Antimicrobial effects of several calcium silicate-based root-end filling materials

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The purpose of this study was to evaluate, in vitro, the antimicrobial effect of iRoot BP, iRoot BP Plus, and mineral trioxide aggregate (MTA) against Enterococcus faecalis and Candida albicans by using direct contact test. The materials were tested immediately after application to the microtiter wells and after setting for 1-day and for 7-days. Ten microliters of microbial suspension was added to each well for direct contact with each material for 1 h at 37°C and 100% humidity. Then fresh media was added and, survival of bacteria and fungi was determined by using 10-fold serial dilution and inoculated onto agar plates. In fresh and 1-day samples all of tested materials showed statistically significant antimicrobial effects compared to control groups (p<0.05). In 7-day samples, there were no significantly differences compared to control groups. MTA, iRoot BP and iRoot BP Plus had similar antimicrobial efficacy against E. faecalis and C. albicans.

Keywords: Antimicrobial, Mineral trioxide aggregate, iRoot BP, iRoot BP Plus

INTRODUCTION

Apical surgery is required to rescue teeth with periradicular lesions in cases which orthograde endodontic therapy is contraindicated or has failed. This surgical procedure includes root-end resection, retrograde cavity preparation and root-end filling. The purpose of retrograde filling is to form an apical obturation at the end of the resected root.

The ideal root-end filling material should provide a complete apical impermeability at the root-end. However, many available filling materials do not satisfy this requirement. If microleakage occurs between the cavity walls and the filling substance, microorganisms may enter the breached area and cause endodontic surgical failure. Therefore, an ideal root-end filling material should also possess antimicrobial activity whereby it can prevent bacterial and fungal growth.

Endodontic infections are caused by anaerobes, facultative anaerobes, aerobes, and fungi. Enterococcus faecalis is the most common causative bacterium of these infections, and Candida albicans is the most common causative fungus; both these organisms can be isolated from teeth with periapical lesions.

Amalgam, gutta-percha, zinc-oxide-eugenol cements, composite resin with and without bonding agent, resin-modified glass ionomers, and calcium silicate-based materials (iRoot BP, mineral trioxide aggregate [MTA], etc) have been used as root-end filling materials.

MTA (Dentsply, Tulsa Dental, Germany) has been favoured as the gold standard because of its high sealing ability and biocompatibility. iRoot BP (Innovative BioCeramix Inc., Vancouver, BC, Canada) is a newly developed, laboratory-synthesised, ready-to-use premixed, injectable bioceramic paste. Further, iRoot BP Plus (Innovative BioCeramix Inc.) is a ready-to-use calcium silicate-based putty material developed for the repair of root canal perforations and root resorption, root-end filling, apexification, and pulp capping. The current literature lacks information regarding the areas of usage of these materials.

The aim of this in vitro study was to evaluate the antimicrobial effect of iRoot BP, iRoot BP Plus, and MTA against E. faecalis and C. albicans by using the direct contact test (DCT).

MATERIALS AND METHODS

The root-end filling materials were prepared according to the manufacturers’ instructions (Table 1).

Test strains and culture conditions

The antimicrobial activity of the test materials was evaluated against E. faecalis (ATCC 29212) and C. albicans (ATCC 10231). The reference strains were grown aerobically in tryptic soy agar (TSA, Merc, Germany) at 37°C for 18–24 h and seeded in 15.0 mL of TSA to produce a turbidity of 0.5 on the McFarland scale, which corresponds to a concentration of 1×10⁸ CFU/mL.

DCT

The antimicrobial activity was tested in 96-well microtiter plates by using the DCT method described by Weiss et al. (Fig. 1). Briefly, equal amounts of each
Table 1 The root-end filling materials used in the study and their constituents

| Materials                        | Constituents                                                                 | Manufacturer                          |
|----------------------------------|-------------------------------------------------------------------------------|---------------------------------------|
| Mineral trioxide aggregate (MTA) | Tricalcium silicate, tricalcium aluminate, tricalcium oxidated, silicate oxide, bismuth | Dentsply, Tulsa Dental                 |
| iRoot BP                         | Calcium silicates, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic | Innovative BioCeramix Inc.            |
| iRoot BP Plus                    | Calcium silicates, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic | Innovative BioCeramix Inc.            |

Fig. 1 Schematic illustration of the DCT experimental setup.

root-end filling material (MTA, iRoot BP, and iRoot BP Plus) were inserted to the walls of the wells by using an appropriate dental instrument. The test samples were divided into three groups. Samples tested 20 min after mixing or the addition of sterile distilled water were designated ‘fresh samples’ (Group 1), those tested 1 day after mixing or the addition of sterile distilled water were designated as ‘1-day samples’ (Group 2), and those tested 7 days after mixing or the addition of sterile distilled water were designated ‘7-day samples’ (Group 3). MTA was mixed and handled according to the manufacturer’s instructions. iRoot BP and iRoot BP Plus, according to the manufacturer’s instructions require presence of moisture to initiate setting reaction. 100 µL of sterile distilled water was added to each pit as described by Lovato et al.\(^5\). All the materials were allowed to set in a 100% moist atmosphere at 37°C before experimenting. A 10-µL microorganism suspension (approximately 1×10^6 cells) was placed on the surface of the fresh, 1-day, and 7-day samples. After incubation for 1 h in a moist atmosphere at 37°C, 240 µL tryptic soy broth (TSB, Merc, Germany) was added to each pit. The solutions were gently mixed with a micropipette for 1 min, and the microbial suspensions from each well were then serially diluted in tryptic soy broth. The survival of the microorganisms was determined by culturing 25-µL aliquots on tryptic soy agar plates after they were serially diluted 10-fold. After incubation for 48 h at 37°C, the colonies on the plates were counted. The viable counts were converted to log10 values, and the CFU/mL value was calculated. *E. faecalis* and *C. albicans* suspensions added to uncoated wells were used as positive controls, and sterile distilled water in test material-coated wells was used as the negative control. Each test was conducted in triplicate.

**Controls for carryover effect**

The procedure used to assess the carryover effect of the root-end filling materials was adapted from Zhang et al.\(^13\). Equal amounts of the materials were coated on the walls of the pits of a 96-well plate, and 10 µL of sterile distilled water was placed on them. After incubation at 37°C for 1 h, 240 µL of TSB was added to each well. The solution was gently mixed with a pipette, and 10 µL of the broth was transferred to an Eppendorf tube containing 970 µL of TSB and 20 µL of the bacterial or fungal inoculum. For controls, the abovementioned procedure was performed without the experiment materials. Then, 10-fold serial dilutions were prepared and incubated onto TSA plates to investigate any possible antimicrobial carryover effect of the root-end filling materials. After incubation at 37°C for 48 h, the survival of the bacteria and fungi was compared in the absence and presence of the test materials. Each test was conducted in triplicate.
Statistical analysis
The results were analyzed statistically using ANOVA followed by Tukey’s test at a 5% significance level (SPSS 11.0; SPSS Inc, Chicago, IL, USA).

RESULTS
The results of the DCT with *E. faecalis* and *C. albicans* are shown in Table 2. Figures 2 and 3 show the detailed results of the DCT for fresh, 1-day, and 7-day samples.

Fresh and 1-day samples of the three root-end filling materials showed similar growth inhibition of the test microorganisms, and the differences were statistically significant compared to the positive controls (*p*<0.05). In contrast, the 7-day samples of the three test materials did not show statistically significant differences in growth inhibition of *E. faecalis* or *C. albicans* when compared with the positive controls (*p*>0.05).

The positive control groups showed bacterial and fungal growth, while the negative control groups showed no microbial growth. Further, the control tests showed no carryover antibacterial and antifungal effects of these materials on the bacterial and fungal strains, respectively.

DISCUSSION
The microorganisms used in this study are pathogenic species associated with endodontic therapy failure14).

|                | Enterococcus faecalis | Candida albicans |
|----------------|-----------------------|------------------|
|                | Fresh 1 day 7 day     | Fresh 1 day 7 day |
| MTA            | 4.24 5.92 6.82        | 4.82 4.82 5.32    |
| iRoot          | 4.45 5.52 6.79        | 4.84 4.91 5.49    |
| Putty          | 4.89 5.46 6.77        | 4.76 4.63 5.35    |
| Control        | 6.95 7.24 6.96        | 5.70 5.70 5.83    |

These pathogens may cause secondary infections after they pass through the root-end to the periapical tissues15). *E. faecalis* is a resilient organism that may implicate the root canal and penetrate the periradicular area. Due to these features it may be held one of the responsible of unsuccessful root canal filling with periradicular lesions14,16,17). *C. albicans* has been found in the infected root canal and periapical pathologic tissues15,19). Both organisms may enter the pulp as contaminants from the oral microflora during root-canal treatment and then enter the periapical region if an adequate root-end sealing is not provided20). Sen et al. showed the penetration of cocci and yeasts into the dentinal tubules through their scanning electron microscopy-based study6). We know that *E. faecalis* is highly robust to several root canal disinfectants containing calcium hydroxide15). Further, detecting yeast-like microorganisms in the root canals of teeth with failed endodontic therapy, suggesting that *C. albicans* is also resistant to endodontic medicaments15). On the basis of these previous results, *E. faecalis* and *C. albicans* were used in this study.

The DCT used in the present study is a quantitative method that simulates the contact of the test microorganisms with retrograde filling materials in the retrograde cavity of resected roots. This procedure allows us to assess the antimicrobial effect of test materials at different stages of the setting reaction5,13). The agar diffusion test (ADT) is another method that may be used...
to evaluate the antimicrobial activity of root-end filling materials, although it has several limitations. The ADT is suitable to evaluate the antimicrobial effects of freshly mixed materials. In this method, the dimensions of the inhibition zone depend on the materials' diffusibility in the medium\(^{16}\). The DCT has been used in this experiment because it is reproducible and appropriate for evaluating the antimicrobial activity of set materials.

A standard method named ISO 22196, could be used for measuring antibacterial effect of hydrophobic plastic materials\(^{21}\). In this technique a 500 dilution of nutrient broth is used. Almost all of microorganisms can survive, but cannot grow. Therefore, *Staphylococcus aureus* and *Escherichia coli* strains used in this method are not suitable for our materials because of they were rarely isolated from periapical lesions\(^{7}\) and hydrophilic properties of root-end filling materials.

MTA is a calcium oxide containing cement, which is converted to calcium hydroxide when it comes in contact with tissue fluid or water. The dissociation into calcium and hydroxide ions results in an increase in the pH and calcium ion release\(^{22}\). The antimicrobial effects of MTA have been widely assessed, but contradictory findings have been reported\(^{1,3,8,18-26}\). An in vitro study showed that MTA exerts antibacterial effects against some facultative bacteria and but not on any species of absolute anaerobes\(^{9}\). Some studies have shown that white MTA\(^{20}\) and grey MTA\(^{23,24}\) exert antifungal effects. However, others have shown that grey MTA has limited or no antifungal effects\(^{19,26}\).

Torabinejad et al.\(^{27}\) reported that the pH of MTA is 10.5 at the time of mixing but 12.9 after 3 h. According to McHugh et al.\(^{28}\), *E. faecalis* is unable to survive at pH values of 11.5 or greater. The antimicrobial effects of MTA against *E. faecalis* and *C. albicans* may be explained by its high pH. Portnier et al.\(^{29}\) showed that *E. faecalis* is more resistant in the stationary phase than in growing cultures. Portenier et al. reported that, mixing the MTA with 0.12% chlorhexidine gluconate may improve its antibacterial efficacy more than with distilled water against *E. faecalis*\(^{29}\).

iRoot\(^{®}\) BP Plus, the putty form of iRoot\(^{®}\) demonstrates remarkable antibacterial and antifungal effectiveness against the test acid bacteria and fungus. However, 7-day samples showed no statistically significant antimicrobial activity. These results are similar with those of Morgental and colleagues\(^{16}\). As the root-end filling materials were maintained in 100% humidity at 37°C, the continued release of antimicrobial components may occur. This may be explained by the fact that under these conditions, calcium ions are released and the pH increases. Duarte et al.\(^{22}\) reported that the pH value and the number of ions are high during the first 3 h after mixing but reduce thereafter.

The results of the present study showed that none of the tested materials completely inhibited the growth of the test organisms. Further studies are needed to investigate the effects of these materials against more bacteria and fungi commonly found in root canals with periapical lesions and to identify ways in which to improve the antimicrobial effects of these materials.

REFERENCES

1) Gutmann JL, Harrison JW. Surgical endodontics. St. Louis: Ishiyaku Euroamerica Inc.; 1994.
2) Eldeniz AU, Hadimli HH, Ataoglu H, Orstavik D. Antibacterial effect of selected root-end filling materials. J Endod 2006; 32: 345-349.
3) Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review — Part I: chemical, physical, and antibacterial properties. J Endod 2010; 36: 16-27.
4) Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. J Endod 1995; 21: 403-406.
5) Lovato KF, Sedgley CM. Antibacterial activity of endocement root repair material and proroot MTA against clinical isolates of *Enterococcus faecalis*. J Endod 2011; 37: 1542-1546.
6) Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol 1995; 11: 6-9.
7) Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001; 34: 429-434.
8) Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 86-93.
9) Maeda H, Hashiguchi I, Nakamura H, Toriya Y, Wada N, Akamine A. Histological study of periapical tissue healing in the rat molar after retrofilling with various materials. J Endod 1999; 25: 38-42.
10) Niederman R, Theodossopoulos JN. A systematic review of *in vivo* retrograde obturation materials. Int Endod J 2003; 36: 577-585.
11) De-Deus G, Canabarro A, Alves GG, Marins JR, Linhares AB, Granjeiro JM. Cytocompatibility of the ready-to-use bioceramic putty repair cement iRoot BP Plus with primary human osteoblasts. Int Endod J 2012; 45: 508-513.
12) Weiss E, Shavlav M, Fuss Z. Assessment of antimicrobial activity of endodontic sealers by a direct contact test. Endod Dent Traumatol 1996; 12: 16.
13) Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. J Endod 2009; 35: 1051-1055.
14) Taschieri S, Bettach R, Lolato A, Moneghini L, Fabbro MD. Endodontic surgery failure: SEM analysis of root-end filling. J Oral Sci 2011; 53: 393-396.
15) Siqueira JF Jr. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J 2001; 34: 1-10.
16) Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Cogo DM, Kopper PM. Antibacterial activity of two MTA-
based root canal sealers. Int Endod J 2011; 44: 1128-1133.

17) Rocas IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004; 30: 315-320.

18) Al-Hezaimi K, Al-Hamdan K, Naghashbandi J, Oglesby S, Simon JH, Rotstein I. Effect of white-colored mineral trioxide aggregate in different concentrations on Candida albicans in vitro. J Endod 2005; 31: 684-686.

19) 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: 309-313.

20) Shahi S, Yavari HR, Rahimi S, Eskandarizadeh M, Shakouei S, Uuchi M. Comparison of the sealing ability of mineral trioxide aggregate and Portland cement used as root-end filling materials. J Oral Sci 2011; 53: 517-522.

21) Standardizations IOF. International Standarts, Plastics-measurement of antibacterial activity on plastic surfaces. International Organisations for Standardizations, Geneva, Switzerland 2007.

22) Duarte MA, Demarchi AC, Yamashita JC, Kuga MC, Fraga Sde C. pH and calcium ion release of 2 root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95: 345-347.

23) Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci 2007; 49: 41-45.

24) Al-Nazhan S, Al-Judai A. Evaluation of antifungal activity of mineral trioxide aggregate. J Endod 2003; 29: 826-827.

25) Mohammadi Z, Modaresi J, Yazdizadeh M. Evaluation of the antifungal effects of mineral trioxide aggregate materials. Aust Endod J 2006; 32: 120-122.

26) Miyagak DC, de Carvalho EM, Robazza CR, Chavasco JK, Leverato GL. In vitro evaluation of the antimicrobial activity of endodontic sealers. Braz Oral Res 2006; 20: 303-306.

27) Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod 1995; 21: 349-353.

28) McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill Enterococcus faecalis in vitro. J Endod 2004; 30: 218-219.

29) Portenier I, Waltimo T, Orstavik D, Haapasalo M. The susceptibility of starved, stationary phase, and growing cells of Enterococcus faecalis to endodontic medicaments. J Endod 2005; 31: 380-386.

30) Stowe TJ, Sedgley CM, Stowe B, Fenno JC. The effects of chlorhexidine gluconate (0.12%) on the antimicrobial properties of tooth-colored ProRoot mineral trioxide aggregate. J Endod 2004; 30: 429-431.