Spectrophotometric Analysis of Crown Discoloration Induced by Two Different Sealers: An In vitro Study

Abstract

Context: Endodontic materials have been assessed with regards to their potential for dental staining. Aims: To evaluate the coronal discol roation effect of Apexit Plus and Resino Seal in mandibular premolars using spectrophotometer. Setting and Design: The study design was original research. Materials and Methods: Forty human single-rooted mandibular first premolar teeth were selected for the study. They were divided into four groups of ten specimens each. Group 1 - Apexit Plus sealer, Group 2 - Resino Seal sealer, Group 3 - positive control (Blood), and Group 4 - negative control (distilled water). Teeth were resected 3 mm below at cemento enamel junction. In all samples, access cavities were prepared, instrumented, and irrigated. Sealers were mixed and placed into the pulp chamber through cervical access and control groups were filled with blood and distilled water. Coronal orifices were sealed using self-cure glass ionomer cement. Samples were analyzed using spectrophotometer to determine the discoloration in each group at 10, 17, and 24 days interval. Statistical Analysis Used: Statistical analysis used Wilcoxon signed-rank test. Results: All sealers used in the present study caused a progressive coronal discoloration over a time period of 17–24 days. There was statistically significant difference between resino seal sealer and apexit plus sealer, with resino seal showing greater coronal discoloration. Conclusion: Resino seal sealer has greater potential to cause discoloration of crown as compared to apexit plus sealer over a period of time.

Keywords: Apexit Plus, Resino Seal, root canal sealers, spectrophotometer, tooth discoloration

Introduction

Most commonly used method for obturation of root canal system is a combination of gutta-percha and endodontic root canal sealer. Sealer ensures fluid tight seal in root canal and occupies inaccessible areas of prepared canal.[1]

Discoloration of posterior teeth is one of the common reasons for patient seeking dental attention after endodontic treatment. This also compromises their smile.[2] One of the main reasons for local intrinsic staining of crown of the tooth is when root canal sealers come in contact with the coronal dentin of the pulp chamber.[3,4]

The other reasons for discoloration of teeth after root canal treatment may be due to pulpal hemorrhage into pulp chamber, pulpal remnant and irrigants, silver amalgam restoration, posts and pins, root canal sealers, and intracanal medicaments.[4] Tooth discoloration is quite a common problem these days after endodontic treatment.[5] Sealers react with dentin over a period of time and result in alteration in the optical and chromatic properties of the dentin.[6]

El Sayed and Etemadi[4] reported that Apexit Plus sealer which is calcium hydroxide-based sealer caused least coronal discoloration as compared to AH Plus sealer and Sultan sealer.

Previous studies reported that other resin-based sealers such as sealer 26, AH 26, and AH Plus caused tooth discoloration.[7] Recently, Resino Seal sealer (Amrith Chemicals and Mineral Agency, Punjab, India) has been introduced as a new epoxy resin-based endodontic sealer. However, there is an absolute lack of information about its effect on tooth color.

The null hypothesis tested was that the distribution of coronal discoloration of tooth across treatment groups is same at each point, and the coronal discoloration of tooth at consecutive time points is same for each treatment group.

Hence, this study was conducted to compare the coronal discoloration using...
root canal sealers such as Apexit Plus and Resino Seal at a short time interval, i.e., 10, 17, and 24 days.

**Materials and Methods**

**Sample selection and preparation**

Forty human single-rooted mandibular first premolar teeth were selected for the study. Teeth with caries, crack, restoration, and coronal stain were excluded from the study. Samples were initially stored in 3% sodium hypochlorite for 24 h to remove debris and then stored in saline until further use. The roots were resected 3 mm below the cementoenamel junction using a diamond disc. Access cavities were prepared in all samples. Gates Glidden drills #1, 2, and 3 were used for coronal enlargement. Pulp chambers were mechanically instrumented manually with hand K-files. All samples were irrigated with 3% sodium hypochlorite solution and final irrigation done with 17% ethylenediaminetetraacetic acid (EDTA) solution (Pulp dent, Watertown, MA, USA).

Teeth were divided into four groups: Group 1 - Apexit Plus sealer, Group 2 - Resino Seal sealer, Group 3 - positive control (blood), and Group 4 - negative control (distilled water).

The chemical composition of these sealers is shown in Table 1.

The sealers were mixed and placed into the pulp chamber through coronal access. Lentulo spiral filler is used to coat internal axial walls with sealer [Figure 1]. The apical access of sample was sealed with sticky wax, and coronal orifices were sealed using self-cure glass ionomer cement (XTRACEM Medicept Dental India). All the teeth were kept in saline in an incubator at 37°C.

**Preparation of blood sample**

In the positive control group, 0.5–10 μl of fresh human blood obtained from a volunteer. To hemolyze the red blood cells (RBCs) and have the breakdown products penetrate the dentinal tubules, a high-speed centrifuge was used to centrifuge the samples at 3000 rpm for 10 min. Lysed RBC was placed in pulp chamber with a micropipette. The pulp chamber filled internally with lysed RBC [Figure 2].

**Measurement of coronal discoloration**

Spectrophotometer equipment (Color i7 Benchtop Spectrophotometer) was used to measure the coronal discoloration of Apexit Plus and Resino Seal. The transparent double tape was used as a guide to ensure that the Commission Internationale de l’éclairage L*a*b* (CIELAB) reading can be recorded from tooth by exactly placing in the same position every time the measurements were recorded.

The resulting color shades were taken directly from the digital screen of computer attached to spectrophotometer device, and CIELAB readings are taken.

Pretreatment color shades and readings of the entire buccal surfaces were taken and considered as baseline data to which the subsequent readings at 10, 17, and 24 days were compared. Spectrophotometer was calibrated according to the manufacturer's instructions before taking each reading. The color differences at each time interval were calculated according to formula.

\[ \Delta E = (|\Delta L'|^2 + |\Delta a'|^2 + |\Delta b'|^2)^{1/2} \]

Where \( \Delta L \) is the difference in lightness calculated from differences in the \( \Delta L' \) readings between two periods. \( \Delta a \) and \( \Delta b \) refer to the difference in chroma. \( \Delta E \) value equal or greater than 3.5 was considered as clinically perceptible color change.[9]

**Statistical analysis**

The quantitative tooth color measurements using spectrophotometer were obtained for samples in four treatment groups. The data was obtained at baseline, 10th, 17th, and 24th day for each sample in each group. The spectrophotometer parameters \( L, a, \) and \( b \) at each time point were and \( \Delta E \) values were obtained as a measure of discoloration. The difference in the discoloration at different times was tested for statistical significance using nonparametric Wilcoxon signed-rank test. All the analyses were performed using SPSS version 20.0 (IBM Inc.) Software and the statistical significance was evaluated at 5% level.

**Results**

In Apexit Plus group, the maximum mean discoloration, i.e., 14.91±3.37 was at 10 days, whereas at 17th day, the mean discoloration was 14.91±3.37, and after 24 days, it was 5.17±3.83 [Table 2]. The significance analysis using Wilcoxon signed-rank test revealed that the difference between 10th and 17th days was insignificant, whereas that of 10th and 24th days, as well as 17th and 24th days was significant.
In the Resino Seal group, maximum mean discoloration was 14.36±4.56 at 17th day, followed by mean of 12.53±3.87 at 10th day and then 10.43±4.29 on 24th day [Table 2]. The difference between the discoloration was statistically insignificant across three time points.

In the positive control group, maximum mean discoloration was at 24th day, i.e., 22.08±4.73, followed by the mean at 17th day, i.e., 15.73±4.60 and then 6.49±1.92 [Table 2]. The difference of discoloration between all the three time points was statistically significant.

Further, in the negative control group, the mean discoloration at 10th day was maximum, i.e., 16.99±2.80, followed by mean of 13.10±4.45 and then at 24th day with a mean of 10.84±6.5 [Table 2]. The difference between the discoloration at 10th and 17th day as well as 10th and 24th day was statistically significant.

**Discussion**

The desire to have white teeth and thus a more pleasant smile has become an important esthetic need for patients today.[10] It has been reported that poor esthetic appearance of treated teeth significantly affects the patient quality of life.[2] Poor dental esthetics compromise the appearance of the patients. The present study was conducted to evaluate the overall coronal discoloration effect of Apexit Plus and Resino Seal sealers.

In the present study, premolar teeth with wide pulp chambers were used as it allows bulk placement of sealer.

In the present study, Apexit Plus showed less coronal discoloration as compared to Resino Seal sealer. It displayed a considerable degree of tooth discoloration after 24 days when compared to baseline data which is statistically significant. These results are in agreement with a study done by El Sayed and Etemadi[4] which concluded that Apexit Plus sealer showed the least coronal discoloration as compared to AH Plus and Sultan sealer. This could be due to the fact that calcium hydroxide-based being a sealer which does not bond to dentin. Although Apexit Plus sealer do not contain silver or any heavy metal, it obviously contains substances that stain dentin. It is also apparent that these substances are changing chemically with time.[14]

Resino Seal sealer showed statistically significant discoloration which increases over a period of time when compared to baseline data. As the epoxy resin sets over time, the presence of natural moisture in the root canal system together with the elevated temperature of the human body triggers a chemical reaction which results in the conversion of the filler to a range of bismuth compounds which shows discoloration of tooth over a period of time. It could be due to interaction between radio-opacifier bismuth oxide, resinous component and the amine i.e methenamine in its composition. This is in agreement with previous

### Table 2: Mean and standard deviation of discoloration for samples in four treatment groups according to time

| Time points (days) | Apexit plus (n=10) | Resino seal (n=10) | Blood (positive control) (n=10) | Distilled water (negative control) (n=10) |
|-------------------|--------------------|--------------------|---------------------------------|--------------------------------------------|
| 10                | 14.91±3.37<sup>a</sup> | 12.53±3.87<sup>a</sup> | 6.49±1.92<sup>a</sup> | 16.99±2.80<sup>a</sup> |
| 17                | 12.95±2.63<sup>a</sup> | 14.36±4.56<sup>a</sup> | 15.73±4.60<sup>b</sup> | 13.10±4.45<sup>b</sup> |
| 24                | 5.17±3.83<sup>b</sup> | 10.43±4.29<sup>c</sup> | 22.08±4.73<sup>c</sup> | 10.84±6.50<sup>b</sup> |

Similar superscripts indicate statistical insignificance. SD=Standard deviation

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**Figure 1:** Sealer placed using lentulo spiral

**Figure 2:** Sample filled with blood
In the present study, the specimens in the positive control (blood) group immediately showed severe coronal discoloration which was significantly different than seen in the experimental groups. Positive control, i.e., blood shows discoloration of teeth but is not statistically significant. These results are in agreement with studies done by Marin et al.[15] and Parsons et al.[12] who commented that the discoloration of enamel by blood components possibly becomes more pronounced with longer exposure time. Although enamel has no tubular morphology, its organic structural features at the dentinoenamel junction may play a role in the discoloration process. Hemoglobin, which is released from degraded RBCs, might cause tooth discoloration. RBCs have a staining capacity. Histochemical analysis has shown erythrocytes to be the blood pigment responsible for tooth staining. The specimen in the negative control group (distilled water) did not show any significant discoloration. The results obtained from the control groups may indicate that the dentin and enamel have the ability to transmit the color of the material placed in the pulp chamber.[4]

The color measurements in the present study were carried out using a X-Rite i7 Spectrophotometer. It is a scientific standardized equipment which gives information about reflectance curve as a function of wavelengths in entire visible range and thus numerically specifies the perceived color of an object. This can also avoid long experimental time period in detecting color changes that are not even clinically observable by human eye. Therefore, standard CIELAB color system was used to express the magnitude of color and relative color changes of all the specimens[17,18] For this reason, it was decided to use spectrophotometric analysis for evaluating tooth discoloration in this study similar to previous studies.[2–4] A spectrophotometer can detect color without the interference of any uncontrolled factors.[18]

In van der Burg et al.’s[16] study, trained visual inspectors analyzed the color difference between samples. It was a subjective method of color analysis which was prone to error due to the individual and environmental factors. In the present study, a more accurate approach using spectrophotometer was used to measure the color at the different times.

Allan et al., Parsons et al., Walsh, and Athanassiadis et al. concluded that root canal cement usually causes discoloration because of the presence of unreacted components or the corrosion of some components owing to moisture and/or chemical interaction with dentin. These findings suggest that cement inside the root canal, which do not have the same appearance when mixed on the glass slab, are more likely to undergo chemical interactions with radicular dentin, in addition to the physical changes that may occur during setting.[19]

Overall, within limitation of this study and in rejection of the null hypothesis:

- There was a difference in the coronal discoloration of tooth across treatment groups. In other words, the coronal discoloration of tooth across treatment groups was significantly different at each time point
- The coronal discoloration of tooth at consecutive times was different for each treatment group. In other words, the coronal discoloration of tooth was significantly different at consecutive time points for each treatment group.

**Conclusions**

Within limitation of this study the following could be concluded:

- Resino seal sealer and Apexit plus sealer showed significant coronal discoloration over 17-24 days.
- Greater discoloration was caused by Resino Seal sealer than Apexit plus sealer.
- In terms of esthetic parameters, the use of Apexit Plus appears to be a favorable choice of material.
- Future research projects may be directed towards investigation of underlying mechanism of tooth discoloration by endodontic sealers and development of nonstaining alternatives.

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**Conflicts of interest**

There are no conflicts of interest.

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