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Evaluation of antibacterial and antifungal activity of calcium silicate based retrograde filling materials

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Abstract
The aim of this study was to compare the antimicrobial and antifungal activity of MTA Angelus, Biodentine and DiaRoot BioAggregate tricalcium silicate based retrograde filling materials with agar diffusion test (ADT) method. *Enterococcus faecalis* and *Candida albicans* standard bacterial strains were used. 100 µl was taken from liquid cultures of *E. faecalis* and planted in Mueller Hinton agar and the same amount of *C. albicans* was planted in sabouraud dextrose agar by means of spread plate technique. Then, MTA Angelus, Biodentine and DiaRoot BioAggregate cements were aseptically filled into the opened pits. Following this procedure, the media were kept in the drying oven at 37 °C for 12, 24, 48 and 72 hours and the diameters of the inhibition zones were measured. Within the limits of our study, retrograde filling materials MTA Angelus, Biodentine and DiaRoot BioAggregate exhibited antibacterial and antifungal effect.

Keywords: MTA Angelus, Biodentine, DiaRoot BioAggregate, Antimicrobial, Endodontics

Introduction

In the presence of pathological lesion that cannot be eliminated by endodontic treatment or in case root canal retreatment cannot be performed or retreatment fails, endodontic surgery procedures are applied [1]. Following apical resection, retrograde filling is performed in order to prevent the transmission of tissue fluid into the canal or the transmission of the microorganisms or the toxins inside the canal into the periapical tissues in the teeth in which root canal filling is performed [2]. To this end, a lot of materials having different physical, chemical and biological properties are used. An ideal retrograde filling material should be biocompatible with the tissues with which it contacts, should have sufficient sealing, should allow for bone development or stimulate bone development and should exhibit antimicrobial effect [3, 4]

Retrograde filling materials cannot provide perfect sealing and thus, there remain microscopic spaces between the retrograde cavity and the filling material [5]. Microorganisms and their products may penetrate into root canal system through these spaces. Therefore, antimicrobial activities of the materials used as retrograde filling are necessary to be advantageous [6].

MTA is one of the main retrograde filling materials frequently used today. Several studies have shown that MTA is a bioactive and biocompatible material [7, 8]. Moreover, in one study, MTA is shown to have better sealing than amalgam, IRM and super-EBA [9]. However, the disadvantages of MTA such as its manipulation being difficult and setting time being long led to the development of new calcium silicate based materials [10].

Biodentine (BD; Septodont, Saint-Maur-des-Fosses, France) is a tricalcium silicate based material, which is developed as dentin restorative material for this purpose and can be used in the endodontic indications of MTA. Likewise, DiaRoot BioAggregate (BA; Diadent Group International, Burnaby, BC, Canada) is a material, which can also be used as newly developed tricalcium silicate based retrograde filling. Unlike MTA, BA does not contain aluminum, but contains calcium phosphate and tantalum oxide.

In the literature search that we made, no study comparing the antimicrobial activity of MTA Angelus, BD and BA is found. For this reason, the objective of our study was to compare the antimicrobial and antifungal activity of MTA Angelus, Biodentine and Dia Root BioAggregate tricalcium silicate based retrograde filling materials against *E. faecalis* and *C. albicans*

Microorganisms frequently isolated in unsuccessful root canal treatment cases with agar diffusion test (ADT) method by evaluating the same in 12, 24, 48 and 72 time zones. The hypothesis of our study was that the antibacterial and antifungal activity of the different tricalcium silicate based retrograde filling materials would not change according to the time zone.

Materials and Methods

In our study, in order to determine the antimicrobial activity of MTA Angelus (Angelus, Londrina, PR, Brasilia), Biodentine (Septodont, Saint-Maur-des-Fosses, France) and DiaRoot BioAggregate (Diadent Group International, Burnaby, BC, Canada) retrograde filling materials with ADT method, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231 standard bacterial strains were used.

Bacteria were planted in Brucella broth medium and incubated at 37 °C for 24 hours. At the end of the incubation period, density of liquid culture was adjusted to 0.5 McFarland standard with McFarland densitometer. 100 µl was taken from liquid cultures of *E. faecalis* and planted in Mueller Hinton agar and the same amount of *C. albicans* was planted in sabouraud dextrose agar by means of spread plate technique. Petri dishes were dried at room temperature. A total of 6 petri dishes were prepared such that each microorganism would have 3 petri dishes. Three pits were formed on the medium provided inside each petri dish in a manner to have 5 mm diameter and 2 mm depth. Then, MTA Angelus, Biodentine and Dia Root Bio Aggregate cement prepared in accordance with the manufacturers' instructions were aseptically filled into the opened pits. Following this procedure, the media were kept in the drying oven at 37 °C for 12, 24, 48 and 72 hours and the diameters of the inhibition zones occurring around the pits at the end of these time zones were measured by means of digital compass. Three measurements were performed for each microorganism and the data were recorded.

Normal distribution control of the data obtained in our study was made with Kolmogorov-Smirnov test and homogeneity control of the group variances was made with Levene's test. Afterwards, for the statistical analysis of data, one-way analysis of variance and for within-group comparisons, Duncan multiple comparison test were utilized. Statistical significance level of the study was adjusted as (α) 5%.

Results

In our study, average inhibition zone and standard deviation values formed by MTA Angelus, BA and BD on *E. faecalis* and *C. albicans* at the end of 12th, 24th, 48th and 72nd hours were shown in Table 1 and 2. Antimicrobial activity of BD versus *E. faecalis* was statistically higher than MTA Angelus and BA ($P<0.001$). Antimicrobial activity of BA versus *C. albicans*, however, was statistically higher than MTA Angelus and BD ($P<0.001$).

Table 1: The means and standard deviations of inhibition zones after 12, 24, 48, 72 hours against *E. faecalis* (in mm).

	12. Hour	**24. Hour**	**48. Hour**	**72. Hour**	***P*-value**
MTA Angelus	7.32 (0.25)^{ax}	8.45 (0.21)^{ay}	6.34 (0.15)^{az}	4.35 (0.15)^{at}	<0.001
Biodentine	13.21 (0.18)^{bx}	13.32 (0.24)^{bx}	13.12 (0.13)^{bx}	10.22 (0.13)^{by}	<0.001
DiaRoot BioAggregate	11.25 (0.2)^{cx}	12.15 (0.25)^{cy}	12.05 (0.2)^{cy}	9.35 (0.14)^{cz}	<0.001
***P*-value**	<0.001	<0.001	<0.001	<0.001	

$^{a, b, c}$: Different superscripts in the same column indicates significantly different

$^{x, y, z, t}$: Different superscripts in the same row indicates significantly different

Table 2: The means and standard deviations of inhibition zones after 12, 24, 48, 72 hours against *C. albicans* (in mm).

	12. Hour	**24. Hour**	**48. Hour**	**72. Hour**	***P*-value**
MTA Angelus	6,34 (0,06)^{ax}	6,45 (0,1)^{ax}	6,15 (0,16)^{ax}	4,35 (0,16)^{ay}	<0,001
Biodentine	10,21 (0,13)^{bx}	10,45 (0,14)^{bx}	10,25 (0,09)^{bx}	5,55 (0,09)^{by}	<0,001
DiaRoot BioAggregate	11,12 (0,15)^{cx}	12,18 (0,2)^{cy}	11,28 (0,08)^{cx}	8,48 (0,25)^{cz}	<0,001
***P*-value**	<0,001	<0,001	<0,001	<0,001	

$^{a, b, c}$: Different superscripts in the same column indicates significantly different

$^{x, y, z}$: Different superscripts in the same row indicates significantly different

When time-related changes of the activities of the materials were compared, antimicrobial activity of BD versus *E. faecalis* was statistically higher at the end of 12th, 24th and 48th hours compared to the 72nd hour ($P<0.001$). It is detected that the best antimicrobial activity of BA versus *E. faecalis* occurred at the end of 24th and 48th hours and this activity statistically decreased at the end of 72nd hour ($P<0.001$). It is found that the best antimicrobial activity of MTA Angelus versus *E. faecalis* occurred at the end of 24th hour and this activity statistically decreased at the end of 72nd hour ($P<0.001$).

It is also found that the best antimicrobial activity of BA versus *C. albicans* occurred at the end of 24th hour and this activity statistically decreased at the end of 72nd hour ($P<0.001$). Antimicrobial activity of BD and MTA Angelus versus *C. albicans* was statistically higher at the end of 12th, 24th and 48th hours compared to the 72nd hour ($P<0.001$).

Discussion

In our study, antimicrobial activities of MTA Angelus, BA and BD on *E. faecalis* and *C. albicans* were examined with ADT method. ADT is a method, which is frequently used in several microbiological studies [6, 11-13]. It is possible to directly compare the effects of the newly mixed materials on the microorganisms tested with these methods. However, determination of antimicrobial properties of the materials in ADT method is based on the factors such as the good contact to be provided by the material and agar gel, the inhibitor agents' capacity of being diffused into the gel, the types of

bacteria, and the cellular density [6, 13, 14]. Since ADT allows for the comparison of the antibacterial activities of the newly mixed dental materials, it was preferred in our study, as well.

It is known that the main reason of the failures occurring after endodontic treatment is the microorganisms. *E. faecalis* was used in our study, mainly the persistent infections after the endodontic treatment, because of the fact that it is a microorganism frequently isolated from the infected root canals, that it is capable of invading the dentin tubules of the microorganism thanks to the "serine protease" and "collagen-binding protein" it contains and that it is resistant to various medicaments such as calcium hydroxide [15-17]. *C. albicans* is a type of fungi which is also mainly isolated in endodontic infections. Siqueira and Sen showed in their study that *C. albicans* can be colonized in root canal walls and can easily penetrate into dentin tubules [18]. Moreover, *C. albicans* was found more resistant to antimicrobial agents such as citric acid and sodium hypochlorite than *E. faecalis* [19]. Due to these reasons, *C. albicans* was used in our study. Moreover, *C. albicans* and *E. faecalis* are the microorganisms frequently isolated in the samples taken after periapical surgical procedures [17, 20]. Therefore, we are of the opinion that examination of the antimicrobial activity of the retrograde filling materials in the market against these microorganisms will shed light on the other studies in terms of clinical use.

Torabinejad *et al*., in the study they made using ADT method, examined the antimicrobial effect of several retrograde filling materials and reported that MTA did not exhibit antimicrobial activity on *E. faecalis* [6]. We think that the difference between the results of our study and this study stems from the differences in the formulation of MTA. Moreover, the results of our study have parallels with the studies by Stowe *et al*. and Tanomaru-Filho *et al*. [13, 14]. In our study, MTA Angelus exhibited antibacterial effect against both microorganisms. In the studies conducted in a manner to support our study, MTA also exhibited antimicrobial effect against both *E. faecalis* and *C. albicans* [21, 22]. However, our results are different from the study by Estrela *et al*. who reported that MTA did not have an antimicrobial activity against *E. faecalis* and *C. albicans* [23]. We think that in our study the antibacterial effect exhibited by MTA Angelus was due to the high pH it formed and the diffusible substances it contains [24].

Our study found that BA exhibited statistically more antifungal effect than the other materials tested. Similar to the results of our study, Dohaithem *et al*. evaluated the antifungal effect of BA versus *C. albicans* with direct contact test and found that BA prevented the proliferation of *C. albicans* at the end of 24 hours [25]. In parallel to the results of this study, it is seen that the largest inhibition zone against *C. albicans* occurred in BA group and at the end of 24 hours in our study. In a study, pH of BA reached the maximum level at the end of 24 hours, which explains the formation of the largest inhibition zone in BA group at the end of 24 hours in our study [26].

According to the results of our study, BA exhibited statistically better antimicrobial effect against *E. faecalis* and *C. albicans* than MTA. The main difference between MTA and BA is the tantalum oxide content contained by BA. Composition of BA substantially contains tantalum oxide [27]. It is reported that tantalum oxide forms a strong inhibition zone in the presence of osteoblast, but despite this it can proliferate well on the fibroblasts [28]. We think that the amount of tantalum oxide contained by BA provides the material to exhibit a better antimicrobial activity.

Our study found that BD formed statistically more inhibition zones against *E. faecalis* than the other materials tested. Bhavana *et al*. examined the antimicrobial activity of ProRoot MTA and Biodentine with ADT method and similar to the result of our study found that BD exhibited a higher antimicrobial effect against *E. faecalis* and *C. albicans* than MTA [29]. Similar to our study results, Hiremath *et al*., in their study, reported that BD exhibited a strong antimicrobial activity against *E. faecalis* and *C. albicans* [30].

Null hypothesis of our study was rejected as it is found that antimicrobial activities of the materials tested in our study results changed in a statistically significant manner between the groups and in time.

Conclusion

Within the limits of our study, retrograde filling materials MTA Angelus, Biodentine and DiaRoot BioAggregate exhibited antibacterial and antifungal effect. However, it is seen that while Biodentine exhibited antimicrobial effect against *E. faecalis*, BioAggregate exhibited antimicrobial effect against *C. albicans* in a broader spectrum.

Acknowledgments

The authors deny any conflicts of interest.

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