



Comparative Evaluation of the Sealing Ability of Three Root Canal Sealers Using Fluid Filtration: An *In Vitro* Study

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Abstract

Aim: The aim of the study was to analyse the sealing ability of three root canal sealers: AH Plus, MTA Fillapex and Resilon/RealSeal SE.

Methodology: Eighty freshly extracted human permanent mandibular first premolars were decoronated using a diamond disc at a level of 14 mm from the root tip in order to maintain uniformity of specimen length. Cleaning and shaping was done using Hyflex CM rotary instruments. The teeth were randomly divided according to the obturating material into three experimental groups.

The coronal part of each root specimen was attached to an apparatus initially proposed by Xu., et al. to assess endodontic micro-leakage.

All specimens were then incubated at 37°C during the observation period. Samples were then analyzed with a glucose kit using colorimetry, and were expressed in mMol/L.

Conclusion: None of the sealers were efficient in preventing microleakage within the root canals. Under the experimental conditions, AH Plus had the least amount of endodontic leakage followed by RealSeal SE and MTA Fillapex.

Keywords: AH Plus; Resilon; RealSeal SE; MTA Fillapex; Hyflex CM

Introduction

In order for one to achieve a higher rate of endodontic success, Complete obturation of the root canal system with an impervious, biocompatible and dimensionally stable filling material is of prime importance [1,2]. Among the various techniques employed in the past to obturate root canals, the most reliable is the use of gutta percha with sealer [3].

A plethora of root canal sealers are available in today's market, a few of which comprise zinc oxide eugenol, calcium hydroxide, glass ionomer and epoxy resin products [4]. Root canal sealers leak to some extent, but its use is found to significantly reduce apical leakage [5].

AH Plus is an epoxy based endodontic sealer that is used with gutta percha in vertical or lateral compaction techniques. It possesses high radiopacity, low solubility, little shrinkage, and good tissue compatibility.

The RealSeal SE system consists of a self-etching methacrylate sealer and Resilon core material. It is claimed to reduce the application steps of the ordinary epiphany system, thus becoming a more user friendly material, and bonds to both the Resilon core and radicular dentin through hybrid layers on both substrates, leading to a monoblock unit, which may prevent leakage and improve root strength [6].

MTA possesses certain advantages like biocompatibility, low cytotoxicity, antimicrobial properties [7,8], low microleakage

[9,10] and its ability to set in the presence of blood or moisture [11]. Hence, MTA Fillapex was proposed as an endodontic filling material [12,13]. It has excellent radiopacity, easy handling, good working time, low solubility and provides sealing through expansion on setting [12].

Establishing an accurate method of studying leakage to evaluate the quality of root canal treatment has been an important component of endodontic research. A wide variety of test methods have been used to assess the seal of endodontic materials including dye penetration, fluid filtration, radioisotopes, electrochemical circuits, bacterial penetration etc. Due to inadequacies associated with these type of testing methods as a result of nonexistence of a universally accepted model, glucose penetration studies might be meaningful and clinically relevant [8].

Aim of the Study

The aim of the present study was to analyze the sealing ability of three root canal sealers: AH Plus, Resilon/RealSeal SE and MTA Fillapex, based on the filtration rate of glucose.

Materials and Methods

The present study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Department of Biochemistry at Seema Dental College, Rishikesh, Uttarakhand.

Eighty human permanent mandibular first premolars extracted for periodontal reasons, collected from the Department of Oral and Maxillofacial Surgery, Seema Dental College were selected for this study.

Sample selection

All samples were checked for the existence of fully formed apex, single apical foramen, no signs of internal resorption, no pulp stones, root canal calcification, obstruction or previous endodontic therapy. The roots with curvature $< 5^\circ$, completely formed apex with patent foramina were selected. Teeth which deviated from such findings were excluded from the study.

Sample preparation

Eighty freshly extracted human permanent mandibular first premolars were cleaned of adherent tissue tags and hard deposits using ultrasonic scalers. The teeth were stored in 0.9% sodium chloride containing 0.2% sodium azide solution for preventing bacterial growth in an incubator at 37°C until used.

All teeth were decoronated with a diamond disk at the level of 14 ± 1 mm from the apex to obtain a relatively standard tooth length (Figure 1). The pulpal remnants were extirpated using a broach. Apical gauging was carried out using size 10, 15 and 20 K-file. The working length was established 1 mm shorter than the length at which a size 10 K-file was visualized at the apical foramen.

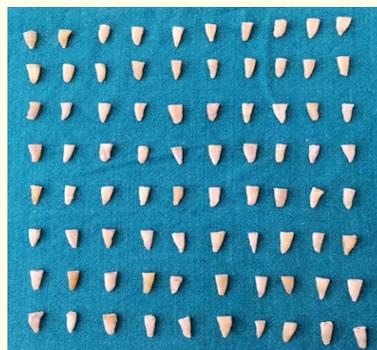


Figure 1: Teeth decoronated with a diamond disk at the level of 14 ± 1 mm from the apex.

Cleaning and shaping was done with Hyflex CM rotary instruments upto size 40/0.04 along with EDTA gel as lubricant. The canals were constantly irrigated with 5 ml of 3% NaOCl solution. After instrumentation, the prepared canals were rinsed with 10 ml of 17% EDTA solution followed by a final flush with 10 ml of sterile saline to remove the smear layer and any traces of NaOCl.

40/0.04 gutta-percha and Resilon cones were placed to the working length and verified radiographically. The canals were dried using absorbent points and the teeth were randomly divided according to the obturating material (Figure 2) into three experimental groups of 20 teeth each and two control groups of 10 teeth each. Teeth in the positive control group were obturated with a 40/0.04 single-cone of gutta percha and without sealer placement to test the maximum fluid flow through the canals. Teeth in the negative control group were obturated with a 40/0.04 single-cone of gutta percha and with AH Plus sealer, but the teeth were dipped in molten sticky wax and further covered with nail varnish to ensure that no fluid flowed through the canals.

Group 1: Gutta percha and AH plus sealer

AH Plus sealer was mixed according to the manufacturer's instructions. A single gutta percha cone of size 40/0.04 was coated with sealer and was placed slowly into the root canal in an up and down motion until reaching the full working length. Gutta percha

was cut at the orifice level with a flame heated ball burnisher and was vertically compacted.



Figure 2: Obturating materials used.

Group 2: Gutta percha and MTA fillapex sealer

MTA fillapex sealer was mixed according to the manufacturer’s instructions and obturation was done similarly as in group 1.

Group 3: RealSeal point and RealSeal SE sealer

RealSeal SE sealer was mixed according to the manufacturer’s instructions. A single RealSeal point of size 40/0.04 was coated with sealer and was placed slowly into the root canal in up and down motion until reaching the full working length. RealSeal point was cut at the orifice level with a flame heated ball burnisher and the coronal surface was light cured for 40 seconds.

Glucose penetration model

The coronal part of each root specimen was embedded in cold cure acrylic resin to form a 4 mm thick cylinder around the root. To enable a leak free contact between the root specimen and the glass pipette, sticky wax was used at this connection.

A hole was created in the screw cap of the glass bottle through which the glass pipette was connected. The assembly was then placed in a sterile 10 ml glass bottle and the cap was screwed and sealed with sticky wax.

A uniform hole was drilled in the screw cap with a #10 diamond bur to ensure an open system at all times. 2 ml of 0.2% sodium azide solution was added into the glass bottle, such that the apex of all the root samples was immersed in the solution. Sodium azide was used to inhibit the growth of microorganisms that might influence the glucose readings.

The tracer used in the study was 1 mol/L glucose solution (pH = 7.0). Glucose has a low molecular weight and is hydrophilic and chemically stable. About 4.5 ml of glucose solution, containing 0.2% sodium azide was injected into the pipette until the top of the solution was approximately 14 cm higher than the top of gutta percha in the canal which created a hydrostatic pressure of 1.5 kPa

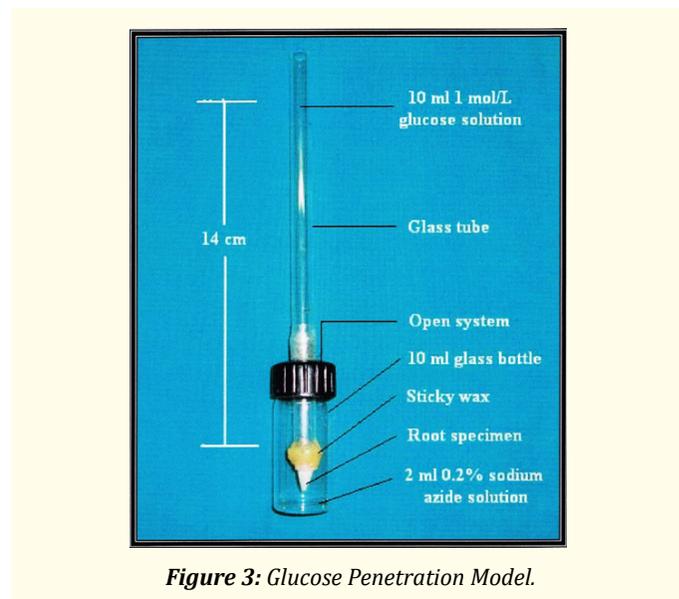


Figure 3: Glucose Penetration Model.

All specimens were then kept in an incubator at 37°C for the duration of the observation period. A 20 µL increment of solution was drawn from the glass bottle using a micropipette at 1, 7, 14, 21 and 28 days. The same amount of fresh 0.2% sodium azide solution was added to the glass bottle reservoir to maintain a constant volume of 2 ml.

The sample was then analyzed with a glucose kit (GOD-POD method) using colorimetry, and was expressed in mMol/L.

Results

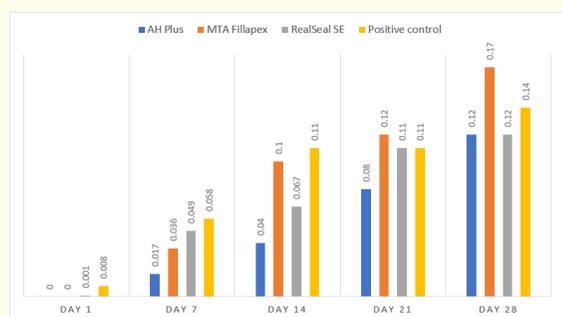
The analysis was done using SPSS software (IBM, v. 20.0). The following tests were employed: QQ Plots, Shapiro Wilk test, Skewness and kurtosis evaluation. Mean leakage comparisons were performed using Repeated Measures ANOVA. Post Hoc analysis was done to test significant differences between specific groups. Significance level was fixed at p < 0.05 (Graphs 1 and 2, Table 1).

Discussion

Three dimensional sealing of the root canal with an impervious, biocompatible and dimensionally stable filling material is one of the



Graph 1: Line diagram depicting mean leakage across the study period for all the materials.



Graph 2: Graph showing mean comparison between materials at different time intervals.

Day	Material	Mean value (\pm SD)	P value
1	AH Plus	0 ^a	0.01*
	MTA Fillapex	0 ^b	
	RealSeal SE	0.0013 (0.002)	
	Positive control	0.008 (0.01) ^{a,b}	
7	AH Plus	0.017 (0.011) ^a	0.004*
	MTA Fillapex	0.036 (0.01)	
	RealSeal SE	0.049 (0.04) ^a	
	Positive control	0.058 (0.04)	
14	AH Plus	0.040 (0.02)	0.001*
	MTA Fillapex	0.10 (0.02)	
	RealSeal SE	0.041 (0.009)	
	Positive control	0.029 (0.009)	
21	AH Plus	0.084 (0.02) ^{a,b}	0.001*
	MTA Fillapex	0.127 (0.01) ^{a,b}	
	RealSeal SE	0.113 (0.03) ^{a,c}	
	Positive control	0.147 (0.02) ^{a,c}	
28	AH Plus	0.121 (0.01) ^a	0.001*
	MTA Fillapex	0.174 (0.02) ^{a,b}	
	RealSeal SE	0.128 (0.03) ^{b,c}	
	Positive control	0.175 (0.01) ^{a,c}	

Table 1: Mean leakage (mMol/L) comparison between groups at various time intervals.

main goals of endodontic treatment. It is essential for preventing reinfection of the canal and for preserving the health of periapical tissues, thereby ensuring success of root canal treatment. However, it has been reported that a complete seal of the root canal system is almost impossible with currently available materials and obturation techniques [13].

The use of gutta percha in conjunction with a sealer is currently one of the most commonly used methods for obturation. Ideally, the root canal sealer should be capable of producing a bond between the core material and the root dentine, effectively preventing leakage. Thus, adequate sealing is considered to be a major prerequisite to improve the outcome of the root canal treatment. Several types of endodontic sealers have been recommended to achieve this goal and consequently, the evaluation of their sealing ability is important [13].

Therefore, in the present study, the sealing ability of newer resin-based and MTA based root canal obturation systems were evaluated under conditions which were standardized as much as possible.

The first resin sealer to be introduced was the epoxy resin-based AH 26 sealer, which showed good sealing ability [14]. Toxicity due to the release of formaldehyde in AH 26 was a major problem. This led to the development of AH Plus sealer, also an epoxy resin-based sealer. In this study, AH Plus sealer was chosen because of its low solubility. Schafer and Zandbiglari (2003) [15] compared the solubility of resin, silicone, calcium hydroxide, zinc oxide-eugenol and glass ionomer-based sealers in water and artificial saliva and reported that AH Plus lost the least amount of weight of all sealers tested in all liquids.

Despite the improved properties of AH Plus, its adaptation to the root canal walls is poor. Findings like this led to the development of sealers which had the ability to bond to dentin. Epiphany (Pentron), EndoREZ (Ultradent), Real Seal (Kerr) and Next (Heraeus Kulzer) were some of the methacrylate-based sealers that were introduced [16].

The reason why we chose an adhesive endodontic sealer with a bondable polymeric root canal-filling material (Resilon/ RealSeal SE) is that they bond throughout the length of the root canal. By creating micromechanical retention via the formation of a thin hybrid layer to the self-etching primer-treated root dentine, and chemical coupling of the UDMA containing Resilon root-filling material to the methacrylate-based sealer, a continuum

is anticipated by the manufacturer that results in creation of a monoblock between the root filling and the intraradicular dentin [17].

MTA Fillapex was created in an attempt to combine the physicochemical properties of a root canal sealer with the biological properties of MTA. Its composition after mixing is basically MTA, salicylate resin, natural resin, bismuth oxide and silica [11].

The use of resin based sealers with greater taper master cones has led to the rejuvenation of the single-cone obturation technique. The single-cone technique consists of a single gutta-percha cone filled at room temperature with sealer layer thicknesses that vary, depending upon the adaptation of the single cone to the walls of the canal. Zmener and colleagues (2005) [16] prepared root canals using a rotary system and obturated them with single-cone and lateral condensation techniques. They reported that with the use of a methacrylate-based sealer, the difference between the single-cone and lateral condensation obturation was not significant but the tapered single-cone technique was faster than lateral condensation. Also, Inan (2009) [18] reported that the results of matched taper, single-cone obturation were comparable with those of lateral condensation and Thermafil techniques.

In the present study, freshly extracted mandibular premolars with a single patent canal were selected because of their ease of availability and to minimize anatomical variation. The teeth were decoronated so as to maintain a uniform length of 14 mm. However, although the teeth used in this study had single straight canals, posterior teeth have narrow and curved canals with complex anatomy, which might present greater challenges. Further study is needed to evaluate the sealing ability of obturation with matched-taper gutta-percha cones in complex canals to determine whether these obturations will have an acceptable apical seal.

The same cleaning and shaping procedure was followed for all the samples so as to obtain a uniform root canal preparation. HyFlex CM instruments are new NiTi rotary instruments with shape memory produced by an innovative methodology that uses a complex heating and cooling treatment which controls the material's memory. These instruments were made from a specific nickel-titanium alloy that has been claimed to have a lower percent in weight of nickel (52%). The manufacturer claims that they are up to 300% more fatigue-resistant and regain their shape after sterilization. If submitted to excessive resistance or stresses they could be plastically deformed and sterilization in autoclave will result in the instrument regaining its shape.

The removal of smear layer may be considered an essential step in the process of successful root canal treatment. It is well known that root filling materials penetrate better into dentinal tubules in the absence of smear layer [19]. Since our aim was to compare the sealing ability, for this reason, to create best possible seal between the root dentin and the obturation material, 3 ml of 17% EDTA followed by a flush with 5 ml of 3% sodium hypochlorite was used to remove the smear layer prior to obturation.

A final rinse of EDTA solution was given, followed by a rinse with distilled water, to eliminate the effect of residual oxygen liberated from NaOCl on polymerization of resin sealers.

The canal was dried with the help of paper points for only 1 - 2 seconds as it has been suggested by the manufacturers of Resilon/RealSeal SE, that complete dehydration of the canals would hinder the penetration of the hydrophilic resins into the dentinal tubules.

For the purpose of standardization, the sealer in all the three groups was applied with the help of a lentulospiral and obturated with a corresponding single cone with 0.04 taper.

The root surfaces of all the specimens in the negative control group were entirely coated with sticky wax and two coats of nail varnish to prevent possible leakage [20].

A quartz-tungsten halogen light source was used to obtain an immediate coronal seal in the case of RealSeal SE. The quartz-tungsten halogen light source was chosen over plasma arc or light-emitting diode light sources as it has been found by Nagas., et al. (2008) [21] that QTH brings about slower polymerization which allows the material to flow in pre gel stage, providing some stress relief during polymerization shrinkage.

Leakage tests are a relevant way to evaluate the apical seal. Methods used to evaluate leakage include dye penetration, electrochemical testing, radioisotope, bacteria, leakage, fluid filtration and glucose penetration [22].

The glucose penetration model, as a new possibility to evaluate the sealing ability of root canal fillings, was introduced by Xu., et al. (2005) [23]. We chose this method for the evaluation of leakage as it has several advantages. Glucose is hydrophilic and chemically stable. It has a low molecular weight of 180 Da and may be used as an indication for toxins that might penetrate the canal. Glucose as a marker in leakage studies has clinical relevance because it is an important nutrient for microorganisms [24]. Sodium azide was used to inhibit the growth of microorganisms that might influence the glucose readings.

In the glucose penetration model, the tooth is continuously subjected to the pressure of the glucose solution in the coronal chamber, for a period of one month. The fluid filtration model detects leakage usually after subjecting the filling to pressure for 3 hours. This enormous time difference might make the glucose test more sensitive, as it may result in detection of small voids in the filling [24].

Souza, et al. (2008) [25] found that the leakage results recorded in the fluid transport model and glucose penetration model were similar. But, in a study conducted by Shemesh and Wesselink (2006) [26] the glucose penetration model was more sensitive in detecting leakage along the root fillings when compared with the fluid transport model.

The results of our study showed that AH plus had the least amount of endodontic leakage (mean = 0.052) followed by Resilon/ RealSeal SE (mean = 0.072) and MTA Fillapex (mean = 0.087).

At the end of the fourth week, all the test materials showed a significant amount of leakage. The inability of these obturation systems to form a "monoblock" may be because of the unfavorable geometry of the canals for bonding, as well as the potential for gapping along the sealer-dentin interface [27].

It is presumed that gutta percha expands in the presence of humidity and closes micro gaps. Expansion of gutta-percha may have occurred in this experiment because of the moisture present during the setting and testing periods. This may have enhanced the sealing ability of the groups tested.

Warm vertical compaction and the single-cone method with AH Plus showed no statistical difference in ability to seal the canal.

The failure of the sealers may be due to their different chemical compositions and physical properties (adhesiveness, dimensional stability, flow, solubility, etc.) Also, obturating techniques, possible presence of smear layer, accessory canals and irregular canals may be responsible for sealing failure [16].

The present study focuses on creation of a successful monoblock in the root canal, that is, mechanical interlocking of the filling material with the dentin, which produces a better seal, thus minimizing leakage. Within the limitation of this *in vitro* study, it may be concluded that the contemporary single cone root canal filling technique does not ensure a durable apical seal against glucose penetration. Although the glucose penetration method appears to be more clinically relevant compared to other

leakage evaluation methods, it is not possible to directly correlate the amount of leakage to the clinical outcomes of the endodontic treatments. Thus, the efficacy of these contemporary single-cone filling endodontic techniques should be further evaluated in randomized clinical trials.

Conclusion

The conclusions drawn were as follows:

1. None of the sealers were efficient in preventing microleakage within the root canals.
2. Under the experimental conditions, AH Plus had the least amount of endodontic leakage followed by RealSeal SE and MTA Fillapex.
3. The variation in mean leakage was significantly different across the time period among AH Plus, MTA Fillapex and RealSeal SE.

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