ABSTRACT

AIM: The objective of the present study was to assess the efficacy of 2.5% sodium hypochlorite and 2.2% glutaraldehyde ('Cidex') and 3% hydrogen peroxide and 5% isopropyl alcohol as sterilizing agents for gutta-percha cones. METHODOLOGY: Gutta-percha cones artificially contaminated with a suspension of Bacillus stearothermophilus (ATCC/7953) were treated with either 2.5% sodium hypochlorite for 5, 10, 15 and 30 min.and 2.2% Glutaraldehyde 5, 10, 15 and 30min and 3% hydrogen peroxide 5, 10, 15, 30 min. and 5% isopropyl alcohol for 5, 10, 15 and 30 min . The cones were then incubated in thioglycollate medium for the determination of microbial growth. I, RESULTS: The results showed that 2.5% sodium hypochlorite and 2.2% glutaraldehyde was not effective after 5, 10 and 15 min, whereas 30 min was necessary to obtain sterilization. . 3% hydrogen peroxide and 5% isopropyl alcohol produced better results, where 10 and 15 min treatment produced higher percentage of disinfection. 100% disinfection of gutta-percha cones was achieved only with 30 min treatment for all the tested disinfectants. CONCLUSIONS: All four chemical disinfectants failed to disinfect gutta-percha cones artificially contaminated with G. stearothermophilus in 5 min in the given condition. 5% isopropyl alcohol produced better results, where 10 and 15 min treatment produced higher percentage of disinfected tubes. 100% disinfection of gutta-percha cones was achieved only with 30 min treatment for all the tested disinfectants.
KEYWORDS: sodium hypochlorite, glutaraldehyde ('Cidex'), G. stearothermophilus.

INTRODUCTION
Control of infection is at most responsibility of the dentist. Adequate universal methods of infection control must be employed to prevent the risk of transmission of infection within dental practice. So sterilization of endodontic instruments and materials is essential in maintaining the chain of asepsis and in preventing introduction of pathogenic microorganisms into root canal system.

No single chemical disinfectant or method will be effective or practical for all decontamination situations. Therefore, when selecting chemical disinfectants and procedures, the purpose for decontamination and the interacting factors must be Considered.

In the study, GP points of 80 no. were artificially contaminated with Geobacillus stearothermophilus and the decontamination of such infected cones was studied using the four disinfectants i.e. sodium hypochlorite, gluteraldehyde, hydrogen peroxide and the isopropanol. The concentrations of all four chemical disinfectants were kept to the usual concentration at which they are used in the laboratory. The aim of the study was to evaluate the effectiveness of different sterilizing agents for the cold sterilization of gutta-percha cone.

Aims & Objectives
Aim: To assess the cold sterilization (chemical sterilization) of gutta-percha by different chemical agents.

Objective: To evaluate the effectiveness of different sterilizing agents for cold sterilization of gutta-percha.

MATERIALS AND METHODS
Materials
A. Gutta-percha cones: No.80, 2%(Dentsply France SAS).
B. Bacteria: Geobacillus stearothermophilus ATCC 7953 as swab sticks (Microbiologics® Inc. USA).
C. Media: Thioglycollate medium (Hi Media Laboratories, India) in the form of lyophilized powder.
D. Chemical disinfectants i.e. Sodium hypochlorite 5%, gluteraldehyde 25%, hydrogen peroxide 10% and isopropanol (absolute) (Qualigens, India.)
E. Glassware: Glassware i.e. test tubes, culture tubes (Borosil, India).

**Scheme of study**

A total no. of 540 gutta-percha cones 80 no. (Dentsply®, Switzerland) ISO colour coded arranged in a package of 20 cones were used for the study. The groups were divided with 10 gutta-percha cones per group. The study was carried out three times at different time intervals hence 30 cones were used in total for each group.

1) Group – I (Negative control): This group of gutta-percha cones were not artificially contaminated. This group represented the microbiological status of packed gutta-percha cones.

2) Group – II (Positive control): This group of gutta-percha cones were artificially contaminated but did not receive any chemical disinfectant. The group represented the optimized bacterial growth on gutta-percha cones.

3) Group - III: This group of gutta-percha cones were artificially contaminated with bacteria and received chemical treatment for 5 min. Based on the chemical disinfectants, this group was further subdivided into four subgroups.

4) Group - IV: This group of gutta-percha cones were artificially contaminated with bacteria and received chemical treatment for 10 min. Based on the chemical disinfectants, this group was further subdivided into four subgroups.

5) Group – V : This group of gutta-percha cones were artificially contaminated with bacteria and received chemical treatment for 15 min. Based on the chemical disinfectants, this group was further subdivided into four subgroups.

6) Group - VI: This group of gutta-percha cones were artificially contaminated with bacteria and received chemical treatment for 30 min. Based on the chemical disinfectants, this group was further subdivided into four subgroups.

**Four chemical disinfectants were used in the study**

1. Sodium hypochlorite: 2. 5% sodium hypochlorite was used in the study. Since sodium hypochlorite was available in 5% concentration, the solution was used without further dilution.

2. Gluteraldehyde: 2.2% gluteraldehyde was used in the study. Gluteraldehyde was available in ............% concentration. To prepare 2% solution, the source solution was diluted ..... times to get 2.2% solution.
3. Hydrogen peroxide: 3% hydrogen peroxide was used in the study. It was available in ..........% concentration. To prepare 3% solution, the source solution was diluted ..... times to get 3% solution.

4. Isopropanol: 5% concentration of isopropyl alcohol was used in the study. To prepare this, 5 ml of absolute isopropyl alcohol was mixed with 95 ml of autoclaved distilled water.

Artificial contamination and decontamination studies were performed under strict aseptic conditions and under a vertical laminar air flow hood (Re-Scholar, India). Gutta-percha cones of each group with 10 replicates were immersed in 1.5 ml spore suspension of *Geobacillus stearothermophilus* ATCC 7953 for 30 min. After 30 min, the cones were aseptically transferred to petri dishes covered with sterile filter paper and kept at 37°C in a dry heat sterilizer until dry.

Gutta-percha cones of group 2 (positive control, 10 cones) were immediately transferred to 5 ml thioglycollate medium in order to confirm the contamination. Gutta-percha cones (80 no.) of group 3 were transferred to their respective chemical disinfectant exactly for 5 min. Out of 40 cones, 10 cones were placed in 25 ml of 2. 5% sodium hypochlorite, 10 cones were transferred to 25 ml of 2. 2% gluteraldehyde, 10 cones were transferred to 25 ml of 3% hydrogen peroxide and 10 cones were shifted to 25 ml of 5% isopropyl alcohol. Exactly after five minutes, the cones were taken out from their respective disinfectant and washed separately with 20 ml of detergent solution (Tween-20, SRL, India) for 5 min and rinsed with 10 ml of autoclaved distilled water for 10 min to neutralize the toxic effects of chemical disinfectants. Each cone was then shifted to 5 ml of thioglycollate medium and tubes were incubated at 60°C. The tubes were checked for bacterial growth every 24 hour till 48 hours.

Gutta-percha cones (80 no.) of group 4 were transferred to their respective chemical disinfectant exactly for 10 min. Out of 40 cones, 10 cones were placed in 25 ml of 5% sodium hypochlorite, 10 cones were transferred to 25 ml of 2% gluteraldehyde, 10 cones were transferred to 25 ml of 3% hydrogen peroxide and 10 cones were shifted to 25 ml of 5% isopropyl alcohol. Exactly after ten minutes, the cones were taken out from their respective disinfectant and washed separately with detergent solution as done for group 3 shown above and rinsed with 10 ml of autoclaved distilled water for 10 min. Each cone was then shifted to 5 ml of
thioglycollate medium and tubes were incubated at 60°C. The tubes were checked for bacterial growth every 24 hour till 48 hours.

Gutta-percha cones (80 no.) of group 5 were transferred to their respective chemical disinfectant exactly for 15 min. Out of 40 cones, 10 cones were placed in 25 ml of 2.5% sodium hypochlorite, 10 cones were transferred to 25 ml of 2.2% gluteraldehyde, 10 cones were transferred to 25 ml of 3% hydrogen peroxide and 10 cones were shifted to 25 ml of 5% isopropyl alcohol.

Exactly after 15 minutes, the cones were taken out from their respective disinfectant and washed separately with detergent solution as done for group 3 shown above and rinsed with 10 ml of autoclaved distilled water for 10 min. Each cone was then shifted to 5 ml of thioglycollate medium and tubes were incubated at 60°C. The tubes were checked for bacterial growth every 24 hour till 48 hours.

Gutta-percha cones (80 no.) of group 6 were transferred to their respective chemical disinfectant exactly for 30 min. Out of 40 cones, 10 cones were placed in 25 ml of 2.5% sodium hypochlorite, 10 cones were transferred to 25 ml of 2.2% gluteraldehyde, 10 cones were transferred to 25 ml of 3% hydrogen peroxide and 10 cones were shifted to 25 ml of 5% isopropyl alcohol. Exactly after 30 minutes, the cones were taken out from their respective disinfectant and washed separately with detergent solution as done for group 3 shown above and rinsed with 10 ml of autoclaved distilled water for 10 min. Each cone was then shifted to 5 ml of thioglycollate medium and tubes were incubated at 60°C. The tubes were checked for bacterial growth every 24 hour till 48 hours.

Microbial growth was analysed after every 24 hour by visualization of turbidity of the culture medium. The growth was scored on the basis of turbidity between no turbidity (negative control) and maximum turbidity (4+). Each set of experiment was performed three times at different time intervals with fresh culture medium as well as fresh chemical disinfectants.

RESULTS

Growth of *Geobacillus stearothermophilus* ATCC 7953

*G. stearothermophilus* is a facultative anaerobe and hence grew at the centre of the medium while inoculated from swab. Once, sufficient growth is achieved, the clump floats on to the surface of semisolid medium.
The growth started to be visualized after 24 hours when incubated at 60°C. After 48 hours, the growth could be clearly seen as a clump (Plate 1).

The bacterium was inoculated on thioglycollate medium for 24-48 hours. Before using the infected broth, the turbidity of the medium was adjusted at 620 nm to 0.13 in order to get $10^6$ to $10^7$ spores per ml of the suspension.

As a negative control, 10 gutta-percha points were directly inoculated in to 5 ml of thioglycollate medium (M191, HiMedia Labs, India) to ensure the previous sterilization. No turbidity in the suspension was observed which states that gutta-percha cones were packed under sterile conditions and unopened cones were still sterile. All three sets of experiments showed that gutta-percha were sterile before starting the experiment.

As a positive control 10 gutta-percha cones were artificially contaminated with inoculated with \textit{G. stearothermophilus} and placed in thioglycollate medium. The turbidity was visualized at 24 and 48 hours subsequently. All 10 positive controls showed turbidity (bacterial growth) after 24 hours which increased after 48 hour observation. The positive control in all three replicate sets confirmed the successful artificial contamination of gutta-percha cones.

Four sets of gutta-percha cones (each having 10 replicates), artificially infected with \textit{G. stearothermophilus} and placed in 2.5% sodium hypochlorite solution. The treatment was given for 5, 10, 15 and 30 min. The treated points were then immersed into 5% Triton-X 100 solution and then washed with 10 ml of sterile distilled water to get rid of the toxic effects of the chemicals itself. All the cones were then immediately placed into thioglycollate medium (5 ml). All the tubes were incubated at 60±2°C in a bacteriological incubator (Jindal, India). The experiment was repeated three times at different time intervals.

After 24 hours, growth could be visualized in 10 out of 10 replicates which received 5 min treatment clearly suggesting that after 24 hours, sodium hypochlorite did not show significant difference between treatments of 5 min in all three runs.

The 10 min treatment with 2.5% sodium hypochlorite produced as much as 8 tubes with positive growth in all three times. The results suggest, though not fully effective, 10 min treatment of sodium hypochlorite produced small degree of disinfection. The same case was with 15 min treatment, where all cones could not be disinfected though the quantity of
disinfected cones increased as compared to 10 min treatment. However, 30 min treatment produced 100% disinfected gutta-percha cones all three times.

Table 1: Bacterial growth obtained after artificially infecting gutta-percha cones with G. stearothermophilus ATCC 7953 and treated with sodium hypochlorite 2.5%. Test was repeated three times at different time intervals. Negative growth in replicates shows level of disinfection at different times of exposure of gutta-percha cones to the chemical.

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>No. of replicates</th>
<th>Test 1</th>
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-ve= no bacterial growth +ve = positive growth of bacteria

Treatment of gutta-percha cones artificially contaminated with G. stearothermophilus and treatment with 2.2% gluteraldehyde showed that treatment with gluteraldehyde for 5 min did not disinfected the contaminated gutta-percha cones. The number of disinfected cones increased with the increase in the time of treatment. The 10 min treatment produced 70-80% tubes with bacterial growth (20-30% disinfection rate) and 15 min treatment was able to produce up to 50% cones disinfected. However, 30 min treatment was able to produce 100% disinfection at all three times (Table 2).
Table 2: Bacterial growth obtained after artificially infecting gutta-percha cones with G. stearothermophilus ATCC 7953 and treated with Glutaraldehyde 2.5%. Test was repeated three times at different time intervals. Negative growth in replicates shows level of disinfection at different times of exposure of gutta-percha cones to the chemical.

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-ve = no bacterial growth  +ve = positive growth of bacteria

Table 3: Hydrogen peroxide 3%. Test was repeated three times at different time intervals. Negative growth in replicates shows level of disinfection at different times of exposure of gutta-percha cones to the chemical

<table>
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-ve = no bacterial growth  +ve = positive growth of bacteria
5% isopropyl alcohol produced somewhat better results, where 10 and 15 min treatment produced large percentage of disinfected tubes, though 100% disinfection was achieved only with 30 min treatment. 5 min treatment failed to produce marked degree of disinfection.

Table 4: Isopropyl alcohol 5%. Test was repeated three times at different time intervals. Negative growth in replicates shows level of disinfection at different times of exposure of gutta-percha cones to the chemical.

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-ve= no bacterial growth   +ve = positive growth of bacteria

When compared the bacterial growth for 24 and 48 hours with or without chemical treatment no marked difference could be observed in the groups receiving 5, 10, 15 or 30 min treatment (Fig 1).
DISCUSSION
Endodontic therapy involves the thorough cleaning, preparation and obturation of the root canal system. The root canal space should be sealed with an inert material that will prevent percolation of fluid and bacteria between the root canal and the apical tissues. Gutta-percha has been accepted universally as the “Gold standard” filling material and it is the most commonly used root canal obturation material. It is compressible, inert, dimensionally stable, tissue tolerant radiopaque and becomes plastic when heated.\(^1\)

*Geobacillus stearothermophilus* ATCC 7953 is used for studies of disinfections and sterilization. The bacterium is thermophillic, able to replicate at the temperatures up to 65°C and have spore forming abilities which makes its use to study the high level of disinfection and sterility.

Contamination of gutta percha cone in single cone obturation techniques causing inadequate monobloc formation and hampered hermatic seal.

Gutta-percha cones are used for obturation of root canals and are meant to provide no space for the microorganisms to reappear into the root canal. Such cones are believed to be sterile when packed and should be sterile till opened. However, Pang et al\(^2\) showed that approximately 20% of gutta-percha cones if left untreated can get contaminated by vegetative bacterial cells. Similar results by Gomes et al.\(^3\) showed 5.5% gutta-percha cones to be infected by *Staphylococcus epidermidis*.\(^4\) showed that at least 8% of the packed gutta-percha cones were infected with *Bacillus* sp. They also highlighted the need of decontaminating gutta-percha by chemical means. According to Linke and Chohayeb\(^5\), gutta-percha may get contaminated by a variety of microorganisms such as cocci, rods and yeasts after exposure to dental operatory environment. Although GP points are prepared under aseptic conditions and usually pre sterilized with ethidium oxide gas before packing, there is a great risk of contamination of GP points due to handling, aerosols and from other sources during storage\(^6\), Gomes *et al*.\(^5\) This does not guarantee the sterility of GP points especially once they are removed from their initial packing. It is quite obvious that the obturation done with an infected GP point may allow the residual bacteria and/or bacteria present on GP point in the roots canal system. In both the cases, the success rate of root canal therapy will fall.

However there is controversy as to whether the sterilization process is necessary for gutta-percha cones firstly because of its own antimicrobial activity due to zinc oxide\(^7\) and
secondly due to the use of sealer during obturation which has considerable antibacterial activity. Nevertheless the sterilization of gutta-percha is often recommended in most of the cases to increase effectiveness of root canal treatment and reduce the chances of reinfection. Since, gutta-percha cones are made up of plant latex, autoclaving or any heat treatment is not advisable for sterilization of gutta-percha cones in order to avoid any change in its chemical and physical properties. The thermoplastic characteristic of gutta-percha cones prohibits sterilization by standard autoclave, which may cause deformation of cones. The chemical sterilization is always the method of choice.\(^8\)

A chemical agent is considered effective in disinfection or sterilization of any given material, if the chemical is able to remove any resistant bacteria in the concentration of \(10^7-10^8\) CFU ml\(^{-1}\) (4). A variety of chemical disinfectants have been used to sterilize gutta-percha cones. Sodium hypochlorite; a strong oxidising agent, has been used in most of the studies in various concentrations and various time of treatment to gutta-percha cones.\(^9\),\(^10\) Apart from sodium hypochlorite, gluteraldehyde and the preparations containing gluteraldehyde have also been used in a variety of studies (L,M). Apart from this chlorhexidine and Chloraprep\(^2\) have been reported. Our results show that for the effective sterilization of gutta-percha contaminated with \textit{G. stearothermophilus} at least 30 min is required for proper sterilization. \textit{Bacillus stearothermophilus} is constantly used in the biotech industry to test the success of sterilization cycles of equipment. Due to the bacteria’s high resistance to heat, it is a suitable Biological Indicator of microbe life after a sterilization cycle. Earlier studies showed that sodium hypochlorite is an effective agent for disinfection of gutta-percha cones. Gomes et al showed the effectiveness of 5.25% sodium hypochlorite for disinfection of gutta-percha cones against \textit{Enterococcus faecalis}. In the study only sodium hypochlorite and chlorhexidine was used. In other study\(^12\), the tested gutta-percha could be sterilized by 2.5% sodium hypochlorite in 5 min when artificially contaminated by \textit{Bacillus subtilis} ATCC 6633. The study also showed the gluteraldehyde (2%) failed to disinfect gutta-percha in 15 min of treatment. The studies have revealed that 2% chlorhexidine and 5.25% sodium hypochlorite have same antimicrobial performance. However, no general agreement exists regarding the optimal time of action for decontamination of gutta-percha cones. Our results show that gluteraldehyde shows efficacy only after 30 min of treatment which is in line with most of the studies, though the target microorganism differed in most of the studies. Certain species of \textit{Bacillus} and \textit{Clostridium} are capable of producing endospores. Such endospores are more resistant to physical and chemical agents in comparison to other vegetative forms. In this way
such species provide a form of survival rather than reproduction. Since, gutta-percha can get contaminated with any of the contaminating bacteria including bacillus and other vegetative genera; we suggest that such disinfection studies should be planned with most resistant bacteria in order to find out best possible chemical agents. Our results are in contrast with the study of Da Motta et al.\textsuperscript{[11]} They described that 2.5\% sodium hypochlorite was effective in 5 min for disinfection of gutta-percha cones contaminated with Bacillus stearothermophilus ATCC 7953. Though they also showed inefficacy of 2.2\% gluteraldehyde for such disinfection. The possible cause may be the difference in culture conditions and/or quality of the chemicals used in the study. Since no similar study is available, we advocate further research in this direction. Similarly, hydrogen peroxide and the isopropyl alcohol have not been used in disinfection studies against Bacillus stearothermophilus ATCC 7953, though during our experiments, such chemicals have provided enough evidences of their potency to disinfect gutta-percha cones. The use of chemical agents to disinfect gutta-percha has also led to the deterioration of gutta-percha cones, and hence the use of excessive chemical agents should be limited. Valois et al\textsuperscript{[10]} showed via atomic force microscopy that exposure of gutta-percha to 5.25\% sodium hypochlorite after 1 min increased the elasticity of gutta-percha cones. Similarly, Pang et al. (saunders\textsuperscript{7}) showed that gutta-percha cones immersed in 5.25\% sodium hypochlorite showed a precipitate with a cubical structure over the entire surface of gutta-percha cones. Other chemicals such as chlorhexidine and Chloraprep showed a shrinkage pattern along the long axis of the cones.

REFERENCES


