Lack of correlation between microbial penetration method and electro chemical technique for assessment of leakage through the root canal fillings

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Abstract

Aims: The purpose of this study was to compare microbial penetration method and electrochemical technique for evaluation of the apical seal. **Materials and Methods:** A total of 28 teeth were prepared using the step-back technique to ISO size 40 master apical files. The specimens were randomly divided into an experimental group, two positive and two negative controls. Root canals in the experimental and negative control group were filled with gutta-percha (GP) and sealer, using lateral condensation technique. In the positive control group, canals were filled with GP without any sealer. The external surface of each tooth was coated with two layers of the nail varnish, except for the access opening and the apical foramen. In the negative control group, the teeth were completely covered with nail varnish. The apical seal was recorded with two methods, each successively used on the same teeth: An electrochemical method and a bacterial penetration method. **Statistical Analysis Used:** The correlation of the electro chemical readings with the results obtained from bacterial micro leakage test was evaluated by Pearson's correlation coefficient. **Results:** Correlation coefficient of the measurements obtained from the two evaluation methods, was 0.23 ($r = 0.23$), so the correlation was not statistically significant ($P = 0.275$). **Conclusions:** This study shows that several studies by different methods are necessary before evaluation of the marginal leakage.

Keywords: Apical leakage, electrochemical technique, microbial penetration, root canal fillings

Introduction

Cleansing and shaping of the root canal system, followed by adequate obturation are the major objectives of endodontic treatment.[1] Root canal obturation provides a seal that prevents microleakage and subsequent reinfection of the canal and the periradicular tissues.[2] For this reason, different endodontic materials and obturation techniques have been applied for decreasing microleakage and improvement of seal.[3,4] Along with these improvements, various test methods have been described to assess the sealing ability of these materials and techniques.[5]

In vitro methods are used, generally can be divided in to two categories: Methods that used a tracer agent penetrating the filled canal and those that rendered without a tracer. Tracers such as, dyes, radioisotopes, bacteria and their products (i.e. endotoxins) are commonly used in microleakage studies. Other methods include fluid filtration technique, electro chemical technique and etc.

Assessment of bacterial leakage might be more biologically relevant than that of dye or radioisotope penetration, but this method has two limitations: Depending on the bacterial species has been used, conclusions might vary and maintaining aseptic condition throughout all steps of the experiment can be difficult.[6,7]

Jacobsen and von Fraunhofer depicted the electrochemical micro leakage method for the first time.[8] In this technique the tooth is immersed in an ionic solution (i.e. NaCl solution). A stainless steel wire (working electrode) is placed into the coronal access cavity, which was filled with the same ionic solution and another stainless steel wire (counter electrode) is submerged in to the solution. The two electrodes are attached to a constant power supply with a multimeter. As the leakage occurs, the solution penetrates through the apical seal. It is assumed that the magnitude of the current detected will indicate the degree of the penetration.[9]

The aim of this study was to compare the results of bacterial and electro chemical micro leakage tests and evaluate any correlation between these tests.

Materials and Methods

Preparation of samples

A total of 28 freshly extracted human maxillary and mandibular anterior teeth with a single, straight root canal

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were selected for this study. The exclusion criteria include: Existing cracks, large carious lesions, open apices and resorptive defects. After removal of bony debris, calculus and soft-tissues on the root surface, the teeth were stored in saline solution. All preparation and obturation procedures were carried out by a trained operator as described below.

The coronal fragments of all teeth were removed with a diamond disks and left roots with 15 mm length.[6] A diamond bur was used to gain a straight-line access to the root canal. Following pulp extirpation, a size 15 K-file (Dentsply, Maillefer, Ballaigues) inserted in to the canal until it was seen at the apical foramen. The working length was determined by subtracting 1 mm from this length. A size 15 file was percolated through the apical foramen before and after the root canal preparation to attain the apical patency. The root canals were instrumented using step-back technique to ISO size 40 master apical file within 1 mm of the apex.

A volume of 2 ml of saline solution were used for irrigation between each file size. After the completion of the preparation procedure, the teeth were randomly divided into one experimental group consisting of 24 samples, two positive and two negative control groups. The canals were dried using paper point (Sina Dent, Iran). The root canals were filled using lateral compaction technique as described below.

The ZOE sealer (Gholchai, Iran) was mixed according to the manufacturer instructions and introduced into the canal using a size 30 file with counter clock wise rotation. Size 40 master Gutta-percha (GP) cones (Diadent, Korea), lightly coated with sealer and was then placed to the full working length. Lateral compaction was achieved using size 25 accessory GP cones and a size B finger spreader (Dentsply Maillefer) that initially reached to within 1 mm of the working length.

The two positive control teeth were obturated as the same manner as experimental teeth but without any sealer. The two negative control teeth were obturated with GP cones and ZOE sealer. After the obturation procedures were completed, all roots were stored at 100% humidity for 24 h in order to allow complete setting of the sealer cement. Before the evaluation of the microleakage, the excess coronal GP was removed with a Peeso Reamer ISO size 3 (approximately 5 mm GP was remained in each root canal.). Apical sealing ability of the obturated canals was then assessed, using electro-chemical and bacterial micro leakage tests.

Electro-chemical micro leakage test

The exterior surfaces of the teeth were completely covered with two coats of nail varnish except for the access opening and the apical foramen. The root surfaces in the negative controls were entirely covered with two coats of nail varnish.

The roots were mounted with silicon through the bottom of plastic cylinders leaving the open access opening inside the cylinder. The cylinders were filled with saline as electrolyte. The cylinders with teeth were mounted in Petri dishes filled with saline electrolyte. Only 2 mm of the root endings were immersed in the solution.

For measurement, a #70 k file (Dentsply, Maillefer, Ballaigues) was placed in each upper chamber and a stainless steel wire was inserted into the Petri dish. The electrode in each upper chamber was separately connected to the electrode in the lower chamber through an electric circuit with an 8-v, DC power supply (Z-IC, 8V1A. Siehe ECA). The electrical conductivity in this circuit was measured in μA with a multimeter (Case, Japan) for each root.

Bacterial microleakage test

The teeth were inserted in to the plastic Eppendorf test tubes with screw caps and then fixed through it. A volume of 2 ml of sterile culture medium (Tripticase Soy Broth (TSB); Merck, Darmstadt, Germany) were added to sterile glass test tubes. The Eppendorf root assembly was mounted inside the test tube with the root tip contacting the culture medium.

A standard *Enterococcus faecalis* bacterium was cultured in Tripticase Soy Agar and then microbial suspension, with 0/5 McFarland score for turbidity, was prepared in TSB.

Using a sterile micropipette, the microbial suspension was placed into the Eppendorf test tubes in contact with the coronal access opening of the filled roots. The test assemblies were incubated at 37°C. The turbidity of the culture medium in the lower chamber was monitored daily for 20 days. As the turbidity was observed, microbial samples were cultured again to confirm the presence of *E. faecalis* bacterium.

The data were analyzed using SPSS software (SPSS ver. 11, Statistical Package for Social Science, IBM Corporation, NY-USA). The statistical significance was set as a 0.05 level. The correlation of the electro chemical readings with the results obtained from bacterial micro leakage test was evaluated by Pearson's correlation coefficient.

Results

Electro chemical method

The teeth in the negative control group showed no flow of electrical current. In the positive control group maximum current flow was recorded (90 μA). The results in the experimental group ranged from 10 μA to 60 μA.

Bacterial infiltration method

As expected; the negative controls showed no significant infiltration during the experimental period. Samples in positive control group showed infiltration after 1 day of contamination. In the experimental group, infiltration occurred between day 4 and day 17.

The results of leakage, obtained by electro chemical method and bacterial infiltration method were compared using Pearson's correlation coefficient [Figure 1]. Since, the correlation coefficient of the measurements obtained from the two aforementioned methods, was 0/23 (r = 0/23), the correlation was not statistically significant (P = 0.275).

Discussion

Various methods have been developed to assess the sealing ability of root canal filling materials.[10-14] The previous studies that measured and compared different methods for evaluation of the leakage, mostly, failed to show any correlation.[10,14] Barthel *et al*.[15] applied the dye leakage test after the bacterial test on the same teeth and found no correlation between these two tests. Pommel *et al*.[14] also compared fluid filtration, electro chemical and dye leakage tests for assessing the sealing ability of two obturation techniques, using the same teeth. They found no correlation among the tests. They described that this result was not surprising because the leakage phenomena are dependent to different factors.

In Modaresi *et al*.[16] study, electrochemical method was compared with dye penetration method. No correlation was found between two techniques. Delivanis and Chapman[9] compared the electrochemical method to the dye penetration or the radioisotope method. They found a correlation, but only at the two ends of the electric score range. Martell and Chandler[17] compared three root end restorative materials using the electrochemical and dye penetration methods and found a correlation between two methods. A study by Wu *et al*.[18] compared bacterial penetration to the fluid transport along root canal fillings. They found no correlation between two methods.

In the present study, the quantitative measurements recorded by electrochemical method and bacterial penetration method gave contradictory results. This may be due to the differences in working principles of various tests methods.

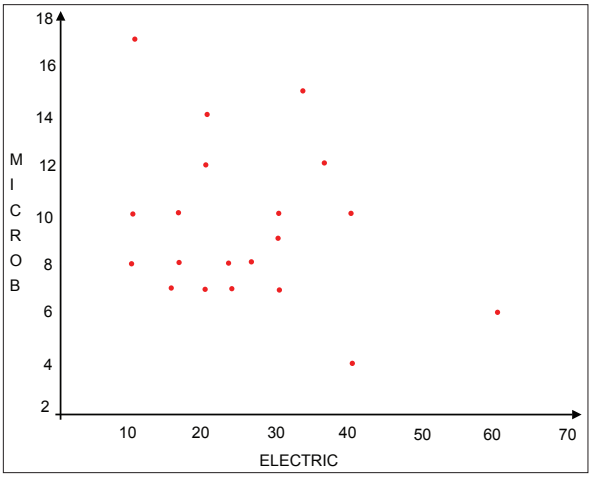

Figure 1: Scattering diagram of microbial penetration results and electrochemical leakage evaluation (r = 0.23)

The electrochemical method is based on the diffusion of ions through very narrow spaces and the outcome of this method likely depends on electrical laws.[14] It is assumed that the magnitude of the electrical current that produced by ions diffusion, between two electrodes is directly proportional to the degree of leakage. Any change in ion concentration can affect the results.

Seidler[19] has emphasized that all sealers undergo dimensional changes, these changes occur upon setting and dissolution in fluids. Dissolution of inorganic salts that used in sealers formulation may affect the ionic concentration.

Another parameter that could be measured in the electrochemical test is electrical resistance. Resistance and leakage related to each other reversely. As the leakage increases, the electrical resistance value declines.

Jacobson and von Fraunhofer[8] applied two different types of metal as electrode (stainless steel and copper). This procedure may lead to an electrical potential that creates between two electrodes and effects on our measurements. The results of electrochemical microleakage tests varied considerably. This may be partly because of differences in the composition of the electrolyte, electrode type and distance between the two electrodes, electrode thickness and electrical conductivity of the ionic solution.

We have used saline solution, as electrolyte, in our study because; osmotic pressure and ionic composition of saline solution are relatively similar to the interstitial fluid and may not interfere with the results obtained by electrochemical tests, as opposed to NaOCL solution. The ionic concentration in hypochlorite solutions is very high especially in thick ones. This may be impact on the electrical conductivity that measured by electrochemical test. Since the electrochemical method did not destroy the tooth structure, we could assess leakage in one sample, repeatedly.

According to Timpawat *et al*.,[7] use of bacteria for assessing the leakage (mainly coronal) is considered to be of greater clinical and biological relevance than the dye penetration method. Many different strains of bacteria have been used to detect marginal leakage and this has led to contradictory results, because the methods depend on the type of bacteria used. A reference *E. faecalis* strain was chosen because they are part of normal oral flora in humans and are frequently found in mixed infections with other aerobes and facultative anaerobes.[20-23] *E. faecalis* is also one of the most commonly isolated microbes from the root canal.[7] In this study, *E. faecalis* was selected due to ease of arrangement and interpretation of the data.

According to the results of this study there was not a significant correlation between electrochemical and bacterial penetration tests for evaluation of the leakage. Thus, the

clinical relevance of leakage evaluation *in vitro* may be questioned. Moreover, lack of the correlation between the two methods, which were applied in this study, is likely related to the differences in criteria. So that it is proposed that several methods of evaluation should be used, to have several sets of data before drawing any conclusion.

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