Evaluation of the sealing ability of silicon-based root canal sealers; an In-vitro study.

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Highlights
- This study evaluates the apical leakage and the adaptability of two silicone-based root canal sealers.
- Shed the light on the importance of sealing ability of root canal filling material.
- Clearing technique and horizontal technique to evaluate the adaptability and presence of void in root canal filling.
- The tested silicon-based root canal sealers could not completely prevent dye penetration.

Aims: To evaluate the apical leakage and the adaptability of two silicone-based root canal sealers.

Methodology: 60 extracted human single-rooted teeth were selected for this study. Teeth were grouped into four groups. Access cavity and canal preparation were performed in all selected teeth by crown down technique. Two polyvinyl siloxane-based root canal filling materials were used in this study [GuttaFlow [GF] and Roeko-Sell Automix (RSA)]. The teeth were randomly divided into 4 experimental groups (15 each). Group I: canals were obturated with GF without any gutta-percha [GP] point. Group II: canals were obturated with GF using the single cone technique [SCT]. Group III: canals were obturated with RSA without any GP point. Group IV: canals were obturated with RSA using SCT. The sealing ability was assessed by clearing technique [CT] and horizontal sectioning technique [HST].

Results: With regards to sealing ability, there was a statistically significant difference (P=0.001) between all groups. Pairwise comparison using Tukey HSD test revealed a statistically significant difference between group III (4290.72±1368.21) and the other three groups. Group II showed the least microleakage (2309.18±976.82). With regards to sealer adaptation, there was a statistically significant (P<0.05) difference between all groups at 4 apical, middle, and coronal thirds of each root.

Conclusion: It was concluded that; none of the sealers used completely prevent dye penetration, significant leakage differences were observed among sealers tested and apical microleakage results showed a moderately strong correlation with a mean percentage of uncoated root canal walls.

1. Introduction

Three-dimensional fluid-tight obturation of root canals is considered as one of the main objectives of root canal treatment, which aims to seal all already cleaned and shaped root canal spaces (Schil-der, 1967), thus preventing or minimizing microleakage after obturation (Seltzer & Bender, 1965) and preventing the ingestion of bacterial and their by-products into the periapical area (Kakehashi et al., 1985). Gutta-percha is the most widely used core filling material (Ø rstavik, 2005) and is used with a paste-like root canal sealer which aids in filling lateral canals, canals irregularities, and inaccessible areas. Furthermore, root canal sealers enhance the sealing ability during compaction (Wu et al. 2000). Many studies have reported that root canals that have been obturated with gutta Pech and a sealer exhibited less leakage (Ole’an et al., 1993; Sen et al., 1996; Tagger et al., 2003). Microleakage along root canal fillings is one of the main causes of failure of root canal treatment, which can occur either between the dentinal wall and sealer, core material and sealer or through the sealer itself. Hence, sealing ability, which is considered as a main essential property of any root canal filling material needs to be investigated (Al-Ghami & Wennberg, 1994). Various techniques have been used to evaluate the sealing ability of root canal filling material via microleakage studies. These techniques include dye penetration; spectrometry of radioisotopes; fluorometric and electrochemical methods; bacterial penetration; and finally, the fluid-transport model (Ainley, 1970; Delivanis & Chapman, 1982; Czontowsky et al., 1985; Wu & Wesselin, 1994). The sealing ability of several root canal fillings and techniques had been investigated by many authors who focused on the evaluation of the coronal leakage (Wu et al., 1993; Roghanizad & Jones, 1996; Chaiterwanitkul et al., 1996; Malone & Donnelly, 1997; Oliver & Abbott 1998).

Owing to the irritation caused by the zinc-oxide and eugenol-based sealers which are widely used by many practitioners, the development of other non-eugenol sealers was necessary. Sealing ability and biocompatibility are considered as the main properties of an ideal root canal sealer (Ø rstavik, 2005). RoekoSeal and Gutta Flow are two polydimethylsiloxane-based root canal filling materials. RoekoSeal comprises polydimethylsiloxane, paraffin-based oil,
specimens were instrumented (Nguyen, 1994) in the first four weeks, which subsequently stays stable, this resulted in the enhancement of its sealing ability (Brestavik et al., 2001). Moreover, it is less cytotoxic in comparison with resin-based root canal sealers (Miletic et al., 2005). GuttaFlow has been introduced as the first non-heated, flowable gutta-percha-based sealer that does not shrink like heated gutta-percha. Its composition resembles RoekoSeal but finely ground gutta-percha particles of a size of <30 µm2 and nanosilver particles were added. The latter to prevent the spread of bacteria. GuttaFlow has very auspicious properties such as insolubility, bio-compatibility, post-setting expansion, great fluidity, and providing a thin film of sealer. The moderate expansion of the GuttaFlow sealer may be beneficial (Blayouit et al., 2005; Monticelli, 2007).

The solubility of a material is defined as its mass loss during a period of immersion in water. The solubility of a root canal sealer should not exceed 3% by mass after 24 hours (ISO specification 6876:2012). The low solubility of root canal filling materials is considered one of the main requirements of root canal fillings. Degradation of root canal sealers and leaching of chemicals over time will result in voids created inside the filling material or interfaces with root canal walls (Qrstavik et al., 2005). While, voids could act as a passage for microorganisms from the coronal to an apical area along the wall of the canal (Nguyen, 1994); the leaching of chemicals may irritate the periapical tissues (Geurtsen & Leyhausen, 1997). Silicone-based sealers have a comparatively low solubility (Schwarze et al., 2002; Özkan et al., 2003; Miletic et al., 2005).

The aims of this study were to evaluate the apical leakage of two silicone-based root canal sealers using a clearing technique and to investigate the adaptability of filling materials and the presence of voids utilizing a horizontal sectioning technique carried out at three levels (coronal, middle, and apical).

2. Materials and Methods:

Sixty extracted human single-rooted teeth were selected for this study.

2.1 Inclusion criteria:

1. Straight root canal.
2. Teeth should be extracted in account of periodontal reasons.

2.2 Exclusion criteria: Any teeth with

1. Resorptive defect
2. Caries
3. Crack
4. Open apices
5. Previous endodontic treatment.

Soft tissue fragments and calcified debris were removed by root planing and scaling. The teeth were checked radiographically to confirm a single canal. The teeth were immersed in 5.25% sodium hypochlorite (NaOCl) for one day to remove the soft tissue from the root canal surfaces. After cleaning, teeth were rinsed and stored in physiological saline solution at 37°C until ready for use.

Access cavities were prepared, and root canals were instrumented with K3 NiTi rotary system (Kerr Corporation, 1717 West Collins, Orange, CA 92867). Instrumentation was completed in a crown-down manner according to the manufacturer’s instructions using a gentle in-and-out motion. The filing was performed with size 40 instruments then size 35, 30, 25, and 20 down to size 15. The step-down between size 20 and size 25 was repeated until size 25 reaches the working length. All specimens were instrumented 1 mm short of the apex according to the measured working length. All root canals were irrigated with 2.5% sodium hypochlorite solutions. Canals were dried with paper points, and the patency of the apical foramen was established during and after the preparation of the root canal bypassing the tip of size 10 K-Flexo file through the foramen. The teeth were stored in a physiological saline solution after instrumentation.

Two polyvinyl siloxane-based root canal filling materials were used in this study (GuttaFlow and Roeko-Seal Automic, both manufactured by Roeko/Colyente/Whaledent, Langenau, Germany). GuttaFlow is a filling system for root canals that combines two products in one: gutta-percha in powder form with a particle size of less than 30µm and the sealer. Roeko-Seal Automic (RSA) has an extremely low film thickness of only 5µm allows the sealer to flow into tiny crevices and dentine tubules.

The teeth were randomly divided into 4 experimental groups (15 each):

- **Group I**: canals were obturated with GuttaFlow without any gutta-percha point.
- **Group II**: canals were obturated with GuttaFlow using the single cone technique (SCT).
- **Group III**: canals were obturated with RSA without any gutta-percha point.
- **Group IV**: canals were obturated with RSA using the single cone technique (SCT).

Gutta Flow and RSA were mixed according to the manufacturer’s directions, placed on a clean slab, and then placed into the canal with a rotary paste filler to full working length. The master cone was coated with the sealer and placed in the canal to the full working length. After root filling, all teeth were radiographed to assure the radiographic quality of obturation. Gutta-percha was removed from the coronal part with a heated instrument, and the access cavities were sealed with Cavit (3M ESPE, Seefeld, Germany). Samples were kept at 37°C for 1 week in 100% humidity to ensure the complete setting of the sealer.

The sealing ability was assessed by two techniques:

1. **Clearing technique** (Robertson et al., 1980) [for apical microleakage]

   Forty teeth were air-dried and prepared for clearing, 10 specimens for each group. The teeth were dried with air-jet and coated with the first layer of nail polish, covering the whole tooth, including the coronal restoration of the access cavity, but leaving a 2-mm area around the apical foramen uncoated. One hour later, a second coat was applied with a polish of a slightly different color than the first, followed by a coating with sticky wax. This coating was necessary to prevent dye penetration into the root canal by other routes than the apical foramen. After one hour of drying, all specimens were immersed in black India ink (Pelikan 4001, Hannover, Germany) for 3 days.

   The roots were dipped in a jar until the surface tension of the dye got broken. No attempt was made to use a vacuum system because teeth were sealed coronally with Cavit, afterward the teeth were rinsed under tap water to remove ink on the external surface. The teeth were cleaned of polish and wax with a scalpel blade. They were inspected under magnification to ensure the complete removal of the acrylic nail polish.

   Teeth were rendered transparent according to the method described by Robertson et al. (1980). The teeth were bathed in 5% nitric acid for a total of 3 days at which time they were determined to be fully decalcified. Teeth were rinsed in running water and placed in numbered containers with distilled water and allowed to soak for 8hrs. The teeth were agitated hourly and the distilled water was changed every 2 hrs.

   Dehydration of the teeth was completed by placing them in ascending concentrations of ethyl alcohol. The teeth were placed in a washed glass of 80% ethyl alcohol and allowed to sit for 12 hrs. The 80% ethyl alcohol was drained and replaced with 90% ethyl alcohol for 2 hrs. After 2 hrs, the 90% solution was similarly drained and replaced with 100% ethyl alcohol and allowed to remain for

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two more hours. The teeth were air-dried and placed in 100% methyl salicylate to complete the clearing process. After 2 hrs the teeth became transparent. The clearing process was completed after 2 weeks and the roots were maintained in the methyl salicylate solution for storage and final evaluation using zoom stereomicroscope Olympus SZ 11 (Olympus Optical Co., LTD, Tokyo, Japan) at a magnification of x18. Apical microleakage was measured by two evaluators unaware of the experimental groupings via the use of a computer-imaging program (Soft Imaging System Gmbh, Analysis 3.1, Munster, Germany). Apical leakage was measured as the distance from the anatomical apex to the deepest extent of ink penetration in the coronal direction. The experimental teeth were viewed in the same plane to ensure dimensional accuracy. The greatest measurement of leakage was recorded for each tooth and submitted for statistical analysis. For each specimen, an average of three readings was taken.

2. Horizontal-sectional technique (Mannocci et al., 1998): [for sealer adaptation]

Twenty teeth were tested by this technique, with 5 specimens for each group. Horizontal sections were made approximately into discs 1.5 mm thick using a low-speed diamond saw. In every root, three sections were made in the apical, middle, and coronal thirds of each root.

2. Horizontal-sectional technique (Mannocci et al., 1998): [for sealer adaptation]

In this study, measurements of maximum linear dye penetration were made to quantify the relative leakage (Fig. 3). Comparison of dye penetration between the 4 groups (Table 1) was analysed by using the ANOVA test. There were statistically significant differences between all groups (P=0.001). Pairwise comparison using Tukey HSD test revealed a statistically significant difference between group III (4290.72±1368.21) and the other three groups (Fig. 4). Group II showed the least microleakage (2309.18±976.82).

\[
\text{% of uncoated canal} = \frac{\sum \alpha \times 100\%}{360^\circ}
\]

Fig. 2. Mathematical transformation of the obtained values

Values of apical microleakage angles uncoated and percent of uncoated canals were tested for normality using the Kolmogorov Smirnov test. Mean values and standard deviations for the four groups were calculated and compared using analysis of variance. A pairwise comparison was performed using the Tukey HSD test. The significance level was set at P≤0.05. Results were graphically represented using bar charts. The Statistical analysis was performed by using SPSS version 20. Pearson correlation coefficient was used to test the correlation between apical microleakage and sealing adaptability.

3. Results

In this study, measurements of maximum linear dye penetration were made to quantify the relative leakage (Fig. 3). Comparison of dye penetration between the 4 groups (Table 1) was analysed by using the ANOVA test. There were statistically significant differences between all groups (P=0.001). Pairwise comparison using Tukey HSD test revealed a statistically significant difference between group III (4290.72±1368.21) and the other three groups (Fig. 4). Group II showed the least microleakage (2309.18±976.82).

![Fig. 1. Horizontal sections at apical, middle, and coronal parts of the root.](image1)

![Fig. 3. Dye penetration of cleared teeth for different groups tested; GF (A), GF and GP (B), RSA (C), RSA, and GP (D).](image2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Apical leakage in microns (mean ± SD)</th>
<th>F test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: GuttaFlow</td>
<td>2772.85±708.31</td>
<td>6.56 (0.001)*</td>
</tr>
<tr>
<td>Group II: GuttaFlow+SCT</td>
<td>2309.18±976.82</td>
<td></td>
</tr>
<tr>
<td>Group III: RSA</td>
<td>4290.72±1368.21</td>
<td></td>
</tr>
<tr>
<td>Group IV: RSA+SCT</td>
<td>2906.77±1053.44</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant at P≤0.05
Pairwise comparison using Tukey HSD test for sealer adaptation between the 4 groups at the apical, middle, and coronal thirds of each root revealed statistically significant difference at $P<0.05$. The comparison of sealer adaptation between the 4 groups in the apical area revealed a significant difference between all pairs except groups I and IV (Fig. 5). Comparison of sealer adaptation in the mid root area revealed a significant difference between group I on one side and groups III and IV on the other side and between group II on one side and groups III and IV on the other side (Fig. 6).
Comparison of sealer adaptation in the coronal area revealed a significant difference between group III from one side and all other groups and between group I on one hand and groups II and III (Fig. 7). Representative photographs from different teeth (Figs. 8-11) showing the 4 groups which were cut at different levels (apical, middle, and coronal).

Fig. 6. The percentage of the uncoated canal in the middle area.

Fig. 7. The percentage of the uncoated canal at the coronal area.

Fig. 8. A cross-section of filled root with GuttaFlow cut at different levels. (A) apical third (M) middle third (C) coronal third (original magnification ×110).
Total uncoated angles and their mean percentage of the uncoated canal were compared in the 4 groups using the ANOVA test. A statistically significant difference was found between the 4 groups (Table 2). Pairwise comparison using Tukey HSD test revealed a statistically significant difference between all groups at P<0.05 (Fig. 12). The greatest percentage of uncoated angles was found in group III (mean % =53.41±3.11), while the least percentage of uncoated angles was found in group II (mean % =32.68±3.22).

Apical microleakage showed a moderately strong correlation (Fig. 13) with a mean percentage of uncoated angles of canal circumference (r=0.566, P<0.0001).

Table 2
Comparison of mean sealer adaptation between the 4 groups in different areas

<table>
<thead>
<tr>
<th>Group</th>
<th>Total uncoated canal circumference Mean±SD (% of the uncoated canal)</th>
<th>F test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (GuttaFlow)</td>
<td>144.76±9.78 (40.21±2.72)</td>
<td></td>
</tr>
<tr>
<td>II (GuttaFlow + SCT)</td>
<td>117.65±11.59 (32.68±3.22)</td>
<td></td>
</tr>
<tr>
<td>III (RSA)</td>
<td>192.28±11.19 (53.41±3.11)</td>
<td></td>
</tr>
<tr>
<td>IV (RSA + SCT)</td>
<td>161.15±14.25 (44.76±3.96)</td>
<td></td>
</tr>
</tbody>
</table>

*statistically significant at p<0.05
4. Discussion

Three-dimensional obturation of root canals and achieving fluid-tight seal at the canal apical constriction, with biocompatible core materials, are the main ambitions of root canal treatment. Approximately 60% of all endodontic failures can be attributed to incomplete or defective sealing of the root canal (Priyanka et al., 2013). It is, therefore, no surprise that studies of the efficacy of sealing cement constitute a major part of endodontic research. Generally, in dentistry, Silicone-based root canal sealers have been utilized for a long time because of their properties. Those properties include low surface tension, which provides an excellent flow rate that allows the materials to fill the inaccessible areas in the canals. Moreover, it expands in a range of 0.6-0.2%, which provides excellent adherence to the walls of the root canals owing to the chemical bond which has been formed between the materials and canal walls (De-Deus et al., 2007). Different methods were utilized to objectively analyse the adaptation of sealers such as radioisotope spectrometry (Czanskiowski et al., 1985), electrochemical methods (Jacobson SM et al., 1976), or the penetration of a radio-labeled isotope (Haikel et al., 1999). Micro-computed tomography [MicroCT] (Hammad et al., 2009; Yan et al., 2018). Micro-CT enables qualitative analyses of images and distinguishes between filling materials, voids, and tooth structures, based on the imaging grayscale (Celikten et al., 2015). Although Micro-CT is a very effective method we could not use it in this study owing to its unavailability.

In view of their simplicity and availability, apical leakage techniques are the most widely employed tests. Assessment of linear
The clearing technique is a common method to explore apical leakage of root fillings after splitting the roots (Alhilali, 1995; Abou Saoud, 2019). The advantage of the clearing technique is that it allows the visualization of dye penetration into the root canal system, enabling the measurement of leakage depth. However, the viscosity of clearing agents, such as chromic oxide or calcium hydroxide, can vary, affecting the measurement of leakage depth. Careful consideration of the viscosity and the selection of the most appropriate clearing technique are essential for accurate measurement of leakage.

In this study, the clearing technique was utilized to examine the leakage of root fillings. The leakage was measured using India ink, which stains dentin to a lesser degree than other dyes, such as methylene blue, and allows clear visualization of the coronal limit of filtration (Woo et al., 1990). Although it has been suggested that methylene blue has a comparable leakage to butyric acid, which is a metabolic product of microorganisms (Kersten et al., 2009), and that methylene blue penetrates more deeply along root-canal filling than India ink (Alhilali et al., 1995). In this study, India ink was used because of the advantages of clearing techniques; the cross-sectional technique allows for a clear observation of dye penetration and the vacuum can generate artifacts, which affect the results (Dickson & Peters, 1993). Hence, in this study, the use of vacuum techniques was avoided.

With regards to dye, the leakage marker used in this study was India ink owing to the fact that it stains dentin to a lesser degree than other dyes, such as methylene blue, and allows clear visualization of the coronal limit of filtration (Woo et al., 1990). Although it has been suggested that methylene blue has a comparable leakage to butyric acid, which is a metabolic product of microorganisms (Kersten et al., 2009), and that methylene blue penetrates more deeply along root-canal filling than India ink (Alhilali et al., 1995). In this study, India ink was used because of the advantages of clearing techniques; the cross-sectional technique allows for a clear observation of dye penetration and the vacuum can generate artifacts, which affect the results (Dickson & Peters, 1993). Hence, in this study, the use of vacuum techniques was avoided.

Changes in the leakage along root fillings containing both gutta-percha and a sealer depend on a variety of factors; the expansion of gutta-percha resulted in diminishing the leakage after filling. Moreover, the dissolution of the sealer may result in an increase in leakage along root fillings containing both gutta-percha and a sealer (Eldeniz & Ørstavik, 2009). Scott and colleagues (1992) who reported that the penetration endpoint of the methylene blue was unclear and suggested that India ink is a better alternative because of its colouring. Conversely, Bhambhami and Sprechman (1994) reported the average penetration of methylene blue was similar to the average penetration of India ink. In the current study, the used India ink particles were less or equal in size to 3 μm and could penetrate a 0.22 μm-bacteria filter. Therefore, bacterial invasion of an apical seal seems to be unlikely if this dye did not penetrate into the root canal via a gap between the root-canal filling and the canal wall (Schafer & Olothoff, 2002).

In this study, India ink was used because of the advantages of clearing techniques; the cross-sectional technique allows for a clear observation of dye penetration and the vacuum can generate artifacts, which affect the results (Dickson & Peters, 1993). Hence, in this study, the use of vacuum techniques was avoided.

The Indian ink is the preferred dye for the clearing method since it allows a three-dimensional evaluation of the root canal walls, providing a clear observation of the root canal system. However, the use of India ink requires careful consideration of the viscosity of clearing agents, such as chromic oxide or calcium hydroxide, which can vary, affecting the measurement of leakage depth. Careful consideration of the viscosity and the selection of the most appropriate clearing technique are essential for accurate measurement of leakage.

This study, it was decided to utilize the clearing technique as it seems to be more precise in detecting leakage than the cross-sectional technique. Rhode and colleagues (1996) highlighted the advantages of clearing techniques; the cross-sectional technique produces the least part of the coronal tissues and dye corresponding to a thickness equivalent to that of the saw blade, as a result of sectioning itself. In addition, clearing assesses the leakage magnitudes in a tenth of a millimeter, compared with the cross-sectional technique which determines whether the leakage is present or not in each section.

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gaps (uncoated canal walls) might be prone to subsequent leakage. A better adaptation to the canal wall reduced the width of the gaps between gutta-percha and the canal wall hence reduced the leakage. The latter was in agreement with another study (Hall et al., 1996) in which the horizontal methods were utilized to examine the different cut sections. In this way, not only the coated and uncoated canal perimeter be measured, but also sealer distribution in the canal space can also be observed.

5. Conclusions
It was concluded that: (i) none of the sealers used completely prevent dye penetration (ii) significant leakage differences were observed among sealers tested (iii) apical microleakage results showed a moderately strong correlation with a mean percentage of uncoated root canal walls.

References