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Bacterial Leakage of Four Endodontic Sealers Using Fresh Human Saliva

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ABSTRACT

Introduction: Bacterial leakage plays a considerable role in endodontic related failures. The aim of this study was to compare the bacterial leakage of bioceramic (including Well Root ST and MTA Fillapex) and resin-based root canal sealers (naming AH-Plus and AdSeal). **Methods and Materials:** Sixty-eight maxillary central incisors were included in this experimental study. These were randomly allocated into four experimental groups, which were obturated with gutta-percha and four different sealers (n=15), and the two control groups (positive, n=4 and negative, n=4). A dual-chamber technique was carried out using fresh human saliva and traced for 90 days. The Chi-square and Long Rank tests were performed for data analysis. **Results:** Bacterial leakage was observed in 33.3% of the samples in Well Root ST, 40% in AH-Plus group, 53.3% in AdSeal group, and 66.6% in MTA Fillapex group. The difference between the Well Root ST and MTA Fillapex groups was significant (P=0.02). The mean leakage time between the Well Root ST (85.6 days) and MTA Fillapex groups (71.2 days) was also significant (P=0.037). **Conclusion:** The results of this *in vitro* study revealed bacterial leakage in all experimental groups. The Well Root ST and the MTA Fillapex groups showed the lowest and highest bacterial leakages, respectively. Then it seems that the Well Root ST is a good substitute for MTA Fillapex in clinical applications.

Keywords: Bacterial Leakage; Bioceramic Root Canal Sealer; Canal Sealer; Epoxy Resins; Root Canal Filling Materials; Saliva

Introduction

The success of endodontic therapy is correlated with three factors: appropriate cleaning and shaping, disinfection, and three-dimensional obturation and sealing of the root canal system. All of these should be confined to the root canal system. Both overfilled and underfilled root canal obturation have shown to reduce the success rate of endodontic treatment. The working length should be less than 0.5 to 1 mm from the radiographic apex [1].

Bacterial leakage plays a considerable role in sustained endodontic related apical pathosis; therefore, obtaining an apico-coronal bacterial tight seal is the main goal in obturation of the root canal system [2]. In addition to the obturation technique, root canal sealers also play a critical role in providing a tight seal against bacterial leakage [3].

As two common resin-based root canal sealers, the sealing ability, physical properties and chemical properties of AH-Plus (Dentsply Maillefer, Tulsa, OK, USA) and AdSeal (Meta Biomed, Cheongju, South Korea), have been evaluated in previous studies [4-6]. Additionally, MTA Fillapex (Angelus, Londrina Parana, Brazil) and Well Root ST (Vericom, Kyunggi-Do, South Korea) are bioceramic-based root canal sealers, the biocompatibility of which has been confirmed in several studies [7-9].

MTA Fillapex is composed of salicylate resin, bismuth oxide, silica, silicon dioxide, titanium dioxide, and set MTA particles.

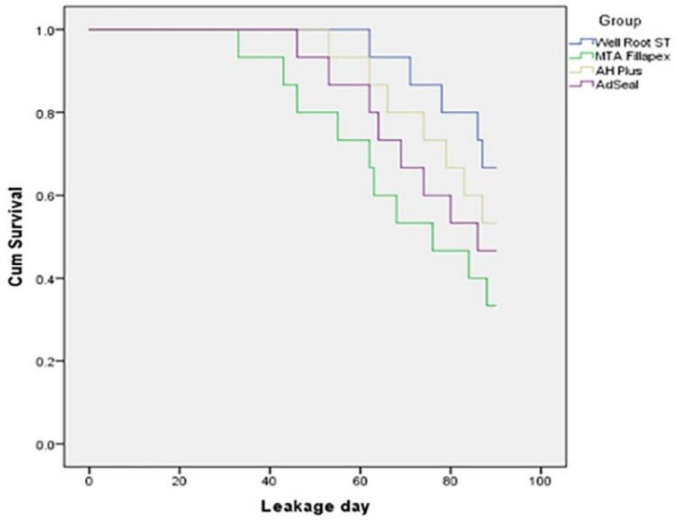

Figure 1. Survival rank diagram of all experimental groups can be seen

Well-Root ST is a premixed, ready-to-use, injectable bioceramic cement paste that includes zirconium oxide, calcium silicate, filler, and thickening agents. The material is hydrophilic and uses moisture in dentinal tubules to initiate and complete its setting reactions. The setting time is 25 min; however, in root canals the setting time can be more than 2.5 h [10].

In vitro bacterial leakage studies cannot mimic actual clinical conditions, though a bacterial leakage model using fresh human saliva in a dual-chamber can better simulate the clinical setting [11].

The aim of this study was to compare the bacterial leakage of obturated root canals using one of the sealers naming AH-Plus, AdSeal, MTA Fillapex, or Well Root STin the presence of fresh human saliva.

Materials and Methods

Sixty-eight single-rooted caries free maxillary central incisors were included in this experimental study. Radiographic evaluation confirmed the absence of calcification and resorption. Furthermore, the apical patency of the roots was checked with a #10 K-File (Dentsply Maillefer, Ballaigues, Switzerland). Teeth with apical constriction larger than a #20 K-File or moderate/severe curvatures (more than 15 degrees) were excluded from the study.

Outer surfaces of the roots were debrided with a chisel and soaked in 5.25% NaOCl for 7 days [12]. Finally, selected teeth were decoronated to reach 16 mm in length. Except for the initial 1 mm of the apical tip, the root surfaces were covered with two layers of nail polish.

A sample size of 15 specimens was determined for each experimental group based on a pilot study of 8 samples each. The specimens were chosen based on inclusion/exclusion criteria and randomly allocated to experimental groups (AH-Plus, AdSeal, Well Root ST and MTA Fillapex) (n=15) and control groups (positive control group with patent root canal and no nail polish coverage, n=4; and negative control group with well-sealed root canal using injectable resin modified glass ionomer and totally covered with nail polish, n=4). Root canal preparation was performed using the ProTaper full sequence (Dentsply Maillefer, Ballaigues, Switzerland) up to F4. Irrigation was carried out using 1 mL of 2.5% sodium hypochlorite during instrumentation intervals. The smear layer was removed with 1 mL of 17% EDTA, followed by 1 mL of 2.5% sodium hypochlorite, each used for one min. The final rinse was executed using 5 mL normal saline. Sealer placement was done using an ultrasonic tip E9 (Woodpecker, Guangxi, China) for 10 sec circumferentially. Obturation was accomplished using the lateral compaction technique (master apical cone #40, spreader #25, and lateral cones #20). A periapical radiograph was taken to confirm the quality of obturation. The accepted specimens were packed in a sterile surgical drape and transferred in an incubator (Shimaz Co., Tehran, Iran) at 37° C and 95% humidity for one week to complete the sealer setting process.

Microbial leakage assessment

A dual-chamber technique was carried out using fresh human saliva. The specimens were fixed and sealed in 1.5 mL Eppendorf tubes with the apical part of the roots exposed outside the tube while the coronal part was within the tube, serving as the upper chamber for Saliva/Brain Heart Infusion (BHI) (Company name, City, Country). The initial complex was packed and sterilized using Ethylene Oxide gas for 12 h. Under a biologic hood, the initial complex was fixed over a sterile glass tube filled with pure BHI solution to compose a dual-chamber complex while the root canal path was the only way between the two chambers.

The fresh human saliva was collected daily in a sterile container from a caries-free person with a normal pH range (determined by Tornosol paper) who had not brushed for at least 12 h. The fresh saliva was mixed with BHI solution in a 3:1 ratio and was replaced every day for up to 90 days [13].

Every day the lower chamber solution was traced for any turbidity using a spectrophotometer (KHB L-3180, China) based on 0.5 McFarland standards (1.5×10^8); then, the lower turbid solution was analyzed for detection of the microbial type which leaked into the lower chamber using the culture method [14].

Statistical analysis

The Chi-square and Long Rank tests were used with SPSS software (SPSS version 19.0, SPSS, Chicago, IL, USA) for data analysis.

Results

Microbial leakage was observed in 33.3% of the Well Root ST, 40% of the AH-Plus, 53.3% of the AdSeal, and 66.6% of the MTA Fillapex samples. While the negative control group showed no leakage throughout the study time (90 days), the positive control group had complete leakage in the first few days. The difference between the Well Root ST and the MTA Fillapex groups was significant (P=0.02). The survival rank diagram is demonstrated in Figure 1. In this diagram influx of specimens (leaked specimens) over the 90 days of the experiment is evident, and the difference between the experimental groups is detectable.

As far as the time needed for maximum microbial leakage, the mean leakage times for the Well Root ST group, AH-Plus group, AdSeal group, and MTA Fillapex groups were 85.6 days, 81.6 days, 77.6 days, and 71.2 days, respectively, and the difference between the Well Root ST and MTA Fillapex groups was significant (P=0.037) (Table 1). Regarding the bacteria species, gram-positive cocci were the most prevalent bacteria that leaked through the root canal filling materials. The exact leakage time and bacterial species in the lower chamber are demonstrated in Table 2.

Table.1. The mean leakage time, leakage positive and negative in different groups in 90 days

Groups	Leakage positive (%)	Leakage negative (%)	Mean (SD)
Well Root ST group (15)	5 (33.3%)	10 (66.6%)	85.6 (2.133)
AH-Plus group (15)	6 (40%)	9 (60%)	81.6 (3.059)
Ad Seal Group (15)	8 (53.3%)	7 (46.7%)	77.6 (3.813)
MTA Fillapex group (15)	10 (66.6%)	5 (33.3%)	71.2 (4.967)
Positive control group (4)	4 (100%)	0 (0%)	1
Negative control group (4)	0 (0%)	4 (100%)	-

Table 2. The leakage time and bacterial species leaked in lower turbid chambers

Groups	Leakage time (day)	Gram positive cocci	Gram negative bacilli	Candida species
AH-Plus group	53	+	-	-
	62	+	-	-
	66	+	-	-
	74	+	-	-
	79	+	-	-
	83	+	+	-
	87	+	-	+
Ad Seal group	46	+	-	-
	53	+	-	-
	62	+	-	-
	64	+	-	-
	69	+	+	-
	74	+	+	-
	80	+	+	+
	86	+	-	+
Well Root ST group	62	+	-	-
	71	+	-	-
	78	+	-	-
	86	+	-	-
	87	+	+	-
MTA Fillapex group	33	+	-	-
	43	+	-	-
	46	+	-	-
	55	+	+	-
	62	+	+	-
	63	+	+	-
	68	+	+	+
	76	+	+	+
	84	+	-	+
	88	+	-	+

Discussion

One of the most challenging aspects of endodontic therapy is bacterial infection. An apico-coronal bacterial tight seal is a critical factor for a successful endodontic treatment [15]. Since there is no chemical or mechanical attachment between core materials and root dentinal walls, sealers play an important role in obtaining a bacterial-tight seal [16]. In the present study, the Well Root ST and AH-Plus groups exhibited a better bacterial seal than the AdSeal and MTA Fillapex groups. Also, leakage in the MTA FIllapex and AdSeal groups began sooner. Gram-positive cocci were the most prevalent species that leaked through the root canal filling materials.

Resin-based root canal sealers are widely used in endodontics because of their good sealing abilities and chemical stability. These properties prevent leakage for an extended period of time. However, their biocompatibility in contact with living tissue is questionable [17, 18]. On the other hand, bioceramic sealers are biocompatible though there are controversies about their chemical stabilities and sealing abilities [8, 10].

The microbial source for this study was fresh saliva from a caries free human because cariogenic microbiota reduces the pH of saliva and may affect microbial leakage by the destruction of sealers/dentine interface [19, 20]. The oral microbiome has been extensively characterized in previous studies [19, 21], so it was unnecessary to characterize the initial microbiota in the fresh saliva.

Among several microleakage studies (dye penetration, dye diffusion, bacterial and endototoxin infiltration, fluid filtration, glucose, caffeine and protein infiltration, radioisotope penetration, animal studies, and electrochemical or 3D evaluation [22]), microbial leakage through fresh human saliva was the method chosen for the current study since this method can provide more biologically and clinically predictable data [11].

Aminsobhani et al. [23] showed that the obturation technique was not a contributing factor for microbial leakage of endodontically treated teeth. The present study used the lateral condensation technique [24, 25]. Moreover, an ultrasonic unit for the enhancement of sealer distribution through the root canal system was utilized [26]. The smear layer was also removed to contribute to sealer penetration into dentinal tubules. The agitation technique of irrigants can also affect tubular penetration of root canal sealers; however, no agitation technique was employed in the present study [27]. The correlation between tubular penetration and the sealing abilities of sealers is not certain [28, 29].

Singh *et al.* [30] and Ahujal *et al.* [31] both employed the dye leakage method, and therefore their results cannot be compared with this study even though some of their results confirm our results. In both studies, the results demonstrated that the MTA Fillapex groups had the worst sealing properties. In both of these investigations, the teeth in the MTA Fillapex groups had more dye leakage compared to the resin based root canal sealer groups.

Jafari *et al.* [32] used bacterial leakage and showed that MTA Fillapex had more bacterial leakage than AH-26, which was in agreement with our results. The anti-bacterial properties of MTA Fillapex are lower than the AH-26 sealer and this phenomenon may affect the efficacy of bacterial leakage in this material [33].

In a scanning electron microscopy (SEM) analysis for sealer-dentinal wall gap detection, Demiriz *et al.* [34] showed that there was no significant difference between dentinal wall adaptation of the MTA Fillapex and AH-Plus sealers using the single cone technique; therefore, greater leakages detected in most studies for MTA Fillapex filled canals may be due to poor sealer-core material adaptation compared to resin-based root canal sealers. Mohammadian *et al.* [6] showed that the sealer-dentin interface had minimal gaps in the AH-Plus group, especially in the middle and coronal parts of the canal. Reszka *et al.* [35] showed the alkaline properties of the Well Root ST group were higher than the MTA Fillapex group, and this phenomenon may affect the antibacterial properties of the sealers as well as their bacterial leakages.

Conclusion

Based on the results of this study, bacterial leakage was observed in all experimental groups; however, the Well Root ST and MTA Fillapex groups, respectively, showed the lowest and highest bacterial leakages. Then it seems that the Well Root ST is a good substitute for MTA Fillapex in clinical applications. In addition, we found that the main bacteria that leaked through root canal filling materials were gram-positive cocci.

Conflict of Interest: 'None declared'.

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