IEJ
Iranian Endodontic Journal

Assessment of Dentinal Tubules Penetration by Hyben X Delivered into the Root Canal System as an Irrigant; An *in Vitro* Study

Leonardo Padilla-Correales [a], José Llanos-Torres [a], Zara Pérez-Quiñones [b*], Antonio Diaz-Caballero [c], Eduardo Covo-Morales [d]

a Endodontics Specialist, University of Cartagena, Cartagena, Bolívar, Colombia; b Dentist, Specialist in Health Management, University of Cartagena, Gitouc Research Group, Colombia; c University of Cartagena, Periodontics Specialist, Master in Education, Ph.D. in Biomedical Sciences, University of Cartagena, Titular Professor, University of Cartagena, GITOUC Research Group, Cartagena, Bolívar, Colombia; d Specialist in Endodontics, Master in Microbiology, Titular Professor, University of Cartagena, Bolívar, Colombia

Article Type: Original Article

Received: 02 Sep 2022

Revised: 21 Nov 2022

Accepted: 05 Dec 2022

Doi: *10.22037/iej.v18i1.33114*

****Corresponding author***: Zara Pérez-Quiñones, Faculty of Dentistry. University of Cartagena, Cra. 50 #24120, Colombia. Postal code: 130015

E-mail: zperezq@unicartagena.edu.co

Introduction: Disinfection of the root canal system is crucial for a successful endodontic treatment. Several factors influence the performance of effective irrigation. Diverse irrigating substances have been used but none has proved to completely penetrate the root canal system. HybenX dries *biofilm* due to its hygroscopic properties; therefore, it is effective in the treatment of *biofilm*-related diseases. This investigation aimed to estimate HybenX's degree of penetration into the dentinal tubules of upper first premolars. **Materials and Methods:** Experimental *in vitro* intervention where 30 extracted maxillary premolars were evaluated to determine HybenX's degree of penetration and 5% sodium hypochlorite in the dentinal tubules using three different irrigation techniques (passive ultrasonic irrigation, dynamic manual irrigation and conventional single jet irrigation technique). After preparation, the root canals were irrigated with fluorescent rhodamine 6G; then 500-micrometer sections were made to be analyzed under a fluorescence microscope. Measurements were made in micrometer in ZEN software to determine the penetration degree of each substance in the different root thirds. **Results:** In the apical third, significant differences between the 3 irrigation techniques were found ($P<0.05$), similarly, for the middle and cervical thirds. Significant statistical differences between the HybenX and sodium hypochlorite were found at the cervical and middle levels when using the conventional Monoject irrigation and passive ultrasonic irrigation techniques. As for the apical level, differences were found between the passive ultrasonic irrigation techniques and the dynamic manual irrigation technique. **Conclusions**: Based on this *in vitro* study, HybenX proved to highly penetrate into the dentinal tubules, especially when using the passive ultrasonic irrigation technique. HybenX may be a useful option for root canal irrigation in endodontics.

Keywords: Biofilms; Hybenx; Root Canal Irrigants; Root Canal Preparation

Introduction

The success of endodontic treatment depends greatly on the bacterial eradication from the root canal system and the prevention of reinfection [1, 2]. This is achieved by combining root canal preparation filing and irrigation to remove infected dentin and pulpal remnants [3]. The main objective of filing is enlarging the root canal to allow penetration of the irrigants [4]. A challenge for irrigation and root canal disinfection is not only the *biofilm* resistance but also the capacity of the irrigant to penetrate the great anatomical complexity of the root canal system and completely eliminate the presence of the smear layer [5-8]. Ideally, irrigants must have the capacity to dissolve organic tissue, be a wide-spectrum antimicrobial (especially against microorganisms organized in *biofilms*), they must be non-toxic when contacting periodontal tissues (with low potential to cause anaphylactic reaction); however, an irrigant with all these properties doesn't exist yet [9, 10].

It is essential that the irrigant sufficiently reaches the apical zone of the canal since 75% of the anatomical ramifications are found there [11]. Meanwhile, 11% and 15% of these ramifications are in the middle and cervical thirds, respectively. These ramifications are potential pathways for bacterial products to reach and damage the periodontal ligament [8]. In addition to the anatomical ramifications of the canal system, other relevant factors that influence irrigant penetration are the size of the apical preparation and its taper, the distance between the needle and the apex during irrigation, the irrigation technique, the volume of irrigation, and the needle dimension. Additionally, the curvature of the root canal has been identified as a limiting potential factor in several studies [8, 12].

Due to the difficulty of evaluating some irrigants' effectiveness such as sodium hypochlorite (NaOCl) during in vivo studies, compared to *in vitro* investigations, the conclusions have not been entirely promising, especially in the apical third, where the anatomical complexity of the root canal system hinders its diffusion and therefore its action [13]. Filing in combination with irrigation should be enough to eliminate pulp tissue and dentinal remnants from the root canal system. Without irrigation, the accumulation of debris would provoke instrument ineffectiveness [14].

The irrigants carry out physical, mechanical, chemical, biological, and microbiological effects. The penetration of each irrigant inside the dentinal tubules plays a crucial role in the antibacterial effect of each solution [15]. Zou, L *et al.* had initial information with micrometric precision about the penetration of sodium hypochlorite (NaOCl) in dentine. In their experiment, the penetration values were varied between 77 and 300 micrometers (μm) [16].

HYBEN X is a semi-viscous, opaque, and purple liquid considered to denaturize tissue. It contains sulfuric acid and sulfonated phenolic compounds. It can eliminate biofilm adhered to soft and hard tissues in the oral cavity, it also possesses hygroscopic properties that absorb water from biofilm quickly and effectively, by precipitation of organic polymers [17]. Additionally, it acts as a desiccant (and not like an acid) by absorbing water from the biofilm and its matrix, causing the collapse of its internal structure, and reducing the possible adverse effects in the periapical region [18, 19]. According to the literature, HybenX® is indicated for the treatment of recurring aphthous stomatitis, resulting in pain relief and healing of the ulcer [20, 21]. It is also effective against the bacterial biofilm in the treatment of chronic periodontitis in adult patients , and lately [22], some authors like Lopez MA *et al.* have evaluated its efficacy in the management of severe or recurring peri-implantitis, concluding that the use of HybenX in the surgical treatment of peri-implantitis shows promising results [23]. On the other hand, Pace R *et al.* evaluated HybenX as an irrigant in combination with NaOCl, affirming that it efficiently removes the smear layer from the root canal system [24]. The main objective of this investigation is to determine the penetration of HybenX® into the walls of the root canals.

Materials and Methods

An *in vitro* experimental intervention study was performed to estimate HybenX®'s degree of penetration into the root canals of first upper premolars extracted for orthodontic purposes. The buccal roots were used for experimental purposes, and the palatal roots as control roots, maintaining the principle of equality between the experimental and control units. Six groups of 10 roots (specimens) each, were created. These were then divided into 3 experimental groups containing buccal roots exclusively for HybenX®, and 3 control groups containing palatal roots exclusively for NaOCl. Additionally, each group of 10 specimens (experimental and control) was organized according to the irrigation technique tested: passive ultrasonic irrigation (PUI), dynamic manual irrigation, and conventional irrigation with Monoject needle. The groups were prepared as follows:

Group A1: conformed by 10 buccal roots irrigated with HybenX® during preparation, using the conventional irrigation technique with a Monoject needle.

Group B1: conformed by 10 buccal roots irrigated with HybenX® during preparation, employing the dynamic manual irrigation technique.

Group C1: conformed by 10 buccal roots irrigated with HybenX® during preparation, through the PUI.

Group A2: conformed by 10 palatal roots irrigated with 5% NaOCL during preparation, using the conventional irrigation technique with a Monoject needle.

Group B2: conformed by 10 palatal roots irrigated with 5% NaOCL during preparation, using the dynamic manual irrigation technique.

Group C2: conformed by 10 palatal roots irrigated with 5% NaOCl during preparation, using the PUI technique.

The crown was removed from each tooth and sectioned by the furcation with a low-speed metallic disc to separate the buccal and palatal roots. Working length was established; later, all roots were set in epoxy resin blocks to ease biomechanical instrumentation.

Root canal preparation and irrigation protocol

The access cavity was prepared with a high-speed handpiece and round diamond burs. Conductometry was taken with a #10 K-file (Dentsply Maillefer, Zurich, Switzerland), passing the file through the apical foramen, to subsequently subtract 1 millimeter and establish this measurement as working length.

All groups of teeth were prepared with a 25.07 Reciprocating System WaveOne Gold (Dentsply Maillefer, Ballaigues, Switzerland) primary taper file. One milliliter of HybenX® was used for biomechanical preparation during the time that the operator takes with each irrigation technique for groups A1, B1, and C1. For the control groups A2, B2, and C2, the conditions were the same as the experimental groups but using NaOCl instead.

After biomechanical preparation, the root canals of the experimental groups were irrigated once again with a 1-milliliter solution of HybenX® diluted in one milliliter of fluorescent rhodamine 6G (Sigma-Aldrich, St Louis, MO, USA) for one min using the selected irrigation protocol for each group. The same was performed on the control samples but using 5% NaOCl instead. All samples were incubated at 37°C, 100% humidity for 24 h.

Rhodamine is a fluorescent heterocyclic compound that, when excited with ultraviolet light, will emit a yellow color, which allows the identification of the areas where HybenX and NaOCl managed to penetrate.

After finishing the irrigation of the root canals, the teeth were sealed with glass ionomer. Then, all roots (experimental and control) were longitudinally sectioned with an Isomet Buehler micro cutter (Isomet, Buehler, Lake Bluff, IL, USA) before microscopic sections of 500 µm were made to be analyzed under a fluorescence microscope. Measurements were made in µm in a ZEN software, to determine the degree of penetration of the evaluated substances.

A Carl Zeiss Axio Vision Zoom fluorescence microscope (Zeiss, Jena, Germany) was used for the analysis of the 500-µm longitudinal sections, to determine the penetration degree of each substance.

Statistical analysis

Initially, the penetration degree (µm) of the HybenX irrigant in the upper premolar roots was determined, taking the three irrigation systems evaluated into account, such as: conventional Monoject irrigation, dynamic manual irrigation, and PUI.

A one-way ANOVA test was performed to compare the penetration degree of the HybenX irrigant in each third, taking into account the irrigation technique used. To verify the distribution of the data, the Kolmogorov-Smirnov test was used in advance.

To compare the penetration degree of each irrigant in each root third, a T-test was performed to verify if there were significant differences ($P<0.05$) using the different irrigation techniques.

Data evaluation was performed using the MINITAB version 16 statistical software Minitab Inc., State College, PA, USA).

Additionally, through fluorescence microscopy, it was possible to qualitatively identify the penetration values using the ZEN software (Carl Zeiss Microscopy GmbH, Jena, Germany) of the Carl Zeiss Axio Vision Zoom V9 microscope.

Ethical considerations

This work was carried out under the considerations of the Resolution 008430 of 1993 of the Ministry of Health of Colombia and the Declaration of Helsinki as ethical benchmarks.

Results

Table 1 shows the mean penetration values of the HybenX irrigant, found in the cervical, middle and apical thirds. The Kolmogorov-Smirnov normality test shows that the data follow a normal distribution at a significant level of 95%. The one-way ANOVA analysis shows that in the cervical third there are significant differences ($P<0.05$) between the three irrigation techniques (conventional Monoject, dynamic manual, and passive ultrasonic), the difference being even greater between conventional Monoject irrigation and PUI ($P=0.015$), the latter achieving greater depth of penetration.

Table 1. Penetration values in µm for the HybenX® irrigant (experimental groups) and the NaOCl irrigant (control groups) using 3 irrigation techniques

	Cervical Third			Middle Third			Apical Third		
	A1	B1	C1 (	A1	B1	C1	A1	B1	C1
CV	0.18	0.21	0.25	0.22	0.27	0.27	0.16	0.25	0.26
Mean (SD)	479.38 ^{a} (89.35)	618.22 ^{b} (138.23)	1015.8 ^{c} (163.07)	522.10 ^{ab} (110.85)	549.47 ^{a} (148.68)	880.08 ^{c} (221.33)	523.41 ^{a} (134.72)	619.98 ^{ab} (172.59)	769.,51 ^{b} (203.48)
	A2	B2	C2	A2	B2	C2	A2	B2	C2
CV	0,15	0,24	0,27	0,20	0,23	0,22	0,26	0,18	0,24
Mean (SD)	744.62 ^{b} (114.99)	707.95 ^{b} (170.72)	658.58 ^{c} (181.23)	516.09 ^{a} (103.43)	459.77 ^{b} (106.41)	460.09 ^{d} (101.74)	653.55 ^{d} (176.34)	606.31 ^{a} (110.61)	566.53 ^{c} (136.95)

SD: standard deviation CV: coefficient of variation. Different letters in the superscripts in the same row and the same root third represent significant differences at P<0.05. Different letters in the superscripts in the same column for the same technique represent significant differences at P<0.05; A1: Hyben X with Conventional Monoject Irrigation; B1: Hyben X with dynamic manual irrigation technique; C1: Hyben X with passive ultrasonic irrigation; A2: NaOCl with Conventional Monoject Irrigation; B2: NaOCl with dynamic manual irrigation technique; C2: NaOCl with passive ultrasonic irrigation

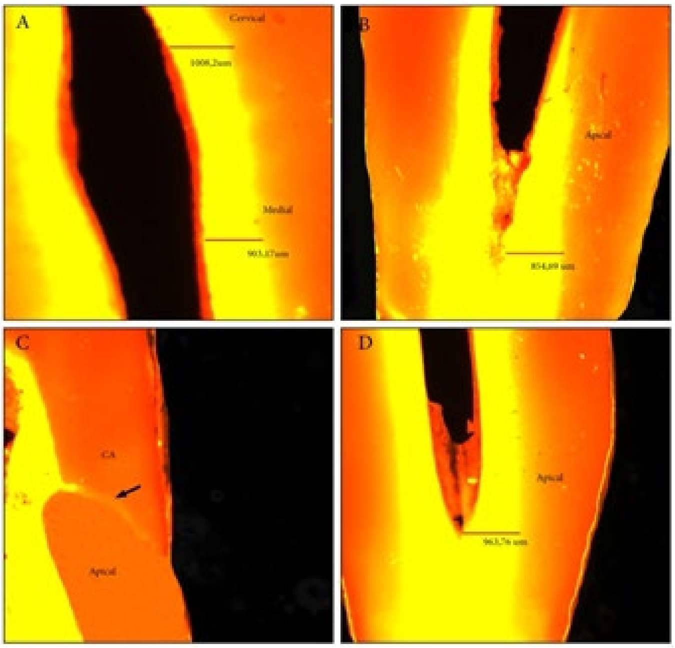

Figure 1. Level of penetration of HybenX® in group C1 using the passive ultrasonic technique by fluorescence microscopy (Rhodamine G) 100× zoom; *A)* Penetration in the cervical and middle third showing levels of 1015.8±163.07 µm and 880.08±221.33 µm respectively; *B* and *D)* Penetration level of 769.514±203.48 µm in the apical third; *C)* HybenX® penetration in the accessory canal; CA: accessory root canal

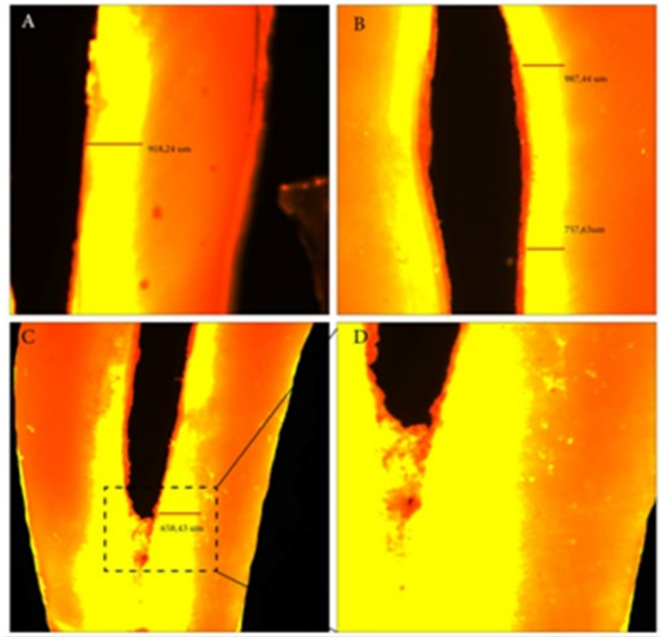

Figure 2. Level of penetration of sodium hypochlorite in group C2 using the passive ultrasonic technique by fluorescence microscopy (Rhodamine G); *A)* level of penetration in the cervical third of 653.55µm; *B)* penetration in the middle third of 606.31µm; *C)* penetration in the apical third of 566.53 µm; *D)* zoom of the apical zone showing a C-shaped root canal

In the middle third, when comparing the conventional Monoject and dynamic manual irrigation techniques, no significant differences were found, but there were differences between the conventional Monoject and passive ultrasonic techniques (P=0.0002). The best results in this third were obtained with the passive ultrasonic technique.

In the apical third, the passive ultrasonic technique continues to show better penetration results. There are no significant differences between the dynamic manual and conventional Monoject techniques (P=0.181), similarly for the passive ultrasonic and dynamic manual techniques (P=0.09), but there are significant differences between the conventional Monoject technique and the passive ultrasonic technique (P=0.005).

The results show that NaOCl presents better results than HybenX, in terms of penetration depth in the cervical third when conventional Monoject and dynamic manual techniques are used. The T-test shows that there are significant differences between NaOCl and HybenX when irrigating the cervical third using the conventional Monoject and passive ultrasonic techniques (P=0.0011), while with the dynamic manual technique, no statistical differences were found (P=0.1431). The HybenX irrigant reached higher depth values than those reached by NaOCl with the passive ultrasonic technique.

When evaluating the middle third, the HybenX irrigant showed better results than NaOCl for all techniques (conventional Monoject, dynamic manual, and passive ultrasonic). Significant differences were found between the passive ultrasonic (P=0.0026) and dynamic manual techniques, while for the conventional Monoject technique, no significant differences (P=0.1383) were found between the two irrigants.

In the apical third, HybenX irrigant reached deeper than NaOCl when irrigating with passive ultrasonic and dynamic manual techniques; however, for dynamic manual irrigation, no significant difference was found (P=0.175). With the passive ultrasonic technique, there are significant differences between the irrigants HybenX and NaOCl (P=0.0212), as with the conventional Monoject technique.

Through fluorescent microscopy and using a Carl Zeiss Axio Vision Zoom V9 microscope, qualitative and quantitative values of penetration were established, supported by ZEN software. Figures 1 and 2, show some of the analyzed replicas of the experimental groups C1 (HybenX® using the passive ultrasonic technique) and C2 (NaOCl using the passive ultrasonic technique) in detail.

Immunofluorescence photomicrographs of the control groups show the penetration of NaOCl with Rhodamine G. Figure 2 shows the penetration level of NaOCl in group C2 using the passive ultrasonic technique.

In Group C1 (passive ultrasonic technique), some aspects of great relevance concerning HybenX® penetration were identified, such as that HybenX® can penetrate accessory canals, as can be seen in Figure 1.

Discussion

In the presented work, an *in vitro* study was carried out to evaluate the penetration of HybenX® (EPIEM Medical) in the dentinal tubules of root canals, and compare it with NaOCl, taking into account the root thirds (cervical, middle and apical) and three different irrigation techniques (conventional Monoject Irrigation, dynamic manual irrigation technique and PUI).

For the root thirds with the HybenX irrigant, statistical differences were found between the three techniques. The PUI technique showed the highest levels of penetration, followed by the dynamic manual irrigation technique and finally, the conventional Monoject irrigation technique showed the lowest levels of penetration. The comparison between both irrigants at the cervical and mid-level showed significant differences between the PUI technique and the conventional Monoject irrigation technique, the first one showing a higher level of penetration. Finally, at the apical level, statistical differences were found between the PUI and dynamic manual irrigation techniques, the latter being the one with the lowest level of penetration.

Since 5% NaOCl is an excellent irrigant, it was chosen as the control substance, due to its antimicrobial action and capacity to penetrate dentinal tubules [25]. On the other hand, HybenX® is a concentrate of a free sulfate aqueous mixture and sulfated aromatics, specifically hydroxy-benzene-sulfonic acid, hydroxy-methoxy-benzene-sulfonic acid, and sulfuric acid [17, 26]. Premolars were selected because they have two homologous roots with similar structural and morphological configuration [27].

The hydroxy benzenes present in HybenX are keratolytic, while the sulfonates and sulfuric acid are hygroscopic and denaturants, giving HybenX a drying capacity, which allows the precipitation and collapse of organic material, thus destroying the biofilm of the oral cavity and dental surfaces. This antibacterial capacity can also be used as an intracanal irrigant, and according to this study, HybenX has the ability to penetrate all root thirds [21, 22, 28].

The results obtained in this investigation show that HybenX® had an average superior value of penetration when compared with NaOCl in all thirds, however, we could identify that the PUI technique, promoted and enhanced the highest levels of penetration in the dentinal tubules. In the same way, Galler *et al.* determined that with the PUI technique a greater depth of penetration is achieved in the apical third [29]. Despite this, other authors like Mohammadi *et al.* state that the superiority of ultrasonic irrigation is still controversial [30].

Other studies have tested the penetration level of different irrigants. Ling Zou *et al.* reported that the maximum penetration depth of NaOCl was achieved when it was used in a 6% concentration, as for this work, 5% NaOCL was used and even though it penetrated in all radicular thirds, it showed lower levels of penetration when compared to HybenX® [16].

A different approach was conducted by Ye *et al.* to assess the anti-biofilm efficacy of different irrigants, including HybenX®, against *in-situ Enterococcus faecalis* biofilm in root canals, isthmuses, and dentinal tubules. Their study concluded that HybenX® showed appreciable biofilm bacteria-killing ability in the root canal system due to its desiccating action; however, it was inferior when compared to 6% NaOCl [19].

Comparing the present study with similar ones is challenging as there is little literature on the subject. Some investigations have focused on evaluating the effect of HybenX® on root canal bleeding during endodontic treatment, showing its capacity to dry the root canal in the presence of serum-hematic blood or exudates [31]. On the other hand, Ballal *et al.* stated that HybenX® can be considered a promising irrigant for root canal treatment of infected teeth, although its antimicrobial efficacy and ability to remove the smear layer were shown to be higher in the cervical and middle thirds. In contrast to this, in the presented study, HybenX demonstrated its ability to highly penetrate into dentinal tubules, even those in the apical third [32].

Conclusions

Based on this *in vitro* study, HybenX® has a higher penetration in the root canal system in comparison to 5% NaOCl, especially when the passive ultrasonic technique is used.

HybenX® proved its efficacy in the cervical, middle, and apical thirds with all the evaluated irrigation protocols, therefore, according to the presented results, it may be a useful option for root canal irrigation in endodontics.

Conflict of Interest: 'None declared'.

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Please cite this paper as: Padilla-Correales L, Llanos-Torres J, Pérez-Quiñones Z, Diaz-Caballero A, Covo-Morales E. Assessment of Dentinal Tubules Penetration by Hyben X ® Delivered Into the Root Canal System as an Irrigant, An *In Vitro* Study Iran Endod J. 2023;18(1): 53-8. *Doi: 10.22037/iej.v18i1.33114.*