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Original Research

Comparative analysis of commercial gutta percha with chitosan impregnated gutta percha and chlorhexidine active gutta percha points for testing anti-microbial efficacy against enterococcus faecalis: An in-vitro study

[1]Ishita Dwivedi, [2]Sonal Dhote, [3]Pankaj Kumar Gupta, [4]Neha Jaju, [5]Sneha

[1]MDS 3rd Year, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh, India;
[2,4]Reader, [3]Professor, [5]Senior Lecturer, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh, India

ABSTRACT:
Background: The success of root canal treatment relies upon complete removal of microorganisms without any odds of reinfection. Enterococcus faecalis is a facultative gram-positive coccus which has been classified as one of the most resistant oral pathogens, especially in secondary and persistent resistant root canal infections. Therefore, to avoid growth of such microorganisms, endodontic obturating materials should ideally have antibacterial effect to prevent reinfection. **Aim:** This study was performed to evaluate and compare the anti-microbial efficacy of Commercial Gutta percha with Chitosan impregnated Gutta percha & Chlorhexidine active gutta percha points against Enterococcus faecalis. **Methodology:** A total of 100 specimens were selected for the study. Assessment of antimicrobial action of gutta percha points impregnated with Chitosan, commercial gutta percha points and Chlorhexidine gutta percha points was done by measuring the inhibition of E. faecalis growth. Agar plates were inoculated with E. faecalis and four wells were made on the plates. The materials to be tested wereGROUP 1:Commercial Gutta-percha, GROUP 2:Chlorhexidine active gutta percha GROUP 3:Chitosan impregnated gutta-percha and GROUP 4: Normal Saline were placed on the plates. After 24hours incubation; diameter of zone of inhibition around gutta-percha points was considered to assess the antimicrobial activity. **Results:** Chitosan impregnated gutta percha was more effective against Enterococcus faecalisthan Chlorhexidine active-gutta percha points and Commercial gutta percha. **Conclusion:** Enterococccus faecalis was more sensitive to Chitosan impregnated gutta percha than Chlorhexidine active gutta percha points.
Keywords: Enterococcus faecalis, Chitosan, Chlorhexidine active gutta percha points.

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Corresponding author: Ishita Dwivedi, MDS 3rd Year, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh, India

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INTRODUCTION

The line of treatment for endodontic infections is based on mechanical removal of pulp tissue, as well as bacteria located in the cell debris and attached to the wall of the root canal[1].

Moreover, the main reason of root canal re-treatment is the persistence of microorganisms after root canal therapy[2] or inadequate coronal seal[3]. The microbial flora of re-treatment cases has been characterized as mono-infections of predominantly Gram-positive microorganisms with approximately equal proportions of facultative and obligate anaerobes[4].

Enterococcus faecalis is a facultative gram-positive coccus, most frequently isolated species and associated with 22-77% of root canal failure cases. It exhibits virulence factors as well as lytic enzymes, cytolysin, aggregation substances, pheromones and lipoteichoic acid. It adheres to host cells, express proteins that allow it to compete with other bacterial cells and alter host responses. It can suppress lymphocytes which leads to root canal failures[5] and is resistant against multiple antibiotics like β-lactams penicillin, amoxicillin, and clindamycin[6].It has virulence factors that permit the adherence to host cells and extracellular matrix that facilitate tissue invasion, and aggregates with other bacteria and produce biofilms[7].

Grossman in 1940 recommended that an ideal root canal filling material should be bacteriostatic. The zinc oxide content in gutta percha might give some antimicrobial properties, until recently contemporary gutta percha cones were neither bacteriostatic nor antiseptic[8].

Chlorhexidine impregnated gutta percha points also called 'activ points' (Roeko, Langenau, Germany) containing gutta percha matrix embedded with 5% chlorhexidine diacetate has been marketed.It is a cationic bisguanide and regarded as a broad spectrum anti-microbial agent[9]. It can be used as an irrigant and an intracanal medicament because of its ability to disinfect dentinal tubules against E. faecalis[10,11].

Chitin is extracted from marine shell waste with a liner structure and high molecular weight. It is composed of β-(1 → 4)-linked N-acetyl-D-glucosamine units. But due to its hydrophobicity it has limited applications.Its primary derivative is chitosan which is obtained by partial deacetylation of chitin. It has desirable biological properties like bioactivity biocompatibility, biodegradability, non-toxicity, muco-adhesiveness and broad-spectrum antimicrobial activity (against gram positive and negative bacteria)[12,13].

Therefore, the purpose of this study was to evaluate the antimicrobial activity against E. faecalis by comparing commercial gutta percha with chitosan impregnated gutta percha and chlorhexidine active points.

AIM

The purpose of this study was to evaluate and compare the antimicrobial efficacy of Commercial gutta-percha, Chitosan impregnated gutta-percha and Chlorhexidine gutta-percha in inhibiting growth of Enterococcus faecalis.

MATERIALS AND METHODS

Commercial Gutta-percha points (Dentsply), Chitosan impregnated gutta-percha and Chlorhexidine active gutta-percha points (Coltene Roeko) were used, against the bacteria Enterococcus faecalis (ATCC 35550). The Enterococcus faecalis strain was obtained from MTCC, Chandigarh. The strain was dissolved in Nutrient broth to make inoculum and then transferred to solid media (BHI) Brain Heart Infusion to get colonies for further use.

To carry out the chitosan coating, it was dissolved in 1% acetic acid (v/v). After manual coating, the points were left to dry to obtain a chitosan film around the gutta-percha points. Antimicrobial Susceptibility test was carried out using Kirby Bauer Diffusion method. The BHI media was sterilized in Autoclave at 121 °C for 15 minutes, 15 lbs pressure. The petri plates were sterilized in Hot Air Oven at 160 ° C for 1 hour. All microbiological procedure was carried out in Laminar Air Flow. To these sterilized plates, sterilized media was poured using pour plate method and kept inside to set at room temperature.

Inoculating loop was used to four wells were made equidistant to each other. **Group 1** was filled with Commercial Gutta Percha (n=25),**Group 2** with Chlorhexidine active gutta-percha points(n=25), **Group 3** with Chitosan impregnated Gutta percha(n=25) and **Group 4** with Normal saline as Control (n=25). These plates were subjected to incubation at 37 ° C for 24 hours. After 24 hours Zone of Inhibition was measured using Antibiotic Zone Reader scale and the zones of inhibition formed was categorized as Sensitive, Intermediate or Resistant zones. Accordingly, the antimicrobial agents were categorized as per the readings noted.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS (Statistical Package for the Social Science) Version 21. Descriptive Statistics were carried out to obtain mean and frequency for continuous variables. To compare the mean values of microbial growth inhibition between different groups Independent T Test was applied.

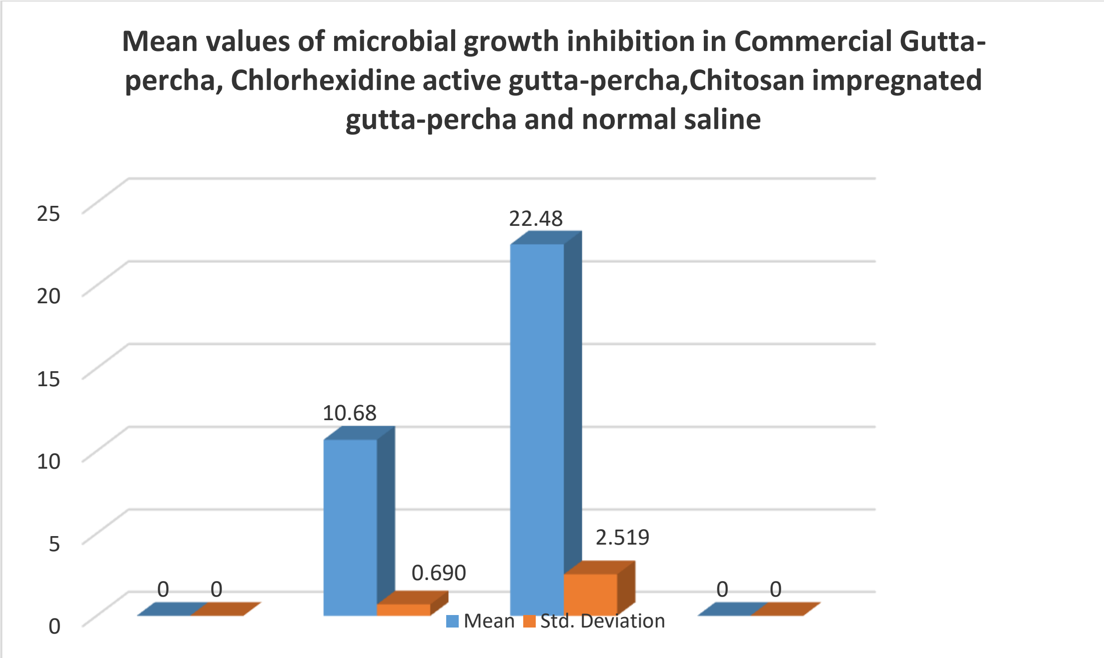
RESULTS

The mean and standard deviations of microbial growth inhibition in Commercial Gutta-percha, Chlorhexidine active gutta-percha, Chitosan impregnated gutta-percha and normal saline are summarized in table 1(A) and comparison of mean values of microbial growth inhibition are summarized in table 1(B).

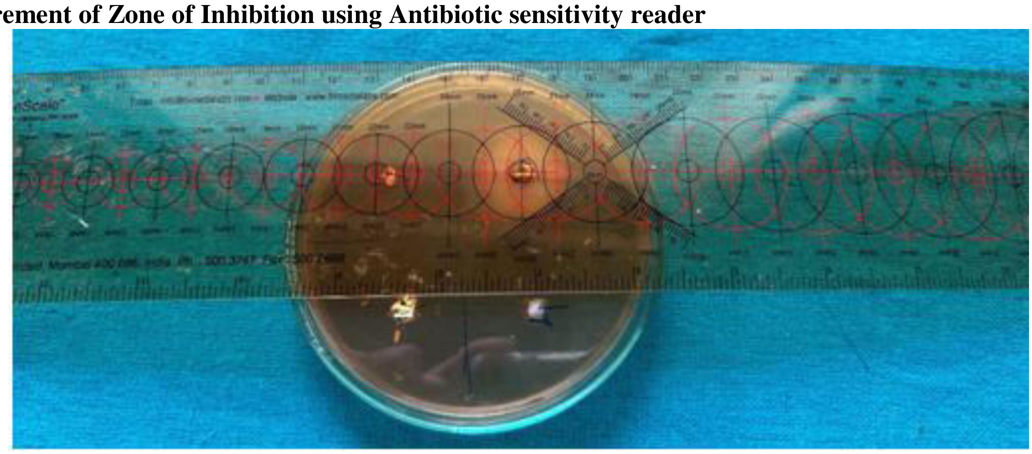
	N	Minimum	Maximum	Mean	Std. Deviation
Commercial gutta-percha	25	.00	.00	.0000	.00000
Chlorhexidine active gutta-percha points	25	10.00	12.00	10.6800	.69041
Chitosan impregnated gutta- percha	25	19.00	27.00	22.4800	2.51860
Normal saline	25	.00	.00	.0000	.00000

	Mean	N	Std. Deviation	Std. Error Mean	Mean difference	P value
Commercial gutta-percha	0	25	0	0		
Chlorhexidine active gutta-percha points	10.6800	25	.69041	.13808	-11.80000	.000
Chitosan impregnated	22.4800	25	2.51860	.50372		

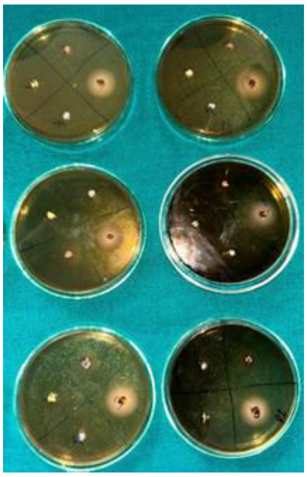
gutta-percha						
Normal saline	0	25	0	0		



Measurement of Zone of Inhibition using Antibiotic sensitivity reader



Zone of Inhibition formed post incubation



No Zone of inhibition seen around Commercial gutta percha. When gutta percha was impregnated with Chitosan the mean values of microbial growth inhibition were 22.48 ± 2.518 and in Chlorhexidine active gutta percha points the mean microbial growth inhibition was 10.68 ± .690.

When comparison was made between Chitosan impregnated gutta percha and Chlorhexidine active gutta percha, it was found that the difference in mean values was -11.800. And this difference was statistically significant ($p<0.05$).

DISCUSSION

Elimination of bacteria from the root canal system is essential for long-term success of endodontic treatment. Additionally, the root filling prevents infection by acting as a barrier to further microbial challenges, entombing any surviving bacteria within the root canal system and stopping periapical tissue fluids from reaching bacterial cells in the root canal[14].

Enterococcus faecalismay survive both chemo-mechanical preparation and intracanal medication and reside in the canal long after therapy. Therefore, further defences such as obturation materials, which prevent infection persistence, could prove valuable[15].

Enterococcus faecalis was chosen due to its potential as a possible microbial factor in therapy resistant apical periodontitis. It is a non-fastidious microbe that is relatively easy to culture and has been shown in-vitro to predictably penetrate dentinaltubules[1,16].It can grow in 6.5% NaCl, at temperatures ranging from 10°C to 45°C and can survive 30 minutes at 60°C and a pH over 9.6. Failed endodontic treatment cases are nine times more likely to contain E. faecalis than primary endodontic infections.It can live in extreme alkaline environment due to its proton pump activity, which makes it resistant to calcium hydroxide medication[2,17].

Agar Diffusion test used in this study is most frequently used methods for assessment of anti-microbial activity of endodontic materials. It allows direct comparisons of the filling materials against the test organisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system[18].

Chlorhexidineacts by adsorbing onto the microorganism cell wall, causing intra cellular component leakage. It has an inhibitory effect against gram-positive and gram-negative microorganisms[7,10].Its efficacy is based on the interaction between positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows chlorhexidine molecule to inhibit bacteria and its toxic effects. Chlorhexidine impregnated gutta percha, ‘activ point’ contain gutta percha matrix embedded with 5% chlorhexidine diacetate which acts as an antimicrobial reservoir that is capable of diffusing onto the surface of gutta percha, inhibiting colonization of bacteria within root canals effects[19].

Chitosan is a natural polysaccharide comprising of co-polymers glucosamine and N-acetyl glucosamine, derived from deacetylation of chitin. It is the only cationic polysaccharide in nature and can be chemically modified to derivatives based on function and application. It is hydrophobic and highly soluble in acidic solvents with pH below 6. The most important property of chitosan is the anti-microbial activity exerted against bacteria, viruses, fungi and even algae. It exhibits anti-bacterial activity by binding to bacterial cell wall and DNA[20].

The results of the present study indicate that, Chitosan-impregnated gutta percha was more efficient in inhibiting E. faecalis than Chlorhexidine activ-gutta percha points, commercial gutta percha and normal saline. The mean value of Zone of Inhibition was greater in Chitosan impregnated gutta percha as compared to Chlorhexidine active-gutta percha points and Commercial gutta percha.

Alejandra et al[21], conducted a study to evaluate and compare the anti-microbial efficacy and mechanical properties of Chitosan impregnated gutta percha and commercial gutta percha against Prevotella bucaae, Porphyromonas gingivalis, Peptostreptococcus stomatis, Enterococcus faecalis and Candida albicans and concluded that Chitosan impregnated gutta percha showed higher and significant anti-microbial and anti-fungal activity and improved mechanical properties when compared to commercial gutta percha points. Ahmed et al[1], who conducted a studyto evaluate the anti-bacterial activity of bioactive bioglass and Chitosan incorporated as fillers in gutta percha against Enterococcus faecalis and concluded that Chitosan was highly effective against E. faecalis when compared to commercial gutta percha. Lui et al[4], also conducted a study to evaluate the anti-microbial effect of Chlorhexidine active gutta percha points against E. faecalis and concluded that Chlorhexidine active gutta percha points did not possess inhibitory activity strong enough to eliminateE. faecalis.

Further in-vitro and in-vivo studies are required to use Chitosan impregnated gutta percha to be used as obturating materials.

CONCLUSION

Within the limitations of the study, the following conclusions can be made:

1. E. faecalis was significantly reduced by Chitosan-impregnated gutta percha and Chlorhexidine active-gutta percha points after 24 hours.
2. On comparing all the groups, Chitosan impregnated gutta percha was highly effective against E. faecalis than Chlorhexidine gutta percha, Commercial gutta percha and normal saline.

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