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DISTRIBUTION OF METAL COMPOUNDS IN THE TISSUES OF THE ROOT OF THE TOOTH WITH APEX-FORESES (IONTOPHORESIS OF COPPER AND SILVER)

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Abstract

We studied the effect of the degree of patency of the root canal on the distribution of metal compounds in the root canal system and hard tissues of the tooth root after apex-phoresis. The study showed that during apex-phoresis, copper and silver ions enter the sound tooth tissues as a result of anodic dissolution of working-active part of the silver-copper electrode. Moreover, the anode in the electrode space formed acids were formed in perielectode zone which may cause the dissolution of calcium hydroxyapatite $Ca_5(PO_4)_3(OH)$ in the adjacent dentine, causing the release of calcium ions. As a result of electrochemical and chemical processes occurring during apex-phoreses, soluble, poorly soluble and sparingly soluble compounds of silver copper, and calcium are formed. The metal salts are chlorides, sulphides, sulphates, phosphates, as well as a number of complex compounds of copper and silver. These salts line the walls of the root canal and fill accessory canals, penetrate dentin.

These processes are strictly local taking place only in the area, where the working-active part of the silver-copper electrode is located during the procedure. Therefore, to ensure the saturation of the apical portion of the canal and apical delta with silver and copper, the root canal must be enlarged for at least half of the working length.

The problem of deep disinfection of dentin, deltas of root canals of teeth and obliterations, including complete ones, can be solved only by using the transport of drugs into the root system using direct current. Improvement of the method of electrophoresis of the root system of teeth for this purpose is of great importance for dentistry.

Keywords: *dentistry, iontophoresis, persistent apical periodontitis, silver, copper, root canal, lateral canal*

Introduction

The improvement of the root canal treatment outcome is a challenging problem of contemporary dentistry [*Efanov OI et al.,2003, Efanov O. et al.,2006, Volkov AG. et al.,2011, Alexandrov MT et al.,2018, Doroshina V.Yu. et al.,2019*]. The main causes of failures and complications in endodontic practice are related to insufficient disinfection of the apical part of the root canal, where multiple accessory canals may be found forming a so-called apical delta [*Efanov OI et al.,2009, Efanov O. et al.,2008*].

Root canals have multiple branches, which makes treatment very difficult [*Tsujimoto Y., 2009*]. Even in the central incisors of the upper jaw, in more than 30% of cases, there is a branched apical delta. The obliterated part of the root canal cannot be treated qualitatively using standard methods. Therefore, in the first half of the 20th century, it was proposed to use a direct electric current for this purpose [*Doering A. et al., 2014*]. Several techniques have been developed: transcanal electrophoresis of iodine, trypsin, lysozyme, transcanal anodalvanization, depophoresis of copper-calcium hydroxide, apex-phoresis using a sil-

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ver-copper conductor [*Volkov AG et al.,2019*]. Japanese researchers studied the root canal wall permeability for Ag (NH3) 2F solution using electrophoresis (ionophoresis) [*Yokoyama K et al., 2013*]. In a study by Ouama T and co-autors, in vitro study examined the antimicrobial efficacy of ionophoresis against Enterococcus faecalis, Candida albicans, Pseudomonas aeruginosa and Bacillus subtilis. The results of this study showed that Ionophoresis with silver diamine fluoride is an effective remedy for the destruction of E. faecalis, C. albicans, P. aeruginosa and B. subtilis, which are often found in the root canals of teeth with apical periodontitis.

In 2001, Peters LB and co-autors conducted a study of two sets of apical periodontitis teeth collected from different geographical locations to study bacteria left in the root dentin tubules [*Peters LB et al., 2001*]. Root dentin of 20 of these teeth was cultured from three locations between pulp and cementum (A, B, and C). In addition dentin from eight teeth was examined histologically. Using the culturing technique bacteria were found in 77% of the dentin samples from set 1 (Amsterdam) and in 87.5% of the dentin samples from set 2 (Glasgow). The dentin layer near the cement contained> 50,000 colony-forming *units/mg* dentin of various microorganisms. The dentin in the layers closer to the root canal contained more gram-positive, anaerobic bacteria and more bacterial species were found. According to the authors, in more than half of the infected roots the bacteria is present in the deep dentin close to the cementum, which confirmed the anaerobic cultivation of dentin.

At present, there is an unresolved problem in endodontics - the preservation of teeth with complete root canals obliteration by disinfecting them [*Connert T et al., 2017*].

In 2015, Paridhi GU et al., considered the etiology, prevalence, classification, diagnosis, as well as options for treatment of teeth with obliteration pulp, as well as different treatment approaches and treatment strategies to overcome potential complications [*Paridhi GU et al., 2015*].

Furthermore, of Yokoyama K and co-autors (2013) has reported that ionophoresis after coating with Ag (NH3) 2F solution leads to the greatest and deepest silver penetration into the root canal wall [*Yokoyama K et al., 2013*].

A novel method of root canal disinfection with the use of direct current through a silver-copper conductor - an apex-phoresis - has been developed at the Department of Conservative Dentistry of I.M. Sechenov First Moscow State Medical University [*E Efanov O. et al.,2006; fanov OI et al.,2003*]. The aim of the method is to enhance the quality of root canal apical part debridement. The antibacterial effect of the apex-phoresis is due to the dissolution of the silver-copper electrode as a result of electrochemical processes occurring at the anode during the procedure, and saturation of the sound tooth tissues with ions of copper and silver. Furthermore, various acids and their salts are formed close to the anode, in particular sodium hypochlorite, also providing bactericidal activity [*Efanov OI et al.,2008, Efanov O. et al.,2009, Volkov AG. et al.,2011*].

The aim of the study was to investigate the effect of the depth of silver-copper electrode insertion into the root canal on the distribution of metal compounds in root canal system and hard tissues during apex-phoresis.

MATERIAL AND METHODS

Twenty-four human teeth were used in order to assess the distribution of the metal compounds in the root canal system *in vitro* after apex-phoresis. The root canals of all teeth were prepared up to size 20 ISO, irrigated with distilled water and filled with isotonic NaCl solution then a special solid silver-copper electrode for apex-phoresis was inserted into a root canal. The electrode is composed of silver-copper metal wire, placed in electrical insulation. The insulation is the entire electrode, except for its working (active) part, at 1 - 2 mm from the end of the conductor where the wire has no insulation. Solid metal conductor is a copper core covered with a layer of silver. Working-active part of the electrode was advanced into the root canal to the maximum possible depth, trying to achieve the apical part. The electrode was fixed in the root canal with the use of dental sticky wax. Silver-copper electrode was connected to the positive charge, "Potok – 1" was used as a direct current generator. Each procedure was limited by the amount of charge of 5 mA per 1 *min*.

The teeth were divided into two groups (n=12). In the first group root canals were scouted and enlarged for more than ½ of working length. In the second group root canals were enlarged to the length of 1/3 to ½ of the working.

Each group was divided into 2 subgroups (n=6) according to the environment in which the apex-phoresis to be conducted. The teeth of the first subgroup were placed in a glass cup with isotonic NaCl solution. The teeth of the second subgroup - into a glass tube filled with gelatine prepared om isotonic NaCl solution. The teeth were immersed in environments up to the cemento-enamel junction and fixed to the cup wall or the tubes with light-curing LC BLOCK. Graphite electrodes were used as the passive electrodes in all groups. Passive electrodes were places at a distance of 10 mm or more, avoiding direct contact with the tooth. Then the procedure of apex-phoresis was performed, as described above. After the apex-phoresis procedures the distribution of the compounds of silver and copper within sound tooth tissues of the roots was studies. Visual inspection or the outer surface of the roots was carried out with the use of operating microscope (x3).

Three teeth in each subgroup were sectioned transversely with the use of diamond discs under water cooling into four parts:

1 - the upper third of the root, which is adjacent to the orifice of the root canal;
2 - the middle third of the root;
3 - the apical third of the root, which is adjacent to the apical part;
4 - the most distal apical part, sectioned from the apical third of the tooth root.

Three remaining teeth in each subgroup were broken in the four described above sites. For this purpose, transverse incisions on the root surface were first made with diamond discs. Then the roots were fractured with forceps in the area of the incisions. These cut and splat parts of the teeth roots, as well as working-active part of the silver-copper electrode used during the procedure were then inspected under the light microscope (x40 – 100). This inspection revealed sites for subsequent electron microscopic examination. Specimens were prepared for scanning electron microscopic examination. Half of the surface of each sample was etched with 37% phosphoric acid (“Ultra etch”, Ultradent, US) for 10 seconds. Then, the samples were washed with running water for 20 seconds. The cuts and splits of teeth were dehydrated, for this purpose they were placed in a vacuum chamber for 15 hours. Dehydrated samples were then gold-coated three times in a vacuum chamber via ion-plasma sputtering. The prepared samples and work-active part of the silver-copper electrode were then examined in a scanning electron microscope Hitachi S-236ON (Japan) at accelerating voltage of 25 *kV*.

RESULTS

The examination of the working-active part of the silver-copper electrodes used for apex-phoresis revealed, that during the procedure, the working-active part of of silver-copper electrode was subjected to anodic dissolution. As a result silver compounds and copper were distributed in the adjacent tissues. The dissolution of the working-active part of the silver-copper electrode is associated with a variety of electrochemical and chemical processes occurring during the procedure on the electrode and in the space close to the electrode.

he most important reactions during the apex-phoresis, in our opinion, are:

1. The generation of active oxygen radicals and chlorine in the perielectrode space, which in turn react and form hypochlorous and hydrochloric acid as well as sodium hypochlorite.

$H_2O - \bar{e} = OH^- + H^+$

$2OH^- - 2\bar{e} = H_2O_2 = H_2O + O$

$2O = O_2\uparrow$

$NaCl = Na^+ + Cl^-$

$Cl^- - \bar{e} = Cl$

$2Cl = Cl_2\uparrow$

$Cl_2 + H_2O = HCl + HOCl$

$NaOH + HOCl = NaOCL + H_2O$

2. The formation of silver chloride on the side surface of the active part of the working electrode.

$Ag^+ + Cl^- = AgCl\downarrow$

Silver chloride is slightly soluble but possesses conductivity. The side surface of the active part of the working electrode consisting of a layer of silver, does not dissolve at this stage, and is covered with silver chloride.

3. The formation of copper chloride on the end surface of working-active part of silver-copper electrode.

The end portion of the electrode contains both silver and copper. However, due to the fact that copper is more active (is to the left from silver in electromotive series of metals) the electrochemical processes occurring at the end of the electrode are mainly associated with copper.

$$2Cu + 4HCl + O_2 = 2CuCl_2 + 2H_2O$$

Copper chloride, formed in this reaction, is a soluble compound, that means than on this stage the copper end portion of the electrode begins to dissolve.

$$Cu\ Cl_2 = Cu^{+2} + 2\ Cl^{-}$$

4. The formation on the side surface of the working-active part electrode of silver sulfide and silver sulfate.

Sulfur is present in root canals. Its sources are primarily protein molecules. During the apex-phoresis acids formed in the perielectrode space begin to dissolve the dentine of the tooth root. This leads to the release of sulfur, which is s part of dentin collagen. At the same time negatively charged protein complexes from the contaminated root canal surface move towards the anode. The destruction of these complexes also causes the release of sulphur ions. On the side surface of the working-active portion of the electrode sulphur reacts with silver:

$$2Ag^{+} + S^{-2} = Ag_2S\downarrow$$

Thus, along with silver chloride less soluble silver sulphide is formed on the lateral surface of the electrode.

The presence of the sulphide layer on the side surface of the electrode in the presence of active oxygen leads to the formation of silver sulphate.

$$Ag_2S + 4O = Ag_2SO_4$$

Silver sulphate compound is more soluble than silver sulphide.

$$Ag_2SO_4 = 2\ Ag^{+} + SO_4^{-2}$$

The formation of silver sulphate results in the dissolution of silver sulphide on the surface of the electrode and in release of silver ions to the surrounding tissue.

5. The formation of copper sulphide and copper sulphate on the end, and on the lateral side (after the dissolution of the silver layer) of the working-active part of the electrode.

Copper reacts with sulphur compounds forming copper sulphide (CuS), which under in turn reacts with oxygen and forms a soluble compound - copper sulphate.

$$CuS + 4O = CuSO_4$$

$$CuSO_4 = Cu^{+2} + SO_4^{-2}$$

The formation of copper sulphate and copper chloride results in the dissolution of the electrode and the release of copper which leads to the distribution of the copper ions in the tissues surrounding the electrode.

Visual inspection of the root surfaces revealed a green-blue staining of the roots by copper salts in both groups irrespective on the environment (saline or gelatine) used during apex-phoresis (Fig.1). In cases where the root canal was enlarged less than 1/2 of the working length, only the upper and the middle part of the root were stained. In group where the root canals were enlarged to a greater depth, the green-blue staining was found in the apical part of the root. The most coronal section of the root in this case was not stained.

Thus, the staining of the root section depended on the degree of root canal patency, and respectively on the depth of the active part of the silver-copper electrode insertion.

On cross-sections of cuts and splits the staining of dentin by copper compounds was also noted (Fig. 2, A-C). The closer the split was to the place

FIGURE 1. The staining of dentin with copper salts after apex-phoresis of teeth with varying degrees of root canal patency

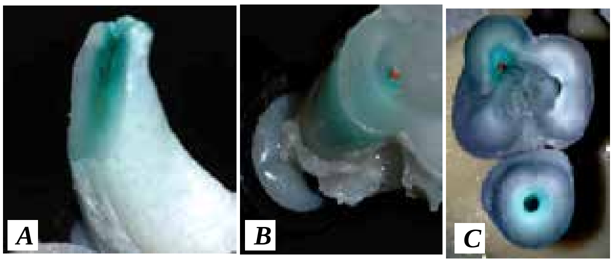

Figure 2. Cross-cuts and splits of the teeth after apexphoresis

where the working-active part of the electrode was located, the more intense was the green-blue staining of dentin. It was found that in the immediate proximity of the location of the working-active part of the electrode, a gray-black staining was also noted along with green-blue, suggesting the presence of silver compounds (Fig. 3).

In the samples where the root canal was enlarged for more than half the working length, in the apical accessory canals were filled with salts of blue-white colour the mail body of which also comprised silver compounds. On next stage of the study scanning electron microscopic evaluation of cross-sections of root cuts and splits was conducted. The root can be sectioned in any desired area and in any direction using diamond burs. Therefore most investigators use cuts of teeth for electron microscopic evaluations. However, the surface of cuts often reflects a distorted picture, since the dentin microparticles contaminate the surface layer of the samples. To get more reliable results in the investigation of cuts, the ½ of the surfaces of each cut sample was etched and washed with water. This made it possible to remove the smear layer, but it led to simultaneous partial removal of silver and copper salts on the surface of dentin.

Figure 3. The staining of dentin with copper and silver compounds in the area of electrode work-active part location

The evaluation of splats roots of the teeth has both advantages and disadvantages. The advantages is that the splits do not need to be etched with acids, so compounds of silver and copper are preserved in the sample. The disadvantage is that it is not always possible to split the root at the desired location and in the desired direction.Thus, to get a reliable information, both cut and splat samples were studied and the results of evaluation of these groups were compared.

The results of canning electron microscopic evaluation showed the deposition of metal salts on the walls of root canals. The the amount of salts increased when approaching the area adjacent to the working-active part of the silver-copper electrode.

In the case where the root canal was enlarged to 2/3 of the working length no metal salt deposits were detected in the coronal part of the canal near the orifice. The root canal was empty, and the openings of dentinal tubules on its were hollow. In the middle third of the root canal, which was close to the location of working-active part of the electrode, deposition of metal salts fragments was determined, on the walls of the root canal obturating the openings of dentinal tubules. In the lower third of the root canal, where working-active part of the electrode was located during the procedure, the dentinal tubules were not visible due to heavy metal salt deposits on root canal walls (Fig. 4. A-C).

The metal salts were determined not only on the walls of the main canal but also in accessory canals (Fig. 5 A, B).

Deposition of metal compounds, Depending on the diameter of the accessory canal, it was partially (Fig. 6) or totally (Fig. 7) obturated by the deposition of metal compounds.

It should be noted that the excessive deposition of metal compounds was observed only in those areas of the tooth roots, which were adjacent or in close proximity to the location of the active part of the working-silver-copper electrode during the procedure. Therefore, in cases where the root canal was enlarged on less than half of the working length, and the working-active part of the silver-copper electrode was placed near the canal orifice, the deposition of metal salts in the apical part was not detected.

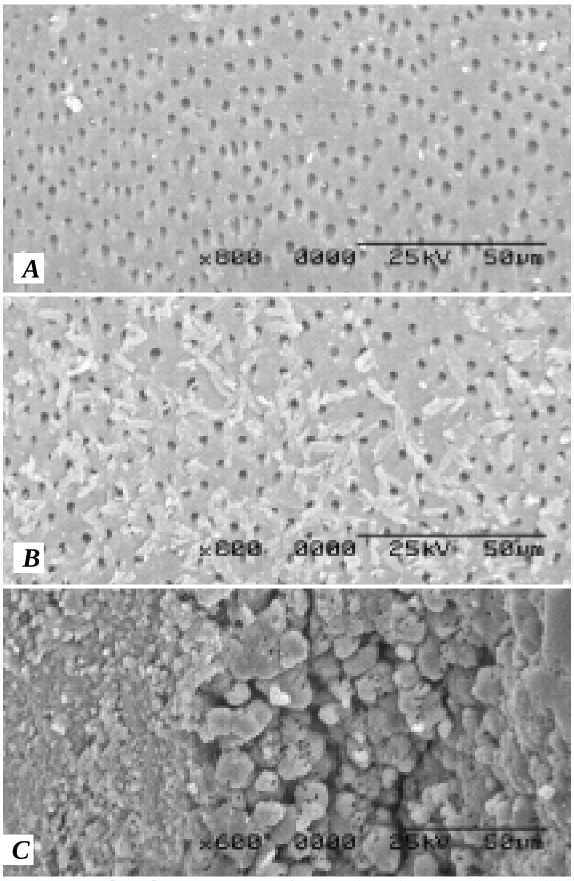

FIGURE 4. The deposition of metal salts on the walls of the root canal after apex-foresis (A - coronal third of the root canal, adjacent to the canal orifice; B - dentin in the middle third of the root canal; C - dentin in the apical third of the root canal).

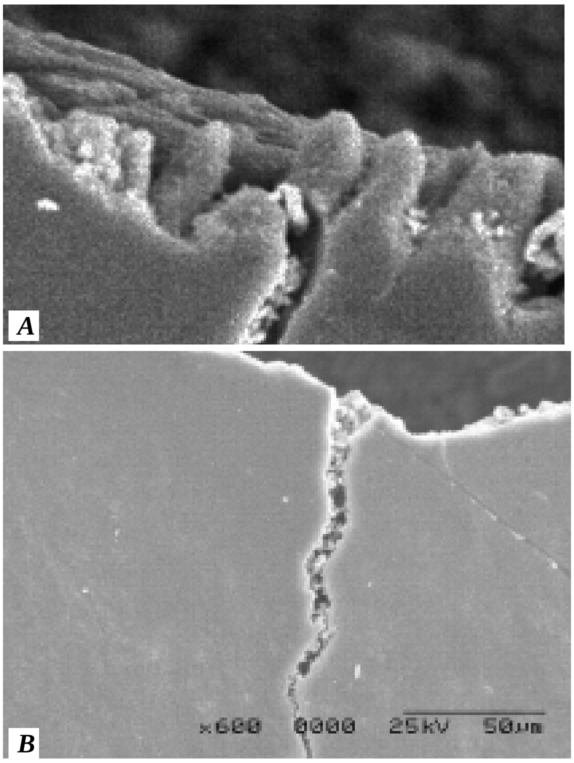

FIGURE 5. The deposition of metal salts in the accessory canal

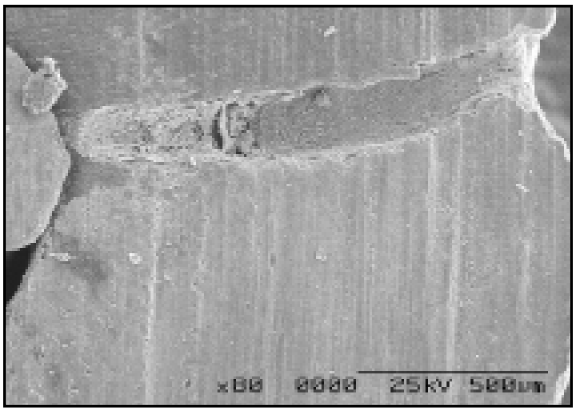

FIGURE 6. Partial obstruction of the accessory canal with metal salts

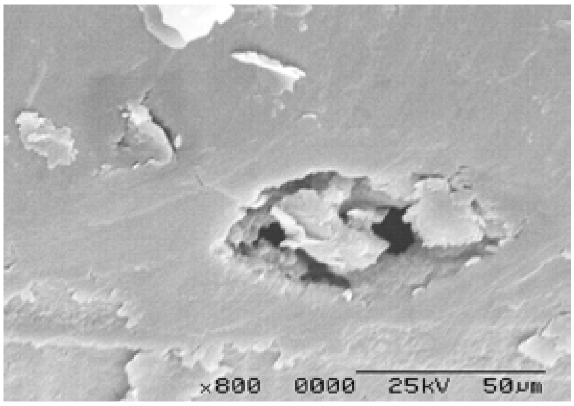

FIGURE 7. The outer surface of the tooth; accessory canal is obturated with metal salts

DISCUSSION

During apexp-horesis, the active part of the electrode placed in the tooth cavity undergoes anodic dissolution, as a result of which metal compounds enter the surrounding tissues. During apexphoresis, these are silver and copper compounds [*Nosov VV, Volkov AG, 2003*].

Metal ions enter into chemical interaction with elements contained in the surrounding tissues. Metal compounds are represented by their salts, namely: chlorides, sulfides, sulfates, phosphates, as well as a number of complex compounds of copper and silver. These salts line the walls of the root canal, fill the microchannels, obturating them, and penetrate the dentin of the tooth root.

In the work of Yokoyama K and co-autors (2013), it was proved that iontophoresis after coating with Ag (NH_3) 2F solution leads to the greatest and deepest penetration of silver into the wall of the root canal. Due to the formation of poorly soluble salts, silver and copper are able to maintain their presence in the hard tissues of the teeth roots for many years. In the presence of silver and copper compounds, unfavorable conditions are created for the development of microflora,therefore, the use of apex-phoresis in the treatment of teeth with difficult root canals allows to achieve the effect of long-term disinfection of the teeth roots. The results of a study by Oyama T and co-autors (2009) showed that iontophoresis with silver diammine fluoride is an effective treatment for *E. faecalis, C. albicans, P. aeruginosa* and *B. subtilis,* which are common in the root canals of teeth with recurrent apical periodontitis. During apex-phoresis, despite the relatively low current strength (0.5 - 1 *mA*) and the short duration of the procedure (5 minutes), a high concentration density of ions and colloidal particles of the drug is created in the apical part of the root - 1.24 - 2, 49 $mAhmin / mm^2$.

This is due to the peculiarities of the methodology for this procedure: the small size of the working - active part of the electrode and its location in the immediate vicinity of the “impassable” apical part of the tooth root. When performing apex-phoresis, there is no current leakage through the “passable” section of the root canal, therefore all electrochemical processes develop near the “impassable” apical part of the tooth root. Knowing the electrochemical equivalent of metals used in apex phoresis, it is possible to calculate the amount of substance entering the tooth during the procedure, as well as its concentration density in the apical part of the tooth root. For silver, the electrochemical equivalent is 1.118 *mg*, for copper - 0, 329 *mg*.

As a result of the calculations carried out, it was found that under ideal conditions, at a dose of apex-phoresis of 2.5 - 5 *mAhmin*, from 0.168 to 0.335 *mg* of silver and from 0.049 to 0.099 *mg* of copper enter the surrounding tissues. In the apical part of the tooth root with apex-phoresis, the concentration density of silver will be 0.083 - 0.168 mg/mm^2, and copper - 0.024 - 0.049 mg/mm^2. Whereas the toxic dose in the body is more than 250 *mg* for copper and 60 *mg* for silver.

During apex-phoresis, electrochemical processes are local in nature and develop in that part of the tooth root where the working-active part of the silver-copper electrode is located during the procedure. To saturate the peri-apical part of the tooth root and the apical delta with silver and copper compounds during apex-phoresis, the root canal must be traversed and expanded by at least half the length of the tooth root. Tsujimot Y and co-autors (2009) examined a large number of extracted teeth to reveal the proportion of teeth, features of the roots and root canals, the presence of lateral branches in them, an apical delta with a large number of small holes in the apex zone [*Tsujimot Y et al., 2009*].

Endodontic therapy is associated with a certain degree of complexity, since we cannot see the root canal from the outside. Therefore, in the authors’ opinion, detailed information about the root canal system is important for endodontic treatment. In this work, the treatment of root canals using microscopy was carried out.

The microscopic images obtained under illumination facilitate easy and precise root canal treatment and reveal structural abnormalities. However, endodontists, anatomists and embryologists need to understand the complex development of the root canal system in order to facilitate precise endodontic therapy, thereby promoting patient health [*Ternovoy SK, Makeeva IM et al.,2010*].

CONCLUSION

During apex-phoresis, copper and silver ions enter the sound tooth tissues as a result of anodic dissolution of working-active part of the silver-copper electrode. Moreover, the anode in the electrode space formed acids were formed in perielectode zone which may cause the dissolution of calcium hydroxyapatite $Ca_5(PO_4)_3(OH)$ in the adjacent dentine, causing the release of calcium ions. As a result of electrochemical and chemical processes occurring during apex-phoresis, soluble, poorly soluble and sparingly soluble compounds of silver copper, and calcium are formed. The metal salts are chlorides, sulphides, sulphates, phosphates, as well as a number of complex compounds of copper and silver. These salts line the walls of the root canal and fill accessory canals, penetrate dentin.

These processes are strictly local taking place only in the area, where the working-active part of the silver-copper electrode is located during the procedure. Therefore, to ensure the saturation of the apical portion of the canal and apical delta with silver and copper, the root canal must be enlarged for at least half of the working length.

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