Molecular Interactions on Ethylenediaminetetraacetic Acid After Mixing With Sodium Hypochlorite

Ekim Onur Orhan, Özgür Irmak, Erol Taşal, *Member, IEEE*, and Murat Tanışlı

Abstract—The aim of this study is 1) to demonstrate the interactions resulting from mixing ethylenediaminetetraacetic acid (EDTA) withsodium hypochlorite (NaOCl) and 2) to monitor the experimental interaction after cold plasma energy transferring mimicking physicochemical activation of solutions. Freshly opened pharmaceutical lavage solutions of 5.25% hypochlorite and 17% EDTA were used in experiments. A mixture of 40 ml was prepared using 5.25% hypochlorite/17% EDTA at a 1:1 ratio. The experimental cold atmospheric-pressure plasma jet (APPJ) treatment was performed using a dielectric barrier discharge (DBD) device for mimicking the activation of lavage solutions. pH measurements of the samples were performed. Spectral changes were analyzed with nuclear magnetic resonance spectroscopy, Fourier-transform infrared spectroscopy, and UV-visible spectrophotometry. N-CH2-COOR and N-CH2-CH2-N were observed in EDTA at 4.65 and 2.90 p/min, respectively. The emergence of new signals at 3.70, 3.07, and 2.91 p/min was observed in the mixture of the EDTA and NaOCl. Decreased peak intensities of EDTA were observed in the APPJ treatment groups at 4.65 and 2.90 p/min with no spectral shift. The main findings are that carbon and nitrogen bonds formed on the EDTA molecule at 2357.08 and 2309.10 cm^{-1} after oxidation with hypochlorite as middle-narrow and mid-wide, respectively. The molecular structure of EDTA is observed to change after the oxidization by hypochlorite. The newly formed peaks attributed to carbon and nitrogen on the oxidized EDTA molecule disappeared after the experimental APPJ application. The obtained absorbance maxima in the APPJ treatment are in the characteristic region for the π-π^* transitions. Within the limitations of an *in vitro* study, the findings of the study demonstrated that the subsequent molecule occurred by the oxidization by hypochlorite or activated hypochlorite is different from each other and also is different from the characteristic structure of EDTA. Experimentally activated hypochlorite by APPJ might have caused the molecular electronic transitions of EDTA.

Index Terms—Dielectric barrier discharge (DBD), ethylenediaminetetraacetic acid (EDTA), hypochlorite, low-temperature plasma, plasma medicine.

I. Introduction

MICROORGANISMS could lead to the development of pulp necrosis and apical periodontitis which would require root canal treatment [1]. To eliminate the microorganisms from the root canal system, chemo-mechanical preparation is performed in root canal treatment [1]. Thus, disinfection of the root canal system is of utmost importance in the outcome of root canal treatment, although entire root canal sterilization is not possible [2].

Intracanal lavage or irrigation is performed for disinfection of root canal space and conditioning of dentin surface [3]. Accordingly, the objectives of irrigation are to eliminate microorganisms, flush out and dissolve the organic debris, and lubricate the root canal instruments [3]. Currently, there is no available single irrigation having organic tissue dissolution ability and demineralizing the inorganic smear layer simultaneously [4]. Therefore, various irrigation agents are used in combination. For standard nonsurgical root canal therapy, a low concentration (0.5%–5.25%) of NaOCl is the goldstandard endodontic irrigation due to its nonspecific microbial killing and tissue dissolving ability [5]. Historically, sodium bicarbonate-buffered 0.5% NaOCl solution was first used for the decontamination of open wounds in World War I [6]. Later, NaOCl irrigation named chlorinated soda was proposed for dental practice for preventing "pericementitis" by Walker [7]. NaOCl is still commonly used as it is inexpensive and readily available in endodontics [3]. As of today, no other solution or material has supplanted the extensive use of NaOCl as an organic tissue dissolving and disinfection [8], [9]. One drawback of NaOCl is that it has a restricted cleansing effect on the dentin substrate due to its inability to dissolve inorganic matter [8], [9]. Therefore, it has been recommended to use calcium-chelating agents in conjunction with NaOCl with an aim of opening the dentinal tubules with the calcium-chelating agent and consequently resulting in improved penetration of NaOCl to the dentine substrate [8], [9].

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Regarding the calcium-chelating agents, the sodium salts of EDTA are commonly used in endodontics. EDTA salt is a white and odorless powder and it exists in a non-hygroscopic crystalline structure. EDTA is slowly soluble in water [10]. In dental practice, either acidic (disodium salt of EDTA-$C_{10}H_{14}N_2Na_2O_8$) or alkaline (tetrasodium salt of EDTA-$C_{10}H_{14}N_2Na_4O_8$ or $Na_4EDTA \cdot 2H_2O$) EDTA are mostly used forms for root canal treatments [11]. The molecular mass of tetrasodium EDTA (CAS No. 10378-23-1) is 336.21 to 372.24 Da [11]. The melting point of disodium EDTA is 240 °C and its flashing point is above 100 °C [11]. Its solubility is >100 g/L in water under normal conditions [11].

In intracanal lavage practice, EDTA is sequentially used with NaOCl. Ideally, EDTA is used only after the root canal is assumed to be fully cleaned and shaped [4]. The goal of EDTA lavage is to remove the smear layer to increase sealer adaptation [9]. The use of NaOCl after this stage is known to cause dentine erosion [9]. Unintentionally, this chemical interaction formed in the root canal space may extrude beyond the periapex, and also may reach the surrounded tissues during the lavage procedure[12]–[15]. More specifically, the possibility of passive irrigant extrusion during the rest period following irrigant delivery has been highlighted in previous studies [12], [13]. If EDTA is mixed or in contact with NaOCl, it interacts with the highly reactive hypochlorite ions of NaOCl which leads to the formation of a blurry liquid [16]. Previously, it has been reported that the interaction negatively affected the tissue-dissolution ability and antimicrobial properties of NaOCl due to the rapid diminishing of free chlorine [16]. Yet, there is no information about the state of the molecular structure of EDTA after oxidizing by hypochlorite or activated hypochlorite.

With this motivation, the background of this study is based on investigating interactions between NaOCl and EDTA as the two most common irrigants in clinical endodontic practice. Preliminarily, understanding the nature of this interaction at molecular grade is fundamental before further cytological observations. We hypothesized that the overall molecular interaction between NaOCl and EDTA was more complicated than reported chlorine-based outcomes. Therefore, the goal of this study wasto demonstrate the interactions after mixing EDTA and NaOCl solutions. Also, the highly oxidizing capacity of NaOCl can be increased by nonspecific physical or physicochemical stimulations using mechanical agitations, ultrasonic, or laser irradiation in clinical practice [17], [18]. Therefore, the second goal of this study was tomonitor the experimental interaction between NaOCl and EDTA after cold plasma energy transferring mimicking physicochemical activation of solutions.

II. EXPERIMENTAL SETUP

A. Pharmaceutical Products

Commercial pharmaceutical products manufactured specifically for dental purposes were chosen in this study. Freshly opened NaOCl (5.25% Promida hypochloride lot#190800; Promida, Eskişehir, Turkey) and 17% EDTA (w/v) (Promida EDTA lot# 190804; Promida, Eskişehir, Turkey) were used.
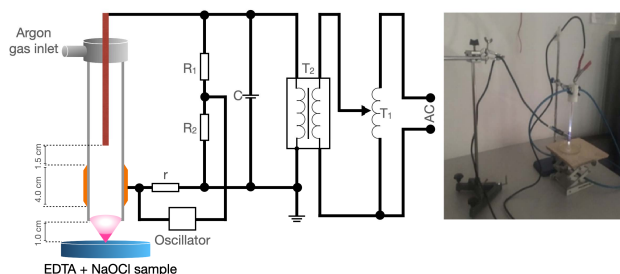

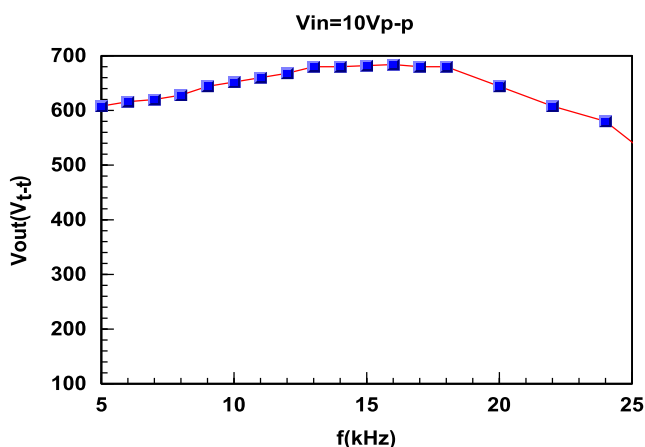Fig. 1.   Experimental sketch of the DBD device used in APPJ treatment.

Fig. 2.   Frequency response of high voltage transformer of the power supply built-in the plasma jet device.

All spectral data were recorded after 1 min of physical mixing procedure.

B. Experimental APPJ Treatment

Plasma-liquid interactions were evaluated experimentally at atmospheric pressure and room temperature. For this purpose, a previously described dielectric barrier discharge (DBD) device was used for experimental atmospheric-pressure plasma jet (APPJ) treatment [19]. The mixture of EDTA and NaOCl was treated with the DBD system. Power supply, argon gas source, a digital flowmeter, electrodes, tubes, and connectors were built-in the device itself. The placement and schematic illustration of components are shown in Fig. 1. Accordingly, the active tungsten electrode was fastened at the center of a 19-cm long quartz-glass tube (0.6/0.8 mm inner/outer diameter). For grounding, a copper electrode was attached to the outer surface of the tube. The active electrode was connected with the frequency controller and then the power supply. An anemometer (Mass-Stream series D-6300; Bronkhorst High-Tech B.V., Ruurlo, Netherlands) was used to measure the flow rate of argon gas constantly at 4 l/min. To ignite the discharges, we applied sinusoidal resonant power to the electrodes at 8 kV and 24 kHz outputs using a power supply. The frequency response of the high-voltage transformer of the power supply built in the DBD device is shown in Fig. 2.

For APPJ, the argon plasma discharge was blown out to liquid samples from a distance of 1.0 cm for 4 and 6 min for experimental activation.
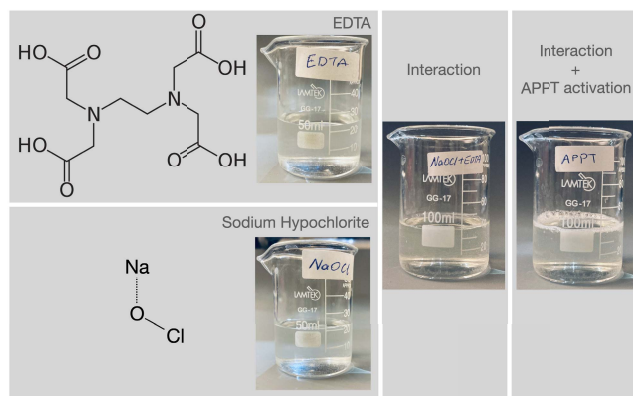
Fig. 3. Molecular structures of endodontic lavage agents with the images of representative test solutions before and after experimental APPJ treatments.

TABLE I
pH MEASUREMENTS OF THE TESTED LIQUIDS

Group	pH	Temperature (°)
NaOCl	11.78	25.0
EDTA	10.05	25.0
NaOCl + EDTA	10.30	25.0
NaOCl + EDTA + APPJ (activation time for 4 min)	10.12	25.0
NaOCl + EDTA + APPJ (activation time for 6 min)	10.25	25.0
NaOCl shows 'sodium hypochlorite', EDTA shows 'ethylenediaminetetraasetic acid', APPJ shows 'atmospheric-pressure plasma jet treatment'.		

The molecular structures of endodontic lavage agents with the images of representative test solutions before and after experiments are shown in Fig. 3.

C. pH Measurements

Liquid samples were transferred in 5-ml polypropylene containers (Lot#104816128; LP Italiana SPA, Milan, Italy) and the pH value was recorded when the sensor-reading was constant for 1 min. The pH of the liquid samples was measured using a digital pH meter (Starter 3100; Ohaus Corporation, Parsippany, NJ, USA) at room temperature. The pH meter was calibrated with buffered solutions with pH of 4.01, 7, and 9.21 (Mettler Toledo; Columbus, OH, USA). The sensor range of the pH meter is between 2 and 16 units and the accuracy is 0.01. The probe was rinsed with interim flushing using ultrapure water for 10 s and blot dried with gauze. The measurements were repeated three times for each sample. The measured values are listed in Table I.

D. Nuclear Magnetic Resonance (NMR) Spectroscopy

Prior to the NMR spectral analysis, samples were undergone a lyophilization process. Deuterium oxide (Lot#S5691166-521; Merck KGaA, Darmstadt, Germany) was used as internal standard material and solvent, respectively. Then samples were placed on the autosampler (JEOL Ltd., Tokyo, Japan). ^{1}H-NMR spectra were collected with JNM-ECZR-500 MHz NMR instrument (JEOL Ltd., Tokyo, Japan).
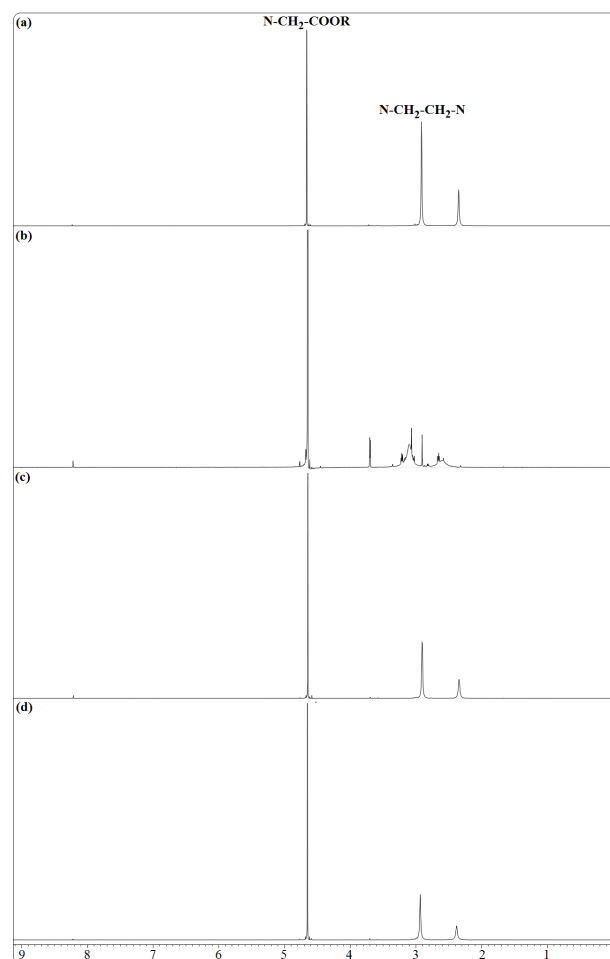

Fig. 4. 1H-NMR spectra of (a) EDTA molecule; (b) mixture of EDTA and hypochlorite; (c) APPJ treatment of the mixture for 4 min; and (d) APPJ treatment of the mixture for 6 min.

E. Fourier-Transform Infrared (FT-IR) Spectroscopy

Spectral changes were detected by FT-IR spectroscopy. For this purpose, the Spectrum 3 FT-IR Spectrometer (Perkin Elmer Inc., Waltham, MA, USA) was used for recording spectral data with the potassium bromide plate method. The spectral range of each record was 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1}. The calibration of the wavenumber axis was conducted by a silicon calibration wafer. Spectra were recorded with a single scan, 10-s exposure for each sample. A fast liquid FT-IR transmission accessory unit (Pearl; Specac Ltd., Orpington, U.K.) was used for liquid samples.

F. UV-Vis Spectroscopy

Absorbance spectroscopies were recorded using a double beam UV-Vis spectrophotometer (UV-2600i; Shimadzu Corporation, Kyoto, Japan) with a spectral wavelength range of 200–800 nm. Standard glass cuvets and a 1-nm slit were used for the measurements. To achieve temperature control at 22 °C, a thermoelectric temperature-controlled cell positioner instrument was used (CPS-100; Shimadzu Corporation, Kyoto, Japan).

TABLE II

CHARACTERISTIC VIBRATIONAL MODES OF EDTA

characteristic vibrational modes	expected wavenumbers ranges (cm^{-1})	observed wavenumbers ranges (cm^{-1})	interpretation
C-N stretching	1100-1400	1402.64	strong
Stretching vibration of C=O group	1600-1800	1634.00	very strong
O-H vibrations modes	3600-3200	3278.50	very strong

III. RESULTS

A. Interpretation of NMR Data

The spectra collected from the NMR analysis are presented in p/min (Fig. 4). The EDTA molecule had two characteristic peaks as N-CH$_2$-COOR and N-CH$_2$-CH$_2$-N at 4.65 and 2.90 p/min, respectively. Also, D$_2$O was observed at 2.34 p/min. Both the decreasing and the widening of characteristic EDTA peaks, as well as the occurrence of new signals at 3.70, 3.07, and 2.91 p/min were observed in the mixture of the EDTA and NaOCl. The observed structural change of EDTA might have been related to oxidation by NaOCl in a highly alkaline environment after the interaction. This might have led to the formation of by-products after the contact between the EDTA and NaOCl.

Interestingly, decreased peak intensities of EDTA were observed in the APPJ treatment groups at 4.65 and 2.90 p/min. The spectral shift did not occur between the two application durations of the experimental APPJ treatment.

B. Interpretation of FT-IR Data

Basically, there are 23 chemical bonds in EDTA Fig. 3. The FT-IR spectrum of the EDTA molecule is shown in Fig. 5. The characteristic vibrational modes of EDTA are listed in Table II.

Regarding the FT-IR spectrum of EDTA molecule, the C-N vibrational modes were observed between 1100 and 1400 cm^{-1} whereas, a strong C-N stretching mode was observed at wavenumber 1402 cm^{-1}. The modes of the C=O group are expected to observe at 1600–1800 cm^{-1}. Accordingly, a very strong C=O band was observed at wavenumber 1634 cm^{-1}. Also, O-H vibrations modes are expected to appear at 3600–3200 cm^{-1}. A very strong O-H band was observed at wavenumber 3278 cm^{-1}.

The vibrational peaks of EDTA after interacting with 5.25% NaOCl are listed in Table III. One of the most prominent spectral observations was noted in the C-N vibration modes at 2357.08 and 2309.10 cm^{-1} as middle-narrow and mid-wide, respectively. These vibration modes were not seen in the characteristic peaks of EDTA. Therefore, newly formed C-N modes as a major finding were attributed to the alteration of the molecular structure of EDTA after its interaction with 5.25% NaOCl Fig. 6. The strong and wide vibration of C-H was seen at the wavenumber 2123.85 cm^{-1}. The middle or narrow vibration modes at wavenumbers 1321.35, 1259.72, 1074.64, and 1065.96 cm^{-1} were attributed to C-O, C-N,

TABLE III

VIBRATIONAL PEAKS OF EDTA AFTER INTERACTING WITH 5.25% SODIUM HYPOCHLORITE

vibrational modes	wavenumbers (cm^{-1})	interpretation
O-H stretching	-	-
C-N stretching	2309.10	medium and widely
	2357.08	narrow and medium
C-Cl and C-C stretching	872.29, 866.97, 852.97, 843.02, 836.87 and 828.87	changing and sharply
	886.08	narrow and medium
C-O, C-N and C-C stretching	1321.35, 1259.72, 1074.64 and 1065.96	narrow and medium
C-O, C-N, C-C and C-Cl stretching	1074.64 and 1065.54	weak and medium
C-H stretching	2123.85	strong and widely

TABLE IV

VIBRATIONAL PEAKS OF EDTA INTERACTING WITH 5.25% SODIUM HYPOCHLORITE AND ATMOSPHERIC-PRESSURE ARGON PLASMA JET TREATMENT

vibrational modes	wavenumbers (cm^{-1})	interpretation
O-H stretching	3901.78, 3853.28	weak and narrow
C-H stretching	2123.39	wide and strong
C=O vibrations	1643.22	wide and strong
C-Cl, C-C stretching	856.45, 837.81, 828.06, 823.98, 850.75, 867.65	changing and sharply
C-O, C-C stretching	1400.85	wide and strong
	1319.39	wide and medium
	1094.82	weak and wide
	1070.56 and 1062.41	weak and narrow

or C-C stretching. Also, weak and medium vibration modes at wavenumbers between 1074.64 and 1065.54 cm^{-1} were attributed to the C-O, C-N, C-C, and C-Cl stretching. Various narrow-sharp vibration modes observed at the wavenumbers of 872.29, 866.97, 852.97, 843.02, 836.87, and 828.87 cm^{-1} were attributed to C-Cl and C-C stretching.

The vibrational peaks of the APPJ-treated mixture are listed in Table IV and Fig. 6. Regarding the FT-IR spectrum of the APPJ-treated mixture, a weak and narrow O-H band was observed at wavenumber 3901.78 and 3853.28 cm^{-1}. The strong and wide vibration of C-H was seen at the wavenumber 2123.39 cm^{-1}. A strong and wide vibration of C=O was observed at wavenumber 1643.22 cm^{-1}. In the spectrum, various modes of a wide and strong stretch at 1400.85 cm^{-1}, a wide and medium stretch at 1319.39 cm^{-1}, a wideband at 1094.82 cm^{-1}, and two weak and narrow bands at 1070.56 and 1062.41 cm^{-1} were attributed to the C-O

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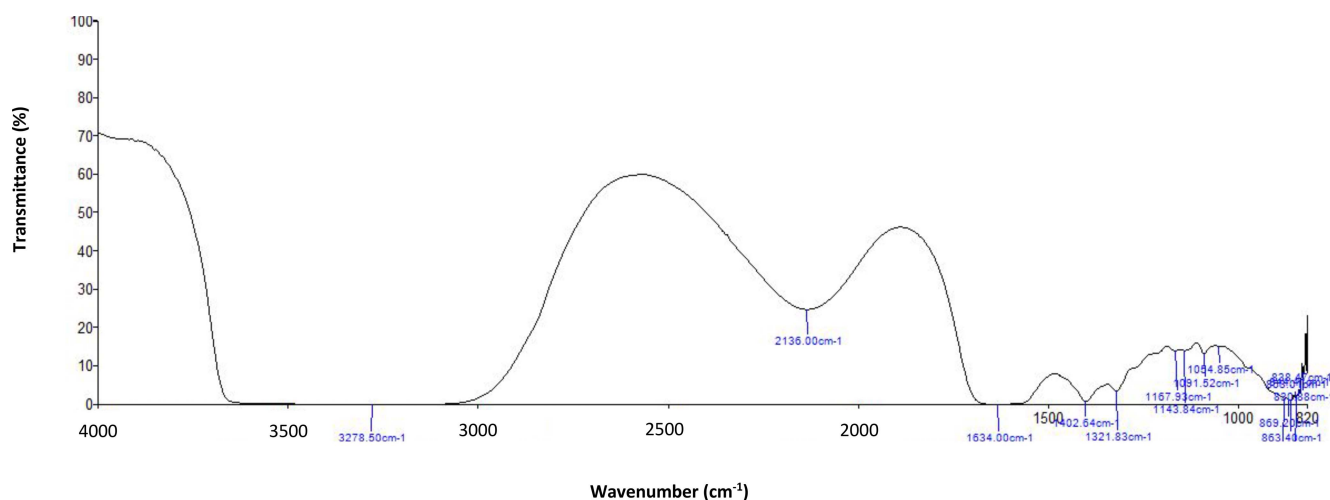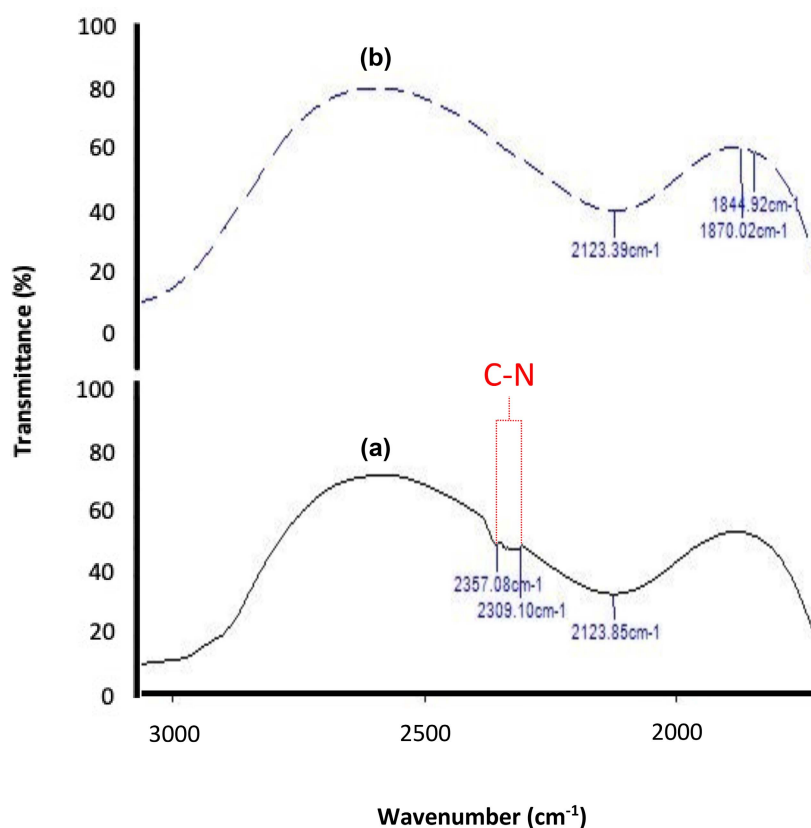
Fig. 5.   FT-IR spectra of the EDTA molecule.

Fig. 6.   FT-IR spectra of (a) mixture of EDTA and hypochlorite and (b) APPJ treatment of the mixture.

and C-C stretching. Various narrow-sharp vibration modes observed at the wavenumbers of 856.45, 837.81, 828.06, 823.98, 850.75, and 867.65 cm^{-1} were attributed to C-Cl and C-C stretching.

Interestingly, the newly formed C-N peaks observed in the mixture disappeared after the experimental APPJ treatment. We speculate that the 1C-2N-3C-4C-5N-6C bonds of the EDTA molecule might have broken off from 3C and 4C. Instead, the 1C-2N-3C and 4C-5N-6C bonds might have occurred. This might have been related to the broken C-N bond. The FT-IR spectra did not

significantly alter after the experimental APPJ treatment between 4 and 6 min.

C. Interpretation of UV-Vis Data

Absorbance UV-Vis spectra of the mixture before and after APPJ treatment are given in Fig. 7. Thus, UV–Vis spectra of 1:1 EDTA and NaOCl were measured in the wavelength region of 200–800 nm, and absorption maxima were detected at 239, 306, 324, and 373 nm wavelengths. Any further absorbance was not obtained in the spectrum. But, in APPJ treatment of the mixture solution, further absorbance maxima

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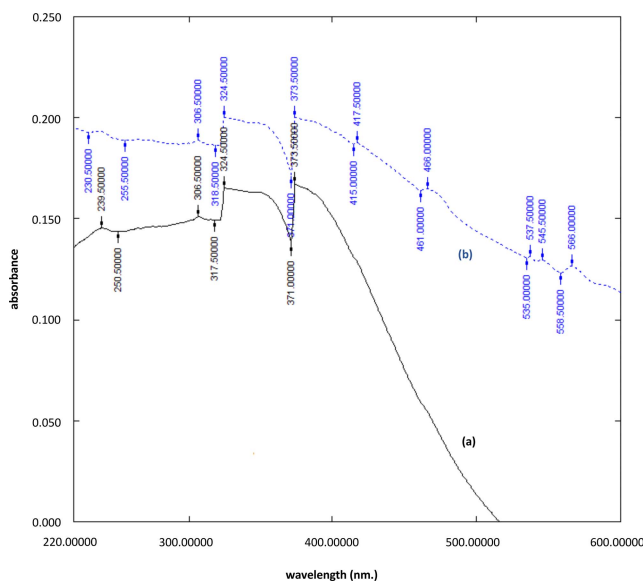
Fig. 7.   UV-Vis absorbance spectra of the mixture: APPJ treatment (a) before and (b) after.

were obtained at wavelengths 417, 466, 537, 545, and 566 nm, respectively.

In theory, if UV and visible radiation interact with the matter, π-π^* or n-π^* electronic transitions which may lead to the isomerization process occurs. The obtained absorbance maxima in the APPJ treatment are in the characteristic region for the π-π^* transitions. We speculate that the APPJ treatment of the mixture might have caused π-π^* electronic transitions. The UV-Vis spectra did not significantly alter after the experimental APPJ treatment between 4 and 6 min. The UV-Vis findings of the APPJ treatment were in agreement with the corresponding FT-IR findings of this study.

IV. Discussion

There is limited information about the molecular structure after the interaction of EDTA and NaOCl in the endodontic literature. This would seem to be the second report on the molecular structural changes after an EDTA and NaOCl mixture. Prior to this study, Grande *et al.* [20] have reported unknown facts regarding the combined use of NaOCl and EDTA after NMR spectroscopic analysis. Regarding their results, it has been reported that the interaction between NaOCl and EDTA leads to the formation of unknown by-products due to oxidation [20]. In addition, it has been stated that the oxidation reaction has a progressive nature over time [20]. In agreement with the previous report, we demonstrated the presence of structural changes in the EDTA molecule after mixing it with NaOCl in this study. All spectral data were recorded after 1 min following mixing. One of the most important findings of this study was that the molecular structure of EDTA has changed after interaction with NaOCl. However, in a previous study, this change in the EDTA molecule due to oxidation did not occur immediately [20]. This disparity might be due to the methodology and utilized instruments. Interestingly, the NaOCl cannot entirely oxidize all of the EDTA molecules when it interacts with it. Regarding the theory of the previous

study: 1) it might have been present in a solution together with oxidized EDTA, unoxidized EDTA, and other possible intermediate combinations and 2) the oxidation process might not only have involved EDTA but also some of its by-products. Accordingly, the amount of EDTA molecules that actually oxidized may decrease even more [20].

Grande *et al.* [20]stated that the functional groups of EDTA could react with oxygen atoms during the oxidation as follows: ($=$N-CH$_2$-C ($=$O) OR) and ($=$N-CH$_2$-CH$_2$-N$=$). It has also been discussed that although oxidation reactions can be analyzed, by-products cannot be identified by NMR spectroscopy [20]. In agreement with the previous study [20], spectral peaks of EDTA corresponding to N-CH2-COOR and N-CH2-CH2-N were observed in our findings at 4.65 and 2.90 p/min, respectively. Notably, both the decreasing and the widening of characteristic EDTA peaks, as well as the occurrence of new signals at 3.70, 3.07, and 2.91 p/min were observed in the mixture of the EDTA and NaOCl. Both the decreasing and the widening of characteristic EDTA peaks, as well as the occurrence of new signals, contribute to the previously described oxidation process theory of the EDTA [20]. Also, the oxidation may not only exclusively impact the EDTA molecule but also its newly formed by-products after interaction with NaOCl; therefore, the characteristic intensities of EDTA molecules may reduce [20]. This might have been the reason for the low peak intensities of the mixture in the 4- and 6-min APPJ treatment, respectively. In addition, the observed characteristic EDTA peaks after the APPJ treatment of the mixture might be attributed to the incomplete oxidization or slow oxidation process of entire EDTA molecules [20].

Besides oxidation shown in NMR spectra, we have shown that the interaction of EDTA with NaOCl is very complex. Regarding the FT-IR spectra of the EDTA-NaOCl mixture in this study, the intensity of the characteristic peaks of EDTA not only changed but also shifted. We speculate that the aliphatic EDTA structure might have been changed due to altered and newly formed C-N bonds in the mixture.

Atmospheric pressure plasmas are non-thermal plasmas that are used in biomedicine [21], [22]. As mentioned earlier, plasma jet applications can maintain at room temperature during tests. Since their temperatures are low, radicals, ions, UV, and electric fields can be controlled. Also, any thermal damage does not occur to the plasma-jet applied material. Notably, due to the stable generation of discharges at low-temperature, atmospheric-pressure plasma can be sustained by using noble gases like argon or helium [21]. Because the gas temperature of the plasma is close to the room conditions, constant and controlled energy can transmit to a sample from the discharge tube of the DBD device. Theoretically, due to its non-thermal property, the interaction observed after APPJ treatment did not affect the temperature-activated oxidation of NaOCl. Although argon atmospheric-pressure plasma is not used clinically for the activation of irrigation materials, it was used to experimentally stimulate oxidized EDTA molecules in this study. In other words, the purpose of the experimental treatment with a DBD device mimicking physicochemical activation of solution was to catch the potential differences between the by-products of oxidized EDTA. Regarding the

APPJ treatment spectral results, the identified differences between spectra might have originated from the NaOCl activation. As mentioned before, the rate of oxidizing reaction of NaOCl rapidly increases when activated with photo-activated systems, lasers, sonic, and ultrasonic energy transfer [17], [18]. Regarding the IR and UV-Vis spectral data, the molecular structure of EDTA changed as a consequence of the first interaction with NaOCl. In contrast, the subsequent interaction after atmospheric-plasma jet treatment was revealed as dissimilar to the former interaction. The hypothesis of this study was not to perform any molecular synthesis using EDTA or its mixture with NaOCl. Such hypotheses or aims can be considered as a subject of a new study.

EDTA is a polyprotic acid. Notably, the sodium salts of EDTA are noncolloidal organic acids that can create nonionic chelates with metals such as calcium ions [16]. Correspondingly, chelation is known as a self-limiting process. Likewise, EDTA has been demonstrated to react rapidly within the first hour of contact with the dentin substrate; however, the equilibrium of the calcium chelation process is approached within 7 h regardless of the surface area exposure [23]. Due to its chelator nature, EDTA adheres to the dentine surface and self-chelation capacity begins inactivated in the root canal system [24], [25].

Adding chelators to NaOCl decreases its pH in a ratio and time-dependent mode [9].The acidic hydrogens of EDTA interact with reactive oxygens of NaOCl when mixed leading to chlorine gas evaporation. Obviously, releasing chlorine gas formed after the interaction can potentially have toxic effects on the human body [26]. The loss of available chlorine in the NaOCl and therefore increase in hypochlorous acid consequently decreases the quantity of the hypochlorite ion [27]. Consequently, tissue dissolution and antimicrobial effectiveness reduce due to a decrease in available chlorine amount in NaOCl [16] and [26]. However, NaOCl does not significantly reduce or alter the calcium chelating or smear layer removal yield of EDTA [9] and [16]. Yoshida *et al.* [28] have reported that EDTA has limited antimicrobial efficiency on necrotic pulp tissues in the root canal as a result of the ion-chelating ability [29]. Though the efficiency of EDTA irrigation is not significantly affected by NaOCl interaction, environments such as dentin substrate or infected dentin presence can limit its chelating activity [16].

The multimethod analytic study was presented by comparing NMR, FT-IR, and UV-Vis spectra before and after argon plasma applications for the first time. Though deductive molecular transformations have been shown in our study, this interaction may represent dozens of different molecular sequences or geometries. Furthermore, the presence of dentin substrate may affect the interaction. Further, molecular studies on this subject may clarify the molecular sequence, toxicity, solubility, and behavior of the variations of EDTA. Also, the pathways of the newly formed or broken molecular bonds in the combination of EDTA and hypochlorite can be determined by analytical methods in subsequent studies.

The molecular interactions after mixing EDTA and hypochlorite were comparatively analyzed. The molecular structure of EDTA changed as a consequence of the interaction

with hypochlorite. The plasma-liquid interaction was caused by the diversity of the EDTA molecule. To better understand the effect of plasma on the interaction of EDTA and NaOCl, different plasma-related parameters that of "plasma power settings" or the "effect of distance" from APPJ to the sample could be investigated in further studies. Also, the interaction of EDTA and hypochlorite is more complex than the previously described theory. Therefore, further studies are needed to understand the toxicity or calcium chelation ability of subsequent molecular changes of EDTA after interacting with hypochlorite.

V. Conclusion

Molecular transitions of endodontic EDTA molecule that occur after hypochlorite interaction is reported. Within the limitations of the study, the following conclusions can be drawn: the molecular structure of EDTA was observed to change after the oxidization by hypochlorite. The subsequent molecule occurred by the oxidization by hypochlorite or activated hypochlorite is different from each other and also is different from the characteristic structure of EDTA. Experimentally activated hypochlorite was observed to cause the molecular electronic transitions of EDTA. Thus, the interaction of EDTA and hypochlorite is more complex than the previously described theory. Since the effect of the molecular changes is unknown on the periapical tissues, EDTA and excited hypochlorite should not be directly stirred during intracanal lavage.

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