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# ASSESSMENT OF CORONAL MICROLEAKAGE FOLLOWING ROOT CANAL OBTURATION WITH CALCIUM SILICATE SEALERS

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## *ABSTRACT*

**Introduction**: This study compared in vitro the coronal sealing ability of four types of endodontic sealers One-Fil, ADSEAL, GuttaFlow Bioseal, BioRoot RCS

**Materials and Methods:** Thirty-two single-rooted mandibular premolars were randomly divided into 4 groups (n=8). After Instrumentation, each group obturated using a different type of sealer as group 1: One-Fil, group 2 ADSEAL, group 3: GuttaFlow Bioseal, and group 4: BioRoot RCS. Teeth were cleared then subjected to a dye penetration test, then evaluated under stereomicroscope.

**Results:** Kruskal-Wallis test was used to compare dye penetration depth among the different endodontic sealers. Post-hoc analysis was conducted using Dunn's multiple comparison test. The mean score of dye penetration could be arranged from the least to the greatest as follows: One-Fil (1.38 ± 0.52) followed by ADSEAL (2.38 ± 0.52) without significant difference between them, however One-Fil recorded lower significant dye penetration compared to GuttaFlow Bioseal (2.5 ± 0.53) and BioRoot RCS which showed the highest mean of dye penetration (2.75 ± 0.46).

**Conclusions**: Premixed calcium silicate sealer revealed the highest significant resistance against dye penetration while hand mixed calcium silicate sealer revealed the least resistance against coronal dye penetration.

**Clinical Relevance:** All types of the investigated sealers allowed dye penetration with variable degrees, therefore, proper coronal and apical sealing with adequate final restoration is the key to successful treatment outcome.

**KEYWORDS:** Coronal microleakage, dye penetration, hydraulic calcium silicate sealers, GuttaFlow Bioseal.

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## INTRODUCTION

The aim of the root canal treatment is to eliminate micro-organisms and prohibit reinfection of root canal system (Ordinola-Zapata et al., 2014). However, failure of root canal treatment usually occurs when the treatment is not up the level of the standard of care (Siqueira, 2001). Bacteria and its by-products are guarded as the major cause of failure of root canal treatment. Therefore, coronal and apical sealing are of paramount importance to prevent the ingress of bacteria from coronal direction or their egress from apical direction. The pathway between the root canal and oral cavity should be sealed to prevent leakage (Zafar et al., 2009). Bacterial leakage coronally may occur preoperative, during treatment or postoperative for many reasons such as: poor isolation, improper temporization, fractured coronal restoration, which may compromise the outcome of root canal treatment (Al-Maswary et al., 2016).

Root canal obturation is executed to deny the space available for bacterial colonization and to entomb the residual bacteria that survived after chemo-mechanical preparation (Abusrewil et al., 2020). Moreover, root canal obturation is important for apical sealing to prevent the percolation of periradicular exudate into the canal, which could act as a nutrients source for the surviving bacteria (Orstavik, 2005).

The rationales of using root canal sealers in addition to the core filling materials are to fill patent accessory canals and multiple foramina, seal any gaps between the core filling material and the root canal wall, and to act as a lubricant to make the placement of the filling core easier (Rathi et al., 2020). Root canal sealers are classified according to their main chemical contents into zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and calcium silicates-based sealers (Abu Zeid et al., 2022). For enhanced adaptation and sealing of root canal space with a bioactive material that would improve the healing of periapical lesion, hydrophilic hydraulic calcium silicate root canal sealers "bioceramic sealers" have been recently recommended (Al-Haddad & Aziz, 2016).

These hydraulic calcium silicate sealers have advantages such as: anti-inflammatory effect, antimicrobial, osteogenic, calcium ion release, biocompatibility, bioactivity, and expansion on setting (Darade et al., 2022), in addition, they promote physical and chemical bonds to dentin by creating hydroxyapatite precipitate at the dentin-sealer interface during the setting. However, one disadvantage of these materials is in the difficulty in removing them from the root canal once they are set for later retreatment or post-space preparation (Washio et al., 2019). Hydraulic calcium silicate sealers are available in two forms: Powder/liquid systems which require manual mixing, or Premixed bioceramics that acquire moisture from the surrounding tissues to set.

Many studies have shown that coronal microleakage occurs through root canal fillings when exposed to the oral cavity directly or indirectly through defective restoration (Shanmugam et al., 2020). In vitro studies have reported dye penetration through all root canal obturation materials within 10–73 days (Torabinejad et al., 1990), while in vivo studies observed bacterial penetration within 21 days (Williamson et al., 2005). Microleakage of filled root canals occurs mostly between the sealer and intracanal wall or between the sealer and gutta-percha cone or cones (Gomes et al., 2007).

Various methods were applied for evaluation of coronal microleakage such as: fluid filtration or transportation, bacteria and toxin infiltration, dye extraction as well as dye penetration method (Kersten & Moorer, 1989, Veríssimo & do Vale, 2006). One of the most popular techniques is the dye penetration since it is easy to use, convenient, and does not require complicated armamentarium (Wimonchit et al., 2002). India ink and methylene blue are usually used in this technique, where part of the sample is submerged inside the dye passively then

monitored for the amount penetration (Spngberg et al., 1989). The penetration of the dye is an indication for the presence of minute space between the canal walls and the filling material which reflects the adaptation and sealing of the filling material with the dentin wall (Goldman et al., 1989).

One-Fil (MEDICLUS, South Korea) is a premixed calcium silicate based bioceramic root canal sealer, it is a bioactive, antibacterial sealer and ready to be applied inside the root canal. ADSEAL (Meta Biomed, Korea) is a biocompatible epoxy resin with excellent sealing ability, and easy-to-mix paste. BioRoot RCS (Septodont, France) is manually mixed hydrophilic calcium silicate root canal sealer in a powder and liquid formula, with outstanding adhesion to dentin and gutta-percha, and GuttaFlow Bioseal is a silicon based obturation system that contain flowable gutta-percha as the previous version of silicon sealer (GuttaFlow 2) but modified by the addition of bioactive glass to improve the healing outcome as claimed by the manufacturer.

The aim of this study was to investigate and compare using dye penetration test the sealing ability of 2 types of calcium silicate sealers (One-Fil and BioRoot RCS), a silicone-based calcium silicate sealer (GuttaFlow Bioseal) versus the most commonly used root canal sealer nowadays Epoxy resin sealer (ADSEAL).

## MATERIAL AND METHODS

### Sample Selection

All the procedures performed were carried out in accordance with relevant guidelines, regulations, and ethical standards of the Institutional Review Board of Beirut Arab University (Exemption code: 2023-H-0115-D-M-0507). Sample size calculation was performed using G*power version 3. 1. 9.5. A sample of 32 (N=32) single-rooted mandibular premolars type I vertucci with root curvature <30° according to the schneider method (Schneider, 1971) were selected for this experiment. Teeth were collected from the department of surgery BAU from patients scheduled for orthodontic extraction after approval of the patients. Teeth with immature open apices, external or internal resorption, cracks on the surface, root caries, or calcified canals were excluded from the study. Calculus and any remnants of periodontal tissue debris were removed, then teeth were autoclaved, and stored in thymol till use.

### Sample Preparation

All the samples were decapitated at the CEJ level with diamond disc double sided 0.2x22mm using a low-speed handpiece to ensure a flat coronal reference point and to standardize the root length at 16 mm, The following procedures were carried out under magnification with surgical operating microscope (PRIMA LABOMED, Essebaan 50, Netherland). A size 15 K-File (MANI, Japan) was introduced passively into the canal until the tip reached flushed with the apical foramen then the working length was established by subtracting 1mm from that length (Shubham et al., 2021). The initial file was standardized to #20 K manual file, samples with initial file larger than #20 were excluded from the study and replaced with other samples. The root canals were prepared using a crown down technique with HyFlex EDM (Coltene, Switzerland) #20, 5% then the preparation was finished with #25, variable taper attached to a rotary motor (E-connect S motor, Eighteeth, China). During instrumentation, 5 mL of a freshly prepared solution of 5.25% sodium hypochlorite (NaOCl) was delivered between each file with irriflex (Acteon, France) irrigation needle (Haapasalo et al., 2014). After instrumentation, all specimens received a final flush of 2 mL 17% EDTA for 1 min and 6 mL 3% NaOCl for 3 min. all root canals were then rinsed with 2 mL of sterile saline and activated using irrisafe tips (Acteon, France) attached to ultrasonic device (Satelec, Acteon, France) in 3 cycles of 20 s each (Abiad et al., 2022) to ensure complete removal of the NaOCl and EDTA since they interfere with the hydration of tricalcium silicate-based materials, finally the canals were dried with paper points.

**Samples grouping**

Teeth were divided into four groups, according to the sealer used in obturation, each group consists of eight teeth (n=8). Group I: One-Fil (MEDICLUS, South Korea). Group II: ADSEAL (Meta Biomed, Korea), Group III: GuttaFlow Bioseal (Coltène/ Whaledent, Altstatten, Switzerland), and Group IV: BioRoot RCS (Septodont, France). (Table 1).

**Method of obturation**

For all groups, teeth was obturated with matched taper single cone gutta-percha technique (Inan et al., 2009) while the sealers were applied to in two different methods, the One-Fil and Gutta-Flow Bioseal applied directly to the root canal whereas the ADSEAL and BioRoot RCS were mixed on a mixing pad and then carried on the master cone to coat the whole walls of the root canals, until seating to full length smoothly and slowly. Teeth were then stored in 100% humidity for 14 days to mimic the oral environment (Thejeswar EP, 2020), Samples were cleared out according to the method described by Rosler (Rosler, 2010)

**Dye application**

All roots were covered by two coats of nail varnish, except the coronal 3 mm. Then all samples were immersed in 2% methylene blue for 48 hours. After removal from the dye, the roots were rinsed in tap water and the nail varnish was completely removed using a Lecron carver. The depth of dye penetration was observed under the stereomicroscope (Olympus SZ61, Tokyo, Japan), images were captured by Olympus Camera hold on the stereomicroscope, thereafter cellSens Entry software (Olympus SZ61, Tokyo, Japan) was employed to analyze the depth of the dye penetration, this was followed by scoring the depth of penetration according to (Davalou et al., 1999) as follows:

Score 1: mean <1.0 mm of dye penetration.

Score 2: mean ≥1.0 < 2.0 mm dye penetration.

Score 3: mean ≥2.0 mm dye penetration.

TABLE (1) Showing the composition, presentation, and method of mixing of the investigated sealer.

| Material | Manufacturer | Composition | Radiopacifier | Presentation | Mixing |
|---|---|---|---|---|---|
| **One-Fil (Calcium Silicate-based)** | MEDICLUS, South Korea | Calcium alumina silicate, Hydrophilic polymer | Zirconium oxide | Syringe | Pre-mixed |
| **ADSEAL (epoxy resin)** | Meta Biomed, Korea | Epoxy oligomer resin, ethylene glycol salicylate, calcium phosphate, bismuth subcarbonate, polyaminobenzoate, triethanolamine, calcium oxide | Zirconium oxide | Two tubes double barrel syringe | Manual |
| **GuttaFlow Bioseal (Silicone Based sealer)** | Coltene, Switzerland | Gutta-percha powder, platinum catalyst silicates, polydimethylsiloxane, silicone oils, silver zinc oxide, bioactive glass, color pigments | Zirconium dioxide | Two tubes double barrel syringe | Pre-mixed |
| **BioRoot RCS (Tricalcium silicate materials** | Septodont, France | Tricalcium silicate, excipients in powder form, calcium chloride and excipients as an aqueous liquid | Zirconium oxide | Powder/liquid | Manual |

**Statistical analysis**

Statistical analysis was conducted using SPSS for windows version 26.0. Descriptive statistics of the dye penetration depth variables, including mean, standard deviation, median, and interquartile range were calculated and reported for different endodontic sealers (One-Fil, ADSEAL GuttaFlow Bioseal, and BioRoot RCS).

**RESULTS**

The normality assumption of the distribution was evaluated using the Shapiro- Wilk test. The results showed that the mean scores dye penetration depth distribution was not normal across all four groups ($p < 0.001$). Therefore, non-parametric tests were used for further analyses. Specifically, the Kruskal-Wallis test which was applied for comparison between different groups of endodontic sealers. Post-hoc analysis was conducted using Dunn's multiple comparison test, with p-values adjusted using Bonferroni correction for multiple testing. The significance level for all analyses was set at $p < 0.05$.

The lowest significant mean score for dye penetration depth was recorded for One-Fil sealer reflecting the lowest coronal micro-leakage then ADSEAL without significant difference between them, followed by GuttaFlow Bioseal, whereas BioRoot RCS demonstrated the highest dye penetration depth indicating higher coronal microleakage without significant difference between BioRoot RCS and GuttaFlow Bioseal. (Table 2)

**Comparing pairs of sealers:** upon comparing One-Fil and ADSEAL, the latter had more dye penetration depth indicating more microleakage. While comparing One-Fil and GuttaFlow Bioseal, Guttaflow showed more dye penetration depth and thus more microleakage with significant differences. One-Fil and BioRoot RCS also showed higher significant mean of dye penetration for BioRoot RCS. Meanwhile there was no significant difference between ADSEAL and GuttaFlow Bioseal, ADSEAL and BioRoot RCS, GuttaFlow Bioseal and BioRoot RCS correspondingly. (Table 3) (Fig 1)

TABLE (2) Description of dye penetration depth score by groups

| Groups | Mean ± SD | Median (25th - 75th percentile) | p-value [y] |
|---|---|---|---|
| ADSEAL[ac] | 2.38 ± 0.52 | 2 (2 - 3) | 0.001* |
| One-Fil[bc] | 1.38 ± 0.52 | 1 (1 - 2) | |
| BioRoot RCS[a] | 2.75 ± 0.46 | 3 (2.5 - 3) | |
| GuttaFlow Bioseal[a] | 2.5 ± 0.53 | 2.5 (2 - 3) | |

***SD: Standard Deviation***

[y] ***Tests the null hypothesis that the distributions of all groups are the same.***

**** Indicates a significant p-value***

***Different superscript letters indicate statistical significance p-value <0.05 (Dunn's test).***

TABLE (3): Dye penetration depth score pairwise comparison of groups (post hoc analysis)

| Group 1 | Group 2 | Test Statistic | Sig. | Adj. Sig.[a] |
|---|---|---|---|---|
| OneFil | ADSEAL | 11.000 | 0.011* | 0.065 |
| OneFil | GuttaFlow Bioseal | -12.687 | 0.003* | 0.020* |
| OneFil | BioRoot RCS | -16.062 | <0.001* | 0.001* |
| ADSEAL | GuttaFlow Bioseal | -1.687 | 0.696 | >0.999 |
| ADSEAL | BioRoot RCS | -5.062 | 0.241 | >0.999 |
| Gutta Flow Bioseal | BioRoot RCS | 3.375 | 0.434 | >0.999 |

***Sig.: Significance, Adj. Sig: Adjusted Significance***

***Each row tests the null hypothesis that the distributions of Group 1 and Group 2 are the same.***

***The Bonferroni correction has been applied to the significance values for multiple tests.***

**** Indicates a significant p-value***

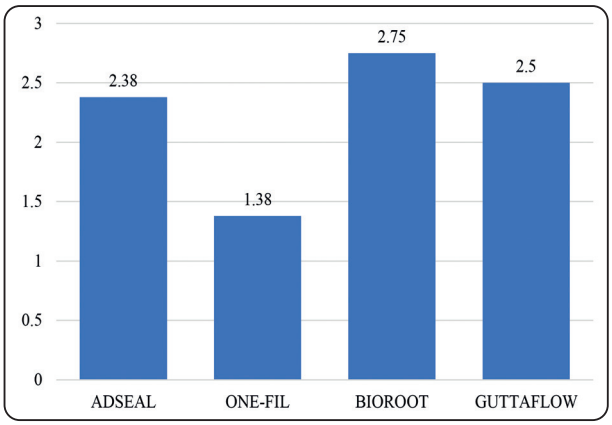


Fig. (1) Bar graph show Mean dye penetration depth score by groups.

## DISCUSSION

Coronal microleakage is a frequent cause of endodontic failure and is defined as the passage of bacteria, fluids, and chemical substances between the internal root canal dentin and the filling material (Roghanizad & Jones, 1996). This Microleakage can occur from coronal direction down the obturated canals to the apical area causing secondary infection or reinfection (Maruoka et al., 2006). it could also occur from an apical direction. Therefore, factors such as root filling technique, root filling material, type of sealer used and the type and quality of bonding to root canal walls have influence on both coronal and apical leakage (Muliyar et al., 2014). Thus, sealing all the pathway between the coronal and apical portions of the root canal system is necessary for the favorable outcome of root canal therapy (Zafar et al., 2009)

In Addition to the factors related to root canal obturation, other factors such as proper temporization and good coronal sealing by final restoration are essential for preventing coronal microleakage (Amlani, 2013). However, loss of temporary filling or delayed final restoration would expose the root canal filling directly to the oral cavity with subsequent coronal microleakage. As microorganisms have been the ability to penetrate through different temporary restorative materials and a supposedly well-obturated root canal system (Davalou et al., 1999).

Mandibular premolars with single canals were selected for this study as they have oval shaped canals, so greater amount of sealer will be needed to fill the space between the rounded gutta-percha and the root canal walls, which will allow investigation of the efficiency of sealing ability of sealers. Teeth were decoronated and the length of all roots were fixed to 16 mm, as that the length of the filling material will be standardized not to affect the amount and rate of dye penetration.

Smear layer was removed to ensure intimate contact between the filling and root canal wall. EDTA solution 17% was used for 1 min only, as increasing the time of exposure makes dentin susceptible to erosion (Kuah et al., 2009). NaOCl was not used as a last irrigant as it is a strong oxidizing agent leaving behind an oxygen- rich layer on the dentine surface, which results in a reduced bond strength to sealers (Wright et al., 2017). So saline was used as a final flush to minimize the compromising effect of NaOCl on the bond strength of root canal sealers to dentin (Doumani et al., 2017). For standardization of obturation technique, matched taper single cone gutta-percha was used in all groups (Schäfer et al., 2012).

In the present study resistance to coronal leakage of 4 types of sealers was investigated; Epoxy resin sealer was chosen as it is considered nowadays the gold standard sealer replacing zinc oxide eugenol in the recent years silicon based modified with bioactive glass root canal sealer was selected as previous studies showed that it provides better sealing ability without the need of using accessory cones as in lateral compaction or thermoplastic techniques(Ruiz-Linares et al., 2019), One-Fil was selected as an example of the pre-mixing type Hydraulic calcium silicate sealers while the BioRoot RCS as a type of the convenient hand-mix which may present different sealing ability than the premixed.

The dye penetration test was evaluated after 48hrs to ensure complete setting of the investigated sealers; as the setting time for ADSEAL is 45 mins, One-Fil 2.5 hours, BioRoot RCS < 4 hours, and Gutta-Flow 12-16 mins according to the manufacturer.

Although coronal and apical dye penetration tests evaluate the microleakage in an opposite directions, coronal seal is way important for the outcome of root canal treatment (Sritharan, 2002). Coronal leakage tests reflect the sealing ability of sealers more than apical leakage test, because the amount of sealer is greater coronal than apical in matched single cone technique, added to the greater surface area of coronal dentin for dye penetration between the sealer the dentin compared to the smaller surface area at the apical end of the root, however both techniques reflect the sealing ability of the filling material (Pereira et al., 2017). Due to lack of studies on coronal leakage of the investigated sealers, coronal and apical leakage studies were compared.

The result of the current study showed that One-Fil and ADSEAL had the least dye penetration which could represent less coronal leakage without significant difference between them, both types of sealers form chemical bond to dentin walls which might provide better sealing ability which in turn should reduce leakage in clinical situations (Neelakantan et al., 2011). The comparable result of ADSEAL to One-Fil may be related to the capability of resin sealer to react with any exposed amino groups in collagen and create covalent bonds after the epoxide ring of resin is opened (Kebudi Benezra et al., 2018). The mechanism of bonding of hydraulic calcium silicate sealer to root dentin could be linked to the following mechanisms; infiltration of the sealer's mineral content into the intratubular dentin resulting in the establishment of a mineral infiltration zone produced after denaturing the collagen fibers with a strong alkaline sealer, Partial reaction of calcium silicate hydrogel with phosphate in a moistened environment leading to the formation of hydroxyapatite with the dentin substrate, and Diffusion of the sealer particles into the dentinal tubules to produce mechanical interlocking bonds added to the chemical bond(Mathew et al., 2019) The results of this study coincide with other studies that found no significant difference between pre-mixed TotallFill biocermic sealer (FKG, Switzerland) and AH Plus resin sealer (DENTSPLY, USA) as both sealers exhibited similar bonding strength to the root canal (Gyulbenkiyan et al., 2020). Also, other studies came up with the same conclusion while investigating the premixed TotallFill, relating their results to the small size particle, followability and expansion of the premixed bioceramic sealer allowing it to penetrate into the dentinal tubules while AH Plus sealer is mild acidic pH which could etch dentin slightly, thereby improving sealer dentin adaptation (Salem et al., 2019). Similarly, Abdelrahman et al rendered the non-significant outcome to the alkaline nature of the calcium silicate-based sealers properties, low film thickness, dimensional stability and denaturation of the dentin collagen which could collectively facilitate the sealer penetration and adaptation to root canal dentin (Abdelrahman & Hassan, 2021). Moreover, another study found no significant difference between iRoot SP pre-mixed sealer (Henry Schein, Hong Kong) and AH Plus as iRoot SP generates interlocking with dentinal tubules lead to strength and more resistance to microleakage while AH Plus had high flowability (Wang et al.,2018).

On the other hand, Asawaworarit et al reported better significant apical sealing of premixed Endosequence sealer than AH Plus linking their results to the shrinkage that occurs in epoxy resin during setting which might crumbled the adaptation of the sealer to the dentine wall, however they investigated the results using apical fluid filtration not coronal dye penetration methodology as in the current study (Asawaworarit et al., 2020)

Gutta-Flow Bioseal exhibited significant inferior dye penetration results compared to One-Fil, this comes in harmony with a previous study which compared premixed Endosequence BC with Gutta-Flow Bioseal (Singla & Panghal, 2021) this could be explained by the heterogeneous composition of the GuttaFlow Bioseal as it is composed of Gutta particle in the nanoscale, polydimethylsiloxane and bioactive glass, although it has the ability to form hydroxyapatite with dentine, its amorphous structure may hinder proper sealing between the silicone part and gutta-percha. On the contrary, Naji et al reported conflicting results with significantly better sealing ability in favor of GuttaFlow Bioseal due to the tag-like integration of GuttaFlow Bioseal into the dentinal tubules. This conflict could be referred to the different method of assessment as they centrifuged the samples after 24h and then measured the optical density of the dyed solution with a spectrophotometer (Naji et al., 2020).

On the other hand, other studies found significantly better results for the Gutta-flow bio seal in comparison to AH Plus, this could be explained by the difference in the method of obturation as they used lateral compaction technique in narrow size canals (the mandibular incisors) and the method of evaluation as they evaluated microbial leakage using the custom-made double-chamber apparatus, while Lee et al used subnanoliter scaled fluid-flow measuring device for measuring microleakage (Lee et al., 2020), and Patil et al used lateral compaction which allow for less volume of sealer in contrast to the matched gutta-percha cone technique which is used in the current study (Patil et al., 2016).

BioRoot RCS manifested the higher dye penetration result, where all samples showed leakage. These results come in agreement with previous studies. (Antunovic et al., 2021, Viapiana et al., 2016, Rashid 2021), this could be related to the method of mixing of BioRoot RCS as it is hand mixed powder and liquid, thus the manual manipulation could include voids in the final mix which could affect the dye penetration and sealing ability of the sealer (Pedullà et al., 2020),moreover BioRoot RCS interacts with the dentine to form a hybrid layer which is rich in mineral , however it was shown that BioRoot RCS When placed in solution, the sealer leaches high levels of calcium ions compared to premixed calcium silicate sealers (Reszka et al., 2016). Also, the contact with wet environment (14 days in the present study ) may have prolonged the setting time (Duarte et al., 2018).This result is in contrast with other studies that found no significant difference between premixed and hand mixed calcium silicate sealers; this could be linked to the difference in the method of sealer application as one of the studies (Dsouza et al.,2020) employed lentulo spiral to apply both types of sealer which could have improved the sealing ability of hand mixed calcium silicate sealers, or the difference in the method of evaluation as in the study by Haji et al where teeth were sectioned then viewed under scanning electron microscope (Haji et al., 2022). Also, contradictory results in favor to BioRoot RCS compared to Epoxy resin sealer were reported by Eid et al different timing for teeth storage and dying could yield this contradiction as they stored the sample in humid atmosphere for 72 hours and assessed the dye penetration after 24 hours while in present study the samples were stored in humid atmosphere 14 days and assessed after 48 hours (Eid et al., 2021).

Although the simplicity and the non-destructive nature of dye penetration test, it possess limitations as it may not reflect the clinical situation of bacterial leakage even if the particle size of the dye is comparable to the common endodontic pathogens, the dye can diffuse through samples supposedly leak-proof to bacteria (De-Deus et al., 2022), therefore further investigation using micro CT is recommended.

## CONCLUSION

Based on the results of the present study we can conclude that although the different types of sealers hindered the coronal dye penetration with different degrees which minimizes the microleakage of oral fluids and bacteria, One-Fil revealed the best resistance against coronal microleakage followed by ADSEAL, then the other sealers. Finally, we emphasize on the importance of Coronal restorative sealing of the orifices, pulp chamber and coronal tissue as a final step that should amplify the protection against coronal leakage for any root canal sealer.

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