



Antibacterial Effect of New Bioceramic Pulp Capping Material on the Main Cariogenic Bacteria

¹Fahiem MM Elshamy, ²Gurpreet Singh, ³Husham Elraih, ⁴Iti Gupta, ⁵Faisal Al Idris

ABSTRACT

Background: The purpose of this research was to assess the antibacterial activity of a new bioceramic pulp capping material (endosequence root repair material [ERRM]) against the main cariogenic bacteria: Salivary *mutans streptococci* (MS) and *lactobacilli* and compare the results with mineral trioxide aggregate (MTA) and calcium hydroxide (Dycal).

Materials and methods: The isolation of MS group bacteria and *Lactobacillus* (LB) spp. from stimulated saliva was performed with in-office caries risk test bacteria dip slide test. Endosequence root repair material, MTA (ProRoot MTA), and Dycal were used as pulp capping materials. *Mutans Streptococci* and LB were scattered on the agar dishes with a swab. The pulp capping materials under study were placed in the wells and prepared in the agar, immediately after mixing. The dishes were incubated for 24 hours at 37°C. The growth inhibition zones were recorded and compared for every material and bacterial strain. One-way analysis of variance test was done to compare the development of growth inhibition of selected bacteria against testing materials. *Post hoc* Tukey honest significant difference was conducted to compare each material group.

Results: All the three selected pulp capping materials were found to inhibit the bacteria LB and MS. The antibacterial activity of ERRM and ProRoot MTA was significantly better than the

Dycal. Against MS, ERRM and MTA showed no statistically significant difference. Mineral trioxide aggregate showed significantly better inhibitory activity against LB.

Conclusion: Endosequence root repair material and MTA had superior antibacterial properties against the main cariogenic bacteria: MS and LB compared with Dycal.

Clinical significance: A pulp-capping agent having good antibacterial properties can have better success rate in maintaining the vitality of the tooth while treating deep carious lesions in patients.

Keywords: Antibacterial, Bioceramics, Cardiac resynchronization therapy, Dycal, Laboratory research, *Lactobacillus*, MTA, *Streptococcus*.

How to cite this article: Elshamy FMM, Singh G, Elraih H, Gupta I, Al Idris F. Antibacterial Effect of New Bioceramic Pulp Capping Material on the Main Cariogenic Bacteria. *J Contemp Dent Pract* 2016;17(5):349-353.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

The vitality of the pulp is important for the maintenance of the structural integrity and normal physiological characteristic of teeth. As our understanding of the significance of pulp in tooth health increases, methods for maintaining pulp vitality throughout caries treatment even after exposure during caries elimination are in great request.^{1,2} Pulp capping is the primary technique for preserving vital pulp; however, the achievement rate of this method at some stage during the treatment of deep caries is low at only 33%.³ The presence of bacteria is the predominant purpose for failure.⁴ Microorganism positioned in deep caries can result in severe inflammatory reactions inside the pulp or even cause pulp necrosis.⁵ So, the prevention of bacterial infections is a critical objective for improving pulp capping treatment in cavities with deep caries.

¹Dental Biomaterials, Faculty of Dentistry, Mansoura University, Mansoura, Egypt

^{2,4}Department of Endodontics, College of Dentistry, Jazan University, Jazan, Kingdom of Saudi Arabia

³Department of Microbiology, College of Dentistry, Jazan University, Jazan, Kingdom of Saudi Arabia

⁵Dental Public Health, College of Dentistry, Jazan University Jazan, Kingdom of Saudi Arabia

Corresponding Author: Fahiem MM Elshamy, Lecturer Dental Biomaterials, Faculty of Dentistry, Mansoura University Mansoura, Egypt, Phone: +966561311593, e-mail: fahiemmm@yahoo.com

The ideal pulp capping material should have antibacterial activity and the capacity to form mineralized tissue.¹ Currently, the most common pulp capping materials used clinically consist of various formulations of calcium hydroxide [Ca(OH)₂] and mineral trioxide aggregate (MTA) combination. Mineral trioxide aggregate has been shown to induce much less pulp inflammation and more dentin bridge formation in addition to providing advanced structural characteristics compared to Ca(OH)₂.^{6,7} The higher performance of MTA in comparison to Ca(OH)₂ may be due to persevered dissolution of Ca(OH)₂ paste, which has a prolonged irritant impact (release of basic ions) on pulp tissues.⁸ Even though Ca(OH)₂ and MTA promote the formation of mineralized tissue, they lack accurate antibacterial residences,^{9,10} and consequently, cannot save from the bacterial infection that typically ends in treatment failure in cases of deep caries. Thus, a unique pulp capping material that gives a combined great antibacterial efficacy and the capacity to induce mineralized tissue formation is highly preferred.

Endosequence root repair material (ERRM) is a new bioceramic pulp capping material that has been recently introduced by Brasseler, USA (Savannah, GA). The term bioceramic material denotes the mixture of calcium phosphate and calcium silicate that is valid for dental application. The material is supplied as a premixed product to the clinician with a reliable and homogeneous material.^{11,12}

The purpose of this research was to assess the antibacterial activity of a new bioceramic pulp capping ERRM against the main cariogenic bacteria: *Salivary mutans streptococci* (MS) and lactobacilli (LB) and compare the results with MTA and calcium hydroxide (Dycal).

MATERIALS AND METHODS

The pulp capping materials in this study were: ERRM (Brasseler, USA), ProRoot MTA (Dentsply DeTrey GmbH, Germany), and Dycal (Dentsply Caulk, Canada). The agar diffusion method¹³ was used to evaluate the antimicrobial activity of the testing materials.

Cariogenic Bacteria Isolation

Caries risk test bacteria chair-side test (Ivoclar-Vivadent, Liechtenstein) was used to isolate MS and LB by means of selective culture media from the saliva. The bright green agar surface and the blue agar surface are designed for the determination of LB and MS in saliva or plaque (MSB Agar) respectively. After taking an agreement from volunteers in dental clinics in the Faculty of Dentistry, Jazan University, paraffin-based sticks (1 minute) were used for stimulating saliva. The latter was collected in

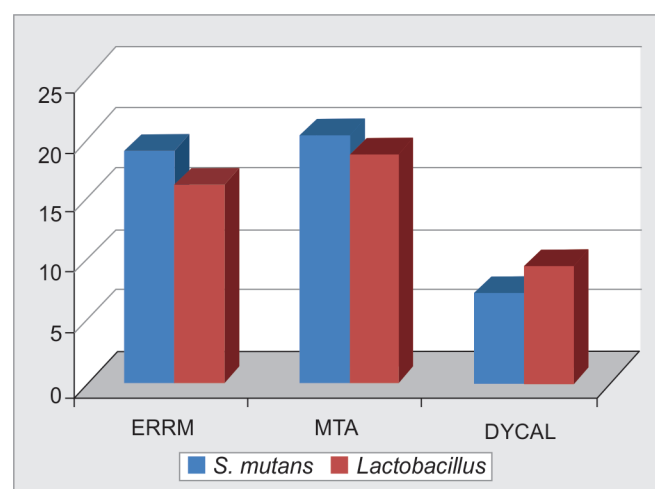
a sterile flask (2 ml on average). At the bottom of the vials the tablets of NaHCO₃ were placed. The vials were then seeded immediately in the laboratory based at Department of Microbiology, Faculty of Dentistry, Jazan University, Kingdom of Saudi Arabia. The test vials were placed in the incubator for incubation at 37°C/99°F for 48 hours. The density of the MS and LB colonies was compared with the corresponding evaluation pictures in the enclosed model chart.

Agar Disk Diffusion Test

Ten petri dishes formerly sterilized were prepared and contain Mueller-Hinton agar in necessary volume to obtain 5 mm thickness. The isolated bacteria were adjusted to equal 0.5 McFarland reaction, and then distributed on the dishes with a swab. For every dish, three wells were made with a sterilized metal tube of 4 mm diameter to receive the testing materials. The materials under study were mixed according to the manufacturer's instructions and positioned inside the wells immediately after mixing. Immediately after setting of the materials, the agar plates were incubated at 37°C for 24 hours. After 24 hours, the formed inhibition zones around the wells containing the materials were recorded. All examinations were made three times, and the outcomes were the mean estimation of the three records. One-way analysis of variance (ANOVA) test was done to compare the development of growth inhibition of selected bacteria against tested materials. *Post hoc* Tukey honest significant difference (HSD) was conducted to compare each material group.

RESULTS

The three studied pulp capping materials showed a considerable inhibitory effect against MS group bacteria and LB (Graph 1).



Graph 1: Means of inhibition zone diameter (mm) of testing materials against selected bacteria (ANOVA=0.000)

Table 1: Comparison of the testing materials on each bacteria separately

		Sum of squares	df	Mean square	F
<i>Lactobacillus</i>	Between groups	224.933	2	112.467	198.471
	Within groups	6.800	12	0.567	
	Total	231.733	14		
<i>Mutans Streptococci</i>	Between groups	509.200	2	254.600	363.714
	Within groups	8.400	12	0.700	
	Total	517.600	14		

ANOVA: Analysis of variance; df^2/F : Test value**Table 2:** Multiple comparisons (Tukey HSD) of selected bacteria against tested pulp capping materials

Dependent variable	(I) Sample	(J) Sample	Mean difference (I - J)	Std. error	Sig.	95% confidence interval	
						Lower bound	Upper bound
<i>Lactobacillus</i>	ERRM	ProRoot MTA	-2.600*	0.476	0.000	-3.87	-1.33
		Dycal	6.600*	0.476	0.000	5.33	7.87
	ProRoot MTA	ERRM	2.600*	0.476	0.000	1.33	3.87
		Dycal	9.200*	0.476	0.000	7.93	10.47
	Dycal	ERRM	-6.600*	0.476	0.000	-7.87	-5.33
		ProRoot MTA	-9.200*	0.476	0.000	-10.47	-7.93
<i>Mutans Streptococci</i>	ERRM	ProRoot MTA	-1.400	0.529	0.052	-2.81	0.01
		Dycal	11.600*	0.529	0.000	10.19	13.01
	ProRoot MTA	ERRM	1.400	0.529	0.052	-0.01	2.81
		Dycal	13.000*	0.529	0.000	11.59	14.41
	Dycal	ERRM	-11.600*	0.529	0.000	-13.01	-10.19
		ProRoot MTA	-13.000*	0.529	0.000	-14.41	-11.59

*The mean difference is significant at the 0.05 level

A one-way ANOVA (Table 1) using Statistical Package for the Social Sciences 20.0 was conducted to know the activity of ERRM, ProRoot (MTA), and Dycal for inhibition of LB and MS. A highly statistically significant difference was obtained ($p=0.000$). *Post hoc* comparisons using the Tukey HSD test (Table 2) indicated a significant difference in the inhibition activity of LB among three materials, with the order of inhibition as ProRoot MTA > ERRM > Dycal (mean difference 2.6, 6.6, 9.2, $p=0.00$). ProRoot MTA was found to have highest inhibitory activity of LB.

A significant difference was found for MS inhibition between ProRoot MTA and Dycal (mean difference 13.0, $p=0.00$) and ERRM and Dycal (mean difference 11.6, $p=0.00$), but ProRoot MTA and ERRM were not significantly different for MS (mean difference = 1.4, $p=0.052$). ProRoot MTA was found to be better than Dycal for MS inhibition.

DISCUSSION

In pulp capping failure the primary etiological agents are represented by bacteria.¹⁴ The antibacterial effect of dental materials has been broadly evaluated with the agar diffusion test.¹⁴⁻¹⁶ The method of agar diffusion test permits direct contrasts of materials against tested microorganisms. However, a major drawback of this method is the impossibility of distinguishing between microbiostatic and microbicidal materials.¹⁷

Streptococcus mutans and LB are significant pathogens creating human dental caries.¹⁸ Consequently, they were decided for assessment of the antibacterial activity of the materials arranged in this study.

The antibacterial action of pulp capping materials has been previously tested. Calcium hydroxide is still viewed as the reference material for pulp capping, because of its antibacterial properties and its ability to affect enzymatic responses, prompting the development of dentin bridge. Recently, MTA has been proposed as a suitable material for pulp capping, on the premise of its natural properties. Mineral trioxide aggregate causes less inflammation than calcium hydroxide and stimulates the differentiation and proliferation of pulp cells, along these lines encouraging the development of a more structured mineralized barrier.¹⁹ Based on these qualities, MTA is currently considered the "best quality" material. However, it has two major disadvantages, i.e., the longer time required for setting and the requirement for moisture during setting.¹⁹⁻²¹

Endosequence root repair material paste (ERRM paste; Brasseler, USA) is a premixed bioceramic material recommended for perforation repair, apical surgery, apical plug, and pulp capping.²² Both ERRM putty and paste have shown similar *in vitro* biocompatibility to both gray and white MTA.^{23,24}

In the present study, all the materials tested demonstrated zones of bacterial inhibition but with various

diameters. Regarding Dycal, these results confirmed the antibacterial activity of calcium hydroxide, as reported in previous studies.^{19,25} However, in this study, this activity was significantly lower than MTA and bioceramics. The antibacterial action of calcium hydroxide-based materials relies on the ionization that discharges hydroxyl particles, bringing about an increase in pH. Cell layer proteins of the microorganism may be reversibly or irreversibly inactivated by a high pH. Furthermore, long-term clinical studies have reported fluctuating achievement rates, because of the persistence of an inflammatory process and cytotoxic impacts.²⁶

According to the findings in the present study, MTA and bioceramics were the best against MS and LB and were significantly different from Dycal. The antimicrobial activity of MTA seems to be associated with elevated pH. Torabinejad et al²⁷ observed an initial pH of 10.2 for MTA, and rising to 12.5 in 3 hours. It is known that the pH levels in the order of 12.0 can inhibit most of the microorganisms, including resistant bacteria, such as *Enterococcus faecalis*.²⁸ These findings can support our results in this study regarding MTA. The antibacterial property of ERRM may be attributed to its high pH value (12.5), hydrophilic nature, and its active calcium hydroxide diffusion.

The new bioceramic ERRM may offer a pulp capping material with antibacterial activity, and this hypothesis was confirmed by the results obtained in the present study.

CONCLUSION

According to the results of the current study, the antibacterial activity of ERRM against MS was comparable between ERRM and MTA and higher than Dycal. *Lactobacillus* was inhibited by ERRM that was superior to Dycal and inferior to MTA.

REFERENCES

- Bergenholtz G. Factors in pulpal repair after oral exposure. *Adv Dent Res* 2001 Aug;15:84.
- Hayashi M, Fujitani M, Yamaki C, Momoi Y. Ways of enhancing pulp preservation by stepwise excavation – A systematic review. *J Dent* 2011 Feb;39(2):95-107.
- Al-Hiyasat AS, Barriehi-Nusair KM, Al-Omari MA. The radiographic outcomes of direct pulp-capping procedures performed by dental students: a retrospective study. *J Am Dent Assoc* 2006 Dec;137(12):1699-1705.
- Momoi Y, Hayashi M, Fujitani M, Fukushima M, Imazato S, Kubo S, Nikaido T, Shimizu A, Unemori M, Yamaki C. Clinical guidelines for treating caries in adults following a minimal intervention policy – Evidence and consensus based report. *J Dent* 2012 Feb;40(2):95-105.
- Bjorndal L, Reit C, Bruun G, Markvart M, Kjaeldgaard M, Näsman P, Thordrup M, Dige I, Nyvad B, Fransson H, et al. Treatment of deep caries lesions in adults: Randomized clinical trials comparing stepwise *vs* direct complete excavation, and direct pulp capping *vs* partial pulpotomy. *Eur J Oral Sci* 2010 Jun;118(3):290-297.
- Damaschke T, Stratmann U, Wolff P, Sagheri D, Schafer E. Direct pulp capping with mineral trioxide aggregate: An immunohistologic comparison with calcium hydroxide in rodents. *J Endod* 2010 May;36(5):814-819.
- Simon SR, Berdal A, Cooper PR, Lumley PJ, Tomson PL, Smith AJ. Dentin-pulp complex regeneration: from lab to clinic. *Adv Dent Res* 2011 Jul;23(3):340-345.
- Tran XV, Gorin C, Willig C, Baroukh B, Pellat B, Decup F, Opsahl Vital S, Chaussain C, Boukpepsi T. Effect of a calcium-silicate-based restorative cement on pulp repair. *J Dent Res* 2012 Dec;91(12):1166-1171.
- Miyagak DC, de Carvalho EM, Robazza CR, Chavasco JK, Levorato GL. *In vitro* evaluation of the antimicrobial activity of endodontic sealers. *Braz Oral Res* 2006 Oct-Dec;20(4):303-306.
- Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. *Braz Dent J* 2000;11(1):3-9.
- Ma J, Shen Y, Stojicic S, Haapasalo M. Biocompatibility of two novel root repair materials. *J Endod* 2011 Jun;37(6):793-798.
- Lieutenant Commander Jeffrey Hilley, Colonel Kathleen McNally. Bioceramics in endodontics. *Clin Update* 2013;35(4).
- Fraga RC, Siqueira JF Jr, de Uzeda M. *In vitro* evaluation of antibacterial effects of photo-cured glass ionomer liners and dentin bonding agents during setting. *J Prosthet Dent* 1996 Nov;76(5):483-486.
- Al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML. The antimicrobial effect of various endodontic sealers. *Oral Surg Oral Med Oral Pathol* 1990 Dec;70(6):784-790.
- Cohen, S.; Burns, RC. Pathways of the pulp. 8th ed. St Louis (MO): Mosby Co.; 2002. p. 293-364.
- Cobankara FK, Altinoz HC, Ergani O, Kav K, Belli S. *In vitro* antibacterial activities of root-canal sealers by using two different methods. *J Endod* 2004 Jan;30(1):57-60.
- Tobias RS. Antibacterial properties of dental restorative materials: a review. *Int Endod J* 1988 Mar;21(2):155-160.
- Takahashi N, Nyvad B. The role of bacteria in the caries process: Ecological perspectives. *J Dent Res* 2011 Mar;90(3):294-303.
- Laurent P, Camps J, De Méo M, Déjou J, About I. Induction of specific cell responses to a Ca (3) SiO (5)-based posterior restorative material. *Dent Mater* 2008 Nov;24(11):1486-1494.
- Pérard M, Le Clerc J, Watrin T, Meary F, Pérez F, Tricot-Doleux S, Pellen-Mussi P. Spheroid model study comparing the biocompatibility of Biodentin and MTA. *J Mater Sci Mater Med* 2013 Jun;24(6):1527-1534.
- Tran XV, Gorin C, Willig C, Baroukh B, Pellat B, Decup F, Opsahl Vital S, Chaussain C, Boukpepsi T. Effect of calcium-silicate-based restorative cement on pulp repair. *J Dent Res* 2012 Dec;91(12):1166-1171.
- Brasseler USA Co. Endosequence root repair material paste, information; [updated 2010 Dec]. Available from: <http://www.brasselerusa.com/display.cfm?pid=newproducts>.
- Alanezi AZ, Jiang J, Safavi KE, Spangberg LS, Zhu Q. Cytotoxicity evaluation of endosequence root repair material. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010 Mar;109(3):e122-e125.
- Damas BA, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. *J Endod* 2011 Mar;37(3):372-375.

Antibacterial Effect of New Bioceramic Pulp Capping Material on the Main Cariogenic Bacteria

25. Lu Y, Liu T, Li H, Pi G. Histological evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide on human pulp tissue. *Int Endod J* 2008 Aug;41(8):643-650.
26. Eldeniz AU, Erdemir A, Hadimli HH, Belli S, Erganis O. Assessment of antibacterial activity of EndoREZ. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006 Jul;102(1):119-126.
27. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. *J Endod* 1995 Aug;21(8):403-406.
28. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill *Enterococcus faecalis* *in vitro*. *J Endod* 2004 Apr;30(4):218-219.