Research Square

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Evaluation of Antimicrobial Activity of A Fast-Setting Bioceramic Endodontic Material

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Research Article

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Abstract

Background: To evaluate the antimicrobial activity of the fast-setting bioceramic iRoot Fast Set Root Repair Material (iRoot FS) and two other calcium silicate cements.

Methods: The antimicrobial activity of iRoot FS, ProRoot MTA and Biodentine against *E. faecalis* and *P. gingivalis* were evaluated in this study. The materials were freshly mixed or set for 1 and 7 days on 5mm diameter sterile filter papers. The agar diffusion test, direct contact test and carry-over effect test were conducted, and the pH values (using a digital pH meter) were also evaluated. The data were analyzed by an analysis of variance and two-way ANOVA (α=0.05).

Results: In the agar diffusion experiment, no obvious inhibition zone was observed for iRoot FS, ProRootMTA or Biodentine at any time interval. In the direct contact test, all three materials showed good antibacterial activity after setting for 20 minutes. The antibacterial properties of the three materials decreased with the increase of setting time. None of the three materials showed carry-over antibacterial effect. The pH measurement showed that the suspension of all the three materials showed high pH values (11-12). With the extension of setting time, the pH of iRoot FS and Biodentine slightly decreased.

Conclusions: Fresh iRoot FS, Biodentine, and MTA killed *E. faecalis* and *P. gingivalis* effectively, and the antimicrobial effect of all the three materials decreased over 1 and 7 days after mixing. All three materials showed a tendency of alkalinity which last for at least 7 days after setting.

Introduction

Endodontic surgery is a future treatment of non-healing apical periodontitis when root canal retreatment has failed or is not possible [2]. The root-end filling materials should possess a good sealing ability and be nontoxic, non-carcinogenic, non-genotoxic, biocompatible, insoluble and show dimensional stability [3]. Also, an ideal root-end filling material should possess antimicrobial activity to inhibit bacteria growth to prevent endodontic surgical failure caused by further microleakage [4].

Mineral trioxide aggregate (MTA), which is the first family of calcium silicate cements, has shown favorable clinical performance and was called “gold standard” for root-end-filling material [5]; however, The long setting time is a major drawback, hence, several kinds of materials with a shorter setting time than MTA have been introduced in recent years [3]. Biodentine is a new version of calcium-silicate based inorganic cement and is commercially claimed to be a ‘bioactive dentine substitute’ [6]. Further, a novel premixed calcium phosphate silicate cement with a short setting time, iRoot Fast Set Root Repair Material (iRoot FS, Innovative Bioceramix) was introduced to the market recently and has been used as a permanent root canal repair material in endodontic treatments and apical surgery [7].

The literature results were variable regarding the antimicrobial activity of these retrograde filling materials. MTA products were investigated in many articles with different methodologies [8–10], while few studies were conducted on Biodentine [11, 12], and none were found for iRoot FS. According to a

previous study, the microbiota of persistent periapical infection is polymicrobial with predominance of *E. faecalis* and *P. gingivalis*, regardless of the method used for microbial identification [13]. Hence, *E. faecalis* and *P. gingivalis* were used in this study to appraise the antimicrobial property of these materials. Therefore, the aim of this study was to evaluate the antimicrobial activity of three retrograde filling materials: MTA, Biodentine, and iRoot FS.

Materials And Methods

Specimen preparation

Five microgram of the ProRoot MTA (Dentsply, York County, PA, USA), Biodentine^{TM} (Septodont, Saint Maur des Fosses, France) and iRoot FS (Innovative Bioceramix, BC, Canada) were prepared according to the manufacturers' instructions and placed on 5mm diameter sterile filter papers. The test samples were divided into three groups as described by Damlar *et al.*[4]. Briefly, samples tested at 20 min after mixing were designated as 'fresh samples', those tested on the first day after mixing were designated as '1-day samples', and those tested on the seventh day after mixing were designated '7-day samples'. All the materials were allowed to set in a 100% moist atmosphere at 37°C before experimenting. For the control groups, 5mm diameter sterile filter papers were immersed in 0.12% chlorhexidine (CHX) and sterile saline respectively for 5 second before each test.

Bacterial Strains And Culture Conditions

Oral microorganism strains, *E. faecalis* (ATCC 19433) and *P. gingivalis* (ATCC 33277) were obtained from State Key Laboratory of Oral Diseases, Sichuan University, Chengdu, China. *E. faecalis* were cultivated in brain heart infusion broth (BHI broth, Becton, Dickinson and Company, US) and BHI agar plate, while *P. gingivalis* were cultivated in BHI broth and blood agar plate supplemented with 0.0005% hemin, 0.0001% vitamin K. Both strains were incubated anaerobically (N_2 80%; H_2 10%;CO_2 10%) at 37°C.

Agar Diffusion Test

Bacterial suspension was prepared for each bacterial strain and the turbidity was adjusted to 0.1 OD, which corresponds to approximately 10^8 colony-forming units (CFU)/mL. Then 100μL of *P. gingivalis* suspension was streaked on blood agar plates, while *E. faecalis* was streaked on BHI agar plates. A sterile scratcher was used to inoculate the bacterial suspension onto the agar plate to achieve a lawn of growth. The plates were dried for 5s in room temperature before the filter papers coated with the materials, sterile saline or CHX were placed on each plate. The plates were cultured anaerobically (N_2 80%; H_2 10%;CO_2 10%) at 37℃ for 48h before the diameter of the halo formed around the materials (inhibition zone) was observed. The tests were conducted in triplicate.

Direct Contact Test (Dct)

The filter papers coated with the materials or sterile saline were placed at the bottom of 96-well plates, followed by 200μL of the bacteria suspension (10^7 CFU/mL) being added in each well in direct contact with the materials. After being cultured at 37℃ anaerobically for 1h, the bacteria suspension transferred from each well were serially diluted. The survival of the microorganisms was determined by culturing 100 μL aliquots on BHI agar plates after they were serially diluted 10^3-10^5 fold. Then the colonies on the plates were counted and the CFU/mL value was calculated. The loss of viability was calculated by the following formula: loss of viability = (CFU control−CFU sample)/CFU control. The tests were conducted in triplicate.

Carry-over Effect Test

The carry-over effects of the retrograde filling materials were assessed with procedures described by Ozcan et al. [14] with some modifications. The filter papers coated with the materials or sterile saline were placed at the bottom of 96-well plates, and sterile saline (20 μL) was placed in direct contact with the materials. After incubation at 37°C for 1 h, 230 μL of culture broth was added to each well. After mixing gently with a pipette, 20μL of the broth was transferred to a tube containing 960μL of culture broth. Then 20μL of the bacteria suspension (1.5×10^8 CFU/ml) was added to the tube. Ten-fold serial dilutions were prepared and plated onto BHI agar plates for colony forming. After incubation at 37°C for 48 h, survival of the bacteria was compared between experimental groups and control group to investigate the antimicrobial activity of the materials. The tests were conducted in triplicate.

The Ph Value Measurement

For the pH value measurement, 25mg of each endodontic material were mixed and evenly spread on the bottom of the 24-well plate and were allowed to set in a 100% moist atmosphere at 37°C before experimenting, then 1ml of distilled water (pH=7.4) were added to each well after 20 minutes, 1 day and 7 days, respectively. After 1 hour, the solution was drawn from the wells and centrifuged at 10,000 rpm for 10 min, and the pH measurement was performed with a Five Easy PluspHFEP20 pH meter (METTLER TOLEDO, Zurich, Switzerland).

Statistical Analysis

The data was analysed with two-way ANOVA and the Tukey's post hoc test for multiple comparisons between the antimicrobial effects of the three retrograde filling materials against each bacterial strain tested. Statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA), and $P< 0.05$ was considered statistically significant.

Results

Antimicrobial activity

No inhibition zone was observed in the agar diffusion test except for the positive control.

The results of the DCT with *E. faecalis* and *P. gingivalis* are shown in Figure 1A and 1B. The negative controls exhibited bacteria growth in all test periods. All three materials presented highest antimicrobial effect against *E. faecalis* and *P. gingivalis* when freshly mixed. Fresh ProRootMTA, iRoot FS and Biodentine inhibited most *E. faecalis* (ProRootMTA 77.5%, iRoot FS 91.2%, Biodentine 80.7%), However, the antimicrobial activity of iRoot FS against *E. faecalis* were lower than the other two materials after setting for 1 or 7 days. As for the antimicrobial activity against *P. gingivalis*, Fresh ProRootMTA and Biodentine inhibited almost all *P. gingivalis* (ProRootMTA 97.9%, Biodentine 98.9%) while iRoot FS inhibited the growth of all *P. gingivalis* (100%). iRoot FS and Biodentine produced almost complete inhibition after setting for 1 day, while the effect of MTA was relatively lower ($p < 0.05$). The 7-day samples of the three test materials showed significantly lower growth inhibition of *P. gingivalis* when compared with the other time interval groups ($p < 0.05$), while Biodentine showed relatively highest antimicrobial effect and that of MTA was lowest ($p < 0.05$).

Carry-over of the antimicrobial effect from the materials was not observed (Figure 2).

The Ph Values Measurements

pH values of the leachate of the materials are shown in Table 1. All three endodontic materials showed significantly strong alkaline effect in all observed time intervals. No significant difference was noticed when freshly mixed among the materials, and Biodentine presented highest pH values after setting for 1 or 7 days.

Table 1
The PH values of MTA, Biodentine, and iRoot FS leachates at different time intervals (mean ± S.D.;n=3)

	MTA	Biodentine	iRoot FS
Fresh	$11.85(\pm0.040)^{A,a}$	$12.11(\pm0.012)^{B,a}$	$11.87(\pm0.027)^{A,a}$
1d	$12.01(\pm0.009)^{A,b}$	$12.07(\pm0.026)^{A,a}$	$11.81(\pm0.030)^{B,a}$
7d	$11.46(\pm0.062)^{A,c}$	$11.66(\pm0.038)^{B,b}$	$11.00(\pm0.075)^{C,b}$

Superscript capital letters represent statistically significant differences in the same row, and superscript lowercases indicate statistically significant differences in the same column. The same letters indicate no significant differences among the compared groups ($P>0.05$) and vice versa.

Discussion

The DCT used in the present study is a quantitative and reproducible method designed to simulate the contact of the microorganism with retrograde filling materials [15]. This procedure allows us to assess the antimicrobial effect of test materials at different stages of the setting reaction. In 2009, Zhang *et al*. reported a modified DCT [16], in which the suspension of MTA was obtained to contact the bacteria suspension. However, the retrograde filling materials were in direct contact with the microorganisms inside the root canals, the DCT applied in the present study might better mimic the clinical situation. The agar diffusion test (ADT) is another method to evaluate the antimicrobial activity of root-end filling materials. Since the outcome of ADT depend on the material diffusibility in the medium [4], the solid root-end filling materials may not be diffusible, which could be a possible explanation for the negative outcome of ADT in the present study. Therefore, DCT seems more appropriate in evaluating the antimicrobial activity of solidified materials.

MTA was introduced innovatively as a root-filling material by Dr. Torabinejad in 1995 [17]. According to previous studies [18, 19], the antibacterial and antifungal properties of MTA were associated with elevated pH value. In the present study, no inhibition zone was observed in agar diffusion test, while direct contact test revealed antimicrobial effect of MTA against both *E. faecalis* and *P. gingivalis*. An *in vitro* study [3] showed that MTA exerts antibacterial effects against some facultative bacteria but not on any species of absolute anaerobes, however, another study by Kim *et al.* [20] found that freshly mixed ProRoot MTA formed a bacterial growth inhibition zone against *P. gingivalis* in disk diffusion test. Since MTA has been tested in many researches but with contradictory results [18] such difference may be attributed to the usage of different methodologies, bacterial strains, aerobic and anaerobic conditions.

Biodentine was developed as dentin replacement material. In addition to shorter setting time and satisfactory strength, it was also reported to be less porosity and less leakage [21], less tooth discolors [22–24] and excellent biocompatibility [25] compared with MTA. In the present study, the antimicrobial effect of Biodentine against *E. faecalis* was similar to that of MTA, and the effect was lower when tested 7 days after setting, which is in accordance with a previous study by Koruyucu *et al* [15].

iRoot FS (Innovative Bioceramix, Vancouver, BC, Canada) was introduced as a root canal repair material. As a premixed material, iRoot FS solidifies only when exposed to a moist environment. Previous studies have reported that iRoot FS has similar apical sealing ability and mechanical properties to MTA [26] and that iRoot FS has a shorter setting time (initial 18 min and final 57 min) than MTA. There are great potentials for the clinical application of iRoot FS as the material is cytocompatible while facilitating cell adhesion, proliferation, differentiation and maintenance of normal cell function [27]. However, the antimicrobial effect of iRoot FS is unknown. In the present study, iRoot FS showed satisfactory antimicrobial effect when tested 20 min or 1 day after setting, and the effect became relatively lower than MTA and Biodentine when tested 7 days after setting, which might be attribute to its shorter setting time.

The pH values measured in this study were between 11 and 12, all the three materials showed strong alkaline pH, which is in accordance with previous studies [18, 28]. However, though Biodentine exhibited

the highest pH value at all time intervals, which might explain its superior antimicrobial effect 7 days after setting, it did not show the strongest antibacterial activity against *E. faecalis*. Therefore, as Zhang *et al*. mentioned in a previous study [29], the antibacterial action cannot be rationally explained by pH alone. Moreover, in clinical situations, a desirable high pH after MTA application cannot be maintained due to the buffering capacity of dentin [20].

The selection of the used bacterial species in this *in vitro* study was intended to represent the poly-micro flora in the periapical lesions. However, the real situation *in vivo* is far more complex and hard to simulate *in vitro*. Further *in vivo* studies are required to better understand the various properties of the retrograde filling materials.

Conclusions

Within the limitations of this study, fresh iRoot FS, Biodentine, and MTA killed E. faecalis and P. gingivalis effectively, and the antimicrobial effect of all the three materials decreased one and seven days after mixing. All three materials showed a tendency of alkalinity 7days of the study.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: MJ designed the work and analyzed the data, was a major contributor in writing the manuscript. YC was a major contributor in conducting the experiment. YW participated in data acquisition and analysis. KX participated in work design and revised the manuscript. XC revised the manuscript. LZ designed the work, acquired the materials and revised the manuscript. All authors read and approved the final manuscript.

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Figures

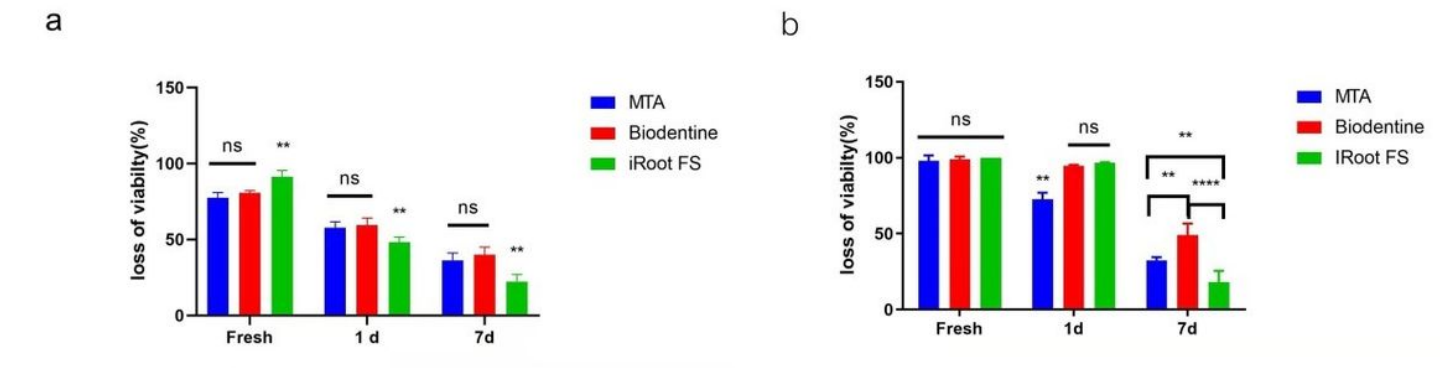

Figure 1

Outcome of direct contact test against (a) E. faecalis and (b) P. gingivalis

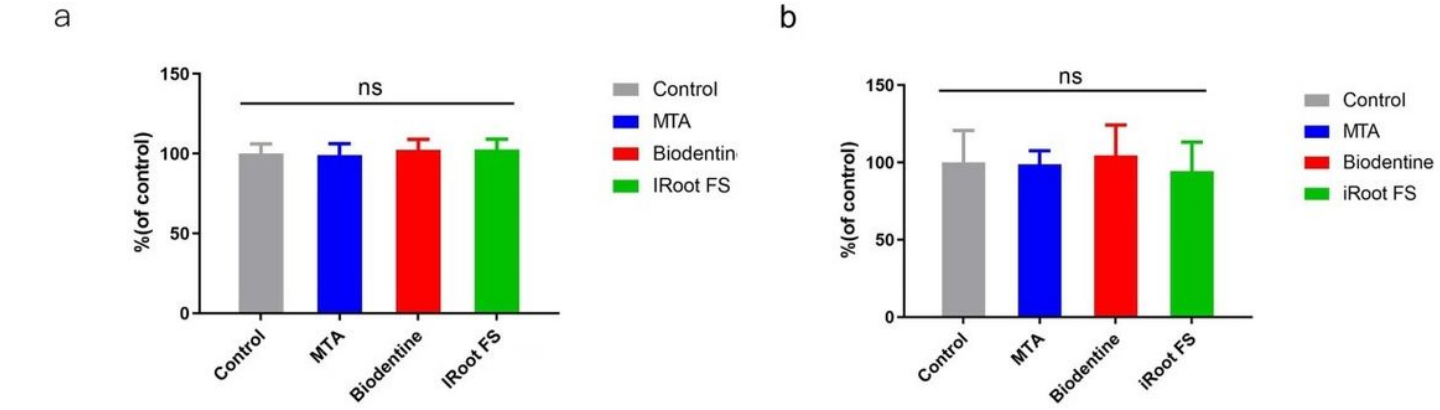

Figure 2

Outcome of carry-over effect test against (a) E. faecalis and (b) P. gingivalis