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Antimicrobial activity of common endodontic materials on *Enterococcus faecalis NEWP 0012*

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Abstract**—** *The elimination of the microorganisms in its totality, during the endodontic therapy, although desired, is hard to be achieved. Even after the canal filling, the microorganisms are able to be into the dentinal mass and into the periapical region. This essay aims to evaluate the antimicrobial activity of endodontic materials against to the Enterecoccus faecalis NEWP 0012. The inoculation of this microorganism in the glass plates was done using swabs pressing the cotton against the wall of the pipe. The bacterial suspension was sowed evenly on the sterile surface of the Ágar MH. The plates were incubated by aerobic conditions, in a constant temperature in the range of 35º a 37°c, for 24 hours. The endodontic materials tested were: Sealer 26, Endofill, AH plus, MTA Filapex, calcium hydroxide paste associated to the chlorhexidine, Maxxion R glass ionomer, MTA and MTA HP. After material manipulation, they were impregnated into absorbent paper discs of 5mm of diameter and distributed sporadically on the plates that contained the microorganism. The experimental data were obtained by the measurement of the inhibition halo in the period of 24h, 72h, 7, 15 and 21 days; thus, the microorganism was classified as resistant or sensible to the different products. From the results, it was possible to verify that the calcium hydroxide paste associated to the chlorhexidine presented superior antimicrobial activity on Enterecoccus faecalis NEWP 0012. It was concluded that the calcium hydroxide paste associated to the chlorhexidine has satisfactory antimicrobial activity against the Enterecoccus faecalis NEWP 0012 in the period between 24h to 21 days.*

Keywords**—** ***Calcium hydroxide, Endodontics, Odontology.***

I. INTRODUCTION

The most common cause of the pulp commitment is the infection by microorganisms due to the dental caries. Even the pulp necrosis that occurs by aseptically reasons, sooner or later, it is infected. The necrotic pulp tissue is very easily infected because the cells and the molecules of the host immune system does not function in necrotic tissue conditions and the apical periodontium cells and molecules cannot reach the inner necrotic root zone [1,16,19].

Many experiences show that the dental pulp can recover itself of an acute inflammation, this is directly dependent of the quantity of microorganisms that will be invading the pulp. In conventional rats and *germ-free* the pulp alterations after the exposition in a mouth area are variable. In the *germ-free* there is formation of dentin bridges and absence of inflammation, although they present necrosis in the exposal area surface. Sometimes the necrosis occurs in the pulp near the exposition to the mouth area and this is considered as caused by the accumulation or impaction of foods. In the conventional rats, after two days there is the presence of an infiltrated of neutrophils in the high layer and beginning of necrosis.

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After seven days the pulp necrosis is more extensive, with periradicular inflammation [13].

The infecting microbiota has the capability of organize itself in a biofilm, changing the own metabolic ways surviving to nutrients scarcity periods or less improper conditions to the development. This association in biofilm do that these microorganisms synthesize and excrete, in the run of the metabolic process, substances with antigenic action that trigger off the host inflammation, that traduce itself sometimes in chronicle clinical manifestations, sometimes in acute manifestations. The organization of the microorganisms in biofilm also offers its way of protection against aggressions and, in this way, the elimination of organized microorganisms in biofilm constitutes a much more complex objective [3,17].

A fundamental stage in the endodontic treatment is the root canals system disinfection. This stage is accomplished by the employ of cut instruments that lengthen the root canal aiming, by the use of an auxiliary irrigator solution, to the removal of the microorganisms that colonized the root canal. As the instruments frequently are not able to cut all the walls of the root canal, the not-touched areas can stay colonized, mainly if there is the presence of biofilm [1-4,16].

In this context the auxiliary irrigation solution takes a fundamental role to reach regions where the endodontic instrument is not able to reach. Therefore, this stage of the endodontic treatment is defined as chemical-mechanical prepare, because it demands the employ of instruments with mechanical action (cut) associated to the employ of an irrigator solution with effective antimicrobial action [10-16].

Many chemical substances have been proposed with the purpose of to provide an effective irrigator solution, but it was not developed still a substance that get together all the desirable qualities. Between the various desirable characteristics to an ideal irrigator solution, its antimicrobial efficiency against bacterial biofilm seems to constitute a fundamental point. In the aid to the combat to the microorganisms to the intracanal medications, endodontic cements and biomaterial can be used [8,12,16,19].

It is observed that the antimicrobial capability of the calcium hydroxide and of the chlorhexidine digluconate is complementary, as the other properties (the virtue of one complement the fails of the other). By this reason, the association of these two substances in the manipulation of the intracanal medication using between sessions is instigator and seems plausible that an addictive or synergic antimicrobial effect can results of this association [5]. In fact, the combination of the calcium hydroxide with the chlorhexidine digluconate in the manipulation of intracanal medications used between seeing being sessions, since a short time, widely analyzed and defended, mainly in cases of persistent apical periodontitis associated to pulp necrosis or to failed endodontic fillings, even with the limited availability of information respect to this association [5,12,19]. The combination of the calcium hydroxide with the chlorhexidine in 2,0% is, at least, as effective as the calcium hydroxide vehiculed in sterile deionized water in relation to the disinfection ("in vivo") of the root canal dentine of teeth with failed endodontic filling. The authors reported that, in fact, the combination of the two active principles presented better results, however, the reduced sample led to the non-significant statistic difference between the two groups [21].

With the advent of this material, this study aims to evaluate the antimicrobial activity of endodontic materials by the diffusion tests in agar and by direct contact. Thus, the objective of this essay was to evaluate the antimicrobial activity of endodontic material to the *Enterecoccus faecalis NEWP 0012*.

II. MATERIAL AND METHODS

It was used reference bacterial strains, used as standard for quality control at Microbiology laboratories and susceptibility tests to antimicrobials, commercialized by NewProv. Each microorganism was stabilized in freeze-dried discs in a concentration above 100.000 UFC/mL. It was used the *Enterococcus faecalis NEWP 0012*.

The discs that contained the microorganisms were revitalized with a flambé and cooled tweezers, the discs were aseptically removed of their original bottles and put into 3 mL of BHI nutritive broth (Brain Heart Infusion). The pipes were identified and incubated in 35°± 2° until the visible turbidity (2 – 3 hours). After, with aid of a 100-microliter calibrated handle, it was inoculated the microorganisms in a non-selective medium plate (Blood agar) by the technique of exhaustion and again they were incubated in 35°± 2° by 24 hours.

It was used 5 different types of endodontic materials commercially acquired by the formulation of gel or solution or freeze-dried: G1 – white MTA Angelus®, G2 - MTA Angelus® HP, G3 - Filapex Angelus® cement, G4 - AH plus cement, G5 – chlorhexidine gel 2% and sodium hydroxide (Farmácia Formula &Ação SP- Brazil). The manipulation of the white MTA Angelus®, of the MTA Angelus® HP, of the Fillapex Angelus® cement and AH plus was accomplished accord to the fabricant instructions. The paste manipulation, gel 2% and sodium hydroxide was accomplished in the 1/1 proportion. after the manipulation of these materials, they were impregnated into absorbent paper discs (Figure 01).

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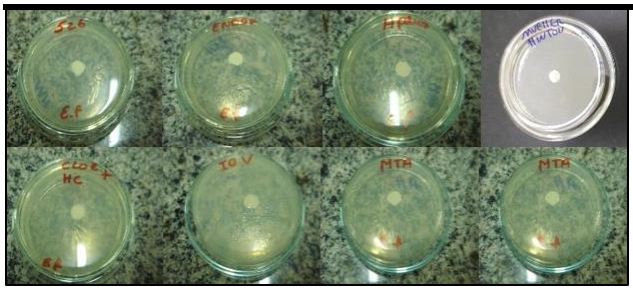


Fig.1: Absorbent paper discs soaked in the endodontic materials in test applied on the petri plates.

2.Test of antimicrobial sensibility in vitro – antibiogram (Diffusion method with Bawer and Kirby discs).

The preparation of plates and growth mediums: the Mueller-Hinton Agar (MH) medium was prepared previously fused, sterilized and cooled in 45-50°C. Then, it should be spilled in petri plate of 150mm of diameter until reach a thickness of about 4mm. In the sequence, it was accomplished the preparation of the inoculums and the pure culture of bacteria (NEWPROV) were cultivated in nutritive broth in 37°C by about 12 hours after the revitalization, enough time that the bacterial suspension presents moderated turbidity. The density of the inoculums from then on, was controlled by dilution with saline to obtain a turbidity density equivalent to that obtained by the addition of 0,5mL of $BaCL_2$-$2H_2O$ (0,048M) solution in 99,5mL of H_2SO_4 0,36N – McFarland Escale.

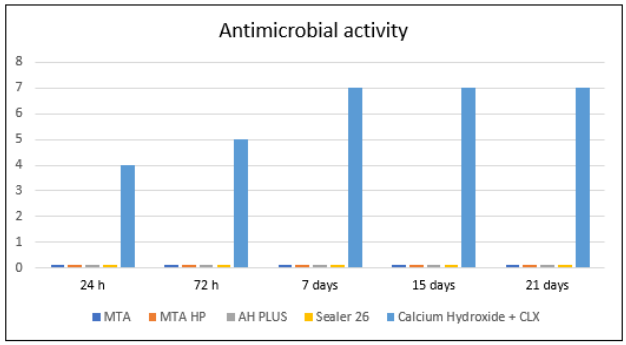
For the inoculation of the plates, cotton swabs were submerged into the bacterial suspension and the excess was removed pressing the cotton against the pipe wall. The bacterial suspension was sowed evenly on the sterile surface of the MH Agar and the discs of antibiotics or of endodontic materials will be sporadically distributed on the inoculums. The antibiogram plates were incubated by aerobic conditions, in a constant temperature in the range of 35-37ºC, for 24 hours and/or 7 days.

III. RESULTS

The obtained results about the antimicrobial activity of the endodontic materials, was measured although millimeter rulers. The inhibition halos were measured and compared in the first 24 hours, and again after 72 hours, they were been evaluated in until 21 days, in three different tests. The *Enterococcus faecalis* demonstrated that it is resistant to the most part of the materials, that did not present any bacterial inhibition halo, and they are: white MTA Angelus®, MTA Angelus® HP, Filapex Angelus® cement, sealer 26, AH Plus® and Maxxion R. in the first test was observed inhibition halo in the materials: chlorhexidine gel 2% and calcium hydroxide (Table 1 and Graphic 1). They were the two-last observed in until 14 days, from this there were decrease of the antimicrobial action. However, the calcium hydroxide and chlorhexidine gel 2% were effective in all tests in until 21 measured days (Figure 2).

Table.1: Inhibition halos of the materials according to the tested time.

Experimental groups	7 days	15 days	21 days
Sealer 26	Absent	Absent	Absent
Endofill	Absent	Absent	Absent
AH Plus	Absent	Absent	Absent
MTA Fillapex	Absent	Absent	Absent
Calcium Hydroxide + Chlorexidine	Present	Present	Present
Ionômero de vidro Maxxion R	Absent	Absent	Absent
MTA	Absent	Absent	Absent
MTA HP	Absent	Absent	Absent



Graphic.1: Antimicrobial action of the substances over the days under test.

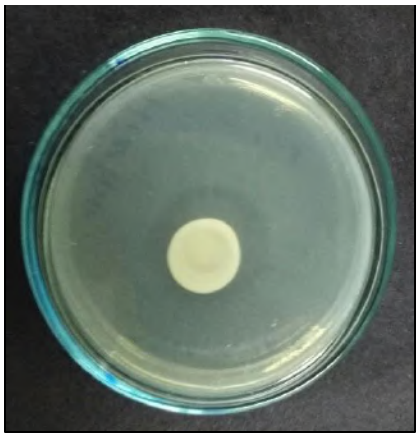


Fig.2: Calcium Hydroxide Paste + Chlorhexidine (Inhibition halo).

IV. DISCUSSION

The chemical-mechanical prepare reduce a big part of the microbiota of the infected root canals [1,12,16,22], however, other authors emphasized the need of the use of

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intracanal measure with the purpose to avoid that the survivor bacteria to the chemical-mechanical prepare, in a enough number and in a favorable environment, multiply themselves in the interval between the treatment sessions [3,25]. Therefore, it becomes evident the need to maintain the canal disinfection obtained by the chemical-mechanical prepare, and this is possible using a proper intracanal medication that presents antimicrobial properties and that functions as physical barrier, of an ideal filling of the root canal systems and of a proper crown sealing. Besides that, the intracanal medication has the intention to reduce the periradicular inflammation, to solubilize organic material, to neutralize toxic products, to control the persistent exudation, to control the inflammatory extern dental reabsorption and to stimulate the reparation by mineralized tissue. With this objective, some options of intracanal medications are been researched. Studies prove the efficiency of ones, more than others, which were present in the clinical routine. It was used the method of diffusion in agar, due to be considerate standard and of easy execution. Other study already used this methodology. The tested materials in this study were Sealer26®, AH Plus®, Fillapex Angelus®, white MTA Angelus®, MTA Angelus® HP, Restorative glass ionomer MaxxionR®, Calcium hydroxide with Chlorhexidine in gel 2% and Cotosol® [29]. But only the Calcium hydroxide with Chlorhexidine has real efficiency against the *EnterococcusFaecalis*, that was the chose microorganism to the accomplishment of the experiments, because it has a considerable resistance to auxiliary chemical substances and commonly used medications in Endodontic, besides being frequently associated to the presence of periradicular lesions and to the failure in the endodontic treatment. It is an anaerobic facultative microorganism, relatively easy to be cultivated and of high clinical relevance [4]. It can be commonly isolated of clinical samples, presents peculiar resistance to the calcium hydroxide, especially, to the elevated level of the pH that results of the dissociation of the hydroxyl ions that occurs in aqueous medium [1,21].

Because it is in powder form, the calcium hydroxide can be associated to other substances to be inserted into the root canal. Usually, the calcium hydroxide used in the endodontic practice is manipulated with saline that is water soluble and as they are an association, that have together chemical characteristics of dissociation, diffusibility and filling ability that are determinant to the biological behavior [13]. However, according to other studies, specific microorganisms, mainly *Enterococcus faecalis*, have been showed resistant to the Ca(OH)2 [6,10] and besides that, the antimicrobial efficiency of the pastes in a long term has been questioned [24]. In this way, researches as this have been developed adding active vehicles with antimicrobial properties associated to the calcium hydroxide increasing this activity, without losing its other characteristics [16].

Estrela et al. (1995) [13], discussed the mechanisms of action of the calcium hydroxide in the treatment of the endodontic infections. The authors highlighted that the calcium hydroxide effect is linked to the increase of the pH resultant of the dissociation, in aqueous medium, in hydroxyl ions and calcium ions. The calcium hydroxide actuates in the activation of the host tissue enzymes as the alkaline phosphatase that participates in the recovery of the mineralized tissues affected by the endodontic infection. In the bacteria, it actuates changing the integrity of essential sites promoting the inactivation of the cytoplasmic membrane enzymes interfering, in metabolic and homeostatic processes, growth and cell division. Therefore, the actions of the calcium hydroxide occur in two fronts, biological, favoring the defense mechanisms and the host affected tissues reparation, as also bacteriological, through the antimicrobial action. This therapeutic action makes that the calcium hydroxide prevails between the active principles of the intracanal medication, but, despite the particular efficiency in the action by direct contact in the main canal, its actuation in the pH into the dentinal tubules and in the cement, region is not so significant, demonstrating a deficiency.

The calcium hydroxide presents some limitations, as for example, a low solubility and low diffusibility, and action only for direct contact, that impedes the arrival of this substance to the hard to reach places in teeth that present anatomic variations, as isthmus, apical deltas, recesses, places where the bacteria are protected of the action of the intracanal medications. The solution of chlorhexidine digluconate 2%, when applied for 10 minutes, previously to the endodontic filling, can enter the dentinal tissue and to provide the antimicrobial action for more than 12 weeks, even if this capability is reduced in function of the time, becoming a great ally to the calcium hydroxide. The use of the chlorhexidine digluconate 2% as intracanal medication can be indicated in cases with primary infection. In retreats, the use of the chlorhexidine can be further important, using alternately as irrigator solution during the chemical-mechanical prepare or as intracanal medication between the sessions [32].

The activity of the chlorhexidine digluconate 0,2% in the reduction of the remaining antimicrobial population after the instrumentation of the canal as delay dressing was showed [8] due to its broad antimicrobial spectrum, the chlorhexidine has been widely used in the Endodontic. Its use has been proposed in form of digluconate salt, liquid or in gel, in different concentrations, as intracanal medication. A recent study

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evaluated the antimicrobial activity of six irrigator against anaerobic bacteria and reported that the chlorhexidine was the most effective. When used as intracanal medication, the chlorhexidine had a better effect than the calcium hydroxide in the elimination of *Enterococcus faecalis* into the dentinal tubules. It is the material that demonstrates most efficiency against this microorganism, in the tests accomplished by this essay [22].

Lenet et al. (2000) [18] compared, in vitro, the residual antimicrobial activity of the chlorhexidine gel 0,2% and 2%, in a system of controlled liberation, and of the calcium hydroxide associated to a saline solution, as intracanal medication, in bovine incisors, for 7 days. After the experimental period, the specimens were inoculated in *Enterococcus faecalis* for 21 days. The results showed that the chlorhexidine gel 2% had absence of viable bacteria in all the dentin depth. The chlorhexidine gel 2% presented most antimicrobial activity. The association of the calcium hydroxide with the chlorhexidine gel 2% decreased the antimicrobial activity of the chlorhexidine, however, potentialized the activity of the calcium hydroxide [30].

Studies demonstrates that the chlorhexidine presents more capability to eliminate the *Enterococcus faecalis* than the calcium hydroxide [31]. Nonetheless, other results were found to be unsatisfactory to chlorhexidine, mainly related to the inability to inactivate the LPS liberated by Gram-negatives bacteria. However, the calcium hydroxide can neutralize bacterial endotoxins, especially, the lipopolysaccharide (LPS) present in the cell wall of the Gram-negative bacteria through the lipid hydrolysis that composes part of the LPS molecule [27]. It highlights that the "Lipid A", that is responsible to the biological or antigenic action of the LPS, is hydrolyzed when is submitted to extremely high pH levels, as the generate by the calcium hydroxide in an aqueous medium [13]. The chlorhexidine digluconate does not change the pH of the medication with calcium hydroxide, it presumes that its antimicrobial action and the inactivation capability of the bacterial LPS is not altered [26,28]. Other effect of the association of the calcium hydroxide to the chlorhexidine is increase the antimicrobial capability of the calcium hydroxide, boosting its penetrability capacity into the dentinal tubules. Also, there was observed in this association, the fact that calcium hydroxide can actuate as physical barrier while the chlorhexidine, in function of its substantivity, maintains the canal free of microorganism [1,14,17-21,28].

Even the calcium hydroxide presenting low solubility in water (1,2 g/L to 25°C) and this limits its diffusibility, it is observed diffusion halos in agar to all the associations based in calcium hydroxide. However, it was verified the inefficiency of the calcium hydroxide itself against the *Enterococcus faecalis* as was observed by other researchers [1,28].

V. CONCLUSION

It concludes that the calcium hydroxide paste associated to the chlorhexidine possess satisfactory antimicrobial activity against *Enterecoccus faecalis NEWP 0012* in a period of 24h to 21 days.

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