Original Article / Özgün Araştırma

A New Method for the Evaluation of pH Changes in Root Dentine by Phenolphthalein Colorimetric Indicator

Kök Dentinindeki pH Değişikliklerinin Yeni Bir Yöntem Olan Fenolftalein Kolorimetrik İndikatörü ile Değerlendirilmesi

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Abstract

Objective: To analyze the effect of two different vehicles and smear layer on the diffusion of hydroxyl ions into the root dentin using phenolphthalein colorimetric indicator.

Materials and Methods: Eighty-eight human mandibular premolars were used. Four randomly selected roots were used as the negative control group. The remaining roots were chemomechanical prepared and randomly divided into 2 main groups according to the final irrigation process: Group 1 (smear-): 4 mL 17% EDTA followed by 4 mL 2.5% NaOCl, group 2 (smear+): 8 mL 2.5% NaOCl. Next, 4 randomly selected roots from group 1 and group 2 were divided into 2 positive control groups. The main groups (n=38) were further divided into 2 subgroups according to the type of vehicle group 1a: CH + glycerin, group 1b: CH + distilled water, group 2a: CH + glycerin, group 2b: CH + distilled water. One week later, root canals were sectioned horizontally. Then, dentin sections were kept in 3% phenolphthalein solution for one day. The stained area, total area and root canal area were measured. The data were statistically analyzed (α=0.05).

Results: The staining pattern was more prominent in the absence of the smear layer ($p<0.001$). In the presence of the smear layer, the CH-glycerin group had more stained areas than the CH-distilled water group ($p<0.01$). In the absence of the smear layer, there was no difference between the vehicles ($p>0.05$).

Conclusion: Both tested vehicles were effective at increasing dentinal pH. Smear layer removal may facilitate the diffusion of hydroxyl ions.

Öz

Amaç: Bu çalışmanın amacı, hidroksil iyonlarının kök dentinine difüzyonu üzerinde iki farklı taşıyıcının ve smear tabakasının etkisini fenolftalein kolorimetrik indikatörü kullanarak analiz etmektir.

Gereç ve Yöntemler: Çalışmada 88 adet insan mandibular küçük azı dişi kullanıldı. Negatif kontrol grubu olarak rastgele seçilen 4 örnek kullanıldı. Kalan örnekler kemomekanik olarak hazırlandı ve son irigasyon işlemine göre rastgele 2 ana gruba ayrıldı: Grup 1 (smear-): 4 mL %17 EDTA ardından 4 mL %2,5 NaOCl, Grup 2 (smear+): 8 mL %2,5 NaOCl. Sonrasında, grup 1 ve grup 2'den rastgele seçilen 4 örnek 2 farklı pozitif kontrol grubuna ayrıldı. Ana gruplar (n=38) ayrıca kalsiyum hidroksit taşıyıcısına göre 2 alt gruba ayrıldı; grup 1a: CH + gliserin, grup 1b: CH

+ saf su, grup 2a: CH + gliserin, grup 2b: CH + saf su. Bir hafta sonra kök kanalları yatay olarak kesildi. Daha sonra dentin kesitleri bir gün boyunca %3'lük fenolftalein solüsyonunda bekletildi. Boyalı alan, toplam alan ve kök kanal alanı ölçüldü. Daha sonra veriler istatistiksel olarak analiz edildi (α=0,05).

Bulgular: Örneklerde, smear tabakasının yokluğunda boyalı alan anlamlı olarak daha fazla izlendi ($p<0,001$). Smear tabakasının varlığında, CH-gliserin grubunda, CH-saf su grubuna göre daha fazla boyalı alan izlendi ($p<0,01$). Smear tabakasının yokluğunda taşıyıcılar arasında anlamlı fark bulunmadı ($p>0,05$).

Sonuç: Test edilen her iki taşıyıcı da dentinin pH'ını artırmada etkiliydi. Ayrıca, smear tabakasının uzaklaştırılması, hidroksil iyonlarının difüzyonunu kolaylaştırabilir.

Introduction

The success of endodontic therapy depends on the complete removal of microorganisms and their toxic by-products from root canal system (1). However, it is difficult to eliminate all bacteria only by means of chemo-mechanical preparation (2). Therefore, intracanal medicaments have been widely used to eradicate the bacteria potentially surviving in the root canal system (3). The most popular intracanal medicament, calcium hydroxide (CH), has been widely used since the 1930s (4). CH exerts a high alkaline pH (~12.5) environment in which most of the endopathogens can't manage to survive (5). However, it is also known that CH has a limited antimicrobial spectrum (6). Although CH is not a panacea (7), it is still the most preferred root canal medicament today.

Diffusion of CH into the root dentine has been widely investigated since the 1980s (8-13). In addition to other techniques (10,11), colorimetric indicators had been used in several studies to observe pH changes in dentine following CH use (9,14). Phenolphthalein is a well-known acid-base colorimetric indicator, in aqueous solutions, it is colorless when the pH is below 8.3 and rapidly turns raspberry-purple at higher pH values. In a very strong basic medium ($pH\geq14$), the indicator becomes colorless again (15). To date, phenolphthalein has never been used as a colorimetric indicator. Therefore, the aims of the present study were to investigate:

- The diffusion depth of hydroxyl ions into the root dentine using phenolphthalein colorimetric indicator,
- The effects of two different vehicles (glycerin and distilled water) on the diffusion of hydroxyl ions through root canal dentine.

Materials and Methods

The data from previous research (16) were used to determine the effect size of this study by using G*Power v.3.1 software. An alpha type error of 0.05 and a beta power of 0.80 were specified and calculated based on these parameters. Thus, the minimum estimated sample size for each group was found to be 19.

Single-rooted human mandibular premolars extracted for orthodontic reasons were collected under the permission obtained from the Human Ethics Committee of Adnan Menderes University (number: 98318678-020, date: 04.04.2018). The teeth were stored in 0.2% thymol solution in 37 °C. Thereafter, two periapical radiographs were taken in mesiodistal and buccolingual directions to confirm the presence of a single canal. Specimen selection was made based on relative dimensions, such as similarity in root morphology. Additionally, teeth with curvatures greater than 10° determined by the Schneider criteria (17) were excluded. As described above, a total of 88 teeth were included in the present study. The teeth were randomly divided into 4 experimental and 3 control groups. Seventy-six teeth were divided into 4 experimental groups (n=19) and 12 teeth were divided into 3 control groups (n=4). The negative control group received no preparation or medication (n=4). Samples in the positive control group were prepared chemomechanically similarly to the samples in the smear layer-negative experimental groups (as group 1) (n=4). Samples in the second positive control group were prepared chemomechanically similarly to the samples in the smear layer-positive experimental group (as group 2) (n=4). CH medication was not applied in any of the control groups.

Then, samples were decoronated at the cemento-enamel junction. The roots were cut further to standardize the root canals to a uniform length of 16 mm. Working length was confirmed by inserting a #10 K-file into the root canal until its tip was visible at the apical foramen.

All the root canals were prepared with ProTaper Universal system (Dentsply, Maillefer) according to the

manufacturer's instructions. All canals were shaped up to F5 in full clockwise rotation and files were renewed after every 5 root canals. During the instrumentation, 2 mL of 2.5% NaOCl irrigation was performed with a 27-gauge notched tip irrigation needle (Endo-Eze; Ultradent, South Jordan, UT) between each file. At the end of the instrumentation procedures, the apex of the samples was sealed with a sticky wax to create a closed-end channel according to Tay et al. (18). Then, all the samples were randomly divided into 2 main groups (n=38 in each group) according to the final irrigation procedure as follows:

Group 1 (smear layer negative): 4 mL 17% EDTA followed by 4 mL 2.5% NaOCl; each continued for 1 minute.

Group 2 (smear layer positive): with only 8 mL 2.5% NaOCl in 2 minutes.

Next, all the samples in both groups were irrigated with 10 mL of distilled water and dried with paper point cones. Then, the main groups (group 1 and group 2) were further divided 2 two sub-groups (n=19 in each), according to the type of the vehicles (distilled water or glycerin) to transport CH as follows:

Group 1a: CH + glycerin [(smear (-)]

Group 1b: CH + distilled water [(smear (-)]

Group 2a: CH + glycerin [(smear (+)]

Group 2b: CH + distilled water [(smear (+)]

Both CH pastes were prepared in a creamy consistency in a powder/liquid ratio of 1/1.5 (w/v) and applied into the root canals using a lentulo spiral. After CH pastes were placed into the root canals, the canal orifice was sealed with cotton pellets and temporary filling material (Cavit; 3M ESPE, USA). All the experimental and control groups were kept in 37 °C and 100% relative humidity for 1 week.

One week later, each root canal was irrigated with 10 mL distilled water. Initially, 1 mm apical parts of all the samples and the sticky wax (both control and experimental groups) were removed to obtain a clear vision in the stereo-microscope visualizations. Then, the remaining 15 mm samples were sectioned horizontally at 3, 6, 9 and 12 mm away from the apex under water cooling to have 4 pieces of dentine sections with a thickness of 3 mm each. After dentine sections were rinsed with distilled water, they were finally kept in 3% phenolphthalein solution (Merck, Darmstadt, Germany) at 37 °C for 24 h. Phenolphthalein solution stained dentine in purple shades because of the alkaline pH of CH (Figure 1). Stained samples were rinsed in distilled water to remove phenolphthalein remnants and then dried with air. The stained area around the root canal was evaluated using a stereomicroscope at x20 magnification (Leica S8 APO, Leica Microsystems, Heerbrugg, Switzerland) and photographed with a microscope attached camera. Images were recorded using the computer program LAS (Leica Application Suite Software, version 2.4.0.R1, Leica Microsystems CMS, Germany). The total area, stained area, and the root canal area of each sample were measured and recorded. Measurements were repeated 3 times and the average of these measurements was taken into consideration. The percentage of stained area in all cross- sectional levels was formulated and calculated as follows:

The percentage of the stained area (mm^2) = [Stained area (mm^2) / Total root area & times; Root canal area (mm^2)] × 100

The control groups were not included in the statistical analysis because no staining or penetration was observed in all of them.

Statistical Analysis

Statistical analysis was performed using the R software. This kind of data does not represent a Gaussian process and it does not meet the assumptions of typical parametric linear models. A widespread solution for analyzing data that do not

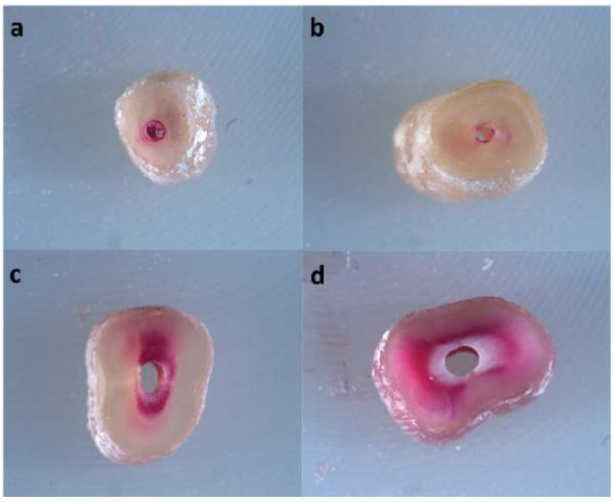

Figure 1. The staining of the same sample in the group 2a by the phenolphthalein colorimetric indicator
(a. 3 mm - cross section, b. 6 mm - cross section, c. 9 mm - cross section, d. 12 mm - cross section)

meet parametric assumptions is to use non-parametric models. However, a common issue of non-parametric models is that they cannot test for interactions among groups. In order to circumvent this issue, data was transformed using a method called align rank transformation (19). ANOVA can be applied to aligned rank transformed data, making it possible to test for both main group effects and their interactions. Align rank transformation and subsequent ANOVA was performed using the R package ARTool (20). Statistical significance was determined at α=0.05. A chi-square test was performed in evaluating the categorical data regarding the presence or absence of staining (α=0.05).

Results

In the statistical analysis, the percentages of staining area, the presence/absence of the smear layer, the type of vehicles (distilled water or glycerin), and the cross-sectional levels were taken into consideration as parameters.

The percentages of the staining area at all cross-sectional levels in the groups are presented in Table 1. The staining pattern was more prominent in absence of the smear layer ($p<0.001$). However, an interaction was found between the smear layer and the vehicle type ($p<0.01$). Accordingly, the type of vehicle had a significant effect on staining pattern only when there was the smear layer ($p<0.01$). In presence of the smear layer, CH-glycerin group had more stained areas than the CH-distilled water group had. However, there was no statistically significant difference by means of the vehicle in absence of the smear layer ($p>0.05$).

When the cross-sectional levels were evaluated, the coronal sections had more stained areas and there were significantly more non-stained samples in the 3 mm group ($p<0.001$). Stained area percentages significantly decreased in the apical sections of the groups (Table 1). The numbers of the stained and non-stained samples at all cross-sectional levels are presented in Table 2.

Discussion

CH, the key intracanal therapeutic agent helping in resolution of periradicular periodontitis (2,5-7). In addition to the research on therapeutic and antibacterial activity of CH, numerous studies were conducted on diffusion dynamics of this medicament (9-12, 21). The first study investigating the pH changes in root canal dentine following the placement of CH was performed by Tronstad et al. (9). They found that pH values ranged between 8 to ~11 in the circumpulpal dentine and 7.4 to 9.6 in the peripheral dentine by utilizing 18 different pH indicators (9).

Table 1. The percentages of the staining areas at all cross-sectional levels

Smear & vehicle		Cross-sectional levels				Mean ± SD
		3 mm	6 mm	9 mm	12 mm	
Smear (+)	Glycerin	10.87±11.62	16.17±20.21	32.79±24.07	42.16±16.20	25.50±14.51
	Distilled water	3.39±10.16	23.08±24.14	31.34±20.91	31.16±10.66	22.00±13.15
Smear (-)	Glycerin	2.80±4.10	27.77±21.70	40.07±24.25	39.47±22.68	27.53±17.43
	Distilled water	15.71±11.00	31.72±19.15	45.34±17.01	55.02±17.74	36.95±17.08
Mean ± SD		8.19±10.94	24.69±21.75	37.39±22.07	42.25±19.16	

SD: Standard deviation

Table 2. The numbers of the stained and non-stained samples at all cross-sectional levels

Smear and vehicle	Cross-sectional levels							
	3 mm		6 mm		9 mm		12 mm	
	Stained (+)	Stained (-)	Stained (+)	Stained (-)	Stained (+)	Stained (-)	Stained (+)	Stained (-)
Smear (+) glycerin	11	8	13	6	15	4	19	0
Smear (+) distilled water	2	17	14	5	18	1	17	2
Smear (-) glycerin	7	12	16	3	19	0	17	2
Smear (-) distilled water	18	1	19	0	19	0	19	0

Then, researchers developed a new method by preparing external cavities on the root canal wall where pH changes and Ca^{+2} ion concentration would be observed simultaneously (10). This relatively direct pH measurement method designed by Nerwich et al. (10) was used in various studies (10,13,21). Although a totally different approach was used, Nerwich et al. (10) declared that their dentinal pH measurements were in agreement with those of Tronstand et al. (9).

Although the method used in the present study was somewhat similar to the study of Tronstad et al. (9), a much simpler method was performed by using a single (phenolphthalein) colorimeter indicator to provide visual information. In this method, there are no limitations such as regional changes of dentinal tubule density and calcification that impede phenolphthalein staining. Thus, the method used in the present study offers a simple analysis model with remarkable visual information, which provides accuracy and precision of the pH measurements. The pH changes in root dentine could be marked by phenolphthalein staining, which enables the observer to be able to trace the pH changes within the root dentine.

Phenolphthalein, an acid base indicator, turns pink between pH 8.3 and 14, and colorless if below pH 8.3 or above 14 (15). It has been declared many times in previous research that the pH range of root canal dentine is 8-11.1 after placement of CH (9,10,21,22). Thus, phenolphthalein presented to be an appropriate colorimetric indicator for determining the pH of the root canal dentine after placement of CH.

The effect of the vehicle used to transport CH has also been previously reported that distilled water or camphorated paramonochlorophenol resulted in better diffusion capacity compared to phosphate-buffered saline or propylene glycol (23). According to Staehle et al. (14), an aqueous suspension allows a more efficient release of hydroxyl ions. Safavi and Nakayama (24), reported that the use of high concentrations of glycerin or propylene glycol as a CH carrier reduces the conductivity of CH, and thus may also reduce its effectiveness. According to the results of this study, in the absence of the smear layer, although there was no statistically significant difference between the two tested vehicles, the percentage of the staining in the distilled water group was higher compared to the glycerin group in all cross-sectional levels. This may be due to the fact that an aqueous suspension allows more effective and rapid release of hydroxyl ions compared to the viscous vehicles, especially in the absence of the smear layer. This result was similar to that of the above-mentioned studies (14,23,24).

One of the limitations of this study was that any direct pH-value could not be given because phenolphthalein colorimetric indicators can only determine pH in a range between 8.3-14.0. Its color changes from pink to fuchsia at a more basic pH, but this does not provide an accurate measurement. In order to overcome this limitation, the total diffusion areas were calculated, and the numerical data were obtained. The results of the present study are consistent with most of the previously published studies on pH changes in the root dentine associated with CH delivery (9-11,21). Therefore, the use of phenolphthalein as a colorimetric indicator can be presented as a promising method when analyzing pH changes in root dentine.

Conclusions

Under the experimental conditions of this study, it can be concluded that both tested vehicles used to transport CH were effective in increasing dentinal ph. Additionally, the phenolphthalein colorimetric indicator, which was used for the first time in evaluating the pH changes of root dentine, appears to be a practical and simple method.

Ethics

Ethics Committee Approval: Single-rooted human mandibular premolars extracted for orthodontic reasons were collected under the permission obtained from the Human Ethics Committee of Adnan Menderes University (number: 98318678-020, date: 04.04.2018).

Informed Consent: Informed consent is not required.

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Authorship Contributions

Concept: H.D.Ö., B.H.Ş., B.Ş., Design: H.D.Ö., B.H.Ş., Data Collection or Processing: H.D.Ö., Analysis or Interpretation: B.Ş., Literature Search: H.D.Ö., B.H.Ş., B.Ş., Writing: H.D.Ö., B.H.Ş.

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