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Evaluating the Antibacterial Activity of White Portland Cement Mixed with various sorts of Sodium Hypochlorite. ¹Saleh Al Kurdi, PHD in Pediatric Dentistry, Faculty of Dentistry, Damascus University, Damascus, Syria ²Nada Bshara, Professor in Pediatric Dentistry, Faculty of Dentistry, Damascus University, Damascus, Syria ³Khalil Khalouf, Masters in Pediatric Dentistry, Faculty of Dentistry, Damascus University, Damascus, Syria **Corresponding Author:** Saleh Al Kurdi, PHD in Pediatric Dentistry, Faculty of Dentistry, Faculty of Dentistry, Damascus, Damascus, Damascus, Syria

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Abstract

Introduction: Portland cement (PC) is recommended as a cheap alternative to MTA. NaOCl improved the antibacterial activity of MTA. This study was designed to evaluate the antibacterial activity of WPC mixed with various sorts of NaOCl.

Materials and Methods: Kirby Bauer disk diffusion test was performed on 35 Petri dishes (n=7), E. faecalis was inoculated over blood sheep agar, then a well of 6*4 mm. was punched in each of them and filled with one of five studied materials: PC + sterile water (control), PC + NaOCl 2.2% liquid, PC + NaOCl 4% liquid, PC + NaOCl 2.2% gel and PC + NaOCl 4% gel. After 24 h. incubation in 37°C. Inhibition zones were measured around studied materials. Finally, One Way ANOVA test followed by LSD post-hoc test were performed on SPSS 17. Package.

Results: Inhibition zones were seen around all groups except control one. One Way ANOVA demonstrated a significant difference between the groups P<0.05. LSD

expressed a significant difference between all groups except PC + NaOCl 2.2% gel () with both PC + NaOCl 2.2% liquid () and PC + NaOCl 4% liquid (). PC + NaOCl 4% gel () was superior to all groups.

Conclusion: Mixing white Portland cement with sodium hypochlorite improved its antibacterial activity toward E. faecalis. Gel types of sodium hypochlorite were superior to liquid ones of the similar concentration in the mixtures.

Keywords: Antibacterial, E. faecalis, NaOCl, Portland cement, Sodium hypochlorite.

Introduction:

Portland cement (PC) has been suggested as a cheaper alternative to MTA as a dental material with multiple usages in dental pulp management for both primary and permanent dentitions $^{1-3}$. It was proved to have same compounds existing in MTA except of the presence of bismuth oxide in the later one $^{4-7}$. In addition to that, PC of various origins proved to be biocompatible $^{8-11}$.

Corresponding Author: Saleh Al Kurdi, ijdsir, Volume – 4 Issue - 2, Page No. 323 - 329

Enterococcus faecalis is associated with pulp system management secondary failure, they are gram positive, anaerobic and highly resistant to antibiotics microorganisms, that can withstand an alkaline pH up to 11.5 enduring multiple types of intracanal medicaments and irrigant solutions ^{12–17}. Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are appropriate intracanal irrigant solutions used in eliminating the amount of E. faecalis during endodontic treatment, regarding NaOCl as the gold antibacterial standard in dentistry ¹⁸⁻²¹. NaOCl is used in different concentrations especially 5.25%, 2.2% and <1%, also it is available in various viscosities ranging from liquid to gel types, preserving its powerful antimicrobial activity in all its variations ^{21–25}.

When MTA comes into contact with NaOCl during final irrigation it allows complete setting of the material without considerable effect on its physical properties ²⁶. It was suggested that mixing MTA with NaOCl reduces setting time considerably, retains its mechanical properties without a cytotoxic reflection ^{27–29}. Furthermore, additives such as NaOCl and CHX improved mineral trioxide aggregate antimicrobial abilities ³⁰.

PC is suggested to be an inexpensive alternative to MTA, so this study was designed to evaluate the antimicrobial activity of white Portland cement when blended with sodium hypochlorite, in addition to the effect of both the concentration and viscosity of the solution used in preparing the mixture.

Materials and Methods:

Four variants of sodium hypochlorite (NaOCl) with white Portland cement (WPC) were examined: NaOCl 2.2% gel/PC, NaOCl 2.2% liquid/PC, NaOCl 4% gel/PC, NaOCl 4% liquid/PC (Table 1). In addition to sterile water/PC as a positive control group. All blended in ratio of 0.4g PC to 0.12ml. of gels and 10ml. of liquids determined by normal consistency of Vicat's Apparatus (H-3050 Vicat Consistency Apparatus/ Humboldt Mfg. Co., Illinois 60176 U.S.A.).

Material	Production		
White	Royal White Portland Cement C150		
Portland	White. El Minya Cement Co./ 7 Moustafa		
cement	Refaat St. – Complex 1135/ Sheraton		
	Buildings, Cairo, Egypt.		
NaOCl	Clorox® Bleach Whitening Gel		
2.2% gel	CX0063HU/ Broadway Oakland CA		
	94612, USA		
NaOCl	Clorox® Regular-Bleach 5813-100/		
2.2% and	Broadway Oakland CA 94612, USA		
4% liquid			
NaOCl 4%	LET'S CLEAN Concentrated Chlorine		
gel	Gel/ D.T.I.C Trade & Industry Company/		
	Damascus – Syria Com Reg 65004 – Ind		
	Reg 17 -36 -55		

Table 1: Showing the tested materials.

Enterococcus faecalis of necrotic pulps was isolated, and the most resistant phenotype was determined using BD Phonex instrument and antibiotic susceptibility disks (BD PhoenixTM 100 Automated Microbiology System 448022/ Becton, Dickinson and Co., Maryland 21152 USA). E. faecalis was subcultured on sheep blood agar medium (Merk, Darmstandt, Germany) and incubated at 37°C. For 24 hours (Bacteriological Incubator 6640-01-071-6596/National Appliance Heinicke Co. Tulatin, USA) prior to test performance. Thirty five Petri dishes (60mm diameter) with sheep blood agar medium to perform Kirby-Bauer disk diffusion test of E. faecalis sensitivity were used (7 dishes for each group). Bacterial density was determined at 0.5 of McFarland standard (1.5X10^8 CFU/mL) using PhonexSpec (BD PhoenixSpecTM nephelometer 440910/ Becton, Dickinson and Co., Maryland 21152 USA). After which a sterile swab was dipped into inoculums tube, then the swab was firmly

^{age}32

pressured into the side of the tube to remove the excess fluid. Bacteria was inoculated over the agar by streaking the swab three times up-down the dish and rotating it in 60° between each stroke, then they were left at 37° C. For 20 minutes to dry. At the center of each dish a well of 6mm diameter and 4mm depth was punched in the agar 21, then the tested material was mixed and freshly transformed to fill its hole. To insure medicaments diffusion through the agar medium dishes were kept at room temperature for an hour before being incubated at 37°C. Under aerobic conditions for 24 hours. Inhibition zones surrounding each medicament were observed by clearance of bacterial colonies around the well. Thereafter the circle was measured with a digital caliper (WEN 10761 Digital Caliper), a higher inhibition zone diameter meant a higher antibacterial activity of the tested mixture (Figure1). Finally, descriptive statistics and statistical analysis were accomplished with SPSS 17 (Statistical Package for Social Science, SPSS, version 17.0, SPSS, Chicago, IL, USA). One Way ANOVA test was carried out, followed by LSD post-hoc test at the confidence level of 95%.



Figure 1: Shows Kirby-Bauer disk diffusion susceptibility test for E. faecalis: (A) control group with microbial growth over the Petri dish; inhibition zone is seen around studied mixtures,(B) NaOCl 2.2% liquid/PC, (C) NaOCl 2.2% gel/PC, (D) NaOCl 4% liquid/PC and (E) NaOCl 4% gel/PC; (F) NaOCl 4% gel/PC mixture with irregularly extended inhibition zone measuring around 18mm.

Results: NaOCl4% gel/PC has shown the highest inhibition zone measuring 14.77±2.5 in comparison to the other studied groups. On the contrary the control group presented with an obvious bacterial growth without any sensitivity toward the mixture (figure2). Kolomogorov-Smirnov normality test offered a normal distribution of the data, so Oneway ANOVA test was performed and showed a significant difference between the groups (P. 000).



Figure 2: A Histogram representing the means and standard deviations of inhibition zone diameter of each group, gels mixtures of NaOCl/PC of each concentration shows top performance over liquids of the same concentration in inhibiting the growth of E. faecalis.

To compare paired groups LSD multiple comparisons posthoc test was completed. LSD displayed a significant difference between the control group and other groups in favor of the studied ones. By contrast NaOCl4% gel/PC was significantly better than other studied groups. However NaOCl2.2% gel/PC acted similar to NaOCl4% liquid/PC group without any significant difference between them (table2).

Multiple Comparisons			
Inhibitions zone Diameter for Entercoccus Faecalis			
LSD			
(I) Group	(J) Group	Sig.	
	NaOCl 2.2% gel/PC	.000	
Control	NaOCl 2.2%	000	
Control	liquid/PC	.000	
	NaOCl 4% liquid/PC	.000	
	NaOCl 4% gel/PC	.000	
	NaOCl 2.2%	056	
NaOCl 2.2%	liquid/PC	.050	
gel/PC	NaOCl 4% liquid/PC	.104	
	NaOCl 4% gel/PC	.000	
NaOCl 2.2% liquid	NaOCl 4% liquid/PC	.001	
/ PC	NaOCl 4% gel/PC	.000	
NaOCl 4% liquid /	NaOCI 4% gel/PC	002	
PC		.002	

Table 2: Representing the results of LSD multiple comparisons post-hoc test of inhibition zones formed around the studied mixtures of PC, NaOCl 2.2% liquid/PC and NaOCl 2.2% gel/PC are on the edge of significance but without enough evidence to prove it. It is obvious that gels has shown a better action in the mixtures at inhibiting E. faecalis comparing to liquids of the same concentration, even the gel of lower concentration acted similarly to the liquid of the higher one.

Discussion

All studied samples showed diffusion zones, yet only the sterile water/PC group samples guarded against the creation of inhibition zones. Portland cement and MTA did not implicate antibacterial effects for Staphylococcus aureus, Enterococcus faecalis and Bacillus subtilis ^{7,31}. Agar diffusion test is appropriate only for diffusive materials to determine their antibacterial activity, the limitations of this test include its inability to determine the

minimal concentration of the tested material to be an © 2021 IJDSIR, All Rights Reserved

effective inhibitor, and its inability to locate whether the material is bactericidal or bacteriostatic ³² those limitations may affect its reliability in determining some dental cements antibacterial characteristics.

It appears that adding NaOCl promoted antibacterial abilities to WPC. PC has an alkaline range of pH between 9.9 - 10.9 33,34 . Guerreiro-Tanomaru found PC to be affective at 10.2 pH value for E. faecalis without any additional antimicrobial impact of radio-pacifying additives 35 . Thanks to E. faecalis membrane durability, some strains were able to grow at an initial pH of 4 -11 36 . It was recognized that alkaline additives to PC and MTA increased their pH 37 , which may increase their antibacterial power.

When NaOCl was used as gel in the mixtures it sounded superior to its identical concentration of the liquid ones. Moreover, NaOCl gel off 2.2% manifested similar action to the 4% liquid and toped the liquid of 2.2%. On the top of that, NaOCl gel 4% outstanded all other mixtures against E. faecalis. As far as it might be recognized that mixing WPC with gels may maintain microorganism's clearance for a longer period of time than liquids do. It was observed that NaOCl gels at concentrations between 2.25% and 5% were as effective as the liquid ones in their antibacterial efficiency 22,24. Additionally, El Sayed suggested the use of NaOCl gel as an intracanal medicament between endodontic sessions for canal disinfection²¹. Then, higher viscosity of NaOCl had no side effects on its pH values and preserved its abilities in eliminating pathogenesis ³⁸. Besides that, the mixture of MTA with NaOCl 3% demonstrated similar bicompabitibale properties with the traditional one of it ³⁹. It seems like that the white Portland cement mixture with sodium hypochlorite 2.2% might be an acceptable blend with fair inhibition power towered E. faecalis.

Conclusion

Mixing white Portland cement with sodium hypochlorite added antibacterial capacity for E. faecalis. It seems that gel variation is superior to liquid in antibacterial properties when used for mixing of Portland cement. The sodium hypochlorite 4% gel surpassed all other groups in eliminating microorganism's growth around studied mixtures, yet 2.2% gel appears to be good enough allowing it to set in a lower concentration and an appropriate antibacterial activity. The mixtures need to be studied deeper prior of clinical usage.

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