Original Article

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The gutta-percha softening efficacy of synthetic D-limonene at different concentrations and contact times

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Objective: To compare the softening efficacy of various concentrations of synthetic D-limonene on gutta-percha at different contact times.

Methods: 210 plastic molds (4 mm diameter, 20 mm deep, tuberculin syringes, Nipro Corporation, Japan) were filled with Cavit (Caviton, GC Corporation, Japan) on the bottom and further filled with thermo-plasticized gutta-percha (B&L -beta Gutta Percha Pellet, B&L Biotech, USA). The molds were stored in an incubator at 37°C to simulate the oral temperature and then randomly divided into 5 experimental groups (97%, 80%, 60% synthetic D-limonene, GP-solvent®, and ethanol) and 2 control groups (chloroform, normal saline). 0.2 ml of each solvent was dropped on the gutta-percha surface using a calibrated dropper and the samples were left for 1, 3, and 5 min. A #30 finger spreader was attached to the upper part of a Universal Testing Machine (EZ-S, SHIMADZU, Japan). The solvent-softening efficacy was measured by the downward force required to move the finger spreader at a constant speed of 5 mm/min 5 mm deep into the gutta-percha, recorded in Newtons. Statistical analysis was performed using IBM SPSS statistics 20 (New York, USA), and the Kruskal-Wallis test at the 95% confidence level.

Results: At each time point (1, 3, and 5 min), the 97% synthetic D-limonene softened the gutta-percha with a similar efficacy to chloroform ($p<0.05$). Chloroform softened the gutta-percha with a greater efficacy than GP-solvent® at all time points ($p<0.05$). At 1 min, there was no significant difference in softening between synthetic D-limonene and chloroform at all concentrations. The data indicated that 60% synthetic D-limonene, which was the lowest concentration used in this study, softened gutta-percha with a similar efficacy to chloroform. However, at 3 and 5 min, the softening efficacy of chloroform was significantly higher than the 80% and 60% synthetic D-limonene groups ($p<0.05$). Furthermore, regardless of the solvent, longer contact time tended to require a greater force to penetrate the gutta-percha.

Conclusion: The 1 min contact time is the optimal contact time to soften gutta-percha for all solvents. The optimal concentration of synthetic D-limonene that yielded the same gutta-percha softening efficacy as chloroform was 60% synthetic D-limonene.

Key words: contact times, softening gutta-percha, solvent concentrations, sythetic d-limonene

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Introduction

The success rate of non-surgical endodontic retreatment over long term follow-ups is 76%-77% [1, 2]. Coronal leakage, missing anatomy, inadequate root canal shaping, cracked tooth, crown fracture, and recurrent dental caries are the causes of failure after initial root canal treatment [3, 4]. Removing the root canal filling material is an important procedure in non-surgical endodontic retreatment. Several mechanical techniques can be used to remove gutta-percha such as an H-file, rotary file, or heat carrier [5, 6]. Therefore, using a mechanical gutta-percha removal technique plays an important role in removing the root canal filling material [7]. However, the use of mechanical techniques alone can lead to canal perforation.

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Previous studies revealed that the combination of solvents and mechanical techniques increases the efficacy of gutta-percha removal [8]. The most common gutta-percha solvents are chloroform, xylene, orange oil, and eucalyptol oil. Chloroform and xylene are considered effective chemical solvents to remove gutta-percha [9-11]. However, chloroform is prohibited by the U.S. Food and Drug Administration due to its carcinogenicity [12, 13]. Although xylene is not considered as carcinogen, it can be harmful to living tissue [10, 11]. Currently, extracted orange oil, an organic solvent, is popular because it is a natural product. Several studies found that orange oil was the least toxic compared with other chemical solvents [14].

The chemical formula of D-limonene is $C_{10}H_6$. It is a clear liquid found in oils extracted from citrus plants, such as grapefruits (92% D-limonene), wild oranges (90% D-limonene), lemons (70% D-limonene), limes (65% D-limonene), and bergamots (30% D-limonene). The code of Federal Regulations list D-limonene as a generally recognized as safe flavoring agent, which is commonly added to food products, such as fruit juices, soft drinks, pudding, ice cream, and baked goods [15].

GP-solvent® (Nippon Shika Yakuhin, Japan) is a commercial brand of D-limonene-containing gutta-percha solvent. This solvent softens gutta-percha and zinc oxide eugenol cement; however, it is expensive and must be imported. Currently, there is a little information regarding the softening efficiency of different concentrations of D-limonene as a gutta-percha solvent. Therefore, the aim of this study was to compare the softening efficacy of different concentrations of synthetic D-limonene as a gutta-percha solvent at different contact times.

Materials and Methods

Plastic tube and gutta-percha preparation

20-mm high plastic molds were made from tuberculin syringes (4 mm diameter, Nipro Corporation, Japan). The molds were divided into three parts. The lower 5-mm of the plastic molds was filled with Cavit (Caviton, GC Corporation, Japan). The middle 10-mm section of the molds was filled with thermo-plasticized gutta-percha (B&L -beta Gutta Percha Pellet, B&L Biotech, USA) by vertical condensation using a #1 Glick instrument until completely dense. The top 5-mm of the mold was left as a space for solvents **(Figure 1)**. The specimens were stored in an incubator at 37ºc for 24 h to simulate the oral temperature. Each gutta-percha-filled plastic mold was placed in a resin block in a PVC ring for stability.

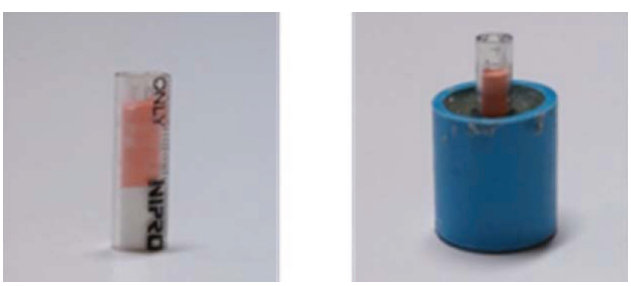

Figure 1 Representative plastic mold specimen and resin in a PVC ring.

Solvent preparation

97% synthetic D-limonene (Chemipan Corporation Co., Ltd, Thailand) was diluted with ethanol to 80% and 60% concentration. The equation $C_1V_1 = C_2V_2$, the rule of three, was used to dilute the solvent. The fresh diluted solutions were prepared by the same chemist.

Testing procedure

There were 10 samples in each group at each time point (**Table 1**). 210 samples were divided into five experimental and two control groups based on the solvent type and concentration: 97% synthetic D-limonene, 80% synthetic D-limonene, 60% synthetic D-limonene, GP-solvent® (Nippon Shika Yakuhin, Japan), and

ethanol (SSCV Corporation Co., Ltd, Thailand). For the two control groups, chloroform (RCI Labscan limited Co., Ltd, Thailand) was used as the positive control, and normal saline solution (Klean & Kare®, A.N.B. Laboratories Co., Ltd, Thailand) as the negative control.

Table 1 The number of samples in each experimental and control group.

Solutions \ Contact time	1 min	3 mins	5 mins
97% synthetic D-limonene	10	10	10
80% synthetic D-limonene	10	10	10
60% synthetic D-limonene	10	10	10
GP-solvent®	10	10	10
Ethanol	10	10	10
Chloroform (+ve control)	10	10	10
Normal saline (-ve control)	10	10	10

After storing in a 37°C incubator for 24 h, 0.2 ml of the respective group's solvent was added into the mold using a calibrated dropper. The solvent was left in direct contact with the gutta-percha for 1, 3, and 5 min. The softening efficacy of each solvent was measured by the force required to move a #30 finger spreader into the gutta-percha. The finger spreader was attached to the upper part of a Universal Testing Machine (EZ-S, SHIMADZU, Japan). The spreader moved down into the tuberculin syringe until contacting the gutta-percha surface and paused at the surface of the gutta-percha perpendicular to, and in the center of, the gutta-percha. Subsequently, the spreader continued to move down towards the gutta-percha at a constant speed of 5 mm/min to a depth of 5 mm (midway). The force exerted to move the spreader through the gutta-percha was recorded in Newtons (N). The numerical data were collected and graphed using the Trapeziumv2 program. All procedures were performed at room temperature.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 20 (New York, USA). The exerted force in each experiment was analyzed using the Kruskal-Wallis test. Comparison between groups was performed using the Mann Whitney U test. The confidence level was set at 95%.

Results

Chloroform and normal saline solution were used as the positive and the negative control, respectively. Ethanol, which was used to dilute the synthetic D-limonene, was included as one of the experimental groups to ensure that it did not have a softening effect on the gutta-percha. We found that the normal saline solution and ethanol did not have a softening effect on gutta-percha.

We determined the median exerted force used to penetrate the #30 spreader 5 mm into the gutta-percha (Table 2). A higher required force represented lower softening efficacy of the solvent. At every time point (1, 3, and 5 min) 97% synthetic D-limonene softened the gutta-percha with a similar efficacy to that of chloroform and the positive control ($p<0.05$). Chloroform softened the gutta-percha better compared with the GP-solvent® at each time point ($p<0.05$).

At 1 min, there was no significant difference in the softening efficacy between the synthetic D-limonene concentrations groups and chloroform. The results showed that 60% synthetic D-limonene, which was the lowest concentration in this study, softened gutta-percha with a similar efficacy to that of chloroform. However, at 3 and 5 min, the softening efficacy of chloroform was significantly higher compared with 80% and 60% synthetic D-limonene ($p<0.05$). We also found that longer solvent contact times tended to require a greater force to penetrate the gutta-percha.

Table 2 Median exerted force (Newtons) used to penetrate into the gutta-percha at different time points.

	Chloroform (n=10)	GP-solvent® (n=10)	97% D (n=10)	80% D (n=10)	60% D (n=10)	NSS (n=10)	Ethanol (n=10)	Independent Samples Kruskal-Wallis Test	Pairwise Comparison Between Solutions
Time = 1 min (T1)									
Median	12.73	14.60	13.18	14.15	14.23	15.22	15.24	p < 0.001	Chlo> GP, Chlo > NSS, Chlo > Ethanol, 97% D > GP, 97% D > NSS, 97% D > Ethanol
IQR	1.34	1.21	0.59	1.03	0.64	1.40	0.56		
Min.	11.61	13.45	11.91	12.83	13.02	13.21	15.03		
Max.	14.27	15.76	13.77	15.26	14.99	15.57	16.02		
Time = 3 min (T3)									
Median	13.12	15.14	13.59	15.04	15.48	14.88	16.48	P<0.001	Chlo>GP, Chlo>80%D, Chlo>60%D, Chlo>Ethanol
IQR	1.47	0.81	1.01	0.74	1.04	0.76	0.73		97%D>GP, 97%D>60%D, 97%D>Ethanol
Min.	12.24	14.35	12.41	13.67	13.73	14.24	16.25		
Max.	13.79	16.11	14.19	15.66	16.50	15.37	17.36		
Time = 5 min (T5)									
Median	13.42	15.80	13.64	15.07	16.14	15.27	16.62	P<0.001	Chlo>GP, Chlo>60%D, Chlo > Ethanol
IQR	0.32	1.24	1.04	1.78	0.65	0.86	0.61		97%D > 60%D, 97%D>GP, 97%D>Ethanol
Min.	13.12	14.58	12.20	13.54	15.26	14.20	15.72		
Max.	13.79	16.69	14.78	16.64	16.77	15.73	17.31		
Pairwise Comparisons of Time Contact in the Same Solutions									
T3-T1	p = 0.31	p = 0.34	p = 0.16	p = 0.054	p = 0.036	p = 0.49	p = 0.001	n.a.	n.a.
T5-T1		p = 0.015		p = 0.036	p < 0.001		p = 0.001	n.a.	n.a.
T5-T3		p = 0.65		p = 1.00	p = 0.24		p = 1.00	n.a.	n.a.

min. = minutes, IQR = Inter-quartile rang (quartile 3rd – quartile 1st), Min. = minimum, Max. = maximum; n.a. = not applicable, > means better softening effect

Discussion

There are many studies that have used various methods, such as immersing gutta-percha cones in solvents and determining the weight loss [16-18], measuring the loading force penetration of an instrument after surface contact with each solvent [18-21], and simulating the clinical situation by evaluating the removal time and radiographic density of the residual gutta-percha in root canals [22, 23], to evaluate the efficiency of solvents to soften gutta-percha. However, the effect of different solvent contact time has not been reported. In this study, the softening efficacy was determined by examining the force required for a #30 finger spreader to penetrate 5 mm into gutta-percha at 1, 3, and 5 min contact time.

Currently, the solvents commonly used to soften gutta-percha are chloroform, xylene, essential oils, eucalyptol oil, and orange oil. GP-solvent® is widely used clinically in retreatment cases because it is safe and non-carcinogenic [14]. The major component of GP-solvent® is orange oil, which is extracted from orange peels, and has D-limonene as its main component [14].

D-limonene is a clear colorless liquid with a pleasant lemon-like odor. D-limonene is soluble in ethanol. It oxidizes to a film in air, an oxidation behavior similar to that of rubber or drying oils (24). Extracting D-limonene from orange peels is difficult and complicated [16]. There are three grades of D-limonene commercially available; untreated technical grade (synthetic D-limonene) (95% pure), food grade (97% pure), and lemon-lime grade (70% pure) (Florida chemical Co., 1991). This study used technical grade D-limonene, which was purified by the Florida Chemical Company, confirming that the D-limonene used in this study was 94-97% pure.

The softening effect of synthetic D-limonene was investigated based on two variables: contact time (1, 3, and 5 min) and concentration (97%, 80%, and 60% synthetic D-limonene).

The contact time results revealed that the required exerted force increased proportionally with the contact time. Jantarat J et al., evaluated the dissolving ability of many essential oils that contain different D-limonene concentrations. Their study used grapefruit oil (90% D-limonene), tangerine oil (90% D-limonene), lime oil (60% D-limonene), and lemon oil (60% D-limonene). The results demonstrated that after a 5 min contact time, the oil that contained a higher percentage of D-limonene had a significantly higher dissolving effect on the gutta-percha [21]. Their results were similar to that of the present study that found that at the 5 min contact time, the 90% synthetic D-limonene group demonstrated a significantly higher softening effect compared with the 60% synthetic D-limonene group ($p<0.05$). In contrast, the results after a 1 min contact time demonstrated that the 90%, 80%, and 60% synthetic D-limonene groups displayed a similar gutta-percha softening effect ($p<0.05$).

In the present study, the results at the 1 min contact time revealed no significant difference in the softening efficacy between chloroform and 97% ,80%, or 60% synthetic D-limonene, which agreed with the results in Gomes et al. Their study evaluated the effectiveness of four different solvents and classified their potential action. Gutta-percha cones were immersed in xylene, eucalyptol oil, orange oil, or chloroform for 1, 2, 3, 4, and 5 min. They found that all solutions caused the highest dissolution of gutta-percha at the first minute and the solvent efficacy decreased with as contact time increased. This may be because gutta-percha contains zinc oxide, waxes, resins and barium sulfate, however, the solvents only dissolve the gutta-percha [25].

During the contact period, there may have been some alteration in the composition of the solvents when in contact with the gutta-percha surface, resulting in a decreased softening efficacy, and therefore a change in the results. Moreover, because the technique of adding the

solvents in this study was different from that in Numan's, where the time taken by a #30 Hedstrom file to pass through the gutta-percha was investigated. In his study, 0.02 ml of tetrachloroethylene, xylene, or eucalyptol oil solvent was sequentially added whenever resistance to penetration into the gutta-percha in the root canal of extracted human lower premolars [26]. In contrast, in the present study the solvent was added once and left for specific amounts of time. Thus, the gutta-percha suspension in the solvent may have negatively impacted the solvent efficiency. Further study on softening depth and solvent volume should be performed.

The present study found that there was no significant difference in softening efficiency between chloroform and GP-solvent®, and between GP-solvent® and each experimental group. GP-solvent® is commonly used in the clinic [13, 14]. Our study indicated that the softening efficacy of 97%, 80%, and 60% synthetic D-limonene was similar to that of GP-solvent®. However, further studies must be performed prior to their clinical use.

Within the limitations of this study, it can be concluded that the softening efficacy of 97% synthetic D-limonene was similar to that of chloroform at 1, 3, and 5 min. All concentrations of synthetic D-limonene have a softening efficiency similar to chloroform within a 1 min contact time.

Conclusions

Within the limitations of this study, the optimal contact time of all solvents to soften gutta-percha was one min.

At one min contact time, the softening efficacy of the different D-limonene concentrations on gutta-percha was similar to that of chloroform. Furthermore, 97%, 80%, and 60% synthetic D-limonene demonstrated approximately the same softening capability compared with GP-solvent®. Chloroform and 97% synthetic D-limonene have a higher softening efficacy than GP-solvent®.

60% synthetic D-limonene with a one min contact time could be optimally effective to soften gutta-percha in endodontic retreatment cases.

References

1. Riis A, Taschieri S, Del Fabbro M, Kvist T. Tooth Survival after Surgical or Nonsurgical Endodontic Retreatment: Long-term Follow-up of a Randomized Clinical Trial. *J Endod* 2018; 44: 1480-6.
2. Ng YL, Mann V, Gulabivala K. Outcome of secondary root canal treatment: a systematic review of the literature. *Int Endod J* 2008; 41: 1026-46.
3. Akbar I. Radiographic study of the problems and failures of endodontic treatment. Int *J Health Sci* 2015; 9: 111-8.
4. Tabassum S, Khan FR. Failure of endodontic treatment: The usual suspects. *Eur J Dent* 2016; 10: 144-7.
5. Tasdemir T, Er K, Yildirim T, Celik D. Efficacy of three rotary NiTi instruments in removing gutta-percha from root canals. *Int Endod J* 2008; 41: 191-6.
6. Yuruker S, Gorduysus M, Kucukkaya S, Uzunoglu E, Ilgin C, Gulen O, et al. Efficacy of Combined Use of Different Nickel-Titanium Files on Removing Root Canal Filling Materials. *J Endod* 2016; 42: 487-92.
7. DUNCAN HF, CHONG BS. Removal of root filling materials. *Endodontic Topics* 2008; 19(1): 33-57.
8. Kaplowitz GJ. Evaluation of Gutta-percha solvents. *J Endod* 1990; 16: 539-40.
9. Gorduysus MO, Tasman F, Tuncer S, Etikan I. Solubilizing efficiency of different gutta-percha solvents: a comparative study. *J Nihon Univ Sch Dent* 1997; 39: 133-5.
10. Spangberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. *Oral Surg Oral Med Oral Pathol* 1973; 36: 856-71.
11. Wourms DJ, Campbell AD, Hicks ML, Pelleu GB, Jr. Alternative solvents to chloroform for gutta-percha removal. *J Endod* 1990; 16: 224-6.
12. Chernichenko IA, Balenko NV, Litvichenko ON. [Carcinogenic hazard of chloroform and other drinking water chlorination by-products]. *Gig Sanit* 2009: 28-33.
13. Reitz RH, Gehring PJ, Park CN. Carcinogenic risk estimation for chloroform: an alternative to EPA's

procedures. *Food Cosmet Toxicol* 1978; 16: 511-4.
14. Magalhaes BS, Johann JE, Lund RG, Martos J, Del Pino FA. Dissolving efficacy of some organic solvents on gutta-percha. *Braz Oral Res* 2007; 21: 303-7.
15. Sun J. D-Limonene: safety and clinical applications. *Altern Med Rev* 2007; 12: 259-64.
16. Kaplowitz GJ. Evaluation of the ability of essential oils to dissolve gutta-percha. *J Endod* 1991; 17: 448-9.
17. Metzger Z, Marian-Kfir V, Tamse A. Gutta-percha softening: "Hemo-De" as a xylene substitute. *J Endod* 2000; 26: 385-8.
18. Hansen MG. Relative efficiency of solvents used in endodontics. *J Endod* 1998; 24: 38-40.
19. Oyama KO, Siqueira EL, Santos M. In vitro study of effect of solvent on root canal retreatment. *Braz Dent J* 2002; 13: 208-11.
20. Pecora JD, Spano JC, Barbin EL. In vitro study on the softening of gutta-percha cones in endodontic retreatment. *Braz Dent J* 1993; 4: 43-7.
21. Jantarat J, Malhotra W, Sutimuntanakul S. Efficacy of grapefruit, tangerine, lime, and lemon oils as solvents for softening gutta-percha in root canal retreatment procedures. *J Investig Clin Dent* 2013; 4: 60-3.
22. Uemura M, Hata G, Toda T, Weine FS. Effectiveness of eucalyptol and d-limonene as gutta-percha solvents. *J Endod* 1997; 23: 739-41.
23. Peraya S NC, Tosaphol P. In vitro study on the efficacy in assisting gutta-percha removal and cytotoxicity of essential oil from Citrus maxima (pomelo oil). *CU Dent J* 2004; 37: 289-98.
24. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res* 2019; 47: D1102-D9.
25. Fabio de Almeida Gomes APBD, Rodrigo Arraes Nunes, Anna Lygia Nunes Fernandes, Claudio Maniglia-Ferreira, Humberto Ramah Meneses de Matos, Taffarel Canuto Nepomuceno. Efficacy of gutta-percha solvents used in endodontic retreatments. *RSBO* 2013; 10: 356-61.
26. Numan FG. The Dissolving Capability Of Various Gutta-Percha Solvents. *Journal of Al Rafidain University College* 2013: 116-24.