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ORIGINAL ARTICLE

Effect of a novel irrigation regimen and root canal filling material versus conventional endodontic protocols on dentin wettability

Tan Fırat Eyüboğlu[1] · Efe Armağan[2] · Ayşe Nur Temel[3] · Mutlu Özcan[2]

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Abstract

The purpose of this study was to compare the effect of different irrigation regimens on the dentin wettability of root canal sealers. The occlusal surfaces of human molar teeth ($N=90$) were removed, polished, and divided into three groups ($n=30$) based on the irrigation regimen: control group (SAL), standard irrigation group (NES), and test group (ODC). Each group was randomly divided into three subgroups according to the root canal sealer: AH Plus (AHP), Total Fill BC Sealer HiFlow (TOT), and OdneFill (ODF). The contact angle was determined using the sessile drop technique. The pendant droplet method assessed the work of adhesion. Surface roughness was measured using atomic force microscopy. Data were analyzed using non-parametric tests ($p<.05$). ODC-ODF showed significantly lower contact angle (34.80°) than ODC-AHP (66.53°, $p<.001$) and ODC-TOT (85.72°, $p<.001$). In NES, no significant difference was found between ODF and AHP ($p>.05$) while both groups presented significantly lower results than TOT ($p<.001$, $p<.001$, respectively). In SAL, the difference between TOT (92.84°) and AHP (83.24°) was insignificant ($p>.05$), whereas ODF presented significantly lower results compared to both ($p<.001$, $p<.001$, respectively). Surface roughness was lowest in ODC (31.54 nm), followed by SAL (62.30 nm) and NES (72.02 nms) which was significantly ($p=0.006$) higher than ODC, whereas the difference between SAL and NES was insignificant ($p>.05$). This study highlights the wettability of a new root canal sealer and the effect of a new irrigation activation system on the wettability of different root canal sealers. OdneFill showed superior wettability compared to other sealers while exhibiting a synergistic effect with OdneClean.

Keywords Contact angle · Dental materials · Endodontics · Epoxy resin · Hydrogel · Inertial cavitation · Wettability

Introduction

A fluid-tight seal within the root canal system is an important achievement for a successful outcome in root canal treatment [1]. Attaining such a goal is difficult due to the complex structure of the root canal system [2]. A core material such as gutta-percha is usually recommended to be used with a root canal sealer cement for optimal sealing [3]. The root canal sealer is required to seal dentinal tubules while entrapping the remaining bacteria within the root canal system [4] and creating adhesion between gutta-percha and root canal dentin [5]. Therefore, the viscosity and wetting ability of a sealer are important and are crucially influencing features in the choice of material during root canal treatment [3, 5].

AH Plus (Dentsply, DeTrey, Konstanz, Germany) is an epoxy resin-amine based sealer that is widely utilized. Total Fill BC Sealer HiFlow (FKG Dentaire, La Chaux des Fonds, Switzerlad) is a calcium silicate-based root canal sealer that can be combined with warm gutta-percha filling techniques [6] with comparable physicochemical properties to AH Plus with the disadvantage of higher solubility [7].

Recently, a new root canal sealer, OdneFill (Lumendo AG, Renens, Switzerland), was developed which is a low-viscosity, injectable, hydrophilic, photo-polymerizable endodontic root canal sealer, forming a hydrogel upon curing. A

Tan Fırat Eyüboğlu
tfeyuboglu@yahoo.com

Mutlu Özcan
mutluozcan@hotmail.com

1 Department of Endodontics, Faculty of Dentistry, Istanbul Medipol University, Atatürk Bulvari, No: 27, 34083 Istanbul, Türkiye

2 Clinic of Masticatory Disorders and Dental Biomaterials, Center for Dental Medicine, University of Zurich, Plattenstrasse 11, CH-8032, Zurich, Switzerland

3 Department of Endodontics, Faculty of Dentistry, Istanbul Medipol University, Istanbul, Türkiye

hydrogel is a crosslinked, hydrophilic polymer with high water content, exhibiting mechanical properties resembling those of natural tissues. Furthermore, hydrogels are known for their excellent biocompatibility, causing minimal inflammatory response and tissue damage. Due to its water-like viscosity and the high hydrophilicity of its uncured state, it can reach irregularities within the root canal system [8].

An optimal root canal preparation requires mechanical removal of the root canal ingredients and disinfection of the root canal system using root canal irrigation solutions [9]. Due to its organic tissue dissolving ability and good antimicrobial properties, sodium hypochlorite (NaOCl) is the most commonly used irrigation solution during root canal treatment [10]. However, since NaOCl cannot remove the smear layer [11] a chelating agent such as Ethylenediaminetetraacetic acid (EDTA) is required [12]. Therefore, the successive use of NaOCl, EDTA, and a final rinse of saline solution to remove all chemicals from the dentin surface as a final irrigation protocol is the most commonly used protocol to obtain optimal adhesion to the dentin surface [13].

A novel irrigation device, OdneClean (Lumendo AG, Renens, Switzerland), has been developed to clean even minimally invasive prepared canals and accessory canals by producing a hydro-dynamic cavitation cloud with a debridement tip as thin as 190 µm using saline solution within the root canal system [14] instead of activating NaOCl in extensively shaped canals [15]. Different irrigation solutions and protocols may alter the chemical and structural composition of dentin surface [16] which in turn affects the adhesion of root canal filling materials [17]. Therefore, it is critical to investigate the effects of irrigation protocols on the mechanical properties of dentine. Hence, the purpose of this study is to compare the effect of three different irrigation regimens on the dentin wettability of AH Plus, Total Fill BC Sealer HiFlow and OdneFill. The null hypothesis tested was that different irrigation regimens or root canal sealers would not affect the wettability and the work of adhesion of the dentin surface.

Materials and methods

Using G Power analysis (v.3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), a sample size of 81 was established with a power of 0.80, p value 0.05, effect size = 0.50, and $\eta^2 = 0.2$. To compensate for any samples lost during processing, one more sample was added to each group, bringing the total sample size to 90. Written informed consent for research purpose of the extracted teeth was obtained by all donors prior to extraction according to the directives set by the National Federal Council. Ethical guidelines were strictly followed and irreversible anonymization was performed in accordance with State and Federal Law (World Medical Association, Declaration of Helsinki, 2013; Human Research Act, 2015) [18, 19].

Specimen preparation

A total of 90 extracted human molar teeth with intact crowns and similar sizes were chosen for the study. The teeth were stored in chloramine-T. Coronal dentin of all specimens was utilized for all investigational purposes, as previously described. [20, 21] To obtain coronal dentin, the occlusal surfaces of all crowns were removed with a low-speed diamond saw (IsoMet; Buehler, Lake Bluff, IL, USA), exposing the dentin surfaces, which were later polished with 600-grit silicon carbide paper underwater to obtain a smooth surface for analysis. Specimens were randomly divided into three groups ($n = 30$) according to the irrigation regimen to be used:

Group 1: 3 mL of saline solution for 1 min (SAL) (Control Group).

Group 2: 3 mL of 6% NaOCl (CanalPro, Colténe, Altstätten, Switzerland) for 1 min + 3 mL of 17% EDTA (CanalPro, Colténe, Altstätten, Switzerland) + 3 mL of saline solution for 1 min (NES) (Standard Irrigation).

Group 3: 3 mL of 6% NaOCl (CanalPro) + OdneClean activation for 1 min with a distance of 6–8 mm of the tip from the dentin surface with an angulation of 30º to the surface. (ODC) (Test Group)(Fig. 1).

After the irrigation regimens were completed and surfaces were gently dried with cotton pellets, each group was further divided into three subgroups ($n = 10$) depending on the root canal sealer used: AH Plus Jet (Dentsply) (AHP), Total Fill BC Sealer HiFlow (FKG Dentaire) (TOT) and OdneFill (Lumendo) (ODF) (Table 1).

Contact angle measurement

Following irrigation, the excessive water on each specimen was gently removed using an air spray for 5 s and subsequently, each specimen was placed on a sample holder of the equipment. The contact angle measurement was performed using the sessile drop method by the Drop Shape Analyzer (DSA) (EASYDROP, Krüss GMBH, Hamburg, Germany) [22]. Before capturing an image, 10 s of spreading time was applied for each measurement to allow the droplet to equilibrate on the dentin surface, ensuring the droplet had reached a stable configuration and the contact angle had reached its equilibrium value.

The image of the droplet was then captured by DSA software to measure the static contact angle of each sealer on the root canal dentine. The Young–Laplace fit was used for each droplet to calculate the contact angle between the sealer and the dentine surface. This model takes into account the balance of surface tension and pressure forces acting on the

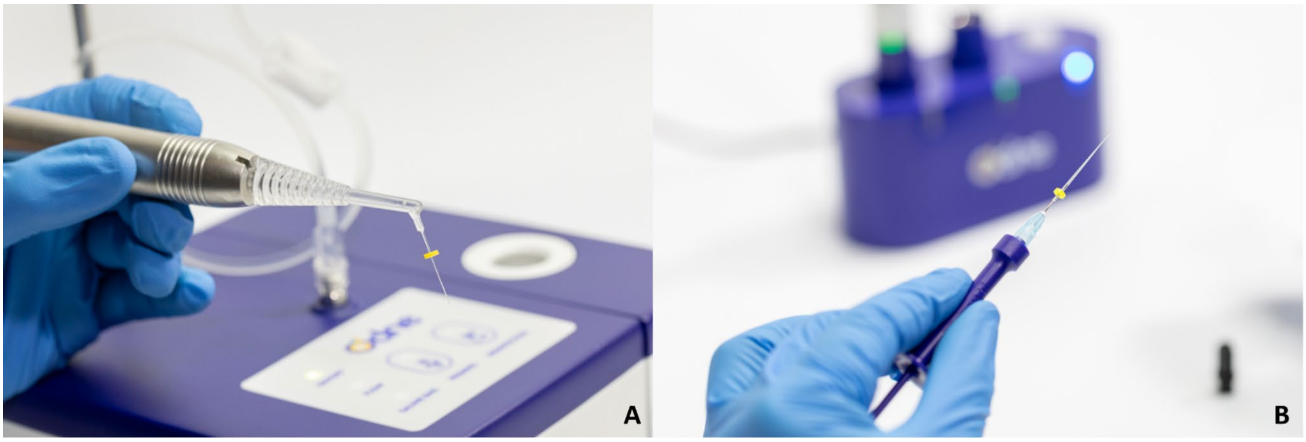

Fig. 1 **A** OdneClean device and handpiece with intracanal irrigation tip, **B** OdneFill syringe with the application tip

Table 1 Composition and preparation information of the tested root canal sealers

Material	Manufacturer	Composition declared by the manufacturer	Preparation
AH Plus Jet	Dentsply DeTrey GmbH, Konstanz, Germany	Epoxide paste: diepoxide, calcium tungstate, zirconium oxide, aerosol, pigment; Amine paste: 1-adamantane amine, *N*,*N′*-dibenzyl-5-oxa-nonandiamin-1,9, TCD-diamine, calcium tongstate, zirconium oxide, aerosil, and silicon oil	With auto mixing tips ready to use
Total Fill BC Sealer	FKG Dentaire, La Chaux des Fonds, Switzerland	Tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, zirconium oxide, tantalum oxide, calcium hydroxide, filler and thickening agents	Ready to use
OdneFill	Lumendo AG, Renens, Switzerland	Zirconium oxide, yttrium Oxide, water, bismeth-acrylated polymer, excipients	Ready to use

droplet, allowing for a more precise determination of the contact angle compared to simpler geometric methods.

Work of adhesion measurement

The work of adhesion between each root canal sealer and the dentin surface was measured using the pendant droplet method [23]. This technique involved determining the interfacial tension of each sealer. Before the measurement, the gravimetric density of each sealer was measured using the pycnometer method. A certain volume of a drop of each sealer was ejected from the micropipette from a distance of 1 cm on the analyzer device which was filled with sealers until it reached a maximum volume before falling off. The work of adhesion between each sealer and root canal dentine was calculated by the formula below;

$$W_{SL}^{A} = \gamma_L(1 + cos\theta)$$

where W_{SL}^{A} represents the work of adhesion between liquid and solid, γ_L is the interfacial tension of liquid and θ is the contact angle between solid and liquid.

AFM analysis

Three samples from each group were randomly selected for atomic force microscopy analysis to evaluate the topography and roughness characteristics of the dentin surfaces following the irrigation regimens.

The atomic force microscope (AFM, Brucker Dimension Icon) was operated in tapping mode to obtain high-resolution images of the dentin surfaces [24]. A silicon cantilever probe with a tip radius of 8 nm (RTESPA-525, Bruker, CA) was used to scan the samples. The included NanoScope software (Bruker, CA) provided precise control and data acquisition capabilities for the AFM system. For each dentin specimen, images were obtained from three separate fields of view, each measuring $10 \times 10\ \mu m^2$. The surface roughness parameters were then measured from these high-resolution scans, and the individual roughness values were averaged to produce a single representative Ra value for each dentine sample. This comprehensive AFM analysis provided detailed quantitative data on the nanoscale surface characteristics of the dentin specimens under investigation.

Statistical analyses

The statistical analyses were completed using IBM SPSS Statistics 29 (IBM SPSS Switzerland). For contact angle and work of adhesion measurements, the homogeneity of the data was evaluated using the Shapiro–Wilk test. The homogeneity of variance was tested using Leven's test. A two-way ANOVA test was adopted for analyses of variance, with a Bonferroni adjustment for pairwise comparisons. For roughness evaluation, the homogeneity of the data was analyzed using Kolmogorov-Smirnov, and one-way ANOVA was adopted for analyses of variance with Bonferroni adjustment. Significance was set at $p < 0.05$.

Results

The data were homogeneously distributed. The groups also have similar variances, according to Leven's test. In terms of irrigation regimens, the ODC group presented the lowest contact angle results (62.35°), followed by NES (64.57°, $p = 0.996$) and SAL (72.39°, $p < 0.001$), respectively. The difference between NES and SAL was also significant ($p = 0.003$) (Table 2). In terms of root canal sealers, the ODF group presented the lowest contact angle results (42.66°), followed by AHP (66.79°, $p < 0.001$) and TOT (89.86°, $p < 0.001$). The difference between AHP and TOT was significant as well ($p < 0.001$) (Table 2). The contact angle was lowest in ODC-ODF (34.80°) and highest in SAL-TOT (92.84°) (Table 2).

Pairwise comparisons presented that in the ODC group, the contact angle of ODF specimens was significantly lower compared to AHP ($p < 0.001$) and TOT ($p < 0.001$). AHP also presented significantly lower contact angle results compared to those of TOT ($p < 0.001$). In the NES group, ODF and AHP presented similar results ($p = 1.00$), while both of the materials had significantly lower contact angle values compared to those of TOT ($p < 0.001$, $p < 0.001$, respectively). In the SAL group, ODF presented significantly lower contact angle values than those of AHP ($p < 0.001$) and TOT ($p < 0.001$) (Table 2, Fig. 2).

The work of adhesion was highest in NES-AHP (88.35 mJ/m^2) and lowest in SAL-TOT (22.41 mJ/m^2). The values presented significant differences both according to sealer type ($p < 0.001$) and irrigation regimen-wise ($p < 0.001$) (Table 3, Fig. 3). The highest work of adhesion values was presented by NES (51.71 mJ/m^2), followed by ODC (50.06 mJ/m^2, $p = 0.188$), and SAL (43.46 mJ/m^2, $p = 0.001$), respectively. There was also a significant difference between NES and SAL ($p < 0.001$). In terms of sealer type, AHP presented the highest work of adhesion values (74.89 mJ/m^2) followed by ODF (46.73 mJ/m^2, $p < 0.001$), and TOT (23.61 mJ/m^2, $p < 0.001$), respectively. ODF also presented significantly higher work of adhesion values than TOT ($p < 0.001$).

Pairwise comparisons in the AHP group presented significant differences between ODC and NES ($p < 0.001$), ODC and SAL ($p < 0.001$), as well as between NES and SAL ($p < 0.001$). In the ODF group, only the difference between ODC and NES was statistically significant ($p = 0.012$), while the differences between ODC and SAL ($p = 0.428$) and between NES and SAL ($p = 0.081$) were not significant. In the TOT group, none of the irrigation regimens presented a significant difference in the means of work of adhesion from each other (ODC-NES, $p = 0.332$; ODC-SAL, $p = 0.184$; NES-SAL, $p = 0.732$). In terms of irrigation regimens, in ODC, NES, and SAL, all sealers presented significant differences from each other (AHP-ODF, $p < 0.001$; ODF-TOT, $p < 0.001$; AHP-TOT, $p < 0.001$).

In terms of surface roughness values, NES (72.02 nm) exhibited the highest mean surface roughness (Ra), followed by SAL (62.3 nm, $p = 1.000$) and ODC (31.54 nm, $p = 0.006$). ODC had significantly lower surface roughness values than NES, whereas the difference between ODC and SAL was insignificant ($p = 0.52$) (Table 4, Fig. 4).

Table 2 Mean contact angle values (°) and standard deviations of all irrigation protocol and root canal sealer pairs

	AHP	ODF	TOT
SAL	$83.24 \pm 9.22^{a,x}$	$41.10 \pm 5.80^{b,x}$	$92.84 \pm 8.46^{a,x}$
NES	$50.60 \pm 8.22^{a,y}$	$52.09 \pm 7.09^{a,y}$	$91.04 \pm 13.79^{b,x}$
ODC	$66.53 \pm 4.78^{a,z}$	$34.80 \pm 8.33^{b,z}$	$85.72 \pm 10.60^{c,x}$

SAL Saline solution, *NES* NaOCL + EDTA + Saline, *ODC* NaOCl + OdneClean, *AHP* AH Plus, *ODF* OdneFill, *TOT* Total Fill BC Sealer HighFlow, a, b, c: different letters indicate significant difference in row; x, y, z: different letters indicate significant difference in columns ($p < 0.05$)

Discussion

The measurement of surface free energy is expressed as contact angle (°) and presents the intermolecular attraction between surfaces [25]. If the contact angle of the material is > 90º, the material is accepted as non-wetting and if the angle is < 90º, then the material is accepted as wetting [26]. According to the results of this study, TOT presented the highest contact angle values compared to ODF and AHP, with barely visible dome-shaped drops on the dentin surface. Previously, a similar problem was also reported due to increased viscosity [27] of the calcium silicate-based root canal sealers [28]. ODF exhibited a considerably lower contact angle than AHP and TOT. Regarding the irrigation protocols, ODC and NES had significantly lower contact

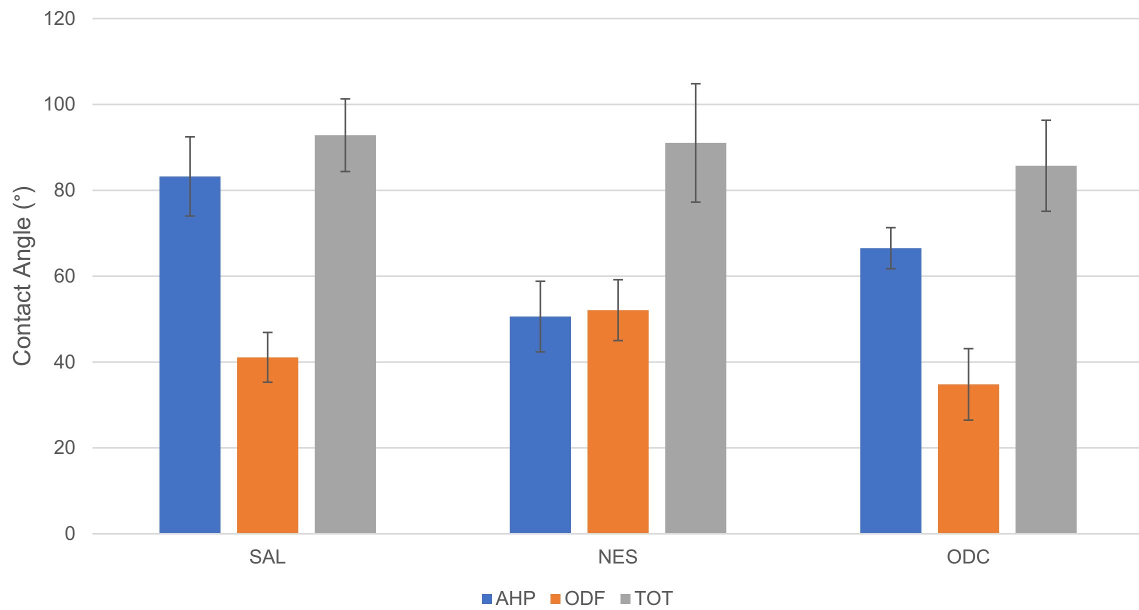

Fig. 2 Contact angle values with their standard deviation according to irrigation regimen groups and root canal sealer material subgroups. *SAL* Saline Solution, *NES* NaOCL + EDTA + Saline, *ODC* NaOCl + OdneClean, *AHP* AH Plus, *ODF* OdneFill, *TOT* Total Fill BC Sealer HiFlow

Table 3 Mean work of adhesion values (mJ/m^2) and standard deviations of all irrigation protocol and root canal sealer pairs

	AHP	ODF	TOT
SAL	$60.56 \pm 8.65^{a,x}$	$47.42 \pm 1.79^{b,x,y}$	$22.41 \pm 3.43^{c,x}$
NES	$88.35 \pm 6.31^{a,y}$	$43.63 \pm 2.58^{b,y}$	$23.14 \pm 5.53^{c,x}$
ODC	$75.76 \pm 4.17^{a,z}$	$49.13 \pm 2.12^{b,x}$	$25.29 \pm 4.31^{c,x}$

SAL Saline solution, *NES* NaOCL + EDTA + Saline, *ODC* NaOCl + OdneClean, *AHP* AH Plus, *ODF* OdneFill, *TOT* Total Fill BC Sealer HighFlow, a, b, c: different letters indicate significant difference in row; x, y, z: different letters indicate significant difference in columns ($p < 0.05$)

angles than the control group. Therefore, the hypothesis is rejected indicating a significant effect of both irrigation regimen and root canal sealer type on the contact angle values.. Moreover, it has also been observed that ODF presented the lowest contact angle values particularly in ODC, while AHP presented the lowest contact angle values in NES, which may be an indication of a synergistic effect between OdneFill and OdneClean as well as between AH Plus and standard irrigation regimen.

The contact angle values of ODF in the saline group are significantly lower than those in the standard irrigation group. In a previous study, the use of EDTA presented significantly increased contact angle values in ThermaSeal Plus and AH Plus compared to those of saline solution, [9] which is partly in agreement with the findings of the present study. Although OdneFill presented similar results to those of ThermaSeal Plus in comparison to EDTA and saline solution, the AH Plus group presented opposite results. This might be due to different study designs, such as the use of coronal dentin instead of radicular dentin, the type of sealers, and irrigation regimens.

The influence of roughness on adhesion has been recognized as a significant factor and has been linked to the concept of wettability in the Wenzel equation. Wenzel reported that the contact angle of root canal sealers decreases with increased surface roughness [29]. However, the present study found no significant difference between SAL and either NES or ODC, despite NES presenting significantly higher surface roughness values than ODC. Due to the increased roughness in the NES group the wettability of all sealers was expected to be increased compared to SAL and ODC groups, respectively. However, it was not the case for all sealers. Although AH Plus presented significantly lower contact angle values in the NES group, roughness was not the determining factor for the contact angle values for the other two irrigation regimens according to the results of this study. For ODF, it was completely reversed whereas the smoothest surface, ODC, has the lowest contact angle and the roughest, NES, has the highest. This discrepancy raised suspicion that the contact angle values could not be solely explained by surface roughness. Moreover, the report on the effect of EDTA on surface roughness was also not conclusive. In a previous study, no significant difference was detected between the use of EDTA and saline solution. This finding is consistent with the present investigation [30].

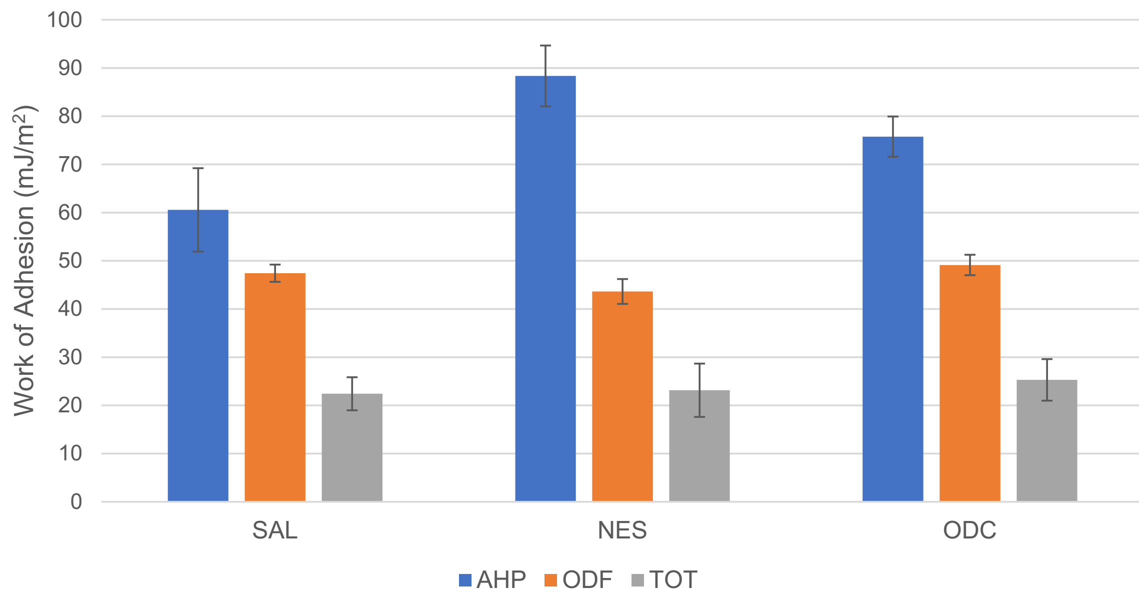

Fig. 3 Work of adhesion values (mJ/m^2) with their standard deviation according to irrigation regimen groups and root canal sealer material subgroups. *SAL* Saline Solution, *NES* NaOCL + EDTA + Saline, *ODC* NaOCl + OdneClean, *AHP* AH Plus, *ODF* OdneFill, *TOT* Total Fill BC Sealer HiFlow

Table 4 Mean surface roughness values (nm) and standard deviations of all irrigation protocols

	Mean surface roughness value (nm)
ODC	31.54 ± 9.57^{a}
NES	72.02 ± 32.552^{b}
SAL	$62.3 \pm 26.16^{a,b}$

*Different letters in the column indicate a statistically significant difference ($p < .05$). Same letters in the column indicate the differences are not significant ($p > .05$)

In previous studies, the use of EDTA was related to increased surface roughness and, therefore, decreased contact angle values due to the removal of the smear layer [24, 31]. To eliminate the smear layer, ODC employs hydrodynamic cavitation, and NES relies on the use of EDTA. The decreased contact angle values seen in both irrigation procedures may be attributed to the elimination of the smear layer, unlike SAL, which does not have an impact on the smear layer.

The removal of the smear layer and the resulting exposure of collagen fibers, which have low surface-free energy [32], could also affect the wettability of the surface negatively [33]. In previous studies, the exposure of collagen fibers was reported to be the reason for increased contact angles [17] in the absence of a surfactant agent [9]. The presence of the

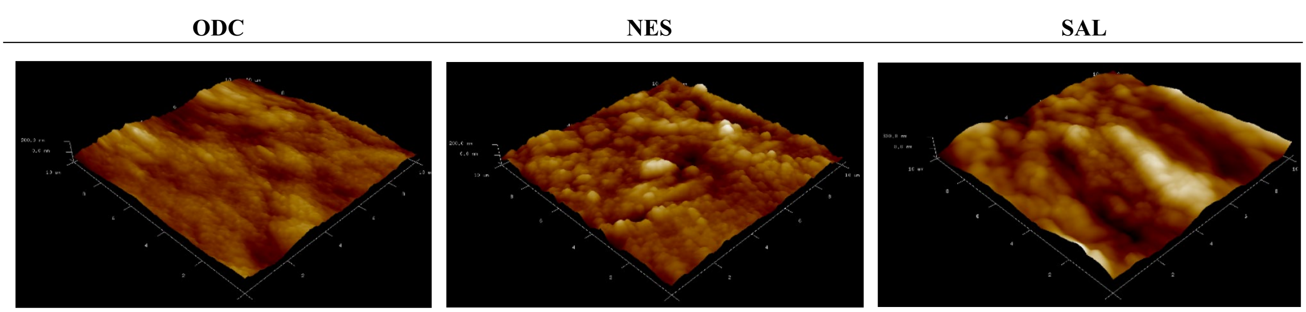

Fig. 4 Representative 3D atomic force microscopy illustrations of dentin after three different irrigation regimens. *SAL* Saline Solution, *NES* NaOCL + EDTA + Saline, *ODC* NaOCl + OdneClean

highest surface roughness yet not the lowest contact angle in NES might be attributed to the presence of a residual thin layer of demineralized collagen fibers on the dentin surface, which could contribute to elevated surface energy and reduced wettability [9, 17, 31]. This could also explain the lower contact angle values of ODF material in ODC than those in the standard irrigation group. In ODC, the use of hydraulic cavitation could help remove the smear layer and any exposed collagen fibers, as was observed in a previous preliminary SEM study, increasing the hydrophilicity of the surface due to exposed dentinal tubules [17] and thus favoring the hydrophilic root canal sealer, ODF, whereas antagonizing the less hydrophilic root canal sealer, AHP, in terms of wettability.

To summarize, the roughness of the dentinal surface with the presence of some hydrophilicity in NES created a synergistic effect for AH Plus, while roughness with increased hydrophilicity in ODC had the same synergistic effect for a more hydrophilic root canal sealer, OdneFill. Although sealers are set or cured rather quickly within the root canal system [34], the primary leakage after root canal filling may play an important role, particularly in root canal fillings completed using root canal sealers with prolonged setting times such as some of the bioceramic root canal sealers [35], Therefore, it is of vital importance to investigate the work of adhesion between the root canal sealers and the dentin surface. Different sealers presented significantly different work of adhesion values with dentin treated with different irrigation regiments. AHP showed significantly higher work of adhesion values compared to those of ODF and TOT, respectively, for each irrigation specimen. Thus, the null hypothesis is also rejected indicating a significant interaction between the irrigation solution and the type of the root canal sealer used in terms of the work of adhesion.

TOT exhibited the most minimal work of adhesion values, irrespective of the irrigation regimen. This can be attributed to the elevated contact angle of TOT due to its viscous characteristics. The increased work of adhesion values of AHP can be attributed to its greater surface tension, which is a result of the material's high density. The ODF, having a density similar to water, exhibited work of adhesion values that fell between those of AHP and TOT. ODF's fast setting time makes it a promising sealer due to the fact that longer setting time and low work of adhesion can raise the risks of microleakage.

The droplet analyses were not completed with the controlled-volume droplet technique due to the differences in the rheological properties of the root canal sealers. However, it has been previously reported that the droplet volume did not affect the advancing contact angle [36]. Hence, the adoption of the sessile droplet technique was concluded as adequate.

From a clinical standpoint, our results suggested that the wettability of the root canal sealer is affected by the interaction between the type of root canal sealer and the irrigation regimen. Therefore, no single irrigation regimen is definitive to ensure optimal wettability, and decisions should be made according to the interaction between root canal sealers and irrigation regimen in a clinical setup. Further studies are required to better understand such interaction between both factors.

In the present study, instead of root dentin, coronal dentin was used setting the angulation of the dentinal tubules to the dentin surface and achieving homogeneity among the samples as the primary factors. The use of radicular dentin [9] could result with almost parallel angulation of dentinal tubules to dentin surface and more material removal from specimen surface to obtain smooth dentin surfaces making the clinical relevance of the specimens questionable. Therefore, the use of coronal dentin should be taken into consideration for the interpretation of these results. The samples were not incubated after the application of irrigation solutions but were only dried with cotton pellets to mimic the clinical conditions as much as possible. The roughness measurements aimed to evaluate material wettability performance without comparing irrigation regimens on dentin surfaces, as no roughness measurements were conducted before implementation.

Conclusions

The wettability of the dentin surface is affected by the irrigation solutions as well as the hydrophilicity, and viscosity of the root canal sealers used. OdneFill presented significantly lower contact angle results, with a possible synergistic effect between OdneFill and OdneClean.

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Data availability The datasets used and/or analyzed during the current investigation are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The directives set by the National Federal Council. Ethical guidelines were strictly followed and irreversible anonymization was performed in accordance with State and Federal Law (World Medical Association, Declaration of Helsinki, 2013; Human Research Act, 2015).

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