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An application of microscopic investigation on extracted premolar with dyschromia, related to the components of endodontic sealer

Ioana Suciu[1], Ecaterina Ionescu[2], Costin Vârlan[3], Oana Elena Amza[1], Sînziana Adina Scărlătescu[1], Ileana Suciu[1], Mihai Ciocîrdel[4], Bogdan Alexandru Dimitriu[1]

[1]Department of Endodontics, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[2]Department of Orthodontics and Dental Facial Orthopedics, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[3]Department of Restorative Dentistry, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[4]Department of Geology, Petroleum–Gas University of Ploiești, Romania

Abstract

The aim of this study was an assessment of the overall optical characteristics changes in volume of the dentin, after the diffusion of the chemical components included in the sealer throughout the endodontic space, in order to demonstrate the discoloration effect of the endodontic sealer on tooth crown of a recently extracted maxillary premolar. Thin sections were studied using the polarized transmitted light microscopy and under a stereomicroscope, operating in transmitted light and reflected light. There were performed serial images of magnitude 40×. Each image was covered by a grid aimed for microscopic sections volumetric measurements. Therefore, all the serial images were merged and resulted a global image of the entire section surface. Thus, we have analyzed a total of three sections of the same tooth. Based on these sets of images, there were made estimations of the areas affected by colorations, determinations that can be extrapolated to total tooth areas, in terms of volumetric color change of dentin. The proportion of low coloristic infiltrated dentin was very close to the deeply impregnated dentin and the overall impregnated dentin covered half of the total dentin analyzed area.

Keywords: endodontic sealer, dyschromia, polarized microscopy.

Introduction

Discoloration of the coronal dental structure represents a major esthetic problem, concerning both the patient and the practitioner in the same time.

Previous studies showed that on dentinal tubules, there has been noticed the presence of substances with semitransparent aspect, that can absorb some of the incident light, while tubular dentin had relatively clear appearance, the latter suggesting that there is no color infiltration or structural changes in the tubular dentin [1, 2].

The polarizing microscope with transmitted light allows qualitative and quantitative studies on any material, but mainly applying on anisotropic materials. The method was mostly used in mineralogy, petrography, chemistry and materials technology. However, the most advanced microscopes used in biological research have also the possibility to produce and analyze the effects of polarized light passing through anisotropic media. Polarizing microscope uses two devices capable of polarizing light (polarizers). One is the path of the light beam prior to manufacture and is called polarizer (in the strict sense) and the other in the path of the light beam, after preparation, named analyzer. This analyzer actually enables highlighting the transition the polarized light produced by the polarizer on the anisotropic medium studied. If one of these devices is removed from the optical path,

then classical microscopic images are obtained, just as simple biological microscopes achieve. This is the so-called mode "natural light". With both polarizer devices introduced in the optical path, if the studied medium is anisotropic, there can be noticed the so-called birefringence colors. This mode is called "crossed polars" [3].

Evaluation of dyschromia on tooth samples can also be assessed by the spectrophotometry method, which is highly used in order to quantify a track on how the color changes or accentuate over time [4, 5].

The aim of this study was to demonstrate the discoloration effect of endodontic sealer on tooth crown. An appreciation of the overall optical characteristics changes of dentin, in volume, after the diffusion of the sealer components from the endodontic space was made by extrapolating the obtained data from a transmitted light microscopic study performed on three parallel longitudinal sections of an extracted maxillary premolar.

Materials and Methods

For this current study, we used one extracted human premolar (3.4). The patient presented in our clinic with an endodontic periodontal pathology on this tooth, but with no local intrinsic discoloration. The endodontic treatment was performed and the sealing of the endodontic space was made using lateral condensation of gutta-percha with

AH Plus sealer (Dentsply, De Trey, Konstanz, Germany). However, after one and a half year the tooth had to be extracted due to the periodontal pathology progress. After extraction and soaking the tooth in 2.5% NaOCl for 15 minutes, to eliminate the organic tissue remnants, and after drying, three micro-slices were carried out using a microtome (Leica RM 2255 Wetzlar, Germany) and for each such slice a thin section was performed. A section consisted of a thin strip (slice) of very thin dental tissue, sandwiched between two glass slides with a special bonding resin (an epoxy resin of known refractive index).

To achieve a thin section, the tooth slice cut with the microtome was first stuck on the front glass slide that was in advance processed. After this clamping, the dental tissue slice is tapered using procedures of sanding rigorously performed, parallel to the gluing surface. Through these sanding, the dental slice is brought to a standard thickness of 30 μm. Then cleaning the polished surface is achieved without contamination of the sample. The thickness of the remaining portion of the tooth is verified by optical methods. Finally, a glass slide is being stuck above the polished surface. The bonding of the slide was achieved with the same epoxy resin as previously used. By adhesion of the slide, the preparation becomes thus a permanent one.

Thin sections were then studied mainly using the polarized transmitted light microscopy and under a stereo-microscope operating in transmitted light and reflected light. In order to accomplish the study on polarized transmitted light, a polarizing microscope Leica DM LP was used, whereas for the stereomicroscopic study, a Krüss stereomicroscope was used.

Birefringence of these crystals is weak and produces white ash color birefringence of order 1 (in accordance with the table birefringence Michel–Levy). Using polarized light enables highlighting of special structures that cannot be studied with conventional biological microscopes. We followed that in this study to capture, at high degrees of magnification, the presence of any anisotropic substances in dentin, other than dentin hydroxyapatite. With the camera attached to the microscope (Leica DMC4500) there were made serial images that allowed quantitative assessments of dentin areas impregnated with sealant.

Moreover, with this polarizing microscope using calibrated micrometer eyepieces, there could be accomplished accurate determinations of distances between microlandmarks, in the section plane (*e.g.*, the distance between the enamel–dentin junction and enamel surface, in an area of interest).

The thin sections obtained were than subsidiary studied with a stereomicroscope, which works both in transmitted and reflected light (Krüss type). By proceeding in this manner, there followed the capture in a single microscopic field of the whole coronal part.

For all the three thin sections examined, obtained from the analyzed tooth, there were observed serial microscopic images at the magnification of 40×. On each of these images, from the optical system of the microscope, there has been overlapped a grid square, having 1600 units of basic area, the smaller squares. Basically, by moving the section, with side steps corresponding to that grid, it was overlaid throughout the whole dental tissue surface from each section. On each of these images, using as a benchmark the basic units of area, it was determined

whether the dentin corresponding areas were affected by color impregnation of the sealant components.

We consider that by performing several parallel sections for each tooth analyzed and assessing on such section the extent of staining on dentin in surface, this data can be extrapolated to determine the three-dimensional extent of discoloration, on the volume.

To give the appearance of the entire surface of the tooth section, the areas of photos on which the grid was overlapped, have been selected and arranged in proper order in the composed image. The reconstruction of the global image of one of the longitudinal sections achieved on the studied tooth was conducted by merging a set of microscopic images, achieved so that the grid used to determine the proportions covered all of its areas (Figure 1).

Figure 1 – *Composed image (from 1 to 35 photos) that reconstructs the maximum sectional area of the examined tooth surface, by joining all the areas of photographs over which was superimposed the determination grid.*

⛶ Results

To assess the dentin staining by impregnation with sealants components, by means of optical microscopy without a polarizer filter of the grid, we conventionally separated on the microscopic fields three types of areas (microdomains), namely:

(a) a deeply chromatic dentin area, characterized by high frequency of impregnated tubules and a relative loaded concentrations of the sealant components, thus causing their appearance of dark gray-brown color;

(b) the low impregnated dentin domain is characterized by either low frequency of the impregnated tubules with sealant components (whether the sealant concentration is high, or decreased in the tubule), or by a relatively high frequency impregnated tubules (*i.e.*, more than 75% in area, but with a low concentration sealant component so that the transparency of the tubule is slightly modified of their interior color); the color is only slightly darker, from gray to brown, from low to moderate intensity.

(c) the presence of dentin area with no impregnation, in which the content of dentin tubules is relatively clear, transparent, and there is no significant change in color compared with the intertubular dentin.

For each section obtained there were performed a set of grids images, which were systematically superimposed over the whole surface of the hard tooth tissue. For each image area, covered by the grid, there was counted a total area of fundamental units (respective squares of the grid), which overlap in such way: (*) over highly infiltrated dentin, (**) over low infiltrated dentin, and (***) over dentin in total (including the one that was appreciated as non-impregnated). The actual values of the area can be appreciated by knowing the order of magnitude used [a grid area is 4 mm^2 and the fundamental unit of area (*i.e.*, a small square) has 0.0025 mm^2]. What interests us most was the evaluation of the percentages of total area, belonging to the deep and low impregnated dentin, so that the proportion of the affected dentin by color impregnation was emphasized (Tables 1–3).

Table 1 – *Results of the assessment areas by impregnation for section of maximum area (the central section) – section 2*

No. of microscopic field area	UA on dentin	UA on deep impregnated dentin	UA on low impregnated dentin	UA on impregnated dentin	UA on dentin without impregnation
1	571	72	52	124	447
2	227	157	70	227	0
3	94	41	16	57	37
4	0	0	0	0	0
5	295	106	56	162	133
6	1504	1105	399	1504	0
7	1583	624	958	1582	1
8	411	34	174	208	203
9	465	101	275	376	89
10	1600	349	344	693	907
11	785	757	28	785	0
12	530	0	481	481	49
13	483	88	334	422	61
14	653	596	57	653	0
15	1591	898	515	1413	178
16	1276	1220	19	1239	37
17	1001	906	11	917	84
18	1600	332	974	1306	294
19	1591	1367	224	1591	0
20	411	411	0	411	0
21	205	205	0	205	0
22	1600	690	674	1364	236
23	1600	95	614	709	891
24	684	271	208	479	205
25	231	186	0	186	45
26	1600	248	712	960	640
27	1600	301	1211	1512	88
28	233	233	0	233	0
29	167	167	0	167	0
30	1600	230	451	681	919
31	1465	1060	404	1464	1
32	1491	0	765	765	726
33	1462	0	174	174	1288
34	1069	0	551	551	518
35	1450	0	0	0	1450
Total	33 128	12 850	10 751	23 601	9527

UA: Fundamental unit of area.

Table 2 – *The final results of the assessment affected areas by impregnation for sections 1 and 3*

Section	UA on dentin by total section	UA on deeply impregnated dentin by total section	UA on low impregnated dentin by total section	UA on impregnated dentin by total section	UA on dentin without impregnation by total section
Section 1	24 020	8887	7422	16 309	7711
Section 3	22 859	7760	6192	13 952	8907

UA: Fundamental unit of area.

Table 3 – *The final results for the whole studied premolar*

Section	UA on dentin by total three sections	UA on deeply impregnated dentin by total three sections	UA on low impregnated dentin by total three sections	UA on impregnated dentin by total three section	UA on dentin without impregnation by total three sections
Total	80 007	29 497	24 365	53 862	26 154

UA: Fundamental unit of area.

Therefore, we have noted with UADPI the number of the fundamental units of area of partially impregnated dentin, with UADSI the number of fundamental units of area of low impregnated dentin and with UATPD the number of fundamental units of total dentin area.

After counting all the analyzed images, we found a total area corresponding to heavily infiltrated dentin of 36.87% calculated by the formula: UADPI × 100/UATPD, the total area corresponding to low infiltrated dentin of 30.45% calculated by the formula UADSI × 100/UATPD, therefore the total area corresponding to infiltrated dentin was 67.32%.

During the microscopic study, while tracking the impregnation changes in dentin, we noticed an additional microscopic aspect of non-physiological nature. At the approximate location of enamel–dentin junction, on all the three sections studied, a coronal fracture was detected. It starts from the enamel, under vestibular cusp tip, at the enamel–dentin adjacent limit, relatively following to the limit, going under the vestibular cusp slope and near the enamel–cement junction, at 1.3 mm from coronal, leaving this limit and crosses the thickness of the enamel towards the exterior (Figure 2). In this place, where the crack comes out, the thickness of the enamel determined in micrometers, on the central section (section of maximum area), is 0.35 mm (Figure 3). This crack communicates with the endodontic space in that it is not perfect located on the enamel–dentin limit, under the vestibular cusp and intersects the terminal portion of the dentin tubules (Figure 4). We categorize this rift as open, in that it communicates with both, endodontic and exterior space.

On the occasion of microscopic inspections of the tooth with transmitted light microscopy, we noticed that the sealant components, which diffused in the dentin tubules, causing both micro and macroscopic changes in appearance, although generally they fail at the enamel–dentin boundary, in this case, however, because of the presence of the crack located at the dentin–enamel limit, they pass through diffusion in this crack, tending to impregnate it.

Besides this cracking described above (the largest expansion crack), other cracks are present in the enamel, which are open towards the outside, but not surprisingly, however, at the intersection enamel–dentin junction or in the dentinal tubules.

By using the stereomicroscope with small magnifying orders (*e.g.*, 20×), it is possible encompassing a larger field of the entire crown (Figure 5). In the center of the image, there is an area of deeply impregnated dentin, which is observed as a relative funnel shape. It is noted that the substance did not emerge to the enamel–dentin limit (with few exceptions, such as the area under the vestibular cusps). Thus, it is observed the persistence of a dentin domain, overall non-impregnated, immediately below the enamel–dentin limit. Such an overview allows a rough assessment of the proportion of impregnated dentin, located beneath the enamel.

The fact that there is an impregnation with sealant components of the dentin tubules can be highlighted if this substance is anisotropic. Anisotropic foreign content of dentin tubules, in the case of the premolar, is evidenced in Figure 6 by the birefringence phenomenon, when the birefringence colors of the intertubular dentin were canceled by the phenomenon of extinction. The tubules containing no sealant, and categorized as impregnated, do not show this birefringence phenomenon and is observed at the right part of the image. By using microscopic techniques for obtaining such images, basically one depicts the three dimensions routes of the impregnated tubules.

Figure 2 – *Image composed of three photos taken serially in transmitted light at 100× magnification, which reveals the position of the cracks localized at the enamel–dentin limit, mainly under the vestibular cusp and on the vestibular slope of it.*
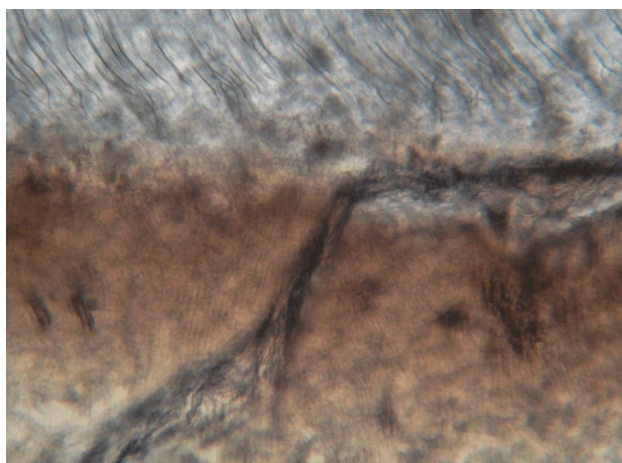
Figure 3 – *Detail on the exterior area of the crack output that runs through the enamel exterior, immediately on the coronal cementum–enamel junction. Natural light, 250×.*
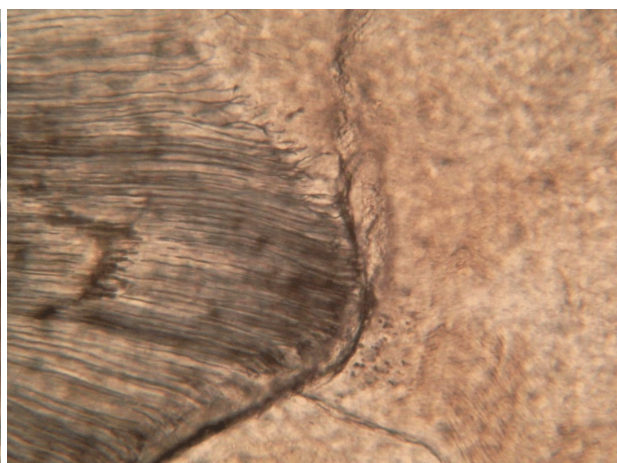

Figure 4 – *Detail on the crack that intersects the terminal portion of the dentin tubules under the top portion of the vestibular cusp. Natural light, 250×.*

Figure 5 – *The premolar crown area investigated by transmitted light stereomicroscopy image (20×).*
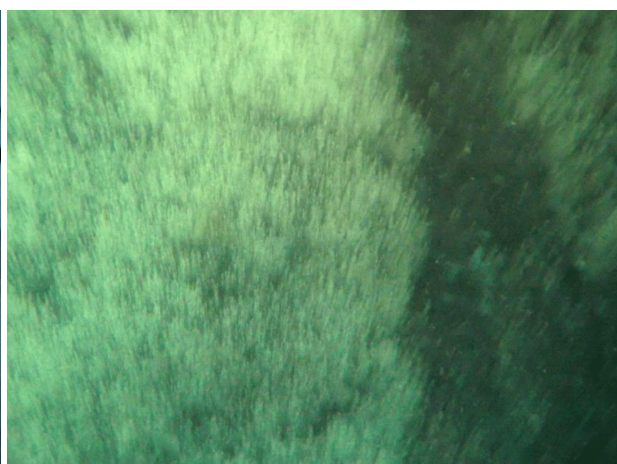

Figure 6 – *An image in which the birefringence phenomenon of the impregnated dentin tubules is shown, with sealant components (image left) compared to the areas with few tubules impregnated (right side of image). Polarized light, 250×.*

⊟ Discussion

Polarized light that enters an anisotropic medium studied and unfolds into two parts, which are polarized in mutually perpendicular planes and traveling at different speeds (corresponding to two different refractive indices). These, after leaving the preparation studied, reach the analyzer and here, 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 birefringence colors seen by the investigator.

The birefringence colors and their intensity depend on: the thickness of the studied specimen (normally constant 30 μm), the specimen structure, the chemical composition of the studied specimen, the transparency of the studied specimen, the way the fundamental units of the specimen are positioned (*e.g.*, crystals) in relation to the directions that polarizers devices polarizes light. For the polarizing microscopes, this direction can be changed by turning the microscope table.

The mineral part of the tooth, being composed of hydroxyapatite crystals, although these are not detectable by this type of microscopy, the tooth is an anisotropic medium that produces birefringence colors. The hydroxyapatite crystals crystallize in the hexagonal system and the refractive index is in the range of 1630–1655 [3].

On earlier studies, that were conducted on many extracted teeth, with previously endodontic treatment on root canal obturation completed, have revealed that some components of the sealer diffuses through the dentin tubules, but generally remained in the dentin domain [6, 7].

In studies previously performed without removal of smear layer, tooth discoloration was less obvious [8–11]. All sealers have a chromogenic effect with a different progression of discoloration over time [12–14].

However, in the current study, it turns out that if there are impregnated tubules, which communicate with open cracks coming from the enamel, the diffusion of the endodontic substances can further achieve the impregnation of these cracks. Basically, in this case, what comes to impregnate, is of endogenous origin (from the endodontic space), but could be confused with exogenous dyschromia.

Only microscopic scale could assess with a high degree of certainty that substances diffused in the enamel cracks

from the endodontic space or from the outside of the tooth, towards the oral cavity.

The criteria in favor of diffusion from the endodontic space are:

(1) intersection of that crack with the dentinal tubules;

(2) same optical appearance of the substances diffused in the cracks with the one noticed in the tubules (the same color and the same degree of transparency, possibly the same birefringence color characteristics) [5–9].

For a given section, the presence of dentin areas heavily impregnated only in the periphery might be something apparently. One such issue might be because the section planes (the dental tissue strips obtained by microtome cutting and following subsequent grinding for thin section preparation) does not completely follow alongside the same tubule from their opening at the pulp chamber and root canal towards the periphery. Therefore, the deeply impregnated tubules (hence the relatively high concentration of foreign substances diffused) may only be encountered at the periphery of the tooth on a given section. The central domains of such tubules were also deeply impregnated, but not captured in the section though. In the domains where the central portions of these tubules would occur in the section plan, there might practically appear other tubules, low impregnated or not impregnated ones, giving the feeling that at the section plane the diffused substance is locally concentrated only in the periphery.

In other words, it would be expected that where a deeply impregnated dentin area is in the periphery (at the enamel–dentin junction, dentin–cement, respectively) tubules with high concentration of diffused substance to give this aspect, to have high concentrations of the substance along the length, towards the opening at the pulp chamber and/or root canals. However, this was not necessarily possible that they have been caught in section along their length, which otherwise unlikely, because some "bundles" of the tubules (adjacent sets of tubules) have curves in several planes.

Nevertheless, a general tendency is observed that deeply impregnated dentin domains to be located towards the central part of the tooth (pulp and inner parts of the root canals).

⊡ Conclusions

Color impregnations of endodontic origin generally stop at the enamel–dentin junction, unless there are cracks that allow the propagation of sealer components, either through enamel or along the enamel–dentin junction, this depending on the orientation of the cracks. The diffusion of the sealant, starting from the pulp chamber or root canal respectively, is especially along the dentin tubules, preferentially on certain sets of tubules and seems not to

involve the intertubular dentin. It seems that the more concentrated the diffused sealer is, the more it becomes less transparent and darker in color, ranging from light brown to dark gray brown. Therefore, we might suggest that the concentration of substances diffused in the dentin tubule is highly dependent on their distance from the opening in the pulp chamber and root canals. Starting from the fact that the proportion of low impregnated dentin is very close to the deeply impregnated one and that the overall impregnated dentin surpassed more than half of the total analyzed area, we may conclude that more than half of the total dentin volume showed discolorations related to endodontic treatment procedures.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding author
Ioana Suciu, Associate Professor, DMD, PhD, Department of Endodontics, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, 17–23 Plevnei Avenue, 010221 Bucharest, Romania; Phone +40722–593 808, e-mail: joa_suciu@yahoo.com