A Comparative Evaluation of Anti-fungal Efficacy of MTA and Biodentine: An Original Research

Jyotsana Sikri[1], *Arpit Sikri[2], Tarun Kalra[3], Sameer Makkar[4], Sandeep Gupta[5] and Geeta Kalra[6]

*Corresponding Author E-mail: **arpitsikri@gmail.com**

Contributors:

[1]SeniorLecturer,[2]Associate Professor, [3] Principal, Professor & Head, [4] Professor & Head, [5] Reader,[6]Professor & Head,[1,4,5] Department of Conservative Dentistry & Endodontics, [2,3] Department of Prosthodontics, [6] Department of Oral & Maxillofacial Surgery, Bhojia Dental College & Hospital, Chandigarh - Nalagarh Road, Budh (Baddi),Teh. Baddi, Distt. Solan, Himachal Pradesh – 173205 India.

Abstract

Introduction: Biodentine has been recently introduced as the "the first all-in-one, bioactive and biocompatible material for damaged dentin replacement". It has been found by the manufacturers that Biodentine has much shorter setting time with better mechanical and handling properties in contrast to Mineral Trioxide Aggregate (MTA) **Aims**: The aim of the study was to evaluate and compare the anti-fungal efficacy of MTA and Biodentine. **Methods and Material**: Candida albicans was grown on Mueller-Hinton agar culture plates. The McFarland 0.5 solution of Candida albicans was made in peptone broth. Sterile swab was used to transfer the growth of Candida albicans from peptone broth to 10 sterile Muller-Hinton agar plates. Two wells of 4 mm diameter and 4 mm height were prepared on each agar plate. MTA and Biodentine were mixed and placed into their respective well in each agar plate and these culture plates were incubated at 37^0C for 24 hours. GIC was used as control group. After 24 hours, zone of inhibition was measured around the wells, following the standard protocol. **Statistical analysis used**: One way Anova and Post hoc turkey test **Results**: Collected data was subjected to statistical analysis which revealed that Biodentine performed significantly better than MTA and GIC against Candida albicans. **Conclusion**: This in vitro study concluded that Biodentine is a better anti-fungal agent than MTA. However, more clinical and laboratory investigations are required.

Keywords: Anti-fungal efficacy, Biodentine, Candida albicans, MTA, Zone of inhibition

INTRODUCTION

Microorganisms are the leading factors which cause progression of pulpal and periradicular diseases[1]. Endodontic treatment success rate is built upon proper cleaning, shaping and obturating the entire root canal system. Teeth with pulpal or periapical pathology have a complex microbial flora consisting of cocci, rods, spirochetes, and fungi[2]. *Candida albicans* is commonly present in post treatment apical pathology[3]. Candida found to be the utmost resistant pathogen by virtue of its ability to penetrate into the dentinal tubules and is resistant to many intracanal medicaments. Endodontic failure cases have to be treated by surgical intervention and placement of root-end filling material to achieve good hermetic seal. It is desirable for root end filling material to have anti-bacterial and anti-fungal efficacy to prevent re-infection. MTA is a popular material due to its low solubility, low cytotoxicity, biocompatibility, anti-microbial, anti-fungal and ability to induce hard tissue formation and it has been used widely in several clinical situations such as sealing the perforations, repair of external/internal root resorption, retrograde filling, pulp-capping agent in vital pulp therapy procedures, apexification etc. However, cumbersome manipulation of MTA makes operator to look for other easy option[4-8]. Recently, new endodontic repair material i.e. Biodentine (Septodont) has been introduced which comprises of Tri-calcium silicate, di-calcium silicate, calcium carbonate, iron oxide, zirconium oxide in powder and liquid contains calcium chloride and hydrosoluble polymer. It is highly indicated as a root end filling material, having similar properties as that of MTA but having better manipulation and decreased setting time overemphasize its use in challenged and compromised endodontic clinical cases[9].

Aim of this in-vitro study was to evaluate the anti-fungal efficacy of MTA (Figure 1) and Biodentine (Figure 2).

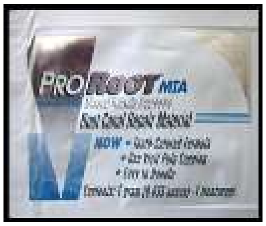


Figure 1: MTA

Figure 2: Biodentine

Materials and Methods:

The study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Department of Microbiology, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, India. Pure culture of Candida albicans (ATCC 10231) was obtained and was grown on Mueller-Hinton agar plate for 24 hours at 37^0C in the incubator. Once the growth was flourished (Figure 3), the colonies of Candida albicans were passed to peptone broth using sterile straight wire as depicted in Figure 4. The turbidity of the peptone broth was matched with 0.5 McFarland Standard (Figure 5). The sample from prepared peptone broth was taken using a sterile swab and a lawn culture was made on new sterile 20 Mueller-Hinton agar plates (Figure 6). Each of 10 agar plates were divided into 2 halves: in each half one well was made of 4x4 mm dimension. The test materials MTA (Dentsply), Biodentine (Septodont) and GIC (Fuji) were taken and mixed as per the manufacturer's instructions. In one well, mixed MTA was placed, while in other, mixed Biodentine was placed (Figure 7, 8). In remaining 10 plates, one well of 4x4 mm diameter was made in the centre of each plate, into which mixed GIC was placed, acting as control group. After 24 hours, zone of inhibition for Biodentine, MTA and GIC was noticed (Figure 9).

Figure 3: Colonies of Candida albicans grown on Mueller-Hinton agar plate

Figure 4: Colonies of Candida albicans passed using sterile straight wire to peptone broth

Figure 5: Peptone broth turbidity matched with McFarland 0.5 standard

Figure 6: Lawn culture made on new sterile agar plate

Figure 7: Wells prepared on agar plates

Figure 8: Materials mixed and placed into their respective wells

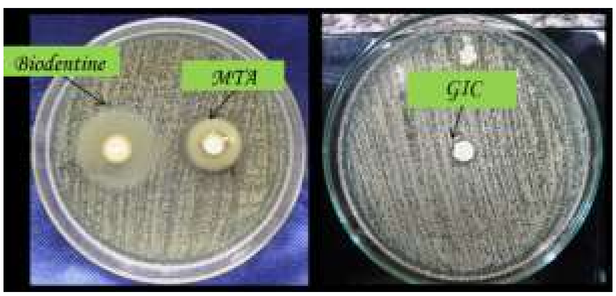
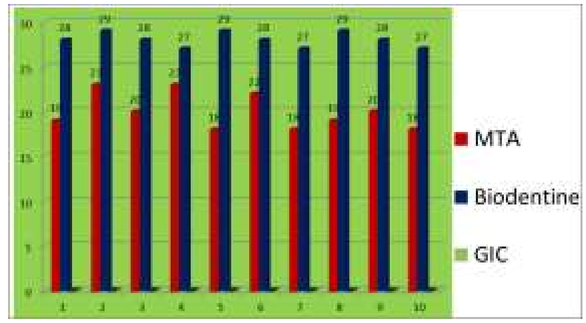


Figure 9: Zone of inhibition for Biodentine, MTA and GIC after 24 hours

<u>Graph 1: Data Collected</u>



Results:

After 24 hours, the zone of inhibition for all samples were measured and determined (Graph 1). Mean zones of inhibition were also calculated for all the sample groups (Graph 2). Results for the anti-fungal efficacy of given materials have been described and calculated using One way Anova and Post hoc turkey test (Table 1 and 2). Biodentine comes out to be significantly (p value <0.0001) better when compared to MTA and GIC against Candida albicans.

<u>Table 1: Statistical analysis</u>

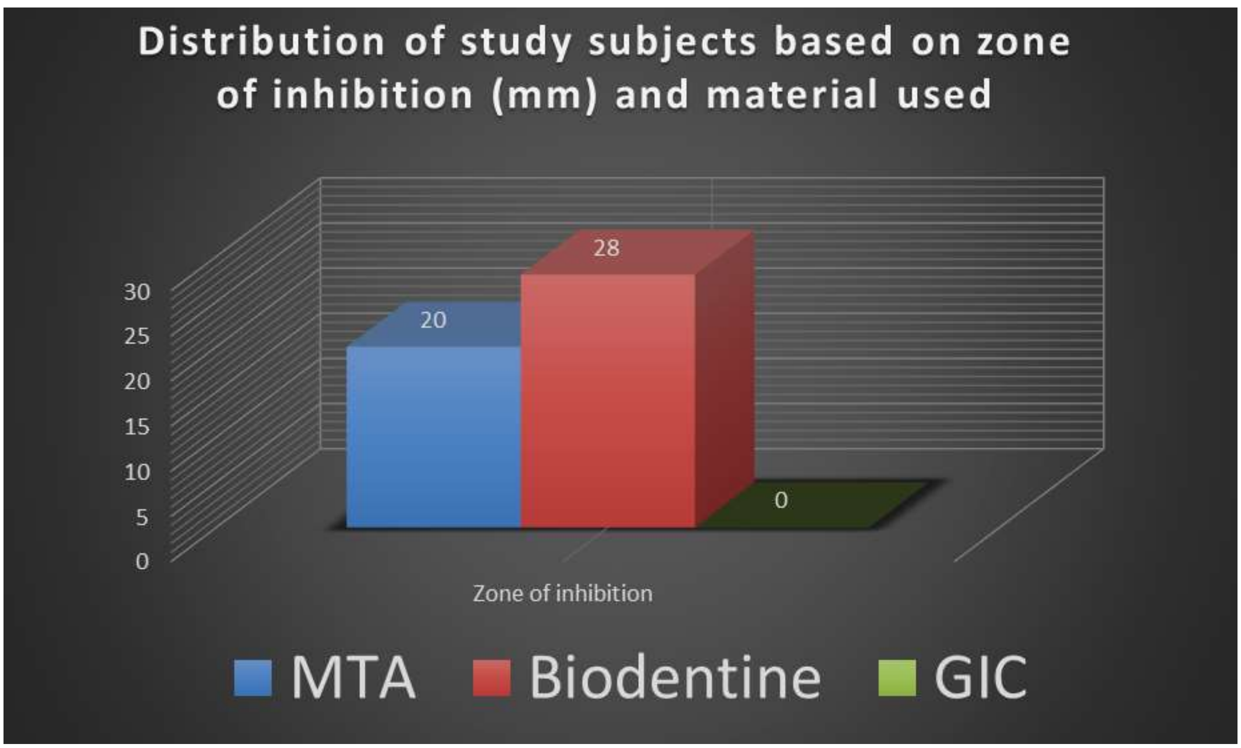
Table- Distribution of study subjects based on zone of inhibition (mm) and material used

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F-value	P-value
					Lower Bound	Upper Bound		
MTA	10	20.00	2.00	0.63	18.57	21.43	1337.14	<0.001
Biodentine	10	28.00	0.82	0.26	27.42	28.58		
GIC	10	0.00	0.00	0.00	0.00	0.00		

Table 2: Statistical analysis

Table- Post hock turkey test to compare zone of inhibition between different groups						
Groups	Groups	Mean Difference	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
MTA	Biodentine	-8.00*	0.56	<0.001	-9.38	-6.62
	GIC	20.00*	0.56	<0.001	18.62	21.38
Biodentine	MTA	8.00*	0.56	<0.001	6.62	9.38
	GIC	28.00*	0.56	<0.001	26.62	29.38
GIC	MTA	-20.00*	0.56	<0.001	-21.38	-18.61
	Biodentine	-28.00*	0.56	<0.001	-29.38	-26.62

Graph 2: Graphical representation of mean value of Zone of Inhibition



Discussion:

Fungi are chemoorganotrophic eukaryotic microorganisms that can take part in endodontic infections and thereby may participate in the etiology of periradicular diseases also[10]. Fungi have occasionally been found in primary root canal infections, but they seem to occur more often in the root canals of obturated teeth in which treatment has failed. Candida albicans are the most commonly found fungi, ranging from 7-18% of the infections[11]. Candida albicans is the focus of consideration because of its various virulence factors, for instance, dentinophilic (affinity for dentin), adaptability to a variety of environmental conditions, adhesion to variety of surfaces, production of hydrolytic enzymes and biofilm formation[10]. The hyphal formation and thigmotropism (movement according to external stimuli) property allow C. albicans to penetrate deep into the dentinal tubules, and also phenotypic alteration of C. albicans help to adapt in ecologically harsh conditions, as in high alkaline environment[12, 13]. C. *albicans* has the ability to form biofilms on different surfaces, and may be involved in cases of persistent and secondary infection[10]. Surgical intervention may be required for failed cases along with placement of biocompatible root end filling material to create an optimal environment for periradicular tissue healing. This is usually accomplished by removing pathology or removing inaccessible parts of the root canal system, and by preventing reinfection from the root canal system[14].

Throughout dental history, a wide variety of materials have been used for retrograde fillings. Although a plethora of materials exist, none of them currently fulfils all of the ideal properties of retrograde filling materials. Metals such as Gold-foil, Silver posts, Titanium screws, Tin posts, Amalgam (with and without bonding agent) and Gallium alloys are some of the materials, which have been commonly used as retro-filling materials. Cements and sealers such as ZOE Cement, IRM, Super EBA, Cavit, Zinc Polycarboxylate, Zinc Phosphate, Glass ionomer cement, Mineral Trioxide Aggregate, Calcium Phosphate cement, bone cement and composite resin have also been employed as root end filling materials[15]. GIC was used as root end filling material because of its adhesive properties forming a chemical bond with dentin, and having significant fluoride releasing property but lesser strength and microleakage, which led to reduction in its use[16]. But none of the material could achieve the ideal property of root end filling material.

MTA was developed at Loma Linda University, USA in 1993, and consists of fine hydrophilic particles of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and other mineral oxides forming a hydrophilic powder which sets in presence of water[17]. MTA provides superior seal when compared with amalgam, IRM and Super EBA. Results from most of the investigations indicated that MTA exhibits significantly less dye leakage in comparison with Super EBA, amalgam and IRM[7]. Manipulation of MTA is quite difficult; this is the major disadvantage of MTA[18].

To overcome the disadvantage of MTA, a newer material came into existence named as Biodentine: Biodentine was developed by Prof Gilles Richard from Septodont's Research Group in 2010 as a new class of dental material which could conciliate high mechanical properties with excellent biocompatibility, as well as a bioactive behavior[19]. The calcium silicate has the ability to interact with water leading to the setting and hardening of the cement. This is a hydration of the tricalcium silicate ($3CaO.SiO_2 = C_3S$) which produces a hydrated calcium silicate gel (CSH gel) and calcium hydroxide ($Ca(OH)_2$). It is highly indicated as a root end filling material. It is suitable as a permanent dentin substitute and temporary enamel substitute[20].

Zone of inhibition for Biodentine was highly significant as compared to MTA and GIC. MTA also has anti-fungal efficacy but GIC was found to be incapable of killing Candida albicans. Anti-fungal property of Biodentine and MTA is due to the release of calcium ions by dissociation of calcium hydroxide[21-23]. Biodentine showed higher release of free calcium ions when compared with MTA, and hence higher alkalinizing capacity. The high release of calcium in Biodentine is attributed to presence of calcium silicate and calcium chloride[24].

Hence, the purpose of our study was to evaluate the anti-fungal property of Biodentine and compare it to that of MTA. Anti-fungal property is an important criteria for any material which has to be used as a root-end filling material because of chances of encountering fungal infection in that area.

Conclusion:

Both MTA (Dentsply) and Biodentine (Septodont) proved to be effective, but GIC showed no efficacy against Candida albicans. This, in vitro study concluded that Biodentine is a better anti-fungal agent than MTA. However, more clinical and laboratory investigations need to be carried out to further establish the antifungal properties of root end filling materials.

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