

EFFECT OF GALLIC ACID ON THE FILLING QUALITY OF AN EPOXY RESIN – BASED ROOT CANAL SEALER

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ABSTRACT

Purpose: our study evaluated the effect of Gallic acid irrigation as a final rinse after sodium hypochlorite (NaOCL) irrigation on the filling Quality of an epoxy resin – based sealers.

Methods and Materials: Twenty single- rooted human teeth were prepared with the protaper system. The specimens were then divided into the following groups: 5.25%NaOCL irrigation (NaOCL group) and 5.25%NaOCL + 10% gallic acid (gallic acid group). The root canals were filled using single cone technique with protaper F₃ cones and Diapro – seal sealer, labeled with rhodamine B dye to allow analysis under a confocal laser scanning microscopy (CLSM). All Samples were sectioned at 3, 5 and 7 mm from the apex and prepared for CLSM analysis. The Penetration segments of the canal were calculated at the apical, middle and coronal thirds. The Mann – Whitney statistical test was used at 5 % significans level.

Results: higher percentage of gaps and voids were observed at all root thirds of the NaOCL group when compared to the gallic acid group ($P < 0.05$). There was a significant increase in the penetration segment of gallic acid group at the coronal and middle root third when compared to the NaOCL group ($P < 0.05$).

Conclusion: our in vitro results showed that the use of gallic acid as an antioxidant agent after NaOCL irrigation promoted a better interfacial adaptation and penetration of epoxy resin – based root canal sealers.

KEYWORDS: confocal laser scanning microscopy; Root canal filling; sodium hypochlorite; gallic acid.

INTRODUCTION

Sodium hypochlorite has a great antibacterial effect and the ability to dissolve organic tissues, so it is the most commonly used irrigant during endodontic treatment ^[1].

The residual oxygen after the use of NaOCL irrigant on the inner dentinal walls disrupts the resin – based sealer polymerization ^[2]. Moreover, the mechanical properties of dentin like modulus of elasticity, microhardness and rigidity are adversely affected ^[3].

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These problems can be overcome by the use of antioxidant agents due to its ability to minimize the prejudicial effect of oxygen liberation after NaOCL use and its harmful effects on root canal sealers ^[4].

Antioxidant agents break up free radicals, reacting and neutralizing NaOCL products. Gallic acid is an antioxidant that has been used as a neutralizing agents of NaOCL, with promising results when used as a reducing agent for sodium hypochlorite – treated dentin ^[5].

The aim of this study was to evaluate the effect of using gallic acid as a final rinse after using NaOCL on the filling quality of an epoxy – based sealer. NaOCL irrigation without antioxidant use was used for comparison. The null hypothesis tested was that there would be no difference in the filling Quality of epoxy resin- based root canal sealer when gallic acid was used after using NaOCL.

MATERIALS AND METHODS

Tooth selection

Completely-formed, single-rooted extracted human teeth (20 teeth total) were selected. The selected teeth should be free off root canal curvatures, cracks, calcification, cavities or restorations.

Endodontic treatment

The tooth crowns were sectioned with a diamond disc to obtain a standard root length of 15 mm for each sample. The root canals were prepared I mm short than the working length with protaper rotary instruments (Dentsply Maillefer, Switzerland) till F₃ at a speed of 350 rpm and torque of 3N. Then, the roots were randomly divided into two equal groups according to the irrigation method used into:

- NaOCL groups : irrigation with 5.25% NaOCL and final rinse with saline

Gallic acid group: irrigation with 5.25% NaOCL and final rinse with 10% gallic acid (sigma – Aldrich, Bangalora, India). The 10 % gallic acid was

prepared by adding 10 gram of Gallic acid powder in 100 ml of distilled water ^[5].

At the beginning, and after each rotary file, the canals were irrigated with 3 ml of 5.25% NaOCL using a 5- ml plastic syringe and 30 gauge Navi tip needle (Ultradent products, south Jordan, USA).

After the F₃ rotary file, the canals [NaOCL group] were rinsed with saline for 5 minutes. The canals of Gallic acid group were rinsed with 10% gallic acid for 5 minutes followed by 17% EDTA for 3 minutes and 10 ml of saline solution again. All canals were dried with paper points.

Root Canal Filling

Diapro-seal (Diadent – Korea) was manipulated according to the manufacturer's instructions. Fluorescent rhodamin B dye (Sigma Aldrich, MO, USA) was added to the sealer mixture at a concentration of 0.1% ^[6] to allow visualization under a confocal laser microscope (Olympus, Tokyo, Japan). Size 30 rotary lentalo spiral was used for a uniform sealer distribution [Dentsply Maille Switzerland]. The root canals were filled using a single gutta percha cone F₃ that matches the final rotary file and fitted to the working length after coating with additional Sealer. The excess filling was removed by a heated plugger.

Vertical compaction was applied to the orifice level. The obturated root samples were stored at 100% humidity and 37°C for one day to allow complete sealer setting.

Specimens Preparation for CLSM analysis

Obturated roots were inserted in acryl, horizontally sectioned at 3, 5 and 7 mm from the apex. So, 3 slices per root were obtained (cervical, Medium and apical root thirds). The spesimena were examined under a confocal microscope. The images were obtained with the absorption and emission wavelengths of 540 and 590 nm, respectively, for rhodamine B. All images were anlysised by a blinded single evaluator with image J soft were (NIH, Bethesda, USA). The polygon selections tool of the image J soft was used to calculate the void area Percentage ^[7].

First, the total area of the canal was delimited for each sample, and the area values were registered. Then, the area of the gutta percha cone was measured. The sealer filled area of the canal was obtained by subtracting the gutta – percha area from the total area of the canal. The voids area was obtained as a percentage from the sealer filled area. A similr method to the one proposed by moon et al [8] was used for penetration segment analysis. The segmented line tool was used (Image J software, NIH, USA). For each slice, first the total perimeter of the root canal was delimited, to obtain the length values. Then, the perimeter with in which sealer penetrates into the Dentinal tubules was measured. In this way, the sealer penetration segment was obtained as a percentage from the total perimeter.

Statistical analysis

The Mann – Whitney test was used for both gaps and root canal sealer penetration segment. Friedman test was used for intragroup comparisons between

the different root slices. A 5% level of statistical significance was applied.

RESULTS

After analyzing the area of voids and gaps (Table 1), the NaOCL group showed a significantly higher percentage of voids and gaps when compared to the gallic acid group in the different root slices ($p < 0.05$). the intra group analysis showed a significant difference only for the NaOCL group where there was a higher percentage of voids in the cervical third when compared to the apical third ($P < 0.05$).

Regarding the root canal sealer penetration segment, the NaOCL group showed a lower segment when compared to the gallic acid group in the coronal and middle root third ($P < 0.05$). No significant differences in the apical third were observed between the groups ($P < 0.05$) (table2). Intra group also demonstrated no significant differences between root thirds ($P < 0.05$). Representative images of each group can be observed in figures; 1 and 2.

TABLE (1) Mean (SD) values of percentage (%) of voids & gaps in the root canal filled teeth:

| Groups | Coronal | Middle | Apical | Over all |
|-------------|-------------------------|--------------------------|--------------------------|--------------------------|
| NaOCL | 30 (17.3) ^{Aa} | 19 (11.3) ^{Aab} | 10.2 (5.7) ^{Ab} | 20.3 (14.6) ^A |
| Gallic acid | 5.7 (2.6) ^{Bb} | 4.1 (2.4) ^{Ba} | 4.5 (2.5) ^{Ba} | 4.8 (2.4) ^B |

Different capital letters in a column indicate statistically significant differences between the groups: different lowercase in the row indicate statistically significant differences between root thirds within the groups.

TABLE (2) mean (SD) values of percentage (%) of penetration segment in the root canal filled teeth:

| Groups | Coronal | Middle | Apical | Over all |
|-------------|--------------------------|--------------------------|--------------------------|-------------------------|
| NaOCL | 54.3(29.8) ^{Aa} | 55.2(10.2) ^{Aa} | 47.2(38.3) ^{Aa} | 51.5(23.4) ^A |
| Gallic acid | 82.5(21.3) ^{Ba} | 83.1(21.3) ^{Ba} | 45.8(40.7) ^{Aa} | 66.8(34.3) ^B |

Different capital letters in a column indicate statistically significant differences between the groups: different lowercase in the row indicate statistically significant differences between root thirds within the groups.

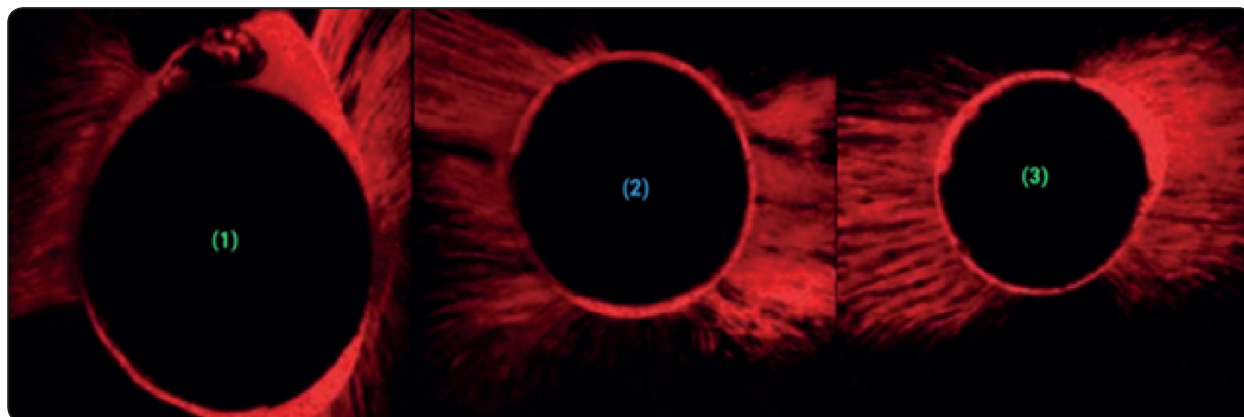


Fig. (1). Representative CLSM images (magnification 10x) of the root slices irrigated with 5,25 %NaOCl at the (1) coronal; (2) middle; (3) apical root third.

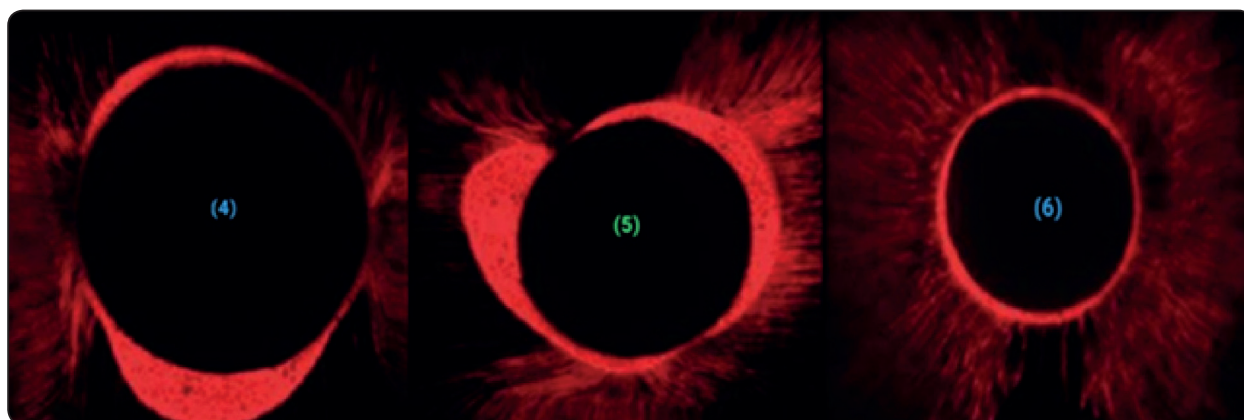


Fig. (2). Representative CLSM images (magnification 10x) of the root slices irrigated with 5,25 %NaOCl+ 10% gallic acid at the (1) coronal;(2) middle;(3) apical root third.

DISCUSSION

Polymerization shrinkage of root canal sealer upon setting lead to gaps between the sealer and root dentin, these gaps allow micro leakage and subsequent bacterial invasion^[9]. In this study, none of tested irrigation protocols at neither of the root thirds, provided a void and gap free obturation.

This result is in accordance with previous investigations^[10-12]. However, the percentage of combined voids and gaps in obturated canals were significantly higher for the sodium hypochlorite group when compared to the gallic acid group all root sections. So, the first null hypothesis was rejected.

The optimal filling gap- free obturation of the chemo mechanically prepared root canal space is associated with more success rate of primary endodontic treatment^[13]. However other studies demonstrated that void – free obturation is a challenge whatever the obturation technique used^[7, 13].

Dia pro- seal is an epoxy resin-based sealer, and it was used in this study due to its acceptable physicochemical properties, biocompatibility, sealing ability and economic price^[14]. The root canal sealer was applied by a rotary lentulo spiral which provided a better sealer wettability and distribution. A previous study demonstrated that the sealer distribution is not affected by sealer placement method^[15].

It has been demonstrated that voids and gaps formation inside irregularly shaped canals is higher when using a single cone technique ^[16].

In addition, single cone technique associated with a higher percentage of sealer and lower of the core material, especially in the cervical third of the oval – shaped canals ^[17]. Since gallic acid is potent antioxidant agent, the lesser formation of gaps and voids observed at gallic acid group, in cervical, middle and apical root thirds, compared to sodium hypochlorite group. Neutralization of sodium hypochlorite on the root dentin promoted by the final rinse with gallic acid. This neutralization allows the complete polymerization of resin-based sealers ^[4]. The gallic acid group provided a higher penetration segment on coronal and middle root thirds, compared to the sodium hypochlorite group since oxygen molecules inhibits resin infiltration into the tubules and inter – tubular dentin ^[18], the oxidizing effect of NaOCL is likely to be responsible for the lack of sealer penetration on dentinal tubules of NaOCL group. The apical root slices did not differ between groups which may be due to decreased number of dentinal tubules, smaller dentinal diameter and tubular obliteration at the root apex ^[19].

Furthermore smear layer removal in the coronal & middle thirds much more the apical one, and the decreased effect on irrigant delivery to the apical area may have some effect ^[20].

A previous study reported that there is no relation between sealing ability and depth of intratubular sealer penetration, and accordingly, the percentage of penetration segment provides a more reliable data ^[21].

It has been suggested that regardless of the depth of penetration, a higher penetration segment combined with antimicrobial properties allows the isolation of remaining bacteria ^[22, 23] and thus prevents microleakage also higher percentage of tubular penetration of the sealer provides a mechanical interlocking improving the root canal

filling retention ^[24]. The results of the present study demonstrated that the percentage of penetration segment was higher when gallic acid was used therefore; the second null hypothesis was also rejected.

In this study, the obturation quality was assessed by using CLSM which have several advantages over (SEM) such as being simpler, easy and nondestructive sample processing, as well as lower tendency to produce artefacts ^[25]. Moreover the images obtained by CLSM allows a quantitative data, unlike the qualitative scores commonly used in SEM images, which may provide less reliability ^[26]. Rhodamine B dye is the standard fluorophore dye used, and it has been previously demonstrated that your use of in low concentrations (0.1%) did not change the sealer properties, especially with epoxy resin-based sealer ^[6, 27].

The lack of a three-dimensional evaluation of the root canal filling might be a limitation of the analysis by CLSM, and micro-CT evaluations have been indicated as superior to the analysis of sectioned roots by digital imaging software ^[11]. A recent research ^[28] obtained a higher incidence of voids after evaluation through stereomicroscopy, when compared with microscopic evaluation of sectioned roots. Moreover, Viapiana et al., ^[29] found a weak correlation between the assessment of sealing ability by micro CT&CLSM, thus this study evaluated the incidence of voids and gaps on the root canal filling through CLSM, as similar researches previously assessed ^[6,10].

CONCLUSION

Our invitro study showed the advantages of gallic acid irrigation after NaOCL irrigation in endodontic treatment with epoxy resin-based sealers using single cone technique, by creating less gap and void formation; we advocate further studies in the field.

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