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Editorial

BRAZILIAN ENDODONTIC JOURNAL

The way the Brazilian government is dealing with the universities in terms of providing sponsorship on research is an important issue to be considered. The editors would call their action " unfair play ". The reduction in the number of scholarships is only one of the many aspects of this situation. The governors try to justify their policies by the old argument of financial problems. Meanwhile, they provide facilities for the car industry that would disappoint alll federal university teachers, who have suffered more than a thousand days without any adjustment in the salary.

The government has also helped substantially a number of private banks and saved them from bankrupcy. At the same time, however, watches it passively the public universities becoming bankrupt. Many of our best teachers and researchers are retiring prematurely, frustrated with their current situation, looking for better prospects.

In general, private schools do not have the facilities to run post-graduate courses, which are usually expensive to mantain. The private car companies and banks keep alive, but the schools... Oh, the schools!...

We are inviting the readers of this journal to consider this topic as a matter of urgency. All we want from the government is "fair play ". The dignity of the Brazilian researcher and teacher must be improved.

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CONTENTS

CHEMICAL ANALYSIS OF CALCIUM CARBONATE PRESENT IN VARIOUS CALCIUM HYDROXIDE SAMPLES

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The objective of this study was to analyze chemically and quantitatively the presence of calcium carbonate in various samples of calcium hydroxide stored for 2 years. Determination of the concentration of carbonate ions was carried out by volumetric analysis of neutralization, using hydrochloric acid. Determination was made visually using methyl orange and phenolphthalein. The level of calcium hydroxide converted to calcium carbonate varied from 5 ± 1% to 11 ± 1%.

Key words: calcium hydroxide, calcium carbonate, intracanal dressing.

INTRODUCTION

The chemical and biologic actions of calcium hydroxide on tissue and bacteria have made this medication indispensable in Endodontics. The effect of calcium hydroxide on bacteria and on tissue is directly related to its ionic dissociation into calcium and hydroxyl ions. ESTRELA et al.[5] explained this action reporting that its high pH inhibits enzyme activities essential to bacterial life, i.e., metabolism, growth and cellular division. The effect of pH on the transport of nutrients and organic components through the cytoplasmic membrane determines its toxic action on bacteria. This also activates the hydrolytic enzyme alkaline phosphatase which is intimately related to the process of tissue mineralization. Thus, this medication presents two fundamental enzyme properties: the inhibition of bacterial enzymes leading to an antimicrobial effect and the activation of tissue enzymes such as alkaline phosphatase leading to a mineralizing effect.

Various studies have reported excellent antimicrobial properties in view of the different types of respiration of bacteria[2,3,6]. Other studies have shown the capacity of calcium hydroxide to hydrolyze bacterial lipopolysaccharides and promote degradation of the residual LPS[18,19]. HOLLAND et al.[11,12] reported its capacity of biological sealing by osteocementum deposit.

ESTRELA et al.[5], studying the effect of pH on the enzyme activity of anaerobic bacteria, reported that calcium hydroxide causes an irreversible (definitive) bacterial enzyme inactivation under extreme pH conditions and a reversible (temporary) inactivation with the return to the ideal pH for enzyme action.

The properties of calcium hydroxide can be neutralized in the presence of a weak oxide acid, such as carbon dioxide.

SELTZER and BENDER[17] tested calcium hydroxide, calcium carbonate, calcium chloride and calcium phosphate in exposed pulps to verify the formation of a mineralized tissue bridge. They reported that, with the exception of calcium hydroxide, the others were harmful to dental pulp.

MAISTO and CAPURRO[14] reported that prolonged contact of calcium hydroxide with atmospheric carbon dioxide or water can inactivate it by carbonatation. SELTZER and BENDER[17] reported that, for clinical use, this medication must be chemically pure, recently manufactured and not containing irritating additives. Prolonged storage causes calcium hydroxide to react with atmospheric carbon dioxide to form calcium carbonate, losing its effectiveness.

Considering that existing literature is lacking in research on the chemical analysis of the reaction of carbon dioxide with calcium hydroxide, the present study chemically analyzes the formation of calcium carbonate in samples obtained from private Endodontic clinics.

MATERIAL AND METHODS

Six samples of calcium hydroxide from private Endodontic clinics were analyzed in this study: Quimis (Mallinckradt Inc., USA), JT Baker (USA), Calen (SS White, RJ, Brazil), Vigodent (RJ, Brazil), Biodinâmica (PR, Brazil), Inodon (RS, Brazil). All samples were stored for a period of 2 years.

The concentration of carbonate ions was determined by volumetric analysis of neutralization with determination made visually using methyl orange and phenolphthalein1. The formation of calcium carbonate was analyzed according to ESTRELA[4].

One liter of solution was prepared with 1 gram of each sample of $Ca(OH)^2$, and 25.0 mL of this solution was titrated with hydrochloric acid (0.0109 mol. L-1) using phenolphthalein. Another 25.0 ml of the same acid was titrated with methyl orange. Cold solutions were used to avoid loss of CO^2. Each experiment was carried out 3 times for each sample.

Considering the volume of acid used in titration with phenolphthalein denoted as v, one notes that in this step all hydroxide was neutralized and the carbonate was converted to hydrogenated carbonate. Considering the volume of acid used in titration with methyl orange denoted as V, the complete neutralization of alkali is observed. Thus, subtracting the volume of acid used in the 2nd titration (V-v), one has the corresponding volume to neutralize half of all carbonate present. To neutralize all carbonate, multiply by 2, which corresponds to 2 (V-v), to calculate the carbonate. The number

of mols of H+ corresponds to double the number of mols of CO3-2. Considering that the number of mols (n) of a specie in solution can be calculated by multiplying the concentration in mol.L-1 (M) by volume, one has: Nco3-2 = 2.mH+.Vc. Because all of this carbonate is present in the 25.0 ml. aliquot taken from solution, the concentration in mol.L-1 (Cm) corresponds to Cm = 40.nCO3-2. Knowing that the standard acid concentration is 0.0109 mol.L-1, thus, Cm = 0.872.Vc.

In order to convert this concentration in g.L-1 (CgL), one must remember that the molecular weight of CaCO3 corresponds to 216.88 g. Thus, the concentration in g/L of carbonate can be calculated (CgL = 189.12. Vc), where the percent of calcium carbonate present in the samples can be obtained as shown in Table 1.

RESULTS AND DISCUSSION

The results showed that the percent of calcium hydroxide transformed into calcium carbonate was small, ranging from 5 ± 1% to 11 ± 1%, as shown in Table 1.

Despite the fact that, in this study, the dimensions of the containers, the number of times and time that the containers remained open and the storage conditions of the samples were not considered, one can confirm that calcium hydroxide in contact with atmospheric carbon dioxide does not change easily. MAISTO and CAPURRO[13] and SELTZER and BENDER[11] reported that prolonged contact of calcium hydroxide with atmospheric carbon dioxide leads to a loss of quality of the product, due to carbonatation. COHEN and LASFARGUES[3] observed in a quantitative chemical evaluation that calcium hydroxide, in either dry powder stored in a closed container or paste in screw syringes, was free from calcium carbonate even after several years. When samples were exposed to air, the rate of carbonatation was extremely slow. Carbonatation, according to these authors, in a period of 0-45 days in an open container, reached as high as 30%, while when stored in a closed container, in an opaque or transparent container and in supersaturated solutions with vehicles of saline, distilled water or an anesthetic without a vasoconstrictor, carbonatation was not greater than 1%. PETOT et al.[15] reported that supersaturation within a liquid was not necessary to avoid carbonatation.

With the objective of avoiding a reaction of calcium hydroxide with atmospheric carbon dioxide, MANFREDI[14], MAISTO and CAPURRO[13] and HOLLAND et al.[12,13] recommended storing calcium hydroxide in a hermetically sealed amber flask.

Estrela4 analyzed chemically the formation of calcium carbonate in calcium hydroxide pastes in vehicles with different acid-base characteristics (anesthetic solution, saline and polyethylene glycol 400) in dog connective tissue. The values in mass of calcium carbonate were small, with an increase for the 3 vehicles up to 30 days and stabilizing at 30 - 60 days. Thus, after the initial reaction of calcium hydroxide with the tissues, there are strong signs to reduce the number of medication changes in the intracanal routine, mainly after the initial inflammatory symptoms.

KONTAKIOTIS et al.[4] studied in vitro the indirect action of calcium hydroxide on the anaerobic flora of the root canal, particularly on obrigatory and isolated facultative anaerobic bacteria in infected root canals. One plaque with bacteria and another with calcium hydroxide were incubated anaerobically for 72 hours, forming an experimental group. Another plaque containing several bacterial species incubated the same way formed the control group. After 72 hours the number of bacteria was counted in each group. The number of bacteria in the control group was significantly greater with no specific resistance to calcium hydroxide detected. The results suggest that the capacity of calcium hydroxide to absorb carbon dioxide may contribute to its antimicrobial effect.

The results of this experiment showed that carbonatation of calcium hydroxide stored in containers under various conditions for a period of 2 years was small. Thus we can confirm that carbonatation of calcium hydroxide which occurs during storage and clinical use is not significant to interfere with its properties.

The velocity of ionic disassociation of calcium hydroxide, influenced by the vehicle added to the paste, characterizes the biologic and antimicrobial properties dependent on elevated pH.

Several studies evaluated the dentin diffusion of hydroxyl ions of calcium hydroxide and showed different pH values in the dentinary mass, which varied depending on the radicular third, the vehicle used in the paste, the experimental period observed, the method used, dentin permeability and degree of calcification[1,10,17,20]. However, the quality control of the calcium hydroxide used, with an elevated quantity of calcium carbonate, could also influence the results. Thus, for clinical use, products of high quality and of reputable origin must be chosen and stored under adequate conditions.

CONCLUSIONS

In all samples analyzed, the level of calcium hydroxide converted into calcium carbonate was small, ranging from 5 ± 1% to 11 ± 1% (Quimis, 5 ± 1%; JT Baker, 6 ± 1%; Calen, 7 ± 1%; Vigodent, 8 ± 1%; Biodinâmica, 9 ± 1%; Inodon, 11 ± 1%).

ACKNOWLEDGMENTS

We thank **CNPq** for financial support for this research (CNPq No. 523384/95-5).

Table 1. Calcium carbonate found in samples.

Products	$CaCO_3$
Quimis	5 ± 1%
JT Baker	6 ± 1%
Calen	7 ± 1%
Vigodent	8 ± 1%
Biodinâmica	9 ± 1%
Inodon	11 ± 1%

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INFLUENCE OF DIFFERENT KINDS OF ROSINS AND HYDROGENATED RESINS ON THE FLOW RATE AND FILM THICKNESS OF GROSSMAN CEMENTS

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In this study, the effect of addition of different kinds of rosin and hydrogenated resin on Grossman cement powder was evaluated over the physicochemical properties of flow rate and film thickness. The experiments were carried out following the American Dental Association specification number 57 for root canal sealers. For this analysis, different Grossman cement powders were prepared using different rosins (X, WW and WG) and hydrogenated resins (Staybelite and Staybelite ester 10). The film thickness study showed that different kinds of rosin and hydrogenated resins do not interfere in the results, and when the flow rate was evaluated, all the samples followed the ADA specifications. The cement obtained from the WG rosin had the greatest flow rate.

Key words: Grossman cements, root canal sealer, resin

INTRODUCTION

In Brazil, until the end of the 80's, the concepts of quality were little diffused and restricted to the industries. In the beginning of the 90's, the final consumer realized that these benefits could be extended to him as well, not being only for industrial products, but also applied to services as a general rule.

Added to this, the publication of the Consumer's Defense Code[5] caused controversy about its application to the Brazilian Dental Service (BDS), as well as other professionals. However, dental treatment is a service and should be included in the Code.

Based on behavioral change and demands of society, dentists began to be asked about the quality of treatment.

For a successful treatment, besides the technique employed, the quality of material is crucial. Thus, the researcher started to play a fundamental role by carrying out scientific research that could be used by the industries in order to produce goods satisfying technical, biological and aesthetic exigencies.

Following this philosophy, much has been researched in the field of Endodontics in order to produce an ideal root canal sealer considering physicochemical, biological and antimicrobian aspects.

The physicochemical properties of root canal sealers have been studied by the Endodontics Research Laboratory of the Ribeirão Preto Dental School, University of São Paulo, in order to evaluate the quality of the products available on the Brazilian market[1,2,3].

The investigation of these properties became standardized since the publication of the specification number 57 of the American Dental Association[4], in 1983, avoiding the problems caused by a lack of standardization of the tests carried out[3], making the research results reproducible and also making accurate comparisons between different materials and research results possible.

The role of each component of the Grossman cement powder on its physicochemical properties has been studied, as well as the effect of the addition of different vegetable oils to eugenol. It is necessary, however, to study the effects of different kinds of rosin and hydrogenated resins on these properties, since Grossman observed the effects of vegetable resins on the setting time. In this investigation, the flow rate and film thickness was studied.

MATERIAL AND METHODS

The root canal sealer proposed by Grossman is based on zinc-oxide and eugenol, as a powder and liquid stored in separated containers. This characteristic places it within specification number 57 of the ADA, which also states that every test must be carried out at 23 ± 2°C and 50 ± 5% relative humidity, which was observed in the experiments of this study. The materials tested were submitted to the ambient conditions 48 hours before the beginning of the tests.

The different kinds of rosins and hydrogenated resins studied are shown in Table 1.

Table 1. Rosins and hydrogenated resins tested, their commercial brands and manufacturers.

Name	*Manufacturer*	*Origin*
Rosin Brontes type X		Eucatex Brazil
Rosin type WG		Madeiteo Brazil
Rosin type WW		Coimbra Portugal
Stabylite ester 10		Hercules USA
Stabylite resin		Hercules USA

The different kinds of powders used in this experiment were dispensed at the Endodontics Research Laboratory of the Ribeirão Preto Dental School, University of São Paulo.

The powders were prepared according to Grossman's[6] specifications, only varying the kind of rosin or

hydrogenated resin used: 42%zinc oxide, 27% rosin or hydrogenated resin , 15% bismuth subcarbonate, 15% barium sulfate and 1% anhydrous sodium tetraborate.

All the chemical components used in the powder preparation were obtained in particle sizes that easily passed through a 100 sieve mesh. All the rosins and hydrogenated resins were pulverized and passed through mesh 60 and 100sieves, as recommended by Grossman[3].After mixing the components, the powder obtained was placed in a rotary mixer for 30 minutes, until the mixture was homogenous. The different powders were then packed in tightly closed plastic containers to avoid contact with air, identified and stored to be used in the physicochemical properties tests.

The powder/liquid relations for the modified cements and spatulation times were obtained as described by SOUSA NETO[13] and are shown in Table 2.

6.0 mm thick, were superinposed, with 2 strips of cellophane paper between them. The thickness of the set was determined by the load device. The cement was then manipulated and 0.5 ml placed on the glass surface, between the two strips of cellophane paper. The second glass surface was then placed over the material. After 180 ± 10 seconds from the beginning of the manipulation, a load of 15 Kgf was applied vertically on the upper glass surface. The cement spread over the glass surface and 10 minutes after the beginning of spatulation the thickness of the set was measured. The difference between the full set and the glass surfaces and cellophane papers alone is the thickness of the film formed by the cement. The arithmetic mean was calculated for 5 repetitions, approximating the results to the nearest 5 micrometers.

Table 2. Powder/liquid relations and spatulation times, in seconds, for the modified cements.

Cements obtained with the following rosins	Grames of powder / 0.20 ml of liquid	Mean values (in g)	Spatulation times (in s)	Mean values (s)
Staybelite ester 10	1.13 1.11 1.18 1.05 1.06	1.11	120 125 130 125 140	128
Staybelite	1.11 0.98 1.10 0.99 1.08	1.05	120 110 120 130 120	120
Kind X	0.92 0.94 0.96 0.93 0.98	0.95	120 110 110 120 120	116
Kind WG	0.90 0.92 0.89 0.94 0.85	0.90	120 120 140 130 120	126
Kind WW	0.80 0.75 0.78 0.82 0.82	0.80	140 120 135 125 115	126

Flow rate

A 3.0 ml Luer glass syringe was prepared, having its tip cut, to hold 0.5 ml of manipulated cement (Figure 1.1). Once the cement was manipulated and had achieved the ideal consistency, 0.5 ml was placed in the center of a clean 10x10 cm glass surface (Figure 1.2). After 180 ± 5 seconds from the beginning of spatulation, a device composed by a glass surface and additional load, which altogether weighed 120 g was carefully placed in the center of the material. (Figure 1.3). After 10 minutes from the beginning of spatulation, the additional weight was removed and the largest and smallest diameters of the disc produced were measured (Figure 1.4) with a digital caliper (TESA, Switzerland) (Figure 1.5).

Two conditions were necessary to validate the tests: the difference between the largest and the smallest diameters could not exceed 1.0 mm and the disc should be uniformly circular. If these conditions were not found, the test was repeated following the same experimental protocol. Five repetitions were carried out for each cement and the arithmetic mean was obtained.

Film thickness

A load device (MLW, Germany) was used to measure film thickness. Initially, two glass surfaces, 200 mm^2 and

RESULTS AND DISCUSSION

Flow test

The data obtained for the flow test, as well as the approximate values, are shown in TabThe data in Table 3 show that all cements tested met the conditions established

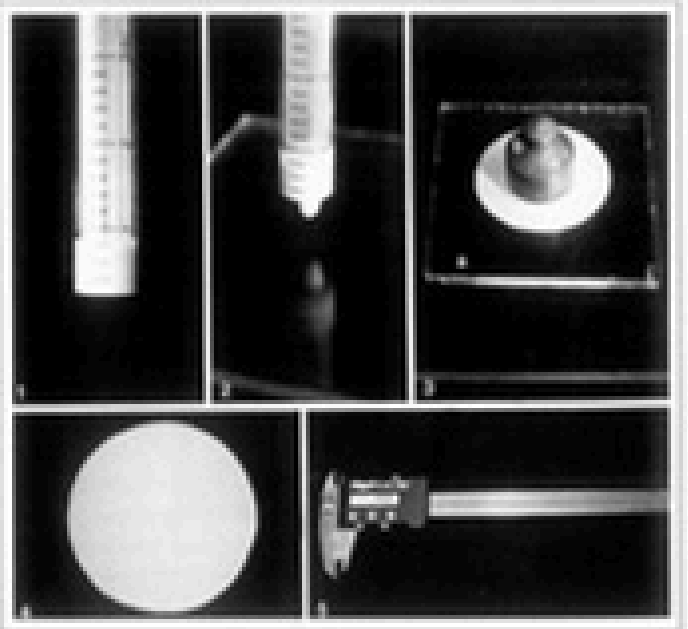

Figure 1. *Flow rate test sequence. 1) Luer syringe adjusted to hold 0.5 ml of cement. 2) Placement of cement on glass surface. 3) Device used in the flow rate test and spatulation time: a. upper glass surface. b. load. The mass of a+b is 120 g. 4) Circular disc obtained with the test. 5) Digital caliper.*

Table 3. Flow test of the studied cements (data in milimeters)

Cements obtained with the following resins and hydrogenated resins	Flow	Mean	Approximation
Staybelite ester 10	21.19 26.07 25.98 26.44 26.99	25.33	25
Staybelite	25.36 26.35 26.74 27.25 26.29	26.40	26
Rosin X type	37.29 36.37 37.75 35.83 37.35	36.92	37
Rosin WG type	41.28 40.18 39.36 39.59 37.98	39.68	40
Rosin WW type	36.96 33.84 36.02 36.99 35.83	35.93	36

by ADA for the flow test, which states that the disc formed by the cement must be at least 25 mm in diameter.

Analysis of the sample parameters showed a non-parametric distribution, which led to the application of the Kruskal-Wallis statistical test wich indicated a 1% significance for an H_0 probability of 0.03%. The sample average comparison was then made, and the results are presented in Table 4.

Table 4. Flow: Comparison between the sample average of the tested cements.

Compared samples (two x two)	Difference between the averages Significance (p<0.01)
Staybelite ester 10 X Staybelite	1.4000 ns
Staybelite ester 10 X Rosin X	12.1000 *
Staybelite ester 10 X Rosin WG	18.2000 *
Staybelite ester 10 X Rosin WW	9.3000 *
Staybelite X Rosin X	10.7000 *
Staybelite X Rosin WG	16.8000 *
Staybelite X Rosin WW	7.9000 *
Rosin X kind X Rosin WG	6.1000 *
Rosin X kind X Rosin WW	2.8000 ns
Rosin WG kind X Rosin WW	8.9000 *

Ns = non- significant

The cements obtained from the Staybelite ester 10 resin and Staybelite resin did not show statistical differences between them. However, when these two kinds of cements were compared with the cements obtained with rosin types X, WG and WW, there were statistical differences at the level of 1%. The cements obtained from the rosin types X, WG and WW did not show statistical differences between them in the flow rate test.

The flow rate of a cement is an important factor for the clinical performance of the material, since it interferes with the ability to penetrate into the small cavities of the dentin and lateral canals.

According to LARA[8], the flow rate can be determined in different ways: viscosity, penetrability, extensibility and extrusion. The method used in this study was the extensibility, which is defined by RICCI et al.[11] as being the average surface obtained when the cement is submitted to a constant load for a determined amount of time. This method refers to the capacity to transform any surface into a flat one when submitted to a load. It also refers also to the easy with which it spreads when traction is applied.

In 1982, GROSSMAN[6] showed that the addition of resin increased the plasticity of the cement, improving its flow rate. SAVIOLI[12] verified an increase in this property when rosin was added to the formula. The cements obtained from hydrogenated resins have a much lower flow rate than those prepared with rosin. In this study the WG rosin promoted the highest values in the flow rate test.

The results point to a tendency that the greater the amount of powder incorporated into the liquid, the lower will be the flow rate. This fact was also observed by ØRSTAVIK[9]. The literature shows that other factors influence the flow time of root canal sealers such as viscosity of the liquid[14] and size of the particles in the powder[1, 16]. In order to standardize the powder and the liquid in this study, the same eugenol was used and the particles passed through a 100 sieve mesh. Almost all cements are approved when submitted the flow rate test standardized by the ADA[10, 17].

Film thickness

The results obtained for the film thickness test are shown in table 5.

Table 5. Film thickness of the tested cements (data in micrometers).

Cements obtained with the following resins	Film thickness	Average	Approximation
Staybelite ester 10	46 49 48 47 46	47	50
Staybelite	49 48 48 47 47	48	50
Rosin type X	47 43 41 44 47	44	45
Rosin type WG	47 47 48 46 46	47	50
Rosin type WW	48 47 47 46 49	47	50

Average were approximated to the nearest 5 micrometers

By observing Table 5, it can be verified that all cements obtained from different kinds of rosins and hydrogenated resins showed a film thickness according to ADA specification 57 , with values equal to or lower than 50 micrometers.

The data was submitted to statistical analysis with a non-parametric distribution leading to the application of the Kruskal-Wallis statistical test which indicated an H_0 probability of 11.23%, showing no statistical significance between the samples tested.

The cement obtained from rosin type X had the smallest film thickness, with an average of 44 µm, and the cement obtained from the Staybelite resin showed the greatest film thickness (48 µm), but all met the ADA specifications.

SOUSA NETO[14] observed that the cement obtained from pure eugenol shows smaller film thickness than those obtained from eugenol mixed with other vegetable oils.

SAVIOLI[12] concluded that 42% zinc-oxide and 27% rosin is necessary to produce a cement having film thickness compatible with the ADA specifications.

WEISSMAN[16] and SILVA[13] verified that an increase in particle size in the powder increases film thickness.

We can observe in this experiment that when there was no difference in the liquid used[14] in the proportion of components of the powder formula[12] and in the size of the particles[13,16] , there were no statistical differences in the film thickness, i.e., different kinds of rosins and hydrogenated resins do not interfere in the film thickness.

CONCLUSIONS

1.The cements prepared with different kinds of rosins and hydrogenated resins have a flow rate that satisfied ADA specification 57.

1.1.The cements obtained from the hydrogenated resins showed a flow rate near the established limit.

1.2. The cements obtained from the rosin type WG showed greater flow rate than the cements prepared with rosin tapes X and WW.

2.The film thickness of the tested cements did not suffer any interference from the different kinds of rosin and hydrogenated resins used to prepare the formulas. All of the cements met ADA specification number 57.

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DENTAL RESTORATIVE MATERIAL BIOCOMPATIBILITY

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The aim of this work is to discuss the biocompatibility of the pulp-dentin complex protective materials. In recent years, Dentistry has observed the appearance of new restorative techniques and materials which deserve, serious, in-depth evaluation before releasing and recommending their use by clinicians. Dentin adhesive systems have been highlighted among these new formulas. Criteria to evaluate the results of a restorative treatment are represented by the physical integrity, absence of marginal infiltration and the biocompatible response of the dentin-pulp complex. Calcium hydroxide is an ideal material for pulpal exposures and very deep cavities due to its properties and intrinsic problems, followed by the preferred restorative system of the professional.

Key Words: Biocompatible materials, calcium hydroxide, dentin adhesive, pulp capping.

INTRODUCTION

In the past years, restorative dentistry has experienced outstanding advances not only in the field of new restorative techniques but especially in the development of new restorative materials and new bonding medium. For this reason, the concepts of pulpal protection and its application have been extensively modified. This chapter in Dentistry is the one that has caused the greatest debates, not only in Brazil but also internationally.

PULPAL PROTECTIVE MATERIALS

The tooth is composed of different tissues of different origins. The enamel, the hardest part of tooth, is almost completely made of calcium. It is the outer layer and the first tissue attacked during demineralization. Caries, the illness that most destroys dental elements, starts when the combination of cariogenic diet, microorganisms and the formation of plaque decreases the pH to approximately 5.5, causing a slow but progressive destruction of the enamel structure, if these factors are not eliminated. In some cases, caries are restricted to the attack on the enamel and may clinically appear as just a white spot without major consequences due to the action of components of saliva (phosphate, calcium and mainly fluorine) buffering the acid pH, and to the remineralization process of the damaged area. According to CARVALHO (personal communication), to treat this or not is just an aesthetic matter. According to SUNFELD et al.[39,40]; ROMANO and TORRIANI[33] and PAIXÃO and HOPNNER[30], treatment with 16% hydrochloridric acid removes the spot and improves the aesthetics.

When the invasive power is greater than remineralization, there is rupture of the enamel protective layer, exposing the dentin. Since dentin is a living tissue, richly innervated by odontoblastic extensions, it immediately indicates the presence of aggression through sensitivity, during the first moments of the process, followed by painful symptomatology as the lesion goes deeper.

Although it is rarer, during this phase the lesion may become chronic instead of remineralized. This process is characterized by dentinal sclerosis, decreased aggression and as bacterial metabolism ends, the process becomes chronic. This is one possibility. The other is the systematic advance of the caries process, increasingly deep invasion and threat to pulpal integrity. During this stage, professional intervention can revert the situation, restoring the loss of dental substances and pulpal normality. Although some studies accept the hypothesis of not restoring cavities with stationary caries, due to cultural characteristics, Brazilian patients seldom accept this idea, since shape and aesthetic aspects are even more valued than function.

EVANS and KASLOFF[20] state that cavity preparation deserves special attention. The cavity preparation residue called "smear layer" is composed of enamel remnants, dentin, blood, saliva, microorganisms and small pieces of instruments used in the process. This layer is only approximately 3 micrometers thick. According to BLACK[3], cavities can be just washed in water. EVANS and KASLOFF[20] suggest that other substances be used, especially those with properties of cleaning the site and at the same time producing the alkalinization of the cavity, improving conditions for pulpal repair. Washing the cavity with calcium hydroxide and water has been considered a decisive and important procedure for the pulpal recovery. MODELLI[25] agreeded that cavities should be washed in alkaline substances and suggested Tergidon as the ideal solution. When the restorations were made of amalgam and composite resin, these materials were known as a synonym of cavity cleansers. But during the second half of the 1980', new materials and methods of retention were discovered and with them new discussion arose. Until this point cavities were classified as flat, middle and deep. According to biological knowledge at that time, all of them should receive a of lining. Conventional and modified varnish were, respectively, the options for flat cavities treated with amalgam and composite resin. In the first case, the major function of the lining to avoid the diffusion of metallic ions and, in the second case, to avoid the diffusion of monomerous residues into the dentinal tubules. For middle cavities, especially in young patients, the use of calcium hydroxide was also recommended in addition to varnish, in order to complement the biological protection. In the case of deep cavities, the procedure was much more rigorous and in addition to calcium hydroxide another material might be used, such as zinc oxide or zinc

phosphate.

For several years this was the standard procedure in restorative dentistry[1,9,11,43,53].

In 1984, dentinal adhesives became available. Before them, the only way of bonding the restorative material (composite resin) to the cavity depended on acid etching ofthe enamel. In 1982, ERICKSON[19] was the first to demonstrate the feasibility of using dentin in the retention process. A phase of intense research started, trying to find a material which did not need mechanical retention and was also able to improve the marginal sealing and reduce the existing diffusion levels of all restorative materials available.

The major problem was the difference between the composition of the enamel (calcium) and dentin (calcium, collagen, dentinal liquid and collagen fibres). It was a complex puzzle. The steps were slow. The presence of smear layer on the walls of the deepest portion of the cavity and of water in the dentin, made this process extremely vulnerable. Retention had a low intensity, attaining at most the mark of 4 Mpa. In order to solve this problem, researchers treated dentin with primers, whose compositions depend on the manufacturer, but are basically made of weak acids with the ability of breaking the structure of the smear layer and favoring a closer contact between the bonding medium and the dentin that was under the smear layer. Although the results were fairly good (the resistence increased from 4 MPa to 12 Mpa) they were not enough. The goal was to attain a figure around 20 Mpa. New research was carried out.

In 1982, NAKABAYASHI[45] developed a highly complex theory to bond dentin to the restorative material based on the removal of the smear layer and the water from dentinal liquid. The first step was to remove all smear layer, conditioning the dentin as was done for the enamel. The results of the use of acids on the dentin were already known through work in endodontics, where these substances were used to promote the decalcification of calcified canals, which under a scanning electronic microscope, showed the patent tubules and the smear layer completely removed. Whether the available acids (37% phosphoric) were used on dentin, the procedure would be the same. The question was the pulpal aggression caused by this methodology which was unconceivable according to old concepts.

In order to allow the use of NAKABAYASHI's[45] tecnique, research was carried out to show that, although the old concepts were not wrong, they resulted from research which used other methodologies and were extremely concerned with the pulpal reaction to such aggression. GWINNETT[24] showed new concepts for the protection of dentin, considering as protection not only the use of biologically compatible materials, but also the sealing of the dentin , not allowing the infiltration of external toxic agents, which means impeding direct access to the pulp. Thus, it was possible to understand that pulpal protection differs from dentinal protection. In the former, it is imperative to stimulate it to form the dentin bridge, since it is exposed. In the latter, although the dentin is sensitive to the contact of any substance and to temperature, if something is interposed between this layer of dentin and the aggressive factor, it could also be considered as pulpal protection. When NAKABAYASHI[45] formulated the hybridization theory, this was his concept.

What is hybridization? It is how the restorative material (composite resin and glass ionomer) is bonded to dentin. The fundamentals for this bonding are the removal of the smear layer and a hydrophilic bonding agent, which contains an organic solvent to dissolve the water in the intertubular dentin or inside the dentinal tubules (intratubular dentin) promoting an interlace with the collagenous not exposed due to calcium removal by etching. This bonding has different characteristics depending on the dentin depth. The superficial dentin has more intertubular spaces, therefore this is the most important part to the adhesive process. Deep cavities have less intertubular dentin and the main adhesion depends on the intratubular dentin, therefore less stable and more difficult to attain. When the cavity is very deep, the space between the bottom wall of the pulpal horns is just 1mm thick, thus hybridization is not recommended and again the pulpal protection has not only a physical but a biological connotation. Indeed, this was the position adopted by the Brazilian Group of Professors in Dentistry, since 1997, after widely discussing the advantages of etching the exposed pulp, as suggested by COX et al.[6]; KANKA III[34] and CHAIN and PRATES[3], and the disadvantages due to its serious risks, as suggested by SOUZA COSTA (personal communication); HOLLAND (personal communication); MONDELLI[41] and ESTRELA[21].

According to the suggestion of PEREIRA[47], the Brazilian Group of Professors in Dentistry provided a new classification for cavities and proposed new means of protection for dentin and pulp (Table 1).

It is important to observe that there are different kinds of remnant dentin. Restorative dentin presents almost as much of calcium, collagen and dentinal tubules as primary dentin. Totally or partially sclerosed dentin has a different balance between calcium and collagen, does not present patent dentinal tubules and is much more friable. As already stated, the kind of dentin depends on the depth of the cavity. Since there are different kinds of dentin and different characteristics of dentin, depending on the depth of the cavity (more or less intertubular dentin), it is necessary to analyze the quality of this substratum in order to choose the most suitable tecnique.

In several cases, the teeth lost their vitality. With this kind of dentin, the collagen is dry and dehydrated and the water-dentin relationship is altered. If hybridization depends on the integrity of collagen and on the presence of humidity, how it will occur in these cases? In these situations, the presence of calcium prevails. Thus, glass-ionomer is the best material to relate with it and the most appropriate technique to be applied is the "sandwich" one. In the previous situations, there is no doubt that hybridization is an uncomparable mechanism and should be used. It increases the retentive capability and diminishes marginal diffusion.

Glass ionomers produce a superficial chelation-of-calcium type linkage that should be understood as a cross linkage of a molecular type, which is very strong especially in adjacent sealing. As far as we know, it can completely inhibit infiltration, notorious in enamel.

Dentinal adhesive produces another kind of linkage. It infiltrates in the intercollagenous spaces (net) and forms an anastomosis in the tubules, producing important

retention. There is no chemical linkage. For this reason, adjacent infiltration may occur, as described by SANO et al.[34].

We agree with the Brazilian Group of Professors in Dentistry, which recommends the use of the hybridization technique in all cavity types that will be restored with composite resin. In very deep cavities (less than 1 mm of remnant dentin) the use of calcium hydroxide and glass ionomer, together or not, is recommended. On the remnant walls, hybridization may complete the proposed technique.

Under the present circumstances and based on recent research, especially by HOLLAND,1996(personal communication), ESTRELA[10], LANZA[19], BUSATO and GABOARDI[3], and also considering primer composition, we do not recommend the acid etching technique of the pulp, even knowing that acid action has little significance. The assumed ease and simplification of the tecnique and of the clinical protocol should not be misunderstood due to the possibility of contamination and loss of pulpal integrity.

BIOCOMPATIBILITY OF THE PULPAL PROTECTIVE MATERIALS

The dentin adhesive systems composed of conditioner, primer and adhesive, are materials that have undergone an evolutionary process. Their main objective is to form an acid resistant layer with the dentinal substratum, in order to reduce the adjacent infiltration in to the tooth-restoration interface and increase the adhesive resistance of the restorative material to the cavity walls and distribute the pressure of the forces over the dental restoration. Over the time, several physical properties of the adhesive systems have been enhanced and we now have the 5th generation.

Much research has been carried out trying to show the excellence of these adhesive systems and to point out new applications for these materials. Currently, despite the recommendation of the manufacturers, some professionals recommend the use of adhesive systems in deep cavities without previous protection of the dentin-pulp complex, using proven biocompatible cements. Other professionals recommend the application of adhesive systems right on to pulp, based on clinical observations and research on animal teeth.

A dental material should present balanced physical and biological properties, in order to efficiently perform its role.

When seeking scientific articles about dentinal adhesive properties, it is easy to note that the number of publications about the biocompatibility of these materials is very small compared to the number of publications about their physical properties. Some of the research on the adhesive system biological properties was carried out using cell culture (*in vitro*) and others using animal teeth (*in vivo*).

Very important in vitro research was carried out by HANKS et al.[26] in 1991. They studied the cytotoxicity of resin components on cultivated 3T3 Balb/c fibroblast monolayers. It was shown that all the components (including Bis-GMA, UDMA, TEG-DMA, CAMP and others) have an inhibiting effect over the synthesis of proteins, even when low concentrations of these components were used. Other research[4,15,17,46,50] also showed that the substances commonly found in adhesive systems (Bis-GMA, TEG-DMA, HEMA, Ghuta-aldeid and UDMA) have cytotoxic effects.

On the other hand, when these components are applied on a dentinal substratum, this mineralized tissue promotes a protective effect which is directly related to its thickness. Thus, the components of the dentinal adhesives can have a smaller cytotoxic effect when a barrier of dentin separates them from the pulp tissue. When in contact with a vivo pulp tissue, its cytotoxic effect can also be smaller due to the high restorative potencial of the dentin-pulp complex.

In 1990, RUEGGEBERG and MARGESON[32] reported that oxygen inhibits the complete polymerization of dentinal adhesive monomers, leaving, a layer of residual monomer on the material, which can exacerbate the toxicity of the material.

The presence of humidity in the dentinal substratum, after the acid etching of dentin, is another factor that interfers with the complete polymerization of the dentinal adhesives. The residual monomers which did not easily polymerize are solubilized and diffuse through the dentinal tubules. The lower the molecular weight of the resin component, the easier and faster it will diffuse, even in the presence of a contrary flux of dentinal fluid (+/- 10 cm of H_2O)[38].

BOUILLAGUET et al.[2] reported that if a 50% HEMA solution (usual concentration in the dentinal adhesives and equivalent to 4.000 mmol/L) was applied to 1mm thick etched dentin for 30 minutes, the dilution factor would be approximately 2500 in the absence of pulpal hydrostatic pressure and 20.000 in the presence of 10 cm of H_2O of pulpal pressure. Thus, the concentration of HEMA in the pulp would be approximately 1.0 mmol/L. Since concentration must range from 2 to 3 mmol/L of HEMA to cause a discrete reaction in the fibroblasts, after 12 hours exposure, the risk of acute toxicity seems to be low. However, if the thickness of dentin was reduced to 0.5 mm, the concentration of HEMA in the pulp would be approximately 2.1 mmol/L which could cause a discrete toxicity, after 12 hours exposure. Considering that a concentration of 4 mmol/L of HEMA is able to reduce the cellular activity (TC50) by 50% after 24 hours, we can deduce that if the 4.000 mmol/L remains for 24 hours on 0.5-mm dentin, approximately 4 mmol/L of HEMA would be on the pulp and this concentration makes it highly cytotoxic.

In cases of resin restorations in human teeth, the components of the adhesive system have long contact with dentin and the accumulated material in contact with pulpal cells could cause a higher cytotoxicity over the time. In 1995, RATANASATHIEN et al.[45] reported that 4 main components of dentinal adhesives have lower toxicity in the following order: Bis-GMA >UDMA>TEGDMA>>>HEMA.

Therefore, the results of in vitro tests show that the components of dentinal adhesives are highly cytotoxic, confirming former results.

In vivo research uses animal teeth (dogs, rats and monkeys). Dogs have a different dental development than humans presenting several lateral canals (delta apical) and their pulpal tissue does not have defined layers (acellular and rich in cells) as do human teeth. Rats do not have peritubular dentin and their teeth are only indicated for tests of drugs that are used in direct contact with pulpal tissue. The cavity openings are also a problem because they are not always standardized and also have dental cavity

extension. Monkey teeth not only have dentinal tubules with different diameters and characteristics than human teeth but also present a positive response to pulpal therapies, which would not be well accepted by humas.

In spite of these facts, in vivo as well as n vitro research is indicated by the FDI as sequence of biocompatibility tests. Nevertheless, the results attained through these methodologies cannot be conclusive and used to recommend the regular application of the material on humans.

Tests of dental materials on human teeth are the main methodology of biocompatibility of materials which are being developed. After tests on animal teeth, tests on human teeth would effectively show the qualities and biological and physical properties of the material.

Researchers and physicians have used radiographic evaluation and temperature fluctuation tests (hot/cold) to verify whether the pulpal tissue is vital even after the application of the adhesive system. But, the vitality of this connective tissue is determined through microscopic and biological analysis of normal cellular histologic and functional characteristics and persistence of the intercellular substance with its physiologically operating macromolecules.

Some physicians use the biocompatibility tests on animals as a basis to recommend the use of adhesive systems in regular practice, even against the manufacturers indication.

Trying to clarify the doubts about the use of dentinal adhesives, professors Josimeri Hebling and Elisa Giro (Pediatric Dentistry – Araraquara) together with the Department of Pathology of the School of Dentistry of Araraquara have carried out research in which the adhesive systems are applied to animal and human teeth. The results of the tests on animal teeth have been negative. The 60-day post-operative follow-up revealed that almost all specimens presented pulpal necrosis.

On human teeth, the results were not satisfactory when adhesive systems were applied on pulpal tissue as well as when applied on 500 mm thick remnant dentin (0.5 mm deep cavities) .

The healing process of the pulpal tissue is different from that observed when calcium hydroxide is used in pulpal therapy. This has confirmed the cytotoxic effect of the components of the adhesive systems, even when applied on a tissue with a high healing potential.

Considering the facts mentioned above and in agreement with the manufacturers of the adhesive systems, these materials have a limited indication due to their irritating effects on the dentin-pulp complex.

Thus, we still recommend the use of P.A. calcium hydroxide, paste or even calcium hydroxide cement on pulpal exposures, followed by clinician's favorite restorative systems chosen by the clinician. Due to the difficulty in determining the thickness of remnant dentin in deep cavities, we always recommend the use of calcium hydroxide cement to line the bottom of the cavity.

The problems resulting from the use of calcium hydroxide are already known, however this is still the best alternative. Research on other materials, with different actions from calcium hydroxide, OP-1 osteoinductive protein for instance, has been carried out with the aim of replacing or even offering another material for direct or indirect capping.

Dentists should carefully choose and apply the adhesive system on the bottom of deep cavities and on the pulp. It is also advisable to wait for the results of new research which can confirm or not the indication of these materials.

CALCIUM HYDROXIDE

Considering the different doubts about the use of calcium hydroxide as a pulp protector, it seems to be appropriate to discuss some of its properties and responses when in contact with pulp tissues and with microorganisms.

Calcium hydroxide was first used in dentistry by NYGREEN in 1838 (Stockholm) to treat dentalis fistule, and by CODMAN in 1851 in radicular amputations of living pulps. In 1920, HERMANN[10] published a study that was considered to be the pioneer in the use of calcium hydroxide and in which there was a formula of a paste (Calxyl-Otto & CO; Frankfurt, Germany), and whose vehicle was physiologic solution[12].

Calcium hydroxide is a strong base obtained from the calcination (heating) of calcium carbonate until its transformation into calcium oxide. Calcium hydroxide is obtained through the hydration of calcium oxide and the chemical reaction between calcium hydroxide and carbon dioxide forms calcium carbonate. It is a white powder with a high pH (12.6) and is slightly soluble in water (solubility of 1.2 g/L, at a temperature of 25°C)[11].

The properties of calcium hydroxide are come from its dissociation into calcium and hydroxyl ions and the action of these ions on tissues and bacterias explains the biological and antimicrobial properties of this substance. Changes in the biological properties can also be understood through the chemical reactions shown below, since calcium hydroxide, in the presence of carbon dioxide, becomes calcium carbonate (weak acid oxide) and this product does not have calcium hydroxide biological properties such as the mineralizing capability.

ESTRELA and PESCE[16] chemically analyzed the release of hydroxyl ions from calcium hydroxide in connective tissue of a dog. From the percentage of calcium ions released, they could calculate the molecular weight of calcium hydroxide and the percentages of hydroxyl and calcium ions found in calcium hydroxide, as follows:

$$Ca(OH)_2 \rightarrow Ca^{2+} + OH^-$$

$$1 * Ca^{2+} = 40.08$$

$$1 * OH^- = 17.0 \quad 2 * OH^- = 34.0$$

$$1 * Ca(OH)_2 = 74.08$$

Taking into account the molecular weight of calcium hydroxide, which is 74.08, through a rule of three , one can obtain the percentage of hydroxyl ions found in calcium hydroxide, which is 45.89%, while 54.11% corresponds to calcium ions.

$$74.08 \rightarrow 100\%$$

$$34.0 - X \qquad X = 45.89\%$$

$$OH^- = 45.89\% \qquad CA^{2+} = 54.11\%$$

Calcium hydroxide has a direct and specific action on enzymes due to its high pH. ESTRELA et al.[16] analyzed the mechanisms of action of hydroxyl ions and calcium ions of calcium hydroxide and highlighted two significant properties of these substances: the inactivation of bacterial enzymes, creating the antibacterial effect, and the enzymatic activation of tissues, creating the mineralizing effect. The enzymes in the cytoplasmatic membrane of bacteria can be affected by high quantities of hydroxyl ions from calcium hydroxide. Its chemical transportation (permeability) and organic component structure and nutrition supply can be altered, causing a toxic effect to bacterial cells. The activation capability of tissue enzymes can be observed from the action of alkaline phosphatase, which favours mineralization when activated by calcium hydroxide.

The optimum pH value for the activation of this enzyme ranges from 8.6 to 10.3, making the release of organic phosphate (phosphate ions) easier , which reacts with calcium ions from the circulating blood, creating a sediment of calcium phosphate on the organic matrix. Indeed, this sediment is the molecular unit of hydroxyapatite[12].

The biological effect of pH on the enzymatic activity of anaerobic bacteria, allowed ESTRELA et al.[17] to formulate the hypothesis of a irreversible bacterial enzymatic inactivation under extreme pH conditions, during a long term, and a reversible enzymatic inactivation (temporary), when the ideal pH for the enzymatic action allows the return to normal activities (level). This fact can be observed during the study of dentinal diffusion of the hydroxyl ions of calcium hydroxide, which maintains the pH high within the radicular canal, and lower on the outer surface of the apical third[19]. This hypothesis can be confirmed by the evaluation of the direct effect of calcium hydroxide on microorganisms, which has shown to be highly effective in direct contact during short terms (1 minute). It has also been effective in indirect contact, however, it requires a longer contact, since the presence of living microorganisms within the dentinal tubules after a 7-day period was verified.

Through histochemical analysis, EDA[5] studied the mechanism of formation of mineralized barriers after the application of direct pulpal protection in dogs teeth, with pastes of calcium hydroxide, magnesium oxide, zinc fluorite and calcium fluorite. After an observation period ranging from 30 minutes to 60 days, the author reported that extremely thin particles which reacted positively to von Kossa dye and were underlying the necrosed layer could be seen during the initial stage of dentin formation. These small grains originated from the reaction of the capping material metal with the tissue carbon dioxide. Magnesium oxide as well as calcium hydroxide showed powerful effects on the formation of new dentin. Nevertheless, SOUZA[18] after morphological study of the dental pulp behavior after pulpotomy followed by protection using magnesium oxide or calcium hydroxide, reported that the possibility of restoration using magnesium oxide was remote. The efficacy of the treatment in the pulps protected with calcium hydroxide was higher, which eliminated the occurence of technical failure during the treatment with magnesium oxide.

HOLLAND[10] analyzed the healing process in the dental pulp after pulpotomy and capping with calcium hydroxide in a morphological and histochemical study carried out on dog teeth. The author reported that in the superficial grainy zone, interposed between the necrosed zone and the deep grainy zone, there was the presence of rough grains with calcium salts, some of them composed of calcium carbonate in the form of calcite and of calcium-protein compounds. These observations demonstrate the active participation of calcium ions from calcium hydroxide used as protective material in the healing process. In this mineral fraction , there was a positive reaction to chloranilic acid and to von Kossa method. Later, SEUX et al.[20] obtained similar results.

Other hydroxides were also evaluated regarding their effects on pulpal tissue. HOLLAND et al.[10] evaluated the effect of calcium hydroxide, barium hydroxide and strontium hydroxide after pulpal capping , using histochemical analysis in dog dental pulp. The results were similar among the three hydroxides and showed deposits of strontium carbonate and barium carbonate grains resembling the graining observed with calcium hydroxide. Since barium and strontium are not naturally present in animals, these grainings originated from the capping material. They also reported that the presence of birefringent large grainings were not observed with the use of other hydroxides such as magnesium hydroxide, or sodium hydroxide, due to the fact that sedimentation only occurs with hydroxides whose solubility is similar to that of calcium hydroxide. Magnesium hydroxide is insoluble and sodium hydroxide is highly soluble in pulpal fluids. Barium hydroxide is slightly more soluble than strontium hydroxide, which can be observed through the fact that barium hydroxide grainings have been found deeper than strontium hydroxide grainings. This research confirms again the active participation of calcium ions from the calcium hydroxide of the protective material in the healing process.

SCIAKY and PISANTI[23] and PISANTI and SCIAKY[22] did not observe that some of the calcium ions in the mineralized barrier (new dentin) came from the calcium hydroxide used in the protection of exposed dog pulp and which contained radioactive calcium (Ca^{45}) or from the intravenous injection in dogs of a solution containing radioactive calcium. Probably it occured due to the methodology used, since the authors used autoradiographies.

WAKABAYASHI et al.[21], using a scanning electron microscope and an X-ray energy dispersion micro-analyzer, evaluated the mechanism of dystrophic calcification induced by calcium hydroxide in the connective tissue of a rabbit auricular chamber. The interactions between the microvessels and the calcium hydroxide were observed immediately after the application and during the following 14 weeks. During the initial phases of tissue reaction, the results revealed the formation of a necrotic layer and calcification seen as a fast sedimentation of crystals through neutralization and its immediate growing in a barrier (dystrophic calcification). They also observed that additional calcium and phosphorus were deposited over the sedimentation particles. They also found that the 24-hour specimen sedimentation showed not only calcium peaks but also weak peaks of phosphorus, sulphur and/or magnesium, in the melted portion among the crystals and suggested that such sedimentation would have the potential

to induce tissue dystrophic calcification, which is in agreement with HOLLAND et al.[30] .

In view of the biological and chemical evaluations of calcium hydroxide, it is possible to note its qualities as a pulpal biological liner.

HOLLAND et al.[33] studied the healing process of dog dental pulp after pulpotomy followed by pulpal protection using calcium hydroxide paste or powder. The results were analyzed histologically after 30 days of treatment. No difference between the experimental groups was observed and almost 90% of the specimens showed a bridge of completely mineralized tissue, protecting the vital dental pulps with no inflammation. Regarding the post-pulpotomy healing process, HOLLAND et al.[37] carried out another study, analyzing pulpotomies and pulpal protection using calcium hydroxide or Dycal. The results showed that the mechanism of healing process of dental pulps protected with Dycal is similar to the one protected with calcium hydroxide, however the use of Dycal has lower efficacy.

LEAL et al.[40] analysed histologically the action of concentrated calcium hydroxide water on the pulpal tissue of 16 dog teeth after pulpotomy. The results showed that irrigation with concentrated calcium hydroxide water caused only partial histomorphologic reactions, over the pulpal remnants which occur during the dental pulp treatment with calcium hydroxide paste.

TRONSTAD[45], studying 64 monkey teeth with exposed pulps protected with calcium hydroxide and Dycal, reported that in the group that used Dycal the formation of mineralized tissue occured just after 30 days, with the presence of chronic inflamatory infiltration near the material, which disappeared over the time and was replaced by mineralized tissue. In the group that used calcium hydroxide , the formation of the mineralized barrier was faster.

Regarding the discussion about the mineralized barrier permeability, HOLLAND et al.[36] observed the formation of a hard tissue bridge after pulpotomy with calcium hydroxide. They performed pulpotomies on 80 monkey teeth using calcium hydroxide. After 30 days, the dressings of 60 teeth were removed, allowing the visualization of the hard tissue bridge. In order to evaluate permeability, the bridges were capped with silicate cement (20 teeth) or, zinc phosphate cement (20 teeth) and 20 teeth were exposed to the oral environment without any protection. The remaining 20 teeth were used as control. The pulpal responses of the experimental groups resembled the ones of the control group. The complete bridges showed a high level of normal remnant pulp. Fragments of dentin were found in the incomplete bridge. Despite the belief of some researchers, the authors reported that these bridges were not permeable, and that the porosity was not a contraindication to the procedures of direct pulpal capping or pulpotomy.

LOPES et al.[43] studied, using scanning electron microscopy, the mineralized bridge after pulpotomy, using different calcium hydroxide pastes. In the observations conducted on the pulpal side, the bridge surfaces presented agglutination of calcospherites, with a small openings. These openings must be blood vessels of the dental pulp which remaineded in the bridge during the calcification process. On the coronal surface, they also observed mineralized structures with shapeless morphology and absence of openings. These results differ from the ones found by GOLDBERG et al.[35], who observed crystals with different shapes, sizes and distribution. These differences are probably due to the removal of the calcium hydroxide particles from the surface of the mineralized bridges caused by the irrigation performed in this study.

Although the literature is clear about the excellent properties of this substance as an ideal material to be applied directly over the pulpal tissue, some of its properties have been the object of doubt.

COX et al.[7] analyzed the permeability of mineralized bridges formed after direct pulpal capping of 235 mechanically exposed teeth, using different calcium hydroxide cements and reported that 192 out of 235 teeth showed hard tissue bridges. A total of 172 out of the 192 hard tissue bridges (89%) showed tunel-type defects and 78 out of the 172 defective hard tissue bridges (45.3%) presented pulpal inflammation and/or necrosis after some time. The authors suggested that the multiple tunel defects clearly showed a morphological rupture of the formed bridge, failing to provide a permanent barrier and thus accessible to bacterial infection. The presence of particles of the capping agent in the pulpal tissue was in a state of progressive disintegration.

CHAIN et al.[6], after critically revising the role of calcium hydroxide as a lining material and as a pulpal capping agent, showed some crucial points and suggested better alternatives of treatment. They listed some disadvantages of calcium hydroxide such as its high solubility, is not permanent, does not stimulate dentinogenesis, no adhesiveness to dentin, does not stimulate sclerotic dentin , does not stimulate the sedimentation of restorative dentin questionable resistance in the presence of amalgam condensation, is associated with tunel-type defects in dentinal bridges, no adhesiveness to composites, questionable resistance to acid etching action, and does not release fluorite. They believe in a new generation of biocompatible materials with enhanced physical properties and most of all, with a better adhesive power to the dentinal structure, allowing a better long-term sealing and avoiding or minimizing microinfiltration. According to CHAIN et al.[6], the modern dentinal adhesives and glass ionomer cements seem to be better alternatives to the use of calcium hydroxide.

In view of such questioning about calcium hydroxide, it is useful to remember that, the likely mechanism of this substance is already known, although it seems that many dentists and scientists have not learned about this. Much research has been carried out in order to better understand the mechanism of biological action of calcium hydroxide[8,13-15,30-32,34,35,40,55,56,61,63] on tissues, while other studies try to explain its mechanism of antimicrobial action[13,15,35,58]. Since the results of these investigations, certify the biological and microbiological excellence of this substance, we cannot reject a material with such properties. It is important to highlight that the studies that showed tunel- type defects in the mineralized bridges formed did not use P.A. calcium hydroxide but cement containing calcium hydroxide, which has almost the same mechanism of action as P.A. calcium

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hydroxide, but with a lower rate of efficacy, according to HOLLAND et al.[37]. Some studies also discuss the permeability of the hard tissue bridge formed, not considering it satisfactory since it could allow the penetration of microorganisms and infect the pulpal tissue in case of a fracture or crack in the restoration. We learned from HOLLAND et al.[36] and LOPES et al.[41] that this mineralized bridge is less permeable than dentin. By analogy, if the permeability of dentin is enough to protect the pulpal tissue then the hard tissue bridge also is. Calcium hydroxide does not have any chemical characteristic that allows the release of fluorite, so there is no scientific meaning to state that this is a disadvantage of using it.

The importance of the physico-chemical properties, such as the adhesive capability and effectiveness, of a restorative material should be considered, however the biological aspect should be not neglected. Scientific investigations should be carried out frequently every moment in order to evaluate materials recommended for use in dentistry. Nevertheless, we should always remember the difference between scientific fact and scientific opinion. Scientific fact originates from an investigation within specific parameters and methodologies, reproducible and well oriented in order to investigate a new material, a new tecnique or to analyze a questionable fact and sometimes established as a quality control. On the other hand, the "scientific" opinion , in several cases, originates from personal opinions, with no scientific criteria, most of the time speculative and sensationalist.

Dentin is considered the best pulpal protective material, and calcium hydroxide has proved, through numerous studies, its capability of inducing the formation of a mineralized bridge over pulpal tissue.

CONCLUSIONS

In view face of the discussion presented here, it is possible to state that:

01. Indication of aN odontological material should be based on its physical and biological properties. In vivo and In vitro research are prescribed by the International Dental Federation as a sequence of biocompatibility tests; however, the results obtained with these methodologies cannot be conclusive but serve as a base to recommend the use of a material under test in the odontological clinic.

02. Calcium hydroxide should be indicated as the ideal material on pulpal exposures and very deep cavities, followed by the clinician's favorite restorative material.

Table 1 . Classification of cavities

FLAT	MIDDLE DEEP	VERY DEEP	PULPAL	EXPOSURE
Hybridization	Hybridization	Hybridization	Calcium hydroxide and/or glass ionomer	Always Calcium hydroxide

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EFFECTIVENESS OF FORMOCRESOL AND A CALCIUM HYDROXIDE / CAMPHORATED PARAMONOCHLOROPHENOL PASTE IN PREVENTING ENTIRE ROOT CANAL RECONTAMINATION BY BACTERIA FROM SALIVA. AN *IN VITRO* STUDY.

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The aim of this *in vitro* study was to evaluate the ability of two medicaments in preventing recontamination of coronally unsealed root canals by bacteria from saliva. The medicaments tested were: formocresol applied on cotton pellets in the pulp chamber and calcium hydroxide/ camphorated paramonochlorophenol (CPMC)/glycerin paste filling the root canal. Root canals were then exposed to saliva and the number of days required for total recontamination was recorded. Canals medicated with formocresol were completely recontaminated within an average of 7 days. Canals filled with calcium hydroxide/ CPMC/glycerin showed total recontamination within an average of 16.5 days. Intracanal medicaments significantly delayed bacterial progression toward the apical foramen ($p<0.05$). Calcium hydroxide/CPMC/ glycerin paste was significantly more effective than formocresol ($p<0.05$).

Key words: Bacterial leakage; intracanal medicaments; root canal recontamination.

INTRODUCTION

The use of an intracanal medicament in the treatment of infected root canals has been advocated for the following reasons: to help eliminate remaining bacteria unaffected by the chemomechanical preparation; to reduce periradicular inflammation and thereby reduce pain; to induce healing by hard tissue formation; to control persistent exudation; to inhibit osteoclastic activity; and to prevent root canal recontamination between appointments[1-3].

Recontamination of instrumented root canals can occur between appointments in some clinical circumstances: leakage through the temporary filling; loss of the temporary filling; fracture of the temporary filling material and/or tooth structure. In these situations, the root canal system is exposed to the oral flora, which allows its recontamination. When intracanal medicaments are used between appointments, the total recontamination of the root canal may be delayed[4].

A plethora of substances has been recommended for use as intracanal medication. Some substances, such formocresol, were used based on a vapor-forming ability. Studies have shown that both formocresol and its vapors possess strong antibacterial effects[3,5,6]. The antibacterial activity of formocresol depends on the combination of the protein-coagulating effects of cresol with the alkylating effect of formaldehyde.

Other substances, such as calcium hydroxide used for filling the root canal,do not release vapors. Several vehicles have been recommended for use in association with calcium hydroxide. Studies carried out by us and others[6,7] have reported that the association of calcium hydroxide with camphorated paramonochlorophenol (CPMC) has a wider antibacterial spectrum and a higher radius of action than when calcium hydroxide is used in association with distilled water, saline or glycerin.

Therefore, based on these premises, the purpose of this study was to evaluate the length of time needed for bacteria from human saliva to thoroughly recontaminate coronally unsealed root canals medicated with either formocresol (applied on cotton pellets) or calcium hydroxide/CPMC/glycerin paste (filling the root canal).

MATERIAL AND METHODS

Forty intact, caries-free, human maxillary incisors and cuspids with straight roots were used in this experiment. During all procedures throughout the study, the teeth were kept moist. Thirty-five teeth were prepared as follows: conventional access preparation was made and a #10 K-type file was introduced into each canal until it reached the apical foramen. The working length was established by subtracting 1 mm from this measurement. The root canals were prepared by the step-back technique using K-files with circumferential filing motion. In order to standardize the diameter, the apical foramen was enlarged to a #25 file. Apical preparation was done at the working length to a #40 file. The coronal portion of the canal was flared with Gates-Glidden burs (#2 through #4), and preparation was completed using the step-back technique of 1.0 mm increments. The patency of the apical foramen was always checked by recapitulation with a #25 K-file after each larger size file. The irrigating used for chemomechanical preparation was 1% NaOCl.

Once preparation was completed, the teeth were randomly divided as follows: *Group 1*: 15 teeth were medicated with formocresol; *Group 2*: 15 teeth were medicated with a calcium hydroxide/CPMC/glycerin paste, mixed to a creamy consistency. For paste preparation, equal volumes of CPMC and glycerin (1:1 ratio) were used. The *positive control* group consisted of five teeth with instrumented canals and access cavities opened

containing only sterile cotton pellets without medicament placed in the pulp chamber. Another five teeth with intact crowns served as the *negative control group*. In all experimental groups no coronal sealing was done.

Calcium hydroxide/CPMC/glycerin paste was used to fill the entire root canal. It was placed into the canal using lentulo spirals in a slow-speed handpiece. Excess paste was condensed by means of a cotton pellet in the pulp chamber until the paste was extruded from the apical foramen. The paste was removed until the length of 10 mm of medicament filling remained in the root canal. A cotton pellet was left in the pulp chamber. Radiographs were then made of all samples to evaluate the quality of paste filling.

Formocresol was applied just after sterilization of the specimens. Cotton pellets were immersed in formocresol, excess medicament removed using dry gauze, and then placed in the pulp chambers of the samples.

Glass vials used to store antibiotic paper disks containing rubber stoppers were adjusted for the purpose of this experiment. By using a heated instrument, a hole was made through the center of each rubber stopper in which the tooth was inserted under pressure up to its cementoenamel junction, so that its crown was outside of the vial and its root within the vial. Cylinders prepared from 10 ml plastic syringes were adapted on the external surface of the stoppers in order to create a chamber around the crown of the tooth.The glass flasks were then filled with Brain Heart Infusion (BHI) broth (Difco, Detroit, MI) so that about 2 mm of the root apex was immersed in the broth.

The testing apparatus were then steam sterilized. Thereafter, cyanoacrylate was applied at the interface between the tooth and the stopper. To ensure sterilization, the whole apparatus was incubated at 37°C for 4 days.

The chamber of each whole apparatus was filled with 3 ml of human saliva, mixed in BHI broth in a 3:1 (v/v) ratio. Saliva was collected from the laboratory staff and replenished every 3 days.

Apparatus were then incubated at 37°C and checked daily for the appearance of turbidity in the BHI broth. The number of days it took for the appearance of bacterial growth was recorded as an indicator of the recontamination of the root canal by bacteria from saliva.

Data were analyzed statistically using the Student's *t* test with the significance level set at 5% ($p<0.05$).

RESULTS

Data related to the number of days necessary for thorough recontamination of the medicated root canals to occur are shown in tables 1 and 2. All specimens of the positive control group presented broth turbidity within 2 days of incubation. By contrast, the specimens of the negative control group remained uncontamined throughout the experiment.

Three specimens of group 3 became contamined after the period of evaluation of sterilization of the apparatus and thus were discarded from the study. These specimens had required additional manipulation after sterilization, because the rubber stopper had been lost from the vials.

Root canals medicated with formocresol (group 1) showed complete recontamination within an average of 7 days (range 6 to 13 days). In group 2, in which the root canals were filled with calcium hydroxide/CPMC/glycerin paste, recontamination was observed within an average of 16.5 days (range 4 to 34 days).

Student's *t* test showed a significant statistical difference between groups 1 and 2 ($p<0.05$) and between each group and the positive control group ($p<0.05$).

DISCUSSION

In the present study, all samples of the positive control group, which received no intracanal medication, showed complete recontamination within 2 days. Statistical analysis of data revealed significant differences between the experimental groups and the positive control group ($p<0.05$). It reinforces the statement that intracanal medication can prevent (depending on time) or at least delay the reinfection of the root canal system between appointments.

Medicaments may retard bacterial invasion of the root canal in a chemical or a physical manner[6]. The former is related to the antibacterial activity of the medicament, killing bacteria that penetrate the canal. The latter depends on the filling ability of the medicament. Formocresol on cotton pellets acts only by the chemical effect, whereas the calcium hydroxide/CPMC/glycerin paste acts both ways.

In addition to providing bacteria, saliva plays another important role in allowing the recontamination of the root canal. It can neutralize medicaments chemically and solubilize the medicament physically. Therefore, the medicament is rendered ineffective and bacteria from saliva can progress toward the apical foramen.

The calcium hydroxide/CPMC/glycerin paste was significantly more effective than formocresol in preventing root canal recontamination ($p<0.05$). As formocresol, it has been reported that this paste has pronounced antibacterial effects[4,7]. Because this paste also has filling ability, it is possible that this property plays a major role in preventing root canal recontamination.

It has been reported that formocresol becomes inactive in the root canal within a short period of time, losing much of its activity within 1 week[6]. This finding corroborates our results, since the average number of days required for recontamination of the root canals was 7. In addition to having a short time of action, volatile medicaments that act by means of vapor release have still other disadvantages such as the difficulty to control distribution, effective concentration and toxicity of the vapors. Thus, their use in modern endodontics becomes questionable.

CONCLUSIONS

The results of the present study indicated that:

1. Intracanal medication is effective in delaying the recontamination of root canals.

2. The calcium hydroxide/CPMC/glycerin paste was significantly more effective than formocresol in preventing bacterial invasion of the root canal system.

Acknowledgments

The authors are grateful to Fernando A.C. Magalhães for his technical assistance.

Table 1 . Rate of complete recontamination of the coronally unsealed root canals medicated with formocresol paste after challenge by saliva.

No. of samples	Cumulative (%)	No. of days
8	53.3	6
5	86.7	7
1	93.3	9
1	100	13

Total: 15 **Average: 7 days**

Table 2 . Rate of complete recontamination of the coronally unsealed root canals medicated with calcium hydroxide/ camphorated paramonochlorophenol/glycerin paste after challenge by saliva.

No. of Samples	Cumulative (%)	No. of Days
2	16.7	4
1	25	7
1	33.3	8
1	41.7	13
1	50	14
1	58.3	15
1	66.7	18
1	75	24
1	83.3	26
1	91.7	31
1	100	34

Total: 12 **Average: 16.5 days**

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"IN VITRO" COMPARATIVE STUDY OF THE EFFICIENCY AND FORMATION OF APICAL ZIP PRESENTED BY THE OHIO MODIFIED, CANAL FINDER SYSTEM AND TEP-10R TECHNIQUES

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Sixty mesio-bucal superior molar roots were used, included in blocks of transparent resin and radiographed with #08 or #10 Kerr type file. The radiographs were projected amplified by ten and drawn on white paper where the contours of the roots, files and floors of the pulp chambers were traced.The teeth were distributed equally according the their level, curvature and anatomic diameter, creating Group I (Ohio Modified technique), Group II (Canall Finder System) and Group III (TEP-10R).

After instrumentation the teeth were radiographed with a #25 final apical file and the pictures projected onto the first drawing, thus obtaining the contour of the root with the projection of the files before and after instrumentation.

The incidence of apical zip was less with the Canal Finder System technique aided by hand instrumentation, although the TEP-10R technique proved more efficient when working time was compared, however, with the disadvantage of greater percentile alterations in odontometry and a high occurrence of apical zip. Automated instrumentation is a valuable aid in chemical and mechanical preparation when used jointly with hand instrumentation.

Key words:
1) Automated instrumentation
2) Hand instrumentation
3) Apical zip

INTRODUCTION

Traditionally, the preparation of the radicular canal system has been done manually, which is time consuming, difficult and frequently stressing. Often, manual preparation does not completely clean the canal, especially the apical third, leaving walls untouched [12, 13]. As the degree of complexity increases, the chances of completely removing its contents decrease and the incidence of steps, transportation and perforations increases [24]. The large number of existing techniques [4, 10] shows the difficulty in preparing the radicular canal and the great concer regarding this stage.

Since 1899, when ROLLINS produced the first hand piece for Endodontics, a large number of automated devices have been introduced in the market and suggestions for a reduction on instrumentation time and greater efficiency in sanification have become imperative. In spite of the numerous investigations showing the limitations of mechanized preparation of radicular canals, its use is more and more frequent [1, 3, 5, 18, 19, 27, 29].

With this in mind, a great number of techniques for manual and mechanized preparation have been proposed. In 1979, MULLANEY [19] described the instrumentation technique employed in the State of Ohio which permits the dilation of the two coronary thirds of the radicular canal with Gates-Glidden drill bits and complementation of the preparation in levels, preserving the position and the original shape of the apical foramen, avoiding operational accidents during instrumentation. As technical advantages, he cites the savings of time, the ease of action with the instruments and the adaptation of the main *gutta-percha* cone.

In 1984, LEVY [14] introduced the Canal Finder System with the purpose of substituting hand instrumentation. This system provides a special movement which allows the file to adjust to the anatomy of the canal, promoting selective drilling, a high level of security and efficiently decreasing the time spent in endodontic treatment.

The CFS has been evaluated in various studies with contradictory results. TRONSTAD and NIEMCZYK [15] consider it to be safe for preparing the S.C.R., maintaining the original shape of the canal. In 1987, comparing the efficiency of CFS and conventional hand instruments in extracted molars with curvature, GOLDMAN and SAKURAI[8] concluded that the CFS maintains the shape of the radicular canal closer to its original than conventional manual preparation. Comparing the efficiency of CFS with sonic and hand instrumentation, HAIKEL and ALLEMANN [7] found better results using hand instrumentation and CFS, considering that the latter saves time in the preparation of canals but tends to rectify their curvature. Other investigations[2,11] have shown adverse results, particularly in the case of zip, reduction of the working length, ledges and apical transport. Studies such as those of VIORA [26], PETSCHELT [18], and WEINFELD[28] report good results in curved and narrow canals, highlighting the fact that it does not cause capacity of not causing apical damage, however, noting the need for complementation when preparing with hand instruments.

Investigations carried out by SYDNEY et al. [22] have shown that little or no apical transportation occurs when using CFS in curved or narrow canals, particularly when used in conjunction with hand instrumentation. According

to SYDNEY et al. [11], these contradictory results are due to the substitutio of because of the attempt of substituting hand instrumentation with automated pieces. It is by exploring the radicular canal with the first instrument that one knows the internal anatomy, the lumen, the direction and the curvature. This is impossible to do automatically. In 1996, SYDNEY et al. [11] concluded, that better results with CFS will begin to be obtained from the moment one understands that nothing substitutes hand instrumentation. They suggest the use of an updated technique in instrumentation with CFS, which consists of associating the manual technique (which begins and ends the preparation) with the automated technique, employing Set-Files, which should be previously curved in order to avoid error in preparing the canal.

The TEP-10R (N.S.K.) is a device appropriate for manual files or lentulos. According to the manufacturer, it offers 1/10 speed reduction, which makes it ideal for endodontic treatment. No reference to this equipment was found in any publication.

Considering the growing use of automated handpieces in Endodontics and in order to reduce the stres of the professional and the time spent in biomechanical preparation, the point to be analyzed in this study is the efficiency and speed of the Ohio Modified, Canal Finder System and automated with contra angle TEP-10R (N.S.K.) Techniques. We have taken as a parameter of evaluation the incidence of apical zip, the time spent in radicular canal instrumentation and the loss of working length .

MATERIAL AND METHODS

Sixty mesio-bucal roots of superior molars conserved in 10% formaldehyde were used. The palatine roots were sectioned and the palatine surface of the crown of the teeth was ground using a carborundum disk, so as to acquire a plane of reference parallel to the greater curvature of the mesio-bucal canal in order to guide the placement of the teeth into transparent resin blocks.

The teeth were radiographed with Kerr #08 or #10 type files at 0.5 mm from the apical foramen, the radiographs were projected amplified ten times and pictures were drawn on white paper whereon the contours of the roots, the files and the floors of the pulp chambers were traced.

The teeth were equally distributed according to level, degree of curvature and anatomic diameter, following the Schneider method as modified by BERBERT and NISHIYAMA [1]. With the results of the level, degree of curvature and anatomic diameter (which is the first file which adapts closely in the working length) the teeth were then divided into three group (table 1).

a) Group I: Ohio Modified Technique (N=20)

The technique formally described as MULLANEY [10] was employed, only modifying the preparation of the apical portion of the canals, instrumented up flexofiles #20 or #25 (according to the anatomy of the canal). The coronary 2/3 were dilated with a GG#02 drill and the final conicity of the preparation was obtained employing echeloned preparation with anatomic draw back. The preparation was refined with a #25 file joining the preparations and a recapitulation with a #10 K-file.

b) Group II: Canal Finder System

The updated technique proposed by SYDNEY et al. [11] which combines hand and automated instrumentation was used in this group.The apical preparation was done manually with #10 and #15 flexofiles at the initial length pre-curved Set-Files #15 to #40 introduced into the radicular canal at 1.0 mm before the working length. When larger diameter instruments (Set-Files #25, #30, #35, #40) were then began to meet resistance in reaching the desired measure, regressive echeloning was done, where the measure was based on the degree of curvature of the radicular canal. The end of the preparation was carried out manually. Generally, a #30 file reached the length with ease. Recapitulation was carried out with manual instruments #15, #20 and #25.

c) Group III: Automated Technique with TEP-10R (N=20)

Due to the lack of published literature and instructions from the manufacturer, the instrumentation used with the TEP-10R was based on the updated technique for instrumentation with the Canal Finder System described by SYDNEY et al. [11] without shortening the working length in in 1mm, because this device does not have variable amplitude and longitudinal movement as does the CFS.

After instrumentation, the teeth were radiographed with a #25 final apical file and the pictures were projected over the first drawing, thus obtaining the contour of the root with the projection of the files made and after instrumentation. New calculations were effected, following the SCHNEIDER method, as modified by BERBERT and NISHIYAMA [1] for determining the modification in the degree and level of curvature. The operational time of the techniques was also observed.

In order to measure the incidence of apical zip, we traced a perpendicular line over the one already traced over the initial file connecting it to the line traced from the final file, also perpendicular to the radicular apex, marking the distance between the pre- and post-operational line traced.

The transportation was measured between these two points in tenths of a millimeter, according to the criterion used by CIMIS [3], where the variation of the transport of the foramen was considered as minimum when smaller than 0.25 mm; moderate when between 0.25 mm and 0.5 mm and serious when greater than 0.5 mm.

RESULTS

In Group I, the Ohio Modified Technique, there was a zip formation in 55% of the samples (11 teeth), with 10% of these (2 cases) showing serious zip formation, 25% (5 cases) moderate zip formation and 20% (4 cases) minimum zip formation. As far as the limit of instrumentation is concerned, working length was maintained in 90% of the samples, while 5% (1 case) went beyond and 5% (1 case) were less than expected. The shortening of the working length in 0.55mm corresponds to one of the samples which presented serious zip.There was no record of instrumentation fracture and the average instrumentation time was about 11.92 minutes.

In Group II, the Canal Finder System, the presence

of zip was recorded in 42.10% of the samples (8 teeth), with 26.31% of these (5 cases) presenting minimum zip and 15.79% (3 cases) moderate zip. The was no serious zip in any of the samples.

There was no shortening in the working length and the average time spent in instrumentation was 11.21 minutes. The was fracture of a Set-File #15 in one sample with a serious 32.5° curvature at the cervical level.

In Group III, the automated technique with the TEP-10R (N.S.K.), there was a zip formation in 63.15% of the cases (12 teeth), with 47.37% (9 teeth) presenting minimum zip, 10.52% (2 teeth) moderate zip, and 5.26% (1 tooth) serious zip.

A total of 15.78% (3 teeth) presented a shortening in the working length. The average time of instrumentation was 8.21 minutes.There was fracture of a Flexofile #30 instrument during the echeloning of a sample with a serious curvature of 40° at the apical level.

The incidence and degree of zip formation for the three techniques are shown in figures 1 and 2.

DISCUSSION

The main criteria for evaluating Endodontic mechanical devices are the cleaning and shaping of the walls of the canal and the security shown during bio-mechanical preparation, no occurrence of fractured instruments, ledges, perforations or shortening of the working length [2, 9].

In this study, therewas a high incidence of zip in the three techniques used, which probably occurred due to the fact that the majority of the samples had serious curvature canals. According to various authors [3, 8, 15, 28], curved canals present several factors which make their treatment difficult, and routine preparation produces forms different from those expected, where a funnel occurs in the direction of the apex which, before reaching the foramen, presents a maximum point of constriction, then widening again and forming the so called "zip". CIMIS et al. [3] relate that 46% of the curved canals show various degrees of apical transportation after instrumentation and that all instruments tend to rectify them.

Among the techniques used, the one that presented less zip incidence was the Canal Finder System with manual complementation presenting 42.10%, compared to 55% with the Ohio Modified Technique and 63.15%, with the TEP-10R. This result, in contrast to the findings of several authors who had greater success with hand instrumentation [5, 7, 17], may be explained by the fact that the Canal Finder System was used in conjunction with hand instrumentation (beginning and ending the preparation) and by the use of Set-Files which, being very flexible and having an inactive penetration guide, decrease the incidence of zip [17]. According to SYDNEY et al. [10, 23], the results obtained improved from the moment that it was understood that no automated device is capable of completely substituting hand instrumentation and that it is through the first manually introduced instrument that one gains knowledge regarding the anatomy of the radicular canals.

Another important factor is the proven success in previous studies [5, 20, 23, 25, 29] of the Canal Finder System in curved and narrow root canals, which made up the majority of the samples in this paper. One should bear in mind that instrumentation with this device offers rotational freedom, which allows a more satisfactory accommodation of the instrument in terms of curvature [11, 12, 25].

With the Ohio Modified Technique, in spite of the widening of the middle and cervical thirds with Gates-Glidden drill bits making apical preparation easier, the incidence of zip was very high, corroborating other studies [5, 28] which show that the majority of the hand instrumentation techniques cause apical transportation of the canal.

In Group III, in spite of the use of flexible instruments (Flexofile), the incidence of zip was high due to the deflection memory of the instruments which, along with the ¼ turn alternative rotation movement, tend to rectify themselves in the interior of the canal, which results in the occurrence of deviations or steps [28]. To make things worse, the greater caliper files (#25 and #30) constantly stuck, which often made it necessary to manually use #25 flexofiles to reach the working length.

The differences in the average time of instrumentation recorded for Groups I and II were practically irrelevant. The TEP-10R had a significantly shorter average time of instrumentation than the previous groups, possibly due to the simplified instrumentation and to the "push-button" system of the counter angle which made changing files easier. The Canal Finder System used in the experiment was the ISO, which requires the change of Set-Files with a file remover and the need of manual complementation of the preparation. With the Ohio Modified technique, one alternatively uses files and Gates-Glidden drill bits, which is time consuming.

In spite of using anti-curvature filing [16, 17] in the three groups, an approximation to areas of danger frequently a occurs, fact which was visually larger in the Ohio Modified technique (due to the use of Gates-Glidden drills), followed by the Canal Finder System and, finally, the TEP-10R technique. No tears or perforations were noted in the bifurcation area.

There was a fracture of a Set-File in Group II, which occurred due to the negligence of the operator who forced the instrument in an apical direction. In Group III, there was a fracture of a Flexofile #30, due to it having locked on to one of the canal walls.

CONCLUSIONS

Considering the results obtained from the experiment, we conclude that:

1- The incidence of zip was less with the Canal Finder System, aided by hand instrumentation.

2- The TEP-10R counter angle was more efficient when comparing instrumentation time, however, with the disadvantage of the incidence of zip being very high.

3- There was no remarkable difference in instrumentation times for the Ohio Modified and Canal Finder System techniques.

4- Group III (TEP10-R) presented greater

alterations in the working length.

5-The incidence of zip was high in all three techniques due to the fact that the samples were canals with serious curvature canals.

6- Accented rectifications in the degree of curvature occured in the techniques, but with no record of tears or perforations.

7- Automated instrumentation is a valuable aid in chemical and mechanical preparation, when used in conjunction with hand instrumentation.

Figura1. Incidence ZIP in the three techniques.

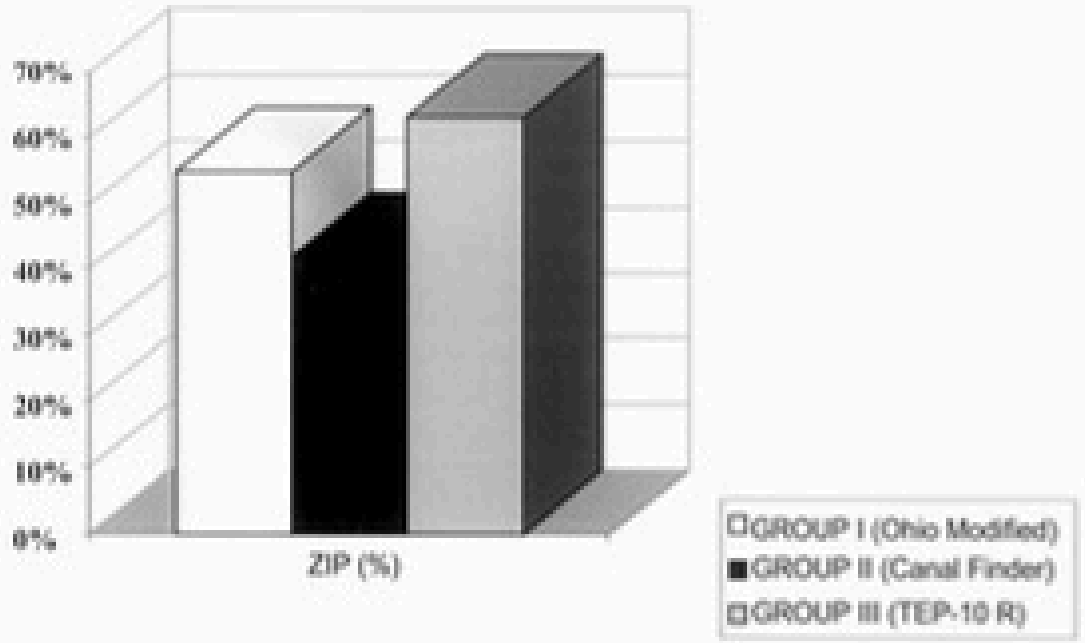

Figura 2. Classification of ZIPformmation in the three techniques.

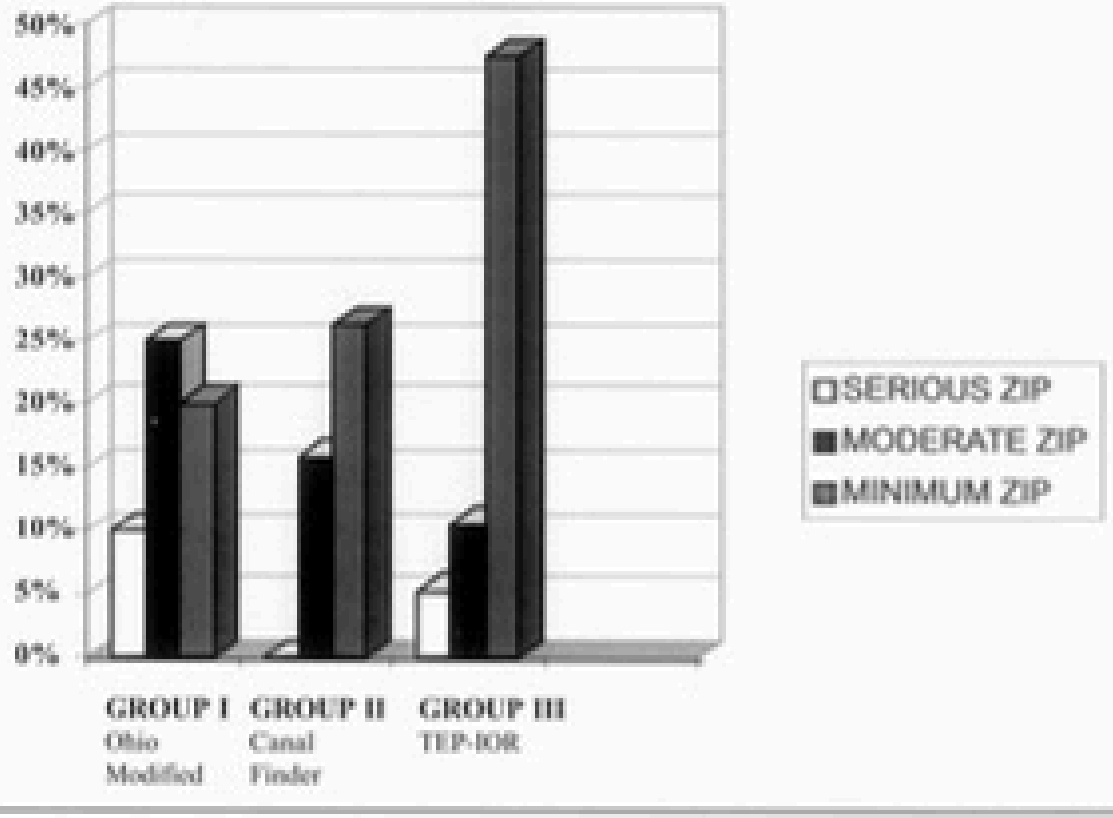

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Table I - Medium qualifyng indexes of radicular curvatures

Index	Group I	Group II	Group III
Location	1.0	1.1	0.8
Angular Magnitude	30.7°	29.3°	29.3°

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EVALUATION OF THE STERILITY OF ABSORBENT PAPER POINTS

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Microorganisms and their products and subproducts play an important role in the etiology and persistence of endodontic diseases. Currently, technical procedures are used to control and maintain the aseptic chain, among them the phase of root canal sealing. The objective of this study was to evaluate the sterility of 96 absorbent paper points of three commercially available brands: Tanari (assorted and individual), Consc and Odahcam. Using sterile forceps, the paper points were removed from sealed packages under a laminar flow hood, immersed in test tubes containing thioglycollate broth and incubated at 37°C for 20 days. Microbial growth was measured daily by the increase of broth turbidity. There was microbial growth with all brands of absorbent paper points tested: assorted Tanari (70.8%), individual Tanari (66.6%), Consc and Odahcam (4.2%). Thus, we conclude that it is necessary to sterilize absorbent paper points before clinical use.

Key words: Absorbent paper points, evaluation of sterility, infection control.

INTRODUCTION

Maintenance of the aseptic chain is essential for successful endodontic treatment[1].

Contaminated dental material used during endodontic treatment can transport microorganisms to the periapical tissues, leading to failure of endodontic treatment[1]. Absorbent paper points have close contact with the walls of the root canal and the apical and periapical regions. Absorbent paper points are also used in microbiological research for the collection of root canal material or to verify antimicrobial activity of solutions or material used in Endodontics[3].

According to Orth[2], the level of microbial contamination depends on the number and type of microorganisms present in the raw materials used. Thus, the microbiological control not only of the final product but also of the raw materials is extremely important.

In Endodontic treatment, it is necessary to evaluate the microbiological quality of all instruments, equipment, and material used. Thus, this study evaluated the level of contamination of 3 different commercial brands of absorbent paper points.

MATERIAL AND METHODS

A total of 96 absorbent paper points, 24 of each brand, were evaluated: assorted Tanari - twelve #15-40 points and twelve #45-80 points; individual Tanari - twenty-four #15-40 points; Consc - twelve #15-40 points and twelve #45-80 points; Odahcam - twelve #15-40 points and twelve #45-80 points. The individual Tanari paper points were enclosed in a sealed package, sterilized by the manufacturer with each number packaged separately.

Using sterile forceps, all paper points were removed from the sealed packages under a laminar flow hood, immersed in test tubes, sealed with a cork, containing approximately 18 ml thioglycollate broth (Difco). These were incubated at 37°C for 20 days and microbial growth was measured daily by the increase of broth turbidity.

RESULTS

Table 1 presents the number and percent of microbial growth of the absorbent paper points.

Table 1 . Level of contamination of 96 absorbent paper points (24 of each brand).

Microbial growth	Tanari (assorted)	Tanari(individual)	Consc	Odahcam
Positive	17 (70.8%)	16 (66.6%)	1 (4.2%)	1 (4.2%)
Negative	7 (29.2%)	8 (33.4%)	23 (95.8%)	23 (95.8%)

DISCUSSION

Absorbent paper points are used for drying the root canal before the application of dressings or before filling. It is expected that the root canal and the periapical region has already been disinfected. It is known that absorbent paper points retain microorganisms[4] and that these are related to endodontic treatment failure. Thus, these paper points must be sterile in order to maintain asepsis of the root canal and the periapical tissues.

In this study we verified that all of the brands tested presented contamination in different percentages. Only the packages of the individual Tanari points contained information concerning sterility. However, these Tanari points presented 66.6% contamination.

Pimenta et al.[5] evaluated the contamination of the following brands of absorbent paper points: Tanari and individual Tanari, Dentsply and individual Dentsply, individual DiaDent and Consc, using thioglycollate broth and incubation at 37°C for 7 days. They verified bacterial growth of 70% for the individual Tanari points and 85%

for the individual Dentsply points. However, no bacterial growth was observed for DiaDent and Conne paper points. Thus, they report the need to sterilize absorbent paper points before clinical use. Our results were similar to their results.

Manufacturers generally do not provide information concerning how absorbent paper points are sterilized after manufacture. Möller et al.[3] reported the presence of formaldehyde in absorbent paper points. Formaldehyde, used for the sterilization of absorbent paper points during the manufacturing process, may remain in the points providing antimicrobial activity[1,4,5] and reducing the possibility of microbial growth[6,7]. However, it has been observed that formaldehyde can cause, *in vitro*, cytotoxicity and hemolytic activity, which can negatively influence the integrity and process of healing of the periapical tissues[8]. In 1966, Möller[3] reported that formaldehyde can be transferred from paper points used for collection of material to the culture media leading to false-negative results due to inactivation of microorganisms present on the paper points. In this study, even though the absorbent paper points presented incorporated chemical agents, this was not sufficient to prevent microbial growth.

Seeking an efficient and safe manner to sterilize absorbent paper points, Holland et al.[1] evaluated the response of subcutaneous connective tissue of rats to absorbent paper points sterilized either in a sterilizer or with formaldehyde tablets for 7 and 30 days. A less intense inflammatory reaction from the points sterilized with formaldehyde tablets compared to the sterilizer at 7 days was found. This initial difference can be attributed to the fixing action of the formol vapors with the paper fibers, contributing to less diffusion in the tissues of irritating elements. At 30 days, the results were similar in both groups.

In this investigation, the need to sterilize absorbent paper points has been proved. Thus, it is indispensable to verify the sterility of absorbent paper points sterilized by the manufacturer when used in endodontic clinics or in microbiological research. Sterilization using an autoclave has been shown to be efficient and safe to control the microbiological quality of absorbent paper points.

CONCLUSIONS

1. Odahcam and Conne absorbent paper points presented the least contamination (4.2%).

2. Individual Tanari paper points, which according to the manufacturer were sterilized, were contaminated in 66.6% of the samples.

3. Independent of the commercial brand, sterilization of absorbent paper points is necessary when used in dental clinics or in microbiological research.

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TISSUE DISSOLUTION ABILITY OF SODIUM HYPOCHLORITE FROM DIFFERENT MANUFACTURERS

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The aim of this study was to investigate the dissolution time of sodium hypochlorite from different manufacturers on "Golden Sirius" Hamsters' tongues simulating pulp tissue. The results showed that the dissolution ability of sodium hypochlorite was directly related to the concentration of chlorine in the solution, thus 5% sodium hypochlorite concentrations exhibited faster dissolving ability when compared to 1% and 0.5% NaOCl solutions. Same samples with equal concentrations, however, displayed different results. This may be due to factors such as storage and stability.

Key words: Dissolution, sodium hypochlorite, pulp tissue.

INTRODUCTION

In Endodontics there are no differentiated treatment phases. Neglecting any of the endodontic therapy steps will ultimately lead to treatment failure. Undoubtedly, however, cleaning and shaping plays a significant role. Sodium hypochlorite is one of the most widely accepted chemical solutions, being advocated by BLESS, applied by WALKER and fully diffused by GROSSMANN[1,3,4,16].

Organic remnants, inorganic debris and microorganisms can be removed by irrigation-aspiration procedures thus creating a clean root canal for filling material.[6]

What makes NaOCl a globally accepted substance is its comprehensive range of properties, such as:

- Low surface tension decrease, i.e. an increase of sodium hypochlorite action as a wetting agent, forwarded by contact chemical reactions which account for cleaning and disinfection.[1,2]

- Bactericidal ability and neutralization of toxic elements[3,4]. Sodium hypochlorite in solution breaks into hypochlorous acid and sodium hydroxide. Sodium hydroxide accounts for the high alkalinity and hypochlorous acid liberates rising chlorine[13] binding with degraded proteins to form chloramines and, secondarily, soaps by fat contamination[3,10,13,16].

GROSSMANN and MEIMANN[7] report that sodium hypochlorite is a pulp tissue solvent. Under normal clinical conditions, however, its usage has been indicated mainly in unpulped teeth.

SCHILDER and AMSTERDAN[19], examining chlorine soda on the connective tissue of a rabbit's eye, observed a mild inflammatory response after 24 hours. Some authors[11,14,15], using sodium hypochlorite at different concentrations and associations, found that a 0.5% solution was less irritating to the dental pulp and periapical tissues. SANT'ANNA[18] analyzed histomorphologically the response of apical and periapical tissues to 0.5% sodium hypochlorite solution in dog's teeth to be. NOLETO[12] compared the response to sodium hipochlorite at different concentrations in dog's teeth by applying it topically for 72 hours and 7 days. He found 1% sodium hypochlorite to be partially aggressive, whereas 4.8% sodium hypochlorite caused large tissue necrosis. SIMÕES et al.[20], using Torneck's method, examined the biocompatibility of 0.5% and 1.0% sodium hypochlorite solutions with test times of 24, 72 and 168 hours and observed a progressive regression of the inflammatory infiltrate after 72 and 168 hours. With regard to pulp tissue dissolution time, COHEN et al.[5] point out that tissues in the deterioration process are more easily dissolved than more intact tissues. Accordingly, cleaning procedures in root canals with vital pulps should last longer to enable greater contact time between the tissue and sodium hypochlorite.

MILANO et al.[11] observed that the tissue dissolution time of pulp "in vitro" in different concentrations of sodium hypochlorite varies from twenty minutes to two hours. ANDERSEN et al.[1] showed that 2% sodium hypochlorite fostered the dissolution of half the pulpal volume in one hour and the remaining tissue in approximately 2 hours. In necrotic tissues, HAND et al.[8] found no significant differences between 1.0% and 0.5% NaOCl, but at 5.25% the solvent effect was more efficient.

The aim of this study was to compare tissue dissolution time of different concentrations of sodium hypochlorite using several trademarks by drawing an analogy between the pulp connective tissue and the connective tissue of the Hamster's tongue.

MATERIAL AND METHODS

Two Golden Sirius hamsters were used in this study. Following anesthesia by administering a subcutaneous injection with 0.1 ml Zoletil 50® (Virbac) on the dorsal part of the animals, their tongues were removed and sectioned using a #15 blade fixed on a #4 scalpel. The two sectioned tongues were then cut into 7 fragments 10 mm long by 2 mm wide simulating pulp tissue.

Different concentrations of commercially available sodium hypochlorite were used in this study (Table 1). Each 0.5 ml of solution was kept in a glass flask previously sterilized, totaling seven flasks. Each tissue fragment was placed in the flasks with the respective solution and covered with a flask's lid. The tissue dissolution time was measured using a stopwatch for each chemical substance. Verification

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time was limited to 48 hours.

RESULTS

Table 1 shows the results of this experiment

Table 1- Dissolution Times of the Samples Examined

Sample	Commercial Name and Manufacturer	Dissolution Periods
1	Dakin Solution (0.5%) - Bellafarma	Over 48 hours
2	Milton Solution (1%) - Inodon	13 hours 20 min
3	Milton Solution (1%) - ULBRA*	2 hours 45 min
4	Milton Solution (1%)-Merrel-Lepetit	12 hours
5	Milton Solution (1%)- Iodontec	2 hours
6	Chlorine Soda (5%)- Inodon	1 hour 40 min
7	Chlorine Soda (5%)- Calendula**	over 48 hours

*Pharmaceutical Laboratory at ULBRA. ** Calendula Manipulation Pharmacy.*

Concentration reported by manufacturer given in parentheses.

DISCUSSION

SIMÕES et al.[20] concluded that both Milton Solution and Dakin Solution have bactericidal ability. However, 1.0% sodium hypochlorite was shown to be more efficient on bacteria found in infected root canals than 0.5% sodium hypochlorite.

MILANO et al.[11] studied the "aging process" (decrease in chlorine concentration) of these solutions. They reported that the chlorine concentration of the solution was directly related to the loss of this element. In addition, they observed a greater decrease in concentration during summer months due to high temperatures. Another highly significant factor is the presence of a stabilizer. In their study they found that sodium chlorite (NaCl) did not act as an efficient stabilizer. We assume that an increase in pH would stabilize the solution. ANDERSEN et al.[3], aiming at creating a canal free of pulp remnants before obturation, reported using sodium hypochlorite as an irrigating solution and calcium hydroxide as a dressing during canal preparation.

HAND et al.[7] confirmed the ability of different concentrations of sodium hypochlorite at to dissolve necrotic tissues compared to saline, water, distilled water and oxygenated water at 3.0%. They found no significant difference between 1.0% and 0.5% NaOCl. Statistical analysis indicated that 5.25% sodium hypochlorite resulted in a significant ability to dissolve necrotic tissues.

This study shows that sodium hypochlorite containing a high chlorine concentration and a low concentration within the root canal after chemo-mechanical preparation should present a more intense tissue degradation.[7]

In this study sample 1 did not totally dissolve the connective tissue within the pre-determined time (Table 1), thus confirming that solutions at lower concentrations have a limited tissue dissolution ability. This is in agreement with several other studies[9,11,14,15,18,20]. The manufacturer reported sample 7 to have a 5% concentration of active chlorine, but it was not able to dissolve the tissue under the pre-determined time. We assume that this may be due to factors such as: storage time and quality, manipulation and absence of stabilizers in the sodium hypochlorite solution that may affect both its concentration and stability [14].

Solution sample 6 agrees with previous studies reporting that the dissolution of organic matter is directly related to concentration[11]. The similar results of samples 3 and 5 completely dissolving the tissue in 2 h 45 min and 2 hours, respectively, seem to agree with the results found by ANDERSEN et al.[3]. Samples 2 and 4 dissolved the organic tissue in 13 h 20 min and 12 hours, respectively, a period of time longer than samples 3 and 5, although all solutions presented identical 1.0% concentrations. This result suggests the above-mentioned factors may significantly alter the properties of sodium hypochlorite.

By analogy, it can be assumed that pulp tissue dissolves more quickly than the tissue in this study because pulp is formed by less fibrous connective tissue than the Hamster's tongue connective tissue.

CONCLUSIONS

The following conclusions can be made from the experimental results:

1. Sodium hypochlorite dissolves the connective tissue of Golden Sirius Hamster's tongues.

2. Tissue dissolution ability is directly related to sodium hypochlorite concentration.

3. Sodium hypochlorite solutions at 0.5% concentration present a limited ability to dissolve the connective tissue of hamster's tongues.

4. It can be assumed that factors affecting both the concentration and stability of sodium hypochlorite cause varying results when different trademarks of this product were used.

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EFECT OF CALCIUM HYDROXIDE P.A. ON HEALING AND MAST CELL POPULATION OF RATS

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Using light microscopy, morphologic and quantitative analyses of the mast cell population in the subcutaneous connective tissue of Wistar rats due to calcium hydroxide effects were carried out as well as morphologic analysis of wound healing evolution at 2, 7, 15 and 30 post-operative days. Morphologically, there were 2 distint forms of mast cell population: elongated-shaped mast cells detached in the superficial layers of the connective tissue, while in the deeper layers, round or oval-shaped cells prevailed. Quantitatively, mast cell population of the experimental group decreased on the 2nd post-operative day, increased subsequently and reached a maximum value at the 15th post-operative day. In the group control, mast cells decreased on the 2nd post-operative day and reached a maximum at the 7th post-operative day. However, statistical evaluation indicated that there were no significant differences. Wound healing developed normally among the groups. Calcium hydroxide caused tissue necrosis, calcification of the muscle fibers and formation of mineralized tissue. These data suggest that calcium hiyroxide does not interfere with the wound healing and mast cell population of rat connective tissue.

Key Words: Calcium hydroxide, wound healing, mast cell

INTRODUCTION

Inflammation is a response of living tissue to injury, through a series of vascular, cellular and neurologic events, in order to destroy or immobilize the aggressor agent with a subsequent cure and restoration of the injured tissue[1,6,23]. Among the cellular types affected by inflammation, the mast cell, a connective tissue cell, plays a relevant role in the inflammatory and reparation processes. This cell exhibits its cytoplasm completely filled with innumerable secretory granules that store various biologically active chemical mediators, most notably histamine, a potential amine acting during the initial phases of inflammation[4,13,23]. Independent of the intensity of the inflammatory reaction, local tissue injuries occur and need to be repaired. Sometimes, inflammation is so intense that it becomes a potentially harmful reaction, which justifies the use of anti-inflammatory drugs.

In this context, calcium hydroxide is a chemical substance extensively used in odontologic procedures, principally for protection of the pulp tissue, with reparative and anti-inflammatory actions[8,11,12,26,27]. Thus, because of its wide use and principally its use to treat inflammatory pulp and periapical lesions by endodontists[3,9-12,25,26,27], we proposed to study wound healing and the mast cell population in rat connective tissue with the use of calcium hydroxide P.A. after surgical trauma.

MATERIAL AND METHODS

In this study, 40 Wistar male rats were randomly divided into two groups of 20 each: an experimental group, that received calcium hydroxide P.A. and another group (control group) that was only submitted to the surgical procedure. Each group was then subdivided into 4 subgroups with 5 animals each, according to the time after surgical procedure until sacrifice, corresponding to 2, 7, 15 e 30 post-operative days.

Following anesthesia, depilatory and disinfection procedures under the median line of the third on the dorsal region of each animal were carried out, a linear incision including skin and deeper tissues was made. The experimental group received approximately 0.015 mg calcium hydroxide P.A. into the wound and, subsequently, the wound was closed by suture. Animals were kept in cages, and fed appropriate food and water *ad libitum*.

By light microscopy, morphologic evolution of the tissue reaction to calcium hydroxide P.A. was evaluated on hematoxylin and eosin-stained slides. Quantitative evaluation of the mast cells was done on toluidine blue-stained slides. Mast cells were counted in 40 successive histologic fields at the epithelium-conjunctive tissue direction, 20 histologic fields where the substance was set and 20 histologic fields at the adjacent regions in order to observe if calcium hydroxide produced **an away effect.**

RESULTS

Morphologic results

1) Hematoxylin and eosin technique

At the 2nd post-operative day, in the experimental group tissue fragments showed a detached epithelial layer at the incision region. Areas of tissue necrosis and intense neutrophil polymorphonuclear inflammatory infiltrate were found in the connective tissue. Calcified muscle fibers were an interesting finding. At the 7th post-operative day, epithelium was restored and connective tissue showed a chronic inflammatory infiltrate, multinucleated giant cells, amorphous and poorly basophilic material (calcium hydroxide), abundant mineralized foci and calcified muscle fibers. Fifteen days afterthe surgical procedure, tissue fragments still exhibited connective tissue moderately infiltrated by mononuclear inflammatory cells, deposition of collagen fibers, young fibroblasts, giant cells, foci of mineralized tissue and calcified muscle fibers. Only on the 30th post-operative day, tissue specimens showed complete wound healing, in spite of focal areas of dystrophic mineralized tissue deep in the specimens.

Evolution of wound healing in the control group included the same phases and aspects observed in the experimental group, except there were giant cells and foci of calcified tissue in the control group.

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2)Toluidine blue technique

This type of staining detaches the metachromatic capacity of the mast cells from the tissue, which become purple cells on the poorly blue histologic fields. In all analyzed slides (control and experimental groups), mast cells located on the superficial layers of the connective tissue were elongated, while mast cells in the deeper layers were more abundant and round or oval. In general, these cells were grouped and located next to blood vessels and fat tissue.

Quantitative results of the mast cells population

The mast cell population of the experimental group showed numeric change at all times. On the 2nd post-operative day, the population of these cells exhibited the smallest numbers (43.6 mast cells/field), which increased continuously until reaching a maximum peak of 92.2 mast cell/field. This cell population did not exhibit any further change, because at the 30th post-operative day, the number of mast cells/field was 85.6 (Table 1). The control group also showed different values in the number of mast cells at the 2nd, 7th, 15th and 30th post-operative days: 57, 98.4, 88.4 and 54.8, respectively (Table 2). The scores of the areas adjacent to calcium hydroxide P.A. revealed that the mast cell population in these regions was always greater than one, excluding the values at the 15th post-operative day experimental group and the 7th post-operative day control group, which, showed smaller averages (Tables 3 and 4). In spite of these data showingt differences among the studied groups and periods, statistical analysis revealed that mast cell number did not differ significantly between experimental and control groups at the 2nd, 7th, 15th and 30th days after the use of calcium hydroxide P.A..

DISCUSSION

The animal organism is able to protect itself from physical, chemical and biological injuries by a diversity of biochemical reactions that occur, basically, in the vascular system, connective tissue and blood, aiming to eliminate any aggressor agent. After this, the defense process, called inflammation, repairs the tissue damage caused by injury and organism reactions, through another series of reactions that occur leading to wound healing. During these processes, there are many cells and chemical mediators involved. In inflammation, the mast cell plays na important role by releasing potential chemical substances, such as histamine and serotonine, which dilate blood vessels and increase vascular permeability[3,4,6,21]. These phenomena represent essential stages of inflammation.

In this study, we verified the effect of calcium hydroxide P.A., surgically placed into the rat connective tissue, on the local and adjacent mast cell populations, and, how wound healing occured after the surgical procedure at the 2nd, 7th, 15th and 30th post-operative days.

At the 2nd day, experimental and control groups exhibited inflammatory infiltrate, where polymorphonuclear neutrophils prevailed and no collagen fibers deposition was observed. These results corroborate the work performed by ROSS and ODLAND[17], who verified these inflammatory cells appearing in wound healing 3 hours after injury and surviving for 48 a 72 hours. According to DeVITO[7], collagen fibers deposition only occurs between the 5th and 15th day. At the 7th and 15th post-operative days, we observed poor and moderate collageneous deposition, respectively. Mononuclear inflammatory cell infiltrate appeared at the 7th day, changing its intensity as time elapsed in both groups. Epithelial layer was interrupted at the 2nd day; nevertheless, a proliferation on the epithelial edges was observed at this time. ODLAND and ROSS[17] reported this proliferation beginning at 24 to 48 hours, corresponding to our results. Complete wound healing in all groups occurred at the 30th post-operative day. Thus, there were not changes in wound healing in the rat, with the use of calcium hydroxide P.A. However, some additional findings which did not interfere with wound healing but which occurred in the experimental group due to calcium hydroxide P.A. At the 2nd post-operative day, foci of mineralization were observed in some muscle fibers, which became more evident and abundant at the 7th day. In this period, numerous giant cells showing fragments of calcium hidroxyde and mineralized material were seen and persisted at the 15th and 30th days. MITCHELL[16] observed pathologic calcification in muscle tissue and giant cells. There are several studies that show calcification areas in tissue due to calcium hydroxide[4,13,28]. Only, TOLEDO et al.[26] did not find this. Therefore, calcium hydroxide induces calcification in tissue, consequently confirming the extensive use of this substance in periapical and pulp tissues[5, 8-12, 18, 225, 28].

There are no reports of the effect of calcium hydroxide P.A. on the mast cell population in subcutaneous connective tissue of rats. Nevertheless, SWIETER et al. [24] , studying mast cell behavior in culture, reported that cells undergo changes because of microenvironmental factors, modifing their role in inflammation. Thus, we proposed to study the influence of calcium hydroxide P.A. on this cell. As repoeted by CARRANZA and CABRINI[2] CORMACK[6] PARODI and DOMINGUEZ[19], and ROBINSON and DE MARCO[20], there are morphologic variations of this cell according to the depth of the connective tissue. In the superficial layers, fusiforms or elongated mast cells are more abundant, while in the deeper layers round and oval-shaped cells prevail. These morphologic variations were also observed in our study.

There is a decrease in the mast cell population at the begining of the inflammatory process, due to expressive degranulation of these cells, delivering several chemical substances and starting the initial inflammatory response. As the aggressor agent is eliminated and wound healing occurs, mast cell population is recovered. Many agents cause degranulation of these cells, that is regulated by free calcium concentration in the cytoplasm and metabolic energy quantity from glucolytic and oxidative processes.

According to ROBINSON and DE MARCO[20], an increase in the inflammation of the gums is followed by a decrease of the mast cell population. PARODI and DOMINGUEZ[19], studying mast cell behavior in the wound healing of the gums after a surgical procedure, concluded that there is an important decrease of these cells at the 10 th day; but, as wound healing progresses, the number of this cell increases, recovering by 30 days. In this study, both experimental and control groups showed a decrease and na increase in mast cells, however, there were no statistical differences. This substance did not cause influence any effect on the areas adjacent to the trauma.

SOUZA et al.[15] report that calcium hydroxide P.A. produces an antiinflammatory effect when in contact with tissue due to: 1) higroscopic action; 2) calcium proteinate bridges formation; 3) phospholipase enzyme inibition and 4) neutralization of acids products. VAZQUEZ[17] affirms that this substance exhibits an elevated concentration of calcium ion, which decreases capillary permeability and, consequently causes an antiexudative effect, aiding wound healing and decreasing post-operative pain.

Based on these results, we verified that calcium hydroxide P.A. does not influence wound healing in the rat, nor does it interfere with the quantity of the mast cells, as observed by CAVALCANTI[3] using corticosteroids in her studies. Thus we suggest other possible mechanisms for this probable antiinflammatory effect of this substance, such as those already proposed by SOUZA et al.[15] and VAZQUEZ[17].

CONCLUSION

1. Calcium hydroxide P.A., in contact with subcutaneous connective tissue of the rat, induced foci of pathologic calcifications. Nevertheless, wound healing occurred normally.

2. The mast cell population at the adjacent and incised areas of Subcutaneous connective tissue did not show any morphologic and quantitative changes with calcium hydroxide P.A.

TABLE 1 - Total number and averages of mast cells in 20 histologic fields at the incised area of the experimental group, at the times studied.

1	2	3	4	5	Total	Average
52	50	55	47	14	218	43.6
50	38	69	52	27	236	47.2
171	100	19	133	38	461	92.2
56	74	46	50	202	428	85.6

Table 2 . Total number and averages of mast cells in 20 histologic fields at the incised area of the control group, at the times studied

1	2	3	4	5	Total	Average
31	49	42	89	74	285	57
57	36	34	242	123	492	98.4
40	131	53	79	139	442	88.4
41	44	20	60	109	274	54.8

Table 3 . Total number and averages of mast cells in 20 histologic fields in the area adjacent area to the incision of the experimental group, at the times studied.

1	2	3	4	5	Total	Average
93	57	78	60	42	330	66
82	33	79	81	98	373	74.6
144	94	12	129	60	439	87.8
82	66	71	57	164	440	88

Table 4 . Total number and averages of mast cells in 20 histologic groups at the area adjacent to the incision of the control group at the times studied.

1	2	3	4	5	Total	Average
74	81	87	87	97	426	85.2
99	30	38	151	101	419	83.8
115	131	91	86	140	563	112.6
24	88	16	83	109	320	64

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**Title page:* The title should be short and clear. The authors full names, highest academic degrees and institutional affiliations should be on this page. A separate page with the name and mailing address of the corresponding author and a suggested running title of three to four words (no more than 40 spaces) should be added.

**Abstract page:* This separate page must not exceed 200 words and must include a concise account of exactly what was done, the results and conclusions.

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a. Thirty references are the maximum permissible. Case reports and short papers should not exceed 20 references.

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c. References follow the style of the Journal, as follow:

one author: ESTRELA [1]

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Journal Articles:

1. ESTRELA C, SYDNEY GB, BAMMANN LL, FELIPPE JÚNIOR O. Mechanism of the action of calcium hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J 6(2): 1-9, 1995.

2. HOLLAND R, SOUZA V, MELLO W: The effect of the filling material in the tissue reations following apical plugging of root canals with dentin chips. **O Surg O Med O Pathol 55:** 398-401, 1983.

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Books:

1. PAIVA JG, ANTONIAZZI JH. Endodontia: Bases para a prática clínica. 2nd. ed. Artes Médicas, São Paulo, 495p, 1988.

Chapter in a book:

1. TROPE M, CHIVIAN N. Root resorption. In: COHEN S, BURNS RC. Pathways of the pulp. 6th ed. Mosby, St. Louis, p.486-512, 1994.

Thesis:

1. SUNDQVIST G. Bacteriological studies of necrotic dental pulps. Thesis, University of Umea, 94p, 1976.

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NEWS AND ANNOUNCEMENTS

01- On August 8, 1997, Dr. M.D. SOUSA NETO obtained his PhD degree at the University of São Paulo-Ribeirão Preto- Brazil, after submeting his these entitled: "Influence of different kinds of resins and hydrogenated resins on the flow rate and film thickness of Grossman cements ", to examining board composed with the following members: Professors Paulo Sapuy, Hidelberto Pesce, Jesus Djalma Pécora, Luiz Fernando Guimarães (University of São Paulo-Ribeirão Preto) and Carlos Estrela (Federal University of Goiás). Professor Pécora was the advisor and the candidate was aproved with grade 10.

From left to right: Professor SAQUY, PESCE, PÉCORA, GUIMARÃES and ESTRELA.

02- Last November 4-7, 1997, Professor CARLOS ESTRELA got his DOCENCE at the University of São Paulo-Ribeirão Preto-Brazil. His These entitled: " Antimicrobial efficacy of calcium hydroxide pastes " to the examining board composed with the following members: Professors VALDIR DE SOUZA (Estadual University Paulista, Araçatuba-Brazil), IZABEL Y. ITO (University of São Paulo-Ribeirão Preto-Brazil), LILI L. BAMMANN (Federal University of Pelotas-Brazil), Jesus Djalma Pécora (University of São Paulo-Ribeirão Preto-Brazil) and Ricardo Garriba (University of São Paulo-Ribeirão Preto-Brazil). The candidate was aproved with grade 10.

From left to right: Professor SOUZA, ITO, BAMMANN, PÉCORA and GARIBA.

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03- Last October, 1997, Professor Carlos Estrela (Federal University of Goiás-Brazil), ministrade a Endodontic Course at the Seminário International of the Universidad de Guadalajara, Jalisco, México.

From left to right: Professor ÁDAM, CARLOS ESTRELA and ÁLVARO CRUZ.

4- Last October, 1997, the Federal University of Goiás promoverd the Symposium of Dental Restorative Material Biocompatibility, at the II Congress Goiano Universitary of Dentistry. The members that participated the simposium were: Professor A.L.S. BUSATO (Federal University of PELOTAS-Brazil), C.A. SOUZA COSTA (Estadual University Paulista-Brazil), C. ESTRELA (Federal University of Goiás-Brazil). The ativators were: Professor E.F. MENDONÇA and A.H.G. ALENCAR.

From left to right: Dr. E.F. MENDONÇA, A.L.S. BUSATO, A.H.G. ALENCAR, C. ESTRELA and C.A. SOUZA COSTA.

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10º CONGRESSO INTERNACIONAL DE ODONTOLOGIA DE GOIÁS

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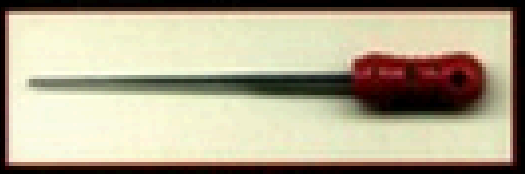

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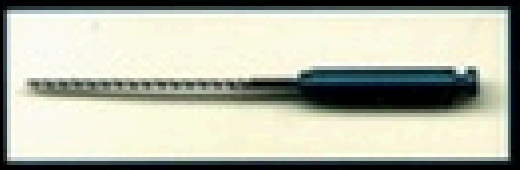

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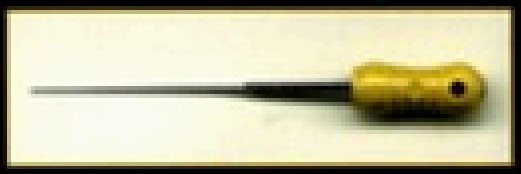

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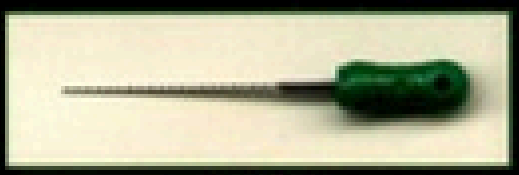

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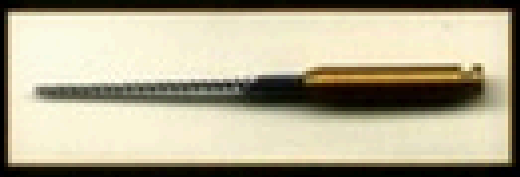

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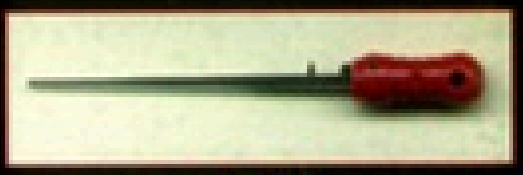

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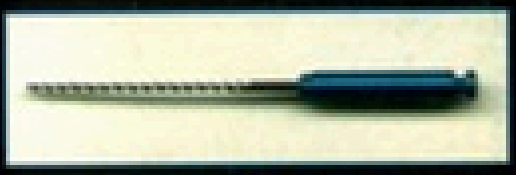

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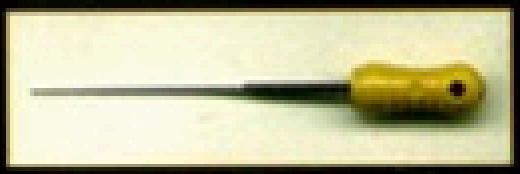

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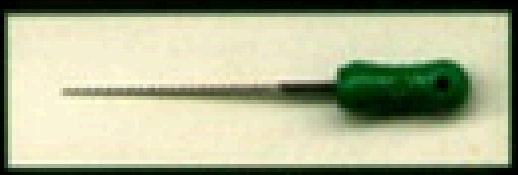

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