Investigating the Antifungal Effects of Mineral Trioxide Aggregate and Indigenously Developed Bioceramic Cement Ceremagnum Plus on Candida Albicans by Invitro Evaluation

Dr. Anusua Mitra[1], Dr. Sonali Sharma[2*]

[1] *Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS) Saveetha University, Chennai-600077, Tamil Nadu, India. Email id: 152206007.sdc@saveetha.com*
[2] *Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India. Email Id: colsonalisharma.sdc@saveetha.com*
[*]*Corresponding Author: Dr. Sonali Sharma*

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ABSTRACT
The aim of this in vitro study was to evaluate the antifungal efficacy and to determine the minimal inhibition concentration (MIC) of MTA and indigenously developed Ceremagnum plus against Candida albicans.
Sabouraud dextrose agar plates containing various concentrations of MTA powder, Ca(OH)2 and indigenously developed Ceremagnum plus were prepared. The plates were inoculated with an overnight culture of C. albicans, then observed for the formation of colonies after incubation at 37 °C for 24. The minimal concentration at which inhibition of the microorganism occurred was measured and noted as the MIC.
There was a statistically significant difference in the antifungal effect between the concentrations of 50mg /ml,25mg/ml,12.5mg/ml,6.25mg/ml,3.12mg/ml,1.56mg/ml,0.78mg/ml,0.39mg/ml,0.19mg/ml and 0.009mg /ml. The MIC of MTA, Ca (OH)2 and indigenously developed Ceremagnum plus against C. albicans.

1. Introduction

Two critical factors for successful endodontic treatment are the removal of microorganisms and the creation of a fluid-tight seal. Many endodontic failures result from insufficient cleaning of the root canal and the infiltration of bacteria and other pathogens into the periradicular area. [1]Along with bacteria, fungi are often found in the root canal, with Candida albicans. being one of the most frequently isolated fungal species in the oral cavity. When initial root canal therapy fails and retreatment is not an option, endodontic surgery is performed. Although fungal infections are far less common than bacterial ones, they can still pose a risk, particularly for immunocompromised individuals. Fungi can infect different parts of the body, leading to various types of infections, including systemic, superficial, cutaneous, and subcutaneous.[2] The most common fungi responsible for human infections include genera such as Aspergillus, Candida, Cryptococcus, Rhizopus,Mucor and Rhizomucor. Over 80% of candidiasis cases are caused by C. albicans, while the most common non-Candida albicans candidiasis (NCAC) are caused by C. glabrata C. parapsilosis, C. tropicalis, C. krusei and C. dubliniensis.[3]

The selection of material plays a crucial role in achieving successful clinical results. For biomaterials to be effective against fungi, they must exhibit a strong alkaline reaction.[4] The use of biomaterials in endodontic treatments has gained popularity in recent years. Biodentine, a fast-setting, calcium silicate-based dentin substitute, is commonly used for direct restorative posterior fillings, furcal perforations, retrograde fillings, and pulp capping. Various in vivo and in vitro studies have demonstrated its bioactivity and successful outcomes in a range of clinical applications.[5]

MTA has become a widely used material for sealing the connection between the root canal system and the external root surface. It is a powder composed of fine hydrophilic particles that, when exposed to water or moisture, form a colloidal gel, which hardens into cement within about 3 hours. [6]The primary ingredients of the gray-colored MTA include tricalcium oxide, tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and calcium sulfate dihydrate. White MTA (WMTA), however, does not contain tetracalcium aluminoferrite.[7]

Certain factors, such as specific intracanal medicaments, local and systemic antibiotics, and prior unsuccessful endodontic treatments, seem to contribute to this process. It has been suggested that reducing particular bacteria

in the root canal during endodontic treatment may create a low-nutrient environment that encourages fungal overgrowth. [8]Additionally, fungi like Candida albicans (CA) may enter the root canal due to coronal leakage.[9]

To assess the antimicrobial properties of materials, various laboratory techniques can be employed, such as the agar dilution method. This approach is effective because it ensures direct contact between microorganisms and the test materials, providing a more accurate representation of their interaction. [10]Using this method, the minimal inhibitory concentration (MIC), which is the lowest concentration of a material that inhibits visible microbial growth, can be determined.

Endodontic surgeries are often performed at the apex of the tooth, where materials are placed to seal the root canal from the periapex. Inadequate cleaning of the root canal and bacterial or pathogen invasion into the periradicular area are common causes of endodontic failure.[11]

2. Materials and methods

2.1 Anti Fungal Activity: Agar Well Diffusion Assay

Sabouraud dextrose broth (SDB) was prepared, two different fungal strain, *candida albicans* strains were inoculated in the SDB. Sabouraud Dextrose Agar was prepared and poured into the sterile petri Allowed to solidify the media. Turbidity of the inoculum was adjusted according to the 0.5 McFarland stand and lawn culture was performed in the Sabouraud dextrose agar plates by using the sterile swab. Four wells with a diameter of 10 mm and a depth of 4 mm were using sterile well cutters.[12] Fluconazole disc was utilized as the positive control, while DMSO served as the negative control. Three samples 100ul (50mg/ml) was added into the well. The plates were then incubated at 37C for 24 hours. Petri plates were observed for zones of inhibition, which were measured using a scale in millimeters.

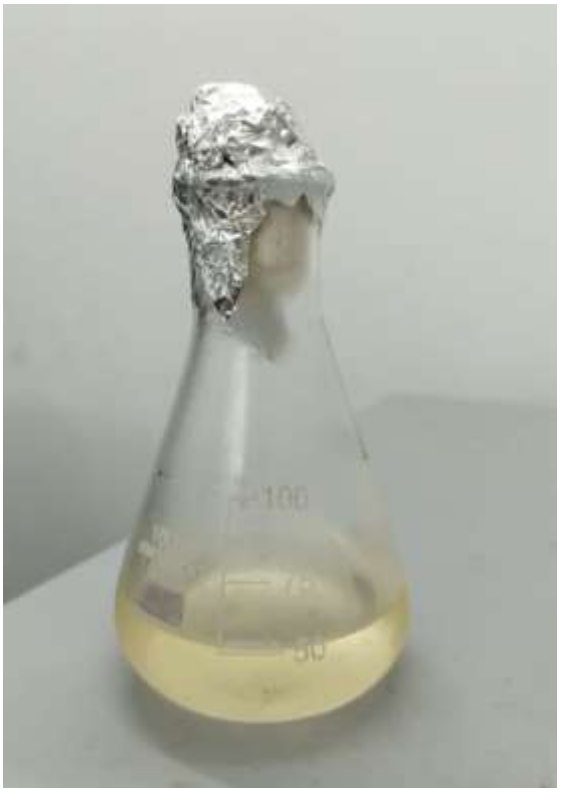

Fig 1: Broth

2.2 Minimum Fungicidal concentration

After determining the minimum inhibitory concentration (MIC) of the samples, 50 µl aliquots were taken from wells showing no observable fungal growth. These aliquots were spread plated onto SDA plates and incubated for 24 hours at 37°C. The minimum fungicidal concentration (MFC) endpoint was determined when 99.9% of the fungal population was eradicated at the lowest concentration of the antimicrobial agent. This assessment involved examining the presence or absence of fungi on both the agar plates before and after the incubation

period.[13]

3. Results

At time period of 24hrs after inoculation the presence of C. albicans colonies was assessed and recorded. The antifungal activities of the different MTA concentrations, Ca(OH)2 and indigenously developed ceremagnum plus were assessed.

3.1 Minimum inhibitory concentration

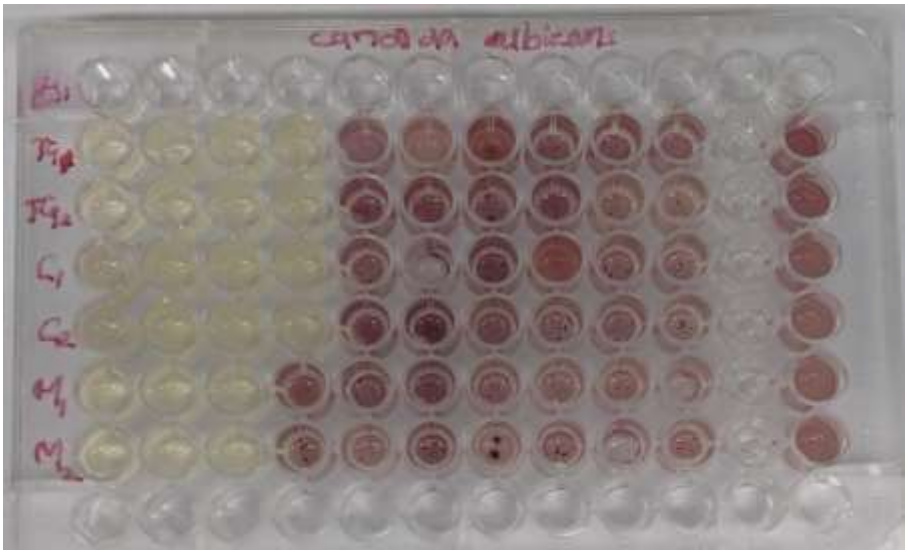

Fig 2: MIC for Ceremagnum plus and Ca(OH)2=6.25mg/ml and for MTA=12.5mg/ml

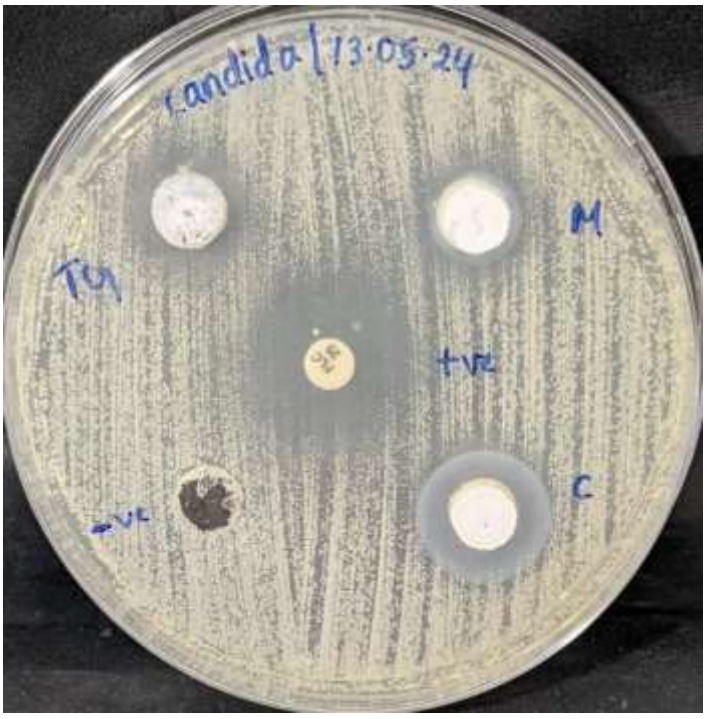

Fig 3: Zone of inhibition

For Candida albicans MTA has highest zone of inhibition as compared to Ca(OH)2 and indigenously developed ceremagnum plus.

4. Discussion

Candida albicans was chosen for this study because it is frequently found in mixed cultures alongside bacteria and is a common microorganism that often withstands mechanical cleaning, chemical treatments, and root canal

medications. The fungal strain used in this study is widely employed in both clinical and laboratory research.[14] Siqueira and Sen reported that C. albicans can colonize root canal walls and easily infiltrate dentin tubules.

Moreover, Demiryürek et al. discovered that all tested retrograde filling materials, including MTA Angelus, Biodentine™, and DiaRootBioAggregate, showed antibacterial and antifungal properties. Other research also concluded that with respect to their antifungal activity, materials such as ProRoot MTA, MTA Plus, and Biodentine™ are effective for use in endodontic treatments.[15] Among these, Biodentine alone demonstrated a slightly stronger inhibitory effect on C. albicans than the Biodentine/chlorhexidine mixture, although the difference was minimal.[16]

Although many studies have explored the antifungal properties of Biodentine™, only one study has reported its minimum inhibitory concentration (MIC) against C. albicans.[17] Hiremath et al. similarly found that Biodentine™ exhibited strong antifungal activity, with an MIC of 2.5 mg/ml.[18]

However, it is important to note that in vitro studies of dental materials may not fully replicate real-life conditions due to factors like saliva, pH fluctuations, temperature changes, food intake, liquids, and chewing in the oral cavity. Therefore, additional in vitro and in vivo studies are necessary to validate the antifungal activity of C. albicans against MTA, $Ca(OH)_2$, and a locally developed material called ceremagnum plus.[19]

In the present study, the methodology enabled the antimicrobial effect evaluation of cements after setting on bacteria organized as biofilm, according to Faria-Júnior et al.[20]

Strong alkalinity is crucial in biological activity, the antibacterial effect against E. faecalis as shown by previous studies McHugh stated that a high alkaline pH of more than 11.5 is bactericidal to E. faecalis.In our study pH of Ceremagnum plus is 14 Estrela et al., demonstrated that MTA had no antimicrobial activity against E. faecalis, but this study proved its antimicrobial efficacy against E. faecalis.[21]

5. Limitations & Future Scope

Conduction of future studies to evaluate MTA's antibacterial efficacy in animal models or human clinical trials is required.

Investigate the persistence of MTA's antibacterial effects over time, including its ability to prevent reinfection or microbial recolonization in clinical settings.

Currently Agar diffusion test results are questionable due the variations in agar medium, bacterial strains, diffusion capacity of inhibitory agents, and cellular density may interfere with the formation of inhibition zones around materials used in antimicrobial testing producing false results.[22]

6. Conclusion

The study confirms the potent antimicrobial activity of indigenously developed Ceremagnum plus against S.mutans, S.aureus, Candida albicans and E.Faecalis.

MIC was determined by lowest concentration that kills 99.9% of initial bacterial population.

Antimicrobial efficacy by Agar Diffusion showed maximum zone of inhibition for Ceremagnum plus to staphylococcus aureus and greater than MTA.

For S.mutans and E.faecalis Calcium hydroxide has shown highest zone of inhibition ,for Candida albicans MTA has highest zone of inhibition whereas ceremagnum plus showed highest for S aureus

Thus indigenously developed bioceramic Ceremagnum plus can be used as an alternative to synthetic antimicrobials.

References

[1] Chong BS, Pitt Ford TR, Watson TF, et al. Sealing ability of potential retrograde root filling materials. Endod Dent Traumatol 1995; 11: 264–269.

[2] Microbiological evaluation of one- and two-visit endodontic treatment of teeth with apical periodontitis: A randomised, clinical trial. Aust Endod J 2004; 30: 161–161.

[3] Poptani B, Sharaff M, Archana G, et al. Detection of Enterococcus faecalis and Candida albicans in previously root-filled teeth in a population of Gujarat with polymerase chain reaction. Contemp Clin Dent 2013; 4: 62–66.

[4] Ozcan E, Yula E, Arslanoğlu Z, et al. Antifungal activity of several root canal sealers against Candida albicans. Acta Odontol Scand 2013; 71: 1481–1485.

[5] Babu NK, Arumugham IM, Kumar RP, et al. Anti-microbial Efficacy of Commercially Available Herbal Dentifrices on Streptococcus mutans and Candida albicans. J Pharm Res Int 2020; 87–94.

[6] Paul S, Mohanram K, Kannan I. Antifungal activity of curcumin-silver nanoparticles against fluconazole-resistant clinical isolates of Candida species. Ayu 2018; 39: 182–186.

[7] Ashwini T, Hosmani N, Patil CR, et al. Role of mineral trioxide aggregate in management of external root resorption. J Conserv Dent 2013; 16: 579–581.

[8] Aravind Kumar S, Rajeshkumar S, Saravana Dinesh SP, et al. Antimicrobial activity of silymarin mediated zinc oxide and hydroxy apatite nanoparticles against oral pathogens. Bioinformation 2020; 16: 863–868.

[9] Jardine AP, Montagner F, Quintana RM, et al. Antimicrobial effect of bioceramic cements on multispecies microcosm biofilm: a confocal laser microscopy study. Clin Oral Investig 2019; 23: 1367–1372.

[10] Hameed AR, Ali SM, Ahmed L. Biological study of Candida species and virulence factor.

[11] Camilleri J, Grech L, Galea K, et al. Porosity and root dentine to material interface assessment of calcium silicate-based root-end filling materials. Clin Oral Investig 2014; 18: 1437–1446.

[12] Anti-fungal activity of chitosan nanoparticle incorporated lycopene against Candida albicans using minimal inhibitory concentration assay. Natarajan k.

[13] Bhavana V, Chaitanya KP, Gandi P, et al. Evaluation of antibacterial and antifungal activity of new calcium-based cement (Biodentine) compared to MTA and glass ionomer cement. J Conserv Dent 2015; 18: 44–46.

[14] Antibacterial activity of novel selenium and zinc nanoparticles toward oral Candida albicans: An in vitro study.

[15] Comparative study of antimicrobial activity of ‘Biodentine’ and ‘Rootdent’ cements and ‘Futura bond HP’ adhesive.

[16] Torabinejad M, Hong CU, McDonald F, et al. Physical and chemical properties of a new root-end filling material. J Endod 1995; 21: 349–353.

[17] Cavalheiro M, Teixeira MC. Candida biofilms: Threats, challenges, and promising strategies. Front Med (Lausanne); 5. Epub ahead of print 13 February 2018. DOI: 10.3389/fmed.2018.00028.

[18] Nowicka A, Lipski M, Parafiniuk M, et al. Response of human dental pulp capped with Biodentine and MTA.

[19] Friedman S. Retrograde approaches in endodontic therapy. Dent Traumatol 1991; 7: 97–107.

[20] Guerreiro-Tanomaru JM, Chávez-Andrade GM, de Faria-Júnior NB, et al. Effect of passive ultrasonic irrigation on Enterococcus faecalis from root canals: An ex vivo study. Braz Dent J 2015; 26: 342–346.

[21] Waltimo TM, Sirén EK, Orstavik D, et al. Susceptibility of oral Candida species to calcium hydroxide invitro.

[22] Ghogre P. Endodontic mycology: A new perspective of root canal infection. research and reviews.