Research Article

Comparative evaluation of commercially available calcium hydroxide with custom-made calcium hydroxide intercanal medicaments on *Enterococcus faecalis*

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ABSTRACT

Aim: The aim of the study is to compare the efficiency of the commercially available calcium hydroxide preparation with custommade calcium hydroxide used as intercanal medicaments. **Background:** Calcium hydroxide is an age-old compound widely used as intercanal medicaments due to its antimicrobial activity. The custom-made calcium hydroxide will have additional properties due to the inclusion of different vehicles proven to be adjunct in contributing to its efficacy. **Materials and Methods:** An *in vitro* evaluation is done using natural teeth, grouping them into control and sample groups and contaminating it with bacteria followed by dressing the canal with medicaments to evaluate their efficacy. **Result:** The efficiency of commercially available and custom-made calcium hydroxide intercanal medicaments are found to be different and custom-made calcium hydroxide intercanal medicaments were found to be more effective.

KEY WORDS: Antimicrobial activity, Calcium hydroxide, Chlorhexidine, Intracanal medicaments

INTRODUCTION

Microorganisms play an important role in causing inflammatory apical lesions, and the goal of endodontic treatment is to prevent and control the infections of pulp and periradicular tissues.[1] Numerous methods have been adopted to reduce the number of microorganism from the root canal system. Mechanical instrumentation alone is not sufficient for elimination of these causative agents; therefore, chemical irrigation and disinfection are necessary to achieve asepsis by the removal of microorganisms and their by-products, pulpal remnants and other debris of the root canal. Intracanal medicaments are materials which are used to disinfect the root canal as a part of controlled asepsis by eliminating the microorganisms present in the canal. They are also used for the prevention and control of the post-treatment pain. In Modern Day Scenario, there are various intracanal medicaments available commercially for treatment.[2] Ideally, per Grossman, an intracanal medicament should be an effective antimicrobial agent with prolonged action, non-irritating to the periradicular tissue should remain stable, should also be active in the presence of blood, and serum and derivatives of tissues. It should posse a low surface tension which does not interfere with the repair mechanism of the periradicular tissues. Most importantly it should not initiate any cell-mediated response and also should not stain the tooth structure. The intracanal medicaments that are widely used are calcium hydroxide and chlorhexidine digluconate. Calcium hydroxide has antimicrobial property and ability to induce, repair and stimulate hard tissue formation. Various other biological properties of calcium hydroxide such as tissue dissolving ability and inhibition of tooth resorption have made it the first choice of root canal dressing material. Calcium hydroxide is a white, odorless powder with

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low solubility and high pH ranging from 12.5 to 12.8. It is available in powder form to be blended with an appropriate vehicle for self-preparation.[3] Commercially available calcium hydroxide is a non-setting paste which is alkaline, radio-opaque, and water soluble. It contains calcium hydroxide as its main constituent along with barium sulfate. Chlorhexidine digluconate (0.2%) is a cationic bisbiguanide which possesses broad-spectrum antimicrobial activity against the common endodontic pathogens. It has both bacteriostatic and bactericidal activity against different bacterial species. Combination of chlorhexidine and calcium hydroxide yields more antimicrobial effect. The aim of this research is to compare commercially available calcium hydroxide with custom-made calcium hydroxide intracanal medicaments.

MATERIALS AND METHODS

Preparation of the Sample

A total of 30 extracted mandibular premolars with single root anatomy were collected from the Outpatient Department of Saveetha Dental College, Chennai. The collected teeth were cleaned using saline. Access opening was done for all the teeth and was autoclaved. Standard strain of *Enterococcus faecalis* was used to test the antimicrobial activity. The bacterial suspension was made by growing the standard strain of *Enterococcus* on brain heart infusion (BHI) agar and suspending in normal saline to the turbidity matching 0.5 McFarland standards. With this, a 100 ml is pipetted into a sterile disposable container containing 25 ml of normal saline and mixed uniformly. This suspension was used to contaminate the teeth by placing the prepared tooth samples for 24 h at 37°C.

Preparation of Calcium Hydroxidepaste

Calcium hydroxide powder and barium sulfate powder were procured from the market and mixed with 2% chlorhexidine digluconate and iodoform.

Formulation of the Paste

About 2 g of calcium hydroxide and barium sulfate was weighed and transferred to a sterile glass slab. 2% chlorhexidine digluconate was added and mixed, and the consistency was matched with the commercially available calcium hydroxide which was about 1.5 ml of 2% chlorhexidine digluconate. This paste was divided further into two portions of which one portion was loaded directly into a 2 ml syringe and labeled as preparation - A. To the other portion, 0.5 g iodoform was added and after through mixing it was loaded into another 2 ml syringe and labeled as preparation - B. The commercial preparation of calcium hydroxide that was used was labeled as Group C.

The teeth sample which was contaminated was separated into 3 Groups A, B, and C of 10 each and the three preparations of calcium hydroxide intracanal medicaments were placed separately in the respective group and placed in a sterile Petri dish. Radiographic evaluation of the packed intracanal medicaments was done to assess the uniformity of the filling and incubated at 37°C for 4 days. After incubation, the canal was scrapped using endodontic file, and the dentinal shavings were collected in a cuvettes containing 1ml of sterile saline. A uniform suspension is made by agitation manually. Then, from each cuvettes 10 ul was transferred to agar BHI and uniformly spread with a sterile loop. The culture plates were incubated at 37°C for 24 h and the colony forming units were counted, and the values were tabulated.

RESULTS

The study revealed the comparison between the custom-made and commercially available calcium hydroxide intracanal medicaments against *E. faecalis*.

It was found that there was a significant reduction in the number of colonizes present on the culture plate of the two custom-made calcium hydroxide groups of intracanal medicaments when compared to the commercially available calcium hydroxide intracanal medicament [Tables 1 and 2].

DISCUSSION

Calcium hydroxide is being extensively used in dentistry for the treatment of vital and non-vital teeth as an intracanal medicament, and it fulfills most of the requirements.[4] In this study, three groups of tooth samples were tested with three different calcium hydroxide preparations. Group A is tested with commercially available calcium hydroxide preparation Groups B and C are custom-made preparations of calcium hydroxide mixing the with chlorohexidine and iodoform with varying combinations. Among the three, Group A and Group B have shown 60% negative in culture of the bacteria done with root canal shavings. The combination which is made with calcium hydroxide, barium sulfate, and 2% chlorhexidine and iodoform has shown comparatively lesser antibacterial effect. In Groups A and B, both the colony count and the number of samples positive for viable culture are more or less the same. In a similar study conducted it was inferred that 2% chlorhexidine gel alone had a better antimicrobial activity than the combination of calcium hydroxide and chlorhexidine or calcium hydroxide alone as an intracanal medicament.[4] On comparison of both, we can conclude that the custom-made preparation with chlorhexidine exhibited equal amounts of antimicrobial activity as of the commercially available preparations. Hence, the custom-made calcium hydroxide preparation mixed with barium sulfate and 2% chlorhexidine can be a

Table 1: The table shows the comparative antimicrobial activity of custom-made and commercially available calcium hydroxide on *E. faecalis*

Commercially available calcium hydroxide	Custom-made calcium hydroxide with 2% chlorhexidine	Custom-made calcium hydroxide with 2% chlorhexidine and iodoform
Positive	Negative	Negative
Positive	Negative	Negative
Negative	Negative	Positive
Negative	Positive	Positive
Negative	Positive	Positive
Negative	Negative	Negative
Negative	Negative	Positive
Positive	Positive	Negative
Negative	Negative	Positive
Negative	Positive	Negative

E. faecalis: Enterococcus faecalis

Table 2: The percentage of tooth samples giving positive growth in the three groups

S.no	Commercially available calcium hydroxide	Custom-made calcium hydroxide with 2% chlorhexidine	Custom-made calcium hydroxide with 2% chlorhexidine and iodoform
Percentage of positivity	40%	40%	50%

suitable replacement for the commercially available intracanal medicament.

Infected root canals exhibit a mixed microbial flora, and the rationale of root canal treatment is the elimination of these microbes which would thereby provide an environment favoring the cessation of the inflammatory responses. Therefore, an ideal antiseptic agent is used as a root canal medicament with non-specific action, active against the most flora and above all biocompatible.[5] *E. faecalis* which is an opportunistic facultative anaerobe which is commonly associated with persistent apical periodontitis in endodontically treated teeth and also highly prevalent in failed root filled teeth. The antimicrobial activity of calcium hydroxide corresponds to the release of hydroxyl ions in an aqueous medium. The lethal effect of the hydroxyl ions on the bacterial cells causes damage to the bacterial cytoplasmic membrane, protein denaturation, and damage to DNA. It possesses anti-endotoxin activity by inactivating the endotoxin that is released by the Gram-negative bacteria during bacterial multiplication or bacterial lysis which causes a series of biological effects leading to stimulation of inflammatory responses and periapical bone resorption.[6] Calcium hydroxide, when placed into the canal, precipitated as calcium proteinate or calcium carbonate crystals, either of which acts as a demarcation between the neurotic and vital pulpal tissue and serves as a suitable matrix for odontoblast alignments.[7] The pH level of calcium hydroxide which is very high due to the free hydroxyl ions in the root canal filling promote a state of alkalinity in adjacent tissues favoring repair and also affect the bacterial cellular metabolism. It has to be placed in close proximity to the appropriate tissue for its maximum efficiency to be obtained. Techniques to deliver dry calcium hydroxide powder alone are difficult when it comes in terms of small and curved canals. The mixing of a liquid vehicle to facilitate the placement of the intracanal medicament is necessary.[8] As calcium hydroxide hydrates only in an aqueous medium, the suitable vehicle to add would be an aqueous solution. The vehicle combined should be tissue compatible, non-irritating to the periapical and periodontal tissues and biological inert and importantly not affect the action of calcium hydroxide.[9-11] The role of water and glycerine as a vehicle for carrying calcium hydroxide intracanal dressing was found to have no statistical difference thereby both being a satisfactory medium to delivery.

Usage of barium sulfate is most common in medicine to increase the radiopacity whereas the use of 2% chlorhexidine as a vehicle enhances the bactericidal property.[12] Chlorhexidine is a cationic bis-biguanide molecule having two chlorophenyl rings and two biguanide chains connected by a hexamethylene bridge.[13,14] It has a wide spectrum of antimicrobial activity against various microorganisms including *E. faecalis*. It is bacteriostatic at low concentrations (0.12%–0.2%) and bactericidal at high concentrations (1.8–2% and above) causing precipitation of bacterial cytoplasm and cell death.[15,16] At optimum ph, it is a di-cationic which enables it to absorb onto the tooth as well as the bacteria and possess the important property of substantively.[17,18] Hence, usage of chlorhexidine along with calcium hydroxide increases the efficiency.

CONCLUSION

From the study, we conclude that custom-made calcium hydroxide preparation with 2% chlorhexidine and barium sulfate proves to be a better alternative for the commercially available calcium hydroxide

intracanal medicament. Using the commercially available calcium hydroxide as an intracanal medicament is easier while the preparation of custom-made calcium hydroxide intracanal medicament paste consumes time yet it proves to be a better cost-effective alternative for the commercially available calcium hydroxide intracanal medicament.

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