

Comparative Evaluation Of Bacterial Contamination Of Bioceramic And Non-Bioceramic Gutta-Percha Cones Before And After Clinical Use And Disinfection Of Gutta-Percha Cones Using Herbal Irrigants – An In-Vitro Study.

Running Title: Gutta Percha Cones Disinfection Using Herbal Irrigants.

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Abstract

Context:

Gutta Percha cones are manufactured under aseptic circumstances; however, contamination occurs due to incorrect storage and handling by the operators. Immersing the GP cones in a 5.25% NaOCl solution for one minute is indicated for disinfection. Recently, the use of phytomedicines derived from natural plants for disinfection has proved various advantages.

Aim: To evaluate and compare the microbial contamination before and after clinical use and chairside disinfection of Bioceramic Gutta Percha cones and Non-Bioceramic Gutta Percha cones, with Sodium Hypochlorite, Calendula Officinalis and Thymus Vulgaris, using culture media – Brain-Heart Infusion agar.

Settings and Design/Methods and Material:

Twelve Bioceramic GP cones and twelve Non-Bioceramic GP cones were collected from freshly opened boxes. Gutta Percha boxes were in clinical use for four weeks. The microbes were evaluated using culture media. In the case of positive result for contamination, for each GP cone, a Chairside Disinfection Protocol (comprising of usage of either NaOCl, Calendula Officinalis or Thymus Vulgaris) was tested. The Gutta-percha cones were again cultured and evaluated for CFUs.

Statistical analysis used:

The data was analysed statistically with Statistical Package for Social Sciences (IBM SPSS Statistic for window, version 21.0. Armonk, NY: IBM Corp.)

Results:

No growth of bacteria was seen with the gutta-percha cones obtained from the freshly opened boxes.

Bacterial contamination was seen to be greater with bioceramic gutta percha than with non-bioceramic gutta-percha.

Highest disinfection was achieved by Sodium Hypochlorite, followed by Calendula Officinalis and Thymus Vulgaris.

Conclusions:

Within the limits of this in-vitro study, it can be concluded that herbal irrigants exhibit significant antibacterial activity against endodontic pathogens and can be utilised as a substitute for sodium hypochlorite, while the latter still remains the gold standard.

Key-words: *Bioceramic, Calendula, Disinfection, Gutta-Percha, Sodium Hypochlorite, Thymus plant*

Key Messages: *There is significant contamination of the gutta-percha cones when in clinical use. Herbal irrigants exhibit significant antibacterial activity against endodontic pathogens.*

INTRODUCTION:

Preventing reinfection of the root canal system (RCS) and minimizing the potential growth of any bacteria that may remain after the chemo-mechanical preparation is the paramount goal of root canal filling.¹

Reinfection can occur even after meticulous endodontic procedures. Using contaminated gutta-percha cones could be one of the reasons for this.² There are studies that have shown microorganisms in freshly opened gutta-percha boxes.^{3,4} Although gutta-percha cones had few bacteria in packaging, clinical use increased their contamination.^{3,4}

Gutta Percha cones are said to be the ideal material for obturating the root canal. Bioceramic Gutta Percha cones are more biocompatible and exhibit improved bonding.¹ Gutta Percha cones are manufactured under aseptic circumstances; however, contamination occurs due to incorrect storage, exposure to aerosols, and handling by the operators.¹

Because it is thermolabile, Gutta Percha cones cannot be sterilized using either wet or dry heat.

For maintaining the aseptic chain, ensuring the sterility of endodontic equipment and materials is essential.⁵ Therefore, it is recommended to use rapid chair-side chemical disinfection for the purpose of disinfecting Gutta-percha cones.⁵ Immersing Gutta Percha points in 5.25% Sodium Hypochlorite (NaOCl) for one minute disinfects them without changing topography.¹

Herbal products have been utilized since ancient times for their antibacterial, antifungal, antioxidant, and anti-inflammatory properties. Plant-based phytomedicines have many advantages over synthetic pharmaceuticals. Although herb therapy is ancient, it has recently gained popularity and importance. In addition to being safe, easy, and affordable, these herbal products have a longer shelf life and no microbial resistance so far.⁶

This in vitro study aimed to evaluate and compare the microbial contamination before and after clinical use and chairside disinfection of Bioceramic Gutta Percha cones and Non-Bioceramic Gutta Percha cones, with Sodium Hypochlorite, Calendula Officinalis and Thymus Vulgaris, using culture media - Brain-Heart Infusion agar.

SUBJECTS AND METHODS:

Gutta Percha cones collection and evaluation of their contamination:

Twelve Bioceramic Gutta Percha cones and twelve Non-Bioceramic Gutta Percha cones (Sure-endo Ltd.) of the same size were collected from freshly opened boxes using sterile gloves and tweezers.¹ Following this, twelve Bioceramic Gutta Percha cones and twelve Non-Bioceramic Gutta Percha cones of the same size as the previous GP cones were collected from boxes that were kept for one month intentionally in chair-side working environment and used by operators who will not be involved in the study.¹

Collection of the Gutta Percha cones in clinical environment:

Gutta Percha were used for 4 weeks after opening. On an average, each box facilitated a total of 8 appointments every week. Boxes were sealed and stored until their next appointment.¹

The Gutta Percha after one month of clinical usage were collected, using sterile gloves and tweezers.¹ Grouping of the obtained Gutta Percha cones were done as follows:

- Group A-Bioceramic Gutta Percha cones (Gutta Percha obtained from freshly opened boxes)
- Group B-Non-Bioceramic Gutta Percha cones (Gutta Percha obtained from freshly opened boxes)
- Group C-Bioceramic Gutta Percha cones (In clinical environment)
- Group D-Non-Bioceramic Gutta Percha cones (In clinical environment)

These were further divided into groups based on the decontaminant used.

Group C was further divided into:

- Group C (I)- Disinfection using NaOCI
- Group C (II)- Disinfection using Calendula Officinalis
- Group C (III) - Disinfection using Thymus Vulgaris

Group D was further divided into:

- Group D (I) - Disinfection using NaOCI
- Group D (II) - Disinfection using Calendula Officinalis
- Group D (III) - Disinfection using Thymus Vulgaris
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Evaluation of Microbes using Culture media:

After overnight cultivation in brain-heart infusion broth, Gutta Percha cones were streaked on agar. They were incubated at 37°C for 24 hours. Colony Forming Units (CFUs) were then measured with a digital colony counter.⁷

Preparation of disinfecting solutions/extracts:

Sodium hypochlorite: 5.25% NaOCI (Prime Dental Limited)

Calendula Officinalis: The Calendula Officinalis extract (SBL CO Mother Tincture Q, SBL Pvt Ltd, India). CO solution was mixed with sterile saline to obtain concentration 30µL/ml).⁸

Thymus vulgaris: 30 g of thyme powder in 300 ml of sterilized distilled water was heated at 90°C for two hours. Filtration of this extract was done using filter paper (Whatman No. 1) and refrigerated at 5°C. 20% concentration of the extract was used.⁶

Chairside disinfection protocol:

In the case of positive result for contamination, for each Gutta Percha cone, a Chairside Disinfection Protocol was tested.

The cones were kept in contact and completely emersed with each disinfectant for one minute in an Eppendorf tube, and then finally rinsed with distilled water.¹

Culture media:

The Gutta-percha cones were again cultured in the same way as mentioned previously and evaluated for CFUs

Statistical Analysis:

The data was analysed statistically with Statistical Package for Social Sciences (IBM SPSS Statistic for window, version 21.0. Armonk, NY: IBM Corp.) at 95% CI and 80% power to the study.

Unpaired t test was applied to check statistical significant difference in the bacterial contamination of gutta percha in clinical environment.

ANOVA with Tukey's post hoc test was applied to check statistically significant difference in Bacterial Contamination after disinfection for 1 minute.

Statistical significance was kept at $p < 0.05$.

RESULTS:

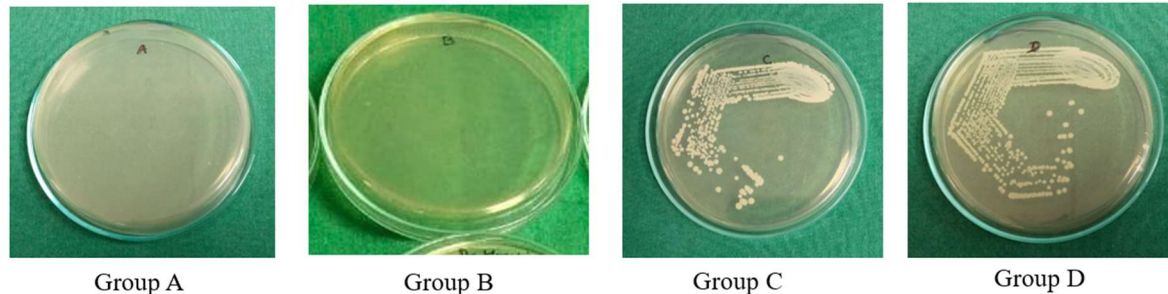


Fig: Evaluation of microbes using culture media

TABLES:

Table 1: Mean Bacterial Contamination in Group A, B, C and D

	Groups	N	Mean	Std. Deviation
Bacterial Contamination	Group A	12	0	0
	Group B	12	0	0
	Group C	12	351.7500	136.79123
	Group D	12	450.0833	81.13564

Table 2: Comparison of Bacterial Contamination after disinfection for 1 minute in disinfectant solutions

Groups		Mean Difference	p value	F value	p value
NaOCl	Calendula Officinalis	-20.00000	.201	83.727	<0.001*

	Thymus Vulgaris	-134.75000*	<0.001*		
Calendula Officinalis	NaOCl	20.00000	.201		
	Thymus Vulgaris	-114.75000*	<0.001*		
Thymus Vulgaris	NaOCl	134.75000*	<0.001*		
	Calendula Officinalis	114.75000*	<0.001*		

Growth of bacteria was seen with the gutta-percha cones obtained from the freshly opened boxes. (Table 1)

Bacterial contamination was seen with the bioceramic and non-bioceramic gutta percha in clinical use, having mean values 351.7500 and 450.0833 and standard deviations 136.79123 and 81.13564 respectively. (Table 1)

Highest disinfection was achieved by Sodium Hypochlorite with mean value of CFUs being 0 and Standard deviation 0, followed by Calendula Officinalis and Thymus Vulgaris. (Table 2)

DISCUSSION:



Disinfection in Sodium hypochlorite solution



Disinfection in Calendula Officinalis solution



Disinfection in Thymus Vulgaris Solution

Fig : Chairside disinfection protocol with Disinfectants

The success of endodontic therapy relies on the strict adherence to a sterile method throughout the whole process, from the access opening to the post-obturation restoration of the tooth.

For optimum infection control, all equipment and materials used should be sterile.⁵

Currently, the most popular root canal core filling material is gutta-percha. Commercially available GP cones have various advantages, including biocompatibility, radiopacity, dimensional stability, ease of removal from root canals, antibacterial properties, etc. Zinc oxide underlies gutta-percha's

antibacterial properties.⁹

Bioceramic gutta-percha is impregnated and coated with a nanolayer of bioceramic particles, which has antimicrobial qualities and enhances adaptability, resulting in a perfect three-dimensional root canal obturation.

The results of this study showed that the cones in the freshly opened gutta-percha packages were not contaminated but microbial contamination occurred upon clinical use of the packages.

Due to its clinical success and simplicity, the cold lateral compaction technique is the most used endodontic obturation technique. This technique can contaminate the box's gutta percha points since the tweezers repeatedly touch them. Cross-contamination is a risk when one box is used in multiple NSRCTs, calling the treatment's success into doubt. It is recommended to use two tweezers: one to pick up and the other to place the GP point into the RCS.¹

Gomes et al. found that 100% of gutta-percha cones handled with gloves had microbial growth, indicating the importance of disinfection.¹⁰ Several studies have investigated cold sterilization agents for gutta-percha.¹¹ The current study tested and compared the antibacterial efficiency of *Calendula officinalis*, *Thymus vulgaris* and 5.25% NaOCl.

Sodium hypochlorite 5.25% has been established to be the gold standard for disinfecting gutta-percha cones. The antibacterial activity of sodium hypochlorite is mostly attributable to hypochlorous acid (HClO) in the solution, which has an oxidative action on the sulfhydryl groups of bacterial enzymes. Several studies demonstrated that 5.25% NaOCl is effective if a 60-second immersion period is used for disinfection of gutta-percha cones, without causing significant topographic changes.^{11,12}

Calendula Officinalis (CO) is an extract of the flowers of pot marigold that is usually prepared as an alcoholic tincture. It has been utilised for its anti-inflammatory, antipyretic, anticancer, and cicatrizing properties. They have flavonoids, coumarins, essential oils, carotenoids, glycosides, sterols, and fatty acids. It possesses potent antimicrobial property.^{13,14,15}

The major ingredients of Thyme are essential oil, tannin, flavonoids, saponins, and Triterpenic acid. They can dissolve Gram-negative bacteria's outer membrane.¹⁶

This study showed that Sodium Hypochlorite is the most effective chair-side disinfectant among the three. This is due to its antibacterial and sporicidal activity related to the liberation of active chlorine.¹⁰

Calendula Officinalis gave better disinfection results than *Thymus Vulgaris*, since *Calendula Officinalis* has phenolic and saponin components which warranty its antibacterial and anti-oxidative activities.⁷

Also, although *Thymus Vulgaris* has tannins and phenols, which affect the enzymatic system of bacteria, it has no antibacterial activity against *B. subtilis* and *S. aureus*, as stated by Mohammed Messaoudi et al.¹⁷

Further, there was statistically significant difference in the bacterial contamination between the bioceramic and non-bioceramic gutta-percha. Antibacterial activity of Bioceramic Gutta percha can be attributed to the development of the nanostructure of the calcium aluminate particles.¹⁸

The results of this study concerning the microbial status of the freshly opened gutta-percha cones align with those of Doolittle, et al.¹⁹ and Pang, et al.²⁰, who observed the gutta-percha cones obtained from freshly opened boxes showed no signs of microbial growth.

However, these results dissent the findings of Montgomery²¹ and Namazikhah, et al.²², who reported

the contrary.

However, these research differ in terms of methodology. Furthermore, the gutta-percha box brands used in these tests are different.

The antimicrobial activity shown by *Thymus Vulgaris* in this study complies with the study by Gupta et al.⁶ *Calendula Officinalis*' antibacterial efficacy results were consistent with Chakraborty's study.¹³ Kavitha et al. stated that the action was due to the flavonoid fraction in the extract.²³

Overall, the findings of this study demonstrated that herbal irrigants exhibit significant antibacterial activity against endodontic pathogens and can be utilised as a substitute for sodium hypochlorite, while the latter still remains the gold standard.

CONCLUSION

Within the limits of this in-vitro study, it can be concluded that there is significant bacterial contamination of the GP cones when in clinical use. Iatrogenically, if the other gutta-percha cones come in contact with the tweezer already contaminated by the patient's saliva, then the gutta-percha box will have to be discarded, or else chairside disinfection protocol will have to be carried out.

Sodium Hypochlorite showed maximum disinfection of Gutta Percha followed by *Calendula Officinalis* and *Thymus Vulgaris*.

Further research can be carried out to compare the contamination among various sizes of gutta-percha. Further, also the quantification and identification of the contaminant species of bacteria can be carried out.

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Figure Legends:

Figure 1 - Colony Forming Units on Brain-heart infusion agar.

Figure 2 – Rapid chair-side disinfection of Gutta Percha Cones.

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