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RESEARCH ARTICLE

EVALUATION OF EFFICACY OF DIFFERENT CHEMICAL AGENTS IN DISINFECTION OF GUTTA PERCHA CONES

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ABSTRACT

Most of the times, gutta percha cones available in the market are non sterilized. Also there is risk of contamination either by handling or aerosols and from physical sources during the storage process. So if we follow the principles of endodontics, then these GP cones should be disinfected prior to using them for obturation. But most clinicians avoid these procedures even though it has a great role in success of root canal treatment. For a chemical agent to be considered effective in the disinfection of a given material, its ability to eliminate resistant microorganisms at a concentration of about 10^7-10^8CFU ml^{-1} must be proved. In the present in vitro study gutta percha cones were contaminated with microbes and thereafter left in disinfecting solutions for different times, and their efficacy in disinfection of gutta percha cones was evaluated.

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INTRODUCTION

The success of root canal therapy relies on thorough disinfection and the use of aseptic techniques. This holds true for obturating materials also. Gutta-percha, has been widely used and accepted as a root canal filling material (Siqueira *et al*., 1998). Because gutta-percha cones come into close contact with periradicular tissues during root canal obturation, they should be free of pathogenic microorganisms. It may be good clinical practice to decontaminate the gutta-percha cones by means of a chemical agent before use (Siqueira *et al*., 1998).

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A contaminated obturating material can reintroduce microorganisms to the root canal system and delay or inhibit healing. Asepsis should be maintained to eliminate chances of possible flare-ups. Studies have shown that gutta-percha cones removed from their packaging are not serlized and get rapidly contaminated by microorganisms in the dental operatory. The various techniques include immersion, wiping or scrubbing and fuming of GP cones in order to disinfect them (Montgomery, 1971). Various chemical agents have been proposed as GP disinfectants, such as sodium hypochlorite (NaOCl), glutaraldehyde, alcohol, iodine compounds and hydrogen peroxide. The appropriate disinfectant should be the one that can be used routinely in dental clinics, providing a fast disinfection without modifying the structure of the cone

(Nabeshima *et al*., 2011). The purpose of this study was to evaluate the effectiveness of four different chemical agents in rendering gutta-percha cones free of Bacillus subtilis spores and Candida albicans.

MATERIALS AND METHODS

In this study, Bacillus subtilis (Fig 1) and candida albicans (Fig 2) were used to infect GP cones as these organisms are commonly found in dental operatory.

Fig. 1. Bacillus Subtilis strain

Fig. 2. Candida Albicans strain

In this study, 0.04 % taper 30 size GP cones (Diadent, Fraser Way, Burnaby, canada) were used (Fig 3).

Fig. 3. 0.04% taper 30 size GP cones

40 GP cones were taken and contaminated with *Bacillus subtilus* (ATCC/7953) and Candidia Albicans (SC5314/ ATCC MYA-2876).

These cones were immersed in 15 ml of microbial suspension in two test tubes and incubated at 37 degree for 30 min (Fig 4).

Fig. 4. GP cones immersed in microbial suspension

After the incubation period, the cones were dried using sterilize gauze and divided randomly into five groups with 8 samples in each group depending upon the chemical agent being used:

- Group 1 – control group
- Group 2 – 3% NaOCl (hyposol Prevest Denpro)
- Group 3 – 0.2% Chlorhexidine (CHX) (Zee laboratories Ltd)
- Group 4 – 5 % povidine iodine (Ovidin)
- Group 5 – eugenol (eugenol Prevest Denpro)

Fig. 5. GP cones immersed in different disinfecting solutions

The disinfecting times to sterlize the cones were 1, 3, 5 and 10 minutes (Fig 5). Out of eight GP cones in each group two were taken out at various time intervals i.e 1 min, 3 min, 5 min, and 10 min and were inoculated on McConkey's agar and incubated at 37 degrees for 72 hrs (Fig 6).

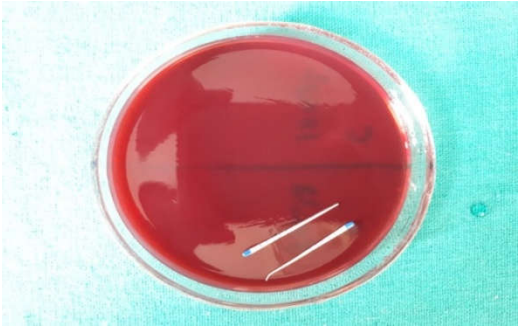

Fig. 6. GP cones inoculated on McConkey's agar

This was repeated for each group based on the disinfectant used. Bacterial growth was evaluated by presence or absence of bacterial colonies and colony counting on the McConkey's agar plate (Fig 7). The presence or absence of colony forming units was evaluated and the results were statistically analysed by Kruskal–Wallis test. Statistical significance level was established at $P < 0.05$.

Fig. 7. Agar plates showing bacterial colonies

RESULTS

The comparison between the bactericidal activities of the chemical agents in disinfecting GP cones in this study is shown in Table 1.

One of the most efficient, reliable, convenient, inexpensive, and used methods of gutta-percha sterilization is the rapid-sterilization technique proposed by Senia *et al*. (2003). It consists of a 1-min immersion time of gutta-percha cones in undiluted (5.25%) sodium hypochlorite. It was found to accomplish sterilization against a variety of Gram-positive, Gram-negative, and spore-forming microorganisms. Spangberg recommended that after sterilization gutta-percha should be rinsed in ethyl alcohol to remove crystallized sodium hypochlorite before obturation. Because of the thermoplastic characteristics of these materials, they cannot be sterilized by conventional autoclaving or in a hot air oven; therefore, they require rapid decontamination before use to maintain the aseptic chain, an essential factor in successful endodontic therapy (Subha *et al*., 2013).

Sodium hypochlorite is widely used as an endodontic irrigant and has been used as sterilizing agent for GP cones, mainly when used at a concentration of 5.25%. Sodium hypochlorite has strong antibacterial and sporocidal effects, which depend on the liberation of active chlorine, a powerful oxidizing agent that inactivates enzymes containing functional sulfhydryl groups. Chlorine compounds may inhibit the germination and outgrowth of bacterial spores (Siqueira *et al*., 1998). A 1-minute immersion of gutta-percha in 5.25% sodium hypochlorite (NaOCl) has been shown to be effective against a variety of gram-positive, gram-negative, and spore-forming microorganisms. A recent study showed that disinfecting solutions increased the surface free energy, promoting high interaction between gutta-percha and sealers. Short *et al*, in a scanning electron microscopic study, revealed that sodium chloride crystal formation could occur after the rapid disinfection of gutta-percha with NaOCl.

Table 1. Results

	Bacillus Subtilus				Candida Albicans			
	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min
Group 1 (control)	+	+	+	+	+	+	+	+
Group 2 (Na OCl)	-	-	-	-	+	+	+	-
Group3 (CHX)	+	+	+	-	+	+	+	+
Group 4 (povidone iodine)	-	-	-	-	-	-	-	-
Group 5 (eugenol)	+	+		+	+	+	+	+

It was observed that growth was present in the control group thus ensuring that the GP cones were infected with microbial suspension. NaOCl was effective against B. Subtilus but effective against candida only after 10 min of immension. CHX suppresses the growth of B. Subtilus only after 10 min and ineffective against C. Albicans. Providine iodine was effective against both B. Subtilus and C. Albicans. Whereas eugenol does not suppress the growth of either of the two.

DISCUSSION

The dentist is occasionally faced with problems of reinfection of root canal that occur after obturation of the root-canal space. One possible explanation of this phenomenon may be the introduction of contaminated gutta-percha cones into the root canal[3]. Gomes *et al*. reported that 5.5% of the GP cones taken from their boxes were contaminated (Pang *et al*., 2007). Gutta-percha taken from sealed packages may be contaminated by a variety of microorganisms after exposure to the dental operatory environment (Subha *et al*., 2013). Because the placement of gutta-percha is the final step in the obliteration of the root-canal space, sterility is of utmost importance[4].

Spangberg showed that the crystals could be removed by rinsing the gutta-percha with 96% ethyl alcohol, 70% isopropyl alcohol, or distilled water (Short *et al*., 2003). Valois *et al*. reported that 5.25% NaOCl had resulted in surface deterioration of GP cone and that it was because of loss of GP cone components by the oxidizing agent (NaOCl). Deep irregularities formed through deterioration of GP cones can create interfacial gaps between the GP cone and the root canal wall, increasing the risk of leakage (Pang *et al*., 2007). However, the significance of crystal formation on the obturation seal is not known. Chlorhexidine is another irrigant that is commonly used during cleaning and shaping procedures. Chlorhexidine kills vegetative bacteria by disrupting the membrane integrity and inducing the precipitation of the cytoplasm. Nevertheless, it has been reported that chlorhexidine is ineffective against spores except at high temperatures (Siqueira *et al*., 1998). Interest regarding the antimicrobial activity of CHX is increasing in endodontic practice. Chlorhexidine gluconate is a cationic bisguanide that appears to act by adsorbing onto the cell wall of microorganism and causing the leakage of intracellular components.

Table 2. Kruskal-Wallis Test

Group		N	Mean Rank
Bacillus(1min)	1	3	12.67
	2	3	3.67
	3	3	9.33
	4	3	3.33
	5	3	11.00
	total	15	
Bacillus(3min)	1	3	13.33
	2	3	4.00
	3	3	8.33
	4	3	3.00
	5	3	11.33
	Total	15	
Bacillus(5min)	1	3	12.83
	2	3	3.50
	3	3	8.00
	4	3	3.50
	5	3	12.17
	Total	15	
Bacillus(10min)	1	3	12.83
	2	3	4.00
	3	3	7.00
	4	3	4.00
	5	3	12.17
	Total	15	
Candida(1min)	1	3	12.50
	2	3	8.00
	3	3	5.67
	4	3	2.33
	5	3	11.50
	Total	15	
Candida(3min)	1	3	13.17
	2	3	6.67
	3	3	6.33
	4	3	2.33
	5	3	11.50
	Total	15	
Candida(5min)	1	3	13.17
	2	3	7.00
	3	3	5.33
	4	3	2.67
	5	3	11.83
	Total	15	
Candida(10min)	1	3	12.83
	2	3	4.83
	3	3	6.67
	4	3	3.50
	5	3	12.17
	Total	15	

CHX has additional properties such as substantivity and biocompatibilty compared with NaOCl (Pang *et al*., 2007). A study observed that 2% chlorhexidine was effective in decontaminating gutta-percha after 10 minutes of exposure. The decontamination of gutta-percha with 2% chlorhexidine has also been shown to increase the adhesion force between these materials and root canal sealers[1]. In Ozalp *et al*'s study, gutta-percha cones contaminated by B. subtilis were decontaminated after 5 minutes of contact with 2.5% hypochlorite. They used only 4 samples per group. Another study also showed that 2% chlorhexidine was not effective with 1 minute of treatment (Subha *et al*., 2013). Povidone iodine have also been studied for the rapid disinfection of gutta-percha (Subha *et al*., 2013). It is also used in the dental operatory in scrubbing and operating field disinfection[1]. Iodine compounds have been used for decades for the disinfection of surfaces, skin and operating fields; they are known as fast-acting and efficient bactericidal, fungicidal and sporicidal agents, where the molecular iodine is responsible for the antimicrobial activity (Nabeshima *et al*., 2011). Iodine is considered by many surgeons to be the best antiseptic available, but it has the disadvantages of staining and irritating the skin and mucous membranes[3]. Because of these adverse effects, it has not been widely used in dentistry. When iodine is combined with polyvinylpyrrolidone, however, the germicidal action of the iodine is prolonged and the danger of irritation, sensitization and burning is minimized (Montgomery, 1971). Eugenol has a strong antibacterial, antifungal, insecticidal and antioxidant properties. In addition, clove oil is used as an antiseptic in oral infections. This essential oil has been reported to inhibit the growth of molds, yeasts and bacteria. The high levels of eugenol contained in clove essential oil are responsible for its strong biological and antimicrobial activities. It is well know that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast. According to this study povidone iodine has been proven to be effective in disinfecting GP cones after 1 min of immersion followed by NaOCl, so both these chemical agents are recommended to be used for chair side rapid sterilization of GP cones

Conclusion

The efficacy of a disinfectant depends on a sufficient length of treatment time for which disinfectant is being used. According to this study povidone iodine was able to disinfect the gutta percha cones completely within 1 min of immersion. Whereas NaOCl killed B. subtilus spores in 1 min. and C. albicans after 10 min. of immersion and CHX was effective against B. subtilus only after 10 min. Eugenol was not able to disinfect GP cones.

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