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Comparative evaluation of effectiveness of sodium hypochlorite, chitosan nano particles and neem extract for disinfection of gutta percha cones - An in-vitro study

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Abstract

Objective: The present study was carried out to assess the efficacy of 5.25% sodium hypochlorite, chitosan nanoparticles and neem extract for disinfection and the effect of these agents on the surface topography of Gutta Percha (GP) cones.

Materials and method: A total of 60 GP cones were immersed in the solution of Enterococcus faecalis and then divided into four groups. Group 1 – GP cones were immersed in 5.25% sodium hypochlorite solution, Group 2 – GP cones were immersed in chitosan nanoparticles, Group 3 – GP cones were immersed in neem extract, Group 4 – positive control, contaminated cones. Group I, II and III were again subdivided into subgroup A, B and C where immersion time was 30 seconds, one minute and 5 minutes respectively. The contaminated cones were then cultured and colony forming units were assessed. The surface topography was evaluated at 1000X under a Scanning electron microscope to evaluate surface topography. Statistical analysis was done using one-way anova.

Results: Sodium hypochlorite was found to be completely effective against E. faecalis followed by neem extract and chitosan nanoparticles. The SEM evaluation revealed surface irregularities after immersion

in disinfecting agent which was not found after rinsing in the distilled water.

Conclusion: Sodium hypochlorite is an effective disinfecting agent for GP cones;however, a final rinse is necessary to remove surface deposits.

Keywords: Chitosan nanoparticles, Disinfection, Gutta percha cones, Neem extract, Sodium hypochlorite

Introduction

For a successful endodontic therapy, proper aseptic conditions should be followed throughout the procedure.[1] After gaining straight line access, canals have to be irrigated thoroughly to eliminate micro organisms that might hinder the outcome of the root canal treatment.

Disinfection of the obturating material is just as crucial as the chemo-mechanical preparation of the root canal. Gutta-percha (GP) cones have proven to be the best material for obturation. GP cones are made up of 18–22% polymer derived from Sapotaceae plants, 59–75% zinc oxide, and 1.1–17.2% barium sulfate. [2,3] At the time of manufacturing, GP cones are made sterile. It is a biocompatible material and it can effectively adapt to and efficiently seal the root canal.[4]

Since it is a thermoplastic material, as the temperature rises it becomes soft and pliable. So, it is not possible to employ conventional sterilizing methods such as moist or dry heat methods of sterilization as these methods require high temperatures.[1] Therefore, chemical method of disinfection is preferable before using GP in obturation to sterile the contaminated gutta-percha cones. Contamination of Gutta-percha cones with aerosols can occur due to inappropriate handling. [5,6]

Amongst the chemical method of sterilization, sodium hypochlorite (NaOCl) is one of the most frequently used, effective and inexpensive antimicrobial agents. NaOCl can be used in various concentrations to achieve proper disinfection. However, the antiseptic effect is primarily dependent on the concentration of the solution and the time at which the microorganisms are in contact with this solution. At a concentration of 5.25%, sodium hypochlorite is effective against various endogenous and exogenous endodontic bacteria for an exposure period of around 1 minute.[7]

Apart from NaOCl, nowadays natural herbal formulations have also been tried for the disinfection of gutta-percha. One of the popular herbal antiseptics is Azadirachta indica (neem). It has been known for its antibacterial, antifungal, antiviral and antioxidant effects.[8] Several studies have proved the effectiveness of this neem extract for the disinfection of GP.

Chitosan is a natural polymer, which is extracted by the deacetylation of chitin. It has bio-adhesive, bio-compatible and antimicrobial properties.[9] The antiseptic property of chitosan has been under investigation; the basis of which is its effectiveness against oral bacteria.[10] Thus, the present study was carried out to evaluate the efficacy of sodium hypochlorite, neem extract and chitosan nanoparticles for the disinfection of gutta percha cones.

Materials and Method

The study involved microbiological evaluation of artificially contaminated Gutta-percha cones and evaluation of the surface changes using SEM after immersing the contaminated Gutta-percha cones in disinfecting solution. The organism used was Enterococcus faecalis. The present study was performed on 60 samples of gutta percha cones which were divided into four different groups. The GP cones were artificially contaminated using E. facecalis and then divided into four groups of 15 samples each.

- Group 1: Contaminated samples were immersed in 5.25% sodium hypochlorite solution.

- Group 2: Contaminated gutta percha cones were immersed in Chitosan nano particle.
- Group 3: Contaminated gutta percha cones were immersed in Neem extract.
- Group 4: Positive control: Contaminated gutta percha cones were directly cultivated in thioglycollate media as a positive control without immersing in any disinfectant solution.

The contaminated GP cones in Group 1, group 2 and group 3 were further divided into sub group A, sub group B and subgroup C; depending on the time of immersion in the respective disinfectant.

- Group 1A, Group 2A, Group 3A – GP cones are immersed in the solution for 30 seconds
- Group 1B, Group 2B, Group 3B – GP cones are immersed in the solution for 1 minute
- Group 1C, Group 2C, Group 3C – GP cones are immersed in the solution for 5 minutes

The group 4, positive control, the GP cones were directly taken from the thioglycolate media and examined under SEM without immersing in any disinfectant solution.

Preparation of neem extract

Neem extract (A. indica) was prepared using fresh mature leaves of the tree. They were washed using sterilized water. They were crushed and then added to 50ml of ethanol. They were kept in the solution and mixed for 1-2 minutes and then filtered. The alcohol part was separated in the water bath to obtain 10ml of neem solution.[11]

Preparation of chitosan solution

Commercially available Chitosan nano particles were used (Aura Biotechnology, Pvt Limited, Chennai). The powder was dissolved in 2% w/v acetic acid solution at a concentration of 0.1% w/v. The pH of solution was kept at 5 with 1 mol/L NaOH. The concentration of chitosan particles was 10 mg/ml.

Artificial Contamination of Gutta-Percha Cones

Microbial suspension of Enterococcus faecalis (ATCC29212) of approximately 108 CFU/ml in BHI broth (HiMedia Laboratories) was used for this study. Gutta percha points were artificially contaminated by immersing in a 10ml microbial suspension of E. faecalis for 30 minutes. The cones were then transferred to sterile paper pads and allowed to air dry for 10 minutes.

Disinfection of Gutta-Percha Cones

The cones were immersed in the respective solution of each group after being contaminated. The stipulated time for immersion as disinfecting protocol was strictly followed. The cones were then individually transferred to sterile test tubes containing 10 ml of thioglycolate media (HiMedia Laboratories) and incubated at 37°C for 7 days. Samples were then washed with sterile Phosphate Buffered Saline (PBS) and plated on to Brain Heart Infusion (BHI) Agar plate. The plates were then incubated for 48 hours aerobically at 37°C and the colony forming units were counted with a digital colony counter.

Topographical Examination of Gutta-Percha Cones

Scanning electron microscope (FEI Quanta 200 ESEM FEG) at 100X, 1000X and 5000X magnification was used for the topographical examination of gutta-percha cones. For the Positive control group, GP cones were directly taken from the thioglycolate media without immersing in a disinfectant solution and examined under SEM. The GP cones from Group I, Group II and Group III were also examined under the same magnification of SEM. For all three test groups, the topographical examination of the cones was done two times. First, they were examined without rinsing with distilled water and one after rinsing with distilled water.

Statistical analysis: The data obtained were subjected to statistical analysis and was carried out using SPSS (Statistical Package for Social Sciences) IBM version 21. The microbiological analysis was done for Group I, Group II and Group III for all the three immersion times of the respective groups. The positive group showed too much microbial colony which was difficult to count and thus was not compared with the tests' groups.

Result

The results were expressed in terms of mean and standard deviation. The colony forming units (CFU) were counted for all the groups. In case of the positive control group, the CFUs were too dense and numerous to be counted and hence were excluded from statistical analysis. There was no colony-forming unit observed with Group I (Figure 1). Group II showed maximum count followed by Group III (Table 1 and Graph 1). The count obtained was maximum for Group IIA and Group IIIA i.e. when the GP cones were inserted for 30 seconds only, followed by Group IIB and Group IIIB (immersion for 1 min) and minimum CFUs were observed in Group IIC and Group IIIC (immersion for 5 mins). The intergroup comparison of all the subgroups with each other was statistically significant. (Table 2 and 3)

The topographical examination was done using Scanning Electron Microscope (SEM) (figure 2) for all the groups at the aforementioned magnifications. For the positive control group, some granules were observed without any bacterial adhesion. For Group I – sodium hypochlorite group, surface of gutta percha was irregular and some crystalline deposits were also observed when examined under SEM without rinsing in distilled water. When rinsed with distilled water, GP cones of the same group showed surface irregularities. In the case of Group II – an irregular surface was observed when examined without rinsing, however, when examined after rinsing, no significant change in surface was observed. Group III – irregular surface was observed when examined without rinsing while not much difference was observed after rinsing.

Discussion

The use of a chemical method of sterilization for the GP cones is advisable and required as they tend to get contaminated mostly from the vegetative forms of bacteria rather than from the heat-resistant bacterial spores.[12] The GP cones are made free of the bioburden using a disinfectant solution as it ensures complete asepsis, which is mandatory for the predictable outcome of endodontic therapy. As the "straight out of the box" GP cones have also shown the presence of micro-organism[5], sterilization can become imperative as it not only decreases the chance of reinfection but also enhance peri-radicular healing.[13] The scientific evidences also favour the use of such an agent which will not affect the inherent properties of the GP cones and make them sterile.[14] The present study evaluated the effect of three different chemical solutions for the disinfection of GP cones as well as the effects of the solutions on the surface of these cones. The test organism used in this study was Enterococcus faecalis, as it is one of the most commonly found resistant oral micro-organisms found which needs to be eradicated. E. faecalis have been found to be a predominant micro-organism in failed endodontic cases;[15] the in vitro studies have shown the presence of E. faecalis into the dentin tubules up to 100 mm from the canal lumen making it an appropriate choice for the study.[16]

The anti-microbial action of sodium hypochlorite is associated with the liberation of active chlorine content; this has been shown to inhibit germination and spores of various bacteria of endodontic origin.[17] In this study, use of 5.25% of NaOCl was used at various time

intervals of 30 seconds, 1 minute and 5 minutes. For all the time intervals, no growth of bacteria has been observed. This finding is supported by other scientific evidence obtained in the studies of Berber et al[18] and Siqueira et al.[19]Siqueira et al have shown the reduction of bacterial load of E.faecalis using 1%, 2.5% and 5.25% of NaOCl.[19]

Chitosan has been shown to have an antibacterial effect. The proposed mechanism for this is by binding to the negatively charged bacterial cell wall resulting in disruption of the cell and alteration of membrane permeability. Chitosan then gets attached to DNA, halting DNA replication and subsequent cell death.[20] Divya et al proposed that chitosan can act as a chelating agent which can selectively bind to trace metal elements resulting in toxin production and microbial cell death.[21] The chitosan immersed GP cones showed a mean growth of 11.80±1.3 CFU within 30 seconds immersion which reduced to 5±0.7 CFU within 5 minutes. The reduction can be attributed to proposed antibacterial mechanism.

Group III showed a reduction from 2.40±0.54 CFU at 30 seconds to 1.2±0.44 CFU at 5 minutes of immersion. This can be attributed to the antibacterial properties of neem extract. Numerous studies have identified phytochemicals such as azadirachtin, gedunin, and nimbolide for the anti-microbial activity of neem.[22] The results obtained in the present coincide with that of the previous studies where A.indica was effective against common endodontic bacteria and inhibited their growth.[23]

Topographical examination revealed some deposits on the surface of GP cones but was removed after rinsing with distilled water. Thus, suggesting that the use of chemical agents does not substantially affect the GP cones. Though the short sample size can be considered a shortcoming of the study hence similar studies with a larger sample size can be done to further validate the results.

Conclusion

Within the limitations of the study, it can be concluded that sodium hypochlorite is effective in the disinfection of GP cones leading to the complete eradication of resistant endodontic microflora. The neem extract can be a suitable option; however, there was evidence of growth with the same hence more immersion time should be considered. Chitosan nanoparticles have shown growth of the bacteria when GP cones were immersed only for 30 seconds which is reduced when immersion time is increased to 5 minutes, thus more immersion should be considered. A final rinse with distilled water should be considered to remove surface irregularities to avoid any change in the properties of GP cones.

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Table Legends

Table 1: Table showing microbiological count in terms of mean and standard deviation obtained from various groups.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
30 sec	Group IA	5	.0000	.00000	.00000	.0000	.0000
	Group IIA	5	11.8000	1.30384	.58310	10.1811	13.4189
	Group IIIA	5	5.2000	.83666	.37417	4.1611	6.2389
1 min	Group IB	5	.0000	.00000	.00000	.0000	.0000
	Group IIB	5	11.0000	.70711	.31623	10.1220	11.8780
	Group IIIB	5	2.4000	.54772	.24495	1.7199	3.0801
5 min	Group IC	5	.0000	.00000	.00000	.0000	.0000
	Group IIC	5	5.0000	.70711	.31623	4.1220	5.8780
	Group IIIC	5	1.2000	.44721	.20000	.6447	1.7553

Table 2: Table showing significant difference amongst various groups using One-way Anova.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
30 sec	Between Groups	349.733	2	174.867	218.583	.001*
	Within Groups	9.600	12	.800		
	Total	359.333	14			
1 min	Between Groups	334.533	2	167.267	627.250	.001*
	Within Groups	3.200	12	.267		
	Total	337.733	14			
5 min	Between Groups	68.133	2	34.067	146.000	.001*
	Within Groups	2.800	12	.233		
	Total	70.933	14			

Table 3: Table showing Post hoc Tukey to compare mean CFUs of various groups.

Multiple Comparisons							
Tukey HSD							
Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
30 sec	Group IA	Group IIA	-11.80000*	.56569	.001*	-13.3092	-10.2908
		Group IIIA	-5.20000*	.56569	.001*	-6.7092	-3.6908

	Group IIA	Group IA	11.80000*	.56569	.001*	10.2908	13.3092
		Group IIIA	6.60000*	.56569	.001*	5.0908	8.1092
	Group IIIA	Group IA	5.20000*	.56569	.001*	3.6908	6.7092
		Group IIA	-6.60000*	.56569	.001*	-8.1092	-5.0908
1 min	Group IB	Group IIB	-11.00000*	.32660	.001*	-11.8713	-10.1287
		Group IIIB	-2.40000*	.32660	.001*	-3.2713	-1.5287
	Group IIB	Group IB	11.00000*	.32660	.001*	10.1287	11.8713
		Group IIIB	8.60000*	.32660	.001*	7.7287	9.4713
	Group IIIB	Group IB	2.40000*	.32660	.001*	1.5287	3.2713
		Group IIB	-8.60000*	.32660	.001*	-9.4713	-7.7287
5 min	Group IC	Group IIC	-5.00000*	.30551	.001*	-5.8150	-4.1850
		Group IIIC	-1.20000*	.30551	.005*	-2.0150	-.3850
	Group IIC	Group IC	5.00000*	.30551	.001*	4.1850	5.8150
		Group IIIC	3.80000*	.30551	.001*	2.9850	4.6150
	Group IIIC	Group IC	1.20000*	.30551	.005*	.3850	2.0150
		Group IIC	-3.80000*	.30551	.001*	-4.6150	-2.9850
*. The mean difference is significant at the 0.05 level.							

Graph Legends

Graph 1: Graph showing microbiological count in terms mean obtained from various groups.

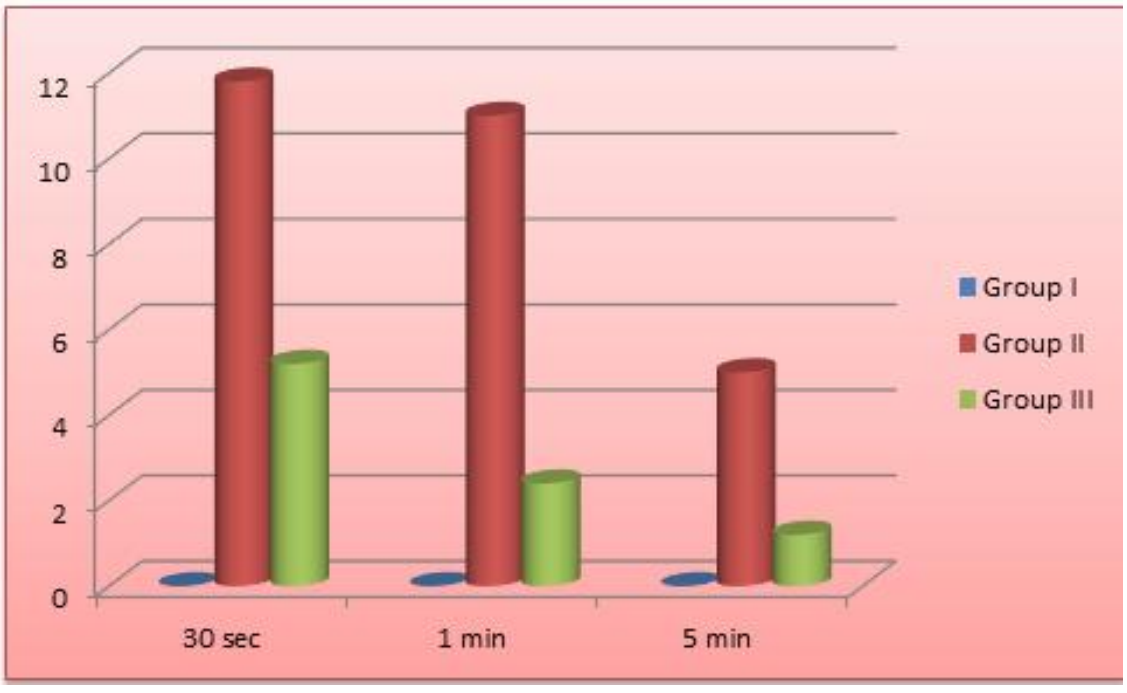
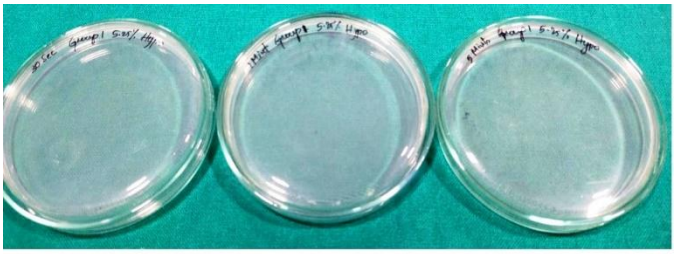


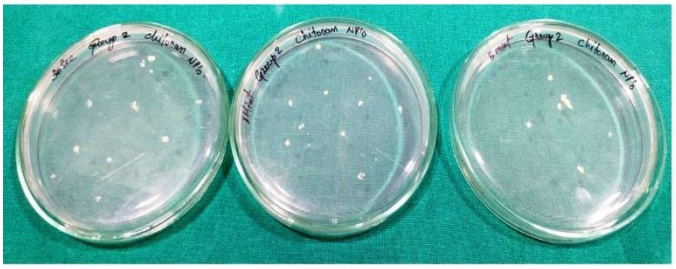
Figure Legends

Figure 1: Composite figure showing microbiological culture of various groups.



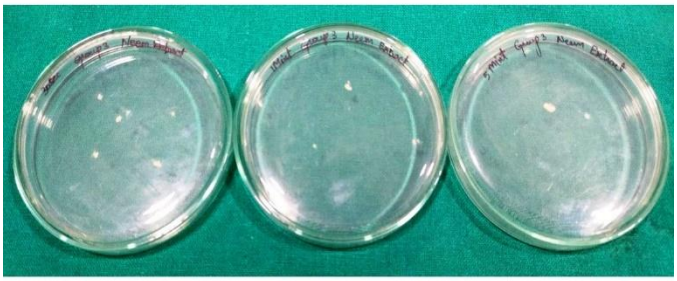
Group A Group B Group C

Microbiological culture after sodium hypochlorite immersion of gutta percha at various time intervals



Group A Group B Group C

Microbiological culture after chitosan nano particle immersion of gutta percha at various time intervals

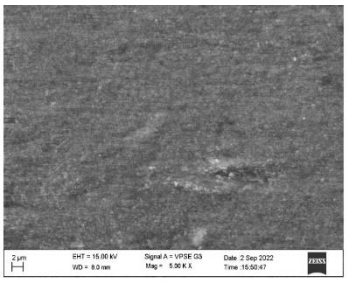


Group A Group B Group C

Microbiological culture after neem extract immersion of gutta percha at various time intervals

Microbiological culture of contaminated gutta percha in positive control group

Figure 2: Composite figure of SEM topographical examination of gutta-percha of various groups.



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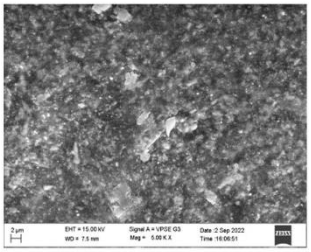

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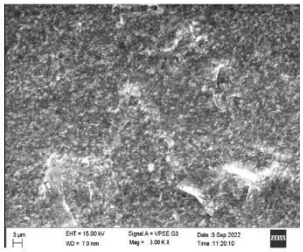

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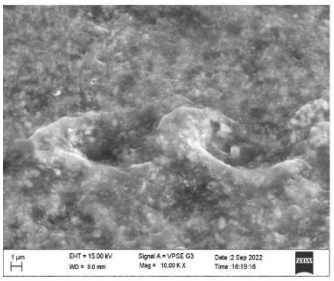

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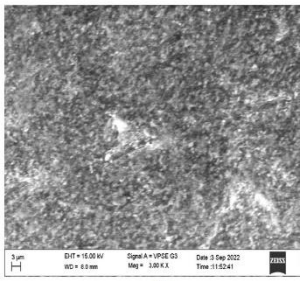

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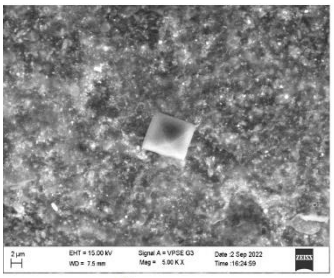

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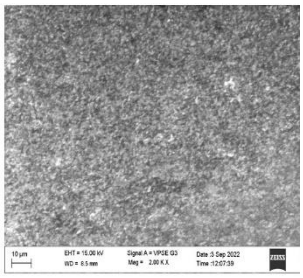

G

SEM Topography A. Contaminated Guttapercha cones: Positive control, B. Contaminated guttapercha immersed in Sodium hypochlorite without rinsing, C. Contaminated guttapercha immersed in Sodium hypochlorite after rinsing, D. Contaminated guttapercha immersed in Chitosan nano particle without rinsing, E. Contaminated guttapercha immersed in Chitosan nano particle after rinsing, F. Contaminated guttapercha immersed in neem extract without rinsing G. Contaminated guttapercha immersed in neem extract after rinsing