

Examining the Influence of the Smear layer on Permeability of Dentin Tubules in Human Teeth Using Dye Penetration Method

Mahdi Daneshi,¹

Department of Operative Dentistry, School of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran. Email: daneshmehdi6@gmail.com Orchid code:0000-0001-9574-9150

Seyedeh Faezeh Miryousefiata,²

Faculty of Biochemistry, Islamic Azad University Science and Research Branch, Tehran, Iran. Email: faezeh.ataee@yahoo.com Orchid Id: orcid.org/0000-0002-0615-7813

Corresponding Author: Mahdi Daneshi, Email: daneshmehdi6@gmail.com

Abstract

Background and aim: The influence of the smear layer on human dentin tubules has been a considerable issue in endodontic treatments. This study aims to measure the dye penetration depth into dentin tubules in the presence and absence of smear layer.

Materials and methods: Thirty healthy human teeth were used in this experimental study. First, a block with a length and diameter of 5 mm was prepared from the tooth roots. Then, their canals were widened using a burr with 1.4 mm diameter to form the Smear layer. The lateral, upper and lower surfaces of the blocks were coated with 2 layers of nail polish except for the entrance of the canals. Blocks were divided into two groups of 15. Specimens of the first group were placed in %17 EDTA for two minutes and then in 25.5% sodium hypochlorite for 20 minutes to remove the Smear layer. The Smear layer was not removed in the second group. Then, both groups' specimens were placed in 2% basic Fuchsin dye. After removing the specimens from the dye and washing them in water, they were all divided into two halves by a horizontal cut and images were taken from their upper surface. Images were then transferred to the computer and dye penetration was calculated by the Photoshop program. The results were analyzed using the Mann-Whitney Test.

Results: No significant difference was found in penetration into dentinal tubules in the presence or absence of the Smear layer ($p=0.120$).

Conclusion: Results of the study showed that removal of the Smear layer has no effect on dentinal penetration depth and dentinal sclerosis plays an important role in this regard. More studies are recommended using other methods such as microbial penetration into the teeth with specific age.

Keywords: Smear layer, EDTA, dye penetration

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Introduction

Dental cleaning is the most basic and important part of the treatment and any mistake made in this process may lead to the treatment failure. Cleaning can be accomplished either chemically or mechanically. Mechanical cleaning is carried out through filing. Since all surfaces of the root-canal system and the space inside the dentine tubules are not in contact with the file and yet microorganisms and contaminated substances can penetrate into the dentine tubules, it becomes necessary to use such compounds as Calcium Hydroxide for chemical cleaning as well. Several factors such as the number and diameter of dentin tubules, the presence of sclerotic dentin as well as the smear layer can affect the dentin permeability and prevent the proper penetration of antimicrobial substances into the dentine tubules ¹.

During canal preparation using mechanical instruments, amorphous, granular, and irregular layer consisting of dentin minerals and organic materials such as pulp residuals, odontoblastic wastes, saliva, blood cells and bacteria cover the surface of the canal which is called as the Smear layer². The Smear layer plays an important role in the tooth root treatment because of its effect on the penetration of canal fillers and sealers as well as the penetration of cleaning materials and disinfectants into the dentinal tubules. Moreover, microbes in the Smear layer can also cause infection. This issue has long been a concern for investigators and several studies have been conducted on the necessity of removing the smear layer and the role it plays in successful treatment of the root. Meta-analysis conducted by Shahravan et al (2007) showed that removal of the smear layer can enhance the sealability of canal obturation³. Laboratory studies conducted by Foster (1993), Guignes (1996), Berutti (1997), Calt & Kokkas (1999) and Yildirim (2008) showed increased permeability of the dentin and more penetration of cleaning materials, sealers and Calcium Hydroxide into the dentinal tubules following the removal of the smear layer ⁴⁻⁹. However, some investigators including Paque (2006) and Engle (2005) do not approve this result ¹⁰⁻¹¹. Various methods have been proposed for smear layer removal including chemical, mechanical, Ultrasonic, and Lasers. ¹²⁻¹⁴. other materials such as MTAD and Maleic Acid are also suggested in this study ¹⁹⁻²⁰.

Because of disagreement over the role of smear layer in dentin permeability and the necessity for its removal, this study was conducted to find the effect of smear layer removal on the permeability of dentinal tubules using the dye penetration method.

Materials and methods

Thirty healthy human teeth with single roots and one root canal with no crack were selected in this experimental study. No information was reported about the age, sex or the reason for tooth extraction. All the teeth were stored in 10% ethyl alcohol solution until the beginning of the study (8). Periodontal fibers were removed from the external surface of teeth roots by Scale; then the teeth roots were removed from the crown at CEJ, the coronal and apical portion of the roots

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were cut by a diamond disc (Tizkavan, Iran) so that a 5 mm cylinder was left from the middle part of each root.

In the next phase, root canals were widened to a diameter of 1.4 mm using a conical cylinder diamond bur (Tizkavan, Iran) to form the smear layer. Lateral, upper and lower surface of roots, except for the entrance of canals, were coated with two layers of nail polish so that the dye solution to be used in the next stage could not penetrate into the dentinal tubules.

The prepared teeth were randomly split into two groups of 15. Teeth of the first group were stored in %17 EDTA for two minutes and then in %5.25 NaOCl for 20 more minutes to remove the smear layer. The smear layer remained intact in the second group. Then, all specimen were washed using physiological serum and they were stored in %2 basic Fuschin dye solution for 24 hours. The teeth were washed in running water for 30 minutes, and their canals were dried by an air blower. At the end, each root was split in half horizontally using a diamond disc (Tizkavan, Iran). Photos were taken from the split portions by a digital camera (Olympus S550, made in Indonesia) (figure 1).

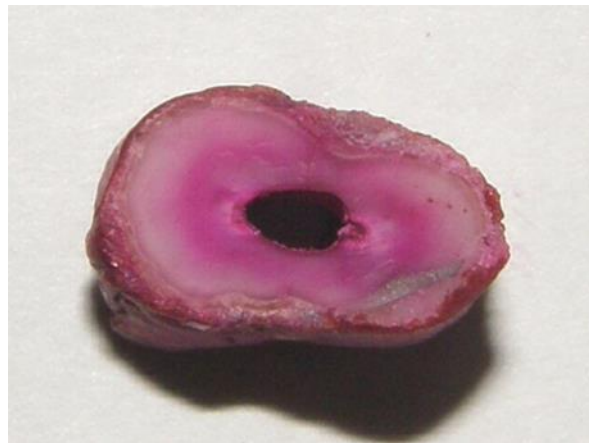


Figure 1-different depths of dye penetration into dentinal tubules

Images were transferred to the computer and magnified 18 times using the Photoshop program and they were placed in a checkerboard. The proportion of color penetration to the entire surface of the teeth was then measured. To do this, the number of colored sub-squares was divided by the number of the entire squares, whether painted or unpainted, and the results were expressed as percentages. Finally, the results were analyzed by Mann-Whitney test using SPSS. The type I error was considered as <0.05 .

Results: According to the results, the average of the percentage of the painted to unpainted surfaces in group 1 (with smear layer) and group 2 (without smear level) were 30.28 %, %39.24 respectively (with standard deviation of 29.33 and 26.89, respectively). Minimum and maximum penetration rate was 2.96% and 73.14%, in group 1, respectively; these amounts were 5.62% and 80.91% in group 2, respectively.

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Mann-Whitney test analysis indicated no significant difference in dye penetration depth in two groups (P-value=0.120).

Discussion: The smear layer is composed of organic material and minerals which is formed and covers the dentin surface during the preparation of the canal using mechanical instruments. Several studies prove the presence of bacteria in dentin tubules¹⁹⁻²⁰. The rate of bacteria penetration into the dentin tubules depends on several factors including the number and type of bacteria, the exposure time, density and diameter of dentin tubules. Meanwhile, the role of smear layer in permeability of dentin tubules and, therefore, penetration of microbes and disinfectants into the dentin tubules have been discussed in the past years.

Most studies, including the ones conducted by Kokkas (2004), Guignes (1996), Calt (1999) and Berutti (1997) have reported reduced permeability of the dentin in the presence of the smear layer⁵⁻⁸. On the other hand, some studies like Paque (2006) and Engel (2005)^{10& 11}, indicate that removal of the smear layer has little effect on penetration of ions after filling the canal with Calcium Hydroxide-the finding which conforms to the results obtained in this study. Different results obtained from the studies conducted on the effect of the presence of smear layer on dentin permeability can be related to the different methods used regarding the type of the material under investigation and the methods used to measure the permeability.

These studies often calculate the penetration of sealer²¹ or disinfectants^{13&14} into dentin tubules. Obviously, difference in the molecular size of the material, their chemical and physical properties, how and how long they remain in canal, Hydraulic forces formed during filling the canal and capillary action are important factors that may affect study results. Considering the importance of adjusting the variables and to ensure adequate penetration of EDTA and NAOCL into the canals, they were widened by a bur, which looks like the use of big gates gliddens in canals. Concerning the calculation of permeability, various methods such as injection of radioactive materials, hydraulic transfer and electron microscope are available; however, dye penetration is still used as a simple and reliable method to study how molecules diffuse into the dentin. Though it should be noted that this method cannot be used to follow the penetration of dye into a particular dentin tubule and the obtained result will indicate permeability of the entire dentin not a particular dentin tubule. For more accurate examination and observing the details electron microscope can be used which can reflect both details of dentin tubules and properties of the surface of material. A problem with electron microscope is that, unlike dye penetration method, it deprives the researcher of accessing a general view because of little magnification of the surface under study. Moreover, during the preparation stages for SEM, Artifact may be created.

Concerning the dye penetration method, though it may seem in theory that the observed dye penetration depends on the path and the angle of dentin tubules in the section under study, the observed dye includes deeper layers as well because the dentin is to some extent transparent;

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therefore, the path of dentin tubules in the section will not be effective in calculations. One problem with dye penetration method is reduced color intensity in the areas far from the dentin (toward the cementum) which is caused by reduced density and diameter of dentin tubules in these areas. Magnification can solve this problem to some extent.

A stereomicroscope is usually used to see and calculate the dye penetration depth. However, in this study, digital photos were taken from the surface of the specimen; they were transferred to the computer and then the percentage of the painted surface was calculated using Photoshop program. Image magnification in such cases may cause problem in the values obtained from studies on calculating the linear dye penetration. But no problem occurred in this study because we aimed at calculating the percentage of the painted surface of the root in proportion to its total surface.

Like most of the previous studies, EDTA and NaOCl were used to remove the smear layer ^(22& 23). Little information is available about the effect of EDTA application time and its influence on dentin permeability but in the studies similar to the present one, the specimens are stored for some minutes in EDTA. Applying EDTA for more than 20 minutes may result in excessive demineralization and occlusion of dentin tubules ¹⁰.

The application time for storing in dye has been different in previous studies. We applied EDTA for 24 hours to provide enough time for the paint to penetrate into the dentin tubules and reach the maximum possible expansion. It is noteworthy that the paint was less expanded to the mesial and distal surfaces of the teeth compared to the Buccal and Lingual sides and this conforms to the results of paque (2006) ¹⁰. This occurs because sclerotic dentin is found in these areas. The effect of this phenomenon on dentin permeability has been known for a long time, but its importance in endodontics was first proposed by Weis (2004) ²². Butterfly shape of the dye penetration is due to the dentinal sclerotic areas where paint is not penetrated and this is the typical view of dentinal sclerosis. This is reflected in such studies as Peters (2001) ²³ and Shovelton (1964) ²⁴. Weis (2004) ²² and Mamootil (2007) ²⁵ also reported better penetration of sealer in the Buccal and Lingual surfaces of the root. Since sclerotic dentin occurs due to excessive physiological accumulation of dentin around the tubule which begins from the third decade of life from epical areas of the root and extends to the coronal area with increasing age, the age of the patients should be considered in the studies on permeability of dentin. The clinical significance of the issue is that since the teeth used in the studies often belong to patients with advanced periodontal problems or unsuccessfully treated tooth roots, they are commonly over 30 years old and this causes increased possibility of sclerotic dentin which may spread in their teeth. Mjor (2001) ²¹ indicated dentin is not permeable to disinfectants and bacteria in the apical third of most treated teeth. Nair (2005) ²⁶ reported that in the teeth with acute periodontitis, most of the bacteria are found in the apical opening of the root, where tissue fluid is accessible. Therefore, reduced probability of penetration of microbes into the dentin tubules with increasing

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age, as well as their obstruction by the smear layer in the dentin tubules will question the results of the studies conducted on contaminated dentin tubules in laboratories; though this finding cannot lead us to come to the certain conclusion that the smear layer should not be removed.

Conclusion

Although the results obtained from this study indicate that removal of the smear layer has no effect on the dentin permeability to dye and dentin sclerosis plays an important role in this regard, and since various methods used to conduct these studies may produce different results, more studies are suggested to be carried out on the teeth with specific age using other methods such as examining the microbial permeability.

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