Electrochemical Impedance Spectroscopy (EIS) Investigation on the Action of Dental Endodontic Lavage Substances

VIRGIL PENTA[1]*, CRISTIAN PIRVU[1]

[1] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Polizu, 011061, Bucharest, Romania

The paper is focused on using the electrochemical impedance spectroscopy (EIS) technique for enhancing cleaning and disinfecting of the root canal structure. The research investigated both NaOCl and EDTA (ethylenediaminetetraacetic acid) effects, establishing the optimal time of EDTA (ethylenediaminetetraacetic acid) application.

Keywords: Sodium hypochlorite, EDTA, ProTaper, AH plus

In the last decade, physical, chemical and engineering procedures have been used extensively in dental medicine, not only for biomaterial characterization [1,2], but for the understanding and improvement of various clinical aspects as well. One of such aspects is root canal morphology and the good quality cleaning and disinfection of this structure. Without a good quality debridement of root canal dentine it is virtually impossible to obtain a correct sealing of the endodontic space reducing the risk of further infectious problems [3]. The present article uses EIS technique to convey results about dental endodontic washing solutions and their effect on the dental canal. It is very difficult to quantitatively regard the effect of sodium hypochlorite vs. the effect of EDTA [4,5], and our work was concentrated on quantifying the action of the two dental solutions. We wanted to observe the cleaning efficacy of sodium hypochlorite in regard to the organic component of the smear layer. Current research shows that it cannot fully clean the complex dental root structure [6]. Initial EIS data after enlarging the root canal in a sodium hypochlorite environment were obtained and discussed in order to underline the debridement qualities of this cleaning agent [7]. Furthermore in order to observe the effect of EDTA [8], a 17% gel was placed in the dental canal and by varying time of action and registering EIS data we could observe the effect. In this way we could see a change in electrical diffusion of the root complex. EIS is a very useful laboratory procedure broadly applied in our days especially in characterizing electrode processes and complex interfaces [9] also used in dentistry [10]. EDTA has been utilized in chemical cleaning of endodontic tooth structure for a long time, but despite some recommendations [5] or some practices, the ideal time needed for cleaning the tooth structure has not yet been established. This will be an original merit of the present study with practical future application. The root canal system has countless secondary canals and dentinal tubules that should be theoretically filled by sealer paste [11]. SEM can offer a surface view with almost no in depth information. Clinical practice has proven that quality filling of the root system is difficult to obtain, partially because of root canal complexity and also because of operator's training and skill. The novelty using the EIS technique consists in its ability to offer a unique view of the whole root structure from the endodontic point of view. In other words one can practically view the different layers of the tooth cementum, dentine, smear layer and the manner in which electrical current passes through, in the form of equivalent electrical circuit phases. The electrochemical techniques have been used intensively for dental materials stability [12] characterization.

The original experimental part has two major steps. The first part concerning EIS observations regarding the effect of NaOCl on the root canal wall and the second concerning quantification of EDTA action and optimal time of application.

Experimental part

EIS Measurements

For laboratory application of EIS, three groups of 5 teeth were exposed to the action of EDTA varying duration from 1 min to 3 min to 5 min. All the groups contain similar maxillary front teeth. The teeth used were prepared using the following steps:

-after extraction, teeth were thoroughly cleaned of any remaining debris using a dental scaler, and then were disinfected using 0.5% chloramine T solution;

-then the teeth were placed into separate recipients filled with distilled water and refrigerated at 4 degrees Celsius for a period of up to one week.

In order to prove the effectiveness of EDTA the human extracted teeth were endodontically treated using ProTaper rotary file system. Working length was established radiologically using the Kodak 6100 digital sensor. The teeth had then received root canal therapy using copious irrigation with NaOCl 3%. After completing the treatment the apex was sealed with composite resin and we placed a thin platinum electrode wire inside each root.

The EIS cell was comprised of a reference Ag/AgCl electrode, a platinum counter electrode and a working electrode. For the working electrode, tooth samples (whole tooth and dentin) were used. Data obtained is expressed graphically using Nyquist and Bode plots. The EIS analysis was carried out using an Autolab PGSTAT 302 N potentiostat with NOVA 1.8 software. Testing was done in SBF (table 1) [13].

Clinical trials.

The in vivo experiment is in regard to the clinical application of EDTA. This part of the study has been conducted after the patients signed the informed consent file and all the procedure details had been carefully

* Tel.: 0757110184

explained. The study group was comprised of 9 patients divided in 3 groups varying the EDTA action time from 1 minute in the first group to 3 minutes in group two and 5 min in group three. After final filling of the root canal all patients received radiographs using the Kodak 6100 digital sensor and sealer penetrability had been judged visually on the final radiographs. All clinical cases were treated by a general practitioner not an endodontic specialist with the routine use of the rubber dam setup and the rotary ProTaper® system for root canal shaping. Clinically a 3% sterile NaOCl solution Parcan® by Septodont® was used for irrigation. The final filling of the root canal was done by the cold lateral condensation technique with ProTaper gutta-percha by Dentsply Meillefer and plus sealer paste auto mixed in order to obtain the same rheological properties.

Results and discussions

Hypochlorite application

The first observations done concerning the effect of sodium hypochlorite on the root canal wall showed incomplete secondary root canal structure permeabilization. After enlarging the canal with rotary Ni-ti instruments and acquiring the initial EIS data the fitting circuits presented three phases corresponding to the natural tooth tissues and to smear layer on the inner part of the root canal wall.

The very high electrical resistance and diffusion effect seen on Nyquist and Bode plots quantified by the fitting circuits and Bode values show a rather limited action of the sodium hypochlorite organic oriented action. Taking into account the complexity of the smear layer, being inorganic and organic this pattern of action could be understood.

All radiographs in the group with only sodium hypochlorite action showed no sealer penetration into secondary root structure. To summarize on the action of this cleaning solution, it is targeted on organic debris being incapable of removing the complex smear layer.

For the second part of the study, the laboratory testing of EDTA on extracted teeth, the most striking result of the EIS interpretation was a markedly decrease in tooth electrical resistance. The teeth, although all frontal teeth were quite unique, it is difficult to subject such variation in structure, size, length, and width to standardization. The results following EIS testing showed a three-phased circuit describing the natural tissues and intra canal debris (fig.1).

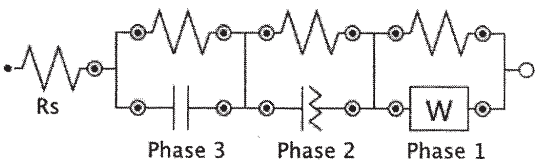


Fig.1 The circuit used for Nyquist fitting

The first resistance, Rs is the equivalent of the physiologic solution, SBF, used for testing in the electrochemical cell. The third phase corresponds to the capacitive behaviour of cementum, this layer being a very thin one of 10-50μm cervically and 50-200μm apically. The second phase corresponds to dentine. Dentine has many dentinal tubules in its structure that should confer a permeable effect if they were accessible. Often they are filled with debris following nerve mortification and mechanical action on intra-canal dentine. The first phase corresponds to the smear layer that blocks the internal end of the dentinal tubules. This smear layer results from filing dentine during endodontic treatment and could be removed if the proper lavage substances were used.

EDTA action

All groups subject to EDTA action have shown a reduction in tooth impedance values proportional to exposure time. Dentine has shown a decrease in resistance, furthermore changes in its character from diffusion to permeability are in relation to partial or complete removal of smear layer. At first the dentine presented a diffusion effect, meaning that there was no permeability in its structure and the conduction effect was decreased. After the EDTA application again proportional to the time of action and smear layer removal the dentine diffusion effect gradually changed to permeability, meaning that the porous dentine structure was gradually chemically cleaned. This can be visualized by the removal of one of the circuit phases.

The EDTA effect is even more interesting; it cleans the lateral canaliculi and secondary root canals as well [14,15]. So the decrease in tooth electrical resistance is very much the result of a more permeable structure. To support this, our results show that after the application of EDTA a change appears on the Nyquist graphs, from diffusion- hinting to a low permeability into increased permeability. An exact quantification of the decrease in impedance values was done using the Bode plots for each tooth in part. The Bode diagrams show the variation of electrical resistance versus electrical current frequency.

Although for the first 1 min group there is little change in the penetration of sealer in secondary large canals for the 3 and 5 minute group there is a significantly increased penetration into lateral canals thus hinting to a better chemical cleaning of the root system. There is a small discussion to be made here, on the one side the EIS shows a relatively clean structure, by showing a great reduction in electrical resistance and on the other side the clinical cases for 3 min testing still show incomplete cleaning, this could occur because of particularities in each of the five clinical cases but it could also mean that an inappropriately wide frequency setting of the Autolab probe was used [6]. The five-minute group shows an even greater reduction in tooth electrical resistance and the clinical group shows complete sealer penetration into the complex root system.

First group – 1 min EDTA tooth testing time

Teeth in this study group have been subject to EDTA for a total period of 60 s. This is a very small amount of time for the substance to make a noticeable effect. Still, after this short period there is a clear quantifiable modification of impedance values and the electrical resistance decreases rendering a more permeable structure. The initial Nyquist plots display an inhomo-geneous dispersion describing a low quality penetration of ionic species through the tooth structures (fig.2).

Based on the Bode diagram a high electrical resistance is characteristic to the untreated enamel and dentin layers (fig.3). With the application of EDTA the character changes revealing a more penetrable construct. We believe this effect is a result of cleaning the superficial part of the smear layer and opening of dentinal tubules. Our experimental data showed a relation between time of action and quality of smear layer removal.

The general structure of the three-phased circuit changes very little in shape; there is a decrease in the electrical resistance in phase two and one (fig.1). So the character of modification relates closely to our model, the reduction in electrical resistance by removal of smear layer and repermeabilization of dentinal tubules.

The Warburg corresponds to non-ideal layer geometry of the structure changing into a constant phase element

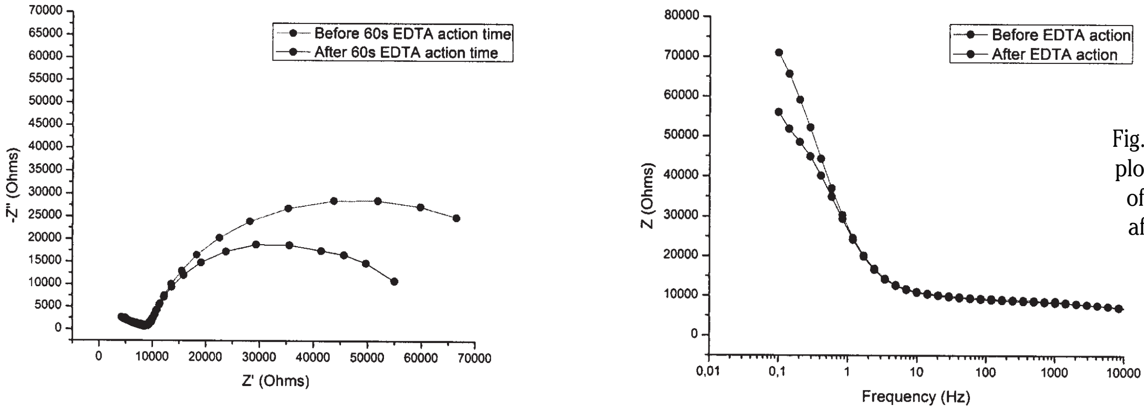
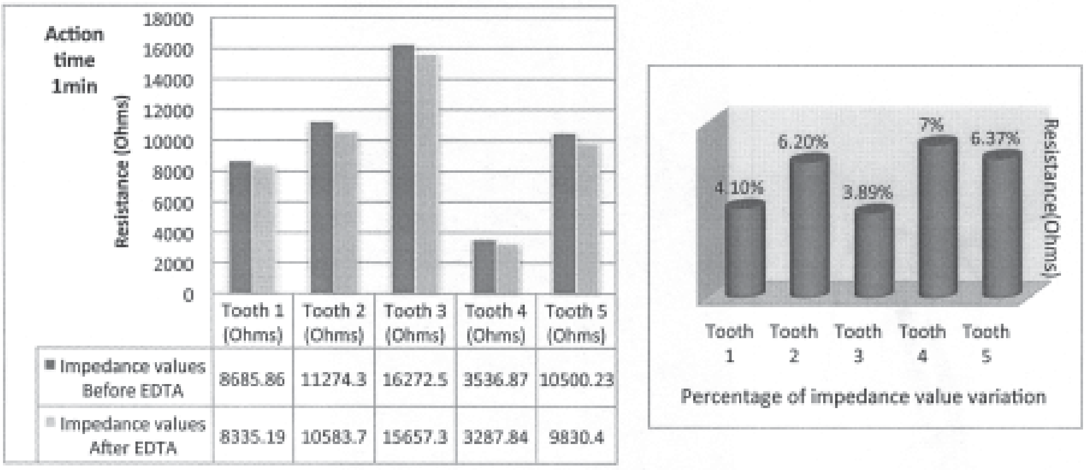


Fig.2. Nyquist and Bode plots showing variation of impedance values afer 60s EDTA action time



	Tooth 1 (Ohms)	Tooth 2 (Ohms)	Tooth 3 (Ohms)	Tooth 4 (Ohms)	Tooth 5 (Ohms)
Impedance values Before EDTA	8685.86	11274.3	16272.5	3536.87	10500.23
Impedance values After EDTA	8335.19	10583.7	15657.3	3287.84	9830.4

Fig.3 Variation of impedance values before and after the application of EDTA for 60s.Right side - percentage of resistance variation befor and after EDTA application

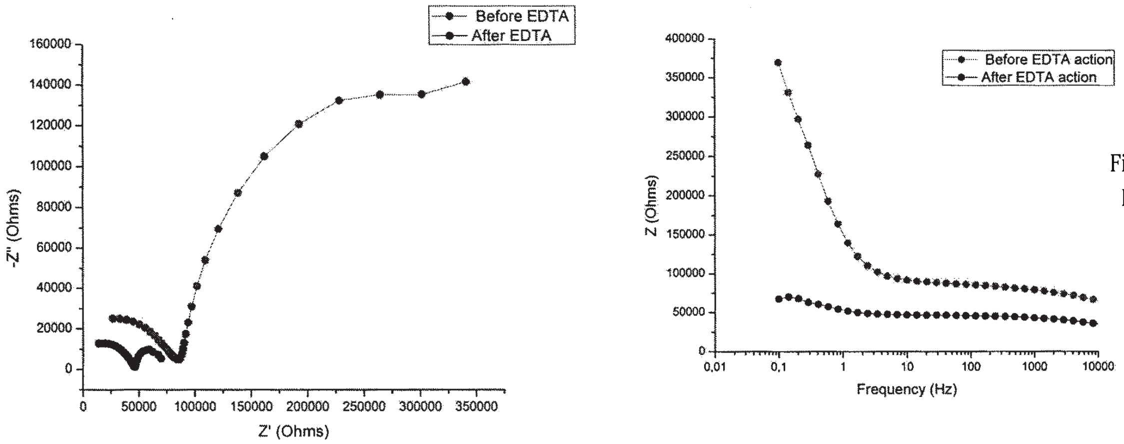


Fig.4 Nyquist and Bode plots for 3 min EDTA tooth action time

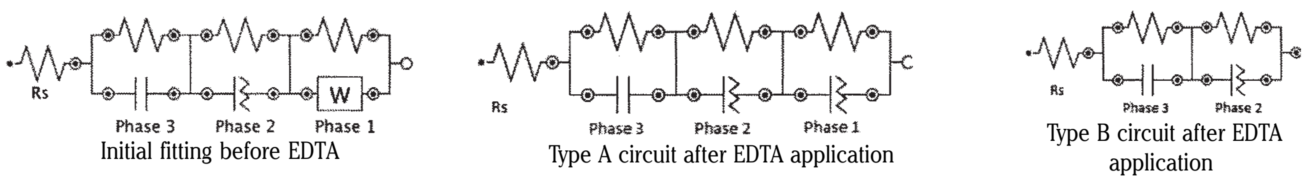


Fig. 5

(CPE) hinting to capacitance -conductiveness- after EDTA application. This change practically means a partial cleaning of the intracanal debris and smear layer structure modification.

The exact quantification of impedance values was done using Bode Plots and is presented in figure 3.

The 60s EDTA tooth action time is limited to partial cleaning of the Smear layer and very low permeabilization of the dentinal structure. Practically there was no noticeable sealer penetration into the dentinal structure as shown on patient's radiographs.

Second group - 3 min EDTA tooth testing time

The teeth in the second testing group were subject to EDTA action for 3 min. This period has produced an important variation on impedance values and has almost halved the tooth electrical resistance in some cases. Nyquist and Bode plots show reduction of tooth electrical resistance and increase tooth permeability effect (fig.4).

The circuit used for initial data fitting, before EDTA, was the three-phased circuit already described.

After the application of EDTA in this second group there were two types of circuit variations for data fitting (fig.5) corresponding to superficial and partially profound smear layer removal.

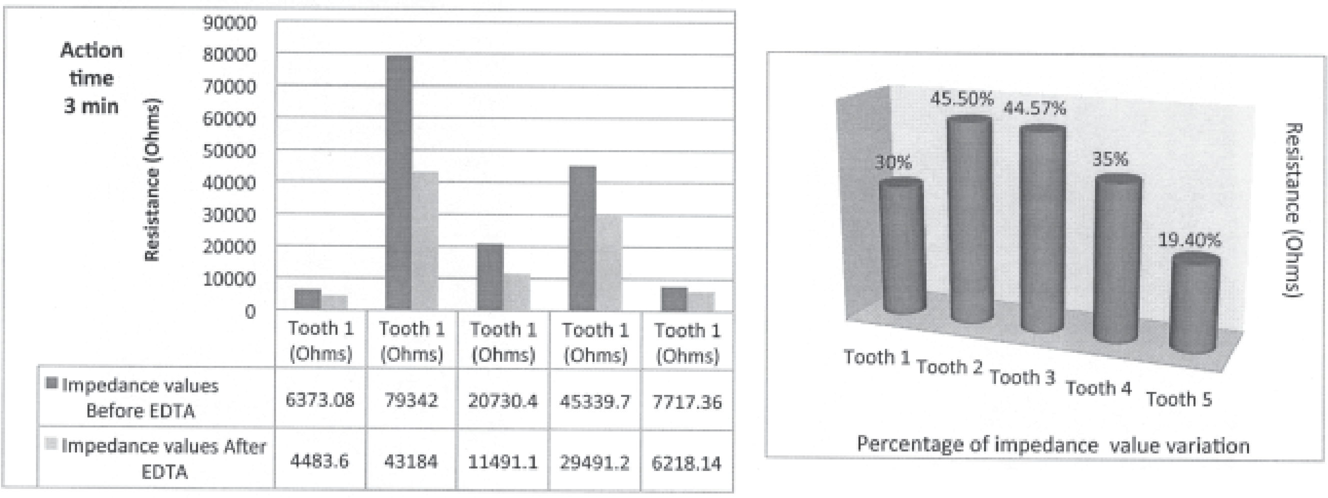


Fig.6. Upper table variation of impedance values before and after EDTA 3min action time, lower table procetage of ohmic resistance difference between initial and final tooth values

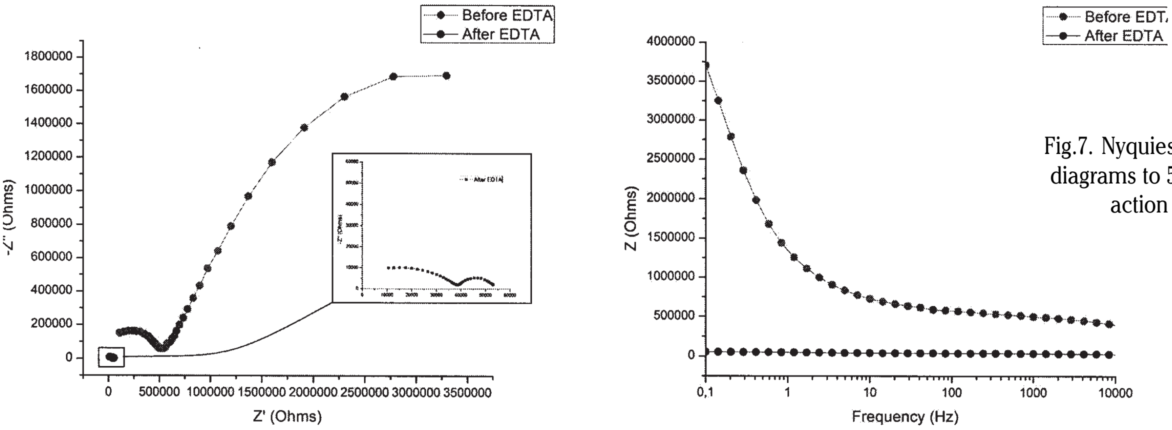


Fig.7. Nyquiest and Bode diagrams to 5 min EDTA action time

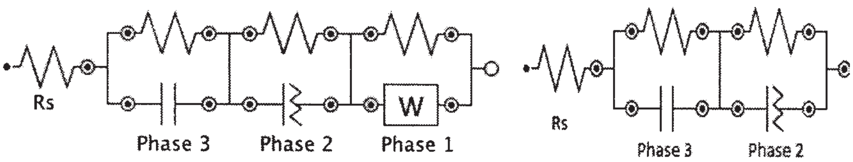


Fig.8. Left side - initial fitting electrical circuit and - the right side- fitting circuit after the application of EDTA for 5 min

Type A circuit presented all the phases of the initial circuit but showed a change from Warburg dispersion to Constant phase element hinting a capacitive effect and a decrease in electrical resistance in phase two. Type B circuit presented the loss of the first phase and a reduction of resistance in the second phase.

Also the graphs shift from a diffusion effect to more capacitive behaviour showing a more permeable dentinal structure. The cleaning effect is more intense with greater time application; the compact smear layer resulted after root canal therapy being partially cleaned. For a better quantification of the tooth electrical resistance variation we used the Bode plots and the results are shown in figure 6.

Third group – 5 min EDTA tooth testing time

This group has been subject to 5 min EDTA action time. The initial fitting circuit was the classic three-phased diagram. After applying the EDTA the circuit evolved into a two-phased representation.

These phases presented a markedly reduction in electrical resistance corresponding to the complete smear layer cleaning and also a permeabilization of the dentinal structure corresponding to opening the tubules and secondary large canals. The Nyquist and Bode diagrams show a change from diffusion to permeability of the root with a very high reduction of ohmic resistance (fig.7).

The fitting circuits show a phase modification in correspondence to cleaning efficiency of the EDTA solution [8].

The removal of the first phase corresponds to removal of superficial smear layer, reduction of resistance in the second phase corresponds to removal of the smear layer compacted in dentinal tubules (deep layer) (fig.9). The modification of the final fitting circuit should have a beneficial effect on the end result of the permanent root filling and correlates with clinical data.

Clinical study

Control group using just sodium hypochlorite and no EDTA for root canal therapy. Clinical case radiographs presenting no lateral sealer penetration into secondary canals root structure after using sodium hypochlorite as sole cleaning agent (fig.10.a).

Clinical case 1, example of group one, 1 minute EDTA action time. Pacient presented two large carious lesions on teeth 2.1 and 2.2 (fig.10.b). Upon clinical examination of the carious cavities teeth 2.1 and 2.2 presented a gangrenous condition and subsequent therapy was conducted. After finishing the mechanical enlargement

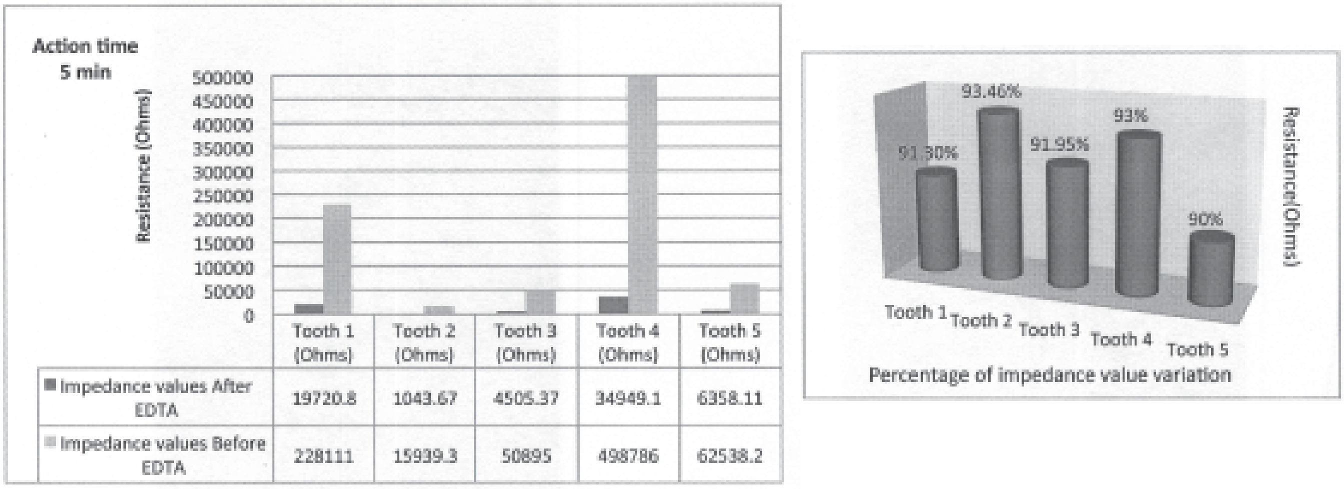
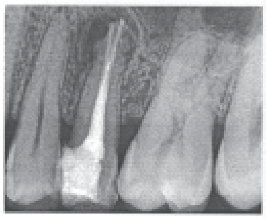


Fig.9. The effect of 5 min EDTA action time. Upper table the variation of impedance values before and after application.Lower table procentual difference before and after resistance



a. No sealer penetration

b. No sealer penetration after 1 min EDTA action

c. Partial sealer penetration into accessory canals

d. Complete sealer penetration into root canal accesory structure

Fig.10. Radiographs

Table 1
IONIC CONTENTS OF SBF

Ion	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO^{3-}	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

of the root canal up to ProTaper file F3 for 2.1 and F2 for tooth 2.2 a 17% EDTA gel was placed with a file inside the endodontic space for 60 s. After the minute of action there was a thorough rinsing step with sodium hypochlorite solution Parcan 3% . Then after drying the canal with paper cones the final filling was done by cold laterally compacted gutta-percha technique. The result was a uniform root canal filling but no sealer penetration into the dentinal structure was visible.

Group 2, three minutes EDTA action time (fig.10.c). Patient presented a gangrenous pulp condition on tooth 2.1 as a result of carious lesions on proximal sides. The tooth was enlarged to ProTaper F2 using copious sodium hypochlorite irrigation. The next step was the 3min application of EDTA into the endodontic space. After this application the root canal was again thoroughly rinsed using sodium hypochlorite and dried using paper cones. The final filling was done using the cold lateral gutta-percha condensation technique. On the final radiograph sealer penetration was evident in the initial part of the dentinal complex but the penetration was incomplete

Group 3, five minutes EDTA action time (fig. 10.d). Patient presented teeth 1.1 and 1.2 under a metal framework with periapical and periodontal problems. After removal of the metal framework the state of the teeth was assessed and a gangrenous pulp condition was found in both teeth. After the mechanical instrumentation up to ProTaper file F2 in both teeth using copious sodium hypochlorite irrigation a 17% EDTA gel was placed in the endodontic space for 3min (tooth 2.2) and 5 min (tooth 2.2). After that time the teeth were thoroughly cleaned using Parcan 3% sodium hypochlorite irrigation and the root canal was dried using sterile paper cones. The final step was the final root canal filling using cold lateral gutta-percha condensation. Sealer penetration was observed in the dentinal structure, tubules and secondary large canals. Five minutes were ideal in the effort to obtain a prone endodontic structure for good quality final filling.

Conclusions

The initial EIS data acquired after sodium hypochlorite cleaning of the root canal show an incomplete cleaning of the smear layer. The EIS investigation suggests that this technique is efficient in monitoring the demineralization process of teeth and understanding their structural changes. The fitting circuits were the equivalent of a 3-phased electrical circuit. Each phase represents a different layer of the tooth structure, cementum, dentin and smear layer.

The initial high tooth electrical resistance is understandable taking into account the type of material that the tooth is comprised of.

Regarding EDTA action time, from our current research we can conclude that a minimum 5 minutes of EDTA cleaning time has the greatest benefits in chemical debridement of the endodontic system found in teeth. The effect of EDTA offers good removal of intra-canal debris and also offers good lubrication for the rotary files preventing the "screwing in" effect and breakage.

Root canal therapy has a very high degree of complexity and uniqueness; EIS can offer a novel view in understanding fundamentals of chemical cleaning of the endodontic space. Thus having interesting implications on further knowledge of tooth morphology and diagnosis.

Acknowledgements : This research was funded by POSDRU project POSDRU/107/1.5/S/76909

References

1.RUSU L., ARDELEAN L., Rev. Chim. (Bucharest), **63**, no. 5, 2012, p. 513

2.VOICU G., BADANOIU A.,ANDRONESCU E., BLEOTU C., Rev. Chim. (Bucharest), **63**, no. 10, 2012, p. 1031

3.SOLOVYEVA A., DRUMMER P., Int. Endod. J., **33**, 2000, 494–504

4.GRANDE N., PLOTINO G., FALANGA A., POMPONI M., J. Endod, ,**32,** 2006, p 460-4

5.TEIXERA CS, FELLIPE MC, FELIPPE WT, Int. Endod. J., **38,** 2005, p. 285-290

6. DADRESANFAR B., KHALILAK Z., DELVARANI A., et al., Int. J. Oral Sci., **53**, 2011, 31-36

7. COHEN S., HARGREAVES K, Pathways of the pulp IX edition, Mosby, Elsevier 2006.p 339

8. CHEUNG G., DARVELL B., Dent. Mater., **24**, 2008, 753–759

9.MAN I., PIRVU C., DEMETRESCU I., Rev. Chim.(Bucharest), **59**, no. 6, 2008, p 615

10. PENTA V., STOIAN B., U.P.B. Sci. Bull. Series B, **74**, 2012,p 161-170

11.CLEGHORN B., CHRISTIE W., DONG C., J. Endod, **32**, 2006, p 813-821

12.POPA M., DEMETRESCU I., VASILESCU E., DROB P., IONITA D., VASILESCU C., Rev.Roum. Chim., **50,** 2005, p. 399 -406

13.MULLER L, MULLER F., Acta Biomater, **2**, 2006, p.181-9.

14.YILMAZ Z, BUZOGLU B., GUMUSDERELIOGLU M., Oral Surg Oral Med Oral Pathol Oral Radiol Endod; **111**, 2011 p.109-14

15.MISHRA L., KUMAR M, SUBBA RAO C., IJOART, **1**, 2012, p 45-50

Manuscript received: 4.07.2013