

EVALUATION OF ENDODONTIC IRRIGANTS EFFECTS ON THE ROOT CANAL DENTIN WALL

Corina Alexandra (Concită) Brînză¹, Mihaela Sălceanu¹⁺, Anca Melian¹⁺, Raluca Dragomir^{1*}, Elena Gabriela Hitruc², I. Taraboanta¹⁺, C. L. Giuroiu^{1*}, S. Andrian¹

Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universitatii Str., 700115 Iasi, Romania; corina-alexandra.concita@umfiasi.ro (C.A.B.C) salceanu.mihaela@yahoo.com (M.S) meliananca@yahoo.com (A.M.) ionut-taraboanta@umfiasi.ro (I.T.); sorin.andrian@umfiasi.ro (S.A.); giuroiu.cristian@umfiasi.ro (C.L.G) draluca@gmail.com (R.D)

Romanian Academy "Petru Poni" Institute of Macromolecular Chemistry. Physics of Polymers and Polymeric Materials Department. Atomic Force Microscopy Laboratory. Aleea Grigore Ghica Voda, 41A, Iasi - 700487, Romania; gabihit@icmpp.ro (E.G.H)

*Correspondence: authors: Email: giuroiu.cristian@umfiasi.ro (C.L.G) draluca@gmail.com (R.D.)

+ All authors have the same scientific contribution and equal rights

Abstract

The associations of ethylenediaminetetraacetic acid (EDTA) and citric acid (AC) with sodium hypochlorite (NaOCl) are combinations used for cleaning and disinfecting of the root canals. The aim of this in vitro study was to evaluate by AFM the effect of 20% EDTA and 40% AC associated with 5,25% NaOCl in root canal SL removal and on root dentine surface roughness. Fifteen extracted single-root teeth were randomly divided into 3 groups according to the irrigation method: in Group 1, samples were irrigated with 5 mL of 5.25% NaOCl followed by 5 mL of 20% EDTA solution for 1min; in Group 2, samples were irrigated with 5 mL of 5.25% NaOCl solution followed by 5 mL of 40% AC solution for 1min; in Group 3 samples were irrigated with 5 mL of 0.9% physiological serum. AFM was used to obtain 2D and 3D images and roughness parameters (Sa, Sq). The data was statistically analyzed using significance F-test, ANOVA and post-hoc Bonferroni test. The images obtained by AFM highlighted free dentinal tubules in the coronal and middle third of the root canal in Group 1, partially free dentinal tubules in the coronal and middle third in Group 2, while in Group 3 opening of the dentinal tubules were not observed. The lowest mean Sa values were recorded by Group 3. Group 1 and 2 presented significantly higher mean values of Sa, Sq, in comparison with the Group 3 in all areas of the root canal ($p < 0,05$). Also, in the Group 1 the mean values of Sa, Sq, were higher than Group 2, especially in the coronal and apical third where the differences being statistically significant ($p < 0,05$). The association of 20% EDTA with 5.25% NaOCl solutions in the irrigation of the endodontic system is more efficient in removing SL in the coronal and middle thirds of the root canal and increases the root dentin surface roughness in all areas in comparison with 0,9% physiological serum and in the coronal and apical third in comparison with 40% AC with 5,25% NaOCl.

Keywords: canal irrigants; smear-layer; dentinal roughness; atomic force microscopy

1. Introduction

The major objective of endodontic therapy is to clean and disinfect the root canals, as well as remove the smear layer (SL) with endodontic irrigation solutions [1, 2]. SL is an amorphous layer made up of dentinal tissue remnants that basically contains minerals such as calcium, necrotic tissues, microorganisms and bacterial products. Also, the presence of SL prevents the

penetration of irrigants, drugs or endodontic sealants at the level of the dentinal tubules [2, 3]. There are different canal irrigation solutions with various smear layer removal ability [4]. The ideal irrigation solution should provide: strong antimicrobial action on a wide spectrum of microorganisms, inactivation of bacterial virulence factors, disorganization or removal of the biofilm, dissolution and removal of pulp tissue residues and of the

SL that occurs after instrumentation or prevention of their formation, lack of adverse effects such as irritation of surrounding tissues, cytotoxicity, allergic reactions [5, 6].

There is no evidence to date for a single irrigant able to fulfil all these requirement [7]. In absence of an optimal irrigant currently is used a combination of irrigants in various protocols, which, in addition to the complete elimination of SL, generate minimal alterations in the chemical composition of the root dentin [8].

Due to the many irrigation procedures available, standardized protocols have not yet been defined for the actual clinical context and irrigator penetration can be influenced by a number of factors such as application time, volume, activation methods and retention time of irrigators, as well as irrigation flow, needle type and needle placement depth [9]. NaOCl is the most frequently recommended used endodontic irrigant widely and has some advantages such as the ability to dissolve organic tissue and antimicrobial properties [3, 8, 10]. Due to the fact that NaOCl only interacts with the organic components of SL, it is associated with demineralizing agents, which can eliminate the anorganic component of SL [8, 11]. Furthermore, NaOCl has some disadvantages such as: disagreeable taste and smell, when accidentally injected into periradicular tissue it has significant toxicity, chewing of metal objects, risk of bleaching clothes and significantly influences the mechanical properties of dentin on roughness, microhardness, bending strength, elastic modulus and inorganic content and organic-inorganic ratio [9, 12,]. NaOCl also has different concentrations, ranging from

0.5 to 8.25%, although there is still no consensus on the optimal concentration [5]. It is recommended that NaOCl be associated with calcium chelating agents, which have a mechanism of action based on their reaction with calcium ions and the formation of soluble calcium chelates, thus leading to dentin decalcification [8]. EDTA is a calcium chelating agent that forms a stable complex with calcium ions and is the most used irrigation solution, with a concentration of 15-17%, application time of 1-5 min in order to remove the SL, especially in the coronal and middle third of the canal [13], even so it has some limits such as reduced antimicrobial action due to the short action time [14]. Although EDTA does not exhibit a significant antimicrobial effect, it does have the ability to disrupt the polysaccharide matrix of the biofilm, thus potentiating the anti-biofilm effect of NaOCl [8].

The AC is also used as an irrigating solution for its ability to remove the anorganic component of SL and its potential to decalcify dentin comparable to EDTA [11, 15], remove the smear layer, react with NaOCl and decrease its effect [8]. To remove both the superficial smear layer and the intratubular plugs it is recommended to use AC solutions with different concentrations between 1% and 50% [9, 12].

The association between NaOCL and EDTA in different concentrations is preferred to achieve a more effective reduction in bacterial load [16, 17]. It has also been reported that this combination causes surface and structural changes of the dentin [18]. Irrigation with NaOCl and EDTA solutions can influence dentine surface roughness and the diameters of the

dentinal tubules, which can interact with the adhesion of the endodontic sealant to root dentin [18, 19]. However, prolonged activity of calcium chelating agents may have undesirable effects on the inorganic dentin structure resulting in apical leakage [20, 21] and a decrease in tooth microhardness, ultimately leading to root fractures [6, 13]. The change in the Ca/P ratio affects the initial proportion of organic versus inorganic components, a fact that determines the appearance of dentin surface roughness [2, 6].

The roughness determined by the irrigants has clinical implications both in terms of fixing the sealant as tight as possible in the dentinal tubules but also on the dentinal microhardness, risking vertical root fracture [22].

Since it was invented by Binning in 1986, AFM evolved and it enables the mapping of sample topography by tracing an atomic force field on a surface by non-destructive probes [22]. As a result, a detailed three-dimensional topographic image of the surface of an object is obtained with vertical and lateral resolution within the nanometer scale [17, 20].

The irregularities determined by the use of irrigants are quantified by measuring the surface roughness which is evaluated by the deviation of an ideal surface from the real surface [18]. The 2D and 3D images obtained by AFM can show the effect of different irrigation regimes on the thickness of the SL and on the roughness of the dentine surface, by analyzing different roughness parameters such as Sa and Sq [23]. AFM works equally well on conducting and insulating surfaces and may be employed under ambient conditions in

air, liquid, and in vacuum. [18]. Regarding the analysis of the three-dimensional parameters of the roughness on the dentinal surface obtained by AFM, the literature states that the most used parameter is Sa (average roughness), not because it is the most reliable, but because it is the most well-known parameter and has historical value [17].

The aim of this in vitro study was to evaluate the effectiveness of two irrigation solutions based on 20% EDTA and 40% AC, used in association with 5,25% NaOCL in removing the SL from the root canal dentinal and tubules and their influence on the dentine surface roughness by AFM analysis.

The null hypothesis evaluated in this study was that there is no significant difference in the effectiveness of 20% EDTA with 5,25% NaOCL, 40% AC with 5,25% NaOCL, and 0,9% physiological serum in the removal of SL and in the production of roughness on the dentinal surface. and that is no significant difference in the effectiveness of 20% EDTA and 40% AC with 5,25% NaOCL in the elimination of SL and in the increasing the root dentin surface roughness.

2. Materials and methods

2.1. Sample preparation

The sample size was calculated by using G*Power software (Heinrich-Hein Universität Düsseldorf, Germany) using with an effect size of 0.6 which is a high effect according to Cohen's classification, an alpha value of 0.05 and power of 80%. The obtained results estimated a minimum number of 5 samples required in each subgroup. Fifteen human maxillary central

incisors extracted for orthodontic or periodontal purposes were selected. The criteria for inclusion in the study were: monoradicular and healthy teeth from patients aged between 18 and 40 years. The exclusion criteria were: devitalized teeth, calcifications of the root canal, internal or external resorptions, incompletely formed apex. The teeth were randomly and equally divided into 3 groups, according to chosen root canal irrigation solution: Group 1 (n=5) the root canal was irrigated with 5 mL of a 5.25% NaOCl solution (CHLORAXID 5.25% CERKAMED, Stalowa Wola, Poland) for 1 minute after each modeling sequence and then irrigated using 5 mL of a 20% EDTA solution (EDETAL LIQUID 20% TEHNODENT GmbH, Dreieich, Germania) for 1 min; Group 2 (n=5) the root canal was irrigated using 5 mL of a 5.25% NaOCl solution (CHLORAXID 5.25% CERKAMED, Stalowa Wola, Poland) for 1 minute after each modeling sequence and then irrigated with 5 mL of a 40% AC (CITRIC ACID 40% CERKAMED, Stalowa Wola, Poland) for 1 min; Group 3 (n=5) Control group the root canal was irrigated using 5 mL of 0.9% physiological serum (Sodium Chloride 0.9% B. BRAUM MELSUNGEN A, Timișoara, Romania) for 1 min. A 5 ml syringe and a 0,3 gauge side vented needle with closet end (ENDO-TOP-CERKAMED, Stalowa Wola, Poland) were used. The tip of the needle was inserted into the canal till 1mm from the apical foramen. The root canals were prepared by crown-down technique (progressive telescoping technique) for 5 minutes, which involved modeling the canal in a conical, telescoping form, starting from the coronal third, progressing towards the apical third with rotating NiTi files (Orodeka FilePlex -V,

Nürnberg, Germany) the last file being 25/04. New sets of files were used for each sample.

The teeth were then sectioned transversely at the cemento-enamel junction and longitudinally under a continuous water jet using a diamond disc 0.17x22 mm (Flexible Double Side, BesQual Diamond Disc, Meta Dental, NY, USA), using a contraangle hand piece, at 20,000 rpm. The diamond disc was used for both sectioning and finishing of the sample. The sectioned teeth were stored for 1 minute in a 20% EDTA solution (group 1) and in a 40% AC solution (group 2), then the AFM evaluation was performed.

2.2. Root dentin surface roughness evaluation

The samples were analyzed in three areas of the root canal: coronal, middle, apical third by AFM evaluation (Scanning Probe Microscope Solver Pro-M, NTEGRA Spectra Atomic Force Microscope, NT-MDT, Zelenograd, Russia). For the accuracy of the images, Nova v.19891, Solver software (NT-MDT, Zelenograd, Russia) was used.

The surface images were obtained with a NTEGRA scanning probe microscope (NT-MDT Spectrum Instruments, Zelenograd, Russia), in AFM configuration. Rectangular silicon cantilevers NSG10 (NT-MDT, Zelenograd, Russia) with tips of high aspect ratio (sharpened pyramidal tip, angle of nearly 20°, tip curvature radius of 10 nm and height of 14-16 µm) were used in order to minimize convolution effects. The samples, were acquired in air, at room temperature (23°C), in tapping mode, with the velocity of 6 mm/s. For image acquisition, the Nova v.19891 for Solver

software (NT-MDT, Zelenograd, Russia) was used. For each sample, the average roughness (Sa) and root mean squared roughness (Sq) were analyzed.

2.3. Statistical analysis

Data were systematized and centralized in an SPSS 18.0 database and processed using the appropriate statistical functions. A 95% confidence interval was used in data presentation.

For the statistical analysis of the parameters, F test, ANOVA One-way and

post-hoc Bonferroni tests were used with a significance level set at 0.05.

3. Results

3.1. AFM qualitative analysis results

When analyzing the obtained 2D and 3D images, the following aspects were observed: In the coronal third of the root canal of samples in Group 1, unoccluded dentinal tubules (black shades) were observed, in Group 2, partially unoccluded dentinal tubules were found, while in Group 3 no unoccluded dentinal tubules were observed (Fig. 1).

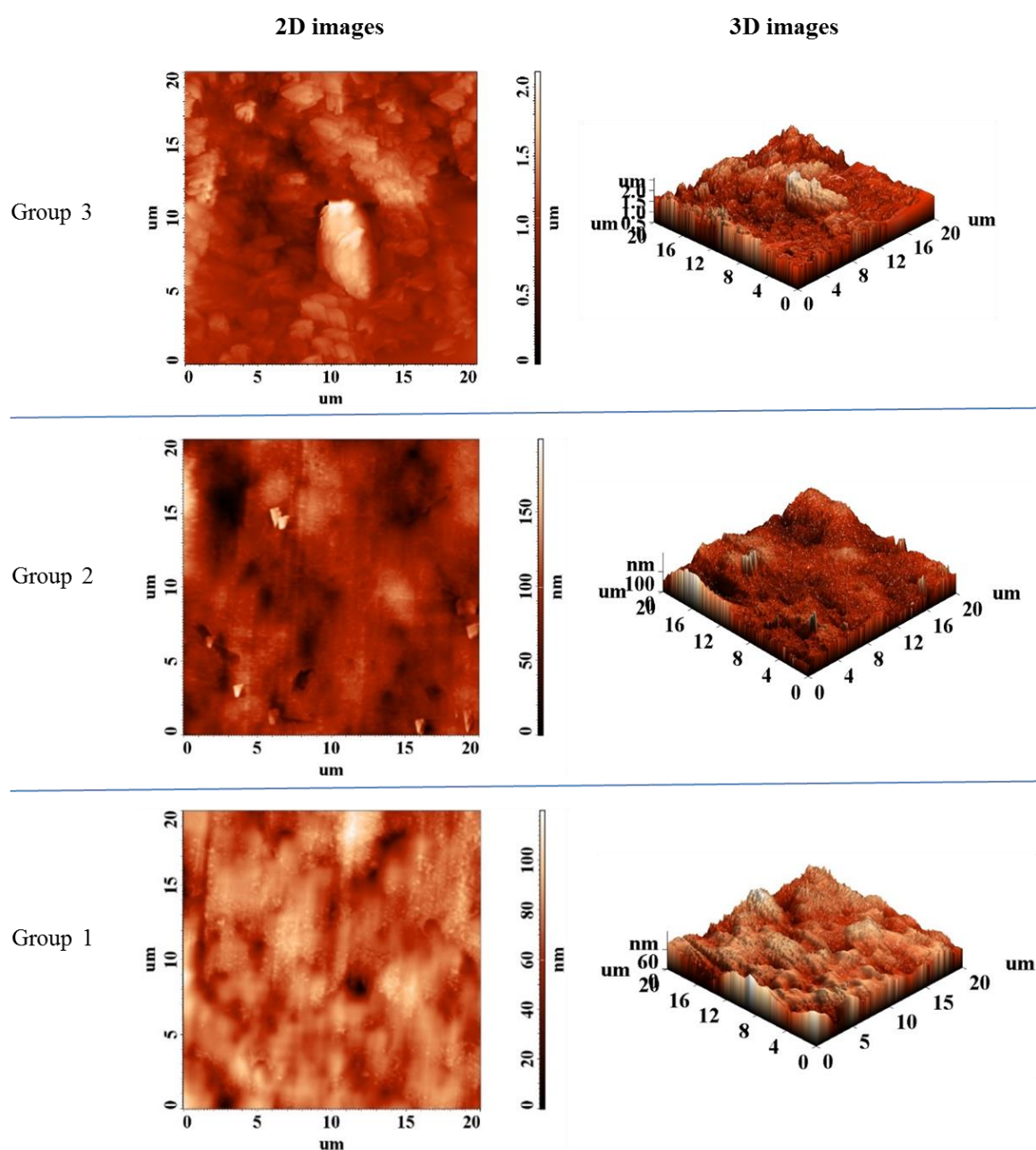


Figure 1. 2D and 3D AFM topography images obtained by scanning on 20x20 μm^2 area on sections from the coronal third of the root canal in the Group 1, 2, 3.

In the middle third of the root canal of samples in Group 1, unoccluded dentinal tubules were observed, in Group 2 partially occluded dentinal tubules were found while in Group 3 the dentinal tubules were totally occluded (Fig. 2).

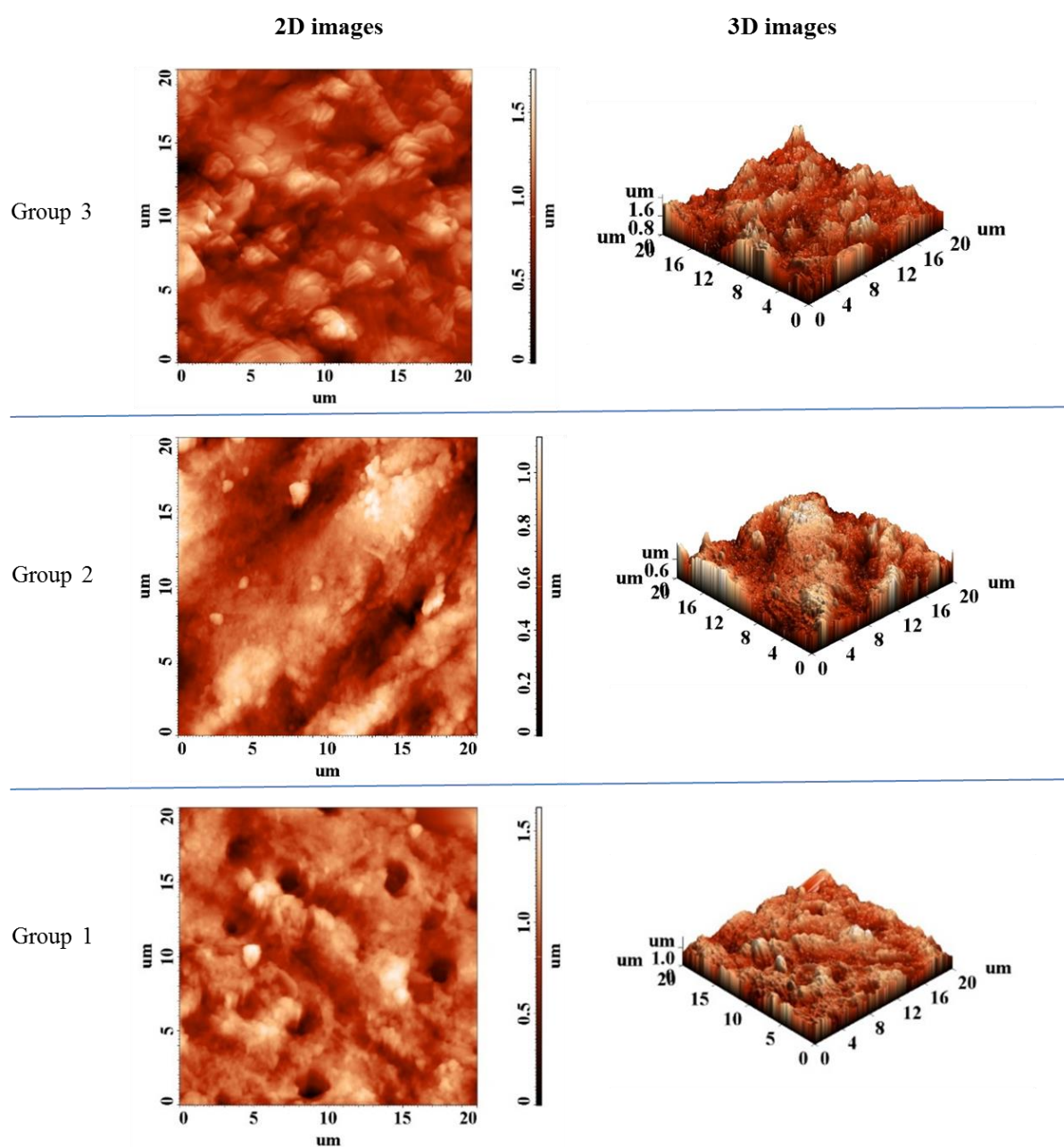


Figure 2. 2D and 3D AFM topography images obtained by scanning on 20x20 μm² area of some sections from the middle third of the root canal in the Group 1, 2, 3.

In the apical third of the root canal of samples in Groups 1, 2 and 3, totally occluded dentinal tubules were observed (Fig. 3).

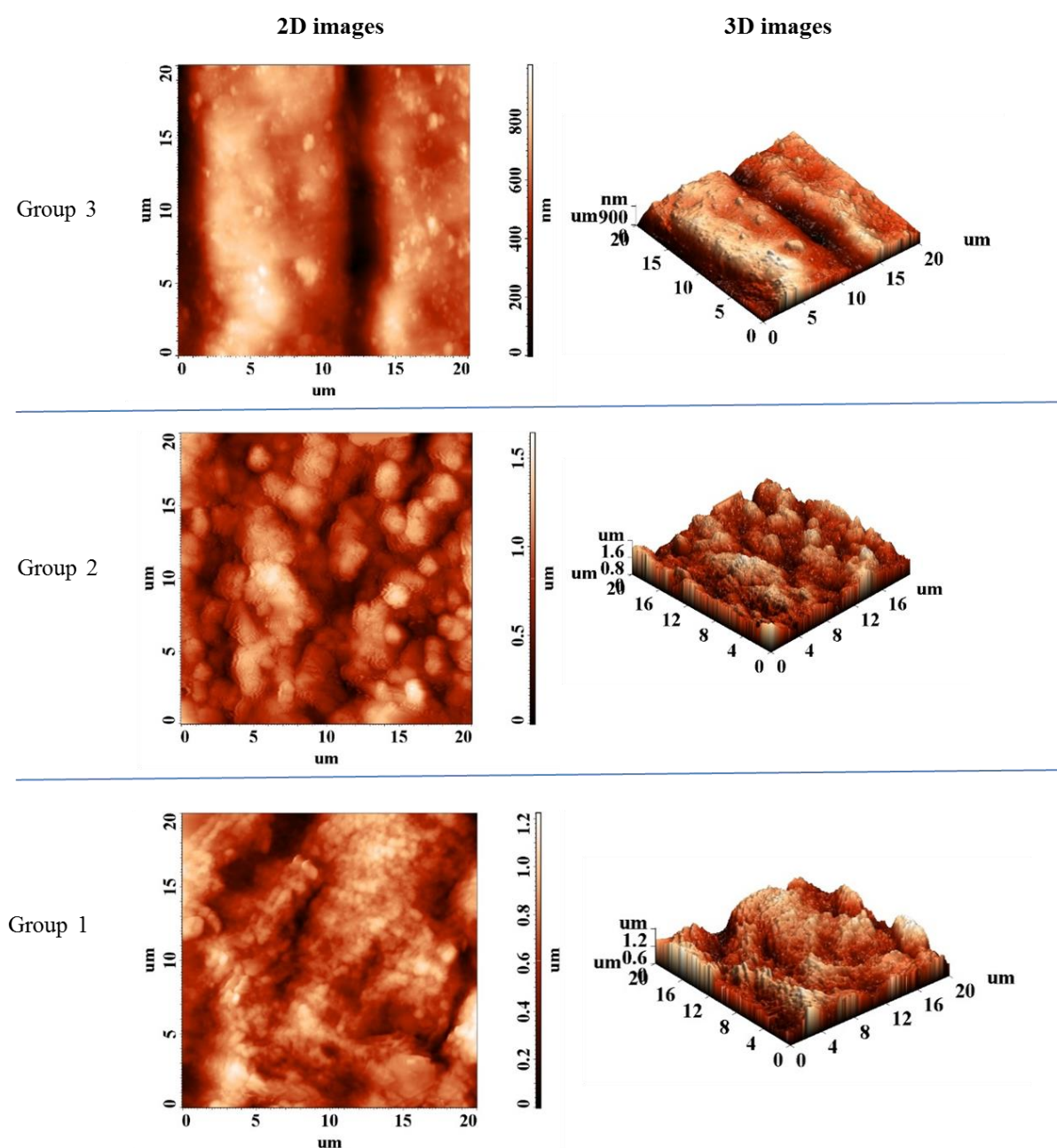


Figure 3. 2D and 3D AFM topography images obtained by scanning on 20x20 μm^2 area of some sections from the apical third of the root canal in the Group 1, 2, 3.

3.2. AFM quantitative analysis results

We evaluated the mean values of the roughness parameters Sa and Sq for coronal, middle and apical third for each group included (table 1)

Table 1. Mean values of the roughness parameters (Sa, Sq) on the 3 study groups.

The Roughness Parameters	Group 3 (n = 5)	Group 2 (n = 5)	Group 1 (n = 5)
1/3 coronal			
Sa [nm]	77,75±80,48	150±2,0 a)	185,32±82,37a)b)
Sq [nm]	97,53±96,17	191,12±12,2 a)	232,27±95,39 a) a)
1/3 middle			
Sa [nm]	51,12±50,50	149,94±12,0 a)	126,33±55,98 a) a)
Sq [nm]	62,95±66,53	191,08±10,0 a)	158,10±68,21 a) a)
1/3 apical			
Sa [nm]	52,18±9,16	149,94±12,0 a)	179,23±27,60 a) a)
Sq [nm]	68,44±13,48	191,08±10,0 a)	226,81±31,81 a) a)

a) $p < 0,05$. b) $p > 0,05$.

When analyzing the obtained mean Sa and Sq values, it can be observed that the lowest values in each of the analyzed segments were recorded by Group 3.

The mean values of Sa were: 60,32 nm for Group 3, 149,96 nm for the Group 2 and 163,62 nm for the Group 1. The mean value of Sq was: 76,30 nm for Group 3, 191 nm for the Group 2 and 205,7 nm for the Group 1.

The lowest mean Sa values were recorded in the Group 3 compared to those recorded in the Group 1, both in the coronal third (77,75±80, 48 nm vs 185, 32±82,37 nm; $p < 0,05$), as well as in the middle (51,12±50, 50 nm vs 126,33±55,98 nm; $p < 0,05$) and the apical thirds (52.18 ± 9,16 nm vs 179.23 ±27,60 nm; $p < 0,05$). The differences were statistically significant in the all areas ($p < 0,05$).

The lowest mean Sa values were recorded in the Group 2 compared to those recorded in the Group 1 in the coronal (150±2,0nm vs 185,32±82,37 nm; $p > 0,05$) and apical thirds (149,94±12,0 nm vs 179,23±27,60 nm; $p < 0,05$). In the apical third, the difference being statistically significant ($p < 0,05$).

The lowest mean Sa values were recorded in the Group 3 compared to those recorded in the Group 2, both in the coronal third (77,75±80, 48 nm vs 150±2,0 nm; $p < 0,05$), as well as in the middle (51,12± 50,50nm vs 149,94±12,0 nm; $p < 0,05$) and apical thirds (52,18±9,16 nm vs 149,94±12,0 nm; $p < 0,05$), the difference being statistically significant in all areas.

The lowest values of Sq were recorded in Group 3, compared to the mean values recorded in Group.1, both in the coronal (97.53 nm vs 232.27±95,39 nm; $p < 0,05$), in

the middle (62.95 ± 66.53 nm vs $158.10 \text{ nm} \pm 68.21$ nm; $p < 0.05$) and in the apical third (68.44 ± 13.48 nm vs 226.81 ± 31.81 nm; $p < 0.05$), the differences being statistically significant in all areas

The lowest values of Sq were recorded in Group 3, compared to Group 2 in the coronal (97.53 ± 96 nm vs 191.12 ± 12.2 nm; $p < 0.05$), middle (62.95 ± 66.53 nm vs 191.08 ± 10.0 nm; $p < 0.05$) and apical thirds (68.44 ± 13.48 nm vs 191.08 ± 10.0 nm; $p < 0.05$), the differences being statistically significant in all areas.

The lowest mean Sq values were recorded in the Group 2 compared to the mean values recorded in Group 1 in the coronal (191.12 ± 12.2 nm vs 232.27 ± 95.39 nm; $p < 0.05$) and apical (191.08 ± 10.0 nm vs 226.81 ± 31.81 nm; $p < 0.05$) thirds, the difference being statistically significant.

The lowest mean values of Sa, Sq were recorded in Group 3 in all three regions. The highest mean values of Sa and Sq were recorded in a more extensive area in Group 1 (in the coronal, middle and apical thirds), comparing to Group 3 and more extensive area comparing Group 2 (in the coronal and apical thirds). the differences being statistically significant ($p < 0.05$).

4. Discussions

Although EDTA and NaOCl are the most recommended irrigants of the dental canal for the removal of SL they can cause alteration in the chemical composition of dentin. This causes a significant decrease of dentin's microhardness and elasticity and an increase of its permeability, solubility and surface roughness. [17,25].

The aim of our study was to evaluate the effectiveness of two irrigation solutions

based on 20% EDTA and 40% AC with 5,25% NaOCl for 1 minute to removing the SL from the root canal and tubules and their influence on the dentine surface roughness, by AFM analysis. The results of this study highlighted the fact that 20% EDTA and 40% AC with 5,25% NaOCl removed SL compared to the group treated only with 0.9% physiological serum. SL was removed more efficiently in the group treated with 20% EDTA and 5,25% NaOCl, in the coronal and middle thirds of the root canal as observed on the 2D and 3D images from the AFM analysis (black shades of unoccluded dentinal tubules).

The arithmetic average height parameter (Sa) is the most universally used roughness parameter and it is defined as the average absolute deviation of the roughness irregularities from the mean line over one sampling length. The root mean square roughness (Sq) represents the standard deviation of surface heights and it is more sensitive than Sa. [25].

In our study the highest mean values of Sa were in the group treated with 20% EDTA with 5,25% NaOCl (163,62 nm) compared to group treated with 0.9% physiological serum (60,32 nm), on the extended area (the coronal, middle and apical thirds), the differences being statistically significant ($p < 0.05\%$).

The highest mean values of Sq were in the group treated with 20% EDTA with 5,25% NaOCl (205,7 nm) compared to group treated with 0.9% physiological serum (76,30 nm), on the extended area (the coronal, middle and apical thirds), the differences being statistically significant ($p < 0.05\%$).

The mean values of Sa are also higher in the group treated with 40%AC with 5,25% NaOCl (149,96nm) compared to the group treated with 0.9% physiological serum (60,32nm) on the extend area (the coronal, middle and apical thirds), the differences being statistically significant ($p < 0,05\%$).

The mean values of Sq are higher in the group treated with 40%AC with 5,25% NaOCl (191 nm) compared to the group treated with 0.9% physiological serum (76,30 nm) on the extend area (the coronal, middle and apical thirds) the differences being statistically significant ($p < 0,05\%$).

The mean values of Sa are higher in the group treated with 20% EDTA with 5,25% NaOCl (163 nm) compared to the group treated with 40% AC (191 nm) with 5,25% NaOCl, in the apicals third the differences being statistically significant ($p < 0,05\%$).

The mean values of Sq are higher in the group treated with 20% EDTA with 5,25% NaOCl (205,7 nm) compared to the group treated with 40%AC (149,96 nm) with 5,25% NaOCl, in the coronal and apical thirds the differences being statistically significant ($p < 0,05\%$).

The results of our study show that 20% EDTA with 5,25% NaOCl removes SL more effectively than 40% AC with 5,25% NaOCl and 0.9% physiological serum in the coronal and middle thirds of the root canal. Both combinations cause roughness on the dental surface, which they are more important in the coronal and apical thirds in the group treated with 20% EDTA with 5,25% NaOCl then 40% AC with 5,25% NaOCl and in the all areas in the group treated with 20% EDTA with 5,25% NaOCl, compared with 0.9% physiological serum, fact supported by the increased

average values of the roughness parameters(Sa and Sq).

In the literature there are few AFM studies that used 15-17% EDTA which highlighted an efficient elimination of SL and production of roughness in the coronal and medial thirds of the root canal and with a variable change in dental microhardness. [17, 21, 23, 24]. Few studies evidenced that microhardness decreased with increasing time of application of chelating solutions. The effect of the chelator is already apparent after 5 min and cannot be significantly increased by extending the working time to 24h [26]. Furthermore, in our study we use the maximum concentration of 20% EDTA available on the market and we applied as final irrigant till 1 min to produce roughness on dentinal surface, especially in apical third of root canal. Also, we reduced the working time to 1 min. We chose 20% EDTA because the side effects of it are already known, following that the results of the study should be extrapolated in the clinical activity

Tsenova-Ilieva et al., analyzed by AFM the effect of different irrigation protocols on the roughness of the root canal dentine and concluded that all tested irrigants increased the roughness of the root canal dentine including 15-17% EDTA [17].

Xiaoli Hu DDS, MS, et al, using Sa by AFM study, analyzed the effect of applying the following irrigants for 10 minutes: 17% EDTA, 5.25% NaOCl, 3% H₂O₂, and distilled water as a control. They observed a significant increase in roughness in the 17% EDTA Group compared to the control group, from 85.39 nm to 140.49 nm ($P < 0.05$). [21].

The null hypothesis can be rejected because, the mean values of the roughness parameters of the dentine surface recorded statistically significant differences ($p < 0.05$).

This study has a number of limitations such as: small number of teeth, subjective analysis of 2D and 3D AFM images, few AFM studies with 20% EDTA and 40% AC with NaOCl, AFM is laborious, expensive and allows the evaluation of only relatively small surfaces.

This study needs to be completed with: studying others roughness parameters: Peak-to-peak (Sy) Ten point height (Sz) Surface skewness (Ssk), Coefficient of Kurtosis (Sku); SEM / EDX studies to see the chemical changes in dentin as well as dentin hardness tests using different concentrations of irrigants and also Vickers microhardness tester. [17, 23, 27]

The limitations of this study are represented by the impossibility of an in vitro model to perfectly replicate the oral environment. Future in vivo studies are needed in order to validate the results of this study.

5. Conclusions

The association of 20% EDTA with 5.25% NaOCl solutions in the irrigation of the endodontic system is more efficient in removing SL in the coronal and middle thirds of the root canal and increases the root dentin surface roughness in all areas.

comparison with 0.9% physiological serum, and in the coronal and apical thirds in comparison with 40% AC with 5.25% NaOCl.

Author Contributions: Conceptualization, C.A.B.C., M.A., I.T., S.A. and C.L.G.; methodology, C.A.B.C., M.A., M.S., S.A., and C.L.G.; validation, C.A.B.C., C.L.G., S.A., and A.M.; formal analysis, C.A.B.C., I.T.; investigation, C.A.B.C., E.G.H., S.A., and R.D.; resources, E.G.H. and C.A.B.C.; writing—original draft preparation, C.A.B.C., E.G.H.; writing—review and editing, S.A., I.T., C.L.G. and R.D. visualization C.A.B.C., I.T., and S.S.; supervision, C.L.G. and S.A.; project administration, C.A.B.C.; funding acquisition, G.C.L., and R.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi (334/16/07/2023).

Informed Consent Statement: applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest

Reference

1. Mozo S, Llena C, Forner L. Review of ultrasonic irrigation in endodontics: increasing action of irrigating solutions. *Med Oral Patol Oral Cir Bucal*. 2012 May 1;17(3):e512-6. doi: 10.4317/medoral.17621. PMID: 22143738; PMCID: PMC3476090.
2. Lima Nogueira BM, da Costa Pereira TI, Pedrinha VF, de Almeida Rodrigues P. Effects of Different Irrigation Solutions and Protocols on Mineral Content and Ultrastructure of Root Canal Dentine. *Iran Endod J*. 2018 Spring;13(2):209-215. doi: 10.22037/iej.v13i2.19287. PMID: 29707017; PMCID: PMC5911296. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5911296>
3. Iqbal A. Antimicrobial irrigants in the endodontic therapy. *Int J Health Sci (Qassim)*. 2012 Jun;6(2):186-92. PMID: 23580897; PMCID: PMC3616947. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3616947/?report=reader#>
4. Mohammadi Z, Shalavi S, Yaripour S, Kinoshita JI, Manabe A, Kobayashi M, Giardino L, Palazzi F, Sharifi F, Jafarzadeh H. Smear Layer Removing Ability of Root Canal Irrigation Solutions: A Review. *J Contemp Dent Pract*. 2019 Mar 1;20(3):395-402. PMID: 31204334. <https://pubmed.ncbi.nlm.nih.gov/31204334/>
5. Boutsoukis C, Arias-Moliz MT, Chávez de Paz LE. A critical analysis of research methods and experimental models to study irrigants and irrigation systems. *Int Endod J*. 2022 Apr;55 Suppl 2(Suppl 2):295-329. doi: 10.1111/iej.13710. Epub 2022 Mar 6. PMID: 35171506; PMCID: PMC931484, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9314845/?report=reader#>
6. Tsenova-Ilieva I, Karova E. Effect of Endodontic Irrigants on Root Dentin Microhardness: A Systematic Review *Int J Sci Res* 2020;9: 49-6. https://www.researchgate.net/publication/340593080_
7. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. *Br Dent J*. 2014 Mar;216(6):299-303. doi: 10.1038/sj.bdj.2014.204. PMID: 24651335. <https://pubmed.ncbi.nlm.nih.gov/24651335>
8. Mikheikina A, Novozhilova N, Polyakova M, Sokhova I, Mun A, Zaytsev A, Babina K, Makeeva I. Knowledge, Attitude, and Practice towards Chelating Agents in Endodontic Treatment among Dental Practitioners. *Dent J (Basel)*. 2023 Jun 21;11(7):156. doi: 10.3390/dj11070156. PMID: 37504222; PMCID: PMC10378705 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10378705/?report=reader#>
9. Tonini R, Salvadori M, Audino E, Sauro S, Garo ML, Salgarello S. Irrigating Solutions and Activation Methods Used in Clinical Endodontics: A Systematic Review. *Front Oral Health*. 2022 Jan 31;3:838043. doi: 10.3389/froh.2022.838043. Erratum in: *Front Oral Health*. 2022 Mar 24;3:876265. PMID: 35174355; PMCID: PMC8841673. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8841673/?report=reader#>
10. Zehnder M. Root canal irrigants. *J Endod*. 2006 May;32(5):389-98. doi: 10.1016/j.joen.2005.09.014. PMID: 16631834 n, <https://doi.org/10.1016/j.joen.2005.09.014>
11. Arslan H, Barutçigil C, Karatas E, Topcuoglu HS, Yeter KY, Ersoy I, Ayrancı LB. Effect of citric acid irrigation on the fracture resistance of endodontically treated roots. *Eur J Dent*. 2014 Jan;8(1):74-78. doi: 10.4103/1305-7456.126248. PMID: 24966750; PMCID: PMC4054036, <https://www.thiemeconnect.com/products/ejournals/abstract/10.4103/1305-7456.126248>
12. Ali A, Bhosale A, Pawar S, Kakti A, Bichpuriya A, Agwan MA. Current Trends in Root Canal Irrigation. *Cureus*. 2022 May 8;14(5):e24833. doi: 10.7759/cureus.24833. PMID: 35698671; PMCID: PMC9184175. <https://doi.org/10.7759/cureus.24833>
13. Gandolfi MG, Taddei P, Pondrelli A, Zamparini F, Prati C, Spagnuolo G. Demineralization, Collagen Modification and Remineralization Degree of Human Dentin after EDTA and Citric Acid

- Treatments. *Materials* (Basel). 2018 Dec 21;12(1):25. doi: 10.3390/ma12010025. PMID: 30577625; PMCID: PMC6337713. <https://doi.org/10.3390/ma12010025>
14. Muana HL, Nassar M, Dargham A, Hiraishi N, Tagami J. Effect of smear layer removal agents on the microhardness and roughness of radicular dentin. *Saudi Dent J*. 2021 Nov;33(7):661-665. doi: 10.1016/j.sdentj.2020.05.001. Epub 2020 May 29. PMID: 34803316; PMCID: PMC8589586. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8589586/?report=reader#>
 15. Malhan, S., Bansal, C., & Johar, S. (2021). Root canal irrigants: A review. *International Journal of Health Sciences*, 5(S1), 134–142. <https://doi.org/10.53730/ijhs.v5nS1.5406>
 16. Kolosowski KP, Sodhi RN, Kishen A, Basrani BR. Qualitative Time-of-Flight Secondary Ion Mass Spectrometry Analysis of Root Dentin Irrigated with Sodium Hypochlorite, EDTA, or Chlorhexidine. *J Endod*. 2015 Oct;41(10):1672-7. doi: 10.1016/j.joen.2015.06.010. Epub 2015 Aug 6. PMID: 26255964. <https://pubmed.ncbi.nlm.nih.gov/26255964>
 17. Tsenova-Ilieva I, Simeonova S, Karova E. Atomic force microscopy study on the effect of different irrigation regimens on the surface roughness of human root canal dentin. *Niger J Clin Pract*. 2022 Mar;25(3):261-266. doi: 10.4103/njcp.njcp_1379_21. PMID: 35295046. <https://pubmed.ncbi.nlm.nih.gov/35295046>
 18. Elbahary S, Haj-Yahya S, Khawalid M, Tsisis I, Rosen E, Habashi W, Pokhojaev A, Sarig R. Effects of different irrigation protocols on dentin surfaces as revealed through quantitative 3D surface texture analysis. *Sci Rep*. 2020 Dec 16;10(1):22073. doi: 10.1038/s41598-020-79003-9. PMID: 33328515; PMCID: PMC7744534 <https://www.nature.com/articles/s41598-020-79003-9>
 19. Rosales JI, Marshall GW, Marshall SJ, Watanabe LG, Toledano M, Cabrerizo MA, Osorio R. Acid-etching and hydration influence on dentin roughness and wettability. *J Dent Res*. 1999 Sep;78(9):1554-9. doi: 10.1177/00220345990780091001. PMID: 10512390. <https://doi.org/10.1177/00220345990780091001>
 20. Raj Narkedamalli, Sandya Kini, Shubha Chhaparwal, Nidambur Vasudev Ballal. Effect of SmearOFF and ethylenediaminetetraacetic acid on the surface roughness and microhardness of human root canal dentin – An ex vivo study. *Saudi Endodontic Journal* 2022 .12 .175-1799 https://doi.org/10.4103/sej.sej_162_21
 21. Hu X, Ling J, Gao Y. Effects of irrigation solutions on dentin wettability and roughness. *J Endod*. 2010 Jun;36(6):1064-7. doi: 10.1016/j.joen.2010.03.007. Epub 2010 Apr 9. PMID: 20478467. <https://pubmed.ncbi.nlm.nih.gov/20478467>
 22. F Öztekin, The effects of different irrigation agents on root canal dentine, *Internationala Dental Research*, vol. 9, no. 1, 2019 https://doi.org/10.5577/intdentres.2019.vol9.no1.table_of_contents
 23. Huang L, Zhang X, Shao J, Zhou Z, Chen Y, Hu X. Nanoscale chemical and mechanical heterogeneity of human dentin characterized by AFM-IR and bimodal AFM. *J Adv Res*. 2019 Dec 30; 22: 163-171. doi: 10.1016/j.jare.2019.12.004. PMID: 32055426; PMCID: PMC7005426. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7005426/?report=reader#>
 24. Veeraiyan M, Kumar YP, Chandhar CY, Priyanka Y, Jaiswal M, Kemasaram D. Evaluation of Smear Layer Removal and Micro Hardness Alteration of Radicular Dentin after Using Various Chelating Agents - An Atomic Force Microscopic Study. *J Pharm Bioallied Sci*. 2023 Jul;15(Suppl 1):S582-S587. doi: 10.4103/jpbs.jpbs_29_23. Epub 2023 Jul 5. PMID: 37654348; PMCID: PMC10466611. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10466611/?report=reader#>
 25. E.S. Gadelmawla a, M.M. Koura b, T.M.A. Maksoud c, I.M. Elewa a, H.H. Soliman, Roughness parameters, *Journal of Materials Processing Technology*, volume 123, Issue 1, 10 April 2002, Pages 133-145. https://www.researchgate.net/publication/255857862_Roughness_parameters

26. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. *Eur J Dent.* 2013 Sep;7(Suppl 1):S135-S142. doi: 10.4103/1305-7456.119091. PMID: 24966721; PMCID: PMC4054072.
27. Jawad, Akram. (2020). *AFM Handbook; Theoretical Principles and Experimental Parameters.* Chapter 2, P,29-49