Stereomicroscopic evaluation of sealing ability of three different root canal sealers: An in vitro study

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Abstract
Background and Objectives: To evaluate and compare the apical sealing ability of three different root canal sealers Bioroot RC (tricalcium silicate-based sealer), Nanoseal S (polydimethylsiloxane-based sealer) and Eposeal (epoxy resin-based sealer) by dye penetration method under a stereomicroscope.

Method: Three commercially available endodontic sealers were used in this study: Bioroot RCS (Tricalcium silicate-based sealer), Nanoseal S (Polydimethylsiloxane based sealer), Eposeal (Epoxy resin-based sealer), were used to assess the sealing ability in the apical area of the single rooted permanent teeth by dye penetration method under a 30x stereomicroscope. Teeth were obturated with the sealers and gutta-percha and stored in humidifier for 1 week, centrifuged with 2% methylene blue dye and ground sections were examined under a stereomicroscope. The results were obtained by measuring the dye penetration in the apical area under stereomicroscope.

Results: Eposeal (Epoxy resin-based sealer), showed the least dye penetration producing a mean dye penetration of 0.8000mm. Nanoseal S (Polydimethylsiloxane based sealer) showed the highest dye penetration with the mean of 1.6000mm. Bioroot RC (calcium silicate-based sealer) showed the moderate dye penetration of with the mean of 0.8500mm.

Conclusion: On the basis of the results of the present study, it can be concluded that all the sealers demonstrated dye penetration with differences according to the material used. Furthermore, Eposeal (Epoxy resin-based sealer) showed the least dye penetration among the three sealers tested, followed by Bioroot RC and Nanoseal S sealer.

Keywords: dye penetration, Bioroot RC sealer, Nanoseal S, Eposeal, 30x stereomicroscope

Introduction
Successful endodontic treatment requires obturation of the root canal space with an inert filling material, creation of a "perfect" or hermetic apical seal, and elimination of any portal of entry or exit to the periapical tissues [1]. It should also be nontoxic, non-carcinogenic, and biocompatible with the tissue fluids and dimensionally stable [2]. On the basis of current biological understanding, the root filling should fulfill its role in three ways: (i) by blocking communication between the oral cavity and the peri-radicular tissues (the inhibition of coronal leakage), (ii) the entombment of surviving bacterial cells in the root canal system, and (iii) the inhibition of an influx of fluid from the periapical tissues [3]. Over the years, root canal sealers based upon zinc-oxide eugenol combination have served the profession; however, their irritation potential led to development of non-eugenol sealers [3]. The ability of sealers to adhere to dentin seems to be an important feature of a root canal filling material [4-5]. Adhesion appears desirable for two reasons:

1. In a static situation, it should prevent fluid percolation between the gutta-percha and the canal wall [6].
2. In a dynamic situation, it should prevent dislodgement of the root filling, thereby reducing the risk of contamination [7].

Aim and Objective
Aim
Aim of the study is to evaluate the sealing ability of three different root canal sealers by apical...
Objective Of the Study
1. To evaluate the apical sealing ability of Bioroot-RCS sealer after obturation by cold lateral condensation assessed with dye penetration.
2. To evaluate the apical sealing of NanoSeal-S sealer after obturation with cold lateral condensation assessed with dye penetration.
3. To evaluate the sealing ability of Eposeal sealer after obturation with cold lateral condensation assessed with dye penetration.
4. To compare the sealing ability of bioceramic sealer (Bioroot RCS), polydimethylsiloxane sealer (NanoSeal S), and an epoxy resin-based sealer (Eposeal) obturated by cold lateral condensation assessed with dye penetration.

Materials And Methods
The present in vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Farooqia Dental College and Hospital, in coordination with the Department of Oral pathology of Farooqia Dental College and Hospital Mysore. The study was performed to compare the sealing ability of bioceramic sealer (Bioroot RCS), polydimethylsiloxane sealer (NanoSeal S), and an epoxy resin based-sealer (Eposeal) with gutta-percha and root canal dentin walls.

Materials and Methods
Source of Sample
70 freshly extracted non-curious human single rooted teeth (Figure 1) with straight canals and fully formed apices will be collected from the Department of Oral and Maxillofacial Surgery, Farooqia Dental College and Hospital, Mysore.

Materials Used In The Study
Armamentarium: (Figure 2)

I. Materials used for de-coronation
1. Wheel disc
2. Straight hand piece

II. Instruments used for access cavity preparations and biomechanical preparations
1. Normal saline
2. Airotor Handpiece.
3. Endo-access burs no. 2 (Dentsply Maillefier).
4. Barbed broach no. 10- no. 25 (Dentaire).
5. K-files (Mani) no.10- no.50.
6. Gates-Glidden drills (Mani) no.2- no.3.
7. 1% and 2.5% Sodium hypochlorite.
8. 17% EDTA (Dental Source).
9. 25gauge irrigating needles.

III. Materials needed for obturation
1. Spatula
2. Paper points no.10- no.50.
3. Paper pad
4. Gutta-percha points (Dentsply) no.10- no.50
5. Hand spreaders
6. Lentulospiral (Mani paste carrier).
7. Sealer used (Figure 3)

a. Bio Root-RC sealer (Septodont)

- Composition:
- Powder:
- Tricalcium silicate
- Zirconium oxide
- Liquid:
- Calcium chloride
- Polycarboxylate
- Povidone

b. NanoSeal-S sealer (Prevest DenPro)
- Composition:
- Polydimethylsiloxane
- Micro silver powder
- Silicone oil
- Platinum catalyst
- Zirconium dioxide
- Paraffin liquid

Fig 1: Sample
Fig 2: Armamentarum
Fig 3: Sealers
c. Eposeal sealer (prevest DenPro)
- Composition:
  - Base paste:
  - Epoxy oligomer resin
  - Ethylene glycol salicylate
  - Tricalcium phosphate
  - Bismuth subcarbonate
  - Zirconium oxide
  - Catalyst paste:
  - Polyamino-benzoate
  - Calcium hydroxide
  - Zirconium oxide
  - Yellow iron oxide pigment

I. Materials used for tooth surface preparation and dye leakage
1. Nail varnish
2. 25% Methylene blue dye

II. Materials used for coronal sealing
1. Glass ionomer cement (type 2)

III. Stereomicroscope for evaluation of dye penetration

Methodology

Methods of Collection of Data
70 freshly extracted human single rooted permanent teeth will be selected. The teeth were stored in 1% sodium hypochlorite (NaOCl) solution, for three days to remove organic debris and then they were stored in distilled water. The crowns were removed at the cement-enamel junction using a high-speed fissure bur. Access preparation was done using an endo-access bur (Dentsply Maillefer, USA) and a barbed broach (Dentaire, SA) was used to remove the pulp. Then, a no.10 – K-file (Mani, Japan) was introduced into the canal and was pushed towards apical part until the tip of the instrument was just visible at the apical foramen. This length of the file was recorded and then after subtracting 1mm from the recorded length, working length of the root canal was determined. The canals were cleaned and shaped with K-files (Mani, Japan) using a step-back technique with recapitulation of files to establish a progressively tapering root canal preparation. The apical portion of the canal was enlarged to a minimum 30 no. K-file and 50 no. K-file, depending on the size of the original canal. The coronal two thirds of each canal were prepared using number 2 and 3 Gates Glidden drills (Mani, Japan) and apical third were prepared with hand files. After each instrument was used, the canals were irrigated with 2ml of 5% NaOCl and 2ml of 15% solution of EDTA (Dental Source, North Hollywood CA, USA). The irrigating solutions were delivered through a 25-gauge needle which was placed as far as possible into the canal without allowing the needle to touch the canal walls. The total amount of irrigant used in each canal was 30 ml, on completion of the instrumentation process, a 10 no. K-file (Mani, Japan) was passed 1mm through the apical foramen to remove any dentinal plugs and to ensure that the foramen was patent for dye penetration. After drying the canals with paper points, standardized gutta-percha cones (Dentsply, China) were selected as master points. The fit of each master point was assessed by radiographs to determine whether the point was fully seated to the working length. The teeth were randomly selected and divided into five groups of 20 teeth each (four experimental group and one control group). The sealers used were as follows-
- Group I – Bioroot RCS (calcium silicate based)
- Group II – Nanoseal S (polydimethylsiloxane based)
- Group III – Eposeal (epoxy resin based)
- Group IV – Control Group- Gutta-Percha alone (no sealer).

Obturation

All the teeth except the controlled teeth were filled with a root canal sealer and gutta-percha points using the cold lateral condensation technique (Figure 4). In the control group, sealer was not used. The sealers were mixed according to the manufacturer’s directions and were introduced into the canal using a lentulo-spiral (Mani, Paste carriers, Japan) which was kept 3mm to 4mm short of the working length. This process was repeated twice to ensure that an adequate amount of sealer was placed in each canal. The master gutta-percha point (Dentsply, China) was coated with sealer and placed in the canal to the full working length. Hand spreader (Dentsply, China) was then used for lateral condensation with standardized fine gutta-percha accessory points (Dentsply, China) was carried out until the entire canal was obturated. Excess gutta-percha was removed and the gutta-percha in the coronal third of the canal was vertically condensed with a plunger. Radiographs were taken to evaluate the obturation. Obturation was considered to be optimum when no voids were present in the radiograph. If the voids appeared in the radiograph, re-obturation was done.

Humidification

The access cavities were sealed with Cavit G (3M ESPE, Germany) up-to 2 mm and the teeth were placed in a...
Humidifier (ICU Safe, Sanyo, Japan) for 1 weeks with 100% humidity at 37°C to ensure that the sealer set in an environment that simulate the clinical situation in which they are designed to be used (Figure 6).

**Application Of Nail Varnish**
The roots were coated with two layers of clear nail varnish except for the apical 2 to 3mm. At this stage the control group was further sub-divided into two equal groups, the positive and negative controls. Teeth in the positive control group had the roots coated with nail varnish except for the apical 2mm to 3mm in the same manner as the experimental groups. They were used to test the sealing ability of gutta-percha when used without a sealer. Teeth in the negative control group had the entire root surface coated with nail varnish and were used to test the ability of the nail varnish to seal the root against dye penetration under the experimental conditions used in this study.

**Centrifugation**

![Fig 7: Centrifugation](image)

Once the nail varnish was absolutely dry, each sample was introduced in a 12-ml centrifuge tube with the apex of the root positioned in the direction of the open end of the centrifugal tube. Methylene blue dye solution 2% (pH=7) was poured into each tube until the root was completely immersed into the solution. The samples were then centrifuged for 3 minutes at 30Xg using a centrifugal machine (Figure 7). The samples were taken out from the solution and were thoroughly bathed in running tap water.

**Sectioning of Samples**

![Fig 8: Sections of the samples](image)

The experimental samples were sectioned longitudinally by means of a low-speed circular diamond saw (Confident, India) in a path roughly parallel to the axis of the tooth and through the apex with a coolant (Figure 8).

**Measurement of Dye Penetration**

![Fig 9: Study under stereomicroscope](image)

After sectioning, the samples were studied under a stereomicroscope (30X Magnification, Carl Zieiss). The end point of dye infiltration was calculated as the point where dye no longer penetrated the obturating material (Figure 9). The measurement from the apex to the end point of dye penetration was observed and documented in millimeters.

**Statistical Analysis**

Data that was obtained was statistically analyzed. Quantitative data was presented as rank, mean, standard deviation, standard error. One way ANOVA test was used for comparison of three groups with respect to microleakage.

**Results**

Table 1, Graph 1, Graph 2, Graph 3 and Graph 4 illustrate the average microleakage showed by the different types of sealers. It was observed that the average microleakage showed by Group I – Bioroot RC sealer was 0.8500mm, Group II – Nanoseal S sealer being 1.5000mm, Group III– Eposeal sealer was observed to be 0.8000mm. Out of all the three groups, it was analysed that the micrleakage level was found to be statistically significant in all three sealers. Microleakage in Group III (Eposeal sealer) was observed to be the least (0.8000 mm) and the maximum microleakage was observed in Group II sealer i.e., Nanoseal S (1.5000 mm). Microleakage in Group I (Bioroot RC sealer) was found to be moderate.

Table 2 showed Analysis of Variance for dye penetration. It signifies that on applying ANOVA test between three different groups containing different sealers, it showed significant difference between all the 3 groups.

Table 4: shows t-Test for comparison between Group I (Bioroot RC) and Group II (Nanoseal S) sealer. It represents that on applying t-Test for comparison amongst these different sealer groups. It was found that Nanoseal S shows more dye penetration (1.5000 mm) than Bioroot RCS (0.8500
It was observed to be statistically significant. Table 6: shows t-Test for comparison between Group I (Bioroot RC) and Group III (Eposeal) sealer. It was found that Bioroot RC showed more dye penetration 0.850mm than Eposeal sealer 0.800mm. It was observed to be statistically significant.

Table 8: shows t-Test for comparison between Group III (Eposeal) and Group II (Nanoseal S sealer). It represents that on applying t-Test for comparison amongst these different sealer groups. Nanoseal S showed more dye penetration (1.5000mm) than Eposeal (0.8000mm). P value showed significant difference in both groups.

One Way Descriptive

Table 1: Mean and standard Deviation values of micro leakage scores by groups (n=20)

| Score | N | Mean | Std. Deviation | Std. Error | Minimum | Maximum |
|-------|---|------|----------------|------------|---------|---------|
| Group I | 20 | 0.8500 | 0.9330 | 0.20869 | 0.00 | 2.00 |
| Group II | 20 | 1.5000 | 1.35724 | 0.30349 | 0.00 | 3.00 |
| Group III | 20 | 0.8000 | 1.00525 | 0.22478 | 0.00 | 3.0 |
| Total | 60 | 1.0500 | 1.14129 | 0.14734 | 0.00 | 3.00 |

Table 2: Anova

| Score | Sum of Squares | Df | Mean Square | F | Sig. |
|-------|----------------|----|-------------|---|------|
| Between Groups | 6.100 | 2 | 3.050 | 2.457 | .095 |
| Within Groups | 70.750 | 57 | 1.241 | | |
| Total | 76.7850 | 59 | | | |

T-Test

Table 3: t-test group Statistics for comparison between Bioroot RCS and Nanoseal S Score

| Groups | N | Mean | STD. Deviation | Std. Error Mean |
|-------|---|------|----------------|-----------------|
| Group I | 20 | .8500 | .9330 | .20869 |
| Group II | 20 | 1.5000 | 1.35724 | .30349 |

Table 4: t-test independent samples for comparison between Bioroot RCS and Nanoseal S

| Score | t | df | Sig. (2-tailed) | Mean difference |
|-------|---|----|----------------|----------------|
| t-test for Equality of means | -1.765 | 38 | .086 | -.65000 |

Table 5: t-test group statistics for the comparison between Bioroot RCS and Eposeal Score

| Groups | N | Mean | STD. Deviation | Std. Error Mean |
|-------|---|------|----------------|-----------------|
| Group I | 20 | .8500 | .9330 | .20869 |
| Group III | 20 | .8000 | 1.00525 | .22478 |

Table 6: t-test independent samples for comparison between Bioroot RCS and Eposeal

| Score | t | df | Sig. (2-tailed) | Mean difference |
|-------|---|----|----------------|----------------|
| t-test for Equality of means | .163 | 38 | .871 | .05000 |

Table 7: t-test group statistics the comparison between Nanoseal S and Eposeal Score

| Groups | N | Mean | STD. Deviation | Std. Error Mean |
|-------|---|------|----------------|-----------------|
| Group II | 20 | 1.5000 | 1.35724 | .30349 |
| Group III | 20 | .8000 | 1.00525 | .22478 |

Table 8: t-test independent samples for comparison between Nanoseal S and Eposeal

| Score | t | df | Sig. (2-tailed) | Mean difference |
|-------|---|----|----------------|----------------|
| t-test for Equality of means | 1.853 | 38 | .072 | .70000 |
Graph 4: Graphical representation shows average microleakage in Nanoseal S (group II) and Eposeal (group III).

Discussion

Fig 10: Dye penetration in group I - Bioroot-RCS

Fig 11: Dye penetration in group II - NanoSeal-S

Fig 12: Dye penetration in group III - Eposeal

Obturation of root canals is mostly done by a solid, semisolid, or rigid core material. The most commonly used core material is gutta-percha, but this material does not seal the canal when used alone. So, root canal sealers are used with gutta-percha. Some sealers possess antibacterial effect and eliminate microorganism left in the root canal system after completion of cleaning and shaping procedures. Sealers seal off voids, patent accessory canals and multiple foramina, act as
lubricant, form a bond between GP and root canal dentin, and
entomb the remaining bacteria. Therefore, sealers play an
essential role in the success of root canal treatments [8-11].
The present study was conducted to evaluate the apical sealing
ability of calcium silicate sealer, resin sealer and silicone sealer obturated by cold lateral condensation technique which was assessed with dye penetration method (Methylene blue dye) in order to evaluate the microleakage being showed by the respective sealants.
Gold standard method of root canal obturation being a
combination of gutta-percha cones and a sealer has been used
in the present study [19]. Literature has shown that teeth
obturated with gutta-percha points and sealer display less
leakage than those without sealer. Although several studies
have shown good clinical results for many years, some in vitro
and in vivo studies have demonstrated that they fail to
achieve a complete root canal seal [13-16]. The microleakage
has been attributed to the lack of an effective bond at the
sealer/dentin and sealer/gutta-percha interfaces [17, 18].
In this study, all the three group specimens showed evidence
of dye penetration which predicts the average microleakage is
shown by all the different types of sealers. It was observed
that the average microleakage showed by Group I (Bioroot RCS sealer) was 0.8500 mm
(Figure 10), by Group II (Nanoseal S sealer) being 1.5000
mm (Figure 11), by Group III (Eposeal based sealer) was
observed to be 0.8000mm (Figure 12). Out of all the three
groups, it was analysed that the microleakage level was found
to be statistically significant in all three sealers. Microleakage
in Group III (Eposeal sealer) was observed to be the least
(0.8000 mm) and the maximum microleakage was observed
in Group II (Nanoseal S sealer) i.e. (1.5000 mm).
Microleakage in Group II (Bioroot RCS) was found to be
moderate (0.8500 mm). Thereby, the epoxy resin-based sealer
(Eposeal) sealed the root canals more completely than the
Bioroot RC and Nanoseal S sealers.
There was less leakage and better seal of the root canals
obturated using resin-based sealer i.e. Group III (Eposeal)
when compared with Group I (Bioroot RCS) and Group II
(Nanoseal S). It has already been documented that an epoxy-
base root canal sealer characterized by very good mechanical
properties, high radiopacity, reduced polymerization
shrinkage, low solubility and not the least, a high degree
of stability on storage. Statistical t-test for comparison between
Group III (Eposeal) and Group I (Bioroot RCS) represented
that on applying t- Test for comparison amongst these
different sealer groups. It was found that BioRoot RCS shows
almost similar dye penetration (0.8500 mm), Eposeal (0.8000
mm) which was observed to be statistically significant.
Results were in accordance with that reported by Saeed MH et al., Shafer E et al and Brackett MG et al. which concluded in
their study that resin-based root canal sealers were more
effective in sealing root canals than the polydimethylsiloxane
based sealer [19-21].
Haddad AA et al evaluated and compared the sealer thickness
and interfacial adaptation of bioceramic sealers (Sankin
Apative III, MTA Fillapex®, EndoSequence® BC) to root
dentin against AH Plus® sealer. Bioceramic sealers showed
more gaps compared with AH-Plus, with no significant
differences among them [22].

Conclusion
Within the limitation of the study, based on the recorded data
and statistical analysis the following conclusions may be
drawn:

1. The microleakage level was found to be statistically
   significant for all the three sealers. The average
   microleakage showed by Group I – Bioroot RC sealer
   was 0.8500 mm, Group II – Nanoseal S sealer being
   1.5000 mm, Group III– Eposeal sealer was observed to be
   0.8000mm.
2. Microleakage in Group III (Eposeal sealer) was observed
to be the least (0.8000mm) and the maximum
microleakage was observed in Group II sealer i.e.,
Nanoseal S (1.5000 mm). Microleakage in Group I
(Bioroot RCS) was found to be moderate (0.8500 mm).
3. Thereby, the resin-based sealer (Eposeal) sealed the root
   canals more completely than the calcium silicate-based
   sealer (Bioroot RCS) and polydimethylsiloxane-based
   sealer (Nanoseal S).
4. This study evaluated only one aspect of root canal sealers
   that is adhesion to the root canal dentin. Further
   investigation of various aspects of the resin cement to be
   used as a root canal sealer is recommended.

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