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Comparative Microbiological Evaluation of Different Commercially available Calcium Hydroxide Iodoform Formulations Against E. *Fecalis* using Direct Contact Assay.

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Abstract

Endodontic treatment is essentially directed towards eradication of microbes from root canal and periapical tissues by biomechanical preparation and use of medicaments. The outcome of endodontic therapy depends on the complete elimination of these microorganisms. Total eradication of bacteria or sterility is a difficult task to accomplish, but the use of intra canal medications such as calcium hydroxide with iodoform has been thought to maximize the chances of eradicating pathogens from micro endodontic system. This study aims to evaluate the antibacterial efficacy of commercially available formulations of calcium hydroxide against E. faecalis.

The antimicrobial efficacy of five different commercially available calcium hydroxide iodoform formulations was assessed against E. faecalis which is one of most common anerobic organisms seen in infected root canals is done using direct contact assay or technique. The Calcium hydroxide iodoform formulations used were Calpex, Metapex, Calplus, Calform RC and Calgene. The antimicrobial activity was evaluated by measuring the optical density upto 24 hours.

Calplus was found to be most effective against E. faecalis followed by Calplus and Metapex. Further in vivo and in vitro trails would be beneficial to assess the antibacterial properties of the various brands of calcium hydroxide iodoform combination.

Key Words: Calcium hydroxide, Iodoform, E. faecalis, Direct contact assay, Optical Density

INTRODUCTION

The objective of an endodontic treatment is to prevent and intercept pulpal/periradicular pathosis and to preserve the natural dentition when affected by pathosis. The aim of pulp therapy in deciduous teeth is to preserve the integrity and health of the teeth and their supporting tissues.[1]

Endodontic treatment of deciduous teeth is more challenging than that of their permanent counterparts; this is because of the anatomical complexities of their root canal systems and their proximity to the developing permanent tooth, coupled with the difficulty in behaviour management in children. [2,3]

Early loss of primary teeth can cause number of problems like alteration in the path and sequence of eruption, space loss for the succedaneous teeth, development of aberrant habits such as tongue thrusting, alterations in speech, and impairment of function.[2,3] Thus it is important that primary dentition should be maintained in the dental arch, provided it can be restored to function and remain free from pathologies. An intact tooth successfully disinfected and restored clinically is a superior space maintainer than any appliance.[4]

Total elimination of micro organsims from the root canal is necessary for the prevention of subsequent reinfection. This is achieved by careful biomechanical preparation of the root canals followed by the complete obturation of the canal space. The ultimate goal of endodontic obturation has remained the same for more than half a century: to create a fluid-tight seal along the length of the root canal system.[5]

The complex morphology of the root canals in primary teeth makes it difficult to achieve proper cleansing by biomechanical preparation alone.[6] So, the use of a root canal filling material with antimicrobial properties will aid in increasing he success of the endodontic treatment.[7]

Since the 1930s, Zinc Oxide Eugenol has been the material of choice as a non-vital pulp therapy medicament in deciduous teeth. But owing to its disadvantages like slow resorption, irritation to periapical tissues and alteration in the path of eruption of succedaneous tooth, it does not fulfil the requirements of an ideal pulpectomy medicament.[2,3]

Iodoform paste alone or in combination with Calcium Hydroxide has been used as an alternative.[4,8] The mechanism of actions of calcium hydroxide results from the ionic dissociation of the Ca^{2+} and OH^- ions and their effect on vital tissues, such as inducing hard tissue deposition and being antibacterial.[9] Iodoform has been added to $Ca(OH)_2$ due to its antibacterial effect, healing properties, and ability to be resorbed when in excess.[10]

Recent studies investigating bacteria in teeth with an unsuccessful restoration have revealed a certain group of microorganisms including Gram-positive facultative aerobics, especially Enterococcus faecalis.[11]

The purpose of this study was to evaluate the antimicrobial efficacy of different calcium hydroxide iodoform formulations used in pediatric dentistry using direct contact technique or assay.

MATERIALS AND METHOD

Microorganism

The microorganism used was strain of E. faecalis (ATCC2912) cultured on Muller Hington broth (media) (Figure 1).

Figure 1: E. *faecalis* (ATCC2912) cultured on Muller Hington broth

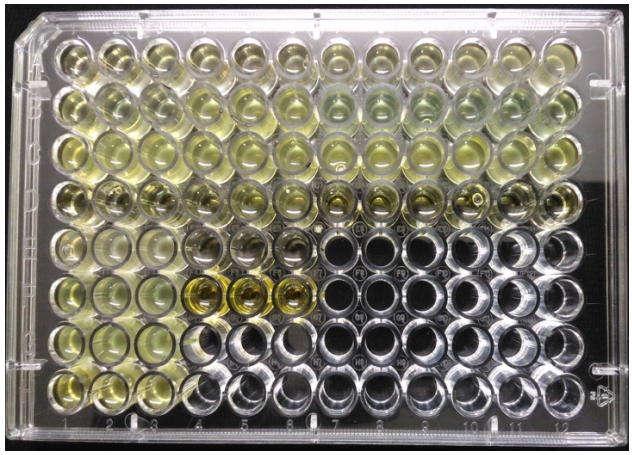


Figure 2: Microplate reader

Medicament formulations

Five different commercially available calcium hydroxide iodoform formulations were used; namely Calform RC (Ammdent), Calpex (Ammdent), Calplus (Pevest Denpro), Metapex (Meta Biomed), Calgene (DEOR).

Methodology

The sides of the wells of a vertical 96 well microtiter plate was coated uniformly with the test compounds and left to dry for 30 min at 37 °C. The wells were then inoculated with 10 µL of an overnight inoculum directly on the coating and incubated for 1 hour at 37°C in static condition. After incubation, 200 µL of liquid media were added to each well and the optical density (OD600) was recorded at one-hour interval (0 – 10 hours and 24hours) using a microplate reader (FLUOstar, BGM Labtech) (Figure 2). The experiments were performed in triplicates.

RESULTS

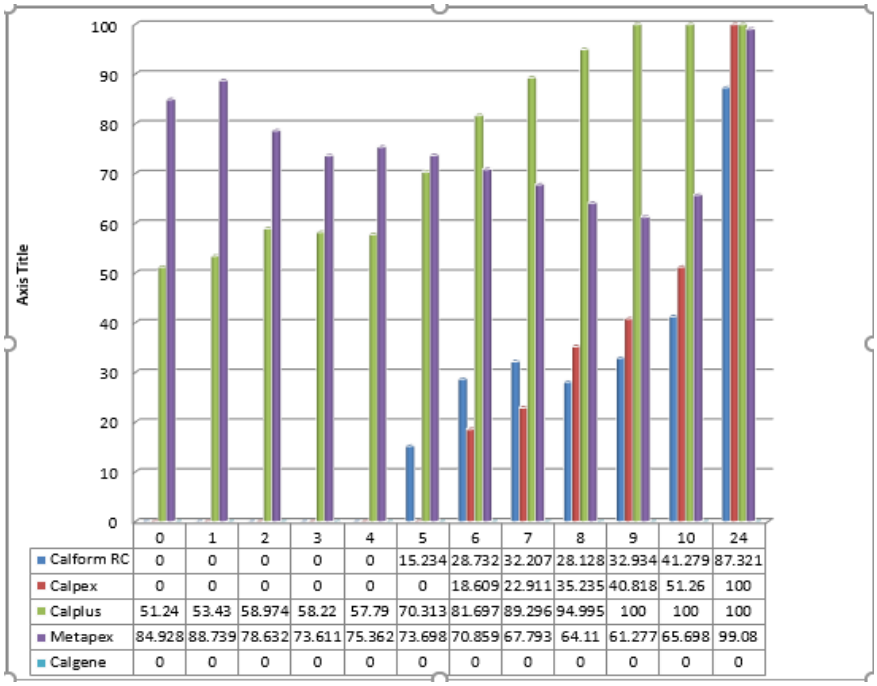
The results of the direct contact test of various calcium hydroxide iodoform formulations for the time period of 24 hours are shown in charts. (Chart 1)

Calplus showed 100 percentage antibacterial efficacy from 9th to 24 hours followed by Calpex. Metapex showed 99 percentage at 24 hours.

Calform RC showed 87% antibacterial efficiency at 24 hours and Calgene showed 0% antibacterial efficiency during 24-hour time period.

DISCUSSION

Endodontic management of primary teeth with severe pulpal necrosis evidenced by nonvital pulp tissue, fistula, and loss of alveolar bone is generally discouraged. Though several reasons have been cited for this, the main one being the variation in root morphology of the primary teeth which is not suitable for biomechanical preparation.[12]



	0	1	2	3	4	5	6	7	8	9	10	24
Calform RC	0	0	0	0	0	15.234	28.732	32.207	28.128	32.934	41.279	87.321
Calpex	0	0	0	0	0	0	18.609	22.911	35.235	40.818	51.26	100
Calplus	51.24	53.43	58.974	58.22	57.79	70.313	81.697	89.296	94.995	100	100	100
Metapex	84.928	88.739	78.632	73.611	75.362	73.698	70.859	67.793	64.11	61.277	65.698	99.08
Calgene	0	0	0	0	0	0	0	0	0	0	0	0

Chart 1: Hourly antibacterial efficacy for each material

Sterilization of the root canal and periradicular region results in good healing of the periradicular region and a results in better prognosis.[13] Microorganisms which are present the root canals of an infected tooth may be easily removed by conventional biomechanical preparation; but those which remain in the deep layers of root canal dentin may later on enter into the periapical region and cause pathosis. Application of antibacterial drugs to endodontic lesions is one of the clinical procedures that can be used to sterilize such lesions.[4]

Enterococcus faecalis is a Gram – positive, facultative anaerobe commonly found in failed pulp therapy. They are able to withstand nutrient depletion, is capable to suppress lymphocyte and form biofilm.[14]

Calcium hydroxide is widely used as root canal filling materials in dentistry due to its ability to eliminate bacteria which cannot be destroyed by biomechanical preparation alone. Calcium hydroxide also possess remineralization capabilities in dentin. It also has ability in accelerating the healing of periapical lesions. Iodoform is incorporated to improve the antibacterial properties of the material.[14,11]

Microplate direct contact assay is based upon optical density assessment. In the Microplate direct contact assay, the antimicrobial activity is measured by the turbidimetric determination of bacterial growth following exposure to the test material. This method was chosen over the more traditional agar diffusion assay as the later possesses numerous limitations in interpretation.

There are various forms of calcium hydroxide iodoform formulations commercially available in different names. In the present study the different commercially available calcium hydroxide iodoform formulations were checked for antibacterial efficiency against E.faecalis in which Calplus showed 100% efficiency at ninth hour and Calgene showed 0% antibacterial efficiency during 24 hour time period.

CONCLUSION

A large number of Calcium Hydroxide – iodoform combination are available now. But the antibacterial properties and efficacy of the products are in question. Among the five easily available Calcium Hydroxide iodoform formulations we compared, Calplus was found to have better antibacterial properties against E. faecalis followed by Calpex. Further in vivo and in vitro trails would be beneficial to assess the antibacterial properties of the various brands of calcium hydroxide iodoform combination.

Conflicting Interest: Nil

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