

Original Research

In vitro evaluation of antibacterial efficacy of different formulations of calcium hydroxide

Divyangana Thakur¹, KC Thakur¹, Pranav Thakur¹, Ambika Thakur¹

¹Private Practitioners at Trikuta Dental Clinic, Udhampur, J & K

ABSTRACT:

Aims: The purpose of this study was to compare the effectiveness of various medicaments, including calcium hydroxide alone, calcium hydroxide with iodoform, calcium hydroxide points, calcium hydroxide with 2% chlorhexidine gel against *Enterococcus faecalis* in vitro. **Methodology:** Seventy five extracted single-rooted human maxillary teeth were used. After removing the crown, each root was instrumented up to size 50 by using a conventional technique and were infected with *E faecalis*. Subsequently, the roots were divided into 4 treatment groups: group 1 was treated with calcium powder hydroxide, group 2 was treated with calcium hydroxide with iodoform, group 3 was treated with calcium hydroxide points, and group 4 was treated with calcium hydroxide with 2% chlorhexidine gel, group 5 was treated with 0.9% sterile saline serving as negative control. Microbial samples were taken after 7, 15, and 30 days. After incubation, dentine chips were obtained from each root canal and examined microbiologically. The microbiological samples were plated to count colony-forming units in per milligram of dentin. **Results:** The results showed that the calcium hydroxide with 2% chlorhexidine gel was significantly more effective than calcium hydroxide alone, calcium hydroxide with iodoform, calcium hydroxide points and control saline solution. **Conclusion:** Under the conditions of this study, calcium hydroxide with 2% chlorhexidine gel is effective in the elimination of *E faecalis* from the root canal system. However, to support this in vitro observation, further in vivo studies are needed.

Key words: Calcium hydroxide, Chlorhexidine

Received: 24 November, 2020

Accepted: 20 December, 2020

Corresponding author: Dr. Divyangana Thakur, Private Practitioners at Trikuta Dental Clinic, Udhampur, J & K

This article may be cited as: Thakur D, Thakur KC, Thakur P, Thakur A. In vitro evaluation of antibacterial efficacy of different formulations of calcium hydroxide. Int J Res Health Allied Sci 2021; 7(1):44-47.

INTRODUCTION

Endodontic infections are polymicrobial with a synergistic relationship between bacteria. Complete disinfection of root canal space cannot be achieved by chemomechanical instrumentation alone because of the anatomic complexity and diversity of root canals.¹ The use of an intracanal medication remains an important adjunct in the total elimination of bacteria remaining even after chemomechanical instrumentation during endodontic treatment. Calcium hydroxide is used as intracanal medicament; it has many properties of an ideal root canal dressing material such as antibacterial action, tissue dissolving ability and induction of hard tissue.²⁻⁴

Medicament is an effective antimicrobial agent which is placed inside the root canal between treatment appointments in order to destroy remaining microorganisms and prevent the growth of any new

arrivals.⁵ Various vehicles like water, saline, glycol, etc. have been tried to carry calcium hydroxide to achieve maximum efficiency but still search of better vehicle is going on. Vehicles can speed up or slow down the ionic dissociation, substances that aid the filling of pulpal cavity by means of their consistency, substances used as antimicrobial medium and media that enhance radiopacity.³

Chlorhexidine consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain. Chlorhexidine is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism. Its efficacy is due to the interaction of the positive

charge of the molecule and the negatively charged phosphate groups on the microbial cell walls, thereby altering the cells' osmotic equilibrium.⁷ This increases the permeability of the cell wall, which allows the chlorhexidine molecule to penetrate into the bacterial cell. CHX is a base and is stable as a salt. The most common oral preparation, chlorhexidine gluconate, is water-soluble and, at physiologic pH, it readily dissociates and releases the positively charged chlorhexidine component. At low concentration (such as 0.2%), low molecular weight substances—specifically potassium and phosphorous—will leak out. At higher concentrations (e.g. 2%), chlorhexidine is bactericidal and precipitation of cytoplasmic contents occurs which results in cell death.⁷

Metapex, is a silicone oil based calcium hydroxide paste containing 38% iodoform. Iodoform based calcium hydroxide paste, all over the years has been used exhaustively as it is antiseptic due to iodine release in nascent state when it comes in contact with secretions or endodontic infections. Iodine action gives them a high reactivity by precipitating proteins and oxidizing essential enzymes and it is used as disinfectant as well as antiseptic.³

Despite of its several advantages, removal of calcium hydroxide from the root canal can be time consuming and has been shown to interact with sealers, so an innovative solution to these drawbacks is the introduction of calcium hydroxide points as an intracanal medicament (Hegde M N et al 2006). The points are 28mm in length and a distinctive brown colour differentiates the calcium hydroxide points from the gutta percha points. They serve as an effective alternative to calcium hydroxide paste and are available in ISO sizes of 15 to 140. Calcium Hydroxide Points are made of 52 % calcium hydroxide, 42% gutta percha, sodium chloride and surfactant and colouring agents. When used as an intracanal medicament in endodontic therapy, moisture in the canal activates the calcium hydroxide and the pH in the canal rises to level of 12+ within minutes. The average treatment time is 1-4 weeks. Calcium hydroxide points maintain an outer dentin pH above 9.5 when compared to aqueous calcium hydroxide.⁸⁻¹⁰

Thus this pioneer study was done to compare the effectiveness of various medicaments, including calcium hydroxide alone, calcium hydroxide with iodoform, calcium hydroxide points, calcium hydroxide with 2% chlorhexidine gel against *Enterococcus faecalis* in vitro.

MATERIALS AND METHODS

Experimental root canal infection

Seventy five extracted teeth were collected and were cleaned with periodontal curettes to remove periodontal tissue and stored in 10% formaldehyde for 2 weeks, then they were washed under running water, blot dried and stored in 0.9% sterile saline at 46°C for no longer than 7 days. After removing the crown, each root was instrumented up to size 50 by using a

conventional technique. The root canal was irrigated with ethylenediamine tetra-acetic acid (EDTA) solution to remove smear layer. Then, roots were infected with *E faecalis* strains obtained from IMTECH, Chandigarh which was grown on tryptone soya agar for 72 hrs. Subsequently, the roots were divided into five treatment groups:

Group 1 was treated with calcium powder hydroxide (Ivoclar),

Group 2 was treated with calcium hydroxide with iodoform (Metapex),

Group 3 was treated with calcium hydroxide points,

Group 4 was treated with calcium hydroxide (Prevest) with 2% chlorhexidine gel (Chlor X Gel),

Group 5 was treated with 0.9% sterile saline serving as negative control.

Microbial samples were taken after 7, 15, and 30 days. After incubation, dentine chips were obtained from each root canal and examined microbiologically. The microbiological samples were plated to count colony-forming units in per milligram of dentin.

RESULTS

The results of our study show that application of two percent chlorhexidine gel with calcium hydroxide was the most effective agent against *E faecalis* (Fig. 1), achieving 100% kills. After 7, 15, and 30 days of treatment with 2% chlorhexidine gel in calcium hydroxide, bacterial counts were significantly reduced compared with the control. In the control group 5, root canals without intracanal medication were highly positive for *E faecalis*. Our results indicate that Ca(OH)₂ with sterile water was completely ineffective against *E faecalis*.

We found that the medicament causing least operative pain are ranked according to superiority as Calcium hydroxide with chlorhexidine > Calcium hydroxide points > Calcium hydroxide paste > calcium hydroxide with iodoform. The 2% chlorhexidine gel in Ca(OH)₂ performed significantly better than the Ca(OH)₂ paste alone. At 30th day significant difference was found between group 2 (calcium hydroxide with iodoform), group 5 (control group) and group 4 (calcium hydroxide in chlorhexidine gel). In this study group 4 with time interval of 15 days and 30 days showed statistically significant results.

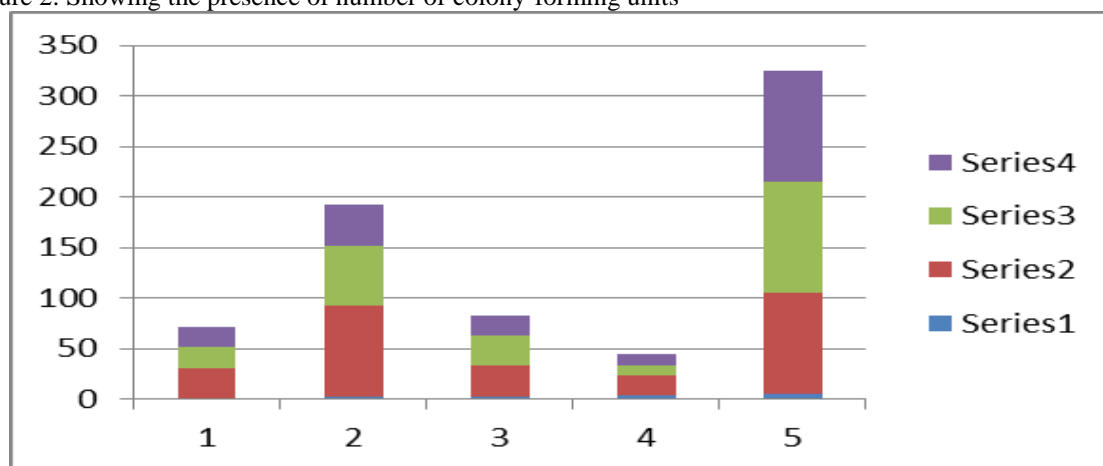
DISCUSSION

E faecalis is considered to be the most resistant microorganisms in endodontic infections and are implicated as a possible cause of root canal treatment failure. There are various methods to evaluate the antimicrobial activity of intracanal medicaments. Although calcium hydroxide is used routinely used as an intracanal medicament, however, several studies have shown that *E faecalis* and *C albicans* are resistant to Ca(OH)₂, and recent studies question the efficacy of Ca(OH)₂ in reducing the number of bacteria in the root canals, even after a prolonged time of interappointment medication.¹¹⁻¹⁵

Figure 1: Showing CFU Units mg⁻¹ Count

Variable	7 DAYS	15 DAYS	30 DAYS
Calcium hydroxide paste	30	20	20
Calcium hydroxide with iodoforn	90	60	40
Calcium hydroxide points	30	30	20
Calcium hydroxide with chlorhexidine gel	10	10	10
Sterile saline	100	110	110

Figure 2: Showing the presence of number of colony-forming units



Apexcal is a viscous polyethylene glycol based paste which contains calcium hydroxide 29%, bismuth carbonate 22% and excipients (polyethylene glycol, glycerine, and water) 49%. It showed very homogenous and constant consistency overtime. Estrela et al described that liberation of calcium and hydroxide ions was faster and more significant when used as calcium hydroxide distilled water paste.¹⁵⁻¹⁹

The temporary calcium hydroxide points are new device for calcium hydroxide delivery for intracanal dressing which contain calcium hydroxide at concentration of 50- 54% that can be easily inserted and removed from the pulp space when their role is accomplished with presence of few residues.³

Calcium hydroxide combined with 2% chlorhexidine has been shown to eliminate *E faecalis* effectively and has been suggested as an alternative to calcium hydroxide alone. De Rossi et al. demonstrated that Ca(OH)₂ was a powerful disinfectant when mixed with 1% chlorhexidine. In our study, Ca(OH)₂ with 2% chlorhexidine mixture was used, and it completely inhibited growth of *E faecalis* 30 days. These findings are in agreement with some earlier reports. However, Sukawat and Srisuwan evaluated the antibacterial effect of calcium hydroxide mixed with 0.2% chlorhexidine and found it ineffective against tested bacteria.¹⁸

As per the available literature, various calcium hydroxide preparations have been tried so as to utilize its antibacterial potential. The reason that chlorhexidine with calcium hydroxide showed minimum colony forming units might be that combined use of chlorhexidine and Ca(OH)₂ in the root canal may generate excessive reactive oxygen species, which may potentially kill various root canal

pathogens. Furthermore, it has been demonstrated that the alkalinity of Ca(OH)₂ when mixed with CHX remained unchanged.¹⁶⁻¹⁸ The results of our study are in concurrence with Zerella et al 2005, Manzur A et al 2007, Zamany A et al 2003 who founded that chlorhexidine is more efficacious in disinfecting the canal.

Thus under the limitations of this study calcium hydroxide with chloorhexidine remains the material of choice when compared to other forms of calcium hydroxide.

CONCLUSION

Thus from this study it concludes that chlorhexidine is an effective antifungal agent especially against *E faecalis*. Mixing chlorhexidine with Ca(OH)₂ may enhance its antimicrobial activity. days. On 15th day and 30th day calcium hydroxide with chlorhexidine showed minimum colony forming units, thus showing that its most efficacious among all groups.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulp in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol. 1965;20:340-9.
2. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981;89(6):475-84.
3. Thakur et al. Comparative evaluation of post-operative pain with different calcium hydroxide formulations when used as intracanal medicament in root canal treatment- In vivo study .Ind J Cons Endod. 2020;5(3):100-104
4. Sundqvist G. Ecology of the root canal flora. J Endod. 1992;18(9):427-30.

5. Jansson L, Ehnevid H, Lindskog S, Blomlof L. Development of periapical lesions. *Swed Dent J.* 1993;17(3):85–93.
6. Greenstein G, Berman C, Jaffin R. Chlorhexidine. An adjunct to periodontal therapy. *J Periodontol.* 1986;57(6):370–7
7. Hess W. Anatomy of root canals in the teeth of the permanent dentition. New York: William Wood and Co; 1925. pp. 36–9.
8. Peters OA, Laib A, Gohring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. *J Endod.* 2001;27(1):1–6.
9. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res.* 1981;89(4):321–8.
10. Weine F. *Endodontic Therapy.* 6th Edition. St. Louis: CV Mosby; 2004. pp. 226–228.
11. Hermann B. Calcium hydroxid als Mittel zum Behandeln und Füllen von Wurzelkanalen [Thesis]. Würzburg; 1920.
12. Farhad A, Mohammadi Z. Calcium hydroxide: a review. *Int Dent J.* 2005;55(5):293–301
13. Spangberg LSW, Haapasalo M. Rationale and efficacy of root canal medicaments and root filling materials with emphasis on treatment outcome. *Endodontic Topics.* 2002;2(1):35–58.
14. Estrela C, Pesce HF. Chemical analysis of the liberation of calcium and hydroxyl ions from calcium hydroxide pastes in connective tissue in the dog. Part I. *Braz Dent J.* 1996;7(1):41–6.
15. Estrela C, PESCE HF. Chemical Analysis of the Formation of Calcium Carbonate and its Influence on Calcium Hydroxide Pastes in Connective Tissue of the Dog-Part II. *Braz Dent J.* 1997;8(1):49–53.
16. Siqueira JF, Jr., Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J.* 1999;32(5):361–9.
17. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol.* 1985;1(5):170–5.
18. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. *J Endod.* 1983;9(9):372–4.
19. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short term intracanal dressing. *Int Endod J.* 1991;24(3):119–25.
20. Lin S, Tsesis I, Zukerman O, Weiss EI, Fuss Z. Effect of electrophoretically activated calcium hydroxide on bacterial viability in dentinal tubules—in vitro. *Dental Traumatol.* 2005;21(1):42–5.