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An optical investigation of dentinal discoloration due to commonly endodontic sealers, using the transmitted light polarizing microscopy and spectrophotometry

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Abstract

The aim of this study was to establish the degree of tooth crown staining by commonly used endodontic sealers. Crown discolorations by tooth canal sealers [AH Plus (Dentsply DeTrey Gmbh, Konstanz, Germany); Endofill (Produits Dentaires SA, Vevey, Switzerland); Apexit (Dentsply DeTrey Gmbh, Konstanz, Germany); and MTA Fillapex (Angelus, Londrina, Brazil)] were tested on extracted human premolars. The samples were divided into five groups of five samples each, after root canal sealing. Five teeth were used as control groups. The spectrophotometric method was performed in order to quantify in terms of color change of the coronal part (it was also recorded a track on how the color changes over time). For the microscopic study of the extracted dental specimens subjected to this study, polarized transmitted light microscopy was used. This method involves the development of special microscopic preparations, called "thin sections". In our case, the thin section was performed on 20 prepared and obturated recently extracted teeth. The degree of discoloration was determined after one week and three months using spectrophotometry and polarized light microscopy. All sealers usually cause some degree of discoloration on the cervical aspect of the crowns that increases in time. AH Plus and Endofill caused the greatest discoloration, followed by Apexit and MTA Fillapex.

Keywords: sealer, crown discoloration, spectrophotometry, polarized light microscopy.

⌐ Introduction

Discoloration of the coronal dental structure represents a major esthetic problem and one of the most common reasons for which patients come to the dentist [1]. The causes of the dentinal discoloration are various ones and there were classified into extrinsic and intrinsic causes [2, 3].

Extrinsic discoloration is caused by the extrinsic agents that act upon the external structures of the tooth. These agents, colored or not, determine color changes in the dentin, as spots or as a whole [4]. The most common agents involved in extrinsic discoloration are: a poor oral hygiene, with an excess of dental plaque and calculus, coffee, tea, other beverages or tobacco colorations [5], drugs used for a longer period of time (Tetracycline, Minocycline, Doxicycline, Chlorhexidine, iron-based drugs) [6–9], etc.

Intrinsic discoloration is more commonly caused by dental caries, pulp pathology or endodontic treatments. There are various factors that may lead to the tooth color change (discoloration) after endodontic treatments; most often, this process occurs due to endodontic material penetration during the dentinal tubules treatment [10, 11]. Due to an anesthetic aspect, about 28% of the adult population in Great Britain [12] and 34% of the USA population [13] are not satisfied with their teeth color.

Starting from the premise that the dentin is considered a permeable medium for the tooth, it would be expected to find variable distribution of sealing components used for root canal filling, at distance from the pulp chamber, or the root canals [14–16]. As a first step, it is therefore appropriate the microscopic detection of the physical substrate of visible color changes, since the macroscopic appearance of the tooth is given at least in part by the phenomena of light passing through transparent media (*i.e.*, enamel).

Transmitted light microscopy shows the changes of a beam of light passing through the specimen (a thin section of this), while other imaging methods commonly used in histological studies on teeth, such as stereo reflective and scanning electron microscopy (SEM) only provides some details of the morphology of the studied surfaces.

In this study, the aim was to detect the presence of coronary plaque color changes and the dentin distribution of sealing components using the spectrophotometry and polarized transmitted light microscopy. In addition, the aim of this study was to demonstrate the discoloring effect on teeth crowns of some commonly endodontic sealers used in the endodontic treatment.

⌐ Materials and Methods

Twenty recently extracted human premolars with

completely root development were used in this study. These teeth were free of discolorations, caries or fractures. The experimental endodontic treatment has been performed immediately after the extraction procedure.

The root canal sealers used in this study were: AH Plus (Dentsply DeTrey Gmbh, Konstanz, Germany), Endofill (Produits Dentaires SA, Vevey, Switzerland), Apexit (Dentsply DeTrey Gmbh, Konstanz, Germany) and MTA Fillapex (Angelus, Londrina, Brazil) (Table 1).

Table 1 – *Root canal sealers used in the study and their composition*

Group	Sealer	Composition
1.	AH Plus (Dentsply DeTrey Gmbh, Konstanz, Germany)	Epoxy-amine resin-based root canal sealer, opacifier – zirconium oxide.
2.	MTA Fillapex (Angelus, Londrina, Brazil)	Resins (salicylate, diluting, natural), bismuth trioxide, nanoparticulated silica, mineral trioxide aggregate, pigments.
3.	Endofill (Produits Dentaires SA, Vevey, Switzerland)	Zinc oxide, hydrogenated resin, barium sulfate, eugenol.
4.	Apexit (Dentsply DeTrey Gmbh, Konstanz, Germany)	Calcium hydroxyde, collophonium, silicone dioxide, disalicylate, bismuth carbonate.

Selection and preparation of extracted teeth

The teeth were preserved for 15 minutes in sodium hypochlorite to eliminate the remaining organic tissue, then the teeth were rinsed in water and external root surface debrided, using an ultrasonic scaler. After preparing the access cavity, the working length was established, using 10K file and after that, the canals were prepared to the working length using a F2, F3, nickel–titanium rotary Pro Taper files (Dentsply Maillefer, Ballaigues, Switzerland) with alternative irrigation, using sodium hypochlorite (Parkan, Septodont, France) and 17% EDTA (Ethylene-diaminetetraacetic acid) (FileCare, Germany). After recording the baseline images, the teeth were divided into four groups, the canals were dried with paper points and the canals were completely sealed, using lateral condensation of gutta percha and sealers, for each group of five teeth: AH Plus, MTA Fillapex, Endofill and Apexit (Table 2).

Table 2 – *ΔE values of groups 1–5 in the examined periods (ΔE – the amount of color change)*

Group	ΔE at one week	ΔE at three months
1. AH Plus	3.06 (1.06)	5.06 (1.85)
2. MTA Fillapex	2.05 (0.81)	3.56 (0.99)
3. Endofill	3.10 (0.93)	3.05 (0.89)
4. Apexit	2.08 (0.84)	2.85 (0.71)
5. Control	0.56 (0.35)	0.95 (0.65)

The excess sealer was carefully removed and no sealer was allowed to be in contact with the external aspect of the root. The teeth were randomly divided into five groups of five samples each for root canal filling with the corresponding sealer and five teeth were used as a control group.

Spectrophotometry

A spectrophotometer (Easyshade® Advance, Bad Säckingen, Germany) was used for the evaluation of the color coordinates (L* a* b*).

The prepared teeth crowns, after recording the baseline images using Olympus Camera C5000 were photographed,

one week to three months after root sealing. The baseline image for each sample represented the reference point to which, after one week up to three months, the color change over the time will be compared. The measurements were repeated twice for each specimen and the mean values were calculated and averaged (considering Δe≤3.5 as a clinically acceptable parameter for color change).

The L* a* b* values were determined, where ΔE was the amount of color change, "L" – lightness, "a" and "b" – chroma (red was +a, yellow was +b, and blue was –b). The color difference was determined by the formula:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The ANOVA tests were used to describe the relation of L* a* b* values for the cervical segment after one week to three months.

Performance of microscopic preparation (the thin section) and the microscopic study

After performing all the spectrophotometric measurements during the study, the investigated teeth have been used for performing the necessary preparations for the microscopic study. These preparations are so-called thin sections, consisting of a very thin strip (slice) of dental tissue, positioned between two glass slides.

For achieving this, the teeth were cut with a microtome (Leica RM2255, Wetzlar, Germany), depending on the area of interest – adjacent to the pulp chamber and the root canals. The resulting cut released more slices and they were fixed with epoxy resin (of refractive index 1.54 or 1.56 standard), on a processed glass slide. The excess resin is removed. The dental slices were rigorously processed in order to be parallel to the bonding surface. Using the grinding method, the dental slice is brought to a standard thickness of 30 μm; furthermore, the polished surface was cleaned, without contamination the sample. Then, the thickness of the remaining portion of the tooth was checked by optical methods. Finally, a glass slide was fixed to the polished surface. Bonding the slide is achieved with the same epoxy resin as previously used. By bonding the slide, the sample becomes a permanent one.

Thin sections were studied using polarized transmitted light microscopy and under a stereomicroscope (transmitted light and reflected light). For the polarized transmitted light study, a polarizing microscope Leica DM LP was used and for the stereomicroscopic study, a Krüss stereo-microscope was used.

With the camera attached to the microscope (Leica DMC4500), in the study there were carried out a series of microscopic images, the most representative were selected and presented as support for those listed in paragraph microscopy.

The stereomicroscope, allowing degrees of magnification lower than the minimum polarizing microscope (40×), was used to obtain some overview images.

Results

Spectrophotometric investigation

Discoloration was significantly greater for AH Plus and MTA Fillapex after three months, and there was no significant difference in the progression of the discolo-

ration between Apexit and Endofill (Table 2). In comparison to the witness group, all the materials used in the endodontic treatment showed high spectrophotometric values, from one week up to three months.

Microscopic investigation

Following the microscopic study, without using a polarizing filter, we noticed three types of dentin microdomains in the dentin:

▪ microdomains with non-impregnated dentin tubules (*i.e.*, clear) (Figure 1a);

▪ microdomains with both, impregnated and non-impregnated dentin tubules, the ratio of the two per unit area being variable (Figure 1b);

▪ microdomains where the majority dentin tubules are impregnated (Figure 1c).

We consider that an impregnated dentin tubule is a tubule where there is found a foreign substance different from the physiologically one and shows a different color (usually darker), different transparency and refractive indices.

The intertubular dentin, regardless of how the tubules are shown (infiltrated or non-infiltrated) has almost, without exception, a clear aspect and does not seem to be structural or coloristic modified.

Due to physiological curvatures of the dentin tubules, a thin section cannot capture a dentinal tubule, only on limited portions. On the sectors captured by section, the tubules can be fully or partially impregnated. Also, for a given tubule, on length, there can be observed variations in color intensity of non-natural origin and associated with

this, various degrees of transparency, suggesting variations in length, concentration of foreign disseminated substances. In some microdomains, were the tubules were captured mainly in length by section, there was a preferential concentration of foreign substance in them, through the main root canal, where the experimentally sealant has been introduced (Figure 2, a and b).

The impregnation in transverse direction is more pronounced toward the axial side, due to the proximity of the dentin tubules, with respect to the main obturated canal. The dentin areas with high proportion of impregnated tubules depend on the angle between the section plane and the direction of the tubules.

In case of dentinal tubules caught on a relatively long section (Figure 3a), there is shown an emphasized aspect. A good portion of incident light does not reach to the observer/the camera, so the overall look is darker. It gives the impression of a deeply colorful dentin.

For a relatively small angle ($20–30^0$) between the tubules dentin direction and the section plane, they are caught on shorter distances (Figure 3a) and a greater part of the incident light reaches to the observer. It creates the impression of low color-impregnated dentin.
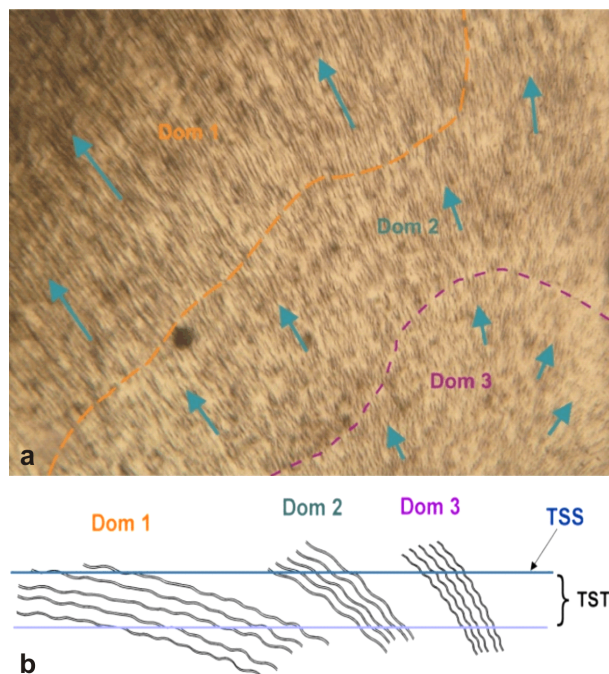
When the angle between the direction of the dentin tubules and the section plane is more pronounced (about $30–50^0$), the captured portions of the tubules are very short on the section thickness (it approaches closer towards the slice thickness) (Figure 3b). All along, the lined appearance disappears and most incident light passes towards the observer. It can create an overall appearance of low impregnated dentin or even non-impregnated.
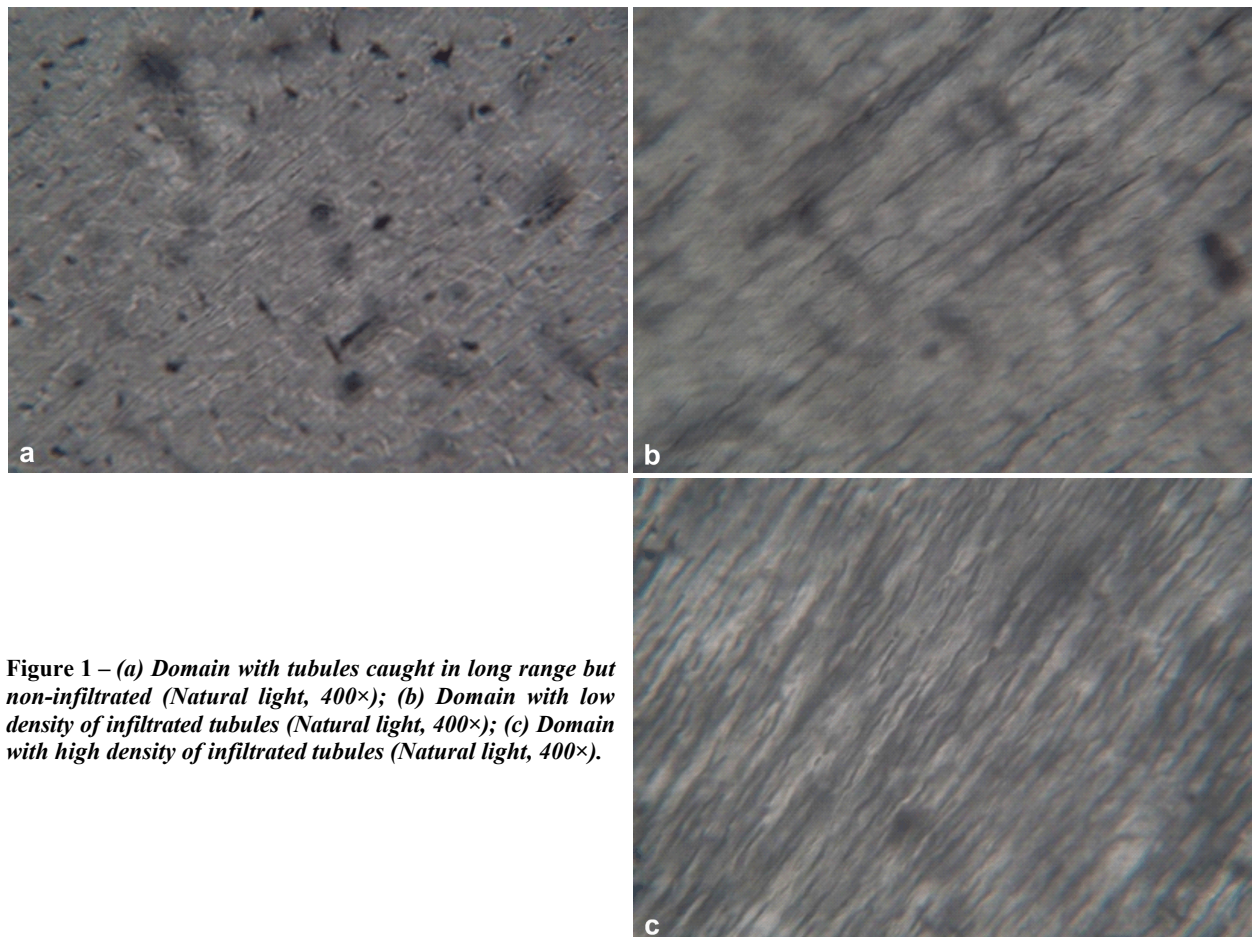
Figure 1 – *(a) Domain with tubules caught in long range but non-infiltrated (Natural light, 400×); (b) Domain with low density of infiltrated tubules (Natural light, 400×); (c) Domain with high density of infiltrated tubules (Natural light, 400×).*
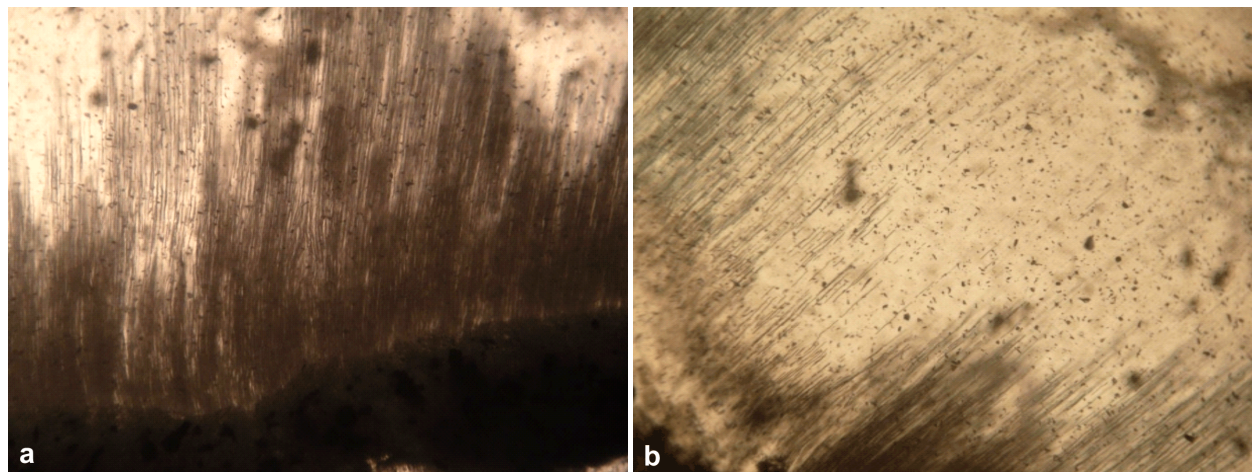
Figure 2 – *(a) Intense color and less transparency of impregnated root tubules, captured on long section, that contains sealant (AH Plus) (Natural light, 100×); (b) Infiltrated tubules, long captured , with the impregnated predominant aspect towards the sealed canal with AH Plus (Natural light, 100×).*

Figure 3 – *(a) The succession of three domains where the angle between the sectional area and the direction of the dentin tubules changes: Domain 1 – relatively long tubules captured; Domain 2 – tubules captured to a relatively small angle; Domain 3 – tubules captured on relatively large angle (Daylight, 100×); (b) Sketch showing the relationship between the direction of dentinal tubules and corresponding section plane. Dom: Domain; TSS: Thin section surface; TST: Thin section thickness.*

The changes of the infiltrated tubules near the enamel–dentin junction: the images captured in long tubules close to this limit show the changes in their microanatomy (impregnation, tapering and branching) (Figure 4, a and b).

In polarized light, at small degrees of magnification (40×), in areas with high density of infiltrated tubules caught in long section, the transmission of incident light is greatly reduced, so there is almost no noted birefringence of intertubular dentin areas (Figure 5). As the density of the impregnated tubules becomes smaller, or their degree of impregnation is lower, the birefringence colors given by the intertubular dentin become apparent. At the bottom of the same image, it can be seen in enamel alternating blue and purple areas, corresponding to the Hunter–Schrager bands. In the present case shown in the image it can be seen, in the dentin, limited to enamel, a thinning of the impregnated tubules, since all the birefringence colors produced by the dentin is unchanged.

It is known from the histology of dental tissue that dentinal tubules narrow towards the periphery of enamel–dentin to the dentin–cement junction. As a result, in the same microscopic field there can be captured on relatively long sectors with thicker tubules segments, because they are closer to the pulp chamber and sectors of thinner tubules segments, once they are further from the pulp chamber. If two such sectors are impregnated and have the same density, microscopic scale color changes appear more pronounced in thicker tubules sectors, because they could retain more scattered foreign substance (Figure 6).

By a microscopic procedure, positioning the preparation so that the birefringence colors of the intertubular dentin be canceled by the phenomenon of extinction, birefringence can highlight any foreign substances present in the tubules. In some of the preparations studied through this process, we were able to highlight the presence of dentinal tubules in anisotropic substances (Figure 7, a and b).

These substances give some low birefringence color and most likely, they were not completely filling the canalicular lumen. The fact that on other non-impregnated tubules present in the microscopic field this birefringence phenomenon does not appear, certifies the fact that is due to the presence of foreign substances and optical phenomena that might occur in peritubular dentin.

⮥ Discussion

The dentin color changes after endodontic treatments may be clinically determined, and more precisely through using spectrophotometry techniques. At present, spectrophotometry is considered a reference method used in dentistry for determining the tooth color changes [17]. In our study, the spectrophotometric analysis showed an early change (after a week) of the tooth color, an aspect that could not be clinically observed.
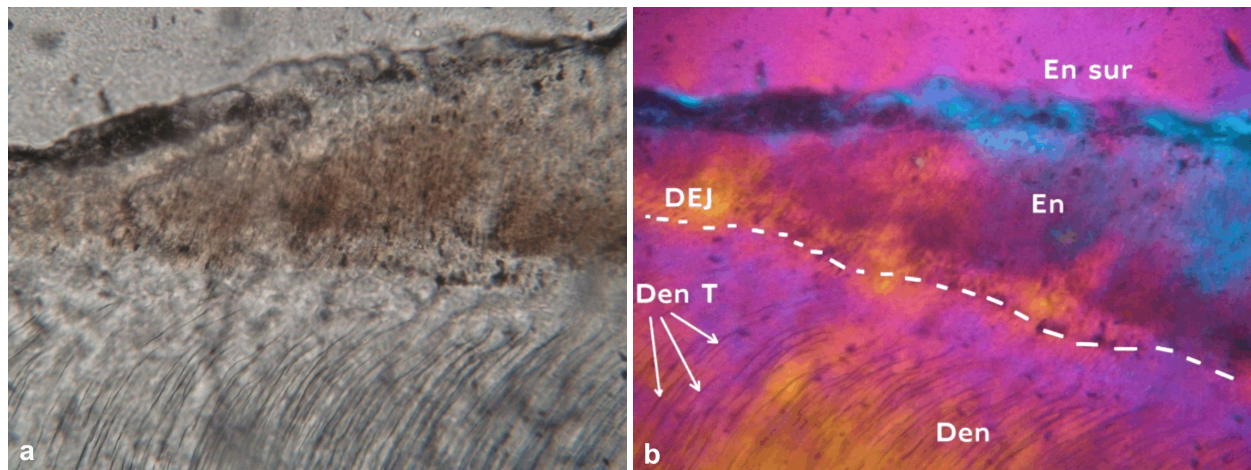
Figure 4 – *(a) Coronal area near the enamel–cementum junction: impregnated dentinal tubules (Endofill), tapers and branches, stop before the enamel–dentin junction (Natural light, 250×); (b) The same microscopic field from previous figure with polarized light and filter colors birefringence (Natural light, 250×). En: Enamel; Den: Dentin; DEJ: Dentin–enamel junction; En sur: Enamel surface; Den T: Dentinal tubules.*
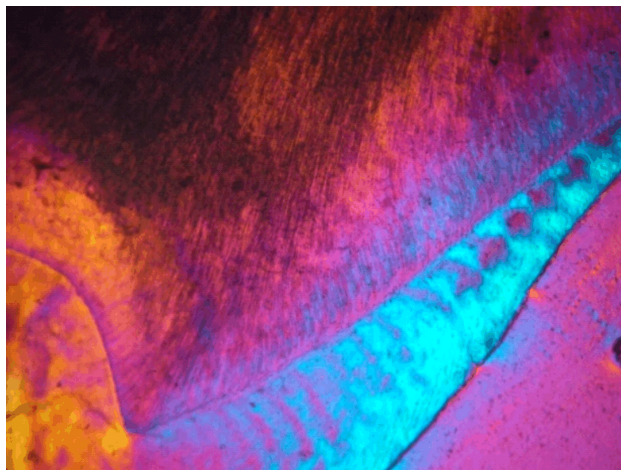
Figure 5 – *Picture of coronal part of the teeth, where the enamel and dentin are captured. At the top left of the image, there is an area densely infiltrated tubules. Here, the spaces with intercanalar dentin can no longer be distinguished (Natural light, 40×) (polarized light color filter, 40×).*
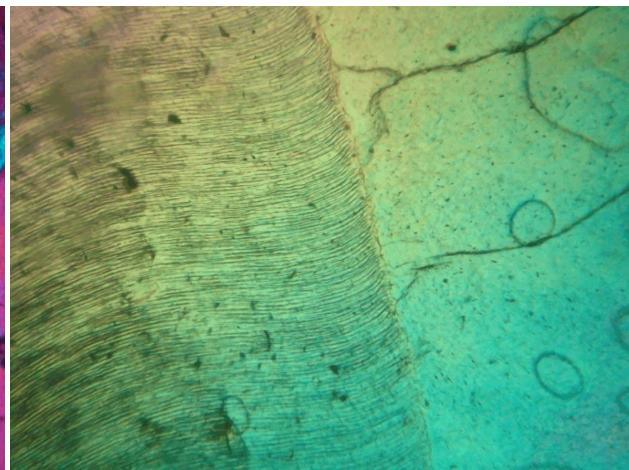
Figure 6 – *Microscopic image comprising enamel and dentin. The dentin is seen on the left and two sections of the canal impregnated with AH Plus sealer but with different thicknesses. The thicker contain more substance diffused per unit length (Natural light, 100×) (polarized light with filter of birefringence color, 100×).*
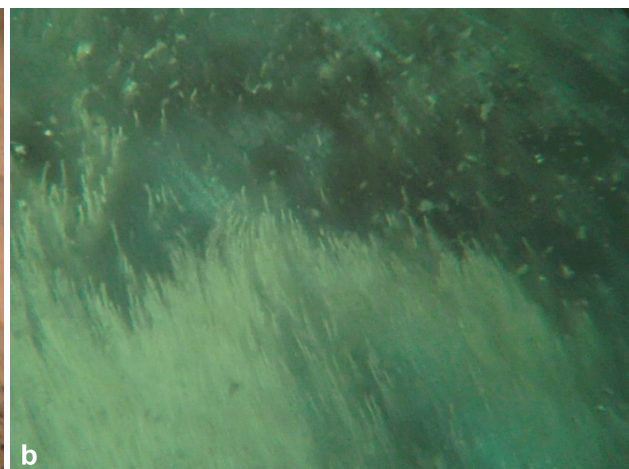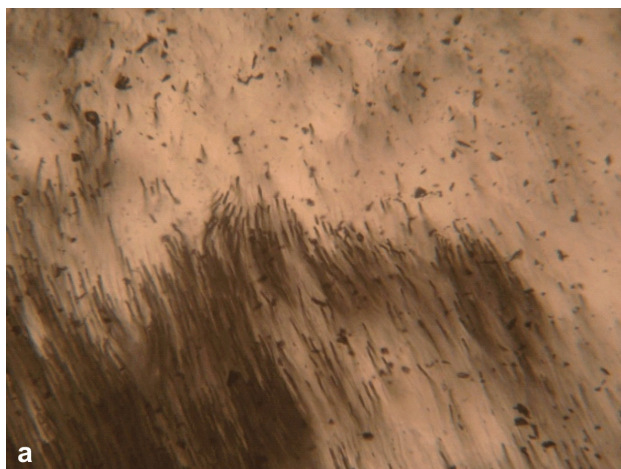
Figure 7 – *(a) Microdomain where tubules are present, both impregnated (brown color) and non-impregnated (translucent). The apparent ends of the tubules are actually the intersection of the tubules and sectional area. Discoloration caused by Apexit (Natural light, 250×); (b) The same microdomain illustrated in previous figure. Inter-tubular birefringence colors of dentin are canceled and only some low birefringence colors appear in the intertubular environment that cannot only be given by the presence of some birefringent substances (Polarized light, 250×).*

The teeth discoloration intensified in time, and thus, after three months since treatment, the spectrophotometric values were higher. Similar data were also obtained by other researchers [18].

Polarized light microscopy gives unique properties for the analysis of the molecular order in the heterogeneous systems, such as living cells and tissues, without using any exogenous coloring agents [19, 20]. Polarized light that enters an anisotropic medium studied unfolds in two parts, which are polarized in planes, mutually perpendicular and running at different speeds (corresponding to two different refractive indices). These, after leaving the preparation studied, there comes the analyzer and some of them are recombined, being made to oscillate in the same plane and thus interfere. This interference can be both destructive and constructive and produce the birefringence colors seen by the investigator.

Birefringence colors and their intensity depends on the thickness of the studied specimen (normally constant = 30 μm), the studied specimen structure, the chemical composition of the studied specimen, the transparency of the studied specimen, how the specimen's fundamental units are positioned (*i.e.*, crystals) relative to the directions of the polarizing devices. On the polarizing microscopes, this direction can be changed by turning the microscope table.

The tooth is an anisotropic medium that produces birefringence colors. Hydroxyapatite crystals crystallize in the hexagonal system and the refractive index is in the range of 1630–1655. Birefringence of these crystals is weak and produces white-ash color birefringence of order 1 (according to Michel–Lévy birefringence table) [21].

Using polarized light enables highlighting of special structures that cannot be studied with conventional biological microscopes. Among these structures are the well known Hunter–Schreger bands of enamel (occurring as a result of changes orientation of enamel prisms, adjacent to the distance). In most mammal species, including the humans, the tooth enamel is characterized by regulated prism strata, alternating in orientation, known as the Hunter–Schreger stripes [22, 23].

In this study, we wanted to capture the high degrees of magnification, the presence of any dentin anisotropic substances, other than the hydroxyapatite dentin. Our study revealed that significant discoloration was identified especially in the cervical segment of the crown after three months.

More studies have shown that tooth discoloration is one of the most common inconvenient of the endodontic material treatment [10, 24, 25], as, in most patients, it determines the emergence of anesthetic coloration of the dentin. According to the studies performed by Asgary *et al.* [26], MTA Fillapex and CEM cement (a bioregenerative material made up of various calcium compounds, frequently used in the endodontic treatment) determine similar histological changes. The MTA Fillapex use in the endodontic treatment determines a grey color of the dentin, mainly due to the tricalcium silicate, bismuth oxide and dicalcium silicate [27]. Other studies, using spectrophotometry techniques, have shown that MTA Fillapex determines a rapid, severe color change a week

after its use [11], aspects that are in accordance with our observations. The mechanisms through which the used materials in the endodontic treatment are not very well known, but most studies show that some chemical components in these materials could enter the dentinal canals [2, 28].

In our study, with respect to the scale tooth, there is observed a lack of homogeneity of distribution coloristic impregnated tubules in lateral and longitudinal way (infiltration favorite group of neighboring tubules on which preferential diffusion occurred) and must be taken into account the different dentin tubules permeability. It was also noticed the general trend of maximum density aria concentration, containing impregnated tubules towards the main root canal, but there sometimes can be found exceptions. The presence of numerous tubules per unit volume creates in terms of microscopic area, a heterogeneous modified dentin aspect, which translates macroscopically in the coronary domain by a changed color with relatively homogeneous appearance.

The greatest color changes in our study were determined in the cervical third of the crown. This is evident, because sealer diffuses through dentinal tubules in a colorless and translucent media, which is thin in the cervical part of the tooth and therefore suitable to dyschromia. Near the main canal, the density of dentinal ducts begins to grow, so that there are differences in coronal-apical direction, in terms of their frequency.

The results were quite similar with other studies that showed crown discoloration after using sealers [29–32].

All these data support the idea that, in the endodontic treatment, the doctor should carefully choose the materials, in order to reduce the negative aspects caused by the discoloration process of the dentin [24, 33, 34].

⊟ Conclusions

All sealers cause a degree of discoloration on the cervical aspect of the crowns that increases in time. Our study showed that the impregnation material is present in the dentin tubules, but not in the intertubular dentin. Microscopy images suggest that the discoloration is caused by sealing components, color impregnating dentinal tubules and not of the microstructural and compositional changes from the intertubular space. Since the diffusion of the endodontic sealing occurs along the tubules, an important factor in determining the degree of infiltration is represented by the accessibility of dentin tubules opening orifice. Regarding the degree of dentin infiltration, through the dentin tubules for a given section, it can certainly be observed microscopically, only where the dentin tubules are relatively long captured by section. One aspect of "non-impregnation" can apparently be caused by a larger angle between the plane and direction of tubules section.

Conflict of interests
The authors declare that they have no conflict of interests.

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