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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL EFFICACY OF CALCIUM HYDROXIDE AND CHLORHEXIDINE GUTTA-PERCHA POINTS AN IN SITU STUDY

[1]Dr. Poonam Singh and [2]Dr. Braj Bhushan Mall

[1]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Awadh Dental College and Hospital, Jamshedpur, Jharkhand

[2]Assistant Professor, Department of Oral and Maxillofacial Surgery, Dental college RIMS Imphal, Manipur, India

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ABSTRACT

Aim*:* To evaluate the effectiveness of different type of medicated and non-medicated gutta-percha points in reducing bacterial colonization in root canal system. ***Methodology***: Three different bacterial strains were used *Staphylococcus aureus*, *Escherichia Coli, Enterococcus Faecalis* **.**Disc was prepared from Whatmann filter paper. Then with the help of tuberculin syringe, 1 ml of the specific solution of gutta-percha points, was added on each disc separately. Then it was allowed to dry at room temperature to avoid excessive moisture accumulation and stored at 2 to 8˚C. Zones of inhibition were measured to nearest mm, and the results were interpreted with the assistance of diameter. ***Result****:* On the basis of zone of inhibition, it was maximum in group IV (Chlorhexidine GP) followed by group III (Calcium hydroxide GP) than group II (conventional GP).Conclusion: Roeko activ (chlorhexidine gutta-percha points) showed higher antimicrobial efficacy than Calcium hydroxide gutta-percha points against the microorganisms tested.

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INTRODUCTION

The aim of root canal treatment is the elimination of infection from the root canal system. Similarly, the main cause of root canal treatment failure is the persistent of microorganisms after therapy or the re-infection of the root-canal system because of inadequate coronal seal. Successful Root Canal therapy relies on the combination of proper instrumentation, disinfection and obturation. Infection of the root canal at the time of obturation has a negative influence on the prognosis of endodontics therapy. However even after conventional root canal preparation, micro-organisms may remain either within the dentinal tubules embedded in the smear layer or bound within the apical dentin plug. The objective of obturating the root canal is the substitution of an inert filling in the space previously occupied by the pulp tissue, to prevent recurrent infection by way of the circulation or through a break in the integrity of the crown of tooth. Microorganisms are considered as main etiologic factor in necrotic pulps and periapical region. Microbial flora associated with infected pulps is polymicrobial and predominantly anaerobic. Calcium hydroxide pastes, commonly used as intracanal medication, have presented antimicrobial action and ability to inactivate endotoxins which facilitate the healing process of periapical tissues Joshua M *et al.*, (2007). Chlorhexidine, used as an irrigating solution, may present substantivity, has antimicrobial action and is able to inhibit most endodontic bacteria Shur AL, Sedgley CM *et al.*, (2007).

****Corresponding author:*** **Dr. Poonam Singh**
Senior Lecturer, Department of Conservative Dentistry and Endodontics, Awadh Dental College and Hospital, Jamshedpur, Jharkhand

MATERIALS AND METHODS

In this study 3 different bacterial strains were used

- ***Enterococcus Faecalis-*** gram positive cocci, facultative anaerobes, mostly isolated species from obturated root canals of teeth exhibiting periapical pathology and periradicular abscesses.
- ***Escherichia Coli*** - gram negative bacteria, facultative anaerobic
- ***Staphylococcus aureus.***-gram positive bacteria, spherical cocci.

Medicated Gutta Percha

Calcium Hydroxide Containing Gutta-Percha: Calcium hydroxide points are time release preparations, which releases calcium hydroxide from a gutta-percha matrix.

Activ Roeko Point: Roeko activ points are time release preparations, which release chlorhexidine diacetate from a gutta-percha matrix.

MATERIALS AND METHODS

A drop of sterile water may be used together with the point for the initial release of ions. However, after inserting the point into the canal, sufficient fluid flows into the space between the point and the canal wall from the dentinal tubules and apical area to activate the $Ca(OH)_2$ even without additional water. The moisture is sufficient to cause dissociation of the ions from the point, so that an alkaline environment develops within the canal and the pH quickly rises above 12. The concentration of the Ca $(OH)_2$ in the point ensures that sufficient $Ca(OH)_2$ is always present in the canal.

Preparation of Sample

2mg of chlorhexidine gutta-percha, 2mg of calcium hydroxide gutta-percha and 2mg of conventional gutta-percha were separately dissolved in10 ml of chloroform in test tube and stored in a sterile condition. All the test tube were labelled according to the type of Gutta-Percha solution.

Preparation of Disc

Disc was prepared from Whatmann filter paper no.3 of 6mm diameter. Then with the help of tuberculin syringe, 1 ml of the specific solution of gutta-percha points, was added on each disc separately. Then it was allowed to dry at room temperature to avoid excessive moisture accumulation and stored at 2 to 8˚C.

Distribution of Samples: Samples were divided in to two main groups:

1. **Control Group:** Group I -Only distilled water (n=5 samples)
2. **Experimental Group:** Were divided into three groups.

Group II- Conventional gutta-percha points (n=5 samples)
Group III -Calcium hydroxide gutta-percha points (n=5 samples)
Group IV -Roeko activ gutta-percha points (n=5 samples).

Control Group

For control group plain nutrient broth was taken in a test tube. Then this nutrient broth was spread over the Mueller Hinton agar plate. Disc containing distilled water was placed on the plate which was incubated at 37^0 C for 24 hours. After 24 hour zone of inhibition was measured using the plastic ruler.

Experimental Group

Mueller Hinton agar plates were taken. Inoculums of *E. coli*, *Enterococcus faecalis* and *Staphylococcus aureus* was prepared in Nutrient broth of 0.5 McFarland turbidity standard in a test tube using stroke's method. 0.5 McFarland turbidity standard is used to usually approximate the concentration of cells in a suspension. Wooden swab stick was dipped in a test tube containing inoculum of the specific strains and was spread on the Mueller Hinton agar plates (lawn culture). After lawn culture, the prepared disc was placed on the Muellar Hinton agar plate and then it was incubated at 37^0 C for 24 hours. The test was repeated 5 times for all the strains. After 24 hours zone of inhibition was measured with the help of plastic ruler in mm and compared with antibiotic zone scale and the results were statistically analyzed.

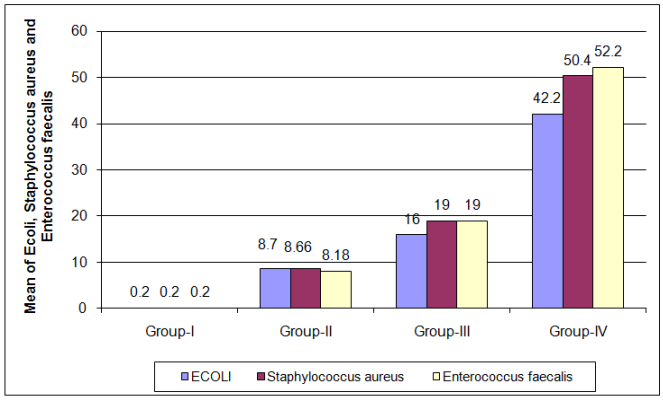
Interpretation: Zones of inhibition were measured to nearest mm, and the results were interpreted with the assistance of diameter. More than 15mm was considered to be sensitive and less than that was considered as resistant.

Observation:
The antimicrobial effect was assessed in terms of zone of inhibition.

On the basis of observations and their analysis, for *Enterococcus Faecalis, Escherichia Coli, Staphylococcus aureus* the order of antimicrobial efficacy was:

Group IV > Group III > Group II > Group I

- On the basis of zone of inhibition ,it was maximum in group IV(Chlorhexidine GP) followed by group III (Calcium hydroxide GP) than group II (conventional GP)
- The anti bacterial effect of medicated and non medicated Gutta Percha points on *Enterococcus Faecalis, Escherichia Coli, Staphylococcus Aureus.* The group IV chlorhexidine containing Gutta percha had maximum mean diameter of zone of inhibition. The Group II of conventional Gutta percha had minimum mean diameter of zone of inhibition.



The histogram compare the anti bacterial efficacy against bacterial strains, used in the study. The chlorhexidine Gutta Percha group (IV) showed maximum anti bacterial against all bacterial strains, while Group III (calcium hydroxide Gutta Percha) and Group II were less effective when compared to Group IV (chlorhexidine Gutta percha).

DISCUSSION

The major goal of successful endodontic treatment is to eliminate bacteria from the root canal and prevent reinfection. However, removing all microorganisms in the canal before obturation has proven to be difficult, and the choice of endodontic materials that have high antimicrobial efficacy can help in decreasing or avoiding growth of the microorganisms that remains. The commonly found micro-organism are E.coli, Enterococcus faecalis, Staphylococcus aureus among several others are able to grow at temperature ranging from 100 to 450

C and pH of 9.6 and can survive extreme environmental challenge. These are the most common organism isolated from failed root canals and are particularly resistant to many conventional antimicrobial agents used routinely. With the aim of providing maximum antimicrobial action, gutta-percha points have been manufactured including several substances such as iodoform gutta-percha, chlorhexidine containing gutta-percha, and calcium hydroxide containing gutta-percha. In the present study a new formulation of calcium hydroxide impregnated gutta-percha (Calcium hydroxide plus point by Roeko) was assessed for its antimicrobial activity. However, antimicrobial activity for gutta-percha containing calcium hydroxide was less as compared to Chlorhexidine but more than conventional Gutta-Percha. 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.

Emre Bodrumlu, Tayfun Alacam (2006) have evaluated in their studies that regardless of time and bacterial strains, medicated gutta-percha, was statistically more effective than regular gutta-percha points. It has been demonstrated in various previous studies (Wilma Brugger 2007; FJ de Souza-Felha *et al.*, 2008; Hale ARI *et al.*, 2010; Vijay R *et al.*, 2010) that the calcium hydroxide gutta-percha were unable to alter the pH and the calcium release was lower in Calcium hydroxide Gutta-Percha. The gutta-percha matrix probably binded the hydroxyl ions and blocked their release at the site of application. This could be the possible reason for calcium hydroxide gutta-percha points showing less zone of inhibition. Chlorhexidine has been used effectively as an irrigant. Studies (Tanomaru *et al.*, 2007, Wilma Brugger *et al.*, 2007, Maria De LosAngeles *et al.*, 2006) have shown that when Chlorhexidine was used as a solution, it has a short antimicrobial effect in the root canals. For long term activity of Chlorhexidine in the root canals, the dentin must be exposed to Chlorhexidine for a longer period than that offered by irrigation. This led to development of Chlorhexidine impregnated gutta-percha points (Active points). In the present study, Chlorhexidine gutta-percha had the highest antimicrobial activity of all the materials tested on. *Enterococcus faecalis, E.coli, Staphylococcus aureus*. It is indicated that Chlorhexidine gutta-percha has an effective antimicrobial efficacy on *Enterococcus faecalis, E.coli, Staphylococcus aureus.* Chlorhexidine impregnated gutta-percha showed the best result under the conditions of the present study.

The use of the best possible material during obturation is of great importance. The ideal obturation should combine antimicrobial action and a capacity to prevent recurrence of periapical pathogen. Chlorhexidine containing gutta-percha has been recommended as a root canal filling material in the treatment of infected root canal (Tanomaru, Juliane Maria Guerreiro *et al.,* 2007). Their result showed that all microbial species used in this study were inhibited by the gutta-percha points containing chlorhexidine which is similar with the result showed by present study. Barthel *et al., (2002)* evaluated the antimicrobial action of gutta-percha points containing chlorhexidine or calcium hydroxide when compared to chlorhexidine gel. Conventional gutta-percha points showed smaller microbial inhibition. Similarly this study showed less zone of bacterial inhibition with conventional gutta-percha, which is in accordance with our study.

Hale ARI *et al.*, (2010) have demonstrated in their study that chlorhexidine-impregnated gutta-percha cones had better antimicrobial effect. In the present study calcium hydroxide containing gutta-percha and conventional gutta-percha points both showed less inhibition zone of bacteria against *Staphylococcus aureus, Enterococcus faecalis* and *E. coli than* chlorhexidine containing gutta-percha cones. The result of our study is in consistent with the result of Daniel A *et al.,* (2010) and Meltem Dartar *et al.,* (2006) who have concluded in their study that the Gutta-Percha containing chlorhexidine have more antimicrobial activity as compared to conventional Gutta-Percha and Calcium Hydroxide containing Gutta percha. Within the limitation of the this study it can be said that chlorhexidine containing Gutta percha points had better antimicrobial efficacy against *Staphylococcus aureus, Enterococcus faecalis* and *E. coli* in comparison to calcium hydroxides containing Gutta Percha and conventional Gutta Percha.

Conclusion

Our study is by far not a conclusive one and more similar study with clinical trials are needed to achieve a definite conclusion. However, within the limits of this study it can be concluded that:

1. Roeko activ (chlorhexidine gutta-percha points) showed higher antimicrobial efficacy than Calcium hydroxide gutta-percha points against the microorganisms tested which were Enterococcus faecalis, Escherichia coli and Staphylococcus aureus.
2. Calcium hydroxide gutta-percha points has better antimicrobial efficacy when compared to conventional gutta-percha points against the microorganisms tested which were Enterococcus faecalis, Escherichia coli and Staphylococcus aureus, but it was far below the calcium hydroxide gutta-percha points.
3. Conventional gutta-percha points showed minimum antibacterial activity against all microorganisms used for the study.
4. The controlled group of distilled water had no antibacterial activity.

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