Dental Materials Journal 2014; 33(4): 453–457

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 *Entercoccus 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[1]. The purpose of retrograde filling is to form an apical obturation at the end of the resected root[2].

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[3]. If microleakage occurs between the cavity walls and the filling substance, microorganisms may enter the breached area and cause endodontic surgical failure[4]. Therefore, an ideal root-end filling material should also possess antimicrobial activity whereby it can prevent bacterial and fungal growth[2].

Endodontic infections are caused by anaerobes, facultative anaerobes, aerobes, and fungi[5,6]. *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[7,8].

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], *e.g*) have been used as root-end filling materials[2,9,10].

MTA (Dentsply, Tulsa Dental, Germany) has been favoured as the gold standard because of its high sealing ability and biocompatibility[11]. 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 1×10^8 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)[12]. Briefly, equal amounts of each

The authors declare that they have no conflict of interest.
Received Sep 17, 2013: Accepted Jan 21, 2014
doi:10.4012/dmj.2013-250 JOI JST.JSTAGE/dmj/2013-250

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.

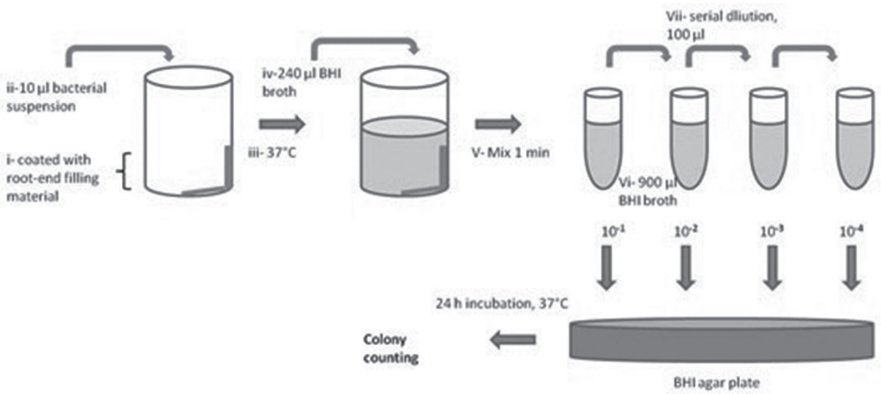

Fig. 1 Schematic illustration of the DCT experimental setup.

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 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 failure[14]. These pathogens may cause secondary infections after they pass through the root-end to the periapical tissues[15]. *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 lesions[14,16,17]. *C. albicans* has been found in the infected root canal and periapical pathologic tissues[18,19]. 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 provided[20]. Sen *et al.* showed the penetration of cocci and yeasts into the dentinal tubules through their scanning electron microscopy-based study[6]. We know that *E. faecalis* is highly robust to several root canal disinfectants containing calcium hydroxide[15]. Further, detecting yeast-like microorganisms in the root canals of teeth with failed endodontic therapy, suggesting that *C. albicans* is also resistant to endodontic medicaments[15]. 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 reaction[5,13]. The agar diffusion test (ADT) is another method that may be used

Table 2 Results of DCT (log10 values of countable colonies)

	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

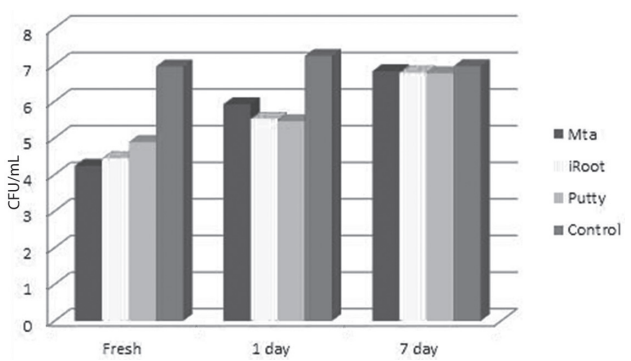

Fig. 2 Antibacterial effects of the test materials.

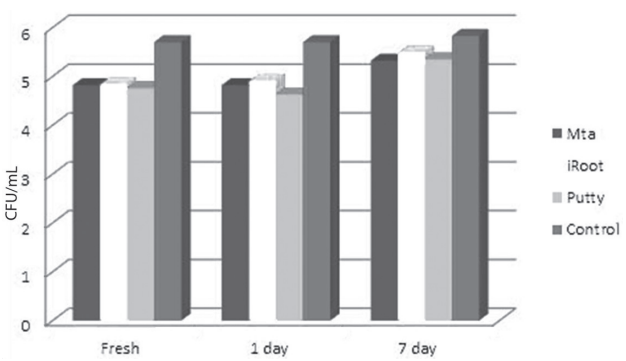

Fig. 3 Antifungal effects of the test materials.

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[3,18,23-26]. An *in vitro* study showed that MTA exerts antibacterial effects against some facultative bacteria and but not on any species of absolute anaerobes[3]. Some studies have shown that white MTA[25] 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 effect 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 is a newly developed ceramic root-end filling material. As it meets water from dentin and periapical tissues, calcium silicates generates calcium silicate hydrogel and calcium hydroxide[13,30]. iRoot® BP Plus, the putty form of iRoot® BP, also requires moisture for setting and hardening. These root-end filling materials require no mixing. Because it is in an injectable form, iRoot® BP is easy to use. Additionally, iRoot® BP Plus has great advantages because it is in a ready-to-use putty form. Because of their ready to use properties, they require no mixing and make the operator saved time during surgical procedure.

The present study showed that the three root-end filling materials tested have similar antimicrobial effects. Fresh and 1-day samples exerted remarkable antibacterial and antifungal effects against the test bacterium 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.

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