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The effect of endodontic materials on the optical density of dyes used in marginal leakage studies

Abstract: The aim of this study was to determine the effect of the exposure of different endodontic materials to different dye solutions by evaluating the optical density of the dye solutions. Seventy-five plastic tubes were filled with one of the following materials: AH Plus, Sealapex, Portland cement, MTA (Angelus and Pro Root) and fifteen control plastic tubes were not. Each specimen of material and control was immersed in a container with 1 ml of each dye solution. A 0.1 ml-dye solution aliquote was removed before immersion and after 12, 24, 48 and 72 hours of each specimen immersion to record its optical density (OD) in a spectrophotometer. Statistical analysis was performed with ANOVA and Tukey tests (5%). No significant difference was found among any of the solution OD values for AH Plus cement. Portland cement promoted different OD values after 12 hours of immersion. MTA-Angelus cement presented different OD values only for 2% rhodamine B and the MTA-Pro Root cement presented different OD values in all 2% rhodamine B samples. Sealapex cement promoted a reduction in the India Ink OD values. Dye evaluation through OD seems to be an interesting method to select the best dye solution to use in a given marginal leakage study.

Descriptors: Dental leakage; Methylene blue; Spectrophotometry; Methods.

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Introduction

Many dyes in different concentrations have been used in leakage studies, such as 5% eosin,¹ India ink,^{1,2} silver nitrate,³⁻⁵ methylene blue^{1,5-8} and rhodamine B.^{5,8-10} However, dyes can chemically interact with sealing materials or dentin, which may influence its diffusion or promote tracer decoloration, impairing an adequate marginal leakage evaluation.^{7,11-13} The purpose of the present study was to determine the different material exposure effects on a 2% methylene blue solution in phosphate buffer at pH 7 (MB), on blue India ink in phosphate buffer at pH 7 (BII), and on a 2% rhodamine B solution in phosphate buffer at pH 7 (RB) after different immersion periods using optical density (OD).

Material and Methods

A methylene blue solution in phosphate buffer at pH 7 (MB) was prepared using 2 g of methylene blue (Synth, Labsynth Produtos para Laboratórios Ltda., Diadema, SP, Brazil) in 100 ml of 0.2 M phosphate buffer at pH 7.0. Blue India ink (BII) (Quink, Parker Pen Holding Ltd., Epping, England, $\text{pH}_{\text{initial}} = 2.47$) was buffered by addition of 5.4 g of sodium phosphate dibasic ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, Reagentes Ecibra, Equipamentos científicos do Brasil, São Paulo, SP, Brazil) and 2.78 g of sodium phosphate monobasic ($\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$, Reagentes Ecibra, Equipamentos científicos do Brasil, São Paulo, SP, Brazil) in 100 ml of India ink. After total salt dissolution, pH was adjusted to 7 with sodium phosphate dibasic, 2 M solution drops. Two percent rhodamine B solution in phosphate buffer at pH 7 (RB) was prepared dissolving 2 g of rhodamine B dye (Synth, Labsynth Produtos para Laboratórios Ltda., Diadema, SP, Brazil) in 100 ml of 0.2 M phosphate buffer at pH 7.

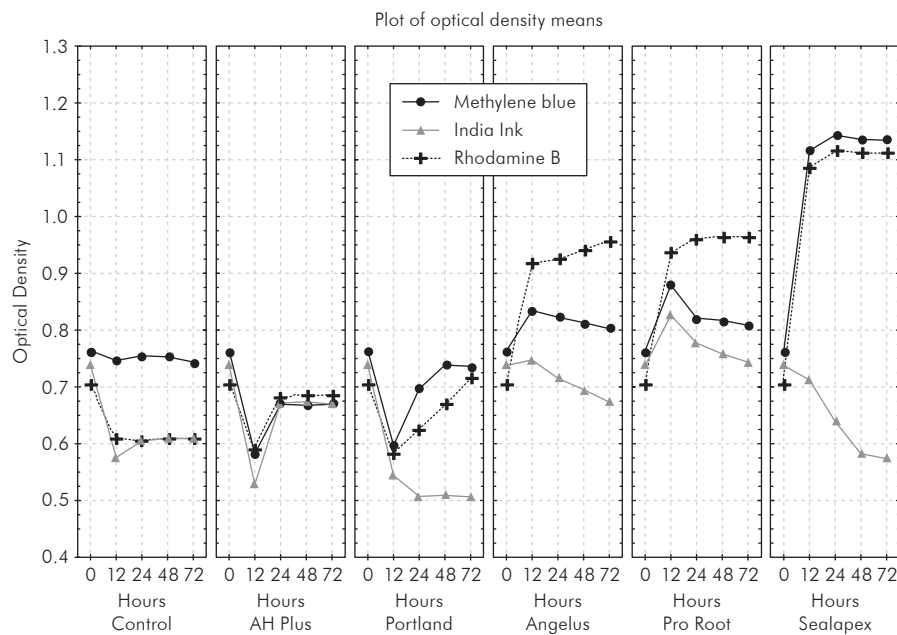
The wavelength at maximum absorption of the dyes was determined with the scan spectrum ranging from 100 to 1,000 nm in a Shimadzu spectrophotometer (Model UV1203, Kyoto, Japan). The methylene blue solution presented maximum absorption at 596 nm, the blue India ink, at 585 nm, and the rhodamine B dye solution, at 566 nm. Ninety 1 mm-thick wall, 2 mm-inner diameter and 10 mm-long standardized plastic tubes (Embramed equipo, Embramed Ind. Com. Ltda., São Paulo, SP,

Brazil) were prepared. Plastic tubes were used because they allow the production of specimens with standardized shape and size according to the pilot study, and serve as a matrix for the different dental filling materials. The materials tested were AH Plus endodontic cement (Dentsply De Trey GmbH, D-78467 Konstanz, Germany), Sealapex (Kerr Corp., subsidiary of Sybron, Orange, CA, USA), Portland cement (CPII type, Votorantim, Votorantim, São Paulo, Brazil), MTA-Angelus (Odonto-Lógica, Londrina, PR, Brazil) and Pro Root-MTA endodontic cements (Dentsply, Tulsa Dental, Tulsa, OK, USA). The materials were manipulated according to the manufacturers' recommendations and the tubes were filled using a lentulo size 4 (Maillefer S.A., Ballaigues, Switzerland) mounted on a contra-angle handpiece (KaVo do Brasil S.A., Joinville, SC, Brazil) running at moderate speed. Fifteen specimens were obtained per material. Specimens were kept in 100% humidity at 37°C for 24 hours to allow the materials to set before dye immersion.⁷ Fifteen plastic tubes were not filled by any test material and served as the control group. Five specimens of each material were immersed individually in the MB, BII and RB solutions. Each specimen was immersed in 1 ml of the dye solution and stored in an amber glass container.

0.1 ml-aliqouts of sample dyes were removed from the glass containers, put in a test tube, diluted in 1.9 ml of deionized water. Optical densities were registered in a Shimadzu spectrophotometer (Model UV - 1203, Kyoto, Japan) at 596, 585 and 566 nm for methylene blue, India ink and rhodamine B, respectively. Optical densities (OD) were registered before (t_0) and after 12, 24, 48 and 72 immersion hours of each material. Absorbance data were submitted to the three-way ANOVA and the Tukey multiple comparison test was used. Both statistical analyses were performed at the 5% significance level.

Results

Graph 1 shows the OD means for each dye before and after each immersion period for each material analyzed. The ANOVA test showed significant variability among all factors analyzed ($p = 0.00$).



Graph 1 - Optical density means observed for each dye in each period analyzed, grouped by material sample.

2% methylene blue aqueous solution in phosphate buffer at pH 7 (MB)

In relation to t_0 , it was observed that the control group did not show significant changes ($p > 0.05$) for OD at any analysis interval. AH Plus and Portland cements decreased OD values. MTA-Angelus, MTA-Pro Root and Sealapex increased OD in relation to t_0 . However, only in the Sealapex group these differences were significant ($p > 0.05$) (Graph 1).

India ink in phosphate buffer at pH 7 (BII)

There was a tendency for decreased OD values in control specimens, and in the AH Plus cement, Portland cement, MTA-Angelus and Sealapex groups. However, these differences were significant only in control specimens ($p < 0.05$), and in those of the Portland cement ($p < 0.05$), and Sealapex groups after 48 hours ($p = 0.00$) and 72 hours ($p = 0.00$) (Graph 1).

Aqueous solution of 2% rhodamine B in phosphate buffer at pH 7 (RB)

In relation to t_0 , immersion of specimens in the control and AH Plus cement groups presented tendency to decrease dye OD values. However, the difference was significant ($p = 0.03$) only for the 12-hour period of AH Plus cement. For the MTA-

Angelus, MTA-Pro Root and Sealapex groups, there was significant OD increase in all time periods ($p < 0.05$) (Graph 1).

Material effect on dye OD

The optical density observed after immersion of the control group in BII (pH 7) and in RB (pH 7) was statistically the same ($p > 0.05$) for each period analyzed (12, 24, 48 and 72 hours) and was statistically different when compared to that observed after immersion in MB (pH 7) (Graph 1). The OD values for all dye solutions after AH Plus cement immersion were statistically similar. A significant OD reduction was observed only for the first 12 hours (Graph 1).

The OD values observed after Portland cement group immersion in MB or RB were statistically the same for each period analyzed. However, for BII the OD values were statistically different ($p < 0.05$) after 24, 48 and 72 hours (Graph 1). In the MTA-Angelus and MTA-Pro Root cement groups, RB OD values were statistically higher than those observed with MB after 48 and 72 hours of immersion and higher than those observed with BII after 12, 24, 48 and 72 hours (Graph 1). In the Sealapex cement group, no statistical differences were observed in OD values after immersion in MB or RB. However, higher significant differences for OD values of MB

and RB were observed when compared to those observed with BII for each period analyzed.

Discussion

Methylene blue is susceptible to oxidation and amino groups are readily hydrolyzed to thional (colorless compound – pH > 7), or can decolorize when in contact with zinc or sulfuric acid solutions.¹³⁻¹⁵ According to Starkey *et al.*¹⁶ (1993) and Wu, Weselink¹¹ (1993), specimen immersion in different solutions of MB with pH ≤ 5, for different periods can demineralize the dentin and allow dye penetration. Phosphate buffer was chosen because it allowed the use of these solutions at pH = 7 and because it prevents pH variation.¹⁷

Wu *et al.*¹³ (1998) and Oztan *et al.*⁷ (2001) analyzed dye stability before and after contact with a variety of dental materials. In their study, five specimens were filled with materials in plastic tubes and were exposed to 0.8 ml of a given dye in one of its extremities to simulate a marginal leakage by dye experiment. In this study, a specimen filled with each material in a plastic tube was immersed in a 1 ml-container filled with one of the three dye solutions evaluated, which allowed material contact with dye in both extremities.

Wu *et al.*¹³ (1998) also used a 596 nm wavelength to determine changes in OD for methylene blue solution at pH 7. However, Oztan *et al.*⁷ (2001) did not take into consideration the type of dye and used a 416 nm wavelength to determine the dye change at optical density. OD dye analysis at t_0 was performed before material immersion, following the same Wu *et al.*¹³ (1998) protocol. However, the difference in methodology, use of buffer and concentration of the methylene blue solution may have contributed to the difference in control group OD values at t_0 in the present study ($OD_{MB\ 2\%} = 0.764 \pm 0.039$) comparing to the results found by Wu *et al.*¹³ (1998) ($OD_{MB\ 1\%} = 0.639 \pm 0.002$).

Oztan *et al.*⁷ (2001) observed different OD values among different experimental groups at t_0 because evaluation was performed immediately after material immersion in dye. The dye solution buffering and the wavelength difference ($\lambda = 416$ nm) used by Oztan *et al.*⁷ (2001) to evaluate dye solution OD could

have contributed for the different control group/MB OD values at t_0 . In the present study, the India ink evaluated was blue while Oztan *et al.*⁷ (2001) used black India ink. The color of the India ink, the buffering, and methodology differences could have contributed to the different OD values observed for the control group in the present study at t_0 .

In the present study, the AH Plus cement group promoted continuous OD reduction of all dye solutions used. Oztan *et al.*⁷ (2001) observed continuous OD increase when AH Plus cement specimens were immersed in 2% methylene blue solution at pH 7. The 2% methylene blue solution buffering in the present study could have avoided the interaction between the dye solution and the AH Plus cement chemical compounds, thus avoiding cement structure particles transference to the solution.

Estrela *et al.*¹⁸ (2000) observed that MTA and two brands of Portland cement contained the same chemical components, except for the bismuth present in MTA. In the present study, there was no statistical difference between MTA-Angelus and MTA-Pro Root specimens immersed in the same solution and for the same period of time (Graph 1). MTA-Angelus and MTA-Pro Root are brand names for mineral trioxide aggregate, which, according to Wu *et al.*¹³ (1998), can decolorize the methylene blue solution when used in marginal leakage studies. MTA has calcium oxide in its composition and, when it reacts with water, it forms calcium hydroxide which dissociates and increases the pH environment, promoting solution decolorizing.^{7,13,19,20} However, differently from Wu *et al.*¹³ (1998), in the present study the dye solutions were prepared with phosphate buffer at pH 7, and when 2% MB aqueous solution (pH 7) and BII (pH 7) were evaluated, no OD value reduction was seen after MTA immersion (both Angelus and Pro Root). Similar results were observed when Portland cement specimens were immersed in 2% MB aqueous solution and in RB for 24, 48 and 72 hours (Graph 1).

Kubo *et al.*⁹ (2005) evaluated the seal of root apexes retrofilled with Pro Root for marginal leakage with RB aqueous solution (pH 7) and dye penetrated 1.89 mm. In previous studies,^{6,8,21} it was observed that the dye solution penetrated from 0.28 to 0.31 mm. For Oztan *et al.*⁷ (2001), the OD means

increase caused by transfer of soluble particles of the material tested to the dye solution interferes in dye penetration evaluation in marginal leakage studies. In the present study, a bigger RB interaction with MTA-Angelus specimens and MTA-Pro Root specimens was observed, thus promoting a significant OD increase after t_0 . On the other hand, a small OD decrease is better than its increase because a small OD reduction indicates dye soluble particle transfer to the material tested, which does not interfere in marginal leakage studies.⁷ However, an OD decrease can also be caused by material soluble particle interaction with the dye solution by formation of a colorless chemical solution.¹³

The Portland cement group presented OD reduction and the lowest OD values after 24, 48 and 72 immersion hours in BII (pH 7) (Graph 1). Moreover, dye coloration intensity reduction could even be visually observed over time.

Sealapex is an endodontic cement, with a calcium hydroxide base containing zinc oxide and reducing agents, and is able to decolorize methylene blue.¹³ Oztan *et al.*⁷ (2001) observed MB OD increase after contact with Sealapex specimens. In the present study, the Sealapex group promoted also significant OD increase in contact with MB and RB (Graph 1). The Sealapex group with BII reduced the dye OD values and visually presented a reduction in coloration intensity over time. Similar results were obtained by Oztan *et al.*⁷ (2001), who observed OD reduction in black India ink when in contact with specimens filled with Sealapex.

Dyes or radioisotopes are used in 82% of marginal leakage studies.¹¹ However, since diffusion and dye coloration through the tooth/obturation material interface can be influenced by the interaction between obturation material, dye and dental structure, it is necessary to analyze the dental filling material influence on the optical density of the dye used in marginal leakage testing.^{5,13} It was observed

that an assorted sample of dental filling materials changed dye OD (Graph 1). OD evaluation seems to be an interesting method to select the best dye solution to use in a given marginal leakage study.

Conclusions

Considering the immersion effect of the evaluated materials on the OD of the three dyes tested, it was observed that:

1. There was no significant statistical difference among the OD values of the three dye solutions evaluated, for each analysis period, when the AH Plus cement was evaluated. An immersion time longer than 24 hours is recommended;
2. Portland cement promoted different OD values after 12 hours of immersion, in all of the three dyes evaluated. After 24 hours, there was difference only for BII. Its use is recommended with MB and RB with an immersion period longer than 24 hours;
3. MTA-Angelus cement presented different OD values only for RB, so its use is recommended with MB and BII at any time interval;
4. MTA-Pro Root cement presented different OD values in all periods with the RB dye and up to 12 hours with the MB dye. BII is recommended for any period of time after 24 hours;
5. Sealapex cement promoted a significant increase of the OD values for MB and RB and a decrease of the OD values of BII, when immersed for 48 and 72 hours. Therefore its use is recommended with BII for 12 and 24 hours.

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