Antimicrobial activity of calcium silicate-based sealer containing antibiotics, tested by means of radial diffusion and minimum inhibitory concentration

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DOI: http://dx.doi.org/10.14436/2358-2545.7.1.060-065.oar

ABSTRACT

Introduction: Replacement of Portland cement by MTA has been recommended in clinical practice due to similarity between their composition, as well as mechanical and biological properties. Adding antibiotics to the cement would provide clinical advantages, provided the antibiotic drug be released before and after the setting of cement. **Objective:** The aim of this study was to assess antimicrobial activity by diffusion and minimum inhibitory concentration (MIC) of pure Portland cement and Portland plus radiopacifier, with addition of amoxicillin and ciprofloxacin, relative to five microorganisms: *S. aureus*, *P. aeruginosa*, *E. coli*, *E. faecalis* and *C. albicans*. **Methods:** For the diffusion test, 30 Petri dishes with Muller-Hinton agar were used, six of which had wells where cements were introduced immediately after manipulation and after a 24-hour setting. For MIC testing, 120 test tubes containing 10ml of brain heart infusion (BHI) broth were prepared, and divided into four groups. Each group was cultured with a fresh inoculum of each organism separately. Tubes were incubated at 37°C and turbidity was measured after 24h. Results were assessed by ANOVA analysis of variance and comparison of groups carried out by Tukey's test, with 5% significance. **Results:** Portland cement showed antimicrobial activity only against *C. albicans*. Amoxicillin showed a slight antibacterial effect, while ciprofloxacin inhibited bacterial growth at all concentrations tested. **Conclusion:** Addition of ciprofloxacin to Portland cement in order to enhance antimicrobial activity appears to be safe; however, results do not support such clinical application, thus requiring further studies to be performed.

Keywords: Root canal treatment. Local antibiotics. Antimicrobial activity tests. Calcium silicate-based sealer.

How to cite: Rocha Júnior HV, Giraldi Neto FO, Freitas RP, Duarte MAH, Silva GF, Weckwerth PH. Antimicrobial activity of calcium silicate-based sealer containing antibiotics, tested by means of radial diffusion and minimum inhibitory concentration. Dental Press Endod. 2017 Jan-Apr;7(1):60-5.
DOI: http://dx.doi.org/10.14436/2358-2545.7.1.060-065.oar

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» The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

Submitted: October 18, 2016. Revised and accepted: January 17, 2017.

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Introduction

When conventional endodontic therapy fails and retreatment is not indicated, one of the solutions to achieve endodontic success and solve the periapical pathological process is paraendodontic surgery through apicectomy associated with retrograde filling.[1]

Thus, the elimination of microorganism from the root canal system becomes an important goal in endodontic therapy, being of paramount importance to place intracanal drug acting against microorganisms. Consequently, the use of a retrograde filling material with some antimicrobial activity would be considered beneficial in the attempt to control the number of microorganisms in the root canal and provide eradication of infections.[2]

Replacement of MTA by Portland cement as a root canal filling material, especially for retrograde filling, has been suggested by some authors. It has become increasingly popular, since the composition of both types of material is very similar, differing mainly in the existence of bismuth oxide in MTA composition, which is a radiopacifier agent.[3-7]

Adding antibiotics to cements proves to be practical and makes a lot of sense.[8] The first reported use of cements containing antibiotics was carried out by Buchholz and Engelbrecht in 1970. Such procedures aim at administration of high doses of low systemic influence antibiotics. The technique success may rely on the antibiotics of choice, which must be guided by susceptibility of pathogens in the region.[9]

The in vitro antimicrobial activity of five antibiotics (amoxicillin, penicillin, clindamycin, metronidazole and doxycycline) added to Kerr EWT endodontic cement, against E. faecalis, was assessed by the agar diffusion method. Results demonstrated the combination of cement-antibiotics containing amoxicillin, penicillin, clindamycin, and doxycycline presented significant difference in mean inhibition zones, when compared to pure Kerr EWT cement.

Therefore, adding antibiotics to cements is effective for prevention and control of infections.[3] This study aimed at investigating the antimicrobial activity of Portland cement, with and without a radiopacifier (zirconium oxide). Additionally, it also aimed at assessing whether adding antibiotics would enhance antimicrobial performance against the tested microorganisms, both pre- and post-setting, as well as assessing the minimum inhibitory concentration of an antibiotic in order to find the best recommended dosage.

MATERIAL AND METHODS

Radial diffusion analysis

Five ATCC strains (American Type Culture Collection) were used in this experiment: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC 10231).

Tests were carried out with Portland cement (Votoran White cement – Votorantim Group, Rio de Janeiro, RJ, Brazil)as follows: pure Portland cement, Portland cement with addition of 50% zirconium oxide (Sigma-Aldrich, São Paulo, SP, Brazil), Portland cement with addition of 10% amoxicillin (Pharmanostra, Rio de Janeiro, RJ, Brazil) weight, pure Portland cement with addition of 10% ciprofloxacin (Pharmanostra, Rio de Janeiro, RJ, Brazil), Portland cement with addition of 50% zirconium oxide and 10% amoxicillin, Portland cement with addition of 50% zirconium oxide and 10% ciprofloxacin. The cements were tested after a 24-hour setting.

A total of 30 Petri dishes were prepared with Müller-Hinton agar, as recommended by NCCLS (National Committee for Clinical Laboratory Standards).[11] Wells with 6-mm diameter were dug in the dishes, as described by Ostrosky et al.[12] Microorganisms were cultured as advocated by NCCLS[11] with 1.5 x 108UFC mL-1 dilution. The wells were filled with a mixture of cement manipulated in a 1g/0.26mL powder/liquid ratio, according to Bortoluzzi et al.[13] In the post-setting test, the cements were handled as previously described, placed in sterile Petri dishes, without a culture medium, and incubated at 37°C for 24 hours. Subsequently, the cement was ground with mortar and a pestle, manipulated in the aforementioned powder/liquid ratio, and inserted into the wells.

After filling the wells, dishes were pre-incubated at room temperature for two hours and then incubated at 37°C for 18 hours, as determined by NCCLS.[11]

The dishes were measured with the aid of a digital caliper in order to measure the inhibition zones.

Results were recorded according to the mean value of the three experiments carried out for each microorganism. ANOVA was applied for analysis of variances, whereas Tukey's test was carried out for a group-to-group comparison, with a 5% significance level.

Minimum inhibitory concentration (MIC) analysis

The minimum inhibitory concentration (MIC) against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Enterococcus faecalis* was determined by means of the tube dilution test, according to, NCCLS.[11]

A total of 120 test tubes containing 10ml of brain heart infusion (BHI) were prepared and divided into four groups. Each group was cultured with 100 microliters of each studied microorganism. The cement combinations in this experiment were the same as those used for the diffusion test. After setting, the final product was ground with mortar and a pestle in order to obtain a fine and homogeneous powder.

The powder obtained by grounding was inserted directly into the tubes of each subgroup, which were mechanically agitated, in order to achieve concentrations of 50 (5mg/ml), 25 (2.5mg/ml), 12.5 (1.25mg/ml), 6.25 (0.625mg/ml), and 3.125 (0.313mg/ml) g/10mL for all groups. The microorganism in the culture medium without cement was used as positive control, whereas the culture medium with cement, but without the microorganism, was used as negative control.

All tubes were incubated at 37°C, and turbidity was assessed after a period of 24 hours. MIC was considered the highest dilution with bacterial growth inhibition. The presence or absence of turbidity was assessed and recorded for each group. Subsequently, 1-ml samples of tubes where turbidity was absent were subcultured on agar plates. The samples that did not show growth on plates were considered the minimum bactericidal concentration (MBC).

RESULTS

Radial diffusion

Table 1 shows mean values of inhibition zones (mm) achieved by the radial diffusion test in the assessed dishes.

Minimum inhibitory concentration (MIC)

After 24-hour incubation period at 37°C, all tubes from pure Portland and Portland 50% groups were turbid, thus revealing microbial growth.

In Portland + amoxicillin and Portland 50% + amoxicillin subgroups, both MIC and MBC were greater than 5 mg/ml for all bacteria.

In Portland + ciprofloxacin and Portland 50% + ciprofloxacin subgroups, both MIC and MBC were lower than 0.313mg/ml for all bacteria. Bacterial growth occurred in all positive controls. In negative controls, no bacterial growth was observed, which excluded the potential for experimental contamination.

Table 1. Mean value of zone size (mm) achieved by diffusion test.

	E. coli		*P. aeruginosa*		*E. faecalis*		*S. aureus*		*C. albicans*	
Pure Portland	0	0	0	0	0	0	0	0	20	14
Pure Portland+ Ciprofloxacin	48	45	50	48	38	38	53	48	20	15
Pure Portlan d+Amoxicillin	37	33	0	0	45	0	65	35	20	15
Portland 50%	0	0	0	0	0	0	0	0	20	15
Portland 50% + Ciprofloxacin	45	45	50	48	38	38	53	47	20	15
Portland 50% + Amoxicillin	35	28	0	0	44	0	65	17	20	14
	Pre-setting	Post-setting	Pre-setting	Post-setting	Pre-setting	Post-setting	Pre-setting	Post-setting	Pre-setting	Post-setting

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in µg/ml of tested material for all analyzed bacteria.

	E. coli		*P. aeruginosa*		*E. faecalis*		*S. aureus*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CSBC	> 5	> 5	> 5	> 5	> 5	> 5	> 5	> 5
CSBCA	> 5	> 5	> 5	> 5	> 5	> 5	> 5	> 5
CSBCC	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313
CSBC50%	> 5	> 5	> 5	> 5	> 5	> 5	> 5	> 5
CSBC50%A	> 5	> 5	> 5	> 5	> 5	> 5	> 5	> 5
CSBC50%C	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313	< 0,313

CBCS: calcium silicate-based sealer
CBSCA: calcium silicate-based sealer + amoxicillin
CBSCC: calcium silicate-based sealer + ciprofloxacin
CBCS50%: calcium silicate-based sealer + 50% radiopacifier
CBCS50%A: calcium silicate-based sealer + 50% radiopacifier + amoxicillin
CBCS50%C: calcium silicate-based sealer + 50%

Discussion

Microorganisms assessed in the present study are pathogens involved with endodontic infection or associated with therapy-resistant cases. Despite the fact that aerobic and facultative aerobic microorganisms are usually the smallest constituents of primary infections, they are more often found in cases of which treatment is longer, in cases of flare-ups and in cases of treatment failure.

E. faecalis is the microorganism most often found in refractory periapical periodontitis, and it is used in many studies on antibacterial properties of disinfectant agents due to being resistant to some medications and having the ability to survive conventional endodontic therapy.[2]

Previous studies have shown that under aerobic atmosphere, MTA (Mineral Trioxide Aggreagte) may produce reactive oxygen species (ROS), with antimicrobial activity. However, under anaerobic atmosphere, a decrease of ROS was observed. The oxygen-rich medium probably favors the antimicrobial activity of MTA.[14]

In a study carried out with *E. coli* and *E. faecalis*, Ribeiro et al[15] showed that E. faecalis was susceptible to GMTA (grey MTA) as much as to calcium hydroxide after incubation under aerobic atmospheric conditions. Nevertheless, strains of *E. coli* were resistant to GMTA and susceptible to calcium hydroxide when incubated under the same conditions. This fact can be explained by studies which demonstrate that pure *E. coli* strains are relatively resistant to ROS. Such a fact is confirmed by the study by Ribeiro et al,[14] which showed it was possible to observe inhibition zones under aerobic conditions promoted by cements studied in two mutant strains of *E. coli*. However, there was no inhibition in wild strains.

It has been shown that colonization by fungi resulting in root diseases is associated with root canal treatment failure. The most commonly isolated fungi is *C. albicans*. This microorganism has been detected in approximately 20% of infected root canals by means of 18S rRNA primer of direct species. *C. albicans* showed an ability to colonize root canal walls and penetrate into the dentinal tubules.[16]

Hendriks et al[8] claimed that antibiotics released by bone cements follow a typically biphasic model.[9] Initially, there is a release peak which is followed by a long period of low release which continues for days and even months. Based on this assumption, Portland cement was tested with and without addition of antibiotics pre- and post-setting. Adding a second antibiotic may also lead to an increase in the release of both antibiotics, which can be convenient when the most appropriate treatment requires synergism between drugs.

Pelletier et al[17] demonstrated that if a high dose of antibiotics is required to eradicate infection, it must be observed that when the proportion of antibiotic in the cement is over 12% by weight, it may present a performance below minimum in required mechanical properties. In their study, one of the cements to which antibiotics were added in the amount of 13% by weight, it was below 70MPa recommended by ADA specifications, with 67.8 + 6.9MPa. The authors further state that high doses of antibiotics incorporated to cements may lead to systemic complications, such as nephrotoxicity, ototoxicity and hepatitis. For those reasons, we can conclude that loading a given cement with antibiotics up to the proportion of 10% by weight does not lead to significant decrease in its mechanical properties.

There is a wide divergence of information in the literature regarding Portland antimicrobial activity. The antimicrobial activity is known to be an extremely desirable property in retrograde filling endodontic material. The literature shows the possibility of increasing antibacterial action of endodontic cements through addition of antibiotics; however, such a possibility has never been extended to Portland cement, a retrograde filling material.

The results of this study showed that Portland cement, with and without zirconium oxide, did not present neither bacterial nor inhibitory effects after setting, regardless of concentration.

Cement with addition of amoxicillin presented poor results, thus denoting bacterial antibiotic resistance. Those results can be extended to the clinical practice, in which amoxicillin is often prescribed in order fight microorganisms resistant to it.

Cements with addition of ciprofloxacin inhibited bacterial growth in all groups and concentrations, thus suggesting low bacterial resistance.

Conclusion

In the present study, we concluded that it is possible to add antibiotics to Portland cement with a view to increasing its antibacterial effect. This is because our results demonstrate that even after setting, antiobiotics keep their antimicrobial action, and this effect is directly related to antibiotic concentration, its action spectrum, and the susceptibility of microorganisms.

Amoxicillin presented a tenuous antibacterial effect for microorganisms tested herein. Ciprofloxacin showed better performance, inhibiting bacterial growth at all concentrations tested.

Therefore, it seems safe to recommend addition of ciprofloxacin to Portland cement with a view to increasing the antimicrobial activity of the cement. As a precaution, we suggest that the physicochemical properties of cements tested in this study be assessed. The results presented are encouraging, but do not yet support their clinical application.

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