Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 993-997 | ISSN:2251-6727

Rapid Decontamination of Gutta-Percha Points- An in Vitro Comparative Study between Different Auxiliary Chemical Substances

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(Received: 07 January 2024 Revised: 12 February 2024 Accepted: 06 March 2024)

KEYWORDS

Chlorhexidine; Gutta-percha disinfectants; Octenidine hydrochloride; Sodium hypochlorite; Zinc Oxide Nanoparticle

ABSTRACT:

Introduction: Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Vitae sapien pellentesque habitant morbi tristique senectus et netus. Dignissim cras tincidunt lobortis feugiat vivamus at augue eget arcu. At risus viverra adipiscing at in. Cras semper auctor neque vitae tempus quam. Sed cras ornare arcu dui. Turpis massa sed elementum tempus. Risus commodo viverra maecenas accumsan lacus vel facilisis volutpat est. Dictum non consectetur a erat nam at. Lorem mollis aliquam ut porttitor leo. Egestas sed sed risus pretium quam.

Objectives: To compare and evaluate the efficacy of 3% Sodium Hypochlorite, 2% chlorhexidine, 0.1% Octenidine Dihydrochloride and 10 h milled ZnO nanoparticle in Disinfection of Gutta Percha Cones against *E. faecalis* in vitro

Methods: A total of sixty gutta-percha cones taken from a freshly opened sealed packet of size 70 and 2% taper were used. They were divided into various groups according to the type of disinfectant. The GP cones were contaminated with microbial suspension of E. faecalis and then decontaminated by immersing in the different disinfectant solutions for 1 min. GPs were transferred to test tubes containing thioglycollate media and incubated at 37°C for 7 days. After 7 days, the media was transferred to a petridish containing brain heart infusion (BHI) agar and incubated for 48 h aerobically at 37°C and the colony forming units (CFU) were counted with digital colony counter. Descriptive data was explored in terms of mean and standard deviation. The data was statistically analyzed by was Kruskal Wallis Test using SPSS version 25.0 with significance level kept at $p<0.05$.

Results: The least number of colonies was seen for 5.25% NaOCL while the maximum mean number of colonies was seen for positive control group.0.1% octenidine dihydrochloride also showed non-significant difference with 5.25% NaOCl.

Conclusions: It was observed that immersion of gutta-percha into 5.25% NaOCL for one minute was found the most effective method to eliminate the selected microorganisms followed by 0.1% octenidine dihydrochloride.

1. Introduction

The utmost motive in root canal treatment is complete bacterial removal [1]. The most widely utilized material for root canal filling has been gutta-percha cones [2].

Thorough cleaning and disinfection are essential for the success of endodontic therapy, Obturation must be done carefully to prevent re-infection by the instruments or filling materials [3]. Even though gutta-percha cones (GPCs) are made in an aseptic environment, they are nevertheless susceptible to contamination from physical

Journal of Chemical Health Risks
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JCHR (2024) 14(2), 993-997 | ISSN:2251-6727

sources, aerosols, handling, and storage processes, hence decontamination is necessary before placing GPCs inside the root canal. The traditional heating approach like moist or dry heat cannot be used to sterilize them due to their thermoplastic characteristics [4,5]. Thus cold sterilizations with various disinfectants has to be used.

Different chemical solutions can be used as disinfectants such as sodium hypochlorite, chlorhexidine, ethyl alcohol, glutaraldehyde, and povidine iodine. An ideal disinfectant should be one that can be used routinely in the dental office with a faster disinfection action without modifying the structure of the cone [6]

NaOCl has the ability to dissolve organic matter and eradicate bacteria is well established. However, concerns regarding its cytotoxicity and potential for tissue damage have led to the exploration of alternative irrigation solutions [7]. It is commonly used to sterilize GP, but due to strong oxidizing effect, it changes the structure of GP [8]. The bond between sealers and canal walls after obturation is hampered by crystal deposition on the GP surface and inside the canals, which results in microleakage [9,10].

Chlorhexidine, a cationic bis-biguanide, is clinically used as an antimicrobial agent. It acts by adsorbing onto the microorganism's cell wall and causing intracellular component leakage. It has antibacterial properties with a broad spectrum and relatively low toxicity [11].

Among the various methods, the widely accepted method for quickly decontaminating gutta-percha is to immerse it in a 5.25% NaOCl solution for one minute. This method is considered the best practice [12,13]. In the field of endodontics, chlorhexidine (CHX) is gaining popularity due to its effectiveness in combating microbial infections [14] as well as an effective gutta percha disinfectant and showed no surface alterations [15].

Octenisept is an antiseptic product designed for various applications, including treating skin burns, disinfecting wounds, and as a mouth rinse. It is composed of octenidine hydrochloride and phenoxyethanol [11] which effectively targets both gram-positive and gram-negative bacteria, fungi, and various viral species [16].

ZnO-NPs exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size leads to enhanced particle surface reactivity [17]. Moreover, it has been reported as a potent applicant in medicine for infectious and non-infectious diseases.

2. Objectives

The objective of the research was to compare and assess the efficacy of 3% Sodium Hypochlorite, 2% chlorhexidine, 0.1% Octenidine Dihydrochloride and 10 h milled ZnO nanoparticle in Disinfection of Gutta Percha Cones against *E. faecali*s in vitro.

3. Methods

A total of sixty gutta-percha cones taken from a freshly opened sealed packet of size 120 (DiaDent) and 2% taper were used. They were divided into various groups according to the type of disinfectant.

Synthesis of 10h milled ZnO nanoparticles

Synthesis of Zinc Oxide (ZnO) nanoparticles were done by milling Bulk Zinc Oxide powder (Merck) in planetary High Energy Ball Milling (Retsch, PM400) using tungsten carbide (WC)

jar (250 ml) and WC ball (10 mm) at 300 rpm with ball to powder ratio 20:1 in ambient atmosphere from 0 to 10h.

Procurement of Microorganism

The microorganism, E. faecalis (ATCC2912) used in this research was procured in freeze-dried form in an air tight glass tube.

Contamination of Gutta-Percha cones

Pre-sterilized test tubes were taken and with the help of micropipette, 5 mL of prepared inocula containing activated E. faecalis was poured in each test tube and were then incubated at 37°C for 30 minutes. The GP cones were taken out from sealed packets with the help of sterilized tweezer and were added into each test tube containing 20 ML of microbial suspension of activated E. faecalis for 30 min. They were subsequently transferred for air drying to sterile dishes containing sterile 4 × 4 gauze pads.

Afterward, they were moved and allowed to air dry on clean, sterile dishes that held sterile 4 x 4 gauze pads.

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JCHR (2024) 14(2), 993-997 | ISSN:2251-6727

Disinfection of Gutta-Percha cones

After artificial contamination,50 GP cones were immersed in the respective disinfectant solutions for 1 min. Based on the disinfectant used, GP cones were subdivided into 6 groups with 10 cones in each group.

Group 1-10 contaminated cones immersed in 5.25% NaOCl

Group 2 -10 contaminated cones immersed in 0.1%% Octenidine

Group 3 - 10 contaminated cones immersed in 2% CHX Solution

Group 4 - 10 contaminated cones immersed in 10h milled ZnO NP Solution

Group 5 - 10 contaminated cones without any disinfectant and served as positive control

Group 6 - 10 uncontaminated cones which served as a negative control

All the cones were individually transferred to sterile test tubes containing 10 ml of thioglycollate media (HiMedia Laboratories) and incubated at 37°C for 7 days.

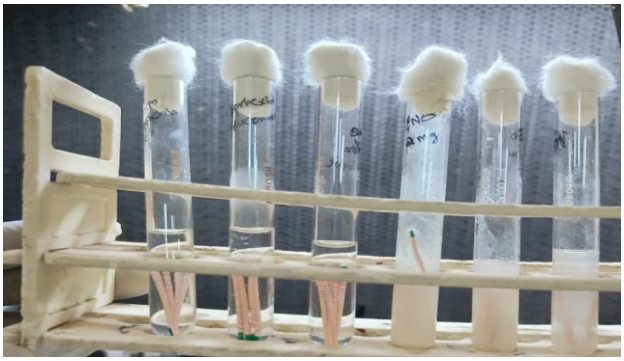

Figure 1: showing incubation of gutta-percha incubated for 7 days

After 7 days, a micropipette was used to transfer the thioglycollate media to a petridish containing brain heart infusion (BHI) agar. A sterile cotton tip was used to spread the thioglycollate media in a thin layer over BHI agar. The plates were then incubated for 48 h aerobically at 37°C and the colony forming units (CFU) were counted with digital colony counter

4. Results

As shown in table 1 and figure 2, the least number of colonies was seen for group A (5.25% NaOCL) while the maximum mean number of colonies was seen for group E (positive control group).

By inter-group comparison in regards to the time interval: it was observed that NaOCL was significantly more effective ($p<0.0001$) than other disinfectants against E. Faecalis at 1-minute time interval followed by 0.1% octenidine dihydrochloride.

Groups	N	Mean	Std. Deviation	Test Statistic	df	P value
A	10	.80	.788	55.554	5	0.0001*
B	10	6.20	1.229			
C	10	18.55	1.643			
D	10	22.40	.775			
E	10	120.20	16.294			
F	10	.40	.516			

Tables 1: showing the differences between the six disinfectants

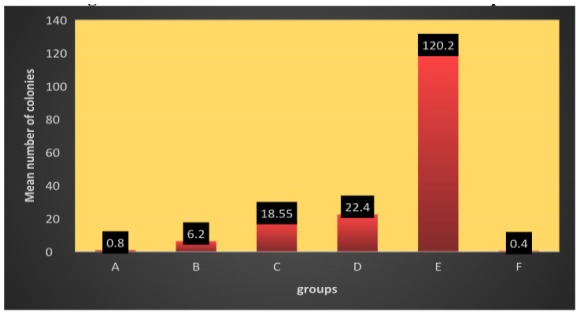

Figure 2: showing the mean number of colonies among six groups

5. Discussion

Gutta-percha cones (GPCs) are not amenable to sterilization through heat. As a result, it is advisable to employ a chemical agent as part of standard endodontic procedures for sterilizing GPCs [11]. When gutta-percha comes into contact with chemical agents during sterilization processes, it may result in significant surface alterations [12]. GPCs have irregular surfaces and root canal sealers 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 [18]. E. faecalis, are facultative gram-positive

Journal of Chemical Health Risks

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JCHR (2024) 14(2), 993-997 | ISSN:2251-6727

cocci-shaped bacteria that were chosen as the test bacterial contaminant because it is a predominant bacterial species in persistent apical periodontitis and it is the most common bacteria associated with the post-treatment infection [19]. NaOCl and CHX are the two common endodontic irrigants and have been used in some studies with varying concentrations [20]. 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 [19]. A 5.25% NaOCl solution kills microorganisms in only 1 minute of exposure [20]. Research has shown that Octenisept exhibits a wide spectrum of antimicrobial effects, effectively combating both Gram-positive and Gram-negative bacteria, fungi, as well as various viral species [14], and the efficacy of octenidine was reported against dental plaque-associated bacteria when compared with CHX [15,16]. Octenisept was chosen as a test solution in this study as the literature suggests that it was used as a root canal dressing material and had antibacterial activity against E. faecalis in the root canal and dentine after one minute [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 [21,22]. In this study, high energy ball milling technique was used to mimic the mechanical milling process used in industrial synthesis for lab scale synthesis of ZnO nanoparticles. Bulk ZnO particles were milled for 10 h in tungsten carbide container with tungsten carbide balls and toluene medium to provide the inert environment for contamination free synthesis of ZnO nanoparticles [17]. In the present study, the effects of irrigation solutions on the GPCs structure were investigated at exposure times of 1minute, respectively. The findings of the present study revealed that 5.25% NaOCL was more effective against E. Faecalis for 1 minute. Subha et al. [6] showed that the time required for antimicrobial property of NaOCl is inversely proportional to its concentration: 1% NaOCl removes E. faecalis in 20 minutes whereas 5.25% NaOCl takes less than 1 minute. While we carefully considered real-world clinical scenarios when determining the immersion time for gutta-percha in various solutions and in the choice of these solutions, it's important to note that the effects of these solutions may vary in actual clinical practice based on the specific root canal environment and the techniques used for root canal filling.

6. Conclusion

Even though gutta-percha cones are usually sterile during storage, they can be easily contaminated if incorrectly manipulated. NaOCl at 5.25% concentration is an effective agent for a rapid high disinfection level of gutta-percha cones.

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