



DECONTAMINATION OF GUTTA-PERCHA CONES WITH DIFFERENT CHEMICAL AGENTS

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ABSTRACT

The purpose of this study was to evaluate the effectiveness of various chemical disinfectants in decontamination of gutta-percha (GP) cones contaminated with *Bacillus subtilis* spores. Sodium hypochlorite solution (4%), glutaraldehyde solution (2%), povidone-iodine solution (5%), and savlon solutions were compared for effectiveness in sterilizing gutta-percha cones contaminated with *Bacillus subtilis* spores. Microbial assays were carried out after immersing the GP in the experimental solutions for 5, 10 and 15 minutes. It was observed that glutaraldehyde decontaminated the gutta-percha cones in 5 minutes, sodium hypochlorite in 10 minutes while povidone-iodine solution and savlon were not effective even at the end of 15 minutes.

Key words: Gutta-percha cones, Decontamination, Chemical agents

INTRODUCTION

Microorganisms are ubiquitous, they cause contamination, infection and decay, and hence it becomes necessary to remove or destroy them from materials or from areas. The process of sterilization prevents contamination by extraneous organisms. The methods of sterilization depend on the purpose for which sterilization is carried out, the material that has to be sterilized and the nature of microorganisms that have to be removed or destroyed.

The success of endodontic therapy is influenced by adequate debridement of the root canal and the use of aseptic techniques. Gutta-percha is a desirable root canal filling material because it does not shrink after insertion unless it is plasticized with a solvent or heat. Aseptic techniques are compromised if microorganisms are introduced into the root canal by gutta-percha cones, thus elimination or reduction of microorganisms within the root canal before and during obturation by chemical and mechanical means is one of the crucial features for successful treatment¹⁻⁴. The gutta-percha cones are damaged by

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standard high temperature sterilization methods, so to maintain the chain of asepsis, chemical methods are used to sterilize gutta-percha cones effectively, inexpensively and rapidly.⁵

Literature registers several methods for rapid decontamination of gutta-percha cones in dentistry. Among others these include the following chemical agents: Polyvinylpyrrolidone-iodine⁶, glutaraldehyde⁵⁻⁷, sodium hypochlorite⁸⁻¹⁰, hydrogen peroxide⁹⁻¹¹, chlorohexidine^{12, 13} quaternary salts of ammonium^{8,9}, iodine-alcohol¹⁴ and paraformaldehyde¹⁵. However, there is still no agreement among national and foreign specialist for the best method.

The purpose of this study was to determine the effectiveness of four commonly used disinfectants in sterilizing artificially contaminated gutta-percha cones, within clinically acceptable exposure times. Today, no such study has been reported from India where the level of contamination of gutta-percha cones is relatively high and no standard protocol is being adopted for decontamination in various set-ups.

EXPERIMENTAL

This study was divided into two groups : experimental group and control group. In the experimental group, four disinfectant solutions were compared. Concentrated 4% sodium hypochlorite solution (Glaxo India Limited), 2% glutaraldehyde solution (PSK Pharma Pvt. Ltd.), 5% povidone-iodine solution (Wockhardt Ltd.) and salvon (Chlorhexidine gluconate solution) I. P. 0.3% v/v and cetrimide solution I. P. 0.6% w/v by (Johnson and Johnson Ltd.). Sterile normal saline served as a control group.

Bacillus subtilis (ATCC 6633) spores were selected to artificially contaminate the cones, as they are highly resistant to chemical and physical means of sterilization. A total of 25 sterile gutta-percha cones were first contaminated, by immersing them for 30 minutes in 10 mL of *Bacillus subtilis* spore suspension (10^7 spore/mL). Contaminated cones were removed from the suspension after 30 minutes exposure and placed on a wire rack to air dry for 30 minutes. All procedures were carried out aseptically under a laminar flow hood to prevent any possible contamination from the environment. Methods were followed as described by Frank and Pellea⁵.

After air-drying, each cone was immersed in a petri dish containing 10 mL of either control solution i. e. normal saline or experimental solutions for 5, 10 and 15 minutes. Five cones were immersed in all the five solutions (i. e. one control solution and 4

experimental solutions) for 5, 10 and 15 minutes. After specified time, the cones were removed from the solution and placed in a test tube containing 10 mL sterile saline solution for microbiological assay. The solution was mixed thoroughly with a vortex shaker. One mL suspension of each control and test solution was diluted with standard ten-fold dilution technique and 10 microliter of each suspension was plated on Trypticase Soy Agar (TSA) (Hi-Media Ltd., India) plates. Another mL of the solution was directly cultured in a test tube containing 9.0 mL of trypticase soy broth solution. All plates and tubes were incubated at 37⁰ C for 24 hours. After incubation, the bacterial colonies growing on each plate were counted and recorded as total number of recoverable colony forming units. The average values were recorded for each solution after visible growth and numbers of colony forming units (CFU) were counted. Broth tubes were evaluated for the presence or absence of bacterial growth and cultured again on trypticase soy agar.

The effectiveness of the disinfectant was determined by comparing the total number of colony forming units recovered from each experimental solution in which the cones were immersed with the number of colony forming units recovered from the control solution.

In addition, 0.1 mL of solution left in the petri plates after the cones were removed was added to sterile normal saline and assayed for the number of recoverable colony forming units in the same manner as stated above. This number represented the number of spores removed mechanically from the cones during treatment period.

To rule out any possibility of false positive results, a control test was also carried out according to the procedure described by Frank and Pelleu⁵.

RESULTS AND DISCUSSION

The microbial effectiveness of the experimental solutions was determined by comparing the total number of colony forming units, recovered from the cones immersed in the experimental disinfectant solutions, with those that were recovered from cones immersed in the control solution.

The effect of the disinfectants on the contaminated cones after being soaked for various time durations is shown in Table 1. Cones exposed to the control solution, showed the highest mean number of colony forming units. The best response was found in cones that were exposed to 2% glutaraldehyde, wherein no colony forming units were present. Recoveries from chemical disinfectant solutions after cone exposure are shown in Table

2.The recoveries represent the number of spores, which were removed from the cones during the exposure period.

Table 1.Effect of disinfectants on artificially contaminated gutta-percha cones

Solutions	Total colony forming units (CFU) recovered after each exposure period		
	5 minutes	10 minutes	15 minutes
Saline (control solution)	21000	23200	19400
Sodium hypochlorite solution	440	0 ± 0	0 ± 0
Glutaraldehyde solution	0 ± 0	0 ± 0	0 ± 0
Povidone iodine solution	5820	2280	2280
Savlon	1520	1360	60

Table 2.Microbial recoveries from chemical disinfectants after cone exposure

Solutions	5 minutes	10 minutes	15 minutes
Saline (control solution)	28780.0 ± 1704.99	22440	19720
Sodium hypochlorite solution	110	0 ± 0	0 ± 0
Glutaraldehyde solution	0 ± 0	0 ± 0	0 ± 0
Povidone iodine solution	8580 ± 861.39	6240	5320
Savlon	7120 ± 1423	5260	2460

Table 3 shows the absence and presence of bacterial growth in broth tubes. No growth was found in the tubes containing 2% glutaraldehyde and 4% sodium hypochlorite solution.

Table 3 Microbial growth in broth tubes

Solution	Microbial growth
Saline (control solution)	+
Sodium hypochlorite solution	-
Glutaraldehyde solution	-
Povidone iodine solution	+
Savlon	+
+ (Present) - (Absent)	

In the present study, we included the chemical agents based on the previously documentation and commonly used in endodontics set-ups. All four disinfectants were able to remove spores from the artificially contaminated gutta-percha cones, but only two of them effectively sterilized the cones within the clinically acceptable exposure time of fifteen minutes or less.

Sodium hypochlorite (4%) solution takes longer time to decontaminate all the cones (10 min) while glutaraldehyde (2%) solution could sterilize all the cones in lesser time (5 minutes). Povidone-iodine solution in 5 % concentration and savlon were not effective in sterilizing all the cones even at the end of fifteen minutes exposure; however, they could reduce the number of contaminating spores.

Gutta-percha cones were decontaminated completely by 2% glutaraldehyde, which was also reported by the Frank and Pelleu⁵. In another study, 2% glutaraldehyde were found to be sporicidal only after 15 minutes of treatment¹⁶. The difference in the timings may be due to the chemical constitution of the tested products and the quantitative methodology. Celso et al.¹⁶ demonstrated the bacterial growth in the inoculated tubes by presence or absence of growth, which was then confirmed by Gram staining. In our study, two methods were used to verify the effectiveness of a chemical agent. The growth in tubes as well as the number of colonies, which were cultured on TSA were counted, followed by Gram stain to confirm the presence or absence of growth.

In this study, sodium hypochlorite killed *B. subtilis* spores after five minutes of exposure; the results are comparable with Seina *et al.*¹⁰, Frank and Pelleu⁵ and Stabholz *et*

al.¹² but they have used 5.25%, 5% and 4.5% sodium hypochlorite solution while we have used 4% hypochlorite. Another difference was that Senia et al.¹⁰ had immersed the contaminated cones in undiluted Clorox (5.25% sodium hypochlorite) for a shorter duration of 60 seconds. In our study, the exposure time was longer but the concentration was much less. Cardoso et al.¹⁷ reported 100% sporicidal effect with sodium hypochlorite solution after treatment of cones for 1-minute exposure time. In present study, we have not tested the efficacy of the disinfectants after 1 minute of exposure so we have compared the results only after 5, 10 and 15 minutes of treatment, which are comparable to other authors.

Iodine in various preparations and in combination with other disinfectants has been used to decontaminate the cones⁶⁻¹⁶ but no bacteriological study has been found in India in which artificially contaminated cones were used to evaluate the efficacy of these agents in endodontic practices. In present study, 5% povidone-iodine solution was found to be completely ineffective at five, ten and fifteen minutes against *Bacillus subtilis* spores. *Bacillus subtilis* spores were seen even at the end of fifteen minutes immersion. The results were similar to that reported by Celso et al.¹⁶ wherein the iodine-alcohol in 0.3% and 1% concentrations showed rapid bactericidal action (1 minute) but no sporicidal activity in 15 minutes, however 10% polyvinyl- pyrrolidone-iodine (PVP-1) was effective in 5 minutes. Montgomery⁶ treated gutta-percha points with 10% polyvinyl- pyrrolidone-iodine (PVP-1) for up to 6 minutes and obtained complete surface sterilization. These conditions were only effective for less resistant microorganisms. Author however did not study the effectiveness of polyvinyl-pyrrolidone-iodine (PVP-1) against resistant organisms like *B. Subtilis* and *Pseudomonas species*. Linke and Chohayeb⁹, reported that betadine solution was not effective in surface sterilization of gutta-percha points infected with *Streptococcus mutans* after five minutes of immersion. We also report the ineffectiveness of povidone-iodine in our study, wherein we had used *B subtilis* spores. The inefficacy of betadine to sterilize was proved in both these studies using different organisms.

Savlon was suggested to be the most effective disinfectant for chair side cold sterilization during endodontic procedures.¹³ However, in the present study, savlon was not found to be an effective disinfectant even after an exposure time of 15 minutes. This could be because of the different concentrations of savlon used in the two-studies. Suchde et al.¹³ used chlorhexidine gluconate B. P. 1.5% v/v together with "Cetavlon" cetrimide B. P. (I. P. 30% w/v) which could destroy *B. subtilis* spores after immersion for half to one minute. In our study, savlon was used in low concentration (Chlorhexidine gluconate solution I. P. 0.3% v/v and cetrimide solution I. P. 0.6% w/v). This concentration was not effective in killing *Bacillus subtilis* spores even after fifteen minutes of exposure.

Our findings showed that the assay procedure was valid and the microbial effect resulted from disinfectants themselves and not from any mechanical or detergent action, which could have washed the spores from the cones during the decontamination procedures.

CONCLUSION

Results from this study; thus, suggest that in our set up, decontamination of gutta-percha cones may be achieved either by treatment with 2% glutaraldehyde solution for 5 minutes or by 4% sodium hypochlorite solution for 10 minutes, which is inexpensive and readily available. Iodine and savlon did lead to a reduction in bacterial counts but were found to be ineffective for complete cold sterilization of gutta-percha cones at chair side. In clinical practice, the microbicidal action probably would be greater than that demonstrated in this study, because the natural contamination of cones in most of the cases would be considerably less and consist mainly of vegetative bacteria rather than resistant spores.

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Accepted : 03.06.2008