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Original Research Article

Evaluation of antimicrobial efficacy of three calcium silicate-based materials using tube dilution: An in vitro study

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ABSTRACT

Background: Periapical pathosis is considered as a persistent microbial infection throughout the treatment period of root canal procedures. Recently the prevalence of endodontic failures is huge and this primarily accounts for the periapical pathogens; mainly Enterococcus faecalis and Candida albicans. Thus, with the advent of newer materials; identification of the most appropriate sealing material with highest antimicrobial efficacy is essential for endodontic success.
Aim and objectives: To evaluate and compare the antimicrobial efficacy of MTA Angelus, Biodentine and Theracal LC against Enterococcus faecalis and Candida albicans.
Materials and Methods: An in-vitro study was conducted using three silicate-based cements; MTA Angelus, Biodentine and Theracal LC. Their antimicrobial efficacy was evaluated using Tube dilution method and their Minimum inhibitory concentration (MIC) and Bacterial counts (CFU's) were assessed and compared. Statistical analysis was done using Paired t-test and ANOVA at 95% confidence interval (p value <0.05).
Results: The MIC was highest and CFU's were lowest with Biodentine in comparison with other two materials. There were statistically significant differences noted between Biodentine and MTA angelus.
Conclusion: Our study results conclude that Biodentine was found to be more anti-bacterial as compared to MTA angelus and theracal LC.

1. Introduction

Microorganisms play an essential role in pulpal and periapical diseases.[1] Periapical pathosis is considered an endogenous infection caused by the oral microflora. Therefore, many investigators have attempted to isolate and identify various microorganisms from root canals or periapical regions.[2] Later, Fabricius et al,[3] in 1982 showed that strict anaerobes succeed over primarily facultative ones in the canals because of the predominant ecology of root canal. Gram positive microorganisms are predominant in re-treatment diseased teeth. They can survive in difficult and less-nutrient environment in obturated teeth. A high prevalence was seen of Enterococci about 29 to 77% in those teeth with persistent and recurrent disease.

Enterococcus faecalis can withstand high pH of intracanal dressings like calcium hydroxide, and is hence found in higher concentration in reinfection cases.[4] Enterococci survive very harsh environments including extreme alkaline pH & salt conc. Other common organism identified in post treatment apical pathology is Candida albicans. Both survive as mono-infection and invade dentinal tubules.[5] Candida albicans also showed the ability of colonization root canal walls and penetration into dentinal tubules. The colonization of fungi in the root canals and various other factors affecting this colonisation are not fully understood.

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The past literature studies have hypothesized that the specific diminution of bacteria in the root canals during endodontic treatment allows overgrowth of fungi in basic environment of low nutrition.[6] Due of coronal leakage, many fungi especially Candida albicans can gain access to the apex of the root canals and lead to initial failures of endodontic treatment. Orthograde retreatment and/or endodontic surgery has helped for the recovery, in such cases. To avoid recontamination in the future and primarily enhance the success of treatment; complete elimination of the infected tissue and the microorganisms is essential to attain the overall root canal system seal.[7]

About 5.5% of the procedures involve perforation repair and endodontic surgery from majority of the 24 million treatments carried out on an annual basis.[8] Those surgeries performed at the apex involve the placement of the material at apical end of the root to seal the root canal from the periapex. Various materials used from amalgam, zinc oxide eugenol, composite resins, & glass ionomer cements. With reference to all these materials, none was able to satisfy the requirements of an ideal root-end filling material. Later in 1990s, mineral trioxide aggregate (MTA) introduced as a root-end filling material. Till date (MTA) used as a material of choice.[9]

The hydraulic self-setting cements involve calcium silicate-based cements (CSC), such as mineral trioxide aggregate (MTA). The powdered form of CSC contains tricalcium and dicalcium silicate. The reaction involves mixing of the powder with water. This leads to the formation of calcium silicate hydrate $Ca(OH)^2$ primarily. Further, this mix results into a colloidal sticky gel i.e. calcium silicate hydrate gel. This gel solidifies to form a hard substance. The regeneration procedures and repair procedures like root-end filling, apexogenesis, pulpotomy, apexification, pulp capping and perforation repair involves usage of calcium silicate-based cements.[10,11] Their biocompatibility and sealing ability synergistic to the physicochemical reaction and interaction with the surrounding local environment contribute to be the specific factors in achieving suitability in difficult clinical situations. Pulp-capping agents have been developed over the years. Most commonly used are calcium hydroxide-based materials.

Till recently, Mineral Trioxide Aggregate (MTA) is also used commonly. But the most popular is calcium hydroxide for the treatment involving direct and/or indirect pulp capping.[12] The high pH (12.5) gives calcium hydroxide an important antimicrobial activity. As stated by Siqueira, the main bactericidal action of $Ca(OH)^2$primarily depends on the maximum release of hydroxyl ions in a clean aqueous environment According to. Siqueira and Lopes evaluated that hydroxyl ions are highly oxidant free radicals that show extreme reactivity with several biomolecules, causing denaturation of proteins and damages to the bacterial cytoplasmic membrane.[13]

The only disadvantage of MTA is the long setting time and poor handling properties. Two new materials MTA like materials; MTA ANGELUS and a calcium silicate-based material; Biodentine can be used as a root-end filling material. A newer calcium silicate-based material for the treatment of pulp capping is Theracal LC. This has found to be advantageous over many conflicting reports and studies stating the antifungal and antibacterial properties of MTA. However, MTA seems to have limited antimicrobial effect against some microorganisms. Thus. the aim of our study was to evaluate and compare the antimicrobial efficacy of three new materials MTA Angelus, Biodentine and Theracal LC using tube dilution method.

2. Materials and Methods

This is an in-vitro study conducted after obtaining relevant permissions from the Scientific Advisory Committee and Institutional ethics committee number-1672/2016-17 dated 23/11/2016. Three materials were used in the study: MTA Angelus, Biodentine and Theracal LC. The antimicrobial efficacy was tested against Candida albicans and Enterococcus faecalis using tube dilution method. The culture media used were Brain heart infusion broth, Mac Conkey's agar, sabouraud dextrose broth, and sabouraud dextrose.

Ten microliter of standardized Enterococcus faecalis was added to tube no. 1-10. These tubes were then incubated at 37°C. Subcultures on Mac Conkey's agar were made on each of the tubes. At the end of 24 hours of incubation, the readings were noted. The antibacterial efficacy of these materials was evaluated in a similar manner, wherein ten different strains of Enterococcus faecalis were analysed. For antifungal efficacy, similar methodology was followed. For dilutions and culture Sabouraud's dextrose agar (SDA) and Sabouraud's dextrose broth (SDB) were used. For the evaluation of antifungal efficacy of these materials, ten different strains of Candida albicans were used.

2.1. *Laboratory analysis*

The outcomes measured were the Minimum Inhibitory Concentration (MIC) and minimal bacterial count {Colony forming units (CFU's)} for all three-calcium silicate material against Enterococcus fecalis and candida albicans.

2.2. *Statistical analysis*

The data entry and sorting was done in Microsoft Excel 2013. Frequency analysis was done of demographic status by using Statistical Package for Social Sciences (IBM SPSS Statistics V21.0) statistical software. Mean and standard deviation of the MIC and Colony forming units was derived. For inter-group comparison; One-way ANOVA was used to determine the difference between the groups followed by Tukey's post hoc test at 95% confidence intervals

Table 1: Comparison of in three materials with respect to mean CFU counts of candida by one way ANOVA

Materials	Mean sum of squares	F-value	p -value
Biodentine vs MTA angelus vs Theracal LC	87.36	0.9474	0.0542*

(*p value <0.05 statistically significant)

Table 2: Pair wise comparison of in three materials with respect to CFU counts of candida by Tukey multiple posthoc procedures

Materials	MTA Angelus	Bio dentine	Theracal LC
Mean	69.68	70.81	75.27
SD	38.38	38.40	43.49
MTA Angelus	-		
Bio dentine	p=0.0979*	-	
Theracal LC	p=0.9482	p=0.9667	-

(*p value <0.05 statistically significant)

Table 3: Comparison of in three materials with respect to mean CFU counts of E.fecalis by one way ANOVA

Materials	Mean sum of squares	F-value	p -value
Biodentine vs MTA angelus vs Theracal LC	406.37	0.5169	0.04021*

(*p value <0.05 statistically significant)

Table 4: Pair wise comparison of in three materials with respect to CFU counts of E.fecalis by Tukey multiple posthoc procedures

Materials	MTA Angelus	Bio dentine	Theracal LC
Mean	46.70	59.27	54.82
SD	24.93	29.76	29.18
MTA Angelus	-		
Bio dentine	p=0.05816*	-	
Theracal LC	p=0.7955	p=0.9331	-

(*p value <0.05 statistically significant)

considering p value <0.05 as significant.

3. Results

An in-vitro study was conducted with the aim to to evaluate and compare the antimicrobial efficacy of three new materials MTA Angelus, Biodentine and Theracal LC using tube dilution method. Inter-group comparison was done to determine the CFU counts using candida one-way ANOVA test. This comparison showed statistically significant differences (p value <0.05) between the three groups. (Table 1).

Pair-wise comparison was done using multiple Tukey's post hoc test. A statistically significant difference was noted between Bio-dentine and MTA Angelus. (Table 2) Inter-group comparison was done to determine the CFU counts using Enterococcus faecalis one-way ANOVA test. This comparison showed statistically significant differences (p value <0.05) between the three groups. (Table 3).

Pair-wise comparison was done using multiple Tukey's post hoc test. A statistically significant difference was noted between Bio-dentine and MTA Angelus. (Table 4) Thus, after evaluating minimal inhibitory concentration and bacterial count (CFU's) for these three-calcium silicate material against Enterococcus fecalis and Candida albicans with tube dilution method; Biodentine found to be more significant in comparison with MTA angelus and theracal LC in our study.

4. Discussion

Our study has evaluated and compared the anti-microbial efficacy of three silicate-based cements. In endodontic disease the primary aetiological agents are represented by microorganisms. In our study Candida albicans and Enterococcus faecalis were primarily focussed upon because Candida albicans is regarded as a potent pathogen which infects the periapical lesions and also has been associated to those infections, which are resistant endodontic non-surgical therapy. It adapts to an extreme range of pH, low oxygen and nutritional environment.[14]

Endodontic failure mainly occurs as major cause because of the persistence of microbial infection in the root canal system. Therefore, to prevent such failures it is important to eliminate the microorganisms, including fungi, from the complex root canal systems. Considering some bacteria, like Enterococcus faecalis and fungi like Candida albicans, it has been found that they are primarily resistant to the antibiotics that are present in irrigating solutions. Thus, causing failure of the endodontic treatment.[15]

The antibacterial activity of dental materials has been widely evaluated with the tube dilution test. This test allows direct comparisons of materials against tested microorganisms. But there is a disadvantage that it is not able to distinguish the between micro-biostatic and microbicidal materials. Furthermore, the control and standardisation of the dilution, evaluation of results, and reading point of inhibition haloes are restricting factors affecting the dynamics and variability of dilution tests.[16] Various studies were conducted to evaluate the antimicrobial efficacy of root canal materials.

Similar to the results of our study, were the conclusions of Gomes et al[17] which showed that Enterococcus faecalis was more frequently recovered from the canals in later appointments after biomechanical treatment procedures; whereas, Sundqvist et al reported that from nine cases in which Enterococcus faecalis was isolated in initial samples during root-canal re-treatment. Molander et al[18] stated that the predominant bacteria which are able to survive at high pH values are Enterococcus faecalis and thus treatment may require use of calcium hydroxide intracanal dressing.

Similar results were shown by Waltimo et al[19] wherein the Candida species are predominantly resistant to the disinfectants and antiseptics, which are commonly used as endodontic medicaments and irrigants. S.Arikan et al stated that MIC value recorded is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested and concluded the lowest MIC for biodentine. These results were similar to those of our study.

According to E. Canton et al; MBC is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24h under a Standardized set of conditions. In the study of Zhang et al[20] the antibacterial effect of MTA and Biodentine at low concentrations was investigated with their suspensions. These showed similar bacterial killing and completely destroyed all bacteria in 1 hour. These results were similar to our study. In our study, biodentine was found to have the highest antibacterial effect as compared to MTA. Similar results were found by Torabinejad et al,[21] which concluded that MTA had no antibacterial activity against E.faecalis and has no effect on the strict anaerobic bacteria.

5. Conclusion

The results of our study can be extrapolated to other population within the country. According to the statistical evaluation within the limitation of this study biodentine has shown to have good antifungal and antibacterial property as compared to MTA Angelus and theracal Lc. Further studies should be conducted using different parameters to evaluate the antibacterial properties of the cements with larger sample size. Moreover, the research studies can be conducted on newer cements to reduce the chances of endodontic failure. Therefore, the evaluation of newer materials will show more biocompatibility of materials when used in vivo.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Hiremath GS, Kulkarni RD, Naik BD. Evaluation of minimal inhibitory concentration of two new materials using tube dilution method: An in vitro study. *J Conserv Dent*. 2015;18(2):159–62.
2. Filho T. In-vitro antimicrobial activity of endodontic sealers, MTA based cements and Portland cement. *J Oral Sci*. 2007;49:41–5.
3. Fabricius L, Dahlen G, Ohman AE, Moller AJ. Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure. *Scand J Dent Res*. 1982;90:134–44.
4. Yasuda Y, Kamaguchi A, Saito T. In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens. *J Oral Sci*. 2008;50(3):309–13.
5. Al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML, et al. The antimicrobial effect of various endodontic sealers. *Oral Surg, Oral Med, Oral Pathol*. 1990;70(6):784–90.
6. Sen BH, Safavi KE, Spangberg LS. Growth patterns of Candida albicans in relation to radicular dentin. *Oral Surg Oral Med Oral Pathol*. 1997;84:68–73.
7. Balouiri M, Sadiki M. Saad KoraichiIbn souda . Methods for in vitro evaluating antimicrobial activity: A. *Rev J Pharm Anal*. 2016;(6):71–9.
8. Dawood AE, Parashos P, Wong RHK, Reynolds EC, Manton DJ. Calcium silicate-based cements: composition, properties, and clinical applications. *J Investig Clin Dent*. 2017;8(2):e12195.
9. Siqueira J, Rocas I, Lopes H, Magalhaes F, Uzeda M. Elimination of Candida albicans Infection of the Radicular Dentin by Intracanal Medications. *J Endod*. 2003;29(8):501–4.
10. Islam I, Chng HK, Yap AUJ. Comparison of the Physical and Mechanical Properties of MTA and Portland Cement. *J Endod*. 2006;32(3):193–7.
11. Mohammadi Z, Modaresi J, Yazdizadeh M. Evaluation of the antifungal effects of mineral trioxide aggregate materials. *Aust Endod J*. 2006;32(3):120–5.
12. Koruyucu M, Topcuoglu N, Tuna EB, Ozel S, Gencay K, Kulekci G, et al. An assessment of antibacterial activity of three pulp capping materials on Enterococcus faecalis by a direct contact test: An in vitro study. *Eur J Dent*. 2015;09(02):240–5.
13. Ghogre P. Endodontic Mycology: A New Perspective of Root Canal Infection. *Res Rev: J Dent Sci*. 2014;2(1):43–50.
14. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *Int Endod J*. 2005;38(8):539–43.
15. Al-Nazhan S, Al-Judai A. Evaluation of antifungal activity of mineral trioxide aggregate. *J Endod*. 2003;29:826–7.
16. Fridland M, Rosado R. Mineral Trioxide Aggregate (MTA) Solubility and Porosity with Different Water-to-Powder Ratios. *J Endod*. 2003;29(12):814–7.
17. Pinheiro ET, Gomes BPFA, Ferraz CCR, Sousa ELR, Teixeira FB, Souza-Filho FJ, et al. Microorganisms from canals of root-filled teeth with periapical lesions. *Int Endod J* . 2003;36(1):1–11.

18. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J*. 1998;31(1):1–8.
19. Waltimo TM, Sen BH, Meurman JH, Ørstavik D, Haapasalo MPP. Yeasts in Apical Periodontitis. *Crit Rev Oral Biol Med*. 2003;14(2):128–37.
20. Zhang H, Pappen FG, Haapasalo M. Dentin Enhances the Antibacterial Effect of Mineral Trioxide Aggregate and Bioaggregate. *J Endod*. 2009;35(2):221–4.
21. Torabinejad M, Hong CU, McDonald F, PittFord T. Physical and chemical properties of a new root-end filling material. *J Endod*. 1995;21(7):349–53.

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