasing the microhardness of dentin compared with desocclusol, which decreased the microhardness of root canal dentin ($p<0.05$). In the meantime, d-limonene had a stronger antibacterial activity than eucalyptol, while desocclusol had no obvious bacterial inhibition.

These three organic solvents are all effective in removing gutta-percha in endodontic retreatment. D-limonene can increase the microhardness of dentin and has a stronger inhibitory effect on E. faecalis than desocclusol and eucalyptol.

Keywords: *microhardness, enterococcus faecalis, desocclusol, eucalyptol, D-limonene.*

Introduction

Although the clinical success rate of endodontic treatment reaches up to 68 to 85th percentile [*Ng Y et al., 2008*]. A certain percentage of cases may still result in failure despite properly preparation and obturation. Interradicular infection caused by microorganisms is the main etiological factor that leads to the failure of root canal treatment [*Siqueira J, 2001; Endo M et al., 2013*]. *E. faecalis* is considered as the most commonly detective species in retreatment cases, which is nine times higher than that in primary endodontic infections [*Möller A, 1966; Sundqvist G et al., 1998; Rôças I et al., 2004*]. The basic objective of non-surgical root canal retreatment is an attempt to completely remove the defective old filling from the root canal system, and obtained an effective cleaning, shaping and re-obturation of canals [*Kvist T, Reit C, 1999; de Chevigny C et al., 2008; Mollo A et al., 2012*]. Therefore, it is crucial important to completely remove old gutta-percha filling and biofilm for effective disinfection and resealing [*Whitworth J, Boursin E, 2000; Mushtaq M et al., 2012*]. Although various available techniques can be used for re-treatment, such as using manual or machine-rotating instruments, lasers, heating equipment or ultrasonic instruments etc. [*Ruddle C, 2004; Fenoul G et al., 2010*], studies have shown that obtaining a root canal system with completely free

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from debris and residual infectious agents by only relying on mechanical removal alone is not feasible [*Wilcox L et al., 1987; Imura N et al., 1996*]. Therefore, additional use of solvents is recommended to facilitate the removal of softened gutta-percha. Since the solvents have an effect on the chemical composition of the dentin surface, it can be speculated that the alterations of dentin microhardness may affect the dentin interaction with materials used for obturation [*Saleh A, Ettman W, 1999*] and even affect the mechanical properties of the root canal [*Seyedmahmoud R et al., 2017*].

The solvents can also help remove Enterococcus biofilm from root canal [*Sundqvist G et al., 1998*]. Studies have shown that organic solvents are bactericidal. D-limonene and eucalyptol have inhibitory effects on *E.coli*, Staphylococcus aureus, Bacillus subtilis, etc. Desocclusol contains wood pomegranate oil, which can kill the Bacteroides melanogaster. However, the inhibitory effect of these three solvents on *E. faecalis* is unclear. Therefore, the purpose of the study is to compare the efficacy of desocclusol, eucalyptol and d-limonene in removing gutta-percha from root canals during retreatment procedures and assessed the effect of three organic solvents on the microhardness of human root dentin. Antimicrobial susceptibility test was used to observe antibacterial properties against *E. faecalis*.

Materials and methods

Samples preparing, shaping and obturation

Forty freshly extracted single-rooted single canal teeth, with fully formed apices and free of any calcifications or internal resorption, were collected due to periodontal or orthodontic reasons. The residues of soft and hard tissue around the teeth were removed mechanically and then immersed in saline and stored at 4°C until use.

The samples were decoronated to obtain a standardized root length of 15 *mm* using water-cooled diamond disc. A size 10 hand K-file (Mani Inc., Tochigi, Japan) was placed in the canal until the file was visible at the apical foramen and the working length (WL) was determined by subtracting 1 *mm* from this measurement. All root canals were prepared with ProTaper universal files (Dentsply Maillefer, Switzerland) up to the size F2 at full WL. During instrumentation, the root canals were irrigated with 2 *mL* of saline frequently.

Root canals were filled with gutta-percha and AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) by means of warm vertical compaction technique. Specimens were controlled with mesiodistal and buccolingual radiographs to confirm complete filling. After that, 2 *mm* of the gutta-percha was cut off from coronal end of the root canal to ensure that the filling length reaches 12 *mm*. Then the canals were coronally sealed with composite resin (3M ESPE Dental, California, USA) and apically covered with temporary filling material (Cavit, 3M ESPE Dental). The specimens were stored at 37°C and 100% humidity for 10 days to allow the sealer to set.

All the above clinical operations were performed by the same operator. Then the teeth were randomly divided into four groups (n = 10 per group). Group A was the control group that free of any solvent. The experimental components were Group B (Desocclusol group), Group C (Eucalyptol group) and Group D (D-limonene group) separately.

Retreatment procedure

The temporary fillings were removed before starting the retreatment procedure. #3Gates-Glidden drill (Mani, Matsutain Seisakusho Co., Tochigi-Ken, Japan) was used till 1/3 of the WL, #2Gates-Glidden drill was used till 2/3 of the WL. Then a #15 K-type file was inserted into the gutta-percha mass by reciprocating, and headed to the apex. The final apical preparation was performed with #20 to #30 H-type files.

In experimental groups, 20 μl of desocclusol (Bilan, France), eucalyptol (Xin Weida Chemical Trading, Jinan, China) and d-limonene (Xin Weida Chemical Trading, Jinan, China) was introduced respectively into the root canal for 3 *min* to soften the gutta-percha before using the #2 and #3 Gates-Glidden drill instruments. A total volume of 100 *ul* solvent was added dropwise into the root canals during the whole retreatment process. When the instruments reached the WL, the root canals were irrigated by distilled water. The retreatment procedure was finalized after no debris of the filling material was visible on the files and irrigation. Thereafter, the roots were dried with corresponding absorbent paper points.

Time required for gutta-percha removal

A chronometer was used for the calculation of

the time required for the removal of gutta-percha. T1 was defined as the time between the start of the insertion of the first file into the root canal to establishing the access of the WL. T2 was the total time required to remove all the filling materials.

Evaluation of residual material

All the samples were digitally radiographed after the retreatment procedure from the mesiodistal and buccolingual directions. The obtained images were analyzed with AutoCAD (Autodesk, San Rafael, CA, USA) software and the proportion of residual filling material in the root canals was calculated.

Weight of apical extrusion

The debris collected in the centrifuge tube was placed in a thermostatic chamber for 10 days to achieve dry material, which was then weighed to calculate the quantity of the apical extrusion.

Microhardness test

Each root was then sectioned longitudinally, from the cervical to the apical area with a low-speed diamond disc（NSK，Japan, so as to separate each root into buccal and lingual segments. The root segments were then horizontally embedded in super hard plaster, leaving the dentinal surfaces exposed. The ascending grades of silicon carbide abrasive papers (800, 1500 and 2000 grit) were used to polish the dentinal surfaces under distilled water. Vickers microhardness was measured by the Vickers diamond indenter at three different locations using a 500-g load and a 20-s dwell time. The tested locations were selected 500 *um* away from the pulp-dentine interface in the coronal, middle and apical thirds along the root canals. The representative hardness value for each specimen at each distance was obtained as the average of the three indentations.

ANTIMICROBIAL SUSCEPTIBILITY TEST

After culture and activation of *E. faecalis* (ATCC 29212) in specific culture media, 0.5 McFarland standard of the bacterial suspension was prepared and cultured on BHI agar culture medium (hap bio, Qingdao, China) by a sterile swab in all directions. Then, the organic solvent and saline impregnated paper disks with a diameter of 6 *mm* were placed on the culture medium. Next, the specimens were incubated at 37°C for 24 hours, and the diameter of the growth inhibition zone was evaluated after culture. Each zone was measured three times, and the mean of the three values was calculated and reported as the diameter of the growth inhibition zone.

Statistical analysis

The mean and standard deviation of the four groups were analyzed first and two-way ANOVA was used to analyze the differences between the effects of organic solvents on microhardness measurements. The diameter of each group of solvent inhibitory zone was analyzed by performing the t-test to at a significance level of p=0.05 with IBM SPSS Statistics 18.0 software (IBM SPSS Inc., Chicago, IL, USA).

RESULTS

The time (T1 and T2) required for retreatment are shown in table 1. The retreatment time (T1 and T2) was significantly shorter in the solvent groups compared to the control group ($p<0.001$). There was no significant difference between the three solvent groups ($p>0.05$).

The proportion of residual filling material in the root canals are shown in figure 1. We calculated the proportion of residual filling material of

Table 1.

Time (in seconds) required to remove gutta-percha

Groups	T_1				T_2			
	Mean	±SD	Min	Max	Mean	±SD	Min	Max
A (no solvent)	14.085	6.628[a]	5.47	24.93	25.457	6.990[a]	18.03	38.18
B (desocclusol)	6.140	2.052[b]	3.92	10.58	13.076	3.030[b]	9.08	18.62
C (eucalyptol)	6.778	1.944[b]	4.02	9.15	14.353	1.801[b]	12.62	17.03
D (d-limonene)	7.466	2.628[b]	4.10	13.22	14.802	2.623[b]	12.09	20.25

NOTE: *SD – standard deviation. Different lower case letters indicate statistically significant differences in the vertical columns. One-way analysis of variance was used to evaluate the parametric data on the time required for filling removal. P<0.05 was considered statistically significant.*

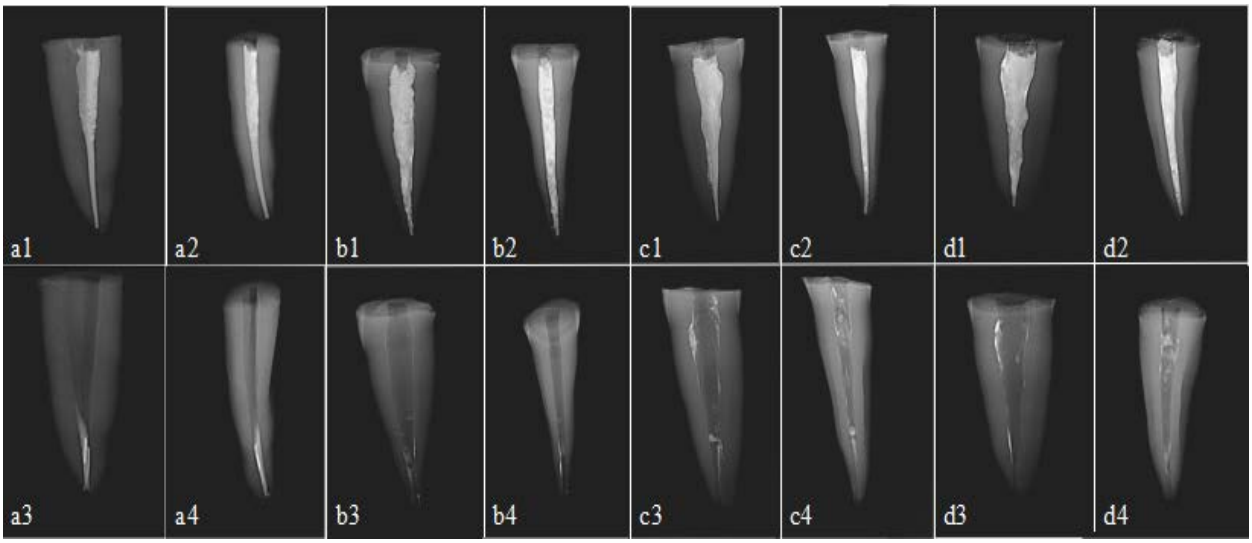

FIGURE 1. *The residual filling material in the root canals after removal of gutta-percha. (a1, a2, b1, b2, c1, c2, d1, d2)*

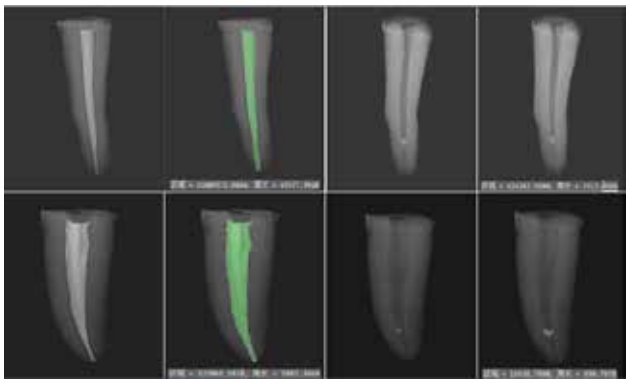

FIGURE 2. *The residual area of the filling material as a percentage of the total area of the root canal was calculated by Auto-CAD software*

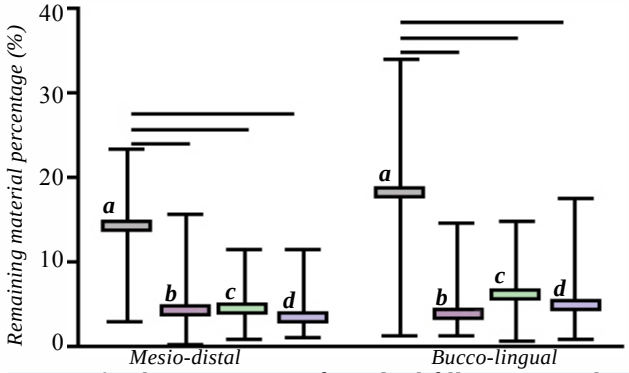

FIGURE 3. *The proportion of residual filling material in the root canals. Where a – no solvent, b – desocclusol, c – eucalyptol, d – D-limonene and * – p<0.05.*

the root canals by AutoCAD (Fig. 2). The solvent groups left significantly less gutta-percha than the control group (Fig. 3) (p<0.05). There was no significant difference between three solvent groups (p>0.05). There was also no significant difference between the same test group at the x-ray photograph in same direction (p>0.05). The quality of apical extrusion in each group was shown in table 2. There was no significant difference between the four groups(p>0.05).

The samples in control, desocclusol, eucalyptol and d-limonene groups were digitally radiographed after filled from the mesiodistal and buccolingual directions (a3, a4, b3, b4, c3, c4, d3, d4). The residual filling material of samples in in control, desocclusol, eucalyptol and d-limonene groups were digitally radiographed after retreatment procedure from the mesiodistal and buccolingual directions.

Three experimental groups were exposed to different organic solvents and tested for microhardness (Fig. 4). The mean and standard deviation values for root dentin microhardness in the solvent groups and control groups are shown in table 3. There was no significant difference between experimental groups and control in the coronal third and middle third of the root (p>0.05), but there was a significant difference between the desocclu-

Table 2.

The weight of apical extrusion (*g*)

Group	weight of apical extrusion			
	Mean	±SD	Min	Max
A(no solvent)	0.001	0.001	0.0003	0.0034
B(Desocclusol)	0.001	0.001	0.0004	0.0033
C(Eucalyptol)	0.002	00.001	0.0002	0.0039
D(D-limonene)	0.001	0.0009	0.0001	0.0031

NOTES: *P<0.05 was considered statistically significant. There was no significant difference between the four groups (p>0.05)*

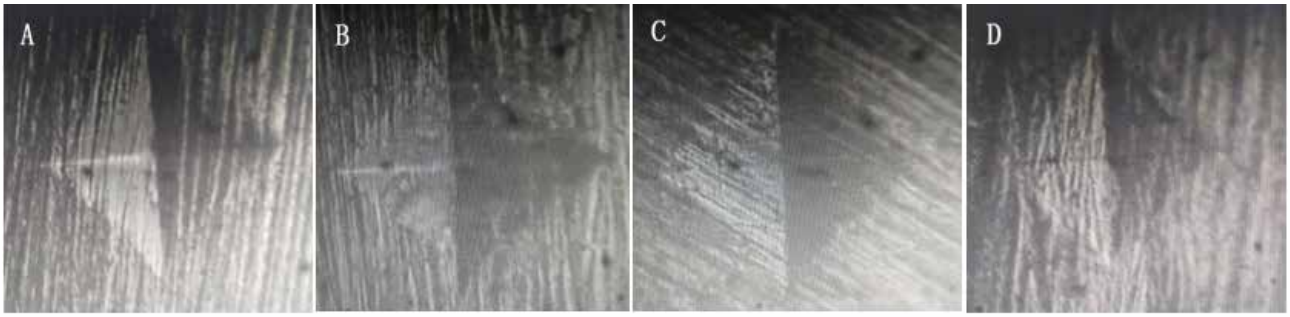

Figure 4. *Vickers microhardness tester Diamond-shaped indentations made on the surface of the dentin with a load of 500g for 20s of group A (no solvent), group B (Desocclusol), group C (Eucalyptol) and group D (D-limonene)*

sol and the d-limonene group ($p<0.05$). The difference in the apical thirds of all groups was not significant ($p>0.05$).

Solvent inhibitory effect

In the disk diffusion test, the growth inhibition zones could be found around d-limonene and eucalyptol disks, but there was bacterial growth around desocclusol and saline disk (Fig. 5). The mean and standard deviation of the diameters of the growth inhibition zone at 24 hours was presented in table 4. The statistical results showed that d-limonene and eucalyptol had inhibitory effects on E.faecalis, and effect of d-limonene was stronger ($p<0.05$). Desocclusol had no significant inhibitory effect on Enterococcus faecalis.

Discussion

There is a growing demand for conserving teeth in endodontic treatment in recent years. Nonsurgical retreatment is more preferred than a surgical procedure (apical surgery or extraction) for a failed root canal treatment [*Kvist T, Reit C, 1999; Paik S et al., 2004*]. *E. faecalis* is an important pathogen in persistent root canal infections. Solvent commonly facilitates the removal of gutta percha and sealer from the root canal system [*Kaufman D et al., 1997*]. It expedites the process of retreatment and decreases the amount of residual material inside root canal [*Hunter K et al., 1991*]. The solvent is also bacteriostatic and can effectively help to remove the biofilm. There are many types of currently reported organic solvents used in root canal retreatment, such as orange oil, eucalyptol, d-limonene, xylene, chloroform, desocclusol, and halothane [*Hulsmann M, Bluhm V, 2004; Saglam B et al., 2014*]. Desocclusol is a successful and reliable solvent applied in clinical endodontics, of which the main component is perchloroethylene. Eucalyptol and d-limonene both have been used for many years on the inner and outer surfaces of the human body with reliability and safety [*Uemura M et al., 1997*]. The former has been used in cough suppressant in which the main component is 1,8-cineole (above 80%), while the latter is used as a flavoring agent in foods and the main component is orange oil (90% to 95%) [*Lewis R, 1993*]. Because of the toxicity of organic solvents, low dissolution efficiency, and changes in the physicochemical properties of the root canal, the aim of the study was to select a more clinically desirable organic solvent.

Table 3.

Vickers microhardness values (Mean, Standard deviation) of root canal dentin after the use of the tested solvents

Group	coronal	middle	apical
A(no solvent)	59.94±5.12	58.67±3.86	57.55±6.36
B(Desocclusol)	54.26±8.82*	54.50±7.66*	56.69±5.58
C(Eucalyptol)	59.02±5.8	59.96±5.31	59.12±4.57
D(D-limonene)	61.86±6.81*	64.31±6.66*	59.77±5.21

Notes: **indicates statistically significant differences in the vertical columns. P<0.05 was considered significant.*

Table 4.

The diameters of the growth inhibition zone at 24 hours (*mm*)

Group	diameter of the inhibitory zone Mean±SD
D-limonene	8.610±1.549[a]
Eucalyptol	6.716±1.161[b]
Desocclusol	None
Saline	None

Notes: *Different lower case letters indicate statistically significant differences in the vertical columns. P<0.05 was considered statistically significant.*

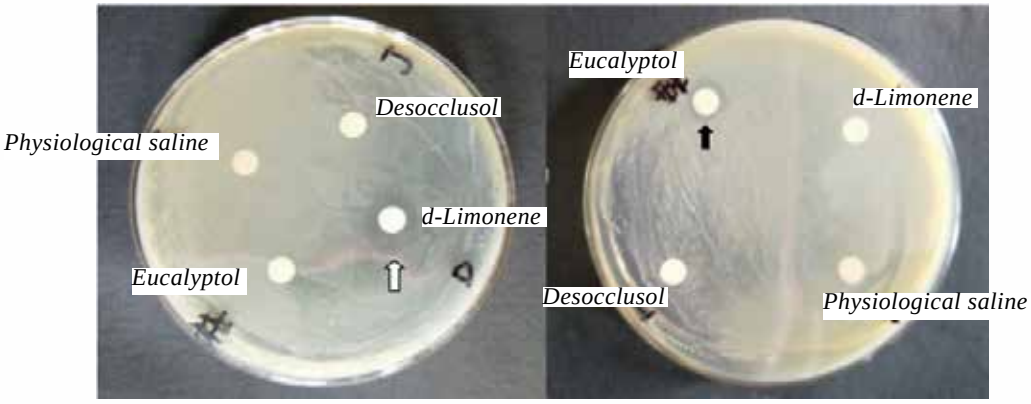

FIGURE 5. *The diameters of the growth inhibition zones could be found around the d-limonene and eucalyptol disk, the white arrow shows the inhibition zone of d-limonene group, and the black arrow shows the inhibition zone of the eucalyptol group.*

All three organic solvents had a strong ability to dissolve gutta-percha in endodontic retreatment. The duration time of reaching the apex and finishing preparation was significantly shortened in three experimental groups. Compared with control, the residual amount of gutta-percha in the root canal was greatly reduced ($p<0.05$) while the quantity of apical extrusion did not increase ($p>0.05$). It seems that desocclusol had the highest efficacy in dissolving gutta-percha, but no difference ($p>0.05$) was obtained compared with the other two groups, indicating that the three solvents have proximal efficiency in dissolving the gutta-percha.

In the present study, the root canal dentin is exposed to the solvents during endodontic retreatment, which may bring changes to the physical and chemical properties of dentin [*Kaufman D et al., 1997*]. The microhardness of dentin is one of its important physical properties. Changes of it can reflect the loss or deposition of minerals in the hard tissue. Studies have shown that the lower the microhardness, the lower the fracture resistance of the root. Therefore, detection the effect of different organic solvents on the microhardness of root canal dentin can provide a theoretical basis for the selection of a gutta-percha dissolver in clinical applications.

Microhardness measurement was done by three methods namely Knoop hardness number, Vickers hardness number and Brinell hardness number. Previous studies showed that Vickers test was not easily affected by surface conditions, by which causing a square-shaped indentation is simpler and more sensitive and accurate when using the same load [*Yassen G et al., 2013*] compared with other measurements of microhardness [*Cruz-Filho A et al., 2011*]. Therefore, the Vickers microhardness test method was selected in this study.

Our results showed that for desocclusol, it required the shortest time to reach working length of root canal with an average time of approximal 13 minutes, but it can decrease the microhardness of the root canal dentin at the coronal third and middle third. For eucalyptol, no significant difference was detected in microhardness compared with control ($p>0.05$). In d-limonene group, the average operating time in the root canal was about 15 minutes longer, while it had a more positive effect on the microhardness of dentin. What's more, in the coronal third and middle third segments of the root canal, d-limonene significantly increased the microhardness compared with desocclusol, in which the microhardness was reduced ($p<0.05$). No obvious microhardness changes in apical third segment among experimental groups in the present study, which may probably because of the short contact time with the organic solvents in apical canals (p>0.05).

Rasoul S and co-authors showed that dentin microhardness was positively correlated with mineral content, negatively correlated with carbonate content, and reduced with the decrease of crystallinity [*Seyedmahmoud R et al., 2017*]. Studies have shown that after removal of root canal sealers (10, 20, and 30 days), desocclusol can lead to demineralization in the inner layer of the root canal (500 *μm* from the pulp–dentine interface), which might because of the organic solvent could change the dentin calcium and phosphorus levels and cause the crystal gap to increase by softening the crown

enamel and dentine, thereby it increased the porosity and permeability of the tissue and reduced its hardness [*Rotstein I et al., 1999*].

In vitro antimicrobial susceptibility test showed that both d-limonene and eucalyptol had inhibitory effect on *E.faecalis*, and d-limonene was stronger in this effect. However, desocclusol had no obvious inhibitory effect on *E. faecalis*. Therefore, during the endodontic retreatment process, additional use of d-limonene will help to remove the *E. faecalis* when re-preparing the root canal.

It is worth noting that organic solvents are volatile and toxic. The strong volatility of dink and its main component tetrachloroethylene has a certain potential harm to health. It is irritating to the respiratory tract and skin mucosa. Furthermore, eucalyptol has been proven that its ability to dissolve gutta-percha being similar to that of chloroform, and also demonstrates cytotoxicity. Therefore, preventive application of rubber barrier isolation should be noticed when using these solvents.

CONCLUSION

Within the limits of this in vitro investigation, it can be concluded that the three solvents in this experiment could effectively remove the gutta-percha and reduce the residue in the root canal. D-limonene could increase the microhardness of the root canal and has a stronger inhibitory effect on *E. faecalis* compared with other solvents.

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