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In vitro study of microbial leakage in roots filled with EndoREZ sealer/EndoREZ® Points and AH Plus sealer/ conventional gutta-percha points

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SUMMARY

Objective. The aim of this study was to determine and compare the microbial leakage of roots filled with EndoREZ sealer/EndoREZ® Points and AH Plus sealer/ conventional gutta-percha points.

Materials and methods. 60 single-rooted teeth were prepared using step-back technique. The smear layer was removed with 18% EDTA. Teeth were divided into two experimental groups (n=25 each group) and two controls (n=5 each group). In AH Plus group root canals were obturated with AH Plus sealer/gutta-percha and in EndoREZ group with EndoREZ sealer/EndoREZ® Points. Five teeth were served as negative controls and five as positive controls. All teeth were inserted into Eppendorf plastic tubes and suspended in glass bottles containing sterile Schaedler broth. The coronal chambers were filled with the mix of human saliva and broth (ratio 3:1). The medium was changed every 7 days. Microbial growth in the broth was evaluated every day up to the end of experiment.

Results. Leakage in the root canals of the teeth from experimental groups occurred between 4 and 75 days. The mean leakage in AH Plus group was 18.86 days, while in EndoREZ group it was 28.28. No statistically significant difference in microbial leakage between two tested filling materials was found.

Conclusion. Both types of root fillings – EndoREZ sealer/EndoREZ® Points and AH Plus sealer/gutta-percha points – showed microbial leakage.

Key words: AH Plus, EndoREZ, microbial leakage, root canal filling.

INTRODUCTION

The three dimensional root canal obturation and the adequate coronal restoration are important barriers to infection or reinfection of the periapex [1]. It has been established that microleakage of root canal fillings might contribute to failure of endodontic treatment [2, 3]. To avoid this problem, a variety of sealers and cements have been tested in combination with gutta-percha for root canal obturation. However, it has been shown that complete seal of the root canal system is impossible with currently available materials [4].

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Sealers based on epoxy resins afford very good physical properties and ensure adequate biological performance. Acceptable apical sealing has been found with epoxy resin-based sealers [5]. AH Plus (DeTrey Dentsply, Konstanz, Germany) has been a sealer of choice for few last decades. Previous studies showed that it is biocompatible, has good tissue tolerance and long-term dimensional stability [5, 6, 7].

In recent studies, a series of methacrylate-based formulations have been tested and have shown promising results [8-10]. Preliminary reports have shown that EndoREZ (Ultradent Products, Inc., South Jordan, Utah, USA), a urethane dimethacrylate resin-based sealer, provides an effective seal when used with lateral condensation [11]. The hydrophilic properties of the sealer allow penetration deep into the root canal walls but not into gutta-percha [11]. The lack of adhesion to gutta-percha constituted a weak point because a path for leakage might be created. In an effort to address this issue and to establish a

Fig. 1. Experimental apparatus

bond between sealer/dentin and sealer/gutta-percha, methacrylate resin coated gutta-percha cones (EndoREZ® Points, Ultradent Products, Inc., South Jordan, Utah, USA) have recently been introduced [12].

The aim of this study was to determine and compare the microbial leakage of roots filled with newly developed EndoREZ sealer/EndoREZ® Points and AH Plus sealer/gutta-percha. The null hypothesis tested was that the microbial leakage does not depend on the type of root canal filling material used for obturation.

MATERIALS AND METHODS

Preparation of teeth

A sample of 60 single-rooted teeth with fully developed root apices were used for the experiment. All of the teeth were extracted for periodontal and orthodontic reasons. After mechanical cleaning, the teeth were stored in isotonic saline solution at 100% humidity and 37°C.

Prior to the study, the crowns of teeth were removed at the cemento-enamel junction using a water-cooled diamond bur. To ensure the same length for all specimens, they were resected 9 mm from the apex.

The root canals were prepared with manual instrumentation, using a step-back technique. The coronal and middle thirds were flared with Gates- Glidden instruments and the apical third was prepared subsequently with sizes 15, 20, 25 and 30 K-Flexofiles (Dentsply Maillefer, Ballaigues, Switzerland) to the full working length. Files were used with in-and-out movements in a circumferential manner. Preparation of the apical third was considered complete when a size 30 file could be inserted without force to the working length. Then, K-files from sizes 35–60, each size 1 mm short of the preceding instrument, were used for final preparation of the coronal and middle third.

Individual instruments were discarded after use in each root canal and irrigation was performed after each change of instrument using 2.0 ml of a 3.0% NaOCl solution (ChlorCid, Ultradent Products, Inc., South Jordan, Utah, USA) followed by 2.0 ml of a 18% EDTA solution (Ultradent Products, Inc., South Jordan, Utah, USA) for the smear layer removing. During instrumentation, the canals were flushed with the irrigation solutions using disposable syringes and 30-gauge needles (NaviTip, Ultradent Products, Inc., South Jordan, Utah, USA), which were placed to approximately 3–4 mm from the working length without binding. Upon completion of instrumentation the root canals were finally flushed for 1 min with 2.0 ml of 18% EDTA solution, which was washed with 2.0 ml of 3.0% NaOCl solution followed by copious rinsing with 4.0 mL saline. Finally the canals were dried with air and paper points (Ultradent Products, Inc., South Jordan, Utah, USA).

Obturation

Teeth were randomly divided into two experimental (n=25) and two control (n=5) groups as follows.

EndoREZ group (25 roots). Root canals were obturated by lateral condensation of EndoREZ® Points and EndoREZ sealer. A size B finger spreader (Dentsply Maillefer), and size 25 EndoREZ gutta-percha points were used for lateral condensation.

AH Plus group (25 roots). Root canals were obturated by lateral condensation of gutta-percha points (Plandent Oy, Helsinki, Finland) and AH Plus sealer (Dentsply Maillefer). A size B finger spreader (Dentsply Maillefer), and size B auxiliary gutta-percha (Dentsply Maillefer), were used for lateral condensation.

The external surfaces of each root from experimental groups, except the apical 2 mm, were covered with two layers of nail varnish.

Positive control group (5 roots). Root canals were neither obturated nor coated with nail varnish.

Negative control group (5 roots). Root canals were filled with gutta-percha and AH Plus sealer by lateral condensation. Root surfaces were completely covered with two layers of nail varnish, including the apex of the root and coronal access.

The sealers were mixed according to the manufacturer's instructions. After obturation, a hot plugger was used to remove excess gutta-percha. After obturation, all specimens were stored in saline solution at 37°C for 3 weeks to allow full setting of the sealer.

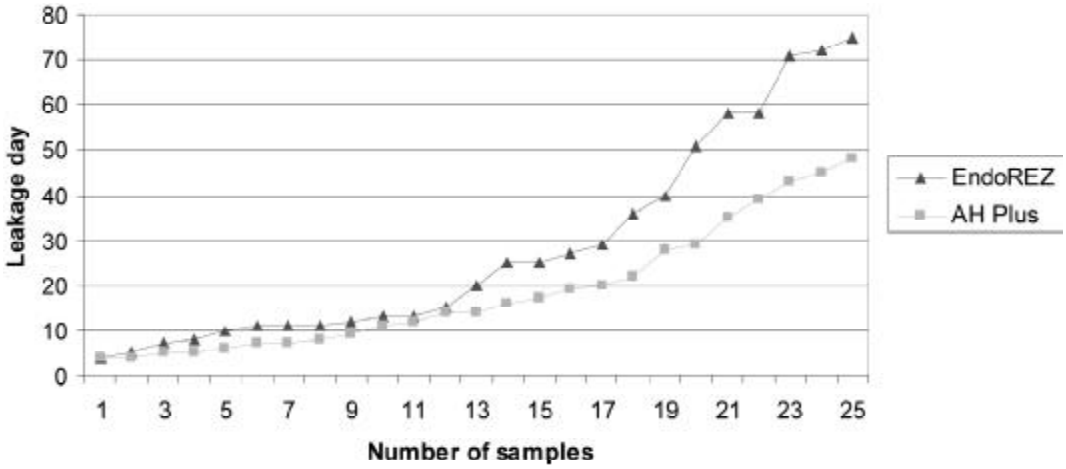

Fig. 2. Leakage differences in experimental groups

After that, they were thermocycled between 5° and 55°C for 300 cycles, for 10 s at each temperature [13].

Preparation of samples

The external surfaces of specimens were covered with two layers of nail varnish. The teeth were inserted individually into an Eppendorf plastic tube (Eppendorf-Elkay, Shrewsbury, MA, USA) with the root apex protruding through the end. The coronal and middle portions of specimens were sealed with cyanoacrylate glue and sticky wax. The system was sterilized using ethylene oxide gas and placed in a 5 ml glass bottle containing 3 ml sterile Schaedler broth (Schaedler anaerobe broth; Oxoid, Unipath Ltd, Basingstoke, UK). Around the entrance to the flask, a layer of cyanoacrylate glue and sticky wax was applied. The model used in this study was refined from a system described by Imura et al. and shown in Figure 1 [14].

Microbial leakage

The coronal chambers were filled with the mix of human saliva and broth (ratio 3:1). The medium with microorganisms was changed every 7 days. The system was stored in an anaerobic incubator at 37°C, and any changes in opacity of the broth in the apical chamber were checked every day.

Statistical analysis of the data

The results were analyzed using statistical software package (SPSS ver.11 for Windows, SPSS Inc.). For statistical analysis of the data Mann-Whitney-Wilcoxon nonparametric test for comparison between the leakage of the experimental groups was used. A probability value equal to or less than 0.05 was considered to indicate significance.

RESULTS

Leakage through the root canals of samples varied from 4 to 75 days. All positive controls exhibited microbial leakage within 48 hrs, whilst the broth in lower chambers used for negative controls remained clear throughout the test period. The mean, minimum and maximum days of microbial leakage in experimental groups are shown in Table 1. The differences in leakage time among experimental EndoREZ and AH Plus groups are shown in Figure 2.

Regardless of the fact that the leakage of AH Plus group in comparison with EndoREZ group was higher, statistical analysis using Mann-Whitney-Wilcoxon nonparametric test revealed insignificant differences between experimental groups ($P<0.05$)(Table 2).

DISCUSSION

Although the potential for an extremely high success rate for endodontic treatment is widely accepted, epidemiological studies demonstrate that success rate varies between 40% - 50% [15, 16, 17].Thus, there is a space for improvement. Conceptually, endodon-

Table 1. Minimum, maximum and mean days at which microleakage occurred

Group	n	Minimum (days)	Mean (days)	Maximum (days)
EndoREZ	25	4	28.28	75
AH Plus	25	4	18.68	48

Table 2. Microbial leakage between experimental groups

Group	Mean leakage (days)	SD	P
EndoREZ	28.28	22.93563	0,084163
AH Plus	18.68	13.8946	

tic treatment is very simple – after optimal cleaning and shaping of the root canal, a filling material should be placed in order to entomb remaining bacteria and block the pathways for additional bacteria to enter root canal system from the oral cavity. However, filling of the root canal with conventional gutta-percha even by most technically proficient operator will not result in a seal that is dependable during long term. In fact, the coronal restoration is more likely to be the main factor of long term success. As shown in numerous *in vitro* studies, gutta-percha/sealer fillings leak at an alarming rate [18, 19].

In vitro leakage assessments may not correlate directly with clinical findings, but are justified for simple comparison and screening of new materials and techniques [5, 20]. The sealing efficacy of different root filling materials has been tested using radioactive isotopes, electrochemical microleakage, dyes or fluids [1]. Bacteria and bacterial products have also been used [21]. Not only bacteria or bacterial cell wall components, but also soluble byproducts from bacterial metabolism or saliva may enter at the junction between the root canal filling and the dentinal wall. Molecular size of the test agents should be representative for bacteria, and/or bacterial cell wall components, and/or nutritive fluids. Whereas dyes represent agents of small molecular size and should be the most critical indicator for penetrability of root canal fillings. There are factors such as ionic charge, pH, temperature changes and the ability of viable microbes to change their shape and size and to move actively, duplicate or grow and this may play a role in the root canal which cannot be represented by an aqueous dye solution. Natural human saliva has some advantages over bacterial cultures. It overwhelms several different bacterial species, high bacterial density and bacterial products, enzymes, proteins and other elements not provided by culture media. The saliva leakage test is a method closely related to the real clinical situation [22]. Due to this reason such leakage model was used in our study.

Under the conditions of our study, nor one of the tested materials produced an effective seal of the root canals. Therefore, the null hypothesis was rejected. The mean leakage value of AH Plus group was higher than that of EndoREZ group, but the difference between mean leakage values of fillings was statistically insignificant. Probably this is a result of the large variability in leakage data, as from the mean leakage data presented in Tables 1 and 2, it is clear that the leakage of AH Plus group specimens is higher about 30%. Furthermore, there is a possibility that the sample size had been larger, the statistical significance level would have been reached. However, the methodology of our research was comparable with other published studies and is acceptable for evaluation of the microbial leakage of root filling materials.

The sealer AH Plus is based on epoxi amine resins and has been used with gutta-percha points for root canal obturation for many years. Miletic et al. reported that AH plus exhibited greater, but not statistically significant, leakage than samples filled with AH 26 [23]. Similar results have been reported by Zmener et al. [24]. This was explained by the faster setting time of AH Plus, which caused shrinkage stress and earlier debonding from dentine walls. Also, some ingredients of AH Plus, such as silicone oils, can affect the sealing ability of this material. Miletic et al. showed that after 1 year, AH plus indicated significantly better sealing ability than Apexit, whereas AH 26 and Diaket had no statistical differences with either sealer [25]. They stated that AH plus showed satisfactory sealing ability. It is difficult to compare the results of our and previous studies because in most of them the dyes as marker were used. However, our results are in agreement with results of Miletic et al. study, where the same testing model and conditions were used [22].

EndoREZ is a new resin based root canal sealer, the active ingredient of which is urethane dimethacrylate resin (UDMA). The manufacturer did not give information about the detail composition of EndoREZ. It has been stated by the manufacturer that EndoREZ may be used on slightly moist canals because of its hydrophilic property. As it is not possible to obtain a completely dry surface throughout root canal surface, this characteristic may be advantageous for the sealer. The results of our study shown, that the leakage of EndoREZ sealer/ EndoREZ® Points fillings is about 30% less than AH Plus sealer/gutta-percha. This is in agreement with the findings of J.A. Von Fraunhofer et al. [26]. O. Zmener et al. showed that the leakage of the conventional gutta-percha/EndoREZ fillings is less in comparison with gutta-percha and Grossman sealer [11].

The better sealing ability of EndoREZ may be attributed to the "mono-block" which is created by deep penetration of the sealer into dentinal tubules and chemical bond between EndoREZ sealer and resin coated EndoREZ gutta-percha points [27]. In contrast, the SEM studies showed, that the conventional gutta-percha filling pulled away from the AH Plus sealer, whereas the sealer remained against the dentin wall with its resin tags penetrating the dentinal tubules [28].This gap between gutta-percha and sealer may be critically important for microleakage in AH Plus experimental group.

The presence of the smear layer on the root canal wall after instrumentation could increase leakage of fillings. In this study 18% EDTA solution was used during and after root canal instrumentation to remove the smear layer and decrease coronal leakage [29]. The adherence of the sealer to the dentin walls is a function of smear layer removal. High bond strengths cannot be achieved unless the smear layer is removed [30]. The bond of the smear layer to the underlying dentin is relatively weak, approximately 5 MPa, and cannot withstand the shrinkage associated with the curing of resins [29]. The resins pull the smear layer from the dentin and provide a way for microleakage.

CONCLUSIONS

The results obtained in this *in vitro* study demonstrated that neither EndoREZ sealer with EndoREZ® resin coated gutta-percha points, nor AH Plus sealer with gutta-percha points prevented microbial leakage. However, root canal fillings in EndoREZ group provide slightly superior resistance to microbial leakage when compared with AH Plus group. Nevertheless, one should take into consideration, that this experiment was conducted *in vitro* with its inherent limitations. Therefore, the clinical extrapolation should be avoided and new root canal filling material should be tested in *in vivo* models.

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