ORIGINAL ARTICLE

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An in vitro comparative study on the antimicrobial effects of bioglass 45S5 vs. calcium hydroxide on Enterococcus faecalis

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INTRODUCTION: An ideal intracanal medicament should be able to eliminate any remaining intracanal microorganism. The aim of this study was to compare the antimicrobial effects of Bioglass 45S5 with calcium hydroxide on *Enterococcus* (*E*) *faecalis in-vitro*.
MATERIALS & METHODS: Direct exposure test (DET) was used to evaluate the antimicrobial effect of Bioglass 45S5, calcium hydroxide and normal saline (control group) on 80 paper cones contaminated with *E. faecalis* suspension. All samples were aseptically transferred into BHI culture medium to quantify microbial concentration in periods of 1, 24, 48 and 72 hours. Turbidity of the culture medium was measured via optical density (OPD) method with a spectrophotometer (wavelength=540nm). Results were then analysed statistically using student t-test.
RESULTS: Mean difference of optical density between Bioglass 45S5 and calcium hydroxide appeared insignificant within 1 hour of the test period ($P>0.05$); however calcium hydroxide showed significantly greater antimicrobial properties after 24 hours ($P<0.05$). Antimicrobial effect of both materials displayed significant increases with time.
CONCLUSION: Although both Bioglass 45S5 and calcium hydroxide exhibited antimicrobial effects against *E. faecalis*, neither attained complete eradication of bacteria. However, calcium hydroxide seemed to have superior disinfecting effect.

KEYWORDS: Bioglass, Calcium hydroxide, Direct exposure test, Root canal medicaments.

Received *September 2010;* ***accepted*** *November 2010*

INTRODUCTION

Bacteria and their by-products play a major role in periradicular diseases and its progress; in fact, they are the main interfering factors in the periapical repair process (1,2). The disinfection of a complicated root canal system is crucial for long term success (3,4). However, even after chemo-mechanical debridement of the root canals, it is difficult, if not impossible, to achieve complete disinfection (5-7).

Recent microbiologic studies demonstrate wide-spread presence of resistant microorganisms such as *E. faecalis* and *Candida Albicans* in persistent canal infections and secondary infections in endodontic failures (4,8-10). Appropriate intracanal medicaments between treatment sessions can offer beneficial antimicrobial effects as they are retained in canal space for relatively longer periods; therefore numerous searches have been performed to find the perfect intracanal medicament (3,5,6,10).

An ideal intracanal medicament should have wide antimicrobial properties, high compatibility with periapical tissue and induce hard tissue repair as well as reduce inflammation (3). Calcium hydroxide is currently known as the gold standard for intracanal medicaments. However, there are doubts about its antimicrobial effect against yeast and *Enterococci* (1,4,10), specially within dentinal

tubules of root canals (1,4-7,10,11).

Bioglass, a silica based melt-derived glass has had wide uses in medicine and dentistry (12,13). This active biomaterial has antimicrobial and anti-inflammatory effects, it has the ability to bond to soft and hard tissues; moreover, when implanted in bone, it displays osteoconductive properties which may assist the repair of bony defects (12-17). The wide-spectrum antimicrobial effect of bioactive glass (BAGs) S53P4 on different oral microorganisms has been reported which justifies its use as an intracanal medicament and intraosseous matrix in endodontics (18-19). Krithikadatta *et al.* compared disinfecting properties of 0.2% chlorhexidine (CHX), 2% metronidazole and bioactive glass S53P4 with calcium hydroxide on dentinal tubules. All groups demonstrated similar results (4).

Bioglass S53P4 is thought to be the most effective glass on pathogenic microorganisms (16). Waltimo *et al.* reported a strong antimicrobial activity for Bioglass 45S5 against *Enterococci* isolated from persistent canal infections (20). Despite different existing formulas available for bioactive glass, the 45S5 appears the best as it consists of 45% SiO_2, 24.5% CaO, 24.5% Na, 2O.6% P_2O_5. These particles are not only osteoconductive, but also have a composition similar to bone in regards to calcium and phosphor ions (13-17).

The aim of the current *in vitro* study was to compare the antimicrobial effect of bioactive glass 45S5 (Nova Bone) and calcium hydroxide on *E. faecalis* using a direct exposure test (DET).

MATERIALS & METHODS

This experimental study comprised of eighty paper cones contaminated with *E. faecalis* (ATCC 19818). Sterile absorbent points #50 were floated in a 10^8 CFU/mL concentration of *E. faecalis* suspension for 5 minutes. The paper cones were then regarded as the study samples. All experimental procedures were carried out under strict aseptic conditions. The contaminated paper cones were randomly divided into four test and control groups as outlined below.

Test group 1 (n=20): 5.1 g bioactive glass 45S5 powder (NovaBone, US Biomaterial Corporation, USA/ Percentage weight rate: SIO_2 (45%), CaO (24.5%), Na (24.5%), P_2O_5 (20.6%)) mixed with 4mL sterile distilled water to form a toothpaste consistency paste.

Test group 2 (n=20): 6.2 g calcium hydroxide $Ca(OH)_2$ (Merk, Germany) mixed with 12mL sterile distilled water to form a paste with the same consistency as the Bioglass mixture.

The control negative group (n=20) was exposed to normal saline and autoclaved, whereas control positive samples were only exposed to normal saline. Direct exposure test (DET) was used for the evaluation of antimicrobial effects of study samples. Each paper cone was separately placed in a sterile petri plate completely covered with experimental materials or saline (control groups). Plates were placed in a 37°C, 100% humidity incubator. A total of 5 samples were aseptically removed from each group at 1, 24, 48, 72 hours intervals and irrigated with normal saline to completely eliminate materials. Each paper cone was then inserted into sterile lab tubes containing 5mL BHI (brain heart infusion broth) and placed in a 37°C incubator for 48 hours. To evaluate the microorganisms in BHI culture medium, turbidity was assessed with a spectrophotometer that measured optical density (OD/100) (at a wavelength of 540nm).The optical density of culture medium is directly related to the total of existing bacterial in the medium. Subculture of blood agar solid medium was used to identify the target microorganism and prove the existence of *E. faecalis*; hence, 0.01mL was pulled out of the liquid culture medium with a sterile loop and subcultured. The morphology of the colonies of *E. faecalis* was evaluated both macro and microscopically (gram staining) after 24 hours. All data were statistically analysed to verify normal distribution; results were then analysed using Student t-test. The level of significance was set at P=0.05.

RESULTS

According to kolmogrov-smirnov test, all data exhibited normal distribution. The absence of microorganism growth in the negative control group proved the aseptic and sterile working

Table1. **Optical Density (Mean±SD) of studied materials at different time intervals (hour)**

Material / Time	Bioglass	Calcium Hydroxide	P value
1	131.8±0.84	125.8±6.83	0.46
24	129.64±1.3	120.68±6.67	0.004
48	126.36±3.61	113.96±3.67	0.000
72	123.52±6.21	108.10±5.25	0.000

condition. Growth of microorganisms in the positive control group indicated bacterial existence within the incubation periods. Therefore, uncertainty regarding any unwanted disturbing factors during the experiment could be eliminated.

There was no significant difference between Bioglass and calcium hydroxide after 1 hour; however, after 24, 48 and 72 hours intervals calcium hydroxide showed superior antimicrobial properties ($P<0.05$). Antimicrobial effect of both materials increased with longer time intervals, both groups displayed significant decrease of optical density (OD/100) after 72 hours (Table1).

DISCUSSION

Many *in vitro* studies have been carried out on the antimicrobial efficacy of different materials (10,11,18,19,21,22).The efficiency of direct exposure test (DET) has been previously established (19,23). *E. faecalis,* a facultative anaerobe, is one of the most resistant microorganisms against disinfecting medicaments in endodontics, and is often present in resistant apical periodontitis. Therefore evaluation of antimicrobial properties of different materials against this microorganism appears reasonable (2,4,10,19,20,24). The current study revealed that calcium hydroxide paste mixed with distilled water has greater antimicrobial efficacy against *E. faecalis*, compared with Bioglass after one day of exposure. Calcium hydroxide, however, could not thoroughly eliminate target microorganisms, concurring with many other studies (4,10,11,19,25). In a recent agar diffusion experimental study, Ballal and Kundabala demonstrated calcium hydroxide paste to have weaker antimicrobial effect on *Candida Albicans* and *E. faecalis* compared to 2% CHX gel (10).

It has been shown that the efficacy of Bioglass 45S5 increases after the first 24 hours (17); this is in agreement with our results.

Previous studies revealed more efficient results for antimicrobial effect of Bioglass S53P4 compared to Bioglass 45S5 (4,18,19). Zehnder *et al.* were the first to evaluate antimicrobial effects of Bioglass S53P4 compared to calcium hydroxide paste in dentinal tubules contaminated with *E. faecalis*. Calcium hydroxide paste appeared completely ineffective and Bioglass suspension was able to reduce infection of the samples after five days (19). In this study, the DET method revealed antimicrobial properties of Bioglass against resistant *Enterococci*, though CaOH was also effective. Krithikadatta *et al.* showed that the antimicrobial properties of Bioglass S53P4 and calcium hydroxide paste are similar (4); our study was only able to demonstrate this in the first 24 hours. These inconsistencies might be due to the difference in particle proportion and percentage, the type of tested Bioglass and also the used antibacterial test. In fact, antimicrobial effect of calcium hydroxide is affected by the buffering effect of dentin, although Bioglass may be less sensitive (19).

A recent study reported that antiseptic effect of bioactive glass (BAG) S53P4 powder was stronger than that of calcium hydroxide. The formulation of Bioglass 45S5 is: Sio2 (45%), Na2o (24.5%), CaO (24.5%), P2O6 (6%), whereas, Bioglass S53P4 is Sio2 (53%), Na2o (23%), CaO (20%), P2O4 (4%).

This effect was apparently not exclusively pH-related. The presence of dentin powder in suspension increased the efficacy of S53P4 against a multiplicity of oral microorganisms (26). The size of Bioglass particles affects antimicrobial property. The smaller the particle, the greater the exposed surface to the liquid. This results in stronger antimicrobial properties (17-20). Commercial brands of Bioglass 45S5 composes of 90-710 micro particles. The new nanometric Bioglass has 20-60 particles which contribute to stronger antimicrobial effects (20). Apparently, with increasing contact surface between smaller particles of nanometric glass, more alkaline

material is released into the liquid medium which induces more antimicrobial effect. Basically, bioactive glasses have calcium oxide, sodium phosphor and silicon oxides in specific proportions which are responsible for the particular characteristics of the material and its strong bond to bone (12-18).Their high tissue compatibility makes them the most appropriate material as a bone substitute in reconstructive surgeries (12,27). The antimicrobial effects of bioactive glass could be justified by the existence of Ca, Si, Na PO_4 ions, cations in liquid medium and their release from glass which contribute to an increase of pH and osmotic pressure (17-18).The activity of released ions and the increase of pH and osmotic pressure could lead to death of microorganisms following exposure to the glass (18). However, the accurate antimicrobial mechanism is not clear yet. Some researchers do not relate the antimicrobial activity of this material to pH as the buffering characteristics of dentine does not seem to decrease antimicrobial effect of Bioglass (19,21,22). On the other hand, human dentin powder has increasing effects on the antimicrobial properties of Bioglass S53P4 against *E. faecalis* (21). It is hypothesized that the degradation of Bioglass particles, particularly SiO_2 is increased when the material is mixed with dentin powder and hence with the release of silica in the environment and an increase of antimicrobial activity is observed (21). It is also reported that bone powder could also enhance this characteristics of Bioglass (22).

Antimicrobial characteristics of $Ca(OH)_2$ is due to the release of OH ions into the environment, the increase of pH and therefore destruction of the microorganism cell wall (11,17,28) along with absorption of CO_2 from the anaerobic environment (29,30). Apparently, $Ca(OH)_2$ mechanism could be pH-dependent so that it was inefficient against resistant microorganisms such as *E. faecalis* that are themselves resistant to high pH . The buffering property of dentine can also neutralize the alkalinity effect of calcium hydroxide when it comes to disinfect contaminated dentinal tubules (11).

A recent study, has reported that the combined effect of dentin powder and BAG is neither a matter of osmolarity or even cellular agglutination, nor is it linked to binding of bacteria to dentin powder in the presence of BAG. It was proved that only combinations of solid BAG and dentin powder and not their supernatants in suspension have an additive effect. Dentin powder with its complex surface acted as a recipient for ions in solution, and hence acted as a catalyst for the dissolution of the glass in aqueous suspension. It was this ionic flow between the glass and dentin powder that appeared to interfere with bacterial viability (26). It should be stated that the antibacterial effect of BAGs is related to their particle size. The lesser effect of the BAG suspension, despite the fact that its initial pH is comparable to that of the $Ca(OH)_2$ counterpart, can be explained by the lesser alkaline capacity of the glass suspension compared to $Ca(OH)_2$. This factor needs to be considered before extrapolating the findings of the study to an *in vivo* situation. The *in vitro* study of Prabhakar and Kumar, BAG was relatively effective against *E. faecalis* and the addition of enamel powder did not significantly boosts its antimicrobial efficacy at the end of 24 hours and three days. However, at the end of five days, this combination exhibited marked increase in antimicrobial activity. The mixture of BAG plus dentin powder increased the antimicrobial efficacy of BAG at the end of 24 hours and three days, which was statistically significant; although it tapered at the end of five days (26).

CONCLUSION

The current study demonstrated that the antimicrobial effect of Bioglass 45S5 against *E. faecalis* was lower than $Ca(OH)_2$, although it tends to increase with time. Considering all desirable properties of Bioglass, the material could well be incorporated into endodontics practise as an intracanal medicament in the future. Further research is recommended to find more effective formulations of Bioglass.

ACKNOWLEDGMENTS

This study was supported by the Endodontic department of Islamic Azad University of Medical Sciences, Dental School, Tehran.

Conflict of Interest: 'none declared'.

REFERENCES

1. Siqueira JF Jr, Rôças IN, Souto R, de Uzeda M, Colombo AP. Actinomyces species, streptococci, and Enterococcus faecalis in primary root canal infections. J Endod 2002;28:168-72.

2. Siqueira JF Jr. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J 2001;34:1-10.

3. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J 1992;25:97-106.

4. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod 2007;33:1473-6.

5. Oguntebi BR. Dentine tubule infection and endodontic therapy implications. Int Endod J 1994;27:218-22.

6. Safavi KE, Spangberg LS, Langeland K. Root canal dentinal tubule disinfection. J Endod 1990;16:207-10.

7. Siqueira JF Jr, de Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. J Endod 1996;22:674-6.

8. Love RM. Enterococcus faecalis-a mechanism for its role in endodontic failure. Int Endod J 2001;34:399-405.

9. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1-7.

10. Ballal V, Kundabala M, Acharya S, Ballal M. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. Aust Dent J 2007;52:118-21.

11. Kangarloo A, Mehrvazfar P, Ghazisaedi K, Asnaashari M, Saffar A. The effect of an antibiotic mixture on the infection of dentinal tubules. Endod practice J 2002;5:17-21.

12. Thomas MV, Puleo DA, Al-Sabbagh M. Bioactive glass three decades on. J Long Term Eff Med Implants 2005;15:585-97.

13. Hench LL. The story of Bioglass. J Mater Sci Mater Med 2006;17:967-78.

14. Xynos ID, Hukkanen MV, Batten JJ, Buttery LD, Hench LL, Polak JM.Bioglass 45S5 stimulates osteoblast turnover and enhances bone formation In vitro: implications and applications for bone tissue engineering. Calcif Tissue Int 2000;67:321-9.

15. Price N, Bendall SP, Frondoza C, Jinnah RH, Hungerford DS. Human osteoblast-like cells (MG63) proliferate on a bioactive glass surface. J Biomed Mater Res 1997 5;37:394-400.

16. Munukka E, Leppäranta O, Korkeamäki M, Vaahtio M, Peltola T, Zhang D, Hupa L, Ylänen H, Salonen JI, Viljanen MK, Eerola E. Bactericidal effects of bioactive glasses on clinically important aerobic bacteria. J Mater Sci Mater Med 2008;19:27-32.

17. Bellantone M, Williams HD, Hench LL. Broad-Spectrum Bactericidal Activity of Ag_2O-Doped Bioactive Glass. Antimicrob agents chemother 2002;46:1940-5.

18. Stoor P, Söderling E, Salonen JI. Antibacterial effects of a bioactive glass paste on oral microorganisms. Acta Odontol Scand 1998;56:161-5.

19. Zehnder M, Söderling E, Salonen J, Waltimo T. Preliminary evaluation of bioactive glass S53P4 as an endodontic medication in vitro. J Endod 2004;30:220-4.

20. Waltimo T, Brunner TJ, Vollenweider M, Stark WJ, Zehnder M. Antimicrobial effect of nanometric bioactive glass 45S5. J Dent Res 2007;86:754-7.

21. Zehnder M, Waltimo T, Sener B, Söderling E. Dentin enhances the effectiveness of bioactive glass S53P4 against a strain of Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:530-5.

22. Waltimo T, Zehnder M, Söderling E. Bone powder enhances the effectiveness of bioactive glass S53P4 against strains of Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans in suspension. Acta Odontol Scand 2006;64:183-6.

23. Estrela C, Rodrigues de Araújo Estrela C, Bammann LL, Pecora JD. Two methods to evaluate the antimicrobial action of calcium hydroxide paste. J Endod 2001;27:720-3.

24. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375-9.

25. Estrela C, Pimenta FC, Ito IY, Bammann LL. Antimicrobial evaluation of calcium hydroxide in infected dentinal tubules. J Endod 1999;25:416-8.

26. Prabhakar AR, Kumar SCh. Antibacterial effect of bioactive glass in combination with powdered enamel and dentin. Indian J Dent Res 2010;21:30-4.

27. Peltola M, Kinnunen I, Aitasalo K. Reconstruction of orbital wall defects with bioactive glass plates. J Oral Maxillofac Surg 2008;66:639-46.

28. Foreman PC, Barnes IE. Review of calcium hydroxide. Int Endod J 1990;23:283-97.

29. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24:119-25.

30. Kontakiotis E, Nakou M, Georgopoulou M. *In vitro* study of the indirect action of calcium hydroxide on the anaerobic flora of the root canal. Int Endod J 1995;28:285-9.