CYPRUS JOURNAL OF MEDICAL SCIENCES

Original Article

DOI: 10.5152/cjms.2021.896

Antimicrobial Effects of Gutta-Percha Points Containing Root Canal Medications against Some Anaerobic Bacterial Species and *Enterococcus faecalis*

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Cite this article as: Öztan MD, Kıyan M, Kermeoğlu F. Antimicrobial Effects of Gutta-Percha Points Containing Root Canal Medications against Some Anaerobic Bacterial Species and *Enterococcus faecalis*. Cyprus J Med Sci 2021; 6(2): 151-156.

BACKGROUND/AIMS
The purpose of this study was to examine the ability of gutta-percha points to reduce bacterial counts.

MATERIAL and METHODS
ISO #40-sized Calcium Hydroxide Plus Points or Activ Points were used for all experiments. Three pieces of each type of gutta-percha points were placed in sterile 0.5-mL eppendorf tubes. After incubating the plates in an anaerobic chamber at 37°C for 2 days, colony forming units' (CFUs) numbers per milliliter of suspension were calculated, and CFU numbers relating to each time interval were statistically analyzed by using Kruskal–Wallis 1-way analysis of variance and multiple comparison tests.

RESULTS
The results showed that Calcium Hydroxide Plus Points or Activ Points have similar effects to kill the tested microorganisms except *Enterococcus faecalis* and *Peptostreptococcus micros*.

CONCLUSION
Calcium Hydroxide Plus Points or Activ Points have similar effects to kill the tested microorganisms and did not kill 100% of *E. faecalis* and *P. micros*.

Keywords: Active point, anaerobic bacteria, antimicrobial activity, calcium hydroxide plus point, *E. faecalis*

INTRODUCTION

Endodontic treatment plays an important role in eliminating bacteria and their substrates by instrumentation, irrigation, usage of intracanal medicaments, and antibacterial obturation materials. If root canal treatment is not performed, the infection can reach the area surrounding the dental root apex and cause an inflammatory response.[1] Several cultural and molecular studies have revealed that more than 500 various bacterial species or phylotypes have been determined in infected root canals.[2] Obligatory anaerobic bacteria predominate the root canals, including *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Peptostreptococcus*, and *Eubacterium*. Some gram-positive facultative anaerobes, such as *Actinomyces*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Gemella*, and *Staphylococcus*, have also been frequently isolated from acute abscesses of endodontic origin.[3]

To achieve local healing and stop the pathologic process, the bacteria need to be eliminated as far as possible from all parts of the root canal system.[4] Tests have been conducted on many substances, which have been utilized for intracanal medication. Calcium hydroxide represents the most frequently utilized intracanal medication. Calcium hydroxide exerts an antimicrobial effect, and it is also able to neutralize bacterial endotoxins.[5–7] Chlorhexidine is an alternative substance for root canal medication that is capable of eliminating gram-negative and gram-positive bacteria,[9,10] but it can not

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Received: 07.03.2019
Accepted: 13.01.2020

TABLE I. Contents of the Gutta-Percha Points Used in this Study

Gutta-percha points	Contents
Calcium Hydroxide Plus Points	52% calcium hydroxide, 42% gutta-percha, sodium chloride, surfactant, coloring agents
Activ Points	5% chlorhexidine diacetate, gutta-percha, ZnO, $BaSO_4$, coloring agents

neutralize bacterial endotoxins.[8] For the selection of an appropriate intracanal medication, the antimicrobial properties of local endodontic disinfectants should be known.

The present research aimed to examine the ability of calcium hydroxide and chlorhexidine-containing gutta-percha points to reduce the bacterial counts of some obligatory anaerobe bacterial species and *Enterococcus faecalis*.

MATERIALS and METHODS

A method presented by Podbielski et al.[11] was used in this study. Gutta-percha points, which were ISO #40-sized, were used. ROEKO Calcium Hydroxide PLUS Points (ROEKO, Langenau, Germany) and ROEKO chlorhexidine diacetate-impregnated active points (ROEKO, Langenau, Germany) were used to test the efficiency of bacterial reduction in all tests. Table I contains information on the points' contents.

Microorganisms

Peptostreptococcus micros (NCTC 11808), *Eubacterium lentum* (NCTC 11813), *Prevotella intermedia* (NCTC 13070), *Prevotella melaninogenica* (NCTC 12963), *Porphyromonas endodontalis* (NCTC 13058), and *E. faecalis* (ATCC 29212) were utilized in the present research. All bacterial strains were cultured in thioglycolate broth (Merck, Darmstadt, Germany) added with 0.5 mg/L of vitamin K (Sigma, St. Louis, USA) and 5 mg/L of hemin (Sigma, St. Louis, USA), and Schaedler agar (BBL, Cockeysville, USA) and *E. faecalis* on 5% sheep blood agar (Lab M, IDG, Lancashire, UK).

Procedure

The bacterial count per milliliter was detected by using a Nano Photometer Pearl spectrophotometer (Implen GmbH, Munich, Germany) to measure the optical densities of cultures at a 600 nm wavelength (OD_{600}) for each strain. Individual standard curves were formed as a result of plotting different bacterial concentrations, demonstrated by viable plate counting of colony forming units (CFUs) against the optical density of the suspension at a 600 nm wavelength. The obtained standard curves were utilized for the spectrophotometric detection of bacterial concentrations utilized in the tests.

Main Points

- There were statistically significant differences between the control group and the experimental groups at 14 days.
- Calcium Hydroxide Plus Points and Activ Points presented antimicrobial activity against all of the strains.
- Calcium Hydroxide Plus Points or Activ Points have similar effects to kill the tested microorganisms and both material did not kill 100% E. faecalis and P. micros.

For quantitative assays, bacteria suspensions at OD_{600} levels previously determined to contain 2×10^7 CFU/mL were prepared. All the test microorganisms were sedimented as a result of centrifuging at 2000 g for 10 minutes using a UniCen 15DR (Herolab GmbH, Wlesloch, Germany) and were then resuspended in 3.1 mL of a mixture containing equivalent volumes of a newly prepared human serum and 0.9% NaCl solution. Serum collection was performed from four different donors. Inactivation of the serum was carried out at a temperature of 56°C for a period of 30 minutes in a water bath, GFL model 1083 (LabortechnikmbH, Burgwedel, Germany), and it was then reduced by incubation in an anaerobic chamber for 4 hours before use. Three pieces of every type of gutta-percha points were put in sterile 0.5-mL Eppendorf tubes (Greiner bio-one GMBh, Frickenhausen, Germany). The pieces were acquired as a result of cutting the points into three parts by utilizing sterilized surgical blades and tweezers. Afterward, aliquots (100 µL) of the bacteria that had been suspended in the diluted serum were put into the Eppendorf tubes. The tubes that contained only bacteria suspensions were used as growth controls. Different tubes for every experimental day (1, 2, 3, 4, 7, and 14 days) were arranged for every condition. The final active compound concentrations were found to be 1 µg/100 µL chlorhexidine diacetate for the active points and 6.29 µg/100 µL calcium hydroxide for Calcium Hydroxide PLUS Points.[12]

For *E. faecalis*, the tubes were kept in an incubator at a temperature of 37°C, and for anaerobic bacteria, the tubes were kept in an anaerobic chamber, Bactron model 1-2 (Sheldon Manufacturing Inc., USA). The removal of tubes was performed at days 1, 2, 3, 4, 7, and 14. At the above-mentioned time, the tubes were vortexed for a period of 15 seconds using the Vortex Mixer MX-S (DragonLab, Beijing, China). The tubes were then opened, and serial dilution of 10-µL aliquots of the bacterial suspensions was performed in a sterile solution of 0.9% NaCl. Aliquots (10 µL) of the dilution steps were streaked onto a solid medium (Schaedler agar or blood agar). Following the incubation of the plates in an anaerobic chamber at a temperature of 37°C for a period of 2 days, CFU numbers per milliliter of the suspension were computed. Every assay was performed again on six independent cases.

Statistical Analysis

Statistical analysis was carried out by the Kruskal–Wallis one-way analysis of variance (ANOVA) and multiple comparison tests. The Kruskal–Wallis one-way ANOVA test was conducted for the purpose of evaluating the alteration in CFU numbers with regard to every time interval; when the *P*-value was statistically significant ($P < .01$), multiple comparison tests were used to evaluate which groups differed from which others. The Bonferroni correction was used for multiple comparisons.

RESULTS

The inhibition of bacterial growth was assessed as a result of employing viable plate counting of cultures that had been grown when the gutta-percha points that contained calcium hydroxide or chlorhexidine diacetate were present. Figures 1–6 demonstrate the susceptibilities of the studied microorganisms to the gutta-percha points that contained the two root canal medicaments.

Calcium Hydroxide PLUS Points were able to kill *P. endodontalis* and *E. lentum* within 1 day and *P. intermedia* and *P.*

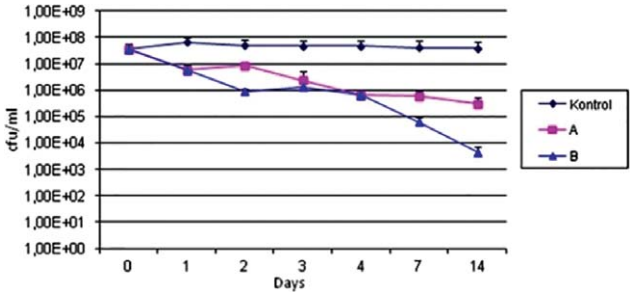

Figure 1. Survival of *Enterococcus faecalis* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

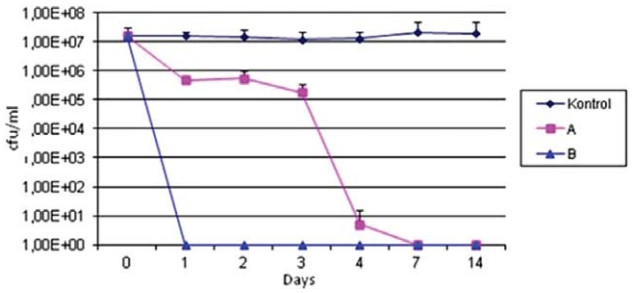

Figure 2. Survival of *Eubacterium lentum* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

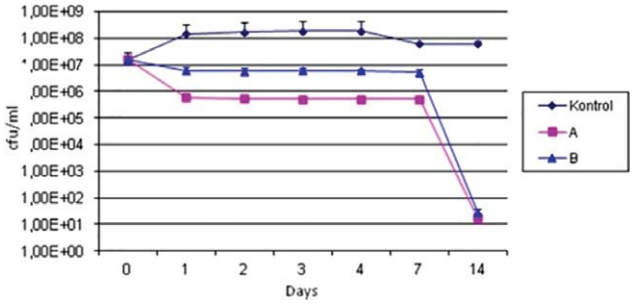

Figure 3. Survival of *Peptosreptococcus micros* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

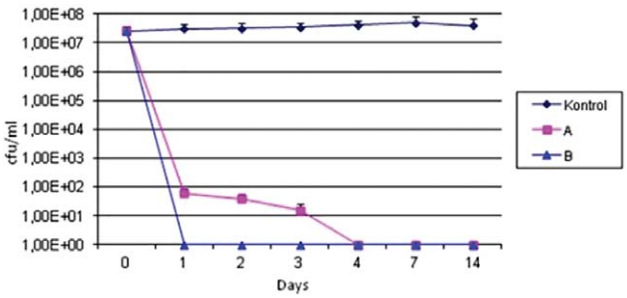

Figure 4. Survival of *Porhyromonas endodontalis* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

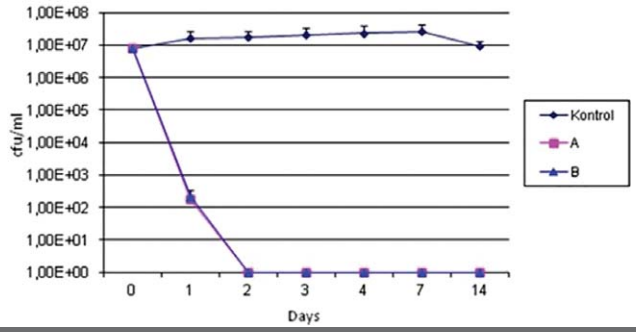

Figure 5. Survival of *Prevotella melaninogenica* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

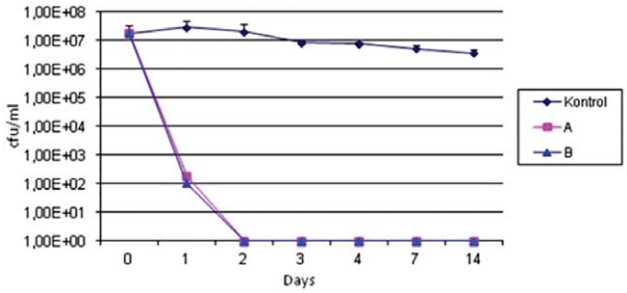

Figure 6. Survival of *Prevotella intermedia* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications. (A) Activ Point (chlorhexidine diacetate); (B) Calcium Hydroxide Plus Points

melaninogenica within 2 days. The active points were able to kill *P. intermedia* and *P. melaninogenica* within 2 days, *P. endodontalis* within 4 days, and *E. lentum* within 7 days. However, neither Calcium Hydroxide PLUS Points nor the active points exhibited adequate antimicrobial activity to kill *E. faecalis* or *P. micros* in a period of 14 days. The results showed that Calcium Hydroxide PLUS Points and the active points have comparable killing effects on the studied microorganisms apart from *E. faecalis* and *P. micros*.

When the antibacterial activity of Calcium Hydroxide PLUS Points and the active points against *E. faecalis* was examined, a statistically significant difference was found between Calcium Hydroxide PLUS Points and the active points at only 2 days ($P = .012$). Statistically significant differences were determined between the experimental groups and the control group for all days ($P < .01$), with the exception of the active points and the control group at 2 days ($P > .05$). For *P. micros*, statistically significant differences were found among the groups at days 1, 2, 3, 4, and 7 ($P < .001$). No statistically significant difference was determined between the two experimental groups at 14 days ($P > .05$). However, statistically significant differences were identified between the control group and the experimental groups at 14 days ($P = .015$). Statistically significant differences were found between the experimental groups and the control group for *E. lentum* for all days ($P < .001$), whereas statistically significant differences were determined at only 1, 2, and 3 days between the two experimental groups ($P < .001$). For *P. endodontalis*, statistically significant differences were identified between Calcium

Hydroxide PLUS Points and the active points at 1, 2, and 3 days ($P \leq .01$). The differences were found to be statistically insignificant at 4, 7, and 14 days for the experimental groups ($P > .05$), but the control group and the experimental groups differed statistically significantly at 1, 2, 3, 4, 7, and 14 days ($P < .001$).

For *P. melaninogenica* and *P. intermedia*, no statistically significant differences were found between Calcium Hydroxide PLUS Points and the active points for the whole experimental period ($P > .05$). The control group and the experimental groups differed statistically and significantly at 1, 2, 3, 4, 7, and 14 days ($P < .001$).

DISCUSSION

The primary goal of successful root canal treatment is eliminating microorganisms from root canals and preventing the following reinfection. Despite the elimination of most bacteria by the biomechanical preparation of the root canal space, a number of microorganisms may survive and increase rapidly in the anatomical complexities of root canals. The microbiota in infected root canals has polymicrobial properties and anaerobic gram-positive cocci dominate it.[13] The bacterial species that were used in our study are most frequently isolated from root canal infections, and *E. faecalis* represents the main pathogen that is associated with root-filled teeth with persistent lesions, since it exhibits resistance especially to numerous conventional antimicrobial agents utilized in clinical practice.[11,14]

Growth conditions that are similar to the common in situ conditions as much as possible represent a significant parameter in assays that investigate antimicrobial effects. Human serum was utilized as the most appropriate test medium in the current research, because a liquid that seeps into the instrumented root canal will represent interstitial fluid and a filtrate of blood components.

Intracanal medicaments are routinely used for root canal disinfection as part of controlling sepsis in root canal treatment.[14,15] The antimicrobial efficiency of the gutta-percha points that contained different medications on different bacterial species was determined in the present research.

Calcium hydroxide has emerged as a popular endodontic intracanal medicament. Calcium hydroxide is available in several formulations due to the number of vehicles that can be used along with it. The most common formulation is calcium hydroxide powder that is mixed with distilled water. A problem associated with it is its incomplete removal of calcium hydroxide remnants from the canal walls.

Calcium hydroxide points are designed for releasing calcium hydroxide from the gutta-percha matrix.[16] Calcium hydroxide ionization and the release of hydroxyl ions promoting the increased pH of the medium and its maintenance determine the antimicrobial mechanism of calcium hydroxide-containing gutta-percha points. Moreover, hydroxyl ions influence some action on bacterial cells as a result of destructing unsaturated fatty acids or phospholipids of the bacterial cytoplasmic membrane.[17]

In vitro studies have shown that gutta-percha points that contain calcium hydroxide may not be effective for calcium hydroxide release.[18,19] It is possible to explain this by the lower hydroxyl-releasing potential of medicated points compared to that of calcium hydroxide paste. Furthermore, it is possible that gutta-percha containing calcium hydroxide will not work as a good physical and chemical barrier as calcium hydroxide paste. Nevertheless, Stojanovic et al.[20] found out that intracanal medication with gutta-percha containing calcium hydroxide efficiently eliminated *E. faecalis* from infected root canals. In our study, Calcium Hydroxide PLUS Points significantly reduced bacteria compared to the control group for all experimental periods.

Chlorhexidine gluconate represents a broad-spectrum antibacterial agent, which functions by adsorbing onto the microorganism's cell wall and leading to the leakage of intracellular components. The formulation of the active points allows large quantities of chlorhexidine to be released from the points' surface.[16,21] Barthel et al.[22] and Lenet et al.[23] found out that gutta-percha points that contain chlorhexidine have a lower antimicrobial impact in comparison with chlorhexidine in other vehicles. In this research, the active points significantly reduced bacteria compared to the control group for all experimental periods.

Some controversies exist about calcium hydroxide effectiveness against *E. faecalis*. A number of theories have been suggested for explaining the survival of *E. faecalis* following treatment using calcium hydroxide.[24,25] It has been demonstrated that *E. faecalis* is capable of maintaining pH homeostasis in a passive and active way by a proton pump. Furthermore, it is capable of surviving in a severe environment, including a high pH value of 11.5, and it can penetrate into the dentin tubules and escape from the medicament's effective concentration. Moreover, the buffering impacts of dentin may ensure that a sufficiently high pH is not obtained in the dentin tubules.[20] Gutta-percha points that contain calcium hydroxide or chlorhexidine have also been found to have no effect against *E. faecalis* in a number of in vitro studies.[12,26,27] Barthel et al.[22] showed the inadequate antimicrobial activity of gutta-percha points that contain calcium hydroxide or chlorhexidine under in situ conditions. In our study, neither Calcium Hydroxide PLUS Points nor chlorhexidine diacetate-impregnated active points exhibited sufficient antimicrobial activity against *E. faecalis* and *P. micros*, even after 14 days. Moreover, Calcium Hydroxide PLUS Points were more effective against *E. faecalis* than the active points at 2 days period.

The effectiveness of chlorhexidine as a potential option to calcium hydroxide as an intracanal medicament when *E. faecalis* is suspected has been shown.[18] Rathke et al.[28] demonstrated that gutta-percha points containing chlorhexidine were significantly more effective against *F. nucleatum* and *P. micros* than gutta-percha points that contained calcium hydroxide. Bozza et al.[29] conducted in vitro research for the purpose of evaluating the antimicrobial activity of gutta-percha that contained calcium hydroxide, gutta-percha that contained chlorhexidine, and conventional gutta-percha points, and concluded that gutta-percha that contained chlorhexidine proved to be effective against most tested species. Tanomaru et al.[17] found out that sufficient antimicrobial activity against *E. faecalis* was not exhibited by gutta-percha containing chlorhexidine inside infected dentine tubules. Identical findings were obtained in the research carried out by Jhamb et al.,[21] who evaluated gutta-percha that contained chlorhexidine, which showed higher antimicrobial activity against *P. aeruginosa, S. aureus*, and *E. coli* than gutta-percha containing calcium hydroxide.

Although some studies have reported gutta-percha containing chlorhexidine as a more efficient intracanal medicament in comparison with calcium hydroxide, Stojanovic et al.,[20]

Podbielski et al.,[11] and Oztan et al.[12] showed that gutta-percha containing chlorhexidine exhibited a similar antibacterial effect against *E. faecalis* in comparison with gutta-percha that contained calcium hydroxide.[11,12,20]

In this research, Calcium Hydroxide PLUS Points exhibited significantly higher efficiency against *P. endodontalis* and *E. lentum* than the active points for 1, 2, and 3 days; *P. micros* for 1, 2, 3, 4, and 7 days. For *P. melaninogenica* and *P. intermedia*, no statistically significant differences were determined between Calcium Hydroxide PLUS Points and the active points during 14 days.

It was assumed that the rapid release of the active ingredients occurred from the gutta-percha matrix. The short-term release in question can be among the reasons why, in this research, the highest antibacterial effect of both gutta-percha containing chlorhexidine and calcium hydroxide was achieved at the beginning, and a decrease occurred in it during the period of 3 days.[28] A considerably higher pH was obtained only in the immediate proximity of the gutta-percha containing calcium hydroxide following 1 day, and it was not possible to detect it following 3 days.[30] The factors that have made the comparison more difficult are associated with the fact that all the studies presented above were carried out in vitro and utilized various methods in order to detect pathogens.[20]

Under the limitations of the in vitro setting, the findings of the present study suggested that Calcium Hydroxide PLUS Points and the active points had an antimicrobial effect against all of the strains utilized in the current research. Calcium Hydroxide PLUS Points and the active points had similar effects to kill the tested microorganisms. However, neither material eradicated *E. faecalis* and *P. micros*.

Ethics Committee Approval: N/A

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.D.Ö.; Design - M.D.Ö.; Supervision - M.D.Ö.; Resource - M.D.Ö., M.K., F.K.; Materials - M.K.; Data Collection and/or Processing - M.D.Ö., M.K.; Analysis and/or Interpretation - M.D.Ö., M.K., F.K.; Literature Search - M.D.Ö., M.K., F.K.; Writing - M.D.Ö., M.K., F.K.; Critical Reviews - M.D.Ö., M.K.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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