Original Article

Effect of Chloroform, Eucalyptol and Orange Oil Solvents on the Microhardness of Human Root Dentin

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Abstract
Objectives: This study aimed to assess the effect of chloroform, eucalyptol and orange oil solvents on the microhardness of human root dentin.
Materials and Methods: Sixty-eight single-rooted single-canal extracted human premolar teeth were used. Tooth crowns were separated from the roots at the cementoenamel junction (CEJ). Roots were buccolingually sectioned into mesial and distal halves. Specimens were randomly divided into 5 groups, with 20 teeth in each solvent group and 4 teeth in each control group. Primary microhardness of specimens was measured using Vickers microhardness tester. Specimens were exposed to solvents for 15 minutes and were subjected to microhardness testing again. Data were recorded and analyzed using repeated measure ANOVA.
Results: No significant difference was found in dentin microhardness before and after exposure to solvents in any of the orange oil, eucalyptol, chloroform or saline groups (P=0.727). None of the experimental groups showed any significant difference in terms of dentin microhardness reduction (P=0.99) and had no significant difference with the negative control group.
Conclusion: This study showed that chloroform, eucalyptol and orange oil as gutta percha solvents did not decrease the microhardness of root dentin. Thus, none of the mentioned solvents has any superiority over the others in terms of affecting dentin properties.
Keywords: Root; Dentin; Hardness; Chloroform; Eucalyptol; Orange oil
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INTRODUCTION

Several root canal filling materials are used for root canal obturation but gutta percha along with a sealer is still the most commonly used material for root filling [1].

Bacteria remaining within the root canal system are an important factor responsible for treatment failure [2, 3]. Nonsurgical root canal retreatment is the first choice to reinstate healthy periapical tissue [4]. It is important to remove as much sealer and gutta-percha as possible for effective disinfection and resealing [5, 6]. Despite various available techniques for re-treatment, studies have shown

that obtaining a root canal system with walls completely free from debris and residual infectious agents is not feasible [7, 8]. Use of solvents is recommended to facilitate the removal of softened gutta percha [9].
These solvents may change the physical and chemical properties of dentin and this issue is clinically important [10] because alterations of dentin surface may affect the dentin interaction with materials used for obturation [11].
Chloroform and Xylene are among the most commonly used gutta percha solvents. Several studies have shown that chloroform dissolves gutta percha efficiently and rapidly[12, 13].
The International Agency for Research on Cancer classifies Chloroform in the group 2B in terms of carcinogenicity [14]. Therefore, several studies have been designed to identify an alternative solvent to soften gutta-percha for removal from obturated root canals [15].
Magalhaes et al. evaluated the solubility of Xylene, chloroform, orange oil and eucalyptol solvents and showed that Xylene had the highest capability for dissolving gutta percha; whereas, chloroform, orange oil and eucalyptol had similar efficacy for this purpose [15]. Orange oil has the highest biocompatibility among the commonly used solvents. Moreover, orange oil has the least cytotoxicity compared to eucalyptol and chloroform [15].
Several studies have evaluated the effect of root canal irrigation solutions and chelators on the root dentin microhardness [11,16-18]; but limited studies have investigated the effect of chloroform, orange oil and eucalyptol gutta percha solvents on root dentin microhardness.
This study aimed to assess the effect of chloroform, eucalyptol and orange oil on the microhardness of human root dentin.

MATERIALS AND METHODS

Sixty-eight single-rooted single-canal extracted human premolar teeth were used. The teeth were evaluated for cracks and the cracked ones were excluded. The teeth were stored in saline solution at 37°C.
The crowns were separated from the roots at the cementoenamel junction using a high-speed hand piece and a bur with water coolant. The roots were longitudinally sectioned in buccolingual direction into mesial and distal halves. Pulp tissue was removed by barbed broach. The roots were mounted in Transoptic powder by the mounting press device (Buehler/ Metaserv, Bradford /UK) in such a way that root canal dentin was evident. For the microhardness testing, canal dentin was polished using a circular grinding machine (Motopol 2000 Grinder Polisher, MI, USA) and 400, 800 and 1200 grit abrasive papers under running water. Specimens were randomly divided into 5 groups, with 20 teeth in each solvent group and 4 in each control group. Solvents used were orange oil (Henry Schein, NY, USA) in group 1, eucalyptol (Sultan Healthcare, NJ, USA) in group 2, chloroform (House Brand, NJ, USA) in group 3, saline (Samen, Mashhad, Iran) in group 4 (negative control) and 37% phosphoric acid (kimia, Tehran, Iran) in group 5 (positive control). Primary microhardness was measured at baseline before exposure to solvents using the Vickers microhardness tester (MV-H210-Akashi) at 400X magnification with 50g load for 15 seconds. According to Ramamoorthi et al study [19], five indents were made at 100µm distance from the canal lumen and parallel to it. The first indent was made at 1000µ distance from the canal entrance and the remaining 4 at 200µ distance from one another. The root canals were then exposed to 50µl of the respective solvents for 15 minutes and then dried with absorbent paper. Microhardness was measured again at the other side of the canal lumen under similar conditions and the obtained data were recorded. The data were analyzed using repeated measure ANOVA.

RESULTS

The mean, minimum, maximum and standard deviation values for root dentin microhardness in the understudy groups after exposure are shown in Table 1. The root dentin microhardness was 50.95 in the orange oil, 53.35 in the eucalyptol, 49.40 in the chloroform, 44.80 in the saline solution and 9 in the phosphoric acid group. In the positive control group (phosphoric acid), a significant difference was found in dentin microhardness after exposure ($P<0.001$). There was no statistically significant difference ($P=0.99$) among the other groups and a significant difference was not found before and after exposure ($P=0.727$).

DISCUSSION

The root canal dentin is exposed to solvents during endodontic retreatment for removing the gutta percha. These solvents may change the physical and chemical properties of dentin and this issue is clinically important [10]. Solvents commonly facilitate the removal of gutta percha and sealer from the root canal system [20] and their use expedites the process of retreatment and decreases the amount of residual material [21]. Microhardness test is a simple non-invasive tool for the assessment of the mechanical characteristics. Previous studies have confirmed the suitability and feasibility of Vickers test for the assessment of hard tissue changes after exposure to chemicals [11, 22]; thus, this method was used in this study.
The microhardness value can be an indirect indicator of mineral loss or deposition in the hard tissue of a tooth [23].
Moreover, a positive correlation exists between the microhardness values and the mineral content of teeth [24].
Some studies have reported that the root dentin microhardness decreases from the surface towards the deeper zones. Increased number of open dentinal tubules and peritubular spaces around the pulp decreases the resistance of dentin to microhardness indenter. Furthermore, Pashley found a reverse correlation between dentin microhardness and tubular density [22].
Microhardness measurement is done by three methods namely the Knoop hardness number (KHN), Vickers hardness number (VHN) and the Brinell hardness number (BHN) [19].
Previous studies used indenter microhardness test, Knoop microhardness test [22,25] and Vickers indenter test [26,27] for the measurement of dentin microhardness and surface changes of the hard tissue. Causing a square-shaped indentation in VHN microhardness test is simple and leads to more accurate measurement of microhardness [28].

Table 1. Vickers microhardness values (Minimum, Maximum, Mean, Standard deviation) of root canal dentin after the use of the tested solvents and in the control groups

Group	Microhardness	N	Minimum	Maximum	Mean	Std. Deviation
Orange oil	Before	20	30.00	67.00	50.5000	8.40739
	After	20	25.00	64.00	50.9500	8.04903
Eucalyptol	Before	20	27.00	77.00	55.500	14.24771
	After	20	25.00	79.00	53.3500	14.67284
Chloroform	Before	20	31.00	80.00	48.6000	11.82059
	After	20	29.00	67.00	49.4000	10.3457
Saline	Before	5	29.00	78.00	52.6000	17.57271
	After	5	22.00	61.00	44.8000	14.75466
Phosphoric acid	Before	3	52.00	59.00	55.5000	4.94975
	After	3	6.00	12.00	9.0000	4.2464

Among the microhardness measurement methods, Vickers test is less susceptible to the surface condition and is more sensitive to measurement errors when using equal loads [16].
In a study by Ballal et al, [17] indentations were made at 0.5 mm distance from the canal wall but in another study [18] using Vickers test for the measurement of microhardness, indents were made at the mid-third of the root canal at 100μm distance from the pulp-dentin interface. In the study by Ballal et al, [17] 200g load with 20 seconds of dwell time was used. Some other studies have used 50g load with 10 seconds of dwell time in order to standardize the specimens for Vickers microhardness testing. Milder load and shorter time were selected because of the reverse correlation between dentin microhardness and tubular density [18]. However, in another study, 50g load for 15 seconds was applied on each specimen [29]. Thus, in the current study, indents were made at 100μm distance from the root canal surface and 50g load for 15 seconds was applied for the microhardness test.
Chloroform is the most efficient solvent for the removal of root canal filling materials [30,31]. Due to its carcinogenic potential, some other materials were tested as alternatives [32]; among which, orange oil was proposed as a suitable solvent for the root canal filling materials [31,33]. Another solvent commonly used clinically is the eucalyptol and has shown to be a good alternative to chloroform [15,34].
Numerous studies have investigated the dissolving efficacy of organic solvents for the root canal filling materials [35,36]. Studies have shown that solvents are capable of softening the coronal enamel and dentin [37]. Although the effect of these solvents on root dentin microhardness has not been well evaluated, several studies have assessed the effect of irrigating solutions and chelators on dentin microhardness in the recent years [11,16-18]. In our study, the effects of chloroform, eucalyptol and orange oil solvents on root dentin microhardness were evaluated.
In a study by Rotstein et al [37], dentin specimens in the experimental groups were exposed to 50μl of the solvents for 5-15 minutes. Erdemir et al. [38] exposed dentin to 20ml of solvents for 15 minutes. In our study, dentin was exposed to 50μl of the solvents for 15 minutes. Erdemir et al found no significant difference between chloroform, halothane and the control group in terms of root dentin microhardness [38]. Their results are in accordance with those of the current study, indicating that use of chloroform, eucalyptol and orange oil as gutta percha solvents did not decrease the root dentin microhardness compared to the control group. In a study by Rotstein, dentin microhardness decreased by 29% after exposure to chloroform for 15 minutes [37]. Under in-vitro conditions, many factors such as the methodology of study and teeth differences may affect the test results [39,40].
No previous study has investigated the effect of eucalyptol and orange oil on root dentin microhardness. Our study found no significant difference in root dentin microhardness before and after using chloroform, orange oil and eucalyptol. Orange oil has higher biocompatibility than chloroform and eucalyptol [12] and has an efficacy similar to that of chloroform and eucalyptol [41]. Thus, orange oil can be a good alternative to these solvents.

CONCLUSION

Based on the results, application of chloroform, eucalyptol and orange oil as gutta percha solvents did not decrease the root dentin microhardness. Thus, in terms of affecting dentin properties, none of the tested solvents had any superiority over the others for endodontic re-treatment.

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