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Evaluating *In Vitro* Performance of D-Limonene as a Solvent for Facilitating Patency in Simulated Root Canals Obturated with a Bioceramic Sealer

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Introduction

Bioceramic sealers associated with single-cone obturation techniques are increasingly prevalent. One outcome study published in 2018 examined 307 teeth with an average follow-up time of 30 months and found a success rate of 90.9% (Chybowski). Even with this optimistic initial outlook for single-cone techniques using bioceramic sealers, clinicians who perform retreatment should expect to see some of these teeth with recalcitrant disease. Conventional retreatment techniques (heat, chloroform, rotaries, and hand files) achieve a measure of material removal (Donnermeyer, Romeiro), but it can be especially difficult to achieve patency through the bioceramic sealer at the apical extent of the canal when the gutta-percha cone was seated short of the apical foramen (Hess, Eymirli). This indicates that there is room for improved retreatment protocols.

Several supplemental approaches to retreatment of bioceramic sealers include sonic and ultrasonic activation of solvents (Pedulla), the use of formic acid with EDTA (Garrib), and the XP-endo Finis her R system (Brasseler USA, Savannah, GA) (Volponi, Aksel). One additional retreatment method is to employ solvents that are alternatives to chloroform (Carpenter). D-Limonene (Prevest DenPro, Lewes, DE), refined orange oil, is an essential oil that has the ability to aid in the removal of gutta-percha and various endodontic sealers during retreatment procedures (Tamse). The aim of this study is to compare the ability of and time required for D-Limonene to facilitate reacquisition of patency in simulated canals obturated 2.0 mm short with

single-cone gutta-percha and EndoSequence BC sealer (Brasseler USA, Savannah, GA). The null hypothesis is that there is no difference when comparing the ability of chloroform, D-Limonene, and saline to facilitate reacquisition of patency.

Materials & Methods

Thirty plastic blocks with simulated J-shaped root canals (Coltene Whaldent, Altstatten, Switzerland) were collected. A glide-path was established in all canals up to a #15 K-file, and the canals were then instrumented using a crown-down technique with Vortex Blue 0.04 tapered rotaries (Dentsply Sirona, Charlotte, NC) to size 30/.04. After each file was used, the canal was irrigated with 1.0 mL of sterile saline through a 30-gauge side-vented needle, and patency was reconfirmed with a #10 K-file. A final rinse was performed with 3.0 mL of saline. The canals were dried with paper points, and patency was reconfirmed with a #10 K-file.

EndoSequence BC Sealer was introduced into all the canals until the sealer was extruded from the apical foramen. In all of the blocks, a size #30/.04 gutta-percha cone (Brasseler USA, Savannah, GA) was trimmed to fit 2.0 mm short of working length, coated with sealer, and inserted into the canal to simulate a short obturation. This approach allowed for an attempt to regain patency through sealer only. The cones in all canals were seared off at the orifice using a System B heat tip (Kerr Dental, Brea, CA). A thin layer of RMGI (Vitrebond, 3M ESPE, Irvine, CA) was placed over the orifices to serve as a secondary seal. The plastic blocks were stored at 37°C in 100% humidity for two weeks to ensure complete setting of the materials.

The 30 blocks were then equally divided into three groups: Group 1 (chloroform), Group 2 (D-Limonene), and Group 3 (saline). The Vitrebond seal was removed with a #4 round bur in a high speed hand piece. A Profile #30/.04 rotary instrument (Dentsply Sirona, Charlotte, NC)

was used to remove the coronal and mid-root gutta-percha to a point 3.0 mm short of working length.

Subsequently, three drops of either chloroform, D-Limonene, or saline were introduced to the canal. An attempt was then made to regain patency using a new #15 K-file. Patency was visually confirmed by placing the #15 K-file out of the apex. If patency could not be re-established within three minutes after placing the solvent, the test was terminated and recorded as a failure. All sample preparations, treatments, and evaluations were performed by a single operator.

The primary outcome of this investigation was the time required to regain patency. We used the Shapiro-Wilk test to assess the normality of the data distribution. Consequently, measures of central tendency and dispersion for time are reported as means with associated standard deviations. An independent samples t-test used for pairwise comparisons and Leven's test was checked for equality of variances between groups. Significance was declared at $P < 0.05$ and all tests were 2-tailed. All data was analyzed by using SPSS version 25.0 (SPSS, Chicago, IL).

Results

A total of 30 samples were examined for this study. Excluded from the analyses were the 10 chloroform samples of Group 1. This was due to a 90% ($n = 9$) failure rate in achieving patency for the group. In contrast, the failure rates for Groups 2 and 3 were 30% and 50% respectively.

Block Number	Chloroform Group 1	D-Limonene Group 2	Saline Group 3
1	NP	NP	NP
2	NP	1:57	2:22
3	NP	1:42	1:35
4	NP	1:48	NP
5	NP	1:25	NP
6	NP	NP	2:03
7	NP	2:55	0:56
8	NP	2:32	NP
9	NP	NP	0:47
10	2:05	0:24	NP

The mean time to regain patency was 109sec ($n = 7$; $SD = 46$) for Group 2 and 105sec ($n = 5$; $SD = 50$) for Group 3. No significant difference was observed between the two groups, $P = 0.88$. Although not explicitly tested, it is worth noting that the single successful chloroform patency reacquisition was achieved in 125sec. Although numerically larger than the means of the other 2 groups, this is within 1 standard deviation of each.

To check whether the non-significant difference in patency time could be due to a lack of statistical power, a post hoc power analyses using the program G*Power v3.1 was performed. The analysis revealed that on the basis of the mean, given the between-groups comparison effect size observed in the present study ($d = .08$), a total sample size 4,114 would be needed to obtain statistical power at the recommended 80% level.

Discussion

The use of bioceramic sealer in a single-cone obturation technique is becoming increasingly commonplace as more short-term outcomes and *in vitro* studies assert the safety and effectiveness of this material. Nonetheless, some teeth obturated using this method require subsequent retreatment. Achieving patency is one of the most significant intraoperative factors affecting tooth survival (Ng). In root canal retreatment, removal of obturation materials is required to maximize the delivery of antibacterial agents to all surfaces of the root canal dentin.

Consequently, a significant prognostic factor influencing outcome of retreatment is the ability to remove or bypass any material that might be obstructing access of irrigants to the apical canal system (Ng). A well-set bioceramic sealer, especially in a canal where gutta-percha was seated short of the working length, may pose a special challenge to achieving patency if that canal requires retreatment. This obstacle calls for more studies investigating supplemental techniques and materials to see if a new approach might be more effective for retreating these cases.

Chloroform is traditionally the solvent of choice for softening gutta-percha and dissolving sealers during non-surgical root canal retreatment procedures. Its general safety and efficacy are well-established when used for this purpose, but side-effects from exposure to chloroform have been reported, and studies have demonstrated that chloroform is possibly carcinogenic to humans (Edgar, Ribeiro). D-limonene is normally used for removing cements and other materials from dental tools, but it also can be used as a solvent during endodontic retreatment. It was found in one study to be more biocompatible than eucolaptol, xylol, chloroform, or halothane, but it also demonstrates less efficiency than chloroform at dissolving gutta-percha (Scelza). Two benefits of considering D-limonene as an alternative solvent are patient acceptance (especially in a patient population that is increasingly conscientious of the materials used for dental procedures) and its inexpensive availability.

This study aimed to assess whether D-limonene would be non-inferior compared to chloroform and saline controls at achieving patency in plastic blocks obturated with gutta-percha coated in EndoSequence BC Sealer and seated 2.0 mm short of working length. This approach allowed for an attempt to regain patency in a J-shaped canal through only sealer. Placing the plastic blocks in 100% humidity for 2 weeks ensured that the material would be completely set prior to initiating the experimental phase of the study. Dividing the blocks into 3 groups allowed

for assessment of D-limonene against 2 controls: saline (which illuminated the effects of a #15 K-file's ability to penetrate through the sealer aided only by the simplest means of lubricating a canal) and chloroform (the most ubiquitous solvent used in root canal retreatment procedures).

The primary outcome of this investigation was the time required to regain patency. The chloroform group was ultimately excluded from data analysis because of a 90% failure rate in achieving patency in under 3 minutes. This failure appears to have occurred not because of the material's inability to dissolve bioceramic sealer but rather because of its corrosive effects on the plastic blocks. Softening of the plastic past the start of the J-shaped curvature produced a context in which the k-files quickly ledged the canals, which prevented further advancement through the bioceramic sealer.

Patency was achieved in under 3 minutes in 7 of the D-Limonene blocks and 5 of the saline blocks, with average times to patency of 109 seconds and 105 seconds, respectively. Although the raw data slightly favors D-Limonene over saline, the differences never reached the level of statistical significance, particularly due to the small sample size chosen for this study. If comparing the similar mean times to patency in both groups, however, an estimated sample size of over 4,100 blocks would be required to obtain statistical power. This indicates that there may be a true lack of statistical difference between the two groups with respect to the time it would take an individual to regain patency.

In addition to the small sample size, another limitation of this study was the use of plastic blocks instead of extracted teeth. While using plastic blocks allowed uniformity across the study, it does not offer as much external validity as an *ex vivo* study using extracted teeth. This is particularly the case because different materials (such as bioceramic sealer, chloroform, D-limonene, and K-files) will interact with tooth structure differently than with plastic. This

limitation especially affected the chloroform group, since the chloroform appeared to dissolve the plastic, resulting in speedy deviation of K-files from the original path and a consequent failure to achieve patency in all but one of the canals in the chloroform group. Future studies designed in a similar vein as this one might improve the methodology by using a large number of extracted teeth having canals with similar working lengths and curvatures to see if a solvent like D-limonene might present a non-inferior or even superior performance compared to chloroform for removing bioceramic sealers from root canals.

Conclusion

According to this *in vitro* study, D-Limonene was not a more effective solvent for regaining patency in canals obturated with bioceramic sealers than saline. Further studies are needed to ascertain whether D-limonene is a workable alternative to traditional solvents like chloroform for removing bioceramic sealers.

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