In vitro Analysis of Apical Sealing Ability of Five Differentsealers using Dye Penetration Method

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Abstract: ***The current concept of endodontic treatment requires that after complete mechano-chemical debridement, a three-dimensional obturation must (should) be done. This becomes a key factor of successful endodontic therapy. It has been reported that a complete seal of the root canal system is possible only with the currently accepted materials and obturation techniques using a combination of gutta-percha and root canal sealer. Ideally, the root canal sealer should be capable of producing a bond between the core material and the dentine of the root canal. It should also be non-toxic and, preferably, have a positive effect on the healing processes of periapical destruction zone. Endodontic sealers based on zinc oxide and eugenols have been used clinically for several decades because of their satisfactory physicochemical properties.Ideally, contemporary root canal sealer should be penetrate into the dentinal tubules, bind to both organic and inorganic phases of dentin, neutralize microorganisms and their products, predictably induce a mineralized tissues - cementum and bone in regenerativehealing processes.***

Keywords: apical sealing, apical microleakage, epoxy resin sealers, calcium phosphate sealers, MTA sealers.

1. Introduction

The current concept of endodontic treatment is to realize a complete mechano-chemicaldebridement and a three-dimensional sealing after that. This becomes a key factor ofsuccessful endodontic therapy [32]. The long-term seal of root canal system plays an important role in supporting the healing of periapical tissues and prevents intracanal recontamination after root canal treatment [38].

It has been reported that a complete seal of the root canal system is possible only with the currently accepted materials and obturation techniques using a combination of gutta-percha and root canal sealer [12].Ideally, the root canal sealer should be capable of producing a bond between the core material and the dentine of the root canal. It should also be non-toxic and, preferably, have a positive effect on the healing processes of periapical destruction zone

[2]. But in contrast sealers can be a cause of root canal treatment failure due to microleakage at sealer-dentin or sealer-core material interface [26].Endodontic sealers based on zinc oxide and eugenols have been used clinically for several decades because of their satisfactory physicochemical properties [1]. The glass ionomers also were introduced in endodontic treatment as sealers because of their satisfactory adhesion to dental hard tissues [25].Recently introduced resin-based sealers, with its active ingredients - UDMA and Bis-GMA, forming the organic matrix of composite resins. Materials containing resin in their composition, adhere adequately to the dentine, but in apical zone there are some unsatisfactory results [30, 20].

An ideal endodontic sealer have to fulfill all requisites for hermetic obturation. The tight seal at the apex can be enhanced,sealerbond chemically to the dentinal wall of the root canal and mild expansion of the sealer improves its adaptation to the canal walls. It should be antibacterialand resistant to dissolution [14].

At present, sealers based on epoxy resins and calcium phosphate compounds, afford very good physical properties and ensure adequate biological performance to periapical tissues. Excellent apical sealing has been found with epoxy resin-based sealers [25]. Previous studies showed that the epoxy resin-based root canal sealer - AH Plus, is cytocompatible,biocompatible and has good tissue tolerance,long-term dimensional stability and good sealing ability [4,21,22,29].

2. Materials and Methods

Fourtytwo extracted human maxillary incisors with one root-anatomy were selected. The teeth with caries, cracks, open apices or resorptive defects were excluded. The selected teeth were stored in 3% sodium hypochlorite (NaOCl) solution for 24 hours.The working lengths were determined by placing a size 10 K-Flexofile (Dentsply Maillefer, Switzerland) into the root canals until it was visible at the apical foramen and then subtracting 1mm from that length. The apical portion of the canals ware enlarged to a minimum ISO size 30 and maximum ISO size 50 file depending on the initial size of the canal. The canals ware prepared using „crown-down" technique with ProTaper (Dentsply Maillefer, Switzerland). For irrigation was used 2.5% NaOCl as the main irrigant and the canal was recapitulated using size 10 K-Flexofile. The final rinsing of the canal was done with

17% EDTA to remove the smear layer and 0.9% saline solution to prepare the dentin walls for sealing. The canal was dried with paper points.

The specimens were divided into 5 experimental groups of 6 samples each (Groups I to V) and 2 control groups of sixsamples each as positive and negative groups. All the groups were obturated using one-cone technique with different sealer for each group, the sealers were mixed according to the manufacturer‟s instruction. AH Plus (Dentsply, Germany) was used as sealer for Group I, Apexit Plus (Ivoclar Vivadent, Liechtenstien) was used as sealer for Group II, Z.O.B.Seal(METABiomedCo, Korea) was used as sealer for Group III, MTA Fillapex (Angelus, Brasil) was used as sealer for Group IV and Gutta Flow 2 (Coltene/Whaledent, Germany) for Group V.The orifices were sealed with flow composite - PermaFlo®(Ultradent, USA) and access cavities were closed with light curing composite Tetric EvoCeram(IviclarVivadent, Liechtenstein). In the control groups, canals were obturated with one-cone technique without any sealer. The obturatedspecimens were stored in 100% humidity at 37°C for 7 days.The samples of the experimental groups and the positive control groups were covered with 2 layers of nail varnish, except at the 1 mm around apical foramen. In the negative control group, the entire surfaces were coated with 2 layers of nail varnish.

Each specimen was placed in a centrifuge tube with the apex toward the open end, 2% methylene blue dye solution was added to each tube until the root was fully submerged. The specimens were centrifuged for 3 minutes at 30×gm. Theallspecimens were washed accurately with tap water, dried and then the nail varnish was removed with a scalpel.Longitudinal grooves were cut in the opposing root surfaces of the specimens, without entering the contents of the obturated space and then separating the specimens into two halves. The one half of the specimens from each group (n=3) were examined using optical microscope and the other (n=3) were examined by scanning electron microscope (SEM). Each groups has a two subgroups – 1 and 2. Subgroup 1 included the specimens for optical microscope and Subgroup 2 included the specimens for SEM observation. The linear vertical extent of dye penetration was measured to the nearest millimeter by two independent observers and the mean value was taken.The assessed gaps between sealer and gutta-percha cones on the one side and sealer and dentin wall on the other were measured by program “Image-Pro Insight”.

Apical leakage examination

The varnish layers were scrapped off and the roots were split longitudinally parallel to the long axis with a diamond disc using a water cooling. The root surfaces of the negative control teeth were entirely coated with two layers of nail varnish and sticky wax to prevent possible leakage.The root of teeth was sectioned longitudinally in the bucco-lingual direction without disturbing the gutta-percha.Thepenetration of the dye was measured in millimeters in each half of each tooth. The vertical measurements of depth of dye penetration was examined and digitalizedunder stereo microscope(Zeiss, Germany) at x40 magnification. The evaluation of the microleakage (depth of dye penetration) values of different root canal sealer wereobtained in units, converted in millimeters for vertical dye penetration and micrometers for horizontal.

Scanning electron microscope examination

Three of specimens from each group were prepared for SEM examination. The roots were sectioned longitudinally also as was mention above. Both preparedhalves of each root were mounted on aluminum stubs.The samples were vacuum sputter with gold dust in an argon medium by using JEOL JFC-1200 fine coater and examined with a scanning electron microscope (JEOL JSM-5510 SEM) at magnification - x500. The magnification - x1000, was appropriate to analizeadaptation to dentinal walls and sealer‟s penetration into dentinal tubules. The samples were examined from the apical to the coronal part.One-way ANOVA was used to analyze statistically the results and the multiple comparisons among the groups was carried out by the Student Newman-Keuls test. The level of significance was fixed at $p<0.05$.

3. Results

The measurements of maximum linear dye penetration were made in order to quantify the relative leakage of each group (Table 1). Dye penetration was observed in all the specimens except the negative control. The lowest mean level of dye penetration was in Group I followed by Group II and Group V. The positive control group showed the highest mean level of dye penetration suggestive of lesser sealing ability of gutta-percha, when used without a sealer. The results of this study showed that Group I showed less microleakage than Groups III-V. There was a statistically significant difference when the three groups were compared.The positive control teeth showed complete leakage throughout the length of the root canal, whereas the negative control teeth revealed no dye penetration. Dye penetration in the experimental groups occurred mainly at the interface of the sealer and the root canal wall in the majority of the specimens.

Table 1: Staistical analysis of vertical apical leakage of specimens in each group (*in mm*).

Vertical apical leakage of specimens in each group (*in mm*)							
Sealer	Group 1	Group 2	Group 3	Group 4	Group 5	Control groups	
Number of specimen	AH Plus	Apexit Plus	Z.O.B.Seal	MTA Fillapex	Gutta Flow 2	Positive Group	Negative Group
1	1.2	3.6	2.3	2.4	2.3	4.2	0
2	3.2	1.5	4.2	4.1	4.5	5.1	0
3	0.5	0.9	1.3	2.1	2.4	3.6	0
4	0.5	2.3	2.5	2.2	2.6	3.2	0
5	1.2	1.8	3.0	3.5	1.4	4.7	0
6	2.1	4.2	2.1	1.2	1.9	3.9	0
Mean	1.45	2.38	2.56	2.58	2.51	4.11	0

Scanning electron microscope examination

The use ofsanning electron microscopy to study dentine adaptability.The SEM examination showed better dentine adaptability for AH Plus (Group I)and Z.O.B.Seal(Group III)thanMTA Fillapex and Gutta Flow 2 (Group III and IV).

Statistical analysis

Analysis of the data showed no significant differences between all groups except between the AH Plus and MTA Fillapex. AH Plus showed significantly less leakage than MTA Fillapex and Z.O.B.Seal. All comparisons of the variances with Fishers F-test in this study were presented in Table 2. Analysis of variance (ANOVA) test was performed and showed that there were very highly significant differences ($p \leq 0.001$). Student‟s t-test (Table 3) was used to determine whether there were significant differences between the means of the five groups at the level of significance ($p < 0.05$).

Table 2: Staistical analysis by Fisher's test.

Fisher's test		
Group	Mean ± SD	ANOVA value
AH Plus	1.45 ± 0.35	F = 2.3414
Apexit Plus	2.18 ± 0.57	p < 0.05
Z.O.B.Seal	2.56 ± 0.36	
MTA Fillapex	2.58 ± 0.39	
Gutta Flow 2	2.51 ± 0.23	
+controlgroup	4.11 ± 0.98	
-control group	0	

SD = *Standart Deviation.*

Table 3: Student's unpared t-test illustrates the range of penetration.

Student's unpaired t-test			
Comparisons	T	P	Notes
Group 1 & 2	0.72	0.156	NS
Group 1 & 3	1.53	0.013	S
Group 1 & 4	1.78	0.009	S
Group 1 & 5	1.89	0.178	NS
Group 2 & 3	0.79	0.256	NS
Group 2 & 4	1.23	0.456	NS
Group 2 & 5	0.89	0.612	NS
Group 3 & 4	1.25	0.094	NS
Group 3 & 5	0.34	0.503	NS
Group 4 & 5	0.78	0.405	NS

***NS** = *Not significant*; ****S**=*Significan*t.

4. Discussion

A root canal sealer haveto provide a highest seal between the core filling material and the canal wall. Therefore it is formed two interfaces, which are of particular importance.The final result of endodontic treatment depends on final sealing of the canal space both apically and coronally. This sealing have to prevent microorganisms or tissue fluids from entering the canal space. Apical and coronal leakages have been shown to be very important reasons for root canal treatment failure [17].Modern endodontics employs mechano-chemical approach to disinfect the root canal and obtain a hermetic seal with complete coronal and apical seal. The good sealing ability of the sealer is one of the factors that determines the seal of a root canal filling [42]. The physical properties of all of the sealers used today are known to be different from those of dentine. The potential for a gap between the sealer and dentin is naturally to exist, espatially in presence of moisture [18,31,43].

The obturation of root canal is done by two materials, one being the core and other is the sealer. This approach is considered as a “golden” standard for contemporary endodontic treatment.Gutta-percha is used as the core material in the contemporary objurgating techniques.Sealer fillempty areas where gutta-percha is unable to fill [8]. Different types of endodontic sealers based on various chemical composition are available [28,37]. Many techniques have been suggested and evaluated precisely. Most of techniques advocating the use of gutta-percha as the core material and with sealers of a different composition to fill the residual gaps between the individual gutta-percha points and between the gutta-percha and the canal wall [3]. Different sealers have been subjected to extensive researchwith respect to their mechanical and biological properties, reflecting the prevailing belief that the appropriate selection of a sealer and its clinical performance may influence, at least in part, the outcome of endodontic therapy [34].

In the present study, AH Plus (epoxy resin based),Apexit(calcium hydroxide based), Z.O.B.Seal(zinc oxide eugenol based), MTA Fillapex (MTA based)and Gutta Flow 2 (silicon based) were used. The sealing ability of materials have been evaluatedby different methodology such as bacterial leakage, human saliva exposure, protein complex, fluid filtration and dye leakage. One of the commonly applied methods to evaluate the sealing ability of different root filling materials and techniques is based on linear measurement of dye penetration[41].The method of centrifuging dye penetration remains a commonly used tool for measuring the quality of root canal fillings [8]. Dyes can chemically interact with sealing materials and dentin, which may influence its diffusion, impairing an adequate marginal leakage evaluation [35,41]. The entrapped air in the filling may alter dye penetration depth. To overcome this theuse of vacuum or centrifugation has been suggested.Methylene blue was used in this study as its molecular size is similar to bacterial by-products such as butyric acid which can leak out of infected root canals to irritate periapical tissues, also it is easy to use, easily detectable under visible light [10], has the ability to maximum diffusion and not absorbable by dentin matrixwhich is added to it advantages [11,24]. It also penetrates voids better than isotopes [27] and has a low molecular weight thereby penetrating more deeply along root canal fillings [3]. Methylene blue dye has the potential to enter into the obstructed canal system of apical third of the root canal also. Therefore in this study we use vertical and horizontal measurements of dye penetration in units by a microscope to achieve more accurate results.

This study assess the sealing property of five different sealers by the centrifuging dye penetration method.The lowest leakage in this study was shown by AH Plus. It is a two-component epoxy resin based material, based on polymerization reaction of epoxy resin-amines [40] and showed good sealing ability when used as the sole filling in a root canal [42]. The resin could shrink in a 7-day period [13], leading to higher leakage values [8]. The highest leakage in this study could be attributed to shrinkage of the epoxy resin based sealer (AH Plus). Second highest leakage was seen in samples which used Apexit as sealer. Apexit is a calcium hydroxide based sealer and showed a significant volumetric expansion during setting due to water absorption [5]. The increases of the solubility of Apexit[7] and can affect the sealing property and the high leakage.AH Plus provides a significantly better seal than others sealers which is also confirmed in few previous studies [6, 23].

Resin based (Resilon Epiphany) obturating system is known for its property of monoblock formation [39]. It uses polymerizable methacrylate carboxylic acid anhydride (4-META) as the acidic resin monomer [16].Resin based sealers have shown to be superior of other sealers [25,33]. These sealers are a dual cure and bonds to a resilon core and root canal dentin simultaneously. But the radicular dentin is a complex organise structure. So, considering the bonding ability, there was no significant difference found by an scanning electron microscope (SEM) study done between bond of sealer to dentin between gutta-percha/AH Plus sealer and resin bond sealer (Resilon Epiphany) [39]. Tay F, et al (2005) concluded that the quality of apical seal achieved with the polycaprolactone-based root filling material and methacrylate-based sealer is not superior to gutta-percha and a conventional epoxy-resin sealer. They concluded also, that a hermetic seal is not established because of biodegradability of Resilon. Whereas gutta-percha is a relatively inert material, polycaprolactone is biodegradable under microbial attack. It is well known that lipases released by bacteria can cleave the ester bonds of polycaprolactone [30, 20].

Only Zmener et al.[44] and Miletic et al.[29] showed that AH Plus exhibited higher leakage values than specimens filled with AH 26. This has been attributed to some components of AH Plus, such as silicon oils, which can affect the sealing ability [9]. The present study results are in disagreement with those of the aforementioned authors. The differences in evaluation methods may account for these contrary results.

Unfortunately leakage cannot be totally eliminated from the fate of the root canal treated teeth. Lateral canals, accessory canals and other anatomical variations play an important role, especially the periapical pressure being the leading factor [36].

5. Conclusion

AH Plus showed significantly less leakage than other sealers and has a better sealing ability compared to Z.O.B.Seal, MTA Fillapex and Gutta Flow. According to results of this study Groups I (1.45mm) had better apical sealing ability and better adaptability to dentine in comparison to Group III, IV and V (2.51 - 2.58mm).Further studies with a larger sample size along with clinical trials, in different canal configuration are needed to evaluate better the sealing abilityaccording to reological characteristicsof sealers.

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