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Comparative Evaluation of Microleakage among Five Root Canal Sealers in Dry and Wet Condition Using Dye Penetration Method

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Abstract: *The aim of this study was to compare the microleakage of five different root canal sealers in dry and wet condition. There were 120 single-rooted teeth chemo-mechanically prepared and randomly assigned to 10 experimental groups, one control group (AH Plus, lateral condensation) (n=10) and positive/negative controls (n=5). The teeth of the experimental groups (a- dry; b- moist) were obturated with Grossman sealer, AH Plus, Gutta Flow 2, Apexit Plus, Tubli Seal in both dry and wet condition. Teeth were centrifuged at 30 G for 3 minutes with 5% methylene blue after sample preparation. Linear dye penetration was measured under a stereomicroscope after sectioning. Under the conditions of this study the best possible apical seal in dry condition was displayed by the sealers in the following order AH Plus, Apexit Plus, Gutta Flow 2, Tubli Seal and Grossman's sealer, whereas in moist condition best possible apical seal was displayed by Gutta Flow 2 followed by AH Plus, Apexit Plus, Tubli Seal and Grossman's sealer. Multifactorial ANOVA test displayed a significant difference in microleakage due to presence of moisture. Presence of moisture in the root canal affected the apical sealing ability of AH Plus, Apexit Plus, Grossman's Sealer adversely and Gutta Flow 2, Tubli Seal favourably.*

Key words*- Obturation, sealers, moisture, dye, microleakage*

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I. Introduction:

The main objective of endodontic therapy is the thorough mechanical and chemical cleansing of the entire pulpal space and its complete obturation with an inert filling material to form an impermeable seal. Schilder[1] described the final objective of endodontic procedure as being "the total obturation of the root canal space". The purpose of this is to avoid leakage from oral cavity and periradicular tissues into endodontic space. "The development of a fluid-tight seal at the apical foramen and the total obliteration of root canal", - an "impermeable seal" must follow the chemo-mechanical preparation to ensure the best chance of long term success. To fill the discrepancies between the fit of the filling material and dentinal wall a root canal sealer is needed which may fill lateral and accessory canals, isthmuses, and irregularities in the root canal system.

The purpose of the study was to compare the sealing ability of five different sealers: an epoxy resin (AH Plus; Dentsply International, York, PA), a calcium salicylate (Apexit Plus; Ivoclar Vivadent, Schaan, Liechtenstein), a novel polyvinylsiloxane- gutta- parcha combination (Gutta Flow 2; Roeko /Coltene /Whaledent , Langenau , Germany), and two zinc oxide eugenol sealer- Grossman type and Tubli Seal; Sybron Endo/Kerr) in both dry or moisture – contaminated root canals using dye penetration method.

II. Materials and Methods

120 non carious, non restored, single rooted mandibular central incisor teeth which were freshly extracted for periodontal cause were selected for the study. After gaining coronal access with the help of Endo Access bur (Dentsply) and air rotor hand piece, apical patency was verified by passing #10 files through the apical foramens. Root canals were then instrumented with Wave One primary file and X-smart Plus endomotor following manufacturer's guideline up to 1 mm short of the anatomic apex. The canals were irrigated with 20 ml 6% sodium hypochlorite (NaOCl) and 2 ml 17 % EDTA solution with ultrasonic activation (P5 Newtron piezoelectric unit and irrisafe). 2ml of Hydrol solution (Septodont, France) was used to dry each canal. Dryness of the canals was checked with two paper points placed consecutively in the canal and retrieved as dry. Fifty teeth were randomly assigned to five groups (groups- 1a to 5a). They were obturated immediately after drying using single cone technique. Another fifty teeth were divided into five groups (groups - 1b to 5b). A wet chamber (Acmas Technologies Pvt Ltd.) was used to rehydrate them (groups - 1b to 5b) at 37 ^{0} C and 100% humidity for 7 days. After 7 days they were obturated using single cone technique. Ten teeth forming control group 6a and were obturated in dry condition using AH Plus with lateral compaction technique. Five teeth each were prepared as positive and negative control.

Table-1: Experimental Groups, Different sealers used, number of samples and type of obturation within different conditions

Groups	Sealers	No of teeth	Condition	Obturation Technique
1a	AH Plus	n=10	Dry	Single cone Technique
1b	AH Plus	n=10	Wet	Single cone Technique
2a	Apexit Plus	n=10	Dry	Single cone Technique
2b	Apexit Plus	n=10	Wet	Single cone Technique
3a	Gutta Flow 2	n=10	Dry	Single cone Technique
3b	Gutta Flow 2	n=10	Wet	Single cone Technique
4a	Tubli Seal	n=10	Dry	Single cone Technique
4b	Tubli Seal	n=10	Wet	Single cone Technique
5a	Grossman Sealer	n=10	Dry	Single cone Technique
5b	Grossman Sealer	n=10	Wet	Single cone Technique
Control 6a	AH Plus	n=10	Dry	Lateral Compaction Technique
Positive control	Nail Varnish	n=5	Dry	
Negative control	-	n=5	Dry	

Glass Ionomer Cement was used to close the access cavity. The teeth were stored for seven days in 100% humidity at 37 ^{0} C in a humidifier (Acmas Technologies Pvt Ltd.). All teeth were coated with two layers of nail varnish. Water cooled diamond abrasive points were used to remove apical part of the roots until the master gutta-percha cones were visible. The negative controls were completely coated with nail varnish to include apical foramen area. The teeth were then put into a test tube containing 5% methylene blue dye and were centrifuged at 30 G for 3 minutes. Serial cross sections were made at 1 mm (level - I to VII) distances using a water cooled diamond saw and micro motor after drying. Stereomicroscope at 40-fold magnification was used to investigate the cross sections for dye penetration.

Measuring system- No penetration of dye within level I was considered as dye penetration of 1 mm i.e. up to level -I (First level from apex without coloration: 1mm). Dye penetration for level I but no penetration within level II were considered as dye penetration of 2 mm i.e. up to level -II (First level from apex without coloration : 2mm). Dye penetration for level I & II but no penetration within level III were considered as dye penetration of 3 mm i.e. up to level -III (First level from apex without coloration : 3mm) and so on. The positive controls showed dye penetration throughout their length as they were not obturated and not coated with nail varnish at the apical foramen and were considered their dye leakage as 8 mm. The negative controls, which were obturated and coated fully with the nail varnish, did not show any dye leakage - were considered their dye leakage as 0.

III. Results and Analysis

Table 2: Dye penetration scores in millimetre – among samples of different groups

	GROUPS										
Sample No	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a
1	1	2	2	2	2	2	4	3	4	6	3
2	2	2	2	2	2	1	4	2	5	5	3
3	1	3	1	3	2	2	4	3	5	5	3
4	2	2	2	3	2	1	4	3	4	5	3
5	1	2	2	2	2	2	4	3	5	5	3
6	2	2	1	2	2	1	4	3	4	5	3
7	1	2	2	2	2	2	5	2	5	4	3
8	2	3	2	3	2	1	4	3	5	5	3
9	1	2	1	3	2	1	4	3	4	5	3
10	1	2	2	3	3	1	4	3	4	6	4

Table 3: Sample size, average and standard deviation among groups obturated in dry condition

	SAMPLES	AVERAGE	STANDERD DEVIATION
1a. AH PLUS (Dry)	10	1.4	0.516
2a. Apexit Plus (Dry)	10	1.7	0.483
3a. Gutta Flow 2 (Dry)	10	2.0	0
4a. Tubli Seal (Dry)	10	4.1	0.316
5a. Grossman Sealer (Dry)	10	4.5	0.527

From the above table it was seen that group 5a i.e. Grossman Sealer used in dry condition had the highest mean value and group 1a i.e. AH Plus used in dry condition had the lowest. For comparison ANOVA table had been constructed as shown below-

Table 4: ANOVA DATA

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SUM OF SQUARES	'F' VALUE
BETWEEN GROUPS	4	83.72	20.93	119.22
RESIDUAL	45	7.90	0.1756	-
TOTAL	49	91.62	-	-

The 'F' value being highly significant ($p<0.001$), critical difference (C.D.) was formed out to compare the averages.

$$C.D. = t_{45} \times \sqrt{\frac{2MSE}{10}}$$ (MSE = 0.1756)

=0.38 / 0.50 /0.66 at p = 0.05, 0.01 & 0.001 respectively.

Table 5: Comparison of averages

	2a	3a	4a	5a
1a	Not Significant	p < 0.01	p < 0.001	p < 0.001
2a	-	Not Significant	p < 0.001	p < 0.001
3a	-	-	p < 0.001	p < 0.001
4a	-	-	-	p < 0.05

Not Significant when p > 0.05. As the table showed: Group 1a & 2a did not differ significantly. Group 2a & 3a did not differ significantly. All other differences in average values were significant i.e. 4a vs. 5a at 5% level, 1a vs. 3a at 1% level, and the rest at 0.1% level.

Table 6: Sample size, average and standard deviation among groups obturated in moist condition

	Sample Size	Average	Standard Deviation
1b. AH Plus (Wet)	10	2.2	0.42
2b. Apexit Plus (Wet)	10	2.5	0.53
3b. Gutta Flow 2 (Wet)	10	1.4	0.52
4b. Tubli Seal (Wet)	10	2.8	0.42
5b. Grossman Sealer (Wet)	10	5.1	0.57

From the above table it was seen that group 5b had the highest mean value and group 3b had the lowest. For comparison ANOVA table had been constructed as shown below -

Table 7: ANOVA DATA

Source	Degree of Freedom	Sum of squares	Mean sum of squares	'F' Value
Between Groups	4	77	19.25	78.75
Residual	45	11	0.244	
Total	49	88		

The 'F' value being highly significant ($p<0.001$), critical difference (C.D.) was formed out to compare the averages.
Critical Differences = 0.32 at 5% level, 0.42 at 1% level and 0.55 at 0.1% level.
Using the Critical Difference values the averages were compared.

Table 8: Comparison of averages

	2b	3b	4b	5b
1b	**Not Significant**	**p < 0.001**	**p < 0.001**	**p < 0.001**
2b	**-**	**p < 0.001**	**Not Significant**	**p < 0.001**
3b	**-**	**-**	**p < 0.001**	**p < 0.001**
4b	**-**	**-**		**p < 0.001**

Not Significant when $p > 0.05$. As shown in the table: Group 1b & 2b did not differ significantly. Group 2b & 4b did not differ significantly. In all the other cases the differences in averages were highly significant ($p < 0.001$).

For comparison within groups between dry and moist condition 'T' tests were applied as shown in the following tables –

Table 9: Comparison between group 1a i.e. AH Plus used in dry condition and 1b i.e. AH Plus used in moist condition-

	Average	Standard Deviation	Significant
1a	**1.4**	**0.516**	**t = 3.80, Degree of Freedom = 18, p < 0.01**
1b	**2.2**	**0.42**	

Table 10: Comparison between group 2a i.e. Apexit Plus used in dry condition and 2b i.e. Apexit Plus used in moist condition -

	Average	Standard Deviation	Significant
2a	**1.7**	**0.483**	**t = 3.53, Degree of Freedom = 18, p < 0.01**
2b	**2.5**	**0.53**	

Table 11: Comparison between group 3a i.e. Gutta Flow 2 used in dry condition and 3b i.e. Gutta Flow 2 used in moist condition -

	Average	Standard Deviation	Significant
3a	**2.0**	**0**	**t = 3.65, Degree of Freedom = 18, p < 0.01**
3b	**1.4**	**0.52**	

Table 12: Comparison between group 4a i.e. Tubli Seal used in dry condition and 4b i.e. Tubli Seal used in moist condition -

	Average	Standard Deviation	Significant
4a	**4.1**	**0.316**	**t = 7.82, Degree of Freedom = 18, p < 0.01**
4b	**2.8**	**0.42**	

Table 13: Comparison between group 5a i.e. Grossman Sealer used in dry condition and 5b i.e. Grossman Sealer used in moist condition -

	Average	Standard Deviation	Significant
5a	**4.5**	**0.527**	**t = 2.44, Degree of Freedom = 18, p < 0.05**
5b	**5.1**	**0.57**	

Significant differences were found in the results between the groups obturated in dry condition and wet condition.

Comparison with Control
Control Average = 3.1
Standard Deviation = 0.316
Confidence limit ($x \pm t_9 \times s / \sqrt{n}$) (2.87—3.33)
Any average which was within this limit was treated as non significant. Others below or above the limit were treated as significant. Therefore, group 4a and group 5a did not differ significantly from the control group in dry condition and among the groups obturated in moist condition only group 5b differ significantly from the control group. The other groups did not differ significantly from the control group.

IV. Discussion

Root canal sealers, used in combination with solid or semisolid core filling materials, are intended to achieve the fluid tight hermetic seal at the entire root canal. **Friedman et al (2000)**[2] reiterated the importance of sealers in root canal obturation and addressed the assessment of leakage of sealers. **Wu, Fan and Wesselink (2000)**[3] investigated the long term success of gutta-percha with and without the use of a sealer and concluded "long term seal of root canal fillings is affected by volume change of both gutta-percha and sealer". Failure of root canal treatment, prolongation of inflammation and infection, lack of healing of periapical lesions, and possibly fracture of roots caused by corrosion of pins and posts fabricated from different materials are the common consequences of poor sealing of the root canals (**Angmar- Mansson B et al -1969**[4]**, Rud J Omnel K-A-1970**[5]). Root canal spaces should be completely dry prior to obturation in order to increase the adhesion of root canal sealers to dentinal walls and the filling materials. **Weine, Cohen** and **Burns** suggested the use of alcohol rinse and paper points to completely dry the root canal system.

In this study 120 mandibular central incisor teeth were selected. This allowed a reliable and almost equal comparison between the different sealers and the effect of moisture on them (**Matthias Johannes Roggendorf, Jhannes Ebert, Anselm Petscheltand, Roland Frankenberger**)[6].

In the present study it was seen that in **dry** condition Grossman's sealer had the highest average dye leakage (4.5; Table-3) and the AH Plus sealer had least dye leakage (1.4; Table-3). According to this study the best possible apical seal in **dry** condition can be obtained by using the sealers in the following order AH Plus, Apexit Plus, Gutta Flow 2, Tubli Seal and Grossman's sealer (Table-3).

In the **moist** condition Gutta Flow 2 had the least dye leakage (1.4; Table-6) and Grossman's sealer had the highest dye leakage (5.1; Table-6). In **moist** condition best possible apical seal can be achieved by using the sealers in the following order Gutta Flow 2, AH Plus, Apexit Plus, Tubli Seal and Grossman's sealer (Table-6).

Under all the testing condition AH Plus appeared to be a good root canal sealer. It exhibited least microleakage in **dry** condition (Average microleakage - 1.4; Table-3) and second least microleakage in **moist** condition (Average microleakage - 2.2; Table-6). Moisture had a significant effect in sealing ability of this sealer (table-9). These results could be attributed to the low film thickness of AH Plus together with its setting reaction. Epoxy resins are vulnerable to traces of moisture, because of its hydrophobic structure.

The setting reaction of calcium hydroxide sealers is effectively accelerated by the presence of moisture. A possible explanation for the increased leakage of Apexit Plus in **moist** canals (Average leakage – 2.5; Table-6) than in **dry** canals (average leakage - 1.7; Table-3, Graph-1) could be related to this decrease in setting time. The presence of moisture in canal could have accelerated the setting reaction of Apexit Plus, preventing it from complete wetting and coating of gutta parcha and the root canal walls leading to poor adaptation to both surfaces as well as the formation of spaces and voids that could have allowed dye penetration to a greater degree symbolizing leakage.

Grossman's sealer was associated with increased dye leakage in the **moist** condition (Average Leakage-5.1; Table-6) than in **dry** condition (Average leakage – 4.5; Table-3). This could be related to the deleterious effect of moisture decreasing its setting time so that the sealer became set before proper wetting of the dentinal wall could happen.

In contrast Gutta Flow 2 and Tubli Seal EWT showed better sealing ability in **moist** condition (Table-11, 12). Moisture could have acted as a lubricant for these sealers that allowed a better attachment to the root canal wall. Thus a complete drying might have adversely affected linear dye penetration. Also this could be attributed to slight expansion of Gutta Flow 2 sealer during setting reaction.

Study by **Sriwalee Limkangwalmongkol, Paul V. Abbott (1992)**[7] correlates with the present study. When the sealers used in dry condition, AH 26 had significantly less dye penetration than Apexit which had less dye penetration than Tubli Seal. The results of the study correlate with the results of the study conducted by **Matthias Johannes Roggendorf, Jhannes Ebert, Anselm Petscheltand, Roland Frankenberger (2007)**[6] , in which they found that moisture affected the sealing ability of AH Plus, Apexit Plus adversely and favourably in case of Tubli Seal and Roeko Seal. Also study of **Alan N Kuhre and Joel R. Kessler (1993)**[8] supports the present study by concluding that the result showed there was a tendency for more leakage in moist canals compared with dry canals, with the group of teeth contaminated with saliva having the most leakage. **Peter**

Portman, Stefan I (2005)[9] in there vitro study evaluating obturation quality after four years of storage at 100% humidity using the non-instrumentation technique found that Apexit showed more dye penetration than AH Plus, which supports the present study. **Miletic, Anic, S. Pezel-Ribaric, S. Jukic (1999)**[10] in their study of leakage of five root canal sealers examined the apical sealing ability of five root canal sealers AH Plus, AH 26, Diaket, Apexit, Ketac Endo using a fluid transport model, found Apexit showed more dye penetration than AH Plus which also supports the present study. **S. Khedmat (2005)**[11] in their study **'**Comparison of the Tensile Bond Strength of Four Root Canal Sealers' concluded that greater bond strength to gutta percha was observed in AH 26 followed by Apexit. The tensile bond strength of AH26 to gutta-percha was significantly higher than Apexit which may influence their apical sealing ability, which also supports the present study. **Sundas H Naser (2015)** [12] concluded that sealing ability of AH Plus was superior than Gutta Flow 2 with single cone technique and canal oburated with only Gutta Flow leaked most among the groups of his study. **Chad W. Lothemer et al**. **(2017)**[13] in their study concluded that the sealing ability of Gutta Flow 2 and AH Plus did not differ significantly. **J Ebert et al**. **(2011)** [14] in their study showed that both Gutta Flow 2 and AH Plus had comparable sealing ability.

The study results o**f Rana et al.** [15] are in contradiction to the present study as they stated that the sealing ability of Gutta Flow 2 was comparable to lateral compaction technique with Zinc Oxide Eugenol sealer. The results of this study are in contradiction with the results obtained by **Mazed M Negm (1989)**[16] as they concluded that Tubli Seal EWT gave better apical seal than AH Plus and there was no effect of moisture or blood on the apical seal when Tubli Seal EWT was used. **Emma Roper (1996)**[17] in her vitro study to compare microleakage in four root canal sealers stated that AH Plus, Sealer 26, Tubli Seal displayed equal ability to prevent microleakage which also contradicts the results of the present study as AH Plus found to seal better than Tubli Seal significantly in both the conditions. These differences could be related to the different experimental conditions like non removal of smear layer, different obturation technique, and different dye penetration method.

The possibility of contaminants being present in the root canal space in daily practice is far from remote. A saliva leak may remain undetected around the rubber dam clamp or the operator may fail to completely dry the root canal space with paper points. Probably the most common cause of contamination is the ingress of a periapical exudates or bleeding of periodontal membrane. This may occur much more frequently than clinician realize. Also the unintentional contamination of the sealers during obturation might cause or speed up its future dissolution. If the sealer is contaminated with NaOCl or other irrigant, there might be a chemical interaction resulting in incomplete setting of the sealer. If the sealer is contaminated with the saliva, bacteria present within the saliva may speed up the breakdown of the sealer. The present study compared the apical seal after 1 week of obturation. Long term studies are required to best understand the sealing ability of the sealers and effects of moisture. According to Seltzer only limited information of clinical significance can be obtained from the vitro studies of the sealing properties of root canal sealers. The biological aspects are not exactly disclosed by such studies. Although every effort is made to simulate the normal biological conditions, minute variation could not be replicated in this study. So further studies are required which may prove helpful in analyzing the effect of moisture on the sealers.

V. Conclusion

Root canal fillings are done to seal the entire root canal system for prevention of reinfection of the periapex. In some cases, it is impossible to dry the root canal space completely before obturation. Over the time, percolation of fluids can be detected through channels connecting pulpal space and periodontium or oral cavity, leading to contamination and failure of the treatment procedures. Moisture influences the setting process and physical properties of root canal sealers and therefore influences their sealing ability. Within the limitation of this study it could be concluded that-

- The best possible apical seal in dry condition can be obtained by using the sealers in the following order AH Plus, Apexit Plus, Gutta Flow 2, Tubli Seal and Grossman's sealer whereas in moist condition best possible apical seal can be achieved by using the sealers in the following order Gutta Flow 2, AH Plus, Apexit Plus, Tubli Seal and Grossman's sealer. Presence of moisture in the root canal affects the apical sealing ability of AH Plus, Apexit Plus, Grossman's Sealer adversely and Gutta Flow 2, Tubli Seal favourably.

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