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ORIGINAL RESEARCH

Evaluation of antimicrobial efficacy of tetracycline gutta percha and calcium hydroxide impregnated gutta percha against enterococcus faecalis- an in vitro study.

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ABSTRACT:

Aim: The purpose of this study was to evaluate the antimicrobial efficacy of tetracycline gutta percha (TGP) and calcium hydroxide impregnated gutta percha against Enterococcus faecalis, an invitro study.

Methodology: Inocula of E.faecalis were spread onto trypticase soy agar medium using sterile glass spreaders. Tetracycline gutta percha, Calcium hydroxide gutta percha and traditional gutta percha cones were aseptically transferred onto each of the inoculated plates using sterile forceps separately. The plates were incubated for 24, 48 and 72 hours at 370 Celsius aerobically. Following incubation the diameters of zone of bacterial inhibition (clear zone) were measured in millimeter for each period of time.

Results: Tetracycline gutta percha had the maximum antibacterial efficacy, exhibiting broader zones of inhibition, where as calcium hydroxide gutta percha had no effect on test bacteria.

Conclusions: Under the conditions of this study tetracycline gutta percha offers maximum antibacterial advantage over Calcium hydroxide and traditional gutta percha.

Key words: ***Brain heart infusion broth(BHI), Calcium hydroxide, Enterococcus faecalis, Tetracycline gutta percha.***

INTRODUCTION

Ever since 1890, when W.D. Miller first observed the presence of microorganisms in pulpal and periapical diseases, microorganisms and their by-products are considered as the primary etiological agents of necrotic pulps and apical periodontitis. The main goal of endodontic therapy is to eliminate microorganisms from the root canal system and the prevention of subsequent reinfection[1]. Although the majority of bacteria are eliminated by biomechanical preparation of root canal space, a few microorganisms might still survive and thus the use of intracanal medication and the use of filling materials with antimicrobial and sealing properties are of essential importance, to avoid the growth of microorganisms.[2]

Attempts have been made to improve the antimicrobial efficacy of the gutta percha points used exclusively as an inter-appointment intracanal dressing by incorporating calcium hydroxide, chlorhexidine and tetracycline.

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Calcium hydroxide is the most commonly utilized and studied intra-canal medication. It has been recommended as an inter-appointment root canal dressing material to control microbial growth as well as to promote healing of periapical tissues.[3] Thus, the use of various formulations and suggestions for mixing calcium hydroxide powder with other substances have been recommended.

In addition to placement, the removal of calcium hydroxide from the root canal without leaving any residue behind is a time consuming and cumbersome procedure. These shortcomings prompted the development of gutta percha points impregnated with calcium hydroxide and tetracycline, which could be easily placed in the root canal, especially in the apical area.[4]

Tetracycline, a broad spectrum antibiotic has also been incorporated into the gutta percha cones to inhibit microbial growth and retards breakdown due to leakage or bacterial contamination. Tetracycline impregnated gutta percha (Tetracycline Gutta Percha, TGP by Medidenta), acts as an antimicrobial reservoir that is capable of diffusing onto the surface of the gutta percha, thereby inhibiting colonization of bacteria on the gutta percha points and within the root canals.[1]

OBJECTIVES OF THE STUDY:

The purpose of this study was to evaluate the in-vitro antimicrobial efficacy of tetracycline and calcium hydroxide impregnated gutta percha against *Enterococcus faecalis*.

METHODOLOGY:

The antimicrobial efficacy of tetracycline gutta percha and calcium hydroxide impregnated gutta percha was investigated using agar diffusion method for different time periods.[1]

TEST MATERIALS USED:

1. Group A- Traditional gutta percha. (Dentsply Maillefer, Switzerland)
2. Group B- Tetracycline impregnated gutta percha (TGP-Manufactured by Medidenta Company, USA) with composition of 20% gutta percha,57% zinc oxide, 10% tetracycline, 10% barium sulphate and 3% Beeswax.[8]
3. Group C- Calcium hydroxide impregnated gutta percha (Calcium hydroxide plus points by Roeko, Germany) with composition of calcium hydroxide 52%, gutta percha 42%, sodium chloride, surfactant, and coloring agents.[10]

MICROORGANISMS:

Standard strains of *E. faecalis* ATCC 29212 were obtained for the study from Department of Microbiology, St. Johns Institute of Medical Sciences and Research Center, Bangalore.

PROCEDURE:

Standard strains of *E.faecalis* were propagated in 5ml of brain heart infusion broth and incubated at 37^0 Celsius for 24 hrs and the broth culture suspension of bacteria was adjusted to No1 Mac Farland standard (approximately 3 x 10^8 cell/ml). Then fifty micro liter of broth suspension containing aliquots of *E.faecalis* were spread on to the Petri dish containing Trypticase soy agar medium using sterile glass spreader. Subsequently, four cones from each test group materials i.e traditional gutta percha (group A), tetracycline impregnated gutta percha (group B), calcium hydroxide impregnated gutta percha (group C), were aseptically transferred onto the inoculated plates using sterile forceps separately.

Then plates were incubated aerobically for 24, 48 and 72 hours at 37^0 Celsius. After incubation, the antimicrobial efficacy of each test materials were determined by measuring the diameter of clear zone/ zone of inhibition around the cones in millimeters from sides of the cones after 24, 48 and 72 hrs. Experiments were performed in triplicate and the average value was determined. The data was subjected to statistical analysis using Kruskal-Wallis test.

RESULTS:

Uniform growth was evident on all control plates. Samples from triplicate trials yielded consistent results. Inhibition of *E.faecalis* in the different materials at each time interval was compared using Kruskal-Wallis test, results are tabulated in table 1 & table 2.

Calcium hydroxide points did not exhibit any antimicrobial effect on the tested microorganisms for all time periods as shown in table 1 & 2.

Tetracycline gutta percha cones inhibited tested bacterial strains for 24 hrs. Regardless of time period TGP was statistically more effective than other test materials ($P<0.05$) as shown in table 1, with a mean diameter of 8.0 mm.

No significant difference was noticed in inhibition of E.feacalis at different time periods ($P> 0.05$).

DISCUSSION:

The practice of Endodontics is a constantly evolving specialty. This is in a large part due to the continued high degree of success achieved with endodontic therapy, attributable to the advancements in technology and techniques. Most pathosis of the dental pulp and periapical tissues are either directly or indirectly related to microorganisms. In this study *E.faecalis* was chosen because of its implication as a possible microbial factor in therapy resistant apical periodontitis. It is a gram positive, facultative anaerobe, and able to grow at temperatures ranging from 10^0 to 45^0 Celsius and pH of 9.6 and can survive extreme environmental challenges.[5]

Although the majority of bacteria are eliminated by biomechanical preparation of root canal space, a few microorganisms might still survive these challenges by residing in anatomical complexities of root canals like cementum crypts, secondary canals, dentin tubules, deltas,. In these locations, bacteria may be unaffected by the chemo mechanical preparation of the root canal, therefore further means such as inter-appointment intracanal medication and use of filling materials with antimicrobial and sealing properties are of essential importance to avoid the growth of microorganisms.[12]

The agar diffusion test used in this study is one of the most frequently used methods for assessment of the anti-microbial activity of endodontic materials. It allows direct comparisons of the filling materials against the test microorganisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the following peculiarities should be taken into consideration when applying such a method: the contact between the tested material and the agar, the weight, size and molecular shape of the antimicrobial agent, the agar gel texture, and the ionic concentration of the medium. All these are relevant factors to the material diffusion in agar.[13]

The lack of standardization of these factors may be considered the main cause of the difference between the present results and those found in comparative studies about antimicrobial properties of tested endodontic materials.

In the present study a new formulation of calcium hydroxide impregnated gutta percha; Calcium hydroxide plus points by Roeko, was assessed for its antimicrobial activity, However, no antimicrobial activity for gutta percha impregnated calcium hydroxide was observed in this study (table 1). The results with Calcium hydroxide gutta percha points in this study co-insides with the results of the previous studies conducted on the material.[2,8] The antimicrobial action of materials containing calcium hydroxide depends on the calcium hydroxide ionization and on the release of hydroxyl ions that promote an increase in the medium pH and its maintenance.

It has been demonstrated in various previous studies that the calcium hydroxide gutta percha was unable to alter the pH and the calcium released was lower in Calcium hydroxide impregnated gutta percha. The gutta percha matrix probably binded the hydroxyl ions and blocked their release at the site of application.[6,7] This could be the possible reason for calcium hydroxide impregnated gutta percha points showing negative results or no inhibition zone in this study.

Tetracycline, a broad spectrum antibiotic has been widely in the treatment of various endodontic infections. The Gutta percha impregnated tetracycline acts as an antimicrobial reservoir that is capable of diffusing onto the surface of the gutta percha, thereby inhibiting colonization of bacteria on the gutta percha points and within the root canals. The important fact is that tetracycline can become incorporated into calcified structures due to its ability to bind to mineralized dentinal matrices and its slow release from dentin makes its antimicrobial effect substantive.

In this study, TGP had the highest antimicrobial activity of all the materials tested on E.faecalis as shown in table 1, and no significant difference was noticed in inhibition of *E.feacalis* at different time periods (P> 0.05) table 2.

The results of the present study with tetracycline impregnated gutta percha were consistent with the previous studies done on the material,[1] indicating that tetracycline gutta percha (TGP) has an effective antimicrobial efficacy on all tested microorganisms for all tested periods.

Tetracycline gutta percha has also been advocated to be used as a final obturating material, as an alternative to regular/traditional gutta percha for root canal obturation. Hence tetracycline gutta percha has dual advantage that can be used as an inter appointment intracanal medicament and also as final obturating material.

Tetracycline gutta percha showed the best results under the conditions of the present study, however before they can used in the clinical situations further in-vivo studies are required to prove their efficacy.

CONCLUSION:

With in the limits of this study, it can be concluded that, Tetracycline impregnated gutta percha showed the maximum antimicrobial efficacy on *E.faecalis* compared to Calcium hydroxide impregnated gutta percha which had no effect on test microorganisms under laboratory conditions.

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Table 1: Comparison of the effect of test materials on the inhibition of E.faecalis at each time period:

Bacteria	Period (hrs)	Group A RGP (Mean±SD)	Group B TGP (Mean±SD)	Group C $Ca(OH)_2$ GP (Mean±SD)	Kruskal-Wallis test Chi-sq	P-Value
E.faecalis	24	0.00	7.67±0.58	0.00	10.485	0.015*
	48	0.00	8.00±0.00	0.00	10.866	0.012*
	72	0.00	8.33±0.58	0.00	10.735	0.013*

Table 2 : Comparison of the effects of each test material on the inhibition of bacteria over different time periods:

Bacteria	Material	24 hrs (Mean±SD)	48 hrs (Mean±SD)	72 hrs (Mean±SD)	Kruskal-Wallis test	P-Value
E. faecalis	Group A	0.00	0.00	0.00	–	–
	Group B	7.67±0.58	8.00±0.00	8.33±0.58	2.667	0.264
	Group C	0.00	0.00	0.00	–	–

RGP- Regular/traditional gutta percha.