Research Article

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A Comparison of two Dyes Used for Leakage Analysis of A Root Canal Sealer

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ABSTRACT

Background: To compare, in vitro, the efficacy of two different dyes used for microleakage analysis of an endodontic sealer based on zinc oxide & eugenol. The two dyes used were Hematoxyline and Propiodium Iodide.

Methodology: 30 single rooted human teeth were selected & cleaned of all debris. The coronal part of each tooth was removed with carborundum disks. They were then divided into 2 different groups, namely, Hematoxyline dye group (15 teeth) and Propiodium Iodide dye group (15 teeth). Each group was further divided into 3 sub-groups: Sub-group A - Negative control (non-instrumented), Sub-group B - Positive control (instrumented), Sub-group C - Experimental (obturated). The specimens of each group were immersed in their respective different dyes. After washing the samples, they were longitudinally sectioned, through the centre of the root, at the cemento-enamel junction. Microleakage was then assessed using a phase contrast and fluorescent microscope.

Results: The mean leakage values, as determined by Propiodium Iodide dye penetration were significantly greater than that quantified by Hematoxyline dye penetration.

Conclusion: Within the limits of this in vitro study it can be concluded that the staining provided by Propiodium Iodide dye was significantly greater than the staining provided by Hematoxyline dye.

Keywords: Propiodium Iodide dye, Hematoxyline dye, Lateral condensation, Endoflux, Dye penetration, Microleakage, Fluorescent microscopy.

INTRODUCTION

The complete obturation of the root canal system by using filling materials with adequate biological & physiochemical properties is one of the main goals of root canal treatment. Sealing of the root canal mostly includes the use of a semisolid core filling material gutta-percha & a sealing cement. The endodontic sealer is required to adhere to the dentin & fill the microspaces between core filling material & dentinal walls. (Garcia-Molina et al 2005). [6]

One of the most widely used methods for evaluating the sealing capacity of any root canal sealer is the in vitro study of microleakage analysis where the tooth is immersed in a dye solution & quantitative measurements are made of the degree of dye penetration observed which would judge the leakage of the sealer being examined.

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The purpose of this study was to investigate, in vitro, two different dyes used for microleakage analysis of an endodontic sealer based on zinc oxide & eugenol. The two dyes used were Hematoxyline dye and Propiodium Iodide dye.

MATERIALS AND METHODS

This in vitro study was carried out on single rooted extracted teeth in the Department of Conservative Dentistry at our institute. The Dyes used were Hematoxyline dye (conventional) and Propidium Iodide dye (fluorescent). Equipment used was Phase contrast and fluorescent microscope.

Teeth:

A total of 30 single rooted, human teeth were randomly selected, carefully cleaned to eliminate tartar and tissue remains, followed by storage in a 0.5% dilute solution of sodium hypochlorite for one week for cleaning purpose and then stored in isotonic saline solution at room temperature until used.

Experimental Groups and Controls:

30 teeth were divided into 2 groups: Hematoxyline dye group (15 teeth) & Propidium iodide dye group (15 teeth)

Each group was further divided into 3 sub-groups:

A - Negative control sub-group (non-instrumented)

B - Positive control sub-group (instrumented)

C - Experimental sub-group (obturated)

Thus the teeth were equally divided into 6 sub-groups (n=5); out of which 2 were experimental groups whereas 4 groups served as controls i.e. 2 positive and 2 negative control groups. The coronal part of each tooth was removed with carborundum disks. Out of the 30 teeth, 10 randomly selected teeth were left untreated & were noninstrumented and served as negative controls. The preparation of the remaining 20 teeth was carried out.

Preparation of Root Canals:

The canals of experimental and positive control sub-groups were instrumented with K-type files, using step-back technique. The working length was established 1 mm short of the apex. After every change of a file size and at the completion of instrumentation, the canals were irrigated with 2 ml of 0.5% solution of sodium hypochlorite.

Obturation of Root Canals:

Only 2 experimental sub-groups were obturated with gutta-percha and sealer by a cold lateral condensation technique. For each canal a standardized mastercone was selected that fitted snugly at the working distance. The teeth were radiographed in a proximal (mesiodistal) view to verify correct obturation.

Assessment of Obturation by Dye Penetration Test

After sample preparation, all the teeth including experimental and control groups were left in physiologic saline solution for 1 week at 37°C. Then all the teeth were dried with an air jet and their root surfaces were coated with a first layer of nail varnish, covering the whole root surface, but leaving a 2 mm area around the apical foramen uncoated. One hour later, a second coat was applied. The root surfaces of the negative controls were entirely coated with two layers of nail varnish to prevent possible leakage. After 1 hour of drying, all samples were divided into 2 groups and immersed in standardized freshly prepared solution of two different dyes after dividing the samples into 3 sub-groups (1 experimental, 1 positive control and 1 negative control) for each dye.3 sub-groups of the samples were immersed in Hematoxyline dye while the other 3 sub-groups of the samples were immersed in Propidium Iodide dye for 25-30 minutes. Dye penetration was assessed following removal of nail varnish. After washing the teeth, the samples were longitudinally sectioned through the centre of the root by using carborundum disks. The resulting sections were then examined under a phase contrast and fluorescent microscope. Samples were observed under 100X magnification. For Propidium Iodide visualization a fluorescent filter was used. For making measurements in micrometers of the dye leakage, an occulomicrometer was used. An image analysis detector was used for accurate detection of area of root canal covered by the dye.

Observations and Results:

The microleakage was analyzed for each group in two ways: the maximum depth of penetration of the dye in the root canal and the percentage of the teeth area covered by the leakage. The positive controls showed dye penetration in all or much of the root canal; in contrast, very less leakage was seen in the negative controls. The Experimental groups also showed some leakage.

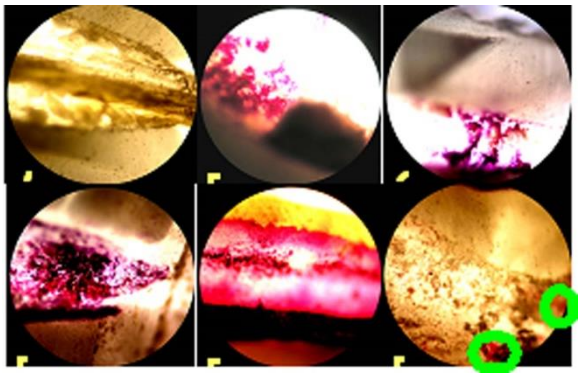

Fig 1: Representative Microphotographs of root canals exposed to Hematoxyline (HE) A: Tooth canal without any exposure to HE; B & C: Un-instrumented root canal; D, E: Instrumented root canal; F: Obturated root canal

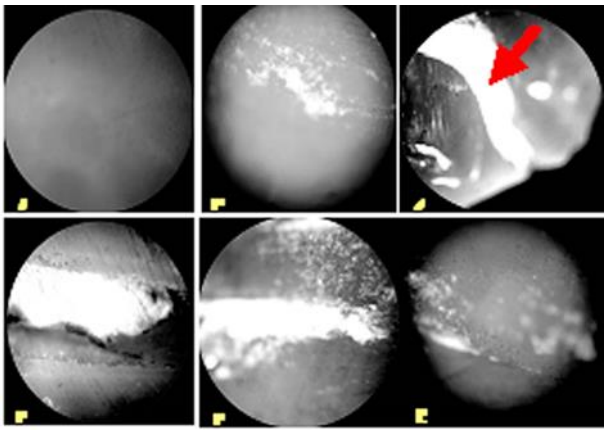

Fig 2: Representative Microphotographs of root canals exposed to Propidium Iodide (PI) A: Tooth canal without any exposure to PI; B: Un-instrumented root canal; C, D, E: Instrumented root canal; F: Obturated root canal.

Statistical evaluation of the results revealed significant differences in leakage between the two dyes. The mean leakage values, as determined by Propidium Iodide dye penetration were significantly greater than that quantified by hematoxyline dye penetration.

Table 1.1: Mean leakage values of the depth of dye penetration of Hematoxyline & Propidium Iodide groups.

S.No	Treatment Group	Mean SE area of dye penetration(mm)	
		Hematoxyline staining	Propidium Iodide staining
1A	Negative control	91.00- 0.966	197.00-13.061
2B	Positive Control	16987.00-182.545	1865.70-132.403
3C	Obturated sub-group	1185.30-31.352	2097.20- 56.312

Table 2.1: Mean leakage values of the percentage of teeth area covered by the dye leakage of Hematoxyline & Propidium Iodide groups.

Group	Treatment Group	Mean SE leakage (% of teeth area)	
	Mean SE area of teeth (mm)	Hematoxyline staining	Propidium Iodide staining
(A)Negative control	92.41-8.538	0.098-0.009	0.232-0.31
(B)Positive Control	91.80-5.80	18.500-2.884	20.252-1.425
(C)Obturated sub-group	93.15-5.583	20.252-2097.	20- 56.312

DISCUSSION

An endodontic filling is required to obtain a hermetic seal of the root canal using sealers which form a fluid tight seal and barrier between the dentin and gutta-percha apically, laterally, and coronally. Filling the root canal with cold or warm gutta-percha without a sealer results in increased apical leakage regardless of the obturation technique used (Schafer & Olthoff, 2002).[16]

The dye leakage evaluation is the most commonly used technique, mainly because it is the simplest method for assessment of the microleakage of a root

canal filling. (Dandakis et al, 1994).[5] The methodology uses tooth immersion in various types of dyes. Next, the teeth are sectioned longitudinally, transversely or cleared and the linear penetration of the dye is recorded. (Zmener et al, 2005).[18]

This dye is significantly more sensitive than a conventional dye. Thus dye penetration can be pinpointed; unlike that in visible dyes, where the relation in dye penetration and its visualization can change which may lead to a misinterpretation of results. This would not happen while using a fluorescent dye, and thus, the quality of observations would not be affected. For dye leakage studies, Rhodamine B fluorescent dye has also been used (Patel et al, 2007,[14] Ainley E. James, 1970.[2])

The aim of this study was to investigate in vitro two different dyes, namely, Hematoxyline and Propiodium Iodide, used for microleakage analysis of a sealer based on zinc oxide & eugenol and then to make a comparison between the two dyes for assessment of the degree of dye penetration afforded by them. Propidium Iodide is a fluorescent dye which stains selectively the apoptotic cells with a red stain. Hematoxyline dye is a biologic dye which is used conventionally for leakage studies & it gives a pinkish-red stain. For this study, propidium iodide has shown better linear penetration since it gives pinpoint results because dye penetration can be accurately seen.

The sealer used was Endoflux (Ammdent, Safe Gronet, India). It is a zinc oxide and eugenol based biocompatible sealer that has broad spectrum antibacterial properties. (Lucena–Martin et al, 2002).[13] assessed the apical sealing afforded by three cements– Endomethasone (ZnOE–based), TopSeal (resin–based) and RoekoSeal (polydimethylsiloxane–based) along with obturation of the root canals by laterally vertically, condensed gutta–percha. This is an interesting consideration because the study compares a recently introduced product (Roekoseal) with materials like zinc oxide & eugenol based sealer whose apical sealing efficacy has been already tested by years of clinical experience and in vitro research (Almeida et al, 2000).[1] Because of the placement of many accessory gutta–percha cones during lateral compaction, the volume of gutta–percha in the root canal is increased; more than 80% of the canal is filled with gutta–percha (Gencoglu et al, 2002).[7] The reason to favour a high gutta-percha/sealer ratio is that most sealers dissolve with time (Peters D D, 1986,[15] Kontakiotis et al, 1997.[10]) and cannot provide long-term seal. Lateral compaction of gutta-percha with sealer is a popular root canal filing technique against which other techniques are measured. In some cases, it has been proved better than other methods. (Hopkins et al, 1986,[9] Lares & ElDeeb, 1990,[11] Chohayeb A, 1992.[3])

In other reports, it was found to be as effective as other techniques. (Luccy et al, 1990,[12] Scott et al, 1992.[17]) But in other citings, it proved not as adequate. (Czonstkowsky et al, 1985,[4] Hata et al, 1992.[8]) Further and more extensive research is needed to corroborate these findings.

CONCLUSION

Within the limits of this in vitro study and according to the methodology and the results drawn, the following statement was concluded: In the experimental as well as the control sub-groups, the staining provided by Propidium Iodide dye was significantly greater than the staining provided by Hematoxyline dye.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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