The Effect of Smear Layer Removal on Internal Apical Baterial Leakage along Root Canal Fillings Using Three Diferent Obturation Techniques

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Abstract

Aim: **This study was conducted to evaluate the influence of smear layer removal on internal apical bacterial leakage of infected root canals obturated with 3 techniques; single cone, lateral compaction and vertical compaction with System-B.** ***Materials and Methods:*** **A total of 140 human teeth were prepared using ProTaper rotary files. The samples were classified into 2 equal groups according to whether the smear layer was removed or not. The root canals were inoculated with Enterococcus faecalis suspension and incubated at 37°C for 1 week. Each group of roots was then subdivided into 3 equal experimental subgroups according to the obturation technique. Following obturation, the samples were suspended in Trypticase Soy Broth (TSB) and the broth was checked on daily basis for turbidity, as an evidence for internal apical bacterial leakage up to 3 months.** ***Results:*** **All obturation techniques leaked more slowly in the absence of smear layer than in its presence, the difference was statistically significant for single cone technique, whereas for lateral compaction and vertical compaction techniques the difference was insignificant. It was also observed that smear layer removal significantly minimized the counts of leaked bacteria through the apical foramen.** ***Conclusions:*** **Smear layer removal minimizes the counts of leaked bacteria through the previously infected root canals and may enhance the sealability of the root canal obturation.**

Keywords: *Apical leakage, Smear layer, E. faecalis, Spectrophotometer*

Introduction

The main purpose of the root canal obturation is the attainment of a fluid-tight seal along the root canal system, to prevent re-infection of the obturated root canals and entomb the remaining viable microorganisms that could not be fully removed from the lateral canals, dentinal tubules or root canal irregularities after the cleaning and shaping procedure[(1)].

Studies have shown that mechanical instrumentation of the root canals produces a smear layer on the surface of dentinal walls. This layer contains microorganisms, odontoblastic processes, and remnants of pulp tissue. Retaining the smear layer on the root canal walls has been considered to be beneficial as it may seal the bacteria inside the dentinal tubules and block the entry of bacteria in contaminated canals into the tubules[(2)]. Despite the debate over keeping the smear layer, it has been demonstrated that it hinders the penetration of medications and irrigants into the irregularities of the root canal system and the dentinal tubules. It also interferes with adhesion and pene-

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tration of sealers into the dentinal tubules; hence it has the potential for compromising the seal of the root canal obturation[3]. However, the influence of the smear layer on microleakage is still conflicting, and it is unclear whether possible beneficial effects of smear layer removal is a general phenomenon or is dependent on the obturation materials and techniques used. It was therefore of interest to examine whether removal of the smear layer aids in preventing apical leakage, using different obturation technique.

Materials and Methods

This study was conducted using 140 human maxillary anterior teeth. The teeth were immersed for 1 hr in 5.25% NaOCl for disinfection. Thereafter, the teeth were decapitated using a fissure bur in such a way that all the roots were 15 mm long. A No.15 K-file was inserted into the canal of each root and the working length was established by subtracting 1 mm from the length recorded when the tip of the file was appeared at the apical foramen. ProTaper rotary files *(Dentsply/Maillefer, Ballaigues, Switzerland)* were used to prepare the root canals in a crown-down approach, at a rotational speed of 300 rpm, using a torque-control electric low-speed motor *(J. Morita USA, INC).* First, SX file was introduced into the root canal until it encountered light resistance, then S1 and S2 files were introduced respectively into the canal to the working length. The apical portion of each root canal was instrumented with finishing files F1, F2, F3, F4 and F5 sequentially. After each instrument use, irrigation of the root canals was performed using 2 ml of 2.25% NaOCl, and a No. 15 K-file was used to assure the patency of the apical foramina.

Smear layer removal:

The samples were classified into 2 equal groups, the smear layer was left intact in group A (Smear +ve), while root canals of group B (Smear –ve) were irrigated with 1 ml of 17% EDTA solution *(MD-cleanser, Meta Biomed. Co., Ltd. Korea)* for 1 min, followed by 5 ml of 2.25% NaOCl to remove the smear layer[4]. All the roots were sterilized by autoclaving for 15 min at 121°C.

Bacteria preparation:

The external surfaces of the roots, except the apical 2 mm were coated with 3 successive layers of nail polish to prevent bacterial leakage through the root surfaces, the apical foramina were sealed with wax, and the root canals were blotted dry with paper points. Isolated colonies of pure culture of *E. faecalis (ATCC 29212, Central health laboratories, Cairo)* were suspended in 20 ml of Trypticase Soy Broth (TSB) *(Oxoid Ltd, Basingstoke, UK)* and incubated at 37°C for 5 hrs until the optical density of the bacterial suspension was adjusted to approximately 3 x 10^8 CFU/ml, by comparing its turbidity to 1 McFarland standard. Ten µl of the bacterial suspension were inoculated into the canal of each root, using a micropipette. The coronal orifices were sealed with wax, and the roots were enclosed in sterile eppendorf tubes and incubated at 37°C for 1 week. After the incubation period, the wax at the coronal orifices and the apical foramina was removed and the patency of the apical foramina was reassured with a No. 15 K-file.

Grouping:

Roots with and without the smear layer were then randomly subdivided into 3 experimental subgroups of 20 samples each. According to the filling technique, and 2 control subgroups of 5 roots each as follows: *Subgroup 1 (SC):* The root canals were obturated with the single cone technique using ProTaper gutta-percha points (Dentsply/Maillefer, Ballaigues, Switzerland) with Tubliseal sealer *(SybronEndo,*

Glendora, CA, USA); Subgroup 2 (LC): The root canals were obturated with the lateral condensation technique, utilizing 0.02 taper gutta-percha cones with Tubliseal sealer; *Subgroup 3 (VC):* The root canals were obturated with the warm vertical condensation technique, utilizing Pro-Taper gutta-percha points combined with Tubliseal sealer; *Positive control subgroup:* The root canals were neither obturated nor coated; and *Negative control subgroup:* The root canals were obturated utilizing the lateral compaction technique, sealed apically with wax, and had their external root surfaces completely coated with nail polish.

Bacterial leakage test:

Sterile Eppendorf tubes were used to suspend the samples in TSB to a level sufficient to cover the apical 3 mm of the root tips. The tubes with the enclosed roots were incubated at 37°C, for different testing periods.

Bacterial leakage evaluation:

TSB in the Eppendorf tubes was checked daily for turbidity up to 3 months. The number of leaking samples after 3, 30, 60 and 90 days was recorded per subgroup. The turbid broth was changed with a new broth at the end of each assigned period. The optical density of each turbid broth was determined using a spectrophotometer at a wavelength of 546 nm, and the counts of leaked bacteria to the broth were calculated using the following equation[5]; *Bacterial count/ml= Optical density of the test/Optical density of the control x* 10^8**.** The nature of the organism of the turbid broth was confirmed by colony morphology, Gram staining and bile aesculin hydrolysis test. The obtained data from the numbers of leaking samples per subgroup and bacterial counts of each turbid broth at the end of each testing interval were statistically analyzed using Chi-Square and Student's *t* tests, respectively.

Results

All samples of the positive control subgroups exhibited broth turbidity within 24 hrs of each assigned period, while TSB of the negative controls remained unaffected throughout the entire monitoring period. All obturation techniques leaked more slowly in the absence of smear layer than in its presence. The difference was statistically significant for the single cone technique (Table 1), while for the lateral compaction as well as the vertical compaction techniques the difference was insignificant (Tables 2 & 3). Also, the results revealed that the counts of leaked bacteria through the apical foramen were significantly lower when the smear layer was removed, for all obturation techniques, at all time intervals (Figure 1).

Table 1: Comparison between internal apical bacterial leakage of smear positive and smear negative single cone subgroups

		Single cone technique (SC)		*P*-value
		Smear +ve	Smear –ve	
Day 3	N (%)	6 (30%)	4 (20%)	0.04*
Day 30	N (%)	14 (70%)	10 (50%)	0.04*
Day 60	N (%)	18 (90%)	12 (60%)	0.03*
Day 90	N (%)	18 (90%)	14 (70%)	0.04*

*A statistically significant difference ($p< 0.05$)

Table 2: Comparison between internal apical bacterial leakage of smear positive and smear negative lateral condensation subgroups

		Lateral condensation technique (LC)		*P*-value
		Smear +ve	Smear -ve	
Day 3	N (%)	2 (10%)	0 (0%)	0.9
Day 30	N (%)	8 (40%)	6 (30%)	0.9
Day 60	N (%)	10 (50%)	9 (45%)	0.9
Day 90	N (%)	12 (60%)	10 (50%)	0.9

Statistically significant difference ($p > 0.05$)

Table 3: Comparison between internal apical bacterial leakage of smear positive and smear negative vertical condensation subgroups

		Vertical condensation technique (VC)		*P*-value
		Smear +ve	Smear -ve	
Day 3	N (%)	0 (0%)	0 (0%)	1
Day 30	N (%)	6 (30%)	3 (15%)	0.6
Day 60	N (%)	8 (40%)	7 (35%)	0.9
Day 90	N (%)	11 (55%)	8 (40%)	0.4

Statistically significant difference ($p > 0.05$)

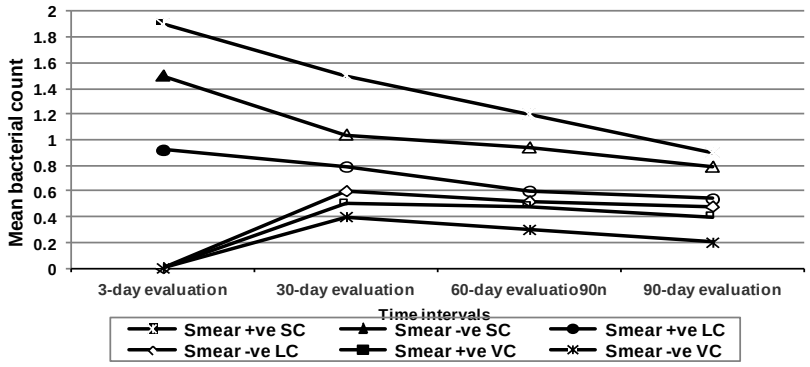

Figure 1: Mean counts of leaked bacteria ($\times 10^8$ CFU/ml) from different subgroups

Discussion

Despite the tremendous improvement of the instrumentation and obturation materials and techniques aiming at complete sealing of the root canal system, a long term fluid-tight seal is not yet fully achievable. To date, all previous studies focused on evaluation of the external apical leakage, whereas the internal apical leakage received no attention, although it is the true cause of the periapical disease. External apical leakage of tissue fluid occurs primarily into the root canals, supplying the latent bacteria inside the lateral canals, dentinal tubules or root canal irregularities with a source of nutrition. Consequently, the nourished bacteria multiply

and leak back in a reverse direction (Internal apical leakage), intimidating the periapical tissue. The alternating use of EDTA and NaOCl has been recommended for efficient elimination of the smear layer from the root canal walls[6]. EDTA is a chelating agent acts upon the inorganic components of the smear layer, causes decalcification of peri-tubular and inter-tubular dentin and leaves the collagen exposed. Subsequently, the use of NaOCl dissolves the collagen, opening up the entrances of the dentinal tubules[7]. Similar to previous studies[8,9], 1 ml of 17% EDTA was used in this study for 1 min to remove the smear layer, as numerous researchers have proved inadvertent erosive effects of EDTA on the intraradicular dentin when applied in higher volumes and longer durations[10]

Wide varieties of methodologies have been used to assess microleakage of root-filled teeth, including electromechanical action, dye penetration, fluid filtration and bacterial leakage models. From a clinical perspective, bacterial leakage method provides a more accurate indicator of the sealing ability since it uses a biological marker. Several bacterial species have been used to evaluate microleakage. Because it is the most prevalently pathogen implicated in persistent root canal infections and its high resistance to antimicrobial agents, Enterococcus faecalis was selected for microleakage evaluation in the current study.

The results of the current study revealed that elimination of the smear layer significantly improved the seal of the single cone technique. These results are consistent with the findings attained by Cergneux et al [11]. Contrarily, Saleh et al.[12] found that smear layer removal increased microleakage of single cone technique. Also, the results showed that smear layer removal had insignificant effect on the sealing ability of lateral condensation as well as vertical condensation technique. These results concur with those reported by Evan and simon[13], Tidswell et al.[14] and Taylor et al[15]. Conflicting with the results of this study, Froes et al[16] found that removal of the smear layer enhanced the sealability of vertical condensation technique. Also, Jacob et al[17] and Xie et al[18] reported that keeping the smear layer significantly improves the seal of lateral condensation technique.

Conclusions: considering the results of the present study it seems that smear layer removal may improve the seal of the root canal obturation, at least for the single cone technique, while it significantly minimizes the counts of leaked bacteria through the apical foramen, for all obturation techniques.

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