ORIGINAL ARTICLE

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The effect of EDTA, phytic acid, and glycolic acid on dentinal tubule penetration of a bioceramic-based paste: A confocal study

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Purpose: In this study, the effects of ethylene diamine tetraacetic acid (EDTA), phytic acid (IP6), and glycolic acid (GA) on the dentin tubule penetration of bioceramic-based sealers were evaluated by confocal laser scanning microscopy (CLSM).

Methods: A total of 60 human mandibular premolar teeth were included. The teeth were divided into four groups (n = 15): Group 1: 5.25% NaOCl + 17% EDTA, Group 2: 5.25% NaOCl + 1% IP6, Group 3: 5.25% NaOCl + 10% GA, and Group 4: 5.25% NaOCl + distilled water (DW). The canals were filled with Bioserra canal paste mixed with 0.1% Rhodamine B.

Results: The penetration depth and percentage of the bioceramic-based paste were significantly higher in all groups than in the control group. However, this difference was not significant for the penetration area.

Conclusion: GA and IP6 are similar to EDTA in terms of their effects on bioceramic paste penetration.

Keywords: Confocal; EDTA; glycolic acid; penetration; phytic acid.

Introduction

Preparation and irrigation play important roles in the disinfection of the root canal system. However, contact with bacteria in deeper tubules cannot always be achieved. By ensuring adequate dentinal tubule penetration of root canal paste, the existing bacteria in the tubule are trapped and neutralized.

The smear layer formed during canal shaping has organic and inorganic contents and covers the dentin tubules on the canal walls (1). This layer prevents the penetration of irrigation solutions, intracanal medicaments, and canal paste into the tubules (2). Although the organic component of the smear layer can be removed with sodium hypoclorite (NaOCl) solution, it is not sufficient by itself because it cannot dissolve inorganic tissue. Therefore, NaOCl should be used together with chelating agents for smear elimination (3).

Depending on the application time, chelating agents may cause a decrease in dentin hardness due to their acidic nature and erosion of root dentin. Additionally, if the chelat-

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ing agent cannot be sufficiently removed from the canal, root canal filling may fail due to microleakage (3).

Ethylene diamine tetraacetic acid (EDTA) is the most commonly used chelating agent to remove smears (3). However, the disadvantages of EDTA, such as its cytotoxic properties and the fact that it causes demineralization if retained for more than 1 minute in the canal, have led researchers to investigate the use of other alternative chelators (4). The effect of the chelator used to remove the smear layer directly affects the penetration of the canal paste (5).

One of the agents that has been investigated to remove smears is glycolic acid (GA). Its organic structure makes it less toxic than EDTA (6,7). In a study in which GA's antibacterial properties were found to be higher than those of EDTA and citric acid, concentrations of 10%, 17%, and 25% were reported to be suitable for alternative use (8). Another chelation agent whose alternative use to EDTA has been investigated is phytic acid (IP6). Nassar et al. (4) first used IP6 as a chelating agent in root canal treatment. In one of the few studies using IP6, it was found to be more effective in canal cleaning than EDTA, and it opened dentinal tubules more than EDTA did (4). In the same study, IP6 was found to be biocompatible for odontoblasts, which play a role in bone healing, and was reported to be an alternative to other chelation agents.

The aims of our study were to visualize the penetration of bioceramic-based root canal paste with confocal laser scanning microscopy (CLSM) in teeth treated with GA, EDTA, and IP6 and to provide a comparison by calculating the paste penetration depth, percentage, and area.

In our study, two null hypotheses (H_0) and one alternative hypothesis were established.

- $^1H_0$: There is no difference between the chelation agents used.
- $^2H_0$: When sections and chelation agents are evaluated together, there is no difference in penetration depth, percentage, and area values.
- H_1: Regardless of the chelation agent used, 7-mm sections have higher penetration values.

Materials and Methods

This study was approved (approval removed for peer review). The study was conducted under the principles of the Declaration of Helsinki.

The tooth sample size in the study was determined as 20 in all groups, with 95% confidence (1-α), 95% test power (1-β), and f = 1.103 effect size. Considering possible sample dropout, 60 teeth (n = 15 teeth per group) were included in the study. Sixty single-rooted, single-canal mandibular premolar teeth extracted for orthodontic or periodontal reasons were included. After final preparation with a RECIPROC® no. 40 file, the teeth were randomly divided into four groups. In all groups, 5.25% NaOCl was used for the final irrigation. In Groups 1–3, 17% EDTA, 1% IP6, and 10% GA (5 ml volumes and 1 minute each), respectively, were used as chelating agents, while distilled water was used in the control group. All solutions used in the final irrigation were activated with a passive ultrasonic irrigation system (Dentac, Meta Biomed, Germany) for 30 seconds.

The irrigated teeth were filled with Bioserra (Dentac, Meta Biomed, Germany) root canal paste labeled with Rhodamine B and R40 gutta-percha. The fillings were supported by lateral condensation. The teeth, embedded in acrylic, were kept in an oven at 37°C with 100% humidity for 1 week (Fig. 1, Fig. 2 & Fig. 3).

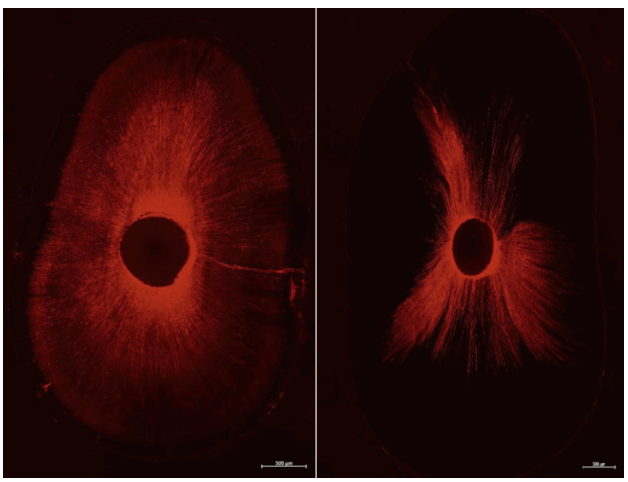

Fig. 1. Confocal images of 7 and 3mm sections of teeth irrigated with 10% Glycolic acid.

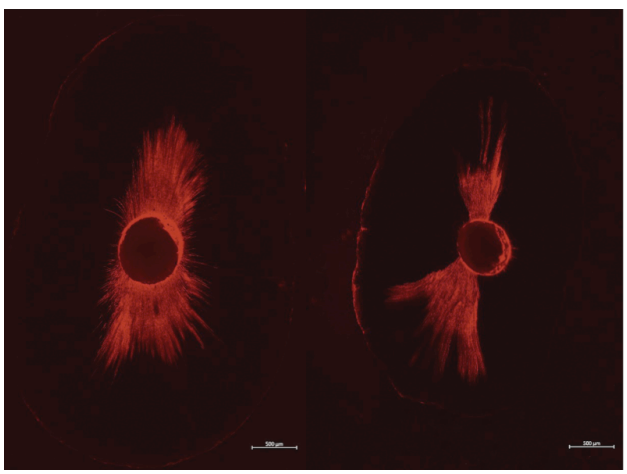

Fig. 2. Confocal images of 7 and 3mm sections of teeth irrigated with 1% Phytic acid.

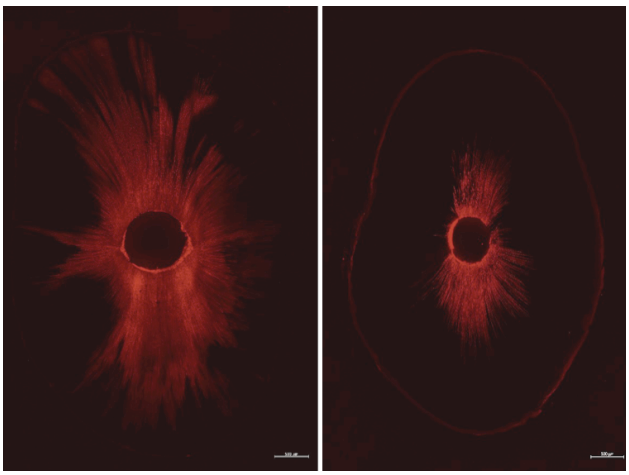

Fig. 3. Confocal images of 7 and 3mm sections of teeth irrigated with 17% EDTA.

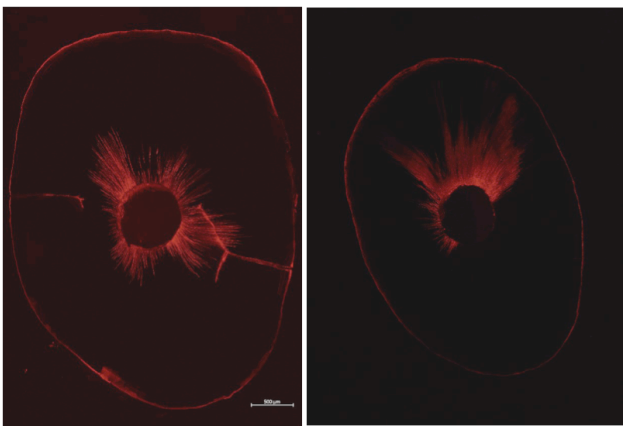

Fig. 4. Confocal images of 7 and 3mm sections of teeth irrigated with Distilled water.

After incubation, the teeth were marked at the apical 3- and 7-mm levels. Horizontal sections 1 mm in thickness were obtained using an IsoMet™ (Zeiss LSM 800, Oberkochen, Germany) device and a diamond disc.

The sample sections obtained were imaged with a Zeiss LSM 800 Confocal Laser Scanning Microscope (CLSM) device (Zeiss LSM 800, Oberkochen, Germany) with 5× lens magnification and a wavelength of 543–590 nm. Each section was divided into four quadrants to calculate the penetration depth in the images. In each quadrant, the longest distance, starting from inside the canal, was measured. The penetration depth was calculated by averaging the measured values. For the penetration percentage measurement, the length of the areas where the paste penetrated the inner canal wall was proportioned to the canal circumference length and multiplied by 100. For the penetration area measurement, the area calculated by drawing around the area where the canal paste penetrated was subtracted from the area calculated by drawing around the canal lumen.

Statistical Analysis

Conformity to normal distribution was examined by the Shapiro–Wilk test. The penetration depth and percentage values according to the irrigation agent and the cross section were analyzed by the Two-Way Analysis of Variance (ANOVA), and multiple comparisons were made with the

Table 1. Analysis of the effect of chelation agent and section on penetration depth, percentage and area

	Penetration Depth		
	F	**p***	**Partial Eta Squared**
Chelation agent	11.64	< 0.001	0.238
Section	137.46	< 0.001	0.551
Chelation agent*Section	0.41	0.748	0.011
	Penetration Percentage		
	F	**p***	**Partial Eta Squared**
Chelation agent	7.88	< 0.001	0.174
Section	39.53	< 0.001	0.261
Chelation agent*Section	0.4	0.756	0.011
	Penetration Area		
	p‡		**Q**
Chelation agent	> 0.05		7.595
Section	< 0.001		60.802
Chelation agent*Section	< 0.001		20.439

*Two-way ANOVA, ǂ Robust Two-way ANOVA. Penetration depth R2=%60.79; Adjusted R2=%58.34. Penetration percentage R2=%36.49; Adjusted R2=%32.52. Q: Robust Two-way ANOVA.

Table 2. Representation of descriptive statistics [mean ± standard deviation and median (minimum-maximum)] of penetration depth, percentage and area according to chelation agent and section

Penetration Depth (µm)			
Chelation agent	**3 mm**	**7 mm**	**Total**
EDTA	596.62 ± 273.39	1319.94 ± 329.58	958.28 ± 473.11[a]
DW	307.57 ± 198.95	862.17 ± 244.84	584.87 ± 357.2[b]
GA	671.89 ± 301.84	1347.27 ± 451.92	1009.58 ± 510.43[a]
IP6	477.93 ± 257.71	1141.49 ± 320.32	809.71 ± 442.12[a]
Total	513.5 ± 289.39	1167.72 ± 388.34	840.61 ± 473.49
Penetration Percentage (%)			
EDTA	79.33 ± 19.16	95.83 ± 7.68	87.58 ± 16.61[a]
DW	61.23 ± 16.73	79.78 ± 9.33	70.5 ± 16.32[b]
GA	80.25 ± 22.9	94.22 ± 10.9	87.24 ± 19[a]
IP6	70.38 ± 20.85	92.81 ± 8.15	81.6 ± 19.29[a]
Total	72.8 ± 21.01	90.66 ± 10.95	81.73 ± 18.94
Penetration Area (µm²)			
EDTA	2021384.12 (1020601.79 – 11362044.58) [A]	11117437.91 (260554.52 – 38711698.94) [B]	5066507.45 (260554.52 – 38711698.94)
DW	1331308.71 (250667.08 – 3076310.67) [A]	6047325.54 (1565457.4 – 11635735.45) [C]	2804246.94 (250667.08 – 11635735.45)
GA	2353328.5 (554528.34 – 8106831.19) [A]	12592987.62 (1863171.99 – 117285757.2) [BC]	6869157.15 (554528.34 – 117285757.2)
IP6	1402217.24 (92618.41 – 6840987.71) [A]	7830419.89 (1235665.65 – 114950053.5) [BC]	4500080.85 (92618.41 – 114950053.5)
Total	1934108.55 (92618.41 – 11362044.58)	8168160.66 (260554.52 – 117285757.2)	4850763.42 (92618.41 – 117285757.2)

[a-b]: There is no difference between columns with the same letter. A-B: There is no difference between columns and rows with the same letter.

Bonferroni Correction. The penetration area values that did not conform to normal distribution according to the irrigation agent and the cross section were analyzed by the Two-Way Robust ANOVA, and multiple comparisons were made with the Bonferroni Correction. The analysis results were presented as mean ± standard deviation and median (minimum–maximum). The significance level was set at $p < 0.05$.

Results

When the penetration depth and percentages of the chelating agents were compared, it was concluded that they showed significantly higher penetration than the control group ($p < 0.001$), but there was no significant difference between EDTA, IP6, and GA (Table 1). When the sections were compared, penetration depth and percentage were significantly higher in apical 7-mm sections than in 3-mm sections ($p < 0.001$) (Table 2).

When the penetration area values were analyzed, the highest value obtained was for 7-mm GA, while the lowest value was for the 3-mm IP6 group, except for the control group ($p < 0.001$). When the sections were evaluated within themselves, no significant difference was found between the chelation agents ($p > 0.05$) (Table 2).

Discussion

One of the most important factors for success in root canal treatment is to make a hermetic canal filling that will prevent microleakage. In the evaluation of the quality of canal filling, parameters such as the depth, percentage, and area of penetration of the root canal paste into the dentinal tubules are important in terms of sealing and antibacterial efficacy. Penetration of the paste into the dentinal tubules is achieved by removing the smear layer covering the tubules with the chelating agents used. Another important factor besides the removal of the smear for paste penetration is the fluidity of the root canal paste and its ability to penetrate the dentinal tubules (9). For this reason, Bioserra canal paste, based on hydrophilic fluid bioceramic with small particle size, was used. When the single cone technique is used during filling, the amount of paste increases, especially in oval canals. The fact that the canal paste shrinks over time and causes gaps is a disadvantage of the single cone technique. For this reason, we preferred the lateral condensation technique in our study (10).

In the literature, different methods have been used to investigate the dentinal tubule penetration of root canal pastes. Kouvas et al. (11), Singh et al. (12), and Schmidt et al. (13) used scanning electron microscopy (SEM) to study penetration, while Furtado et al. (14), Tedesco et al. (15), and Aksel et al. (16) used CLSM. The use of

these methods has advantages and disadvantages. If imaging with SEM is to be performed, the teeth should be subjected to special procedures such as drying in an oven with alcohol and then plating with gold. Samples may be damaged during these processes. Image artifacts occur in damaged specimens (17). However, samples do not need to undergo any special treatment for imaging with CLSM. For this reason, the samples are not damaged, and there is no artifact in the image (18). Thus, the use of CLSM was preferred in our study. To obtain fluorescence images, the canal paste was labeled with 0.1% Rhodamine, as recommended by Gharip et al. (19).

Eyüpoğlu et al. (20) compared the penetration depth and percentage of the coronal, middle, and apical sections of the three root canal paths they used in their study and concluded that each root canal path showed significantly more penetration in the middle third than in the apical third. No significant difference was observed between the coronal and middle regions (20). For this reason, it was thought that the use of apical 3- and 7-mm sections of the roots would be sufficient in our study.

Considering the results of the study, the penetrations were found to be higher at the apical 7-mm level than at the apical 3-mm level for all penetration parameters. With this result, the H_1 hypothesis was confirmed. In the present study and similar studies, the decrease in the penetration depth and percentage of root canal pastes in the apical region may be due to the low density of dentinal tubules and narrow tubule diameter in the apical region.

Although the chelation agents used in our study increased the penetration parameters compared to the control group, there was no significant difference between them. According to this result, hypothesis $^1H_0$ was confirmed. In the study by Eskander et al. (21), the penetration parameters of bioceramic root canal paste were similar for 17% EDTA and 1% IP6 (Fig. 4). However, in their study, the penetration parameters of the group using AH Plus and EDTA were found to be statistically significantly higher than the AH Plus and IP6 group and the groups using bioceramics. In line with the results of this study, when the use of bioceramic canal paste is considered, we suggest that EDTA, IP6, and GA can be used as alternatives to each other due to their similar penetration parameters.

When the chelating agents and cross sections were evaluated together, there was no significant difference in penetration depth and penetration percentage. However, the penetration area values differed significantly, with the highest in the 7-mm GA group and the lowest in the 3-mm IP6 group. The second null hypothesis, $2H_0$, was that there was no difference between the groups in terms of penetration depth, percentage, and area when chelating agents and sections were evaluated together. This hypothesis was confirmed for penetration depth and percentage but rejected for penetration area.

In our study, penetration depth and area values in the groups using 10% GA were numerically higher than those in groups using other chelators. Although this difference is not significant, we argue that when the results of the study by Demirbaş et al. (22) are taken as reference (the bond strength of the bioceramic canal paste used in the groups using 10% GA was found to be higher than that in the groups using 17% EDTA), further studies with larger samples are needed to evaluate whether the use of glycolic acid as a chelation agent is successful or not.

In a study, the effects of 17% EDTA, 1% IP6, and 7% maleic acid solutions on both smear removal efficiency and penetration of AH Plus root canal paste were investigated. According to the results of that study, the smear removal efficiencies of 17% EDTA and 1% IP6 solutions were similar. However, the paste penetration of EDTA samples was significantly higher than that of IP6 and maleic acid (23). This result was different from our study. The reason for this difference may be due to the properties of the paste used and the use of activation differently from our study.

In our study, when the penetration areas were compared, while the numerical values were GA > EDTA > IP6, respectively, this difference was not statistically significant. The penetration area values were close to each other in all groups. In a study conducted by Donnermeyer et al. (24), where they tested the suitability of the use of Rhodamine B dye in the examination of paste penetration with CLSM, they concluded that Rhodamine B can penetrate independently of the paste and overestimate penetration values. This property of Rhodamine B may have affected our study results.

Conclusion

There are studies in the literature comparing GA and IP6 with EDTA. However, there is no known CLSM study comparing the effect of these two chelators on the penetration of bioceramic-based root canal paste with EDTA. For this reason, we believe that our study results will contribute to the literature. However, since the studied teeth belonged to different individuals, differences in age, external factors, and the number and structure of dentinal tubules are limitations of our study. These limitations may have affected the results of our study. For this reason, more alternative studies on the effect of chelating agents on paste penetration are needed.

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Informed consent: Written informed consent was obtained from patients who participated in this study.

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