

## Elimination of *Candida Albicans* Biofilm in Radicular Dentin By Intracanal Medications-*In Vitro* Study

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**ABSTRACT:-** The present study is conceived with an aim to evaluate the effectiveness of different intracanal medications like Ca(OH)<sub>2</sub>/glycerine, Ca(OH)<sub>2</sub>/chlorhexidine, Chlorhexidine/ZnO, Saline solution (control) in the elimination of *Candida albicans* biofilm from infected radicular dentine. It is an *invitostudy*. Freshly extracted human incisors were used for this study.

### I. INTRODUCTION

There is no greater association between a basic science and the practice of endodontics than that of microbiology. The vast majority of diseases of dental pulp and periradicular tissues are associated with microorganisms. After microbial invasion of these tissues host responds with both non-specific inflammatory responses and with specific immunologic responses. Viable microorganisms after root canal preparation and disinfection contribute significantly to failure of endodontic therapy. Fungi are considered a normal inhabitant of oral cavity, but may produce disease when there are local or systemic factors predisposing the individual to infection. The mechanism believed to be involved in the pathogenesis are<sup>2</sup>: adaptability to a variety of environmental conditions, adhesion to a variety of surfaces, production of hydrolytic enzymes, morphogenic transition, biofilm formation, evasion and immunomodulation of host defenses etc.

### II. REVIEW OF LITERATURE

Menezes M.M., Valera M.C., Jorge A.O., Koga Ito C.Y., Camargo C.H., Mancini M.N. in 2004 evaluated *in vitro* the effectiveness of sodium hypochlorite (NaOCl), chlorhexidine (CHX) and five intracanal medicaments on microorganisms within root canals. Calcium hydroxide + CPMC paste was the most effective intracanal medicament for elimination of the 2 microorganisms.<sup>4</sup>

Sawai J., Yoshikawa T. in 2004 did quantitative evaluation of antifungal activity of metallic oxide powder (MgO, CaO and ZnO) by an indirect conductimetric assay. *Candida albicans* NBRC 1060, *Saccharomyces cerevisiae* NBRC 1950, *Aspergillus niger* NBRC 4067 and *Rhizopus stolonifer* NBRC 4781 were used as test micro-organisms. MgO and CaO powders exhibited the antimicrobial activities against all fungi used-, in this study and showed little differences between types of fungi. However although ZnO powder inhibited fungal growth, the value of [ I ]100 were over 100 mg MI/I.<sup>5</sup>

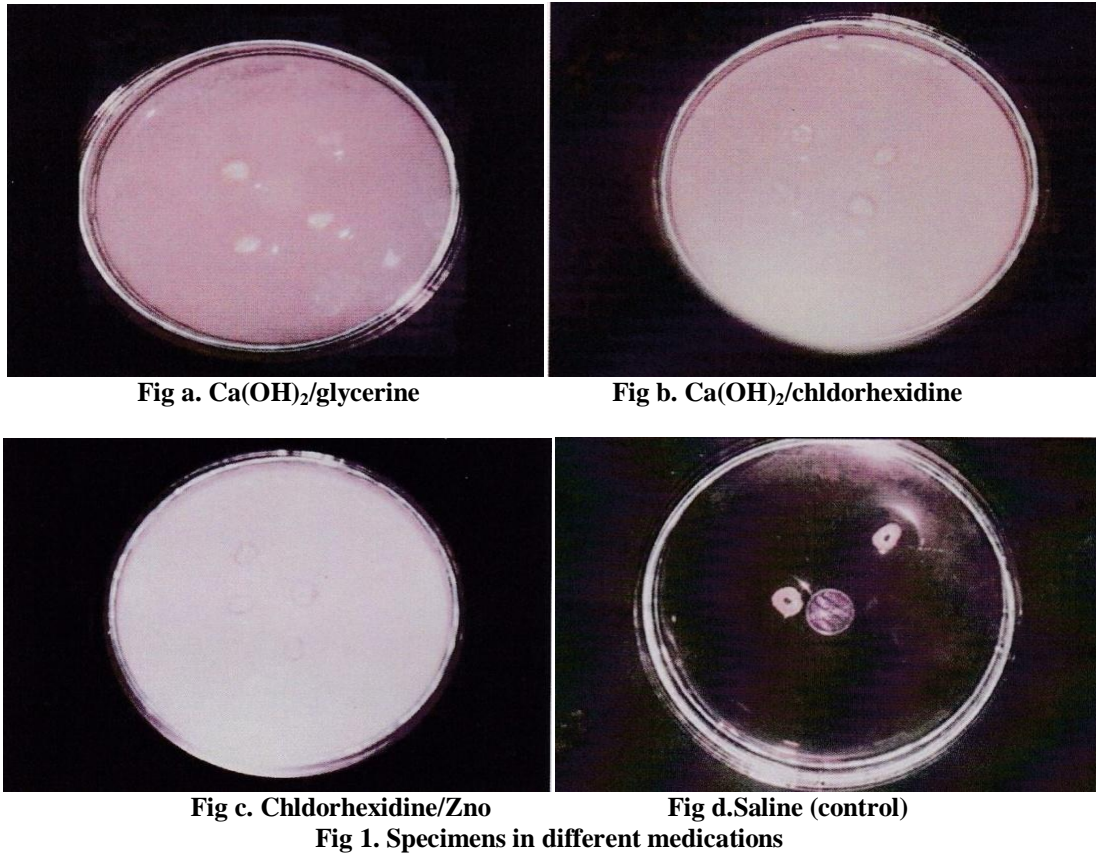
Andrei Barasch et al published the results of a pilot study in 2004. The pilot study evaluated the effect of chlorhexidine (CHX) 0.12% rinses on the clinical and microbiologic manifestations of oral candidiasis in HIV-infected children. This study suggests that the topical disinfectant CHX may be a promising agent for treating and preventing oral candidiasis in HIV infected children.<sup>6</sup>

### III. MATERIALS & METHODS

Freshly Extracted Human maxillary incisors were used for this study. The microorganism used was *Candida albicans* (ATCC 10231)

Materials used for the study are 0.5% Sodium hypochlorite (Hi Media), 10% Citric acid (Merck), Phosphate buffer saline (PBS) (Hi Media), Fluid thioglycollate (FT) (Hi Media), Sterile Ca(OH)<sub>2</sub> powder (Merck), Glycerin (Merck), 0.2% Chlorhexidine digluconate (Hexidine ICPA), Sterile ZnO powder, Sterile saline (Hi Media) and Sabouraud dextrose Agar (SDA) (Hi Media)  
Equipment used are Ultra sonic cleaner (Cole parmer), Autoclave (Sanyo), Incubator (Sanyo), Scanning electron microscope and its accessories (Hitachi), Trinocular research microscope (Leica DMR) and Digital colony counter (Toshiba).

Specimens have been prepared as per the standard protocol and survival rate of biofilm was examined by standard procedures like viable count, fluorescent microscopy and SEM.



**IV. RESULTS**

**Table 1.1<sup>st</sup> hour sample**

Observation			
CG	CC	CZ	S
NT NTNTNT	NT NTNTNT	NT NTNTNT	T
NT NTNTNT	NT NTNTNT	NT NTNTNT	T
NT NTNTNT	NT NTNTNT	NT NTNTNT	T

NT: Notturbid. T: Turbid

**Table 2.2<sup>nd</sup> day sample**

Observation			
CG	CC	CZ	S
NT NTNTNT	NT NTNTNT	NT NTNTNT	T
NT NTNTNT	NT NTNTNT	NT NTNTNT	T
NT NTNTNT	NT NTNTNT	NT NTNTNT	T

NT: Not Turbid T: Turbid

**Table 3.7<sup>th</sup> day sample**

Observation			
CG	CC	CZ	S
NT NTNTNT	NT NTNTNT	NT NTNTNT	T
NT NTNTNT	NT NTNTNT	NT NTNTNT	T
NT NTNTNT	NT NTNTNT	NT NTNTNT	T

NT: Not Turbid T: Turbid

CG :Ca(OH)<sub>2</sub>/Glycerine CC:Ca(OH)<sub>2</sub>/Chlorhexidine CZ: Chlorhexidine/ZnO S: Saline

**Table 1, 2 & 3. Observation of samples in Fluid thioglycollate Medium, after medication exposure**

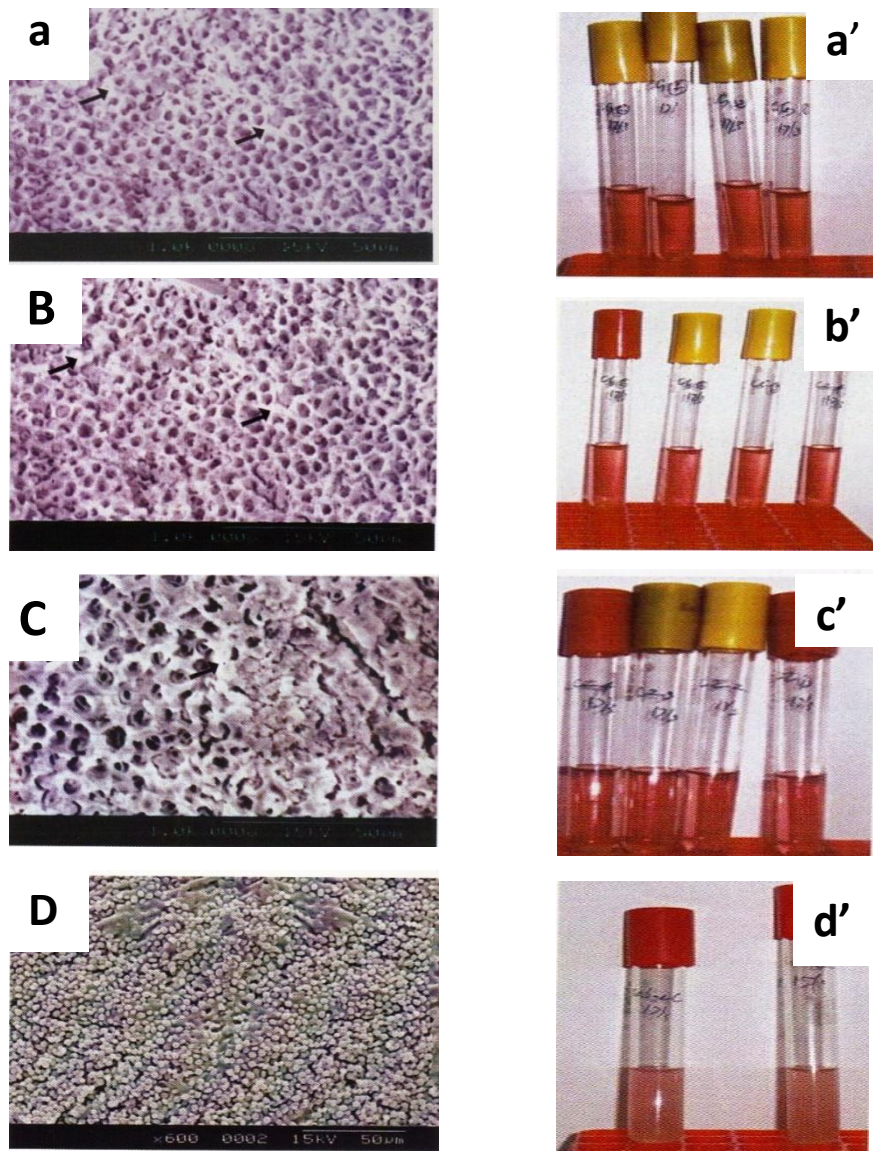


Fig 2.Observations of medicated specimens by SEM & culture (  $\text{Ca(OH)}_2$ /glycerine(a,a') ;  $\text{Ca(OH)}_2$ /chldorhexidine(b,b') ; Chldorhexidine/Zno(c,c') ; Saline (d,d').

## V. DISCUSSION

The goal of endodontics is the complete debridement of the root-canal system to eliminate all microorganisms, their byproducts and tissue debris. Microorganisms present within tubules can be unaffected by the chemomechanical procedures. *C.albicans* has the ability to form biofilms on different surfaces, and this property may be one of the reasons why this species is considered more pathogenic than species that are less able to form biofilms such as *C.glahrata*, *C.tropicalis* and *C. parapsilosis*.<sup>3</sup> According to Donlan and Costerton, a biofilm can be defined as a community of microorganisms irreversibly attached to surface, containing exopolymeric matrix and exhibiting distinctive phenotypic properties.

*Candida* has molecules on its surface that mediate its adherence to tissues, including a receptor homologous to the human CR3 integrin, which binds RGD (arginine-glycine-aspartic acid) groups on fibrinogen, fibronectin, and laminin, and mannose-containing proteins that bind to lectin like molecules on host cells and tissues.<sup>8</sup> The adherence of *C.albicans* to the extracellular matrix proteins, type 1 collagen and fibronectin is dependent upon the presence of extracellular calcium, which is abundant in dentin. It may help to explain dentin colonization by *C. albicans* as observed in this study. *C.albicans* produces hydrolytic enzymes that may be involved in the damage to the periradicular tissues. Enzymes include secreted aspartyl proteinase, collagenase, aminopeptidases, glucosaminidases, acid and alkaline phosphatases, hyaluronidase, and chondroitin sulfatase, all of which have some effects in the degradation of extracellular matrix proteins. It has been shown that a collagenolytic enzymes produced by this fungal species may degrade the human dentine collagen.



Here lies the relevance of PNR study by Nair et al. He observed that even though radio graphically perfect root canal filling was obtained, there are some failures encountered in many of the cases. On investigation he found that the presence of *Candida albicans* is one of the major reasons for failure of endodontic therapy.<sup>9</sup>

The issue of single visit and multiple visit endodontic treatments of teeth with necrotic infected pulp has been the subject of debate for many years. Studies comparing the long term outcomes single visit and multiple visit endodontic treatment have shown that there are significantly fewer failures with 2 visit treatment.

The different medicaments used in this study are Ca(OH)<sub>2</sub>/Glycerine, Ca(OH)<sub>2</sub>/Chlorhexidine, Chlorhexidine/ZnO and Saline(control). Calcium hydroxide is the most used, discussed, and studied intracanal dressing. Estrela et al reported that because the site of action of hydroxyl ions of calcium hydroxide includes the enzymes in the cytoplasmic membrane this medication has a large scope of action, depending on its quantity, and therefore is effective on a wide range of microorganisms, regardless of their metabolic capability. Cytoplasmic membranes are similar regardless of their morphological, staining, and respiratory characteristics that means that this medication has a similar effect on aerobic, anaerobic, Gram-positive, and Gram-negative bacteria. Antimicrobial mechanism of calcium hydroxide was observed as that the enzymes in the cytoplasmic membrane were the targets of pH changes, which can lead to a reversible or irreversible inactivation of aerobic, anaerobic, Gram positive, and Gram-negative microorganisms.

Chlorhexine is a dicationic biguanide with broad antimicrobial activity. Chlorhexidine exhibits wide spectrum of activity encompassing Gram - positive and Gram-negative bacteria, yeasts, dermatophytes and some lipophilic viruses. Its antimicrobial activity is of the membrane activity type (damages the inner cytoplasmic membrane). It has a strong affinity for binding to skin and mucous membrane., CHX has been suggested as a root canal irritant, owing to its unique ability to bind to dentin, its effectiveness as an antimicrobial agent, and its substantivity in the root canal system. While these substantive and antimicrobial properties of CHX found here are promising, it does not have the tissue-dissolving properties of NaOCl. Although NaOCl is still considered the irrigant of choice, the use of CHX may be considered advantageous as a treatment prior to obturation, an alternate irrigant during retreatments, or even incorporated into antimicrobial dressings. In a study by Scquera J.F. Jr, Rocas J.N.1 the addition ZnO with chlorhexidine increases the antifungal activity of chlorhexidine. So in this study we chose these medications.

Root specimens prepared from freshly extracted human incisors were used for this study. The efficacy of the different medications was checked by keeping the infected dentinal specimens in different medications for 1 hr, 2 days and 7 days period. The time required to eliminate *Candida albicans* within dentine *in vitro* cannot be directly extrapolated to the clinical situation because the optimum contact between medication and dentine under *in vivo* condition is not achieved as *in vitro* and because of other variables imposed by a clinical situation and not reproduced in this experimental model. In this study we have repeated the whole experiment 3 times and got the same result. These findings indicate that it is an effective treatment strategy to perform root canal disinfection with any of the medicaments preceding root canal obturation. Thus multiple sittings will be beneficial and prevent prevalence of persistent root canal infection.

## VI. CONCLUSION

The *in vitro* study conducted on radicular dentine of human maxillary incisors with the aim of evaluating the effectiveness of different intra canal medications in the elimination of *Candida albicans*, It can be concluded that

1. All the medications used in this study were equally effective for the elimination of *Candida* from infected tooth sample
2. Even 1 hr exposure of medicament was sufficient for the complete elimination of the *Candida*
3. This study also stresses the importance of multiple visit endodontic treatment

To conclude, use of intracanal medicaments is essential for the elimination of microorganisms including fungi. This justifies multiple visit endodontic treatment with intracanal medicament for complete disinfection of tooth.

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