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ORIGINAL RESEARCH

ANTIMICROBIAL AND STRUCTURAL EFFECTS OF DIFFERENT IRRIGATION SOLUTIONS ON GUTTA-PERCHA CONES*

Farklı Kök Kanalı Yıkama Solüsyonlarının Gütaperka Üzerindeki Yapısal ve Antimikrobiyal Etkileri

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ABSTRACT

Purpose: *To evaluate the alterations on the surface of gutta-percha cones (GPCs) on exposure to the different irrigation solutions and their possible antibacterial effect against Enterococcus faecalis. (E. faecalis)*
Materials and Methods: *Disinfection ability of different solutions (5.25% sodium hypochlorite, 2% chlorhexidine, 1% peracetic acid, and QMix) were tested with 96 GPCs and the time of exposure to each solution was 5 and 10 minutes, respectively. GPCs used in this study were contaminated with E.faecalis. After disinfection, GPCs were placed in tubes containing the medium and incubated at 37˚C for 7 days. All tubes were visually checked for turbidity at 24-hour intervals. About 92 new GPCs were analyzed by means of SEM/EDS to assess the topography and chemical elements present on their surface. The data generated was analyzed using Pearson chi-square test, p<0.05.*
Results: *There were no significant statistical differences in disinfection quality between the irrigation solutions used on GPCs contaminated with E. faecalis (p>0.05). SEM/EDS analyses showed no alteration in the superficial features of GPCs after treating with various irrigation solutions.*
Conclusion: *QMix was found to be an effective agent for rapid disinfection of GPCs as well-known irrigation solutions. Irrigation solutions were found to have sterilized the GPCs after both 5 and 10 minutes of exposure.*

Keywords: *Antimicrobial agents, Energy Dispersive X-ray Spectroscopy, Gutta-Percha cones*

ÖZ

Amaç : *Farklı kök kanal yıkama solüsyonlarının Enterococcus faecalis (E. faecalis) ile kontamine olan gütaperka konları (GPK) üzerindeki antibakteriyel ve yapısal etkilerinin incelenmesidir.*
Gereç ve Yöntem: *Antimikrobiyal etkinliğin tespitinde 96 adet GPK kullanılmıştır. GPK, E.faecalis ile kontamine edildikten sonra, % 5.25'lik sodyum hipoklorit,% 2'lik klorheksidin,% 1'lik perasetik asit ve QMix solüsyonlarında 5 ve 10 dakika boyunca bekletilmişlerdir. Bu sürenin sonunda GPK besi ortamı içeren tüplere konularak 7 gün süreyle 37°C'de inkübe edilmiştir. Tüpler 24 saat arayla bulanıklık açısından kontrol edilerek bulgular kaydedilmiştir. Solüsyonların GPK üzerinde oluşturdukları yapısal ve kimyasal değişiklikler ilk çalışmada kullanılan konlardan bağımsız 92 adet konun SEM/EDS aracılığıyla incelenmesiyle tespit edilmiştir. Veriler Pearson ki-kare testi ile %95 güven aralığında karşılaştırılmıştır.*
Bulgular: *Solüsyonlar arasında bulanıklık varlığı değerlendirmesi sonucu elde edilen antimikrobiyal etkinlik ve SEM/EDS ile ulaşılan yapısal değişiklik açısından anlamlı bir farklılık bulunamamıştır (p> 0.05).*
Sonuç: *Yeni bir yıkama solüsyonu olan Qmix de GPKın dezenfeksiyonunda etkin bir ajan olarak bulunmuştur. Yıkama solüsyonları tüm ölçüm zamanlarında GPKnı sterilize etmişlerdir.*

Anahtar kelimeler: *Antimikrobiyal ajanlar, enerji dağılımlı x-ışını spektroskopisi, gütaperka*

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Introduction

The success of endodontic therapy lies in thorough disinfection and the use of aseptic techniques. Care must be taken during obturation to avoid root canal cross-contamination by the instruments or filling materials. Even though gutta-percha cones (GPCs) are manufactured under aseptic conditions, they can get contaminated during the storage process, (1, 2) while handling, by aerosols, and physical sources etc. Therefore, before inserting GPCs into the root canal, disinfection is required. Because of their thermoplastic properties, the conventional heating method cannot be used to sterilize them (3). Chemical agents such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), peracetic acid (PAA) etc. are used for rapid disinfection of the cones (4-6). The most appropriate disinfectant should provide rapid disinfection without modifying the structure of the cone.

NaOCl is the most widely used disinfecting agent in the endodontic therapy for irrigation and cone disinfection. It is an effective antimicrobial agent, but its effectiveness depends upon its concentration and the time of exposure (4). 1-minute immersion of the gutta-percha cone in a 5.25% NaOCl solution has been shown to be quite effective against a variety of gram-positive, gram-negative, and spore-forming microorganisms (2,5).

CHX is used as an irrigation solution because of its antimicrobial efficiency and substantivity. A study observed that 2% CHX solution was effective in decontaminating gutta-percha after 10 minutes of exposure (7). The physical changes that occur in the GPCs after chemical sterilization have been reported. The efficacy of a disinfectant depends on sufficient length of treatment time and an effective concentration of the disinfectant. As a strong oxidizing agent, 5.25% NaOCl is able to cause extreme topographic alterations in the cones, which results in aggressive deterioration (8). However, 2% CHX did not change gutta-percha cone properties following exposure for up to 30 minutes, suggesting that this substance is less detrimental to the structure of gutta-percha (8).

Peracetic acid (PAA), since its introduction in the market, has been indicated for high-level disinfection and sterilization of the hospital equipment and devices. It has also been studied in the disinfection of acrylic based resins in dentistry. PAA is peroxide and acts rapidly against all microorganisms even at low concentrations (6, 9). A recent study observed that 1% PAA was effective in decontaminating the gutta-percha (6). However, there are no recent studies being done regarding the surface changes in gutta-percha after disinfection with PAA.

QMixTM 2in1 (DENTSPLY Tulsa Dental Specialties, Tulsa, OK, USA), a novel irrigating solution, has been introduced to do both i.e. remove smear layer and kill the bacteria. QMix is a clear solution, ready to use with no chair-side mixing. It contains EDTA, CHX, a detergent and water (10). It has been found to be effective against bacterial biofilms (11). The literature shows no studies regarding the disinfection and the surface changes in gutta-percha after being immersed in QMix.

Therefore, the objective of this study was to compare the effectiveness of QMix, 5.25% NaOCl, 2% CHX, and 1% PAA solutions to disinfect the GPCs contaminated by Enterococcus faecalis, after the immersing in these solutions for 5 and 10 minutes. Surface changes and the alterations in the chemical compositions of GPCs after disinfection were determined by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS).

Materials and Methods

Ninety-six GPCs ISO size 80 GP (Dia Dent) were used to test the disinfection efficacy of different irrigation solutions (5.25% NaOCl, 2% CHX, and QMix and 1% PAA) and the time of exposure to each solution was 5 and 10 minutes, respectively. *E. faecalis* ATCC 29212 as a microbial suspension containing approximately 108 CFU/mL in trypticase soy broth was used to contaminate the surface of GPCs. Sample packets were opened under aseptic conditions. About 80 samples of gutta-percha were selected and contaminated by immersing each pellet for 30 minutes in the bacterial suspension. After immersion, the cones were transferred to a sterile filter paper and allowed to dry in a dry heat sterilizer for 10 minutes at 37°C. Then, for each group, 10 contaminated cones were immersed in separate tubes that contained 2 mL of the chemical agents for 5 or 10 minutes. Experimental groups were, Group 1: treated with 5.25% NaOCl; Group 2: treated with 2% CHX; Group 3: treated with QMix; Group 4: treated with 1% PAA. Positive controls consisting of 8 GPCs, were contaminated with *E. faecalis*, and then immersed in sterile water. Negative controls consisting of 8 uncontaminated GPCs were also immersed in the respective disinfectant solutions.

The cones were then rinsed with 10 mL of sterile

distilled water to neutralize any residual effects of the test agents. Subsequently, the cones were individually transferred to the test tubes containing 10 mL Mueller-Hinton broth and incubated at 37˚C for 7 days. All test tubes were observed at 24-hour intervals and visually checked for turbidity, signifying microbial growth, by holding samples to light. Samples from each experimental and control group were randomly chosen, plated, incubated at 37°C, and checked for growth at 3rd and 7th day. If growth was present, colony morphology and Gram staining confirmed the bacteria. The data obtained was evaluated using the Pearson chi-square test with a P value set at < 0.05.

SEM Findings for GPCs

Separately, ninety-two extra GPCs were selected and subjected to the same disinfecting protocol. Then treated and untreated GPCs were used for SEM and EDS analysis. The cones were mounted individually on aluminum stubs, and the surfaces were analyzed by SEM (QuantaTM 450 FEG, FEI, Oregon, USA) and EDS respectively for the topography and the chemical elements present. Three photographs (x1.500 magnification) of each sample were taken, and an area was randomly chosen to be evaluated using EDS.

Results

Heavy turbidity occurred in all positive controls, and no growth was detected in negative controls. *E. faecalis* growth was confirmed by culturing and Gram staining. The statistics revealed that there was no statistically significant difference between irrigation solutions in the disinfection of samples contaminated with *E. faecalis* for both 5 and 10 minutes (Table 1). All irrigation solutions were found to sterilize the GPCs at all tested specified time points.

Figure 1, 2 and 3 illustrates SEM images and EDS spectrum. SEM analyses showed no alteration in the superficial features of the cones after the immersion in 5.25% NaOCl, 2% CHX, QMix and 1% PAA for both 5 and 10 minutes (Figure 1 and 2). EDS analyses showed no changes in the composition of the cones after exposure to the respective agents, except PAA group. According to EDS analyses the ratio of the component carbon (C) of gutta-percha increased after disinfected with PAA for 5 and 10 minutes

Table 1. *Comparison of disinfection of gutta-percha cones with various disinfectants at different time points: the percentage of samples showing turbidity positive (TP) and turbidity negative (TN) samples.*

Group	Disinfectants	N	Time			
			5min		10min	
			TN	TP	TN	TP
1	5.25% NaOCl	10	70%	30%	80%	20%
2	2% CHX	10	80%	20%	100%	0%
3	QMix	10	60%	40%	70%	30%
4	1% PAA	10	30%	70%	80%	20%

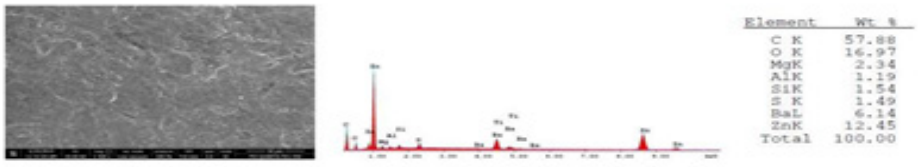

Figure 1. *Scanning Electron Microscopy (SEM) images and Energy Dispersive Spectroscopy (EDS) spectrum of untreated gutta-percha cones*

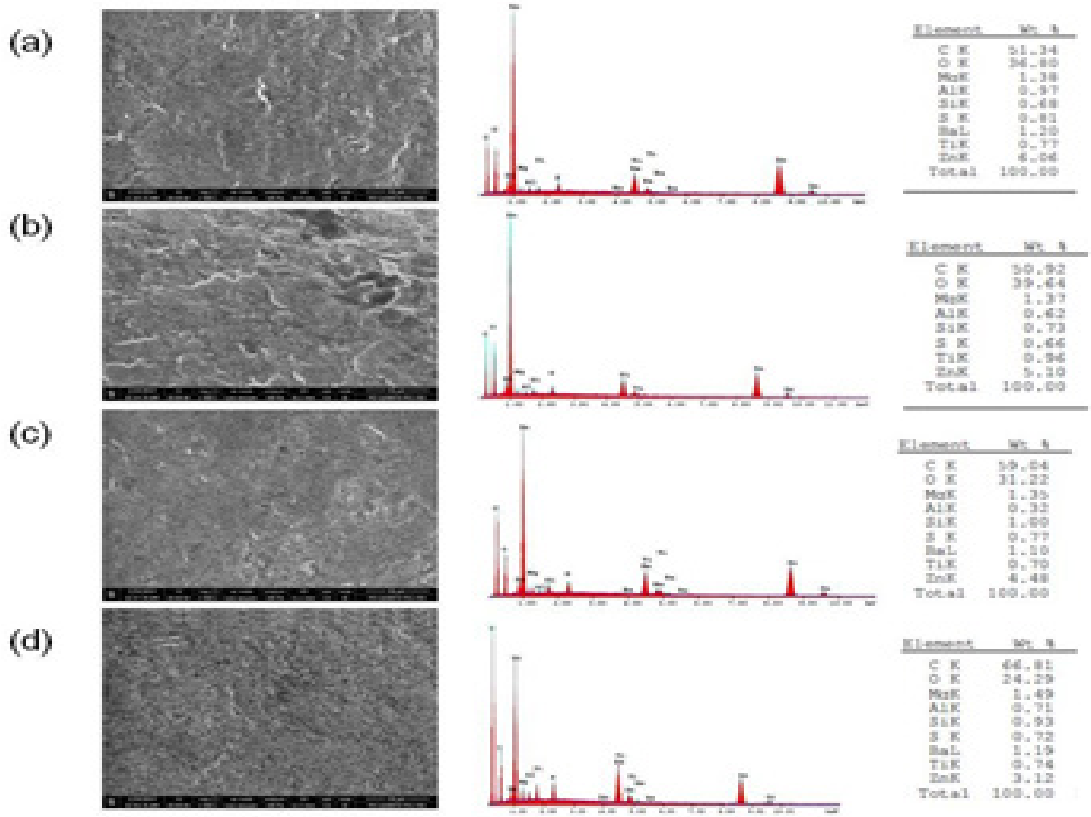

Figure 2. *Scanning Electron Microscopy (SEM) images and Energy Dispersive Spectroscopy (EDS) spectrum of gutta-percha cones immersed in (a) 5.25% NaOCl (b) 2% CHX (c) QMix and (d) 1% PAA for 5 min.*

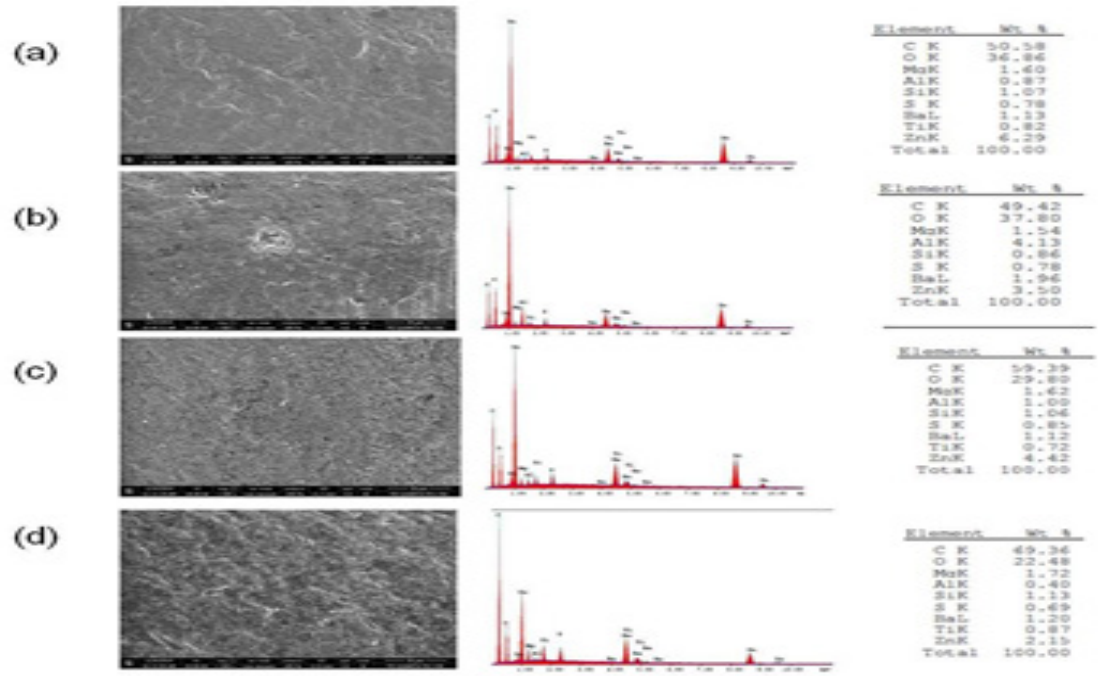

Figure 3. *Scanning Electron Microscopy (SEM) images and Energy Dispersive Spectroscopy (EDS) spectrum of gutta-percha cones immersed in (a) 5.25% NaOCl (b) 2% CHX (c) QMix and (d) 1% PAA for 10 min.*

Discussion

There is a risk of contamination of GPCs due to handling, by aerosols and physical sources during the storage process. GPCs cannot be sterilized by heat. Therefore, a chemical agent should be used in the routine endodontic practice to sterilize the GPCs.

Sterilization procedures with contact between the gutta-percha and the chemical agents can cause extreme surface alterations in the cones. GPCs present irregular regions on their surfaces and root canal sealers are known to fill these irregularities. However, these gap areas could create a large interface with root canal walls, resulting in the leakage of molecules that will serve as nutrients for the microorganisms present in the root canal system (12). Even though atomic force microscope has been introduced as a tool for evaluating topographic and quantitative data (8, 12), SEM has, for long been the standard tool for investigation of the surface characteristics of the GPCs (13, 14). The present study evaluated the changes in the surface of cones after disinfection procedures by means of SEM.

E. faecalis, are facultative gram-positive cocci shaped bacteria that were chosen as the test bacterial contaminant because it has been shown to be a predominant bacterial species in persistent apical periodontitis (15) and it is the most common bacteria associated with the post-treatment infection of the root canal system (16, 17). NaOCl and CHX are the two common endodontic irrigants and have been used in some studies with varying concentrations for chemical disinfection of root canal filling materials (4, 5, 18). Previous studies have shown that the antimicrobial activity of NaOCl is related to its concentration, and higher concentrations have been shown to take less time to inhibit bacterial growth than lower concentrations (1, 19). A 5.25% NaOCl solution has been routinely used as an effective chemical agent for rapid decontamination of GPCs before clinical use, as it kills microorganisms in only 1-minute of exposure (20). Gomes *et al.* (4) reported that 1% NaOCl eliminated *E. faecalis* and *C. albicans* in 20 minutes, but 5.25% eliminated these microbes in 45 seconds. However, no general agreement exists regarding the optimal time of action for the decontamination of gutta-percha by 2% CHX, which usually ranges from 1 minute to more than 10 minutes (20). In the present study, the effects of irrigation solutions on the GPCs structure were investigated at exposure times of 5 and 10 minutes, respectively. The findings of the present study revealed that 5.25% NaOCl and 2% CHX have similar antimicrobial performance against *E. faecalis* for both 5 and 10 minutes. SEM analyses showed no topographic change on the surface of the GPCs for NaOCI and CHX. Additionally, there was no difference in element ratios between the different GPC exposure time points in both solutions. However, Valois *et al.* (8) and Isci *et al.*(21) detected surface alterations after short periods of exposure to 5.25% NaOCl and 2% CHX. Such difference in the findings is possibly due to the different techniques used, while Valois *et al.* (8) and Isci *et al.* (21) used atomic force microscopic analysis, SEM was used for this study.

QMixTM 2in1 a novel endodontic irrigant for smear layer removal with added antimicrobial agents. It contains EDTA, CHX and a detergent. In previous studies, it was observed that QMix proved to be an effective irrigant against *E. faecalis* (10, 22). In this study it was found that QMix was equally effective as 5.25% NaOCl, 2% CHX, and 1% PAA against *E. faecalis* due to the substantial antimicrobial efficiency of CHX. According to SEM analyses no topographic change on the surface of GPCs were found and EDS analyses showed no significant changes in the composition of the cones after immersion in QMix.

The results of our study revealed that 1% PAA was effective against *E. faecalis* for both 5 and 10 minutes. This result is in line with a previous study (6). Subha *et al.* (6) demonstrated that PAA can be used in the rapid disinfection of GPCs, 1-5 minutes immersion was adequate to disinfect them. However, the literature lacks data regarding the action of PAA on the topography of GPCs. According to the results of the present study no topographic changes on the surface of GPCs were found after PAA disinfection. However, EDS analyses showed that the ratio of the component Carbon (C) was increased after immersion in 1% PAA at both time points. PAA is produced by a reaction between hydrogen peroxide and acetic acid. When PAA dissolves in water, it disintegrates into hydrogen peroxide and acetic acid, which further breakdown into water, oxygen and carbon dioxide (23). Thus, it can be concluded that due to this reason, the ratio of C of GPCs might increase after being disinfected with PAA. Further studies should evaluate the effect of PAA on changes in the physical properties of the gutta-percha for clinical relevance.

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None declared

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