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# Assessment of antibacterial activity of MTA Flow® against *Enterococcus faecalis* and *Staphylococcus aureus*: An *In vitro* study

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**Abstract**

**Objectives:** This research evaluated antimicrobial activity of MTA Flow® and assessed whether its bioactivity is preserved in different concentrations and compared to MTA Angelus®.

**Methods:** Methodologies were agar diffusion test (ADT) and direct contact test (DCT) against *Enterococcus faecalis* and *Staphylococcus aureus*.

**Results:** In ADT, no cement revealed bioactivity against *E. faecalis*, while for *S. aureus*, the lower concentration of MTA Flow® showed significantly lower antimicrobial activity when compared to other MTA Flow® concentrations and MTA Angelus®, after 24 and 48 hours. In ADT, the other concentrations of MTA Flow® were equivalent to that of MTA Angelus® in both exposure times. MTA Flow® and MTA Angelus® had no significant antimicrobial effect against *E. faecalis* in DCT. On the other hand, with *S. aureus*, DCT showed that MTA Flow® antimicrobial action was better than MTA Angelus®, at 24 hours for all concentrations. DCT for *S. aureus*, showed that antimicrobial activity of MTA Flow® and MTA Angelus® has increased over time, demonstrating that antimicrobial property is influenced by the contact time of cements/microorganisms.

**Conclusion:** Thus, none of cements had antimicrobial effect against *E. faecalis* neither in ADT nor in DCT. All MTA Flow® concentrations had a better performance than MTA Angelus® against *S. aureus*, in DCT.

**Keywords:** antimicrobial activity; endodontic therapy; MTA; agar diffusion test; direct contact test

## Introduction

Most of endodontic pathologies are caused by microorganisms present in root canal system. Thus, endodontic treatment aims to eliminate these pathogens and prevent re-infection of the root canal by microorganisms that may remain available after chemo-mechanical preparation or may cross the physical barrier formed by filling materials.[1] Sometimes microorganisms that remain within root canal system and dentinal microtubules may cause endodontic treatment failures, leading to apical periodontitis. In these cases, microorganisms must be able to adapt and survive to chemo-mechanical preparation, and resist to antimicrobial effect of intracanal medicaments and cements applied in endodontic therapy [2].

*Enterococcus faecalis* and *Staphylococcus aureus* are some of microorganisms find in pathological situations like necrotic pulp cases and persistent periapical lesions [3]. Researches have shown that, once established within root canal, *E. faecalis* may be considered the bacteria with high resistance to endodontic antimicrobial agents, also playing an important role in failure of endodontic treatments [4, 5]. *S. aureus* has a remarkable ability to adapt and withstand to antibiotics and may be considered responsible for more severe pathologies, such as osteomyelitis, septicemia and mediastinitis [6, 7].

Endodontic sealers are used to assist in complete three-dimensional filling of root canal, eliminating empty spaces, as well as in local control of bacteria.[8] Previous studies have shown that the lower antimicrobial activity of endodontic sealer the higher failure rates in endodontic treatments, especially in clinical situations with recurrent infections by antimicrobial-resistant bacteria [9].

Mineral trioxide aggregate (MTA) (MTA Angelus®, Londrina, Paraná, Brazil) is a powder that consists of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and bismuth oxide. Since its introduction in the 1990s it has been widely used in endodontic clinical practice [10]. MTA Flow® is a novel formulation of MTA, which can be used in different concentrations (Ultradent Products Inc., South Jordan, UT, USA). The assessment of the antimicrobial activity of MTA Flow® is essential to demonstrate if this cement can be safely applied to various clinical situations in which its use is indicated.

There are very few studies in literature that evaluate the antimicrobial activity of MTA Flow®. Thus, this research aimed to analyze the antimicrobial property of MTA Flow®, assessing whether this activity is preserved when the sealer is used in different concentrations and compare them with MTA Angelus®.

## Material and Methods

### Bacterial strains and growth conditions

In this study, antibacterial activity of MTA Flow® and MTA Angelus® were evaluated against *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 29213). Microorganisms were activated, from frozen samples, in liquid Brain Heart Infusion (BHI, Difco®, Detroit, MI, USA), for 24 hours at 37ºC under aerobic conditions. After microbial growth, 100 µL aliquots were inoculated on Mueller-Hinton agar (Difco®, Detroit, MI, USA). Plates were incubated at 37° C for 48 hours. From this culture, bacterial suspensions were prepared with 0.85% sterile saline to match the turbidity equivalent to 0.5 McFarland standard tube ($1.5x10^8$ CFU/mL).

### Endodontic sealers discs

To verify whether different concentrations of MTA Flow® would change its antimicrobial activity, the cement was prepared as recommended by manufactures, in three different concentrations: 0.19 g/50 µL (3.8 µg/µL), 0.26 g/100 µL (2.6 µg/µL) and, 0.19 g/100 µL (1.9 µg/µL). The antimicrobial activity of MTA Angelus® was also evaluated in 2.8 g/50 µL (5.6 µg/µL) powder/distilled water ratio. Cements were prepared and immediately placed into a silicone mold to prepare uniform discs (6 x 6 x 1 $mm^3$).

### Agar diffusion test (ADT)

Plates containing Mueller-Hinton agar were spread with 0.1 mL of bacterial suspensions with turbidity equivalent to 0.5 McFarland standard tube ($1.5x10^8$ CFU/mL) with a sterile Drigalsky loop under aseptic conditions. Cement discs were placed at equidistant points on each plate as soon as they were prepared. The experiments were performed in duplicate for each bacterium (*E. faecalis* and *S. aureus*), for MTA Angelus® and for each concentration of MTA Flow®.

To prove the sterility of MTA, cement discs were placed on a Petri dish over uninoculated medium. A plate containing uninoculated medium was prepared to confirm the medium sterility. Positive controls were antibiotic discs containing ampicillin (10 µg) for *E. faecalis* and vancomycin (30 µg) for *S. aureus*. All plates were maintained at room temperature for 2 hours for prediffusion of the materials and then incubated at 37ºC for 48 hours under aerobic conditions. The diameter of the inhibition halo around the sealers´ discs was measured by two previously calibrated evaluators in two perpendicular directions with a dry point compass and a millimeter ruler with accuracy of 0.5 mm after 24 and 48 hours.

### Direct contact test (DCT)

Direct contact test was performed by measurement of culture medium optical density with a spectrophotometer set at 640 nm wavelength, as described by Domínguez *et al* [11].

Analyzes were performed in duplicate for MTA Angelus® and for each concentration of MTA Flow®. In all, 24 test tubes were prepared. With a sterile forceps, the discs of cements were dispensed into test tubes containing 10 mL of Soy Triptcasein Broth (TSB) (Kasvi, Brazil) and 0.1 mL of bacterial suspension (*E. faecalis* or *S. aureus*) adjusted to match the turbidity standard of 0.5 McFarland scale ($1.5x10^8$ CFU/ mL).

Two tubes did not receive bacterial suspension nor cement discs to control the sterility of culture medium; two tubes received the cements to verify their sterility and calibrate the spectrophotometer and, two tubes containing ampicillin (10 µg) for *E. faecalis* and vancomycin (30 µg) for *S. aureus* received bacteria suspension (positive control); and two tubes received only bacterial suspension (negative control).

The tubes were incubated at 37°C, ensuring direct contact between bacteria and endodontic cements and the analyzes were performed after 24 and 48 hours. To determine the densities of bacterial cultures, readings of absorbance for each suspension were carried out in a spectrophotometer (Femto 600 Plus). The higher the bacterial density, the greater absorbance of the solution and the lesser antimicrobial effect of analyzed substance.

### Statistics

Differences between cements were statistically analyzed by Jamovi® 1.2 software. Data were submitted to One-Way-ANOVA and Tukey Post-Hoc Test for comparisons between groups. For comparisons between exposition times Repeated Measures ANOVA with Tukey Post-Hoc Test were carried out. The significance level accepted was $p < 0.05$.

## Results

### Agar Diffusion Test (ADT)

Diameters averages of growth inhibition halos promoted by *E. faecalis* in ADT are shown in figure 1. Results indicate that none of the tested cements (MTA Angelus® and MTA Flow®) showed antimicrobial effect against *E. faecalis*.

For *S. aureus*, after both times of exposure, the lowest concentration of MTA Flow® (1.9 µg/µL) showed significantly less antimicrobial activity compared to other concentrations of MTA Flow® and MTA Angelus® ($p < 0.05$). There was no significant difference ($p < 0.05$) in inhibition of bacterial growth with 48 hours of exposition when compared to 24 hours (Figure 2).

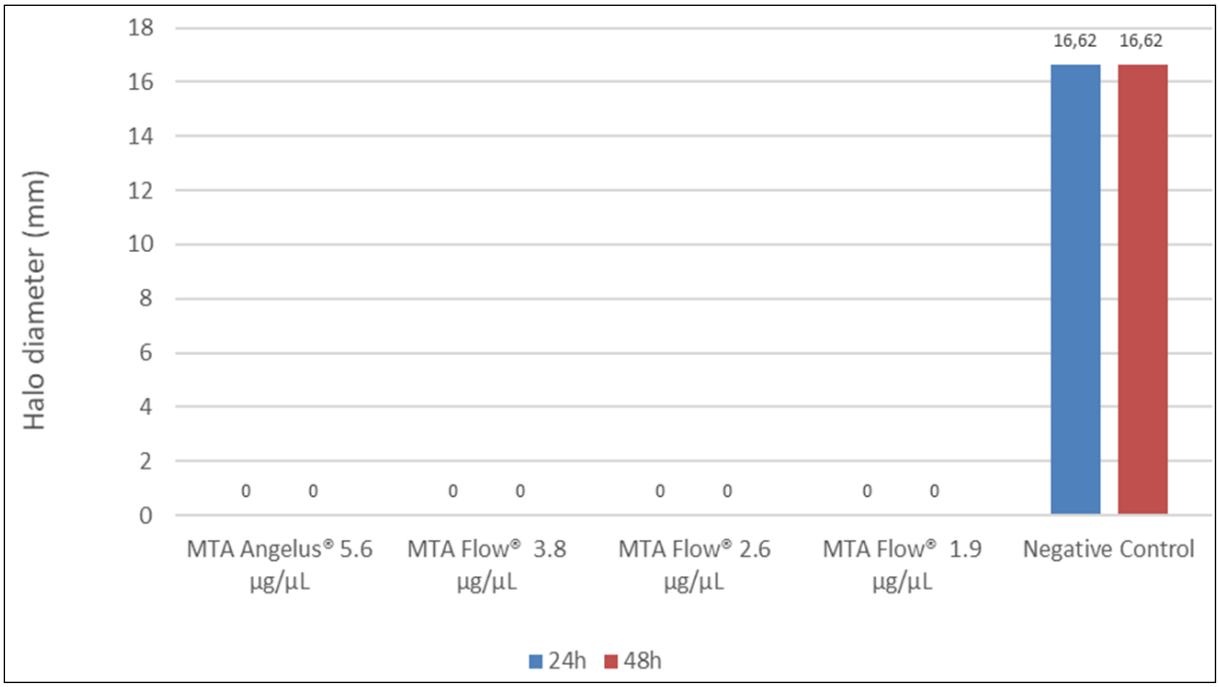


**Fig 1:** Inhibition of bacterial growth (*E. faecalis*) promoted by the three concentrations of MTA Flow® and MTA Angelus® in agar diffusion test after 24 and 48 hours.

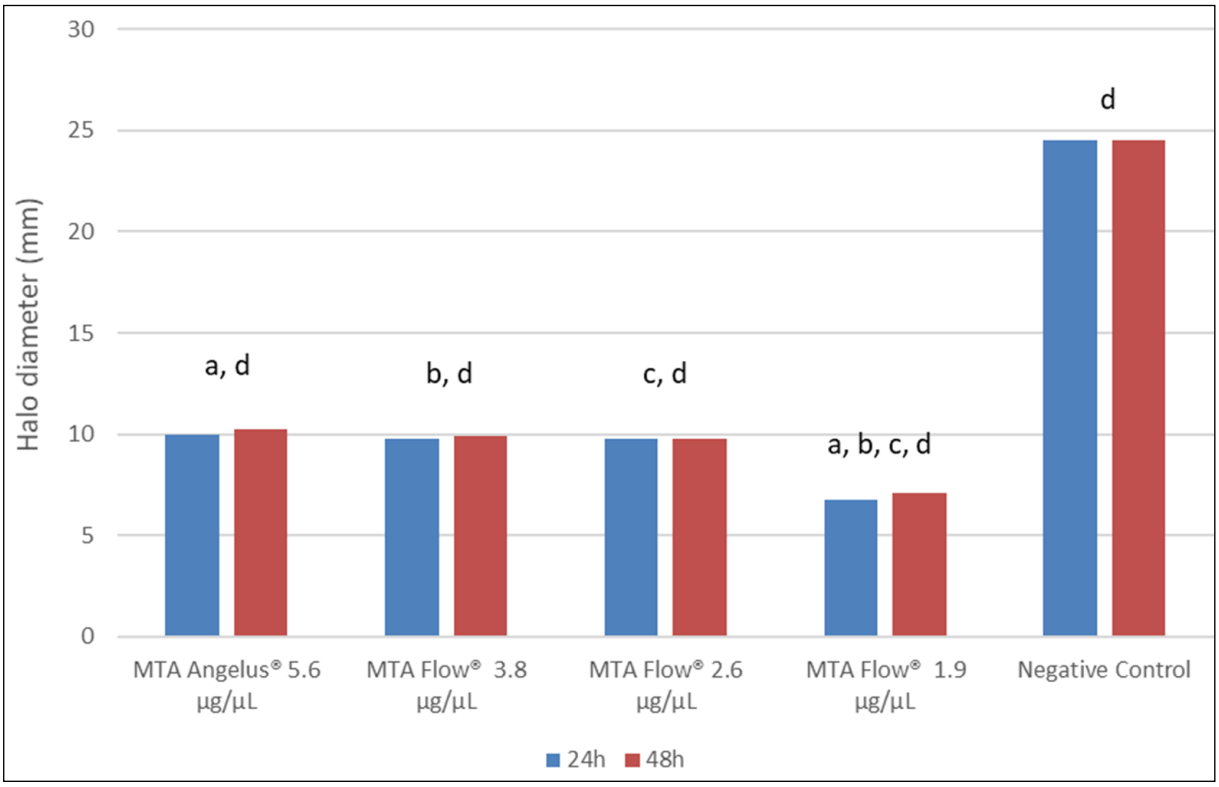


**Fig 2:** Inhibition of bacterial growth (*S. aureus*) promoted by the three concentrations of MTA Flow® and MTA Angelus® in agar diffusion test after 24 and 48 hours. [a, b, c, d] Same letters indicate statistical differences between cements, One-Way-ANOVA and Tukey Post-Hoc Test, $p < 0.05$.

**Direct Contact test (DCT)**

Regarding the inhibition of *E. faecalis* growth by the DCT (Table 1), results show that at 24 and 48 hours of exposure none of evaluated cements reduced bacterial growth significantly when compared to negative control. There were no significant differences ($p < 0.05$) between the antimicrobial activities of MTA Flow® and MTA Angelus® at 24 and 48 hours. In addition, MTA Angelus® did not inhibit bacterial growth at 48 hours of exposure, which can be proven by increase in absorbance values of this sample. However, these results showed no significant difference ($p < 0.05$).

**Table 1:** Absorbance by spectrophotometer analyzes in direct contact test (DCT) promoted by MTA Flow® and MTA Angelus® against *E. faecalis*.

| *E. faecalis* | Sealers | Mean | Standard Deviation |
|---|---|---|---|
| Absorbance - 24h | MTA Angelus® 0.28 g/50 µL (5.6 µg/µL) | 0.453 | 0.0184 |
| | MTA Flow® 0.19 g/50 µL (3.8 µg/µL) | 0.619 | 0.1683 |
| | MTA Flow® 0.26 g/100 µL (2.6 µg/µL) | 0.667 | 0.0962 |
| | MTA Flow® 0.19 g/100 µL (1.9 µg/µL) | 0.552 | 0.0184 |
| | Negative control | 0.648 | 0.0481 |
| Absorbance - 48h | MTA Angelus® 0.28g/50µL (5.6µg/µL) | 0.587 | 0.1640 |
| | MTA Flow® 0.19 g/50 µL (3.8µg/µL) | 0.579 | 0.1534 |
| | MTA Flow® 0.26 g/100 µL (2.6µg/µL) | 0.520 | 0.0134 |
| | MTA Flow® 0.19 g/100 µL (1.9µg/µL) | 0.521 | 0.0156 |
| | Negative control | 0.701 | 0.0184 |

Comparing 24 and 48 hours, there was a reduction in absorbance values for all concentrations of MTA Flow®. Nevertheless, these results showed no statistical difference ($p < 0.05$).
For *S. aureus* (Table 2) results showed that all MTA Flow® concentrations had better ability to inhibit bacterial growth than MTA Angelus® at 24 hours of exposure ($p < 0.05$).

**Table 2:** Absorbance by spectrophotometer analyzes in direct contact test (DCT) promoted by MTA Flow® and MTA Angelus® against *S. aureus*.

| *S. aureus* | Sealer | Mean | Standard Deviation |
|---|---|---|---|
| Absorbance - 24h | MTA Angelus® 0.28 g/50 µL (5.6 µg/µL) | $1.26^{A,a,b,c}$ | 0.02263 |
| | MTA Flow® 0.19 g/50 µL (3.8 µg/µL) | $1.04^{B,a}$ | 0.00354 |
| | MTA Flow® 0.26 g/100 µL (2.6 µg/µL) | $1.07^{b}$ | 0.07283 |
| | MTA Flow® 0.19 g/100 µL (1.9 µg/µL) | $1.03^{C,c}$ | 0.04243 |
| | Negative control | 1.08 | 0.04101 |
| Absorbance - 48h | MTA Angelus® 0.28 g/50 µL (5.6 µg/µL) | $0.959^{A}$ | 0.00566 |
| | MTA Flow® 0.19 g/50 µL (3.8 µg/µL) | $0.649^{B}$ | 0.04101 |
| | MTA Flow® 0.26 g/100 µL (2.6 µg/µL) | 0.863 | 0.14213 |
| | MTA Flow® 0.19 g/100 µL (1.9 µg/µL) | $0.649^{C}$ | 0.19658 |
| | Negative control | 1.061 | 0.02828 |

$^{(A, B, C)}$ Same capital letters indicate statistically difference between exposure times. Repeated Measures ANOVA, Tukey Post-Hoc Test, $p < 0.05$. $^{(a, b, c)}$ Same small letters indicate statistically difference between cements. One-Way-ANOVA and Tukey Post-Hoc Test, $p < 0.05$.

There was a significant increase of antimicrobial activity for both MTA Flow® and MTA Angelus® at 48 hours of exposure ($p < 0.05$). Thus, for *S. aureus*, results show that the higher contact time of bacteria and cements, the higher bactericidal activity ($p < 0.05$).

**Discussion**

Antimicrobial properties and appropriate consistency are two desirable characteristics of endodontic cements, since the presence of residual bacteria, caused by inadequate chemical-mechanical preparation and sealing are the main causes of failure in endodontic treatment [5].
The antibacterial activities of MTA Flow® and MTA Angelus® were evaluated by ADT and DCT, the most used methodologies for assessing the antimicrobial activity of endodontic cements. ADT depends on the solubility and physical properties of the cements; thus, it seems to be more suitable for soluble materials [5, 12]. On the other hand, DCT qualitatively assesses antibacterial activity, even for materials that show low solubility, since microorganisms and the material that is being studied remain in direct contact [1].
In this study, the antimicrobial action of cements was evaluated against *S. aureus* and *E. faecalis*, both present in oral microbiota and associated with the failure of endodontic therapy [13]. *S. aureus* is a gram-positive, aerobic or anaerobic facultative bacterium, normally present on the skin and nasal passages of healthy people. However, it may cause diseases due to direct tissue invasion, primary or secondary bacteremia or, exclusively, by production of toxins [6].
*E. faecalis* is a gram-positive, optional anaerobic bacterium. Several virulence factors of this pathogen contribute to its survival inside root canal system, even after endodontic therapy [14]. *E. faecalis* has the ability to penetrate dentinal tubules, adhere to collagen and withstand long periods without access to nutrients [2].
In fact, previous tests revealed the resistance of *E. faecalis* to different endodontic cements, by ADT methodology [15]. Miyagak *et al* [16]. found that MTA and four other cements used in endodontics, did not exert antimicrobial activity on *E. faecalis*. Another study adds that MTA Angelus®, and other materials evaluated, were also unable to exert an antimicrobial effect on *E. faecalis* [17]. These results are similar to the present study,

which demonstrated that MTA Angelus® and MTA Flow®, even in different concentrations, were not able to inhibit *E. faecalis* growth in ADT (Figure 1).

The antimicrobial inefficiency of some cements containing calcium hydroxide may be related to the low solubility and diffusion capacity of these substances. Previous study demonstrated the inefficiency of endodontic cements containing calcium hydroxide in inhibiting some facultative anaerobic and aerobic bacteria, such as *E. faecalis* [13].

Until now, few studies have analyzed the antimicrobial activity of MTA Flow®. The low solubility of substances can impact the inhibitory effect of microorganisms´ growth, mainly by ADT. Thus, in this study, ADT showed no antimicrobial effect of cements against *E. faecalis.* For *S. aureus,* the ADT showed significantly less antimicrobial activity of MTA Flow®´s lower concentration (1.9 µg/µL) in relation to the other analyzed concentrations and to MTA Angelus®, after 24 and 48 hours ($p < 0.05$). These results showed the low efficiency of MTA in inhibiting microorganism by ADT, especially in low concentration.

For DCT, this study was based on sample's absorbance by spectrophotometric analysis. For *E. faecalis*, results demonstrate that inhibition of bacteria growth was not statically significant for both MTA Angelus® and MTA Flow® (Table 1). On the other hand, *S. aureus* growth was significantly reduced at 48 hours of direct contact with cements (Table 2).

The antimicrobial effect of calcium hydroxide-based cements is related to the release of hydroxyl ions, which raise medium´s pH. A pH greater than 9.0 can inactivate enzymes in microorganisms´ cell membrane, resulting in loss of biological activity or membrane integrity [13].

Results show that the inhibition of bacteria growth was intensified with a longer exposure time to cements. In DCT, statistical analyses (Repeated Measures ANOVA with Tukey Post-Hoc Test, $p<0.05$) comparing exposure time of *S. aureus* to both MTA Angelus® and MTA Flow® showed that the higher contact time the higher bactericidal activity. Similar results observed by Leonardo *et al* [18]. indicate that the antimicrobial activity is proportionally greater to the increase of contact period of endodontic sealers and microorganisms.

As observed in this study, previous research [15]. showed greater efficiency in the evaluation of antimicrobial activity of low soluble cements, such as MTA, by DCT than ADT.

## Conclusion

Thus, within the limitations of this research, results indicate that cements´ antimicrobial activity depends on the vulnerability of the microorganisms. Neither MTA Angelus® nor MTA Flow® showed to be effective against *E. faecalis* in both, ADT and DCT. *S. aureus* was susceptibly to MTA Angelus® and MTA Flow®. Nevertheless, in DCT all concentrations of MTA Flow® showed superior antimicrobial activity when compared to MTA Angelus® at 24 hours. Moreover, antimicrobial activity of MTA Flow® has increased over time, what suggests that this property seems to be influenced by the contact time between cements and microorganisms.

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