To evaluate the influence of smear layer with different instruments and obturation methods on microleakage of root canal filled teeth: *In vitro* study

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Abstract

**Aims and Objectives:** The success of root canal treatment depends on proper debridement, instrumentation, proper accessibility, and proper restoration. The presence of a smear layer is considered to be a significant factor. This *in vitro* study was conducted to assess the effect of the presence/absence of a smear layer on the microleakage of root canal filled teeth using different instruments and obturation methods. **Materials and Methods:** One hundred extracted mandibular premolars with closed apices and single roots were chosen and then divided into six groups, A to F, consisting of 15 teeth each. The control group included 10 teeth; 5 positive and 5 negative. The teeth were decoronated at the cementoenamel junction. Groups A, B, C, and D were instrumented with engine-driven rotary Protaper NiTi files. Groups E and F were instrumented with conventional stainless steel hand files. Groups A, C, and E were flushed with 3 ml of 17% EDTA to remove the smear layer prior to obturation. All teeth were flushed with 5.25% sodium hypochlorite solution and obturated with AH-Plus sealer with lateral condensation technique for Groups C, D, E, F and with thermoplasticized gutta-percha technique for Groups A and B. Using an electrochemical technique, leakages in the obturated canals were assessed for 45 days. The results were tabulated using Student’s *t*-test (paired and unpaired *t*-test) with the Statistical Package for the Social Sciences Software Version 21 (IBM Company, New York, USA). **Results:** Group A showed the lowest mean value at intervals of 10, 20, 30, and 45 days. There was no current flow in the negative controls during the test period. There was leakage in the positive controls within a few minutes of immersion. **Conclusion:** The results showed that rotary instrumentation contributed toward an exceptional preparation of root canals compared to hand instrumentation. Elimination of the smear layer enhanced the resistance to microleakage; thermoplasticized gutta-percha obturation technique produced a better seal compared to the lateral condensation technique.

**Key words:** Apical Seal, Gutta-percha, microleakage, root canal treatment, smear layer

INTRODUCTION

Root canal treatment is influenced by efficient biomechanical instrumentation of root canal to provide a surface free of debris, disinfection, and dissolution of organic matter, to remove bacterial pathogens, and to obtain a three-dimensionally sealed and obturated canal.

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The smear layer contains a thin layer of smeared material covering the canal wall of 1–2 μm thickness. The smear layer has an inorganic particulate debris and organic component from necrotic/viable pulp tissue. The smear layer on canal walls and in dentinal tubules may impede adaptation of root canal fillings and avoid tubular penetration of endodontic filling materials, thereby causing microleakage.

Microleakage is defined as the passage of bacteria, fluids, and chemical substances between root structure and fillings. It was found that microleakage declined after removal of the smear layer, but increased dentin permeability. The application of sodium hypochlorite during instrumentation and high volume final flush using EDTA solution followed by sodium hypochlorite was found to be effective in eliminating superficial debris and smear layer.

Previous studies have aimed to quantitatively assess the leakage potential of root canal filling materials. Studies of seal integrity of filling materials include methods using bacteria, air under pressure, fluorometric assays, radioactive isotope penetration, scanning electron microscopic examination, and dye penetration methods including methylene blue, India ink, Silver staining have been reported. The advantages of the electrochemical technique offer benefits of quantifiable data, speed, accuracy and efficiency, and enabling continuous testing.

MATERIALS AND METHODS

The sample size was similar to previous studies by Osins et al. The sample size was standardized at 15 teeth per group, 10 teeth as control (5 teeth for positive and 5 teeth for negative), with confidence interval of 95% and a power of at least 80%. One hundred freshly extracted single rooted human mandibular premolars with closed apices and straight roots of patients aged between 17–25 years indicated for orthodontic extraction were selected for this study in the Department of Conservative and Endodontics, H.S.R.S.M. Dental College and Hospital, Hingoli. Comparative straight roots, single canal, oval anatomy of the canals are required for uniform biomechanical preparation of canal. The mandibular premolars satisfy the abovementioned prerequisites, and hence were chosen for the study. The debris on the roots was removed by placing samples in 5% sodium hypochlorite for 24 h and using an ultrasonic scaler.

Using a diamond disc in high-speed hand piece under water spray, the teeth were decoronated at the cementoenamel junction, such that all roots are standardized at approximately 12 mm length. The roots were split randomly into six groups, namely, A, B, C, D, E, and F, consisting of 15 teeth each; 5 teeth were used as positive control and another 5 teeth were used as the negative control group.

Access preparation was performed in all teeth using a round bur, and pulp tissue was removed with a barbed broach. No. 10 file was placed 1 mm beyond the apical foramen to ensure canal patency and the working length was calculated. The following techniques were followed under sodium hypochlorite irrigation.

- Group A: Rotary instrumentation + smear layer removed + thermoplasticized gutta-percha obturation
- Group B: Rotary instrumentation + smear layer not removed + thermoplasticized gutta-percha obturation technique
- Group C: Rotary instrumentation + smear layer removed + lateral condensation technique
- Group D: Rotary instrumentation + smear layer not removed + lateral condensation technique
- Group E: Hand instrumentation + smear layer removed + lateral condensation technique
- Group F: Hand instrumentation + smear layer not removed + lateral condensation technique.

Positive Control: Hand instrumentation (step-back method) + smear layer removed + no obturation

Negative Control: Hand instrumentation (step-back method) + smear layer not removed + roots completely sealed with two coats of sticky wax [Table 1].

Smear layer

Groups A, C, E, and positive control received a final rinse with 3 ml of 17% EDTA File-Rite (Pulpdent Corp, USA), which remained in canal for 5 min, followed by irrigation with 10 ml 5.25% sodium hypochlorite to clear the smear layer.

Groups B, D, F, and negative control group received a final rinse of 5.25% sodium hypochlorite after complete instrumentation. Canals of all teeth in the groups were then dried with paper points and placed in 0.9% saline solution before obturation.

Electrochemical circuit

Leakage inside the obturated canal was measured by an electrochemical technique [Figures 1 and 2]. A PVC-insulated copper wire with a 5 mm bare end was placed coronally in the obturated canal of each tooth and

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then sealed with sticky wax. After that, all exposed surfaces and the tooth-wire junction, except the tooth apex, were sealed with three layers of nail varnish. Each tooth was kept immersed throughout the procedure in 1% sodium sulphate, where it created an electrode of a circuit, with a stainless steel strip acting as another electrode. A 10 V DC voltage was passed through the electrodes, and the current flow, indicating the onset of leakage, was measured by \( iR \) drop across a 10 \( \Omega \) resistor kept in series with the electrodes and the power source. When leakage occurred, an electrolytic pathway was established between copper and stainless steel. The extent of leakage was directly proportional to the magnitude of current for each tooth.

Positive controls: A 22-gauge wire was placed inside the root up to the complete working length. With two coats of sticky wax, each wire was then sealed inside the canal to secure it in place ensuring that it was bare in at least apical third.

Negative controls: Biomechanically prepared and completely sealed with two coats of sticky wax roots.

Calculations from the control were done daily with experimental samples for 45 days. The temperature of the electrolytic solution in each container was maintained at ambient temperature (approximately 25°C) during nights and at 25°C during the day to prevent variations in the current flow due to temperature fluctuations. The mean apical microleakage current (\( \mu A \)) values were recorded for all teeth in the groups and were tabularized and regulated for statistical analysis using Student’s paired and unpaired t-test with the Statistical Package for the Social Sciences Software version 21 (IBM Company, New York, USA).

**RESULTS**

Group E showed the highest microleakage value of 9.058 and Group A showed the lowest value of 5.144 at a 10-day interval. Group E showed the highest mean value of 9.537, and Group A showed the lowest mean value of 6.664 at a 20-day interval. Group E showed the highest mean value of 9.545, and Group A showed the lowest value of 7.310 on the 30th day. At the 45-day interval, Group B showed the highest mean value of 9.506 and Group A showed the lowest mean value of 8.044 [Table 2].

When all samples of different groups at different intervals were tabulated, Group A showed the lowest mean value at each interval of 10, 20, 30, and 45 days. Current did not flow with negative controls during the test period. The positive controls demonstrated leakage shortly after a few minutes of immersion and displayed a mean steady leakage current of 9.67 \( \mu A \) during the test period.

| Group   | Method of Preparation                                                                 | Number |
|---------|---------------------------------------------------------------------------------------|--------|
| A       | Rotary instrumentation + smear layer removed + Thermoplasticized gutta-percha obturation technique | 15     |
| B       | Rotary instrumentation + smear layer intact + Thermoplasticized gutta-percha obturation technique | 15     |
| C       | Rotary instrumentation + smear layer removed + Lateral condensation technique          | 15     |
| D       | Rotary instrumentation + smear layer intact + lateral condensation technique            | 15     |
| E       | Hand instrumentation + smear layer removed + Lateral condensation technique             | 15     |
| F       | Hand instrumentation + smear layer intact + Lateral condensation technique              | 15     |
| Positive control | Hand instrumentation + smear layer removed + no obturation                        | 5      |
| Negative control | Hand instrumentation + smear layer intact + roots completely sealed with two coats of sticky wax | 5      |

**Table 1: Various test groups, method of preparation, and number**
Table 2: Mean apical microleakage current (µA) (P-values) and standard deviation and coefficient of variation of all groups at time-intervals of 10, 20, 30, and 45 days

| Groups | 10 days (P-value and SD) | 20 days (P-value and SD) | 30 days (P-value and SD) | 45 days (P-value and SD) |
|--------|--------------------------|--------------------------|--------------------------|--------------------------|
| A      | 5.14±0.123               | 6.65±0.169               | 7.31±0.090               | 8.04±0.068               |
| B      | 7.09±0.170               | 8.53±0.123               | 9.32±0.071               | 9.51±0.050               |
| C      | 6.68±0.110               | 8.08±0.048               | 8.70±0.078               | 8.81±0.050               |
| D      | 8.37±0.234               | 9.06±0.079               | 9.10±0.052               | 9.20±0.070               |
| E      | 9.06±0.169               | 9.54±0.099               | 9.54±0.049               | 9.41±0.066               |
| F      | 8.25±0.297               | 8.87±0.049               | 9.17±0.057               | 9.24±0.071               |

Statistically highly significant difference was noted in all the groups at intervals of 10, 20, 30, and 45 days [Table 2].

This study also showed highly significant difference at different intervals of 10, 20, 30, and 45 days between the pairs among the groups, i.e., Groups A–C, Groups A–D, Groups A–E, Groups A–F, Groups B–C, Groups B–D, Groups B–F, Groups C–F, Groups D–E, and Groups D–F [Table 2].

DISCUSSION

Instrumentation is often planned to eliminate microorganisms and necrotic material and to prepare canal for a root filling. The eventual goal of these chemomechanical steps is to produce a clean, debris free canal for obturation.[1] According to one school of thought, endodontic smear layer serves as a physical barrier obstructing adhesion and penetration of sealers into dentinal tubules, which influences the sealing capability of root canal obturation.[6] Another school of thought states that smear layer serves as a physical barrier to bacteria and bacterial byproducts.

Irrigation is done to eliminate organic component, such gross debris arising from pulp tissue, and inorganic component such as smear layer. Çapar and Aydinbelge[7] studied the consequences of various combinations of EDTA and sodium hypochlorite as a working and/or irrigation solution during and after instrumentation and reported that the combined use of 17% EDTA preceded by 10 ml of 5.25% sodium hypochlorite produced the best results for smear layer removal. Traditional instrumentation with stainless steel files have been shown to yield variations in canal morphology, disregarding the technique or the file type used.[8] Bigger size files straighten within canal and cut indiscriminately, creating ledges, strip perforations, zips, and transportation.[9,10]

NiTi rotary instruments can effectively produce a smooth funnel shape with a reduced risk of ledging and transporting the canal in a short time than it takes with traditional techniques.[11] Studies have demonstrated that all root canal fillings leak and a total sealing of canals is seldom accomplished.[12,13]

Lateral condensation has been demonstrated to be a very acceptable gutta-percha obturation technique. Thermoplasticated gutta-percha was shown to replicate a seal superior to that produced by other obturation methods.[14,15] The outcome of eliminating the smear layer was more conspicuous for the thermoplasticated methods, such that although sealer was used, the entry of thermoplasticated gutta-percha inside tubules was observed.[16]

In this study, it was witnessed that all specimens exhibited leakage throughout the test period. These findings are similar to previous studies that the desirable to achieve a hermetic seal is scarcely obtained in reality.[2,12]

In this study, an electrochemical technique was selected to check the apical microleakage because this method has been demonstrated to be efficient, predictable, and superior, compared with other techniques of dye and radioisotopic assessment of leakage.[17,18]

Mokhtari et al.[18] and Kazemipoor et al.[19] demonstrated an increase in peak value followed by a decline. The microleakage current values seen in the present study showed a long-term continuous increase, which is similar to previous observations.[20]

Group A, C, and E specimens showed less values of mean leakage current at each interval of 10, 20, 30, and 45 days suggesting less extent of leakage comparative to other groups. This decrease leakage in smear-free canals may be because of increased mechanical locking of filling materials inside patent tubules, superior adhesion to cleaner canal walls and increased canal wall sealing surface area.[21]

As we compare the leakage current values of all the Group A, Group B, Group C, Group D, Group E, and Group F, the effect of eliminating smear layer was more noticeable for the Groups with thermoplasticated gutta-percha obturation than for groups with laterally condensed gutta-percha.
These observations were similar to the studies by Homayouni.[22]

Within the limitations of this study, it is clearly evident that elimination of smear layer is advantageous to root canal sealing. Obturation with thermoplasticized gutta-percha promotes a better seal compared to lateral condensation, and canals instrumented with rotary NiTi files decreases the intensity of microleakage in root canals.

Further in vivo studies are necessary to evaluate the electrochemical mode of leakage for long-term analysis.

CONCLUSION
1. The study suggests that rotary instrumentation of the root canals provides a superior preparation in comparison to hand instrumentation
2. Elimination of smear layer boosts the resistance to microleakage
3. Thermoplasticized gutta-percha obturation demonstrates the production of an exceptional seal as compared to lateral condensation.

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Conflicts of interest
There are no conflicts of interest.

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