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POLÍTICA EDITORIAL

El objetivo de **Acta Odontológica Latinoamericana** (AOL) es ofrecer a la comunidad científica un medio adecuado para la difusión internacional de los trabajos de investigación, realizados preferentemente en Latinoamérica, dentro del campo odontológico y áreas estrechamente relacionadas. Publicará trabajos originales de investigación básica, clínica y epidemiológica, tanto del campo biológico como del área de materiales dentales y técnicas especiales. La publicación de trabajos clínicos será considerada siempre que tengan contenido original y no sean meras presentaciones de casos o series. En principio, no se aceptarán trabajos de revisión bibliográfica, si bien los editores podrán solicitar revisiones de temas de particular interés. Las Comunicaciones Breves, dentro del área de interés de AOL, serán consideradas para su publicación. Solamente se aceptarán trabajos no publicados anteriormente, los cuales no podrán ser luego publicados en otro medio sin expreso consentimiento de los editores.

Dos revisores, seleccionados por la mesa editorial dentro de especialistas en cada tema, harán el estudio crítico de los manuscritos presentados, a fin de lograr el mejor nivel posible del contenido científico de la revista.

Para facilitar la difusión internacional, se publicarán los trabajos escritos en inglés, con un resumen en castellano o portugués. La revista publicará, dentro de las limitaciones presupuestarias, toda información considerada de interés que se le haga llegar relativa a actividades conexas a la investigación odontológica del área latinoamericana.

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Although **Acta Odontológica Latinoamericana (**AOL) will accept original papers from around the world, the principal aim of this journal is to be an instrument of communication for and among Latin American investigators in the field of dental research and closely related areas.

AOL will be devoted to original articles dealing with basic, clinic and epidemiological research in biological areas or those connected with dental materials and/or special techniques.

Clinical papers will be published as long as their content is original and not restricted to the presentation of single cases or series.

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Short communications which fall within the scope of the journal may also be submitted. Submission of a paper to the journal will be taken to imply that it presents original unpublished work, not under consideration for publication elsewhere.

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IN MEMORIAM

Prof. Dr. Carlos Eduardo José Bozzini
(1932 -2017)

We deeply regret to announce the passing of Dr. Eduardo Carlos Bozzini, Associate Editor or our journal, on March 24.

For over thirty years, Dr. Bozzini, in collaboration with Dr. Cabrini, drove the idea of creating Acta Odontológica Latinoamericana with the aim of promoting publication of research in Latin America. Since then, he continuously cooperated with editing and publishing papers.

Dr. Bozzini was a Doctor in Dentistry, Buenos Aires University (UBA, 1957) and Doctor in Biological Science (UBA, 1991). After graduating he did full-time teaching and research. He was Full Professor of Physiology at the UBA School of Dentistry from 1971 to 1998, after which he continued as Professor Emeritus. He had a permanent researcher position (Carrera del Investigador) with the Argentine National Research Council (Consejo Nacional de Investigaciones, CONICET), attaining the position of Senior Researcher.

He was distinguished among the Great Masters at UBA and Master of Dentistry by the Argentine Dental Association (Asociación Odontológica Argentina).

His numerous scientific activities, including approximately 200 published papers, direction of over twenty PhD theses and participation in conferences, symposiums and round tables in Argentina, USA, Peru, Bolivia, Austria, Germany, France and England, brought major international prestige to the Department of Physiology and the School of Dentistry in the context of his main line of work: regulation of erythropoiesis and high altitude physiology.

We will always remember him as a great scientist, collaborator and friend.

Con profundo pesar comunicamos el fallecimiento del Dr. Eduardo Carlos Bozzini, Editor Asociado de nuestra revista, ocurrido el 24 de marzo.

Hace ya algo más de treinta años, el Dr. Bozzini, junto con el Dr. Cabrini, impulsó la idea de crear Acta Odontológica Latinoamericana, con el fin de promover la publicación de los trabajos de investigación realizados en Latinoamérica. Desde entonces, ha colaborado siempre en la edición y revisión de trabajos.

Bozzini era doctor en Odontología, Universidad de Buenos Aires (UBA, 1957) y doctor en Ciencias Biológicas (UBA, 1991) Desde su graduación, se dedicó a la docencia y a la investigación con dedicación exclusiva. Fue Profesor Titular de Fisiología de La Facultad de Odontologia, UBA, entre los años 1971 y 1998, continuando luego sus tareas como profesor Emérito. Fue miembro de la Carrera del Investigador del Consejo Nacional de Investigaciones (CONICET), alcanzando la posición de Investigador Superior.

Fue distinguido entre los Grandes Maestros de la UBA y como Maestro de la Odontología por la Asociación Odontológica Argentina.

Sus numerosas actividades científicas, entre las que se destacan aproximadamente 200 publicaciones de trabajos, mas de una veintena de tesis doctorales dirigidas y participaciones en conferencias, simposios y mesas redondas en Argentina, Estados Unidos, Perú, Bolivia, Austria, Alemania, Francia e Inglaterra, colocaron a la Cátedra de Fisiología y a la Facultad, en una importante posición de prestigio internacional en el ámbito de su principal línea de trabajo: Regulación de la eritropoyesis y fisiología de la altura.

Lo recordaremos siempre como un gran científico, colaborador y amigo.

Effect of silver diamine fluoride (SDF) on the dentin-pulp complex. *Ex vivo* histological analysis on human primary teeth and rat molars

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ABSTRACT

The aim of this study was to determine the effect of SDF on the dentin-pulp complex using two models: teeth after SDF application (ex vivo) and experimental animal molars. A descriptive study was performed using two models. In the first model, primary teeth (ex vivo) with enamel-dentin caries, without pulp involvement and previously treated with 38% SDF, were evaluated by means of two techniques: (a) Scanning Electron Microscopy (SEM) and energy-dispersive X-ray detector (EDS) to determine qualitative and quantitative composition, and (b) brightfield optical microscopy (OM) after decalcification. The second model used laboratory animal molars from 12 male Wistar rats. Standardized enamel-dentin cavities approximately 0.5 mm deep were made the distal fossa of the occlusal face of both first lower molars, to one of which a 38% SDF solution was applied, while the other was used as a control. Histological sections were prepared and dental pulp was evaluated qualitatively in both groups. SEM on ex vivo teeth showed areas of hypermineralization in the intertubular dentin and few blocked tubules, while EDS detected Ag in the center of the lesion (7.34%), its concentration declining at the edges (1.71%), with none in the areas farthest from the lesion. OM showed SDF sealing the tubules only at the site where it had been placed, with limited penetration beneath, the tubules appeared normal and the pulp tissue associated to treated caries showed chronic inflammatory infiltrate and formation of tertiary dentin, with no Ag precipitate. In the experimental animal model, pulp histology was not significantly altered in the molar cavities exposed to SDF. The observations using the different techniques on dental tissues suggest that SDF causes minimal adverse effects. The results of this study may contribute to further studies on the suitability of SDF as a cost-effective strategy for treating caries.

Key words: *Dental caries; fluorides; silver diamine fluoride.*

Efecto del diamino fluoruro de plata (DFP) sobre complejo dentino-pulpar. Análisis histológico *ex vivo* en dientes primarios humanos y molares de rata

RESUMEN

El objetivo del trabajo fue determinar del efecto del DFP en complejo dentino-pulpar aplicando dos modelos: piezas dentarias luego de su aplicación (ex vivo) y en molares de animales experimentales. Se realizó un estudio descriptivo aplicando dos modelos: en piezas dentarias primarias (ex vivo) con caries amelodentinarias sin compromiso pulpar que hayan sido sometidas previamente con DFP 38%, mediante dos evaluaciones: Microscopía electrónica de Barrido (MEB) y detector de energía dispersiva de rayos X (EDS) a fin de determinar su composición cuali y cuantitativa y Microscopía óptica de campo claro (MCC) mediante la técnica descalcificación y en molares de animales de laboratorio donde se utilizaron 12 ratas Wistar macho. La técnica fue estandarizada en la fosa distal de la cara oclusal del primer molar inferior, se realizó una cavidad amelodentinaria aprox. 0.5 mm de profundidad, en ambos molares. En un molar se aplicó la solución DFP al 38 % y el opuesto como control. Se realizaron cortes histológicos y se evaluó en forma cualitativa la pulpa dental en ambos grupos. En las piezas ex vivas mediante MEB se observaron áreas de hipermineralización en la dentina intertubular y escasos conductillos obliterados y por EDS se detectó Ag en el centro de la lesión (7.34%), disminuyendo su concentración en los límites (1,71%) y no se detectó en las zonas más alejadas de la misma. En MCC se observó DFP sellando los conductillos sólo en sitio de colocación y con una penetración limitada, por debajo, los conductillos se observaron de aspecto normal y el tejido pulpar asociado con la caries tratada ha mostrado un infiltrado inflamatorio crónico y formación de dentina terciaria, sin observarse precipitado de Ag. En el modelo experimental en las cavidades expuestas con DFP en molares no se alteró en forma relevante la histología pulpar. Las observaciones realizadas con las diferentes técnicas y en tejidos dentarios sugieren que el DFP genera mínimos efectos adversos. Los resultados de este estudio contribuirían a continuar con investigaciones que permitan recomendar el producto como una estrategia costo efectivo para el tratamiento de la enfermedad.

Palabras clave: *Caries dental; fluoruros; diamino fluoruro de plata.*

INTRODUCTION

The antimicrobial agent silver nitrate ($AgNO_3$) was used industrially for over 100 years to make water potable. $AgNO_3$ is used medically in eye drops to prevent infections in newborns, and in dentistry it is often used in stomatological treatments for mouth ulcers[1,2]. In 1969, silver diamine fluoride [$F(NH_3)_2Ag$] (SDF) solution was synthesized for dental treatments[3-5]. Since then, it has been used in Japan as Saforide® Solution (J Morita Company, Japan) for application to caries lesions due to its capacity as an antimicrobial agent and to stabilize caries processes, particularly in primary teeth, thanks to which it has an important role in pediatric dentistry[6,7].

SDF is a colorless solution which is used at 38-40%, pH 8-10. On contacting the caries surface it produces calcium fluoride (CaF_2) and silver phosphate (Ag_3PO_4)[7]. The F:Ag ion ratio is 44,800:255,000 ppm[6,8-12].

SDF is manufactured and marketed in South America as Fluoroplat® (Laboratorios Naf, Buenos Aires, Argentina), in Australia (Creighton Pharmaceutical, Sydney, Australia) and in Brazil as Safluoride di Walter® in 10% solution (Polidental, Río de Janeiro, Brazil).

The mechanism of action of SDF on caries has been related to the formation of silver phosphate by reaction with the tooth enamel surface. When the dentin is compromised, the compound penetrates the tubules, partially or totally, blocking their lumen. In addition, it has an antimicrobial effect, inactivating cariogenic bacteria it contacts [1,2,7-10].

Silver fluoride (AgF_2) is much more soluble in water than other silver halides. Silver diamine fluoride (SDF) contains ammonium in addition to AgF_2. The ammonium ions combine with the silver ions to produce a complex ion called silver diamine ion [$Ag(NH_3)_2$], which is reversible and more stable than AgF_2. It can thus be kept at a constant concentration for a longer time.

Craig et al.[13] and Gotjamanos[14] showed that silver fluoride (AgF_2) is effective in arresting caries in primary molars in children.

Different studies have evaluated the potential toxicity of SDF in children[15-17]. Gotjamanos and Afonso report that commercial 40% AgF_2 preparations contain high concentrations of fluorides and if used for treatment of young patients may cause fluorosis[15]. Western Australia Dental Health Services conducted a study using AgF_2 and found no evidence of its appropriate use causing fluorosis[18]. There is no clinical report of fluorosis as a result of using SDF.

Chu et al.[19] conducted an 18-month study on 375 children and reported 70-83% effectiveness of SDF applied on primary central incisors. Llodra et al [7] found similar effectiveness in a controlled cohort clinical study on a Cuban population of 373 6-year-old schoolchildren over 36 months, finding 80% effectiveness on canines and primary molars, and 65% effectiveness on permanent first molars.

Chu and Lo [19-20] and Zhi et al [21] report that SDF application once or twice a year significantly reduces the incidence of caries and reduces the substantial risk of adverse events. Llodra et al [7] and Chu et al[6] report that SDF applications produce reversible gingival irritation, although this disadvantage is minimized when SDF is applied using an adequate relative isolation protocol. Sharma et al [22] claim that SDF is efficacious for arresting caries lesions.

Gao et al [23] and Mei et al [24] conclude that professional use of 5% sodium fluoride varnish can remineralize enamel caries and that 38% SDF can arrest dentin caries.

In vitro studies on the penetration of SDF into the tooth structure found that it penetrates approximately 2 µm into enamel and 50-200 µm into dentin, while in arrested lesions it reaches a thickness of approximately 150 µm [25].

Different authors have reported the antimicrobial effect of SDF. Among the most relevant studies is Chun et al.[10], reporting that SDF has antimicrobial effect against cariogenic *S. mutans* or *A. naeslundii* biofilm on dentin surfaces. These findings agree with Mei et al., who report the same conclusions with 38% SDF solution[12].

De Almeida et al.[26] confirmed the antimicrobial effect of SDF at commercial concentrations of 12% and 30%, which are lower than the concentration used in our study.

One of the main drawbacks of SDF is esthetic because of the dark stain it produces on the tooth surface. Knight et al.[9-11] therefore conducted *in vitro* studies combining SDF with potassium iodide (IK), which lessened the stain while preserving the antimicrobial properties. Although the product containing SDF and IK emerged on the Australian market, it has not become well known in the rest of the countries that use SDF[27].

SDF is considered a simple, low-cost therapeutic alternative which does not require training for application by health professionals and has a significant benefit for individuals and populations, based on biological sealing[22,28-29].

Although satisfactory results have been obtained using SDF, to date, its potential toxic action and mode of interaction with dental tissues have not been fully elucidated.

Thus, the ***aim*** of this study was to determine the effect of SDF on the dentin-pulp complex by using two models: (a) *ex vivo* teeth after SDF application and (b) molars in experimental animals.

MATERIALS AND METHODS

We performed a descriptive study on the effect of SDF on the dentin-pulp complex by using two models: *ex vivo* human teeth after application and molars in experimental animal.

Histological Study of human teeth treated with SDF

We used 8 human primary teeth obtained by exfoliation or indicated extraction. Inclusion criteria were: teeth with dentin-enamel caries without pulp involvement and previously subject to SDF treatment prior to exfoliation or extraction due to persistence (approximately 1 year after SDF application).

The following protocol was used for applying 38% SDF:

Relative isolation of the lesion, removal of affected dentin using hand instruments, application of 38% SDF by rubbing for 1 minute with a manual applicator soaked in the solution, followed by rinsing with distilled water.

This study was partly performed within the framework of the project "Strategic approach for reconversion of barriers to access to dental care in highly vulnerable groups", which was reviewed and approved by the Ethics Committee at the Buenos Aires University School of Dentistry (UBACYT U20020120100324BA). In order to include children in this study, we obtained informed consent from their legal guardians and formal acceptance from each child. An authorization for donation was attached to the consent forms describing how the tooth would be used, the research aims, and a statement that refusal to participate would not generate any conflict with participation in the project.

Four teeth were cut in half using a diamond disc for Scanning Electron Microscope (SEM) observations. The other four teeth were decalcified for observation under brightfield microscopy.

In each histological analysis of human teeth (*ex vivo*) the zone opposite the treated lesion was used as control.

Evaluation using Scanning Electron Microscopy (SEM)

Sections were prepared from the 4 primary teeth which had been previously cut to expose the lesion. Residue was removed from the sections by ultrasound and they were dehydrated in an alcohol concentration gradient (100, 96, 70 and 50%). Samples were sputter-coated with gold-palladium (using a Termo VG Scientific SC 7620 sputter coater) for SEM observation (Scanning electron microscope model SUPRA 40 Gemini II, Carl Zeiss). One of the teeth was also studied using an energy-dispersive X-ray detector (EDS) in order to determine its qualitative and quantitative composition.

Evaluation by brightfield optical microscopy (OM): Decalcification technique

Teeth were fixed in 10% formalin buffer for at least 48 hrs, after which they were decalcified in 7.5% nitric acid for 7 days. Then they were embedded in paraffin, and mesiodistal histological sections approximately 8 µm thick were cut. Sections were stained with hematoxylin and eosin and a qualitative histological evaluation of dental pulp was performed under brightfield optical microscopy.

Experimental study on laboratory animals

Twelve male 2-month-old Wistar rats weighing 300-350 grams were used. They were anesthetized (ketamine 50ml/kg and xylazine 15 ml/kg i.p.) and placed on an adapted operating table. The lower jaw was isolated and the following standardize technique applied. An enamel-dentin hole about 0.5 mm deep was made in the distal fossa of the occlusal face of each lower first molar (left and right) using a ¼ carbide drill bit at medium speed. The 38% $F(NH_3)_2Ag$ solution was applied to the left molar using a paper point, while the right molar was used as a control (cavity only).

Seven days after treatment the animals were euthanized using 0.2 mg/weight sodium pentobarbital (euthanyle).

Lower jaws were extracted and fixed in 10% buffered formalin for 48 hs, decalcified in 10% EDTA pH 7.2 for 30 days, processed histologically and embedded in paraffin. Histological mesiodistal sections were prepared and stained with hematoxylin and eosin, and qualitative histological evaluation of the dental pulp was performed on both treated and untreated molars under brightfield optical microscopy.

The experimental protocol is in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The procedure described above is shown in Fig. 1.

RESULTS

Histological study on human teeth treated with SDF

Scanning electron microscopy (SEM) - EDS

Fig. 2 shows the lesion treated with SDF, evaluated by EDSwithin the lesion,outside the lesion.

EDS values for the Ca/P ratio were: within the lesion, only Ca was recorded. At the edge of the lesion, the Ca:P ratio was 3.51, and outside the lesion it was 2.275. Ca percentage was 4.07 within the lesion, 27.74 at the edge and 25.63 outside the lesion.

Silver (Ag) was recorded within the lesion (7.34%) and at the edge (1.87%), but none was recorded outside the lesion.

Cross sections of dentin tubules in healthy/ untreated dentin vs. treated dentin were analyzed for each portion of the selected primary teeth. The tubules corresponding to dentin treated with SDF showed areas of hypermineralization of the intertubular dentin and few blocked tubules (Fig. 3).

Brightfield optical microscopy (OM): Technique using decalcification

In the histological sections, SDF was observed sealing the tubules and there was limited microscopically visible penetration (Fig. 4). Beneath the treated lesion, the tubules appeared normal and the pulp tissue associated with the treated caries showed chronic inflammatory infiltrate and formation of tertiary dentin, with no silver precipitate (Fig. 5).

Experimental study on laboratory animals

Analysis of the histological sections of Wistar rat molars with and without SDF treatment showed a precipitate inside the dentin tubules at the application site of the molars treated with SDF.

No silver was found in the pulp, and evaluation of pulp in both groups showed well-organized dental pulp with good vascularization and mild inflammatory infiltrate, with no relevant histological change (Fig. 6).

DISCUSSION

Historically, several lines of work have questioned the idea of surgical treatment of dental caries as the only therapeutic alternative, and reported protocols and results of the use of chemical inhibition of the caries process using different resources [30-34].

Some of these positions were based on the need to respond to the issue of dental caries in countries with high prevalence of the disease and low capacity for its resolution, whether as a result of the healthcare model in place or the incompetence of any of its components[35].

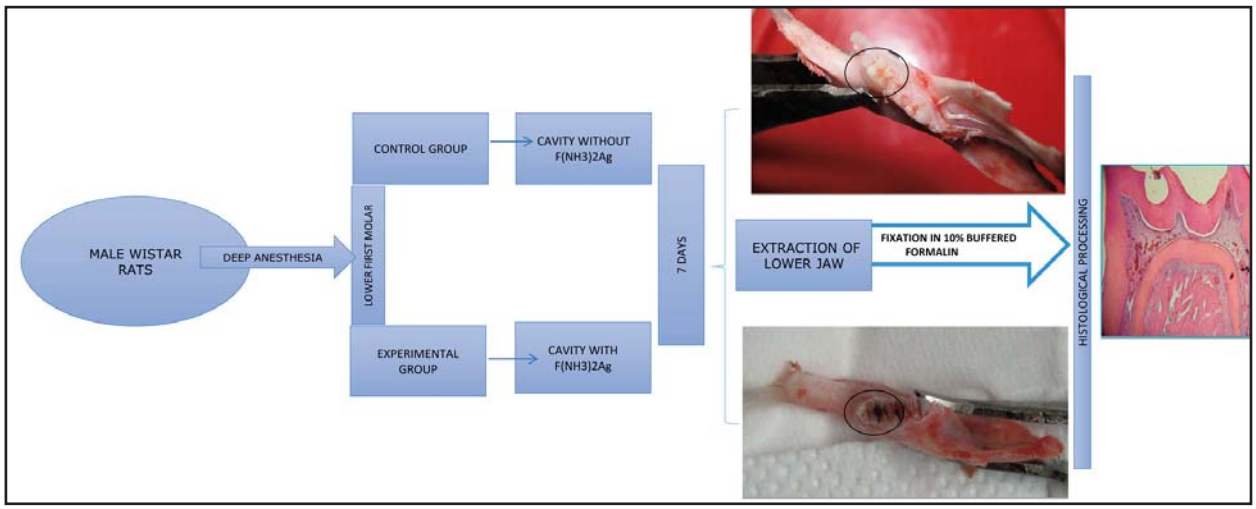

Fig. 1: Experimental study design with laboratory animals.

Nevertheless, before recommending extensive clinical use of SDF on the dentin-pulp organ, its safety must be established.

Mei et al.[12] observed that SDF application on patients with high caries prevalence generates formation and precipitation of silver phosphate. They also found calcium fluoride, silver phosphate and less soluble proteins with silver forming a protective layer that may reduce the loss of calcium and phosphorus from the carious lesion[12,19].

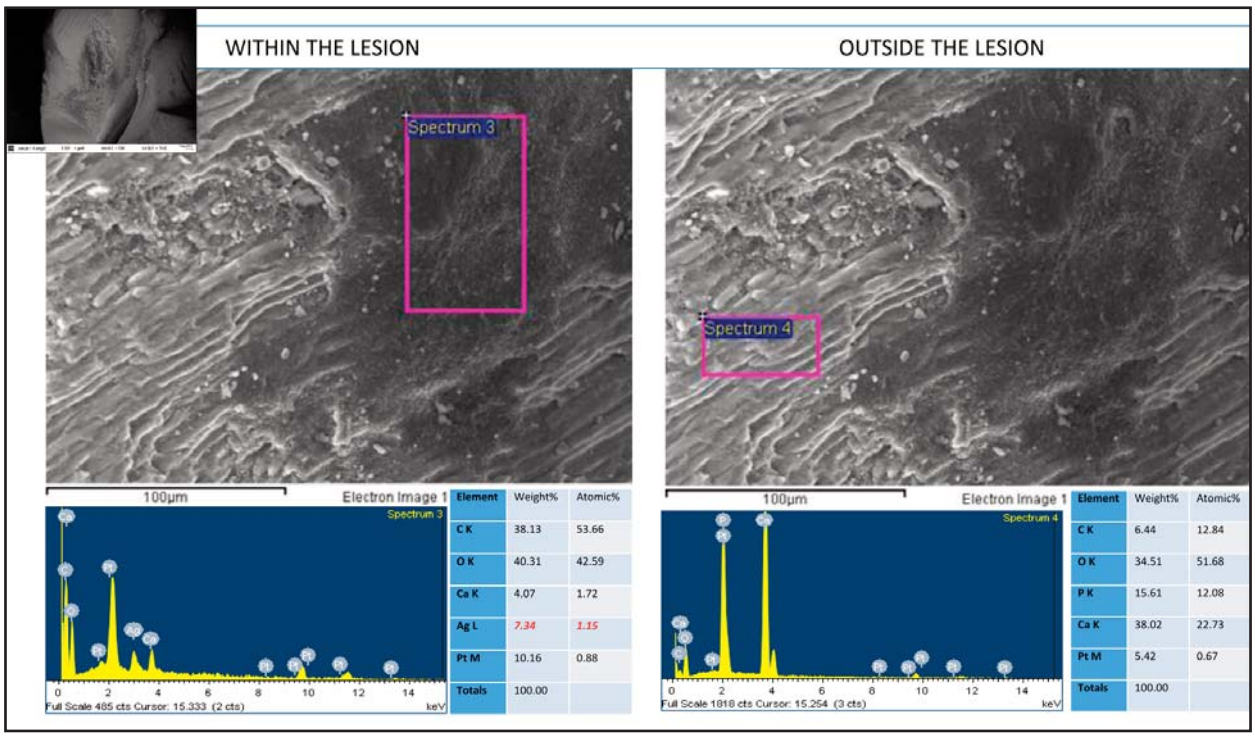

Fig. 2: SEM images show enamel prisms and lesions. Insets show EDS determination inside (left) and outside (right) of the treated lesion. The graphs beneath the figure show the concentrations of elements present in each zone. Note that Ag is only present within the lesion.

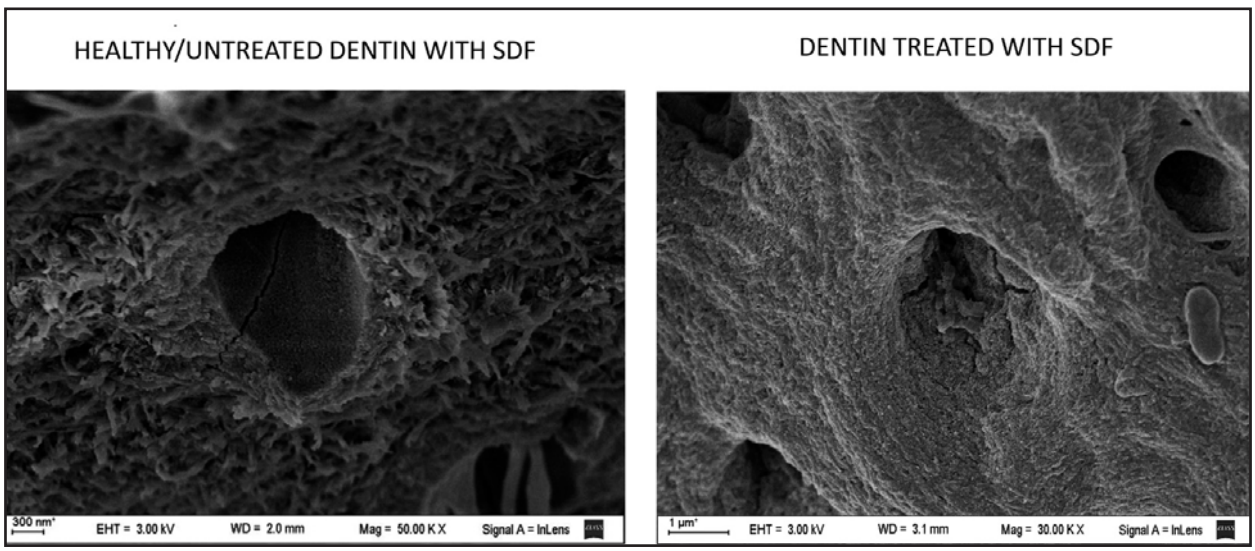

Fig. 3: SEM images showing healthy/untreated dentin tubules (left) and tubules treated with SDF (right). The healthy dentin (left) shows a dentin tubule and intertubular dentin. The treated dentin (right) shows a partially blocked tubule and the intertubular dentin appears to be hypermineralized.

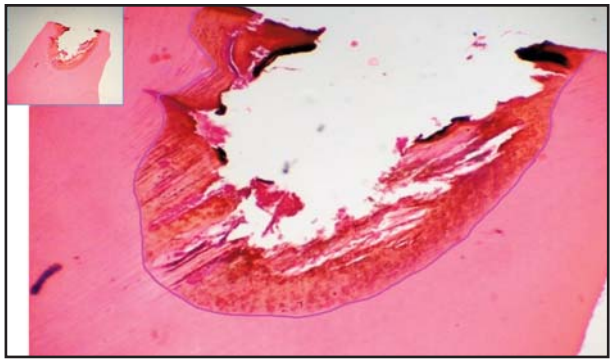

Fig. 4: Histological section prepared using the decalcification technique and stained with H&E, showing dentin tubules and the lesion. The tubules associated to the lesion contain silver precipitate. The marked area shows the delimitation of SDF penetration. 100x

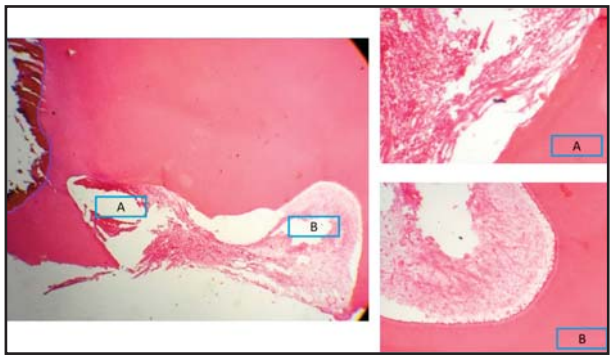

Fig. 5: Left: Histological section of the lesion treated with SDF on human teeth, prepared using decalcification technique and stained with H&E. On the left there is a planar image showing dentin, pulp and the lesion. Zone A shows the dental pulp tissue associated to the lesion; greater magnification (right) shows fibrous pulp tissue with inflammatory infiltrate. Zone B is pulp tissue associated to healthy dentin; greater magnification shows pulp architecture with plentiful blood vessels.

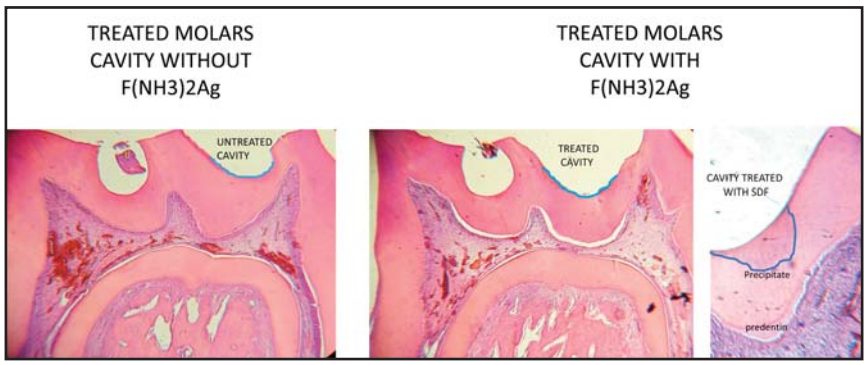

Fig. 6: Histological section of Wistar rat molars, with cavities. Both sections show dentin and pulp. The figure on the right shows greater increase in silver precipitate in the dentin tubules in the treated cavity. It is also clear that the penetration of the solution was limited. Decalcification technique and H&E stain.

The results of our study showed that surfaces treated with 38% SDF and observed using SEM had areas of hypomineralization in the intertubular dentin and few blocked tubules. EDS studies showed that the proportion of silver content is minimal with relation to the composition of the dentin structure. In an *in vitro* study, Mei et al.[36-37] used 38% SDF and found partially blocked dentin tubules, similar to the results of our study[12, 27,36,37].

In another study, Yu et al.[38] found precipitates with high silver and phosphorus content blocking dentin tubules. Rosenblatt et al.[1] published a review of the literature and report results similar to those of Yu et al.[38] and of our study.

Our observations found hypermineralization at the edge of the treated lesion, reflected by the difference in Ca concentration, which was 4.07% within the lesion and 27.74% at the edge. The Ca:P ratio just beyond the edge the lesion dropped to 2.27, increasing to 2.4 as distance increased. It may be assumed that the value would increase up to normal values if it were measured at points increasingly distant from the lesion. These results are similar to those of Mei et al. [24], who observed a highly mineralized zone rich in calcium and phosphate in primary teeth dentin lesions which had been arrested with a single application of SDF. However, they analyzed the lesion as a whole according to depth and did not distinguish the two zones within it. Moreover, the protocol used by Mei et al.[25] applied SDF twice a year, whereas our study examined a single application. They reported that the mineral density was higher in the outer layer of the active lesion than within the body of the lesion, finding a distinct layer 150 µm thick in the arrested lesion, with greater density than the unaffected dentin, which could be considered to be the hypermineralization zone. They did not find very high fluoride levels in the lesion, probably because it was rinsed away with water. For silver, the highest absorption capacity was within the lesion, decreasing noticeably at the edge, with no silver absorption outside the lesion.

In another study, Mei et al.[12] concluded that application of 38% SDF arrests the caries process by reducing demineralization and collagen destruction. In addition, the presence of high fluoride and silver concentrations may inhibit microbial growth of the species present in cariogenic biofilm. It has also been suggested that SDF has an inhibitory effect on metalloproteinases, thereby protecting collagen from destruction in carious lesions, and acting as another form of protection against dentin degradation. In another in vitro study, Mei [2] et al. found that the primary components of SDF appear to react with dentin tissues, forming calcium fluoride, a compound that protects against caries.

Dentin microhardness changes according to its mineral content. Any changes produced by applying SDF may thus also be evaluated by microhardness, and it would be useful to supplement our observations with mechanical determinations on dentin in order to assess its potential correlation[36, 37].

The evaluation of experimental animal molars showed that SDF has limited penetration. We found no major alteration to dental pulp or presence of silver in the pulp of either human teeth or experimental animal molars. These findings agree with Korwar et al.,[39] who reported absence of inflammatory symptoms in *ex vivo* teeth with cavities treated with SDF. Our paper also describes the presence of tertiary dentin adjacent to the treated cavity.

Internationally, Japan has marketed products containing SDF for over 80 years and the US Food and Drug Administration (FDA) approved its use in the year 2014[40].

Dental caries is a highly prevalent disease in children in developing countries[41]. Under many circumstances, conventional methods for prevention and treatment of caries are unavailable or unaffordable to communities in those regions.

The use of unconventional protocols for treatment of caries lesions, including agents that stabilize the process, is essential to the development of dental care programs for highly vulnerable sectors of society and/or sectors with barriers to healthcare access. Traditional approaches for treating caries in populations with these barriers provide temporary benefits due to the high relapse rates in individuals with greater burden of disease.

The results of our study may contribute to establishing the absence of potential adverse effects in dental tissues subject to topical application of SDF and thereby enable continued research which may ultimately enable SDF to be recommended as a low-cost, highly effective strategy for treating caries.

Through the use of different histological study models (tooth substrates and observation techniques) this study suggests that SDF produces minimal adverse effects on the structures described. However, the toxicity and biocompatibility of silver compounds require further evaluation before its safety can be established and its application recommended as a therapeutic measure in programs intended for populations with barriers to conventional dental care.

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Surface detail reproduction and dimensional accuracy of molds: Influence of disinfectant solutions and elastomeric impression materials

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ABSTRACT

This study compared the surface detail reproduction and dimensional accuracy of molds after disinfection using 2% sodium hypochlorite, 2% chlorhexidine digluconate or 0.2% peracetic acid to those of molds that were not disinfected, for four elastomeric impression materials: polysulfide (Light Bodied Permlastic), polyether (Impregum Soft), polydimethylsiloxane (Oranwash L) and polyvinylsiloxane (Aquasil Ultra LV). The molds were prepared on a matrix by applying pressure, using a perforated metal tray. The molds were removed following polymerization and either disinfected (by soaking in one of the solutions for 15 minutes) or not disinfected. The samples were thus divided into 16 groups (n=5). Surface detail reproduction and dimensional accuracy were evaluated using optical microscopy to assess the 20-µm line over its entire 25 mm length. The dimensional accuracy results (%) were subjected to analysis of variance (ANOVA) and the means were compared by Tukey's test (α=5%). The 20-µm line was completely reproduced by all elastomeric impression materials, regardless of disinfection procedure. There was no significant difference between the control group and molds disinfected with peracetic acid for the elastomeric materials Impregum Soft (polyether) and Aquasil Ultra LV (polyvinylsiloxane). The high-level disinfectant peracetic acid would be the choice material for disinfection.

Key words: *Dimensional accuracy; dental disinfectant; dental Impression materials.*

Reprodução de detalhes da superfície e estabilidade dimensional de moldes: influência das soluções desinfetantes e elastômeros

RESUMO

Este estudo comparou a reprodução de detalhes da superfície e estabilidade dimensional de moldes obtidos após desinfecção utilizando hipoclorito de sódio 2%, digluconato de clorexidina 2%, ou ácido peracético 0,2% a moldes que não foram desinfetados com quatro elastômeros: polissulfeto (Light Bodied Permlastic), polieter (Impregum Soft), silicona reação por condensação (Oranwash L) e silicona reação por adição (Aquasil Ultra LV). Os moldes foram preparados sobre matriz contendo linhas de 20, 50 e 75 µm realizado sob pressão com moldeira de metal perfurada. Os moldes foram removidos após a polimerização e desinfetados (utilizando uma das soluções por imersão, armazenados em frascos fechados durante 15 minutos) ou não desinfetados. Assim, as amostras foram divididas em 16 grupos (n=5). A reprodução detalhes da superfície e a precisão dimensional foram avaliadas usando microscopia óptica na linha 20 µm com 25 mm de comprimento, de acordo com a norma ISO 4823. Os resultados de precisão dimensional (%) foram submetidos à análise de variância (ANOVA) e as médias comparadas pelo teste de Tukey com 5% de nível de significância. A linha de 20 µm foi completamente reproduzida por todos os elastômeros, independentemente do processo de desinfecção. Não houve diferença estatisticamente significativa entre o grupo controle e moldes desinfetados com acido peracético para os elastômeros Impregum Soft (polieter) e Aquasil Ultra LV (silicona reação por adição). O desinfetante de alto nível ácido peracético seria o material de escolha para a desinfecção.

Palavras-chave: *Estabilidade dimensional; desinfetante dental; materiais de moldagem.*

INTRODUCTION

High-accuracy impression materials (elastomeric impression materials) were first used in dentistry in the 1950s[1]. Currently, four different elastomeric impression materials are used: polysulfide, polyether, polydimethylsiloxane and polyvinylsiloxane, each of which has specific chemical reactions and setting characteristics[1]. Impression materials should

reproduce hard and soft tissues in order to obtain biologically, mechanically, functionally and esthetically acceptable restorations[2], and in addition to being capable of recording the anatomic topography of the desired area, they should remain dimensionally stable[3]. The dimensional accuracy of a material is usually time-dependent; for example, a material may be highly dimensionally accurate soon after its initial polymerization but less accurate after storage for a period of time[4]. Dimensional changes may occur in the molds as a result of features inherent to the impression materials such as wettability, handling properties and viscosity, or to thickness of the material between the oral structures and tray, method of fixing the impression material to the tray, time elapsed until cast pouring, material's hydrophilicity, by-product loss, polymerization shrinkage, thermal shrinkage due temperature change (from mouth to room temperature), incomplete elastic recovery, and, in some cases, soaking[1].

Disinfection is defined as a clinical stage designed to destroy most microorganisms (viruses, bacteria and spores) from the surface of an impression[5], and is an important biosafety measure. In absence of disinfection, treatment procedures can expose dentists, hygienists and laboratory workers to direct or cross-contamination[5,6]. During the impression procedure, the materials come into contact with fluids such as blood and saliva, which may contain pathogenic microorganisms capable of transmitting infectious diseases such as herpes, hepatitis, tuberculosis or AIDS[5,7].

Disinfection can be accomplished by physical or chemical action. However, physical action may result in temperature increase, which can cause measurable deformations in the molds[5]. For impression materials, the use of solutions with chemical action is recommended[5]. Disinfectants must perform effectively as antimicrobial agents while not adversely affecting the dimensional accuracy or feature fidelity of the impression material and resulting gypsum cast[8]. Disinfection should be carried out with the product that requires the least amount of time for the disinfection process[9]. The most frequently used disinfectants are glutaraldehyde, formaldehyde, alcohol, iodine solution, synthetic phenol, sodium hypochlorite and other chlorine-releasing solutions[5]. Other potential disinfectants may be used to eliminate pathogens, provided they do not alter the properties of elastomeric impression materials. Peracetic acid has been cited in the literature as a promising alternative for disinfection due to its antimicrobial efficiency [10], but there is no report on its use as a disinfectant for elastomeric impression materials.

This study compared the surface detail reproduction and dimensional accuracy of elastomeric molds prepared using polysulfide, polyether, polydimethylsiloxane or polyvinylsiloxane elastomeric impression materials and disinfected using 2% sodium hypochlorite, 2% chlorhexidine digluconate or 0.2% peracetic acid, to those of models produced using molds that were not disinfected. The null hypotheses tested were that surface detail reproduction and dimensional accuracy of elastomeric molds are not affected by either [1] the elastomeric impression material or [2] the disinfectant solution.

MATERIALS AND METHODS

This study used the light-body elastomeric impression materials polysulfide (Light Bodied Permlastic, batch number 1-1311, Kerr, Romulus, MI, USA), polyether (Impregum Soft, batch number 1220700759, 3M Deutschland, Seefeld, Germany), polydimethylsiloxane (Oranwash L, batch number 133520, Zhermack, Badia Polesine, RO, Italy) and polyvinylsiloxane (Aquasil Ultra LV, batch number 100223, Dentsply Caulk, Milford, DE, USA).

Dimensional accuracy and surface detail reproduction were evaluated in accordance with ISO 4823[11]. The molds were prepared on a matrix (38 mm outer diameter and 29.97 mm internal diameter) containing three parallel lines 20, 50, and 75 μm wide and 25 mm long, spaced 2.5 mm apart. Two additional lines marked X and X′ were used to determine the dimensional accuracy and surface detail reproduction on the 20 μm line.

Before the impression procedure, the matrix was cleaned ultrasonically and dried with compressed air. The elastomeric impression materials were prepared according to the manufacturers' instructions. A perforated metal tray (31 mm internal diameter × 5 mm high) was placed on a glass plate and filled with the molding material. The tray was joined to the matrix and a 20 N force was applied using a pneumatic press to simulate the impression process and permit leakage of excess material[5].

The molds were removed 3 min after polymerization of the elastomeric materials (polymerization time was consistent with the minimum time recommended by the manufacturers)[5] and disinfected by soaking for 15 minutes at 37º C in 2% sodium hypochlorite solution (Qboa, batch number L1-1212, Indústria Anhenbi S/A, Osasco, SP, Brazil), 2% chlorhexidine digluconate solution (Riohex 2%, batch number R1202994, Indústria Farmacêutica Rioquímica LTDA, São José do Rio Preto, SP, Brazil), or 0.2% peracetic acid solution (Peresal, bath number 4232AP0504, Ecolab Deutschland GmbH, Düsseldorf, Germany). Control samples were not disinfected. The samples were divided into 16 groups (n=5) according to disinfectant procedure and elastomeric impression material: Group 1: No disinfectant (control group) + polysulfide; Group 2: No disinfectant (control group) + polyether; Group 3: No disinfectant (control group) + polydimethylsiloxane; Group 4: No disinfectant (control group) + polyvinylsiloxane; Group 5: 2% Sodium hypochlorite solution + polysulfide; Group 6: 2% Sodium hypochlorite solution + polyether; Group 7: 2% Sodium hypochlorite solution + polydimethylsiloxane; Group 8: 2% Sodium hypochlorite solution + polyvinylsiloxane; Group 9: 2% Chlorhexidine digluconate solution + polysulfide; Group 10: 2% Chlorhexidine digluconate solution + polyether; Group 11: 2% Chlorhexidine digluconate solution + polydimethylsiloxane; Group 12: 2% Chlorhexidine digluconate solution + polyvinylsiloxane; Group 13: 0.2% Peracetic acid solution + polysulfide; Group 14: 0.2% Peracetic acid solution + polyether; Group 15: 0.2% Peracetic acid solution + polydimethylsiloxane; Group 16: 0.2% Peracetic acid solution + polyvinylsiloxane.

Surface detail reproduction was measured using an optical microscope (SZM, Bel Engineering srl, MI, Italy). The molds were examined under low-angle illumination at a magnification of 4x to 12x to determine whether the 20 µm-line was completely reproduced over the full length of 25 mm between the intersecting reference lines (X and X′), in accordance with ISO 4823[11].

Dimensional accuracy was measured on the molds using an optical microscope (STM, Olympus Optical Co Ltd, Japan) with an accuracy of 0.0005 mm. Dimensional accuracy expressed as a percentage (L) was calculated in accordance with ISO 4823[11] using the equation:

$L = [(L2 - L1) / L1] \times 100$, where L1 is the distance between the lines on the matrix and L2 is the distance between the lines on the impression material.

Then, 100% was added to the results of the equation[12] and the dimensional accuracy results (%) were subject to the Kolmogorov-Smirnov test for normality, two-way ANOVA (material x disinfectant), and the means were compared by Tukey's test at 5% significance levels.

RESULTS

The surface detail reproduction of all the elastomeric impression materials was completely reproduced on the 20 µm line regardless of disinfection procedure (100% of the 5 samples in all 16 groups).

There was a statistically significant difference in the mean values of dimensional accuracy in the interaction between disinfectant procedure and elastomeric impression material ($p = 0.00001$). The dimensional accuracy of non-disinfected Aquasil Ultra LV (polyvinylsiloxane) (Table 1) was statistically higher than that of Oranwash L (polydimethylsiloxane); however Impregum Soft (polyether) and Light Bodied Permlastic (polysulfide) did not differ from the others. There was no significant difference between the control group and the molds disinfected with peracetic acid for the elastomeric materials Impregum Soft (polyether) and Aquasil Ultra LV (polyvinylsiloxane).

DISCUSSION

The success of some forms of dental treatment depends upon the accuracy with which a restoration can be manufactured in the laboratory, using models constructed from impressions[13]. Clearly, the precision of the initial impression, in terms of both dimensional accuracy and detail reproduction, is a prerequisite for success[13]. The risk of cross-infection from a patient to a dental technician is a matter of concern[14], and in order to protect the members of the dental team, a high standard of hygiene and disinfection of dental equipment, including dental impressions, is recommended[6]. A disinfectant has dual requirements: it must be an effective antimicrobial agent yet cause no adverse response to the dimensional accuracy and surface-texture features of the impression material and resultant plaster cast[8]. The most frequently used

Table 1: Mean values for dimensional accuracy (%) for different groups.

	Dimensional Accuracy (%)			
Elastomeric impression material	**No disinfectant (control group)**	**2% Sodium hypochlorite solution**	**2% Chlorhexidine digluconate solution**	**0.2% Peracetic acid solution**
Light Bodied Permlastic (Polysulfide)	99.87 (0.03) AB a	99.62 (0.05) C b	99.70 (0.03) B b	99.71 (0.07) B b
Impregum Soft (Polyether)	99.90 (0.01) AB b	100.12 (0.14) A a	99.99 (0.16) A b	99.96 (0.06) A b
Oranwash L (Polydimethylsiloxane)	99.83 (0.02) B a	99.37 (0.10) D c	99.41 (0.02) C c	99.55 (0.04) C b
Aquasil Ultra LV (Polyvinylsiloxane)	99.98 (0.02) A a	99.82 (0.08) B b	99.88 (0.10) A ab	99.96 (0.06) A a

Mean values followed by different lowercase letters in rows and uppercase letters in columns differed statistically by Tukey's test at 5% level of significance. Standard deviations are provided in parentheses.

disinfectants are glutaraldehyde, formaldehyde, alcohol, iodine solution, synthetic phenol, sodium hypochlorite and other chlorine-releasing solutions[5]. However, there have been few examinations of the interaction between types of elastomeric impression materials and disinfection with peracetic acid solution. The current study used 3 disinfection treatments, consisting of soaking specimens for 15 minutes in 2% sodium hypochlorite, 2% chlorhexidine digluconate or 0.2% peracetic acid.

The current recommendation is to disinfect elastomeric impression materials by immersion in glutaraldehyde[5,14] or sodium hypochlorite[5]. Glutaraldehyde is considered a high-level disinfectant[15] that should eliminate some spores, the bacillus responsible for tuberculosis, vegetative bacteria, fungi and viruses [3]. However, it has been banned in some Brazilian states[3].

Substances containing chlorine, such as 2% sodium hypochlorite, are considered intermediate-level disinfectants that have limited effect on bacterial spores and non-lipid-containing viruses, but are effective against tuberculosis bacilli, vegetative bacteria and most fungi[3]. However, they also have disadvantages, such as toxicity during manipulation by health professionals, causing irritation to the eyes and respiratory system, damage to the environment and incompatibility with some types of materials such as metals.

Chlorhexidine is a cationic bisbiguanide [1,6-di (4-chlorophenyl-diguanido) hexane] agent with broad-spectrum antibacterial (Gram-negative and Gram-positive), some virus and antifungal activities[9]. It is biocompatible with oral tissues[9] and has the ability to remain on a surface and be released gradually[9,16]. Its excellent properties have motivated its increasing use in dentistry. However, microorganism response to it depends, among other factors, on the type of microorganism. A study by Casemiro et al.[9] found that *Pseudomonas aeruginosa* (Gram negative bacilli) showed no response to chlorhexidine, probably because this strain is resistant to chlorhexidine. Thus, chlorhexidine is also considered an intermediate-level disinfectant.

Peracetic acid is a combination formed from the chemical reaction of acetic acid (CH_3COOH) with an aqueous solution of hydrogen peroxide (H_2O_2) or by the reaction of tetraacetylethylenediamine with alkaline hydrogen peroxide solution[17]. In addition to being a high-level disinfectant, it is biodegradable and nontoxic. Therefore, after several debates, the World Health Organization has suggested replacing the disinfectants described above with peracetic acid, which has a broad spectrum of antimicrobial activity and shorter soak time, and is active in presence of organic matter, environmentally friendly and safe for both the professional and the patient.

The main groups of available elastomeric materials differ significantly in rheological properties[12,18] and in their interaction and tolerance of moist surfaces according to their composition[4,13]. Polysulfides and polyethers are considered hydrophilic because they contain functional groups that attract and interact chemically with water molecules through hydrogen[18]. In polyethers, the hydrophilic groups

are the carbonyl (C==O) and ether (COC) groups, while polysulfide, the hydrophilic groups are the disulfide (—SS—) and mercapto (—SH) groups[18]. Our results showed that the 20-µm line was completely reproduced by all the elastomeric materials; however, although there was no change in the 20-µm line for the Light Bodied Permlastic (polysulfide) and Impregum Soft (polyether) elastomeric materials, their surfaces appeared porous when disinfected with sodium hypochlorite. Acceptable methods of measuring the dimensional accuracy of casts include measuring calipers[9,20], micrometers[21], dial gauges[22] and measuring microscopes[3]. A microscope was used in this study due to its high accuracy (0.0005 mm). An ideal impression material would be dimensionally accurate over time, and could therefore be poured at the operator's convenience[23]. One study found that the impression material polyvinylsiloxane presents ideal dimensional stability[23]. Another study found that polyether presented better dimensional precision than the polydimethylsiloxane and polysulfide materials[24], while in another[25], polyether presented intermediate behavior between polydimethylsiloxane and polyvinylsiloxane. Thus, although these studies used different methodologies, by analogy, polyvinylsiloxane appears to have the best dimensional accuracy, followed by polyether. In the present study, for non-disinfected molds, dimensional accuracy (Table 1) was statistically higher for Aquasil Ultra LV (polyvinylsiloxane) than for Oranwash L (polydimethylsiloxane), while Impregum Soft (polyether) and Light Bodied Permlastic (polysulfide) did not differ from the others. The lower dimensional accuracy for Oranwash L may be the result of ethanol being formed as a by-product during its polymerization reaction and being lost through evaporation from the surface of the material before disinfection.

Although polydimethylsiloxane has greater polymerization shrinkage, it is hydrophobic, being less susceptible to water sorption by immersion in disinfectant solutions[5]. Thus, the lower dimensional accuracy results for Oranwash L may be attributed to the time elapsed (15 min) during disinfection. Table 1 shows that the samples immersed in 2% sodium hypochlorite, 2% chlorhexidine digluconate or 0.2% peracetic acid showed no similar patterns after disinfection. The results of this study show no significant difference between the control group and the molds disinfected with peracetic acid for the elastomeric materials Impregum Soft (polyether) and Aquasil Ultra LV (polyvinylsiloxane). For Oranwash L (polydimethylsiloxane) and Light Bodied Permlastic (polysulfide), the significant difference between the control group and the molds disinfected with peracetic acid was probably related to leaching of alcohol or water in the disinfecting solutions. Thus, peracetic acid would be the material of choice for disinfection. As previously mentioned, polyethers can be considered hydrophilic, which was verified in the interaction Impregum Soft – sodium hypochlorite. However, dimensional accuracy of about 0.1 to 0.8% is compensated at some stages during the laboratory steps required in the preparation of the restorations[26]. Despite the diversity of results in the literature regarding the effect of disinfectant solutions on the dimensional stability of elastomeric materials[5], the dimensional variations observed in this study cannot be considered sufficient to create significant distortions which could compromise the accuracy of prosthetic restorations. Disinfection is an essential step which cannot be omitted.

Based on the results of this study, the first null hypothesis was accepted and the second was rejected, as there was no difference in [1] the surface detail reproduction, although [2] significant differences were found in the dimensional accuracy of elastomeric molds. The authors conclude that although there are differences in dimensional accuracy of elastomeric molds when they are disinfected, this change has no clinical affect. Moreover, peracetic acid only promoted a significant difference from the control group (dimensional accuracy) when compared to Oranwash L (polydimethylsiloxane) and Light Bodied Permlastic (polysulfide), which was probably not a result of the use of this disinfectant. Thus, the high-level disinfectant peracetic acid would be the material of choice for disinfection. Further studies are needed to prove its effectiveness in disinfection of elastomeric impression materials.

CONCLUSION

Under the conditions and within the limitations of the current study, it can be concluded that the high-level disinfectant peracetic acid would be the material of choice for disinfection.

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Analysis of the adverse events reported to the office of the clinical director at a dental school in Bogotá, Colombia

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ABSTRACT

Dentistry is interested in identifying and controlling adverse events, understood as involuntary injuries to the patient during dental care. The aim of this study was to analyze the adverse events reported to the Office of the Clinical Director at the School of Dentistry at Pontificia Universidad Javeriana (Colombia) during 2011-2012. It was an observational, descriptive study that evaluated 227 dental clinical records of patients who filed a complaint with the Office of the Clinical Director. Of these, 43 were adverse events and were used as the basis for this study. Of the 16,060 patients who received care during 2011 - 2012, 0.26% (43) filed a complaint involving an adverse event, of which 97.7 % were considered preventable. Most of these (76.18%, n= 32) occurred during clinical management of treatments in different specialties, 9.5% (4) were the result of deficient external dental laboratory quality, and 14.32% (6) were due to failure in document management, soft tissue injury, misdiagnosis and swallowing foreign objects. Of the patients involved, 65.2% (28) received care from postgraduate students, with the highest number of cases in the Oral Rehabilitation speciality. The occurrence of adverse events during dental care, indicates the need for information about their origin in order to establish protection barriers and prevent their incidence, particularly in the educational area under the student dental clinic service model.

Key words: *Dentistry, Reporting events, Reporting incidents.*

Análisis de los eventos adversos reportados a Dirección de Clínicas en una Facultad de Odontología de Bogotá-Colombia

RESUMEN

En odontología existe interés por identificar y controlar los eventos adversos, entendidos como las lesiones no voluntarias que ocurren durante la atención odontológica. El objetivo de este estudio fue analizar los eventos adversos reportados a Dirección de Clínicas de la Facultad de Odontología de la Pontificia Universidad Javeriana durante el periodo 2011-2012. Se realizó un estudio observacional descriptivo para el que se evaluaron 227 historias clínicas de pacientes que reportaron una queja a la Dirección de Clínicas, de las cuales en 43 se evidenció la presencia de eventos adversos, a partir de las cuales se registró la información analizada en este estudio. De los 16.060 pacientes atendidos durante el periodo 2011 y 2012, el 0,26% (43) formularon alguna queja que resultó en un evento adverso, de los cuales el 97,7 % se consideraron prevenibles. El mayor porcentaje 76,18 % (32) se presentó durante la gestión clínica de tratamientos en diferentes áreas. El 9,5 % (4), se debieron a fallas en la calidad del trabajo del laboratorio externo; el 14,32% (6) correspondió a eventos generados por fallas en la gestión documental, lesiones de tejidos blandos, fallas de diagnóstico y deglución de objetos extraños. El 65,2 % (28) de los pacientes fueron atendidos por estudiantes de posgrado, con el mayor número de casos en la especialidad de Rehabilitación Oral. La presentación de eventos adversos durante el proceso de atención en odontología, es indicador de la necesidad de conocer su origen para establecer barreras de protección y prevenir su incidencia, especialmente en el área formativa bajo el modelo de atención docencia servicio.

Palabras clave: *reporte de eventos, reporte de incidentes.*

INTRODUCTION

Due to their complexity, health services are considered a high-risk system. There is concern to identify, control and prevent adverse events, which are understood as involuntary unsafe care which unintentionally harms the patient and can be attributed to the healthcare provided but not to the underlying pathology. Adverse events may be caused by human failure or defects in the system. Although some events are considered unpreventable accidents, most of them are considered preventable.[1-4]

When unsafe care does not cause any damage, it is considered a sign or incident, defined as an event

or circumstance which may warn of increased risk of a failure occurring in healthcare[3].

The World Health Organization (WHO)[5] encourages reporting, monitoring and managing adverse events, and highlights the fact that there is little available documentation in dentistry. Given that patients safety is a global sanitary issue and that adverse events occur at all health centers, the WHO, the International Dental Federation and several researchers have conducted a study on safety culture in the field of dentistry, benefitting both professionals and patients[4].

In Colombia, the Ministry of Health and Social Protectionfosters, by means of a quality assurance system, adverse event management and prevention, which must be applied as from the training stage pursuant to the Ministry of Health decree 2376 of the year 2010, which refers to training practice as "an educational institution's planned, organized pedagogical strategy seeking to integrate academic education andproviding healthcare service, with the aim of strengthening and creating competencies and skills in students training under healthcare programs, within a framework promoting the quality of healthcare, responsible, ethical professional exercise"[6].

The potential harm that a patient may suffer when receiving care from personnel undergoing dental care training has not been measured widely. Further knowledge of the frequency of this kind of error and fostering a culture of systematically reporting incidents will serve as a basis to design new, efficient tools to measure the occurrence of incidents, and most importantly, preventing them.[4,7] The School of Dentistry where this study was conducted provides care to patients under the teaching-service model, where pedagogical practices at all levels of training – low, medium and high – are provided, according to the complexity of the treatments required, at theoretical, pre-clinical and clinical levels. Dental services at the School include a portfolio offering General Dentistry and the specialties Oral Surgery and Pathology, Maxillofacial Surgery, Endodontics, Periodontics, Orthodontics, Pediatric Dentistry and Oral Rehabilitation.

Adverse events occur during or as a result of clinical procedures, which should therefore be subject to management tools and methodologies to reduce or prevent them. This would impact the costs of lack of quality, and contribute to safe, efficient, people-centered care as essentialin training human resources in Dentistry[8,9]. This study was performed as a contribution to the Patient Safety Program, with the aim of analyzing the adverse events filed with the Office of the Clinical Director at the School of Dentistry at Pontificia Universidad Javeriana during 2011-2012.

MATERIALS AND METHODS

During 2011-2012,we analyzed 16,060 patients who received care at undergraduate and postgraduate services at the clinics. Of these, 227 dental clinical records were found in which patients filed a complaint with the Office of the Clinical Director and requested a review of their current treatment condition or their further care. We analyzed them to determine whether there had been any risk situations which mighthave caused an incident or adverse event, finding 63, including20 with signs of unsafe care and 43 with occurrence of an adverse event, defined in the opinion of the experts in the Institutional Technical-Scientific Committee.

Using an *ad-hoc* form, we recorded patient demographics (age, sex, occupation); type of adverse event; complexity of treatment; number of students and their level (undergraduate/postgraduate); specialty of postgraduate students; support services from dental laboratories; failures before, during and after treatment in the clinical, academic and administrative spheres; and management of instruments, supplies and equipment. In addition, we considered time of treatment as a factor of non-conformance and clinical risk affecting the proper evolution of treatments.

After collecting the information, we classified the adverse events detected. The analysis is supported by descriptive statistics.

RESULTS

Of the 16,060 patients who received care during 2011 and 2012, 0.26% (43) filed a complaint which has resulted in an adverse event. Of these,62.7% were female, while regarding age, 4.8% were under 18 years old, 57.1% were between 19 to 59 years old and 38.1% were over 60 years old.

Occupation was classified according to patient's activity at the time of the event, without considering schooling education level, 42.2% working, 48.9% homemakers, students or pensioners, and 7% with no recorded data. None had any physical or mental disability impacting the occurrence of the event.

Of the 43 adverse events detected, only one was classified as unpreventable, in which the treatment

failed because bone regeneration was not viable despite the fact that the morning C-terminal telopeptide (CTX) value was within normal limits. Individual biological response was the determining factor in the occurrence of the event.

Of the adverse events classified as preventable (9.7%), most (52.38%,n = 22) occurred during clinical management of prosthetic treatments, with the most common cause being fracture of prosthetic material after cementing. In second place, 23.8% (10) of the events were related to clinical management in other areas, the most frequent being excessive drilling. In third place, 9.5% (4) were failures in the quality of work from the external dental laboratory, and the remaining 14.32% (6) were caused by failures in document management, soft tissue injury, misdiagnosis and swallowing foreign objects (Table 1).

Regarding the level of the students providing clinical care, 34.8% (15) patients received care from undergraduate students and 65.2% (28) received care from postgraduate students, mainly in Oral Rehabilitation speciality (Fig. 1).

It is important to use and follow clinical and learning guidelines as academic support within the care model at the School. We found that these guidelines had been used to support clinical practice in 97.6% (42) of the cases and not used in 2.3% (1). Regarding the degree to which the recommendations in these guidelines were followed, we found that they had been followed by 11.62% (5), not followed by 53.48% (23), and there was no report in the dental clinical records for 34.8% (15).

Complaints about dissatisfaction with care provided within the teaching-service model at the School were classified according to cause. It was found that the 83.72% (36) of the complaints were due to clinical care, while for 11.62% (5) it was due to clinical-administrative errors caused by students, such as delays in care or evolution of treatment, not calling the patient after the inter-semester period or unpunctuality. Right of petition for clinical and administrative cause were 2.3% each, being a resource used by very few patients, and were filed due to dissatisfaction with the type of clinical care received. Administrative complaints exclusive not were found. (Fig. 2).

Analysis of use of diagnostic aids showed that 88.4% (38) had initial radiographs. Final radiographs were only found in 58.2% (25) of the dental clinical records.

Considering that treatments take longer in the teaching-service care model, we originally considered that the longer the time, the greater the probability of an event occurring, so we checked the average duration of treatments in months. In general, the cases took 1 month to 14 years. In contrast to what we expected, we found that most cases with events had completed the treatment during the first year (20.9%) and almost 70% had been completed within 5 years (Fig. 3).

Under the assumption that the more students taking part in a treatment, the greater the chance of an event occurring, we analyzed the number of students who took part in each adverse event studied. We found that 37% of the cases were conducted by 1 to 5 students, 33% by 6 to 10 students, 19% by 11 to 15 and 11% by more than 16 students. The number of students involved in care of a given patient was not associated to a greater number of adverse events (Fig. 4).

No adverse event wasfound to be related to management of instruments, supplies and equipment.

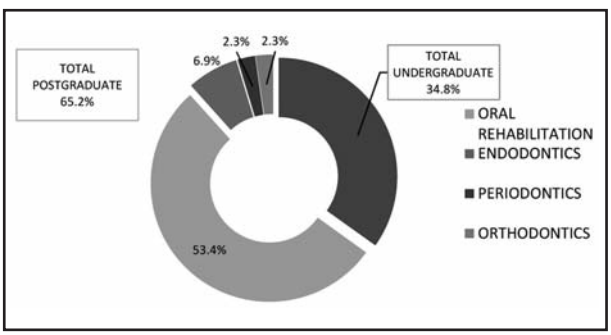

Fig. 1: Distribution of adverse events found according to student training level and specialty.

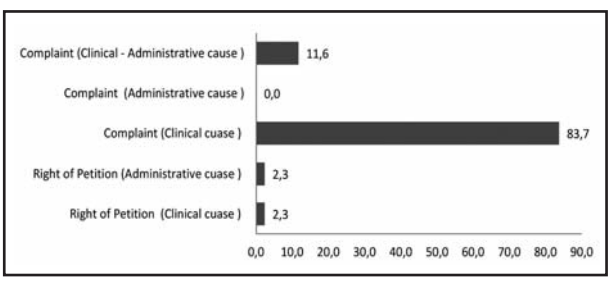

Fig. 2: Classification of complaints filed with the Office of the Clinical Director of the School of Dentistry which correspond to the adverse events found.

DISCUSSION

Given the concern about quality in dental services and that the school of dentistry is a teaching service institution, research has been one of the main purpose since 2008 with the aim of determining the occurrence of incidents or adverse events during care provided by students in different postgraduate courses that could put patient safety at risk, in order to create preventive and control strategies.

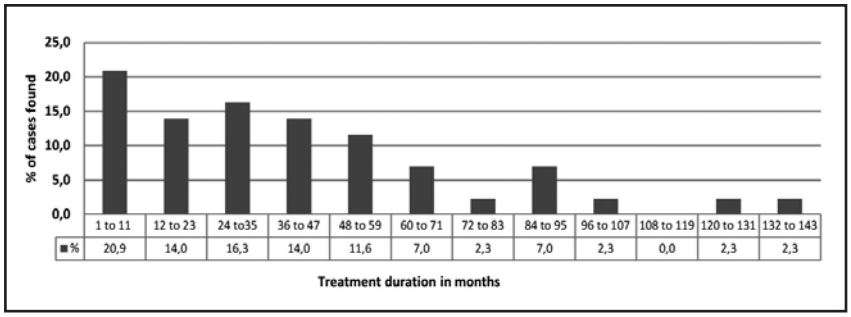

Fig. 3: Percentage of cases according to treatment duration in months.

Worldwide, there is little scientific literature on adverse events in dental care, and the wide variety in both in theory[3] and methodology forstudying adverse events make progression the subject difficult in dentistry.

One of the most relevant results in this study was the low frequency (0.26%) of adverse events found during analysis of clinical records of dissatisfied patients who filed a complaint with the Technical-Scientific Committee, relative to total number of patients who received care at the School clinics. The cases filed were the most severe or those involving legal implications. However, there is consensus in the literature that cases are under-recorded, under-reported and unsystematized, so adverse events cannot always be analyzed in-depth.[10]

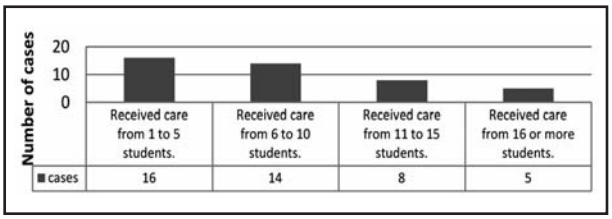

Fig. 4: Adverse event cases classified according to number of students involved in patient care.

One of the problems that creates uncertainty about the real frequency of adverse events is under-reporting, as expressed by Thusu et al.[11], who conducted a one-

Table 1: Preventable adverse events caused by failures before, during and after treatment.

Event according to cause	Type	% (n)
Events in clinical management of prosthetic treatments	Implant screw breakage Fault in hybrid bar design Prosthetic material fracture after cementing Uncemented prosthesis or part of prosthesis Repetition of prosthetic work with unspecified cause Repetition of prosthetic work due to misfit Repetition of prosthetic work due to faulty design	52.38 % (22)
Events in clinical management of treatments in other fields of dentistry	Tooth loss due to endodoncy Excessive tooth drilling Loss of permanent tooth follicle during extraction of temporary tooth Endodontic file breakage Anesthetic needle breakage	23.8 % (10)
Failures in quality of external laboratory work	Loss of ceramic material Broken abutments in removable partial denture Repetition of prosthesis	9.5 % (4)
Failures in document management	Mismanagement of referrals and transfers	4.78% (2)
Soft tissue injury	Burn on lip from electric scalpel Gum injury when placing post-surgical staple	4.78 % (2)
Misdiagnosis	Misdiagnosis	2.38 % (1)
Swallowing foreign objects	Swallowing implant screwdriver	2.38 % (1)

year study based on dental reports from the database of the United Kingdom's National Patient Safety Agency. They found a low rate of reports on dental incidents, possibly due to the voluntary nature of reporting and the reluctance of dental practitioners to disclose incidents for fear of some kind of professional inconvenience.

A study conducted on the database from the Spanish Observatory for Dental Patient Safety (OESPO), analyzed 415 law suits and reported that 40% were caused by errors (conscious event), 40% by complications and 20% by accidents (conceptually defined as an adverse event). It concluded that the use of this source has limitations because dentists report few adverse events since they perceive their procedures as being less complex than medical procedures and not life-threatening, although the same study found 11 cases of death attributed to the dentist's confidence during treatment (allergy, endocarditis caused by lack of prophylaxis, hemorrhages in anticoagulated patients and infections in immunocompromised patients, among others), pointing to the need for detailed clinical records[10,] Obadan et al.[12] report that 24.1% of adverse events required that the patient be transferred to an emergency department, of which 11.1% resulted in death of the affected patient.

In our study, the most severe event (2.38%) of the 43 found was due to swallowing a prosthodontic screwdriver. The patient was taken to emergency room and the device had to be removed under medical care at a hospital. Although events such as this are infrequent, they are important because of their potential complications which may lead to death of a patient. There are reports of a wide range of ingested items, such as fixed prosthesis, orthodontic items[13,14]metal restorations, crowns, cores, endodontic files, and ultrasonic tips, among others[15.]

In their analysis of the those cases, Obinata et al found that ingestions occurred more frequently during treatment of lower molars, and suggest keeping the patient's head inclined towards the side being treated so that objects fall in the buccal pouch. Cases of ingestion occurred more frequently when the procedures were performed by professionals with less than 5 years of experience. Therefore, and considering the risk created by these accidents, dentists should take meticulous precautions and be prepared to deal with this kind of emergency[15.] When there is inhalation, the risk is greater, so it is suggested that dental offices should have emergency protocols for dealing with it promptly[14.] In addition, patients at greater risk of ingestion or aspiration of objects should be identified and extra precautions takento prevent such complications. Zitzmann et al.[16]provide guidelines for managing inhaled or ingested objects during dental treatment.

In addition to the issue of underreporting, the source of information on which an analysis is based modifies the casuistic and results found on the subject. The other methodologies most frequently used for detection and analysis of adverse events are direct review of clinical records and surveys. These methods usually increase the number of events reported. Our research group conducted a study on adverse events in the field of endodontics, finding reports in 74.4% of the records analyzed over two years. It is interesting to note that most of them (81.3%) are considered preventable[17]similarly to the current study, in which only one event was not preventable.

A study by Hiivala et al.[4] used an internet survey of dentists who worked at public and private institutions. It reports 872 patient safety incidents, of which 53% were considered adverse events, 45% incidents and 13% severe events potentially causing permanent damage, as a result of factors caused by application of local anesthesia, allergic reaction, exposure to radiation and extracting wrong teeth, among others. Anotherstudy conducted on the database of adverse events reported to national supervision and administrative institutions in the healthcare sector found that 32% occurred at private dental offices, 62.9% were preventable, 4.1% not preventable and 33% could not be evaluated[18.]

The most frequent types of adverse events have been reported in most fields of dental care. In our study, the highest frequency occurred in clinical management of prosthetic treatments, with 52.4%, followed by another clinical management treatments in other fields of dentistry, 28.8%. These results were similar to those reported by Hivalla et al.[18], who rank prosthodontics in first place with 16.4%, restoration 9.5%, implants 8.4%, endodontics 6.6%, orthodontics 3.6% and periodontics 1.8%. Similarly, Tiwana et al.[19] report events in the field of prosthodontics in first place. Perea-Perez[10] finds the highest frequency of events in the

field of implantology, followed by endodontics, oral surgery, prosthodontics and orthodontics.

A range of factors have been found to influence the occurrence of an adverse event. For example, poor management of the patient's medical records considered relevant to patient safety, as well professional skill during the clinical interview, particularly regarding sensitive issues such as HIV[20.] Our study only found one adverse event as a result of diagnosis (2.38%), which occurred due to the lack of a comprehensive diagnosis. Tiwanaet al.[19]found insufficient or erroneous records in 35.6% and in complete medical recordsin 15.1%. Considering the importance of diagnostic help such as diagnostic support and follow-up of treatments, the School of Dentistry policy is that all patients begin with an initial radiograph.It was found that approximately 10% of the cases did not have radiographs, which the students may have removed to perform the diagnosis and failed to return to the clinical file. The problem has now been overcome by the use of digital clinical records, which were implemented 4 years ago at the School.

Among other predisposing factors for adverse events are patient care by a large number of students[21] attempting to perform procedures that are beyond the professional's technical skill, lack of consultation with experts, overconfidence in own skills and knowledge, ignoring evidence-based medicine, operator fatigue, lack of awareness of risks, lack of communication leading to procedural errors through mismanagement of referrals and transfers[22] and following guidelines[18,20,21,23]. We looked at these factors during this study, but found no association between the occurrence of adverse events and the demographic variable sage, sex, level or any influence of number of students or use of guidelines. Regarding age, adverse events have been reported more frequently in adults (25-60 years), with no difference between sexes.[12]

Regarding timing, we found that most events occurred within the first 5 years of treatment, including the inter-semester periods, given the modality of university service provided. In a study on dentists, Hiivala et al.[18] found a time of 17 months, with adverse events attributed mainly to communication breakdowns in the organization.

With regard to the main factors that contribute to preventing adverse events for professionals and university teachers, Bailey[20] mentions knowledge of the patient's medical record as having the greatest impact, as well as quality and adequacy of the record-taking. The pedagogical model implemented at our School includes in its clinical-administrative competences, knowledge of how to use the dental clinical record as an essential safety factor when providing service.

Ten years ago, the School implemented patient safety and service quality committees with the aim of providing a safety policy contributing to systematic follow-up, implementing safety barriers, research and knowledge management in the area, with the aim of creating an impact on students in the exercise of their future profession.

One of the weaknesses of the patient safety culture is the lack of understanding of the concept of adverse event and the lack of research, which does not allow learning how to prevent adverse events, guidelines for improving quality such as detailed monitoring of critical biosafety processes and sterilization, proper medication prescription, control of unnecessary radiation and checklists for all surgical procedures, among others, to help improve quality and patient safety[10.]

The greatest impact on the culture of patient safety is achieved through training. It is at theacademy that knowledge is consolidated with principles of ethics and responsibility. Methodical, systematic rigor in the adherence to safe practices by all staff involved in patient care will reduce the occurrence of adverse events.

Clear, prompt communication skills with patients are essential for decision making by patients regarding treatments and contribute to creating trust. There is a need in both dental professionals and students who are undergoing training to gain deeper knowledge and research of adverse events in order to prevent them from occurring again, thus contributing to improving patient safety and student training.

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Evaluation of pH and calcium ion diffusion from calcium hydroxide pastes and MTA

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ABSTRACT

The aim of this ex vivo study was to evaluate changes in pH and calcium ion diffusion through root dentin from calcium hydroxide ($Ca(OH)_2$) and mineral trioxide aggregate (MTA) pastes at 7, 30 and 60 days; and the relationship between pH and ion diffusion. Thirty-two human premolars were used. Crowns were sectioned and root canals instrumented and filled in with the following preparations: 1) $Ca(OH)_2$ + distilled water (n=7); 2) $Ca(OH)_2$ + 0.1% chlorhexidine gluconate (n=7); 3) MTA + distilled water (n=7); 4) MTA + 0.1% chlorhexidine gluconate (CHX) (n=7); 5) distilled water (n=2) (control); 6) 0.1% chlorhexidine gluconate (n=2) (control). The apex and coronary opening were sealed with IRM. Roots were placed in Eppendorf tubes with 1 ml distilled water at 37°C and 100% humidity. At baseline, 7, 30 and 60 days, pH was measured with pH meter, and calcium ion content in the solution was analyzed by atomic absorption spectrophotometry. The data were statistically analyzed using ANOVA, simple linear regression analysis and Pearson's correlation test. The highest pH values were achieved with calcium hydroxide pastes at 60 days ($p \leq 0.05$). Calcium ions were released in all groups. The calcium hydroxide paste with distilled water at 60 days had the highest calcium ion value ($p \leq 0.01$). There was a positive correlation between calcium and pH values.

Key words: *pH; calcium hydroxide; mineral trioxide aggregate.*

Evaluación del pH y la difusión de iones calcio de pastas de hidróxido de calcio y MTA

RESUMEN

El objetivo de este estudio ex vivo fue evaluar los cambios en el pH y la difusión a través de la dentina radicular de iones calcio a partir de pastas de hidróxido de calcio ($Ca(OH)_2$) y trióxido mineral agregado (MTA), durante 7, 30 y 60 días; y la relación entre el pH y la difusión de iones. Se utilizaron 32 premolares humanos. Las coronas fueron seccionadas, los conductos radiculares fueron instrumentados y obturados con las siguientes preparaciones: 1) $Ca(OH)_2$ + agua destilada (n = 7); 2) $Ca(OH)_2$ + gluconato de clorhexidina (CHX) al 0,1% (n = 7); 3) MTA + agua destilada (n = 7); 4) MTA + gluconato de clorhexidina al 0,1% (n = 7); 5) agua destilada (n = 2) (control); 6) gluconato de clorhexidina al 0,1% (n = 2) (control). El ápice y la apertura coronaria se sellaron con IRM. Las raíces se colocaron en tubos Eppendorf con 1 ml de agua destilada a 37 °C y 100% de humedad. Se midió el pH inicial y a los 7, 30 y 60 días, con pHmetro, y se analizó el contenido de iones calcio en la solución por espectrofotometría de absorción atómica. Los datos fueron analizados estadísticamente con ANOVA, análisis de regresión lineal y correlación de Pearson. Los valores de pH más altos se obtuvieron con las pastas de hidróxido de calcio a los 60 días ($p \leq 0,05$). Todos los grupos mostraron liberación de iones calcio. La pasta de hidróxido de calcio con agua destilada mostró el valor más alto de iones calcio a los 60 días ($p \leq 0,01$). Hubo una correlación positiva entre los valores de pH y calcio.

Palabras clave: *pH, hidróxido de calcio; compuesto de trióxido mineral.*

INTRODUCTION

Calcium hydroxide ($Ca(OH)_2$) has been widely used as an intracanal medication[1]. It is a strongly alkaline substance (pH 12.5-12.8) with high antibacterial activity against oral bacteria. The effectiveness of $Ca(OH)_2$ for inactivation of microorganisms and tissue healing is directly related to its dissociation into calcium and hydroxyl ions. Hydroxyl ions diffuse through the dentinal tubules and inactivate Gram-negative bacterial lipopolysaccharide (LSP)[2]. In addition, their high pH activates tissue enzymes, causing a mineralization effect[3].

Drug vehicles play an important role in the ion dissociation rate, causing the paste to be solubilized

and resorbed by the periapical tissues from within the root canal[4]. Calcium hydroxide was pioneered by Heithersay[5] and Frank[6] in apexification treatment, which provided adequate apical healing through the induction of an apical barrier. However, long-term calcium hydroxide treatment lasting more than 30 days may reduce dentin fracture strength[7]. Due to its alkaline nature, calcium hydroxide denatures some of the acidic dentin proteins which act as bonding agents, thereby weakening the dentin and increasing risk of fracture[8].

Mineral trioxide aggregate (MTA) powder is basically a mixture of 75% Portland cement, 20% bismuth oxide and 5% gypsum[9]. It was developed in 1990 by Torabinejad at Loma Linda University[10]. MTA has been recognized as a bioactive[11], hard tissue conductive[12], hard tissue inductive, biocompatible material[13]. It has been used for apical barriers, root-end fillings, perforation repairs, regenerative therapy, pulp capping and pulpotomies[14]. During the setting process, MTA pH is initially 10.2, increasing to 12.5 during the first few hours. MTA has also been shown to have antibacterial activity[15].

Chlorhexidine gluconate (CHX) is widely used as an irrigant for the treatment of infected root canal systems to reduce endodontic microbiota[16]. Many studies suggest the use of a combination of CHX with $Ca(OH)_2$ for greater antimicrobial action[17]. Chlorhexidine is a broad spectrum antibacterial agent with effectiveness against *Enterococcus faecalis* and *Candida albicans*[18]. CHX can be adsorbed onto dental tissue, resulting in substantive antibacterial activity[19]. Stowe et al.[15] demonstrated that the substitution of sterile water by 0.12% CHX in tooth-colored ProRoot MTA enhanced the antimicrobial effect of the MTA *in vivo*.

MTA could be an alternative to $Ca(OH)_2$ to halt resorption without the disadvantage of weakening tooth structure[20]. Use of MTA must be considered permanent because it is difficult to remove once it has set[21].

The aim of this *ex vivo* study was to evaluate the relationship between pH and diffusion of calcium ions through root dentin using $Ca(OH)_2$ and MTA pastes with different vehicles for 7, 30 and 60 days.

MATERIALS AND METHODS

Preparation of specimens

Thirty-two single-root human mandibular premolars, recently extracted for orthodontic reasons, were selected on the basis of their similarity in morphology and size. Crowns were amputated at cementoenamel junction level using a high speed bur #2200 (KG Sorensen, SP, Brazil) and water irrigation. The cementum was then removed using Gracey curettes. Root canals were enlarged up to file #45 (Maillefer, East Lansing, MI, USA), and cleaned and shaped using the step-back technique. After each instrument change, root canals were irrigated with 2 ml 1% sodium hypochlorite, and 17% EDTA was used as final irrigation for 2 minutes. Then root canals were rinsed with distilled water, dried with absorbent paper points, randomly divided into six groups and filled with the following preparations: Group I: 1 g calcium hydroxide (Farmadental Lab, Buenos Aires, Argentina) with 1.5 ml distilled water ($Ca(OH)_2$ + DW); Group II: 1 g calcium hydroxide with 1.5 ml 0.1% chlorhexidine ($Ca(OH)_2$ + CHX); Group III: MTA (CPM™, Egeo S.R.L., Buenos Aires, Argentina) with distilled water (MTA + DW). The powder/liquid ratio was 0.33/1 (w/w). Group IV: MTA with 0.1% chlorhexidine (MTA + CHX). The powder/liquid ratio was 0.33/1 (w/w). Group V: distilled water as a control group (DW); Group VI: 0.1% chlorhexidine (CHX), prepared from a 20% chlorhexidine solution, as a control group.

In the experimental groups, the paste was placed using a Lentulo spiral (Dentsply, Mailifer, Switzerland). Once the root canal was filled completely, the apical foramen and root canal openings were sealed with restorative temporary cement, IRM™ (Denstply, USA). The roots were then stored in individual plastic tubes (Eppendorf) containing 1 ml distilled water (Fig. 1). They were kept at 37 ºC and 100% relative air humidity throughout the testing period. After 7, 30 and 60 days, the water was assessed for pH and calcium ion release.

Analyses of pH and calcium ion release

pH readings

The pH was determined with a digital pH meter (Broadley-James Irvine, California, USA) for small volumes (sensitivity: 0.01 pH units), calibrated to pH 7 and 4 with standard buffer solutions before use. The pH was determined by placing the refillable calomel electrode in 30 µl of sample on a slide for 10 seconds. The electrode was washed with distilled water and wiped dry between readings.

Fig. 1: Experimental groups were stored in individual plastic tubes (Eppendorf).

Calcium ion release readings

To determine the concentration of calcium ions, 970 µl of each sample were transferred to a 5 ml flask, and 5 g/l of potassium chloride and 5g/l of lanthanum chloride were added to eliminate interferences by acidifying the samples with hydrochloric acid. The final volume was completed with distilled water. A calibration curve was prepared with standard solutions of 1.5, 2.5 and 5 mg/l of Ca (Certipur Merck to NIST).

Atomic Absorption Spectroscopy (Perkin Elmer AAnalyst 100) with acetylene flame was used. Calcium in the samples was determined at 422.7 nm wavelength. Each value was expressed in mg/l. Measurements were performed at 7, 30 and 60 days.

Statistical analysis

Differences between study groups were statistically analyzed by ANOVA at a significance level of 5%. Simple linear regression analysis and Pearson's correlation test were used to evaluate the relationship between calcium ion release and pH over time.

RESULTS

Table 1 shows mean pH values for the pastes at the different experimental times. The pH values increased over time for all groups. At 7 days, all test groups differed significantly from the control groups. There was no statistically significant difference between treatments and controls at 30 days. At 60 days, Group I presented the highest pH value. There was a considerable difference between $Ca(OH)_2$ and MTA groups, but no difference between Groups I and II ($p>0.05$).

Groups III and IV did not differ significantly either at different time intervals or from each other (statistical data not shown).

Mean values for calcium ions released for all groups are shown in Table 2. Calcium ion diffusion occurred in all groups. At 7 days, there were statistically significant differences between Groups I and II and the control Groups ($p\leq 0.05$). Group II presented the highest value for calcium ion release at 7 days and was the only group that differed significantly from the rest of the experimental groups ($p\leq 0.05$).

At 30 days, Group I showed the highest value for calcium. There was no significant difference between Group II and Group IV ($p>0.05$).

Moreover, Groups III and IV did not differ significantly from the control group.

Group I released the most calcium ions and achieved the highest mean at 60 days, differing significantly from all the other groups ($p\leq 0.05$). Group II also differed considerably from the rest of

Table 1: Mean values for pH and standard deviations for all periods and groups.

Groups	Initial	7 days	30 days	60 days
I. $Ca(OH)_2$ + DW	6.48 ± 0.00	7.55 ± 0.05^b	8.93 ± 0.78^a	12.2 ± 0.26^b
II. $Ca(OH)_2$ + CHX	6.48 ± 0.00	7.66 ± 0.23^b	8.64 ± 0.84^a	11.4 ± 1.00^b
III. MTA + DW	6.48 ± 0.00	7.72 ± 0.11^b	7.89 ± 0.24^a	8.27 ± 0.26^a
IV. MTA + CHX	6.48 ± 0.00	7.84 ± 0.11^b	8.00 ± 0.30^a	8.00 ± 0.23^a
V. DW	6.48 ± 0.00	7.17 ± 0.14^a	7.78 ± 0.02^a	7.53 ± 0.08^a
VI. CHX	6.48 ± 0.00	7.18 ± 0.10^a	7.83 ± 0.05^a	7.47 ± 0.05^a

DW: distilled water; $Ca(OH)_2$: calcium hydroxide; CHX: chlorhexidine; MTA, Mineral trioxide aggregate. Different letters in the column indicate statistically significant differences between the groups in each study period ($p \leq 0.05$).

the pastes. MTA pastes did not differ significantly from each other or from the control group.

There was no significant difference between 7 and 30 days in Group II, although a difference appeared at 60 days (statistical data not shown).

At all the experimental times, the MTA groups showed the lowest values for calcium ion release (Table 2).

There was a positive correlation between calcium ions and pH in experimental groups I, II, III and IV (Fig. 2), with calcium ions increasing as pH values increased. According to Pearson's correlation test, the correlation between pH and calcium ion diffusion was 69% at 7 days, 98% at 30 days and 97% at 60 days.

Control groups V and VI showed a negative correlation, with calcium ions decreasing as pH values increased (Fig. 2).

DISCUSSION

The therapeutic effects of calcium hydroxide depend on the dissociation of calcium and hydroxyl ions and the availability of hydroxyl ions to increase the pH of the medium[22]. The greater the number of hydroxyl ions, the higher the pH. Many components are mixed with $Ca(OH)_2$ including various vehicles, such as propylene glycol, distilled water, anesthetic solution, saline solution, chlorhexidine, chitosan and antibiotics[23]. Propolis[24] and *Aloe vera*[25] have also been proposed as vehicles for $Ca(OH)_2$.

Considering that the efficacy of distilled water (DW) as a vehicle for $Ca(OH)_2$ has been demonstrated in the literature[3], it was used in this study as reference group for comparisons.

Nerwich et al.[26] measured pH changes in root dentin over a four-week period, which they considered to be a reasonable time interval in which to expect effective therapeutic benefits from $Ca(OH)_2$ based materials. However, long-term and short-term

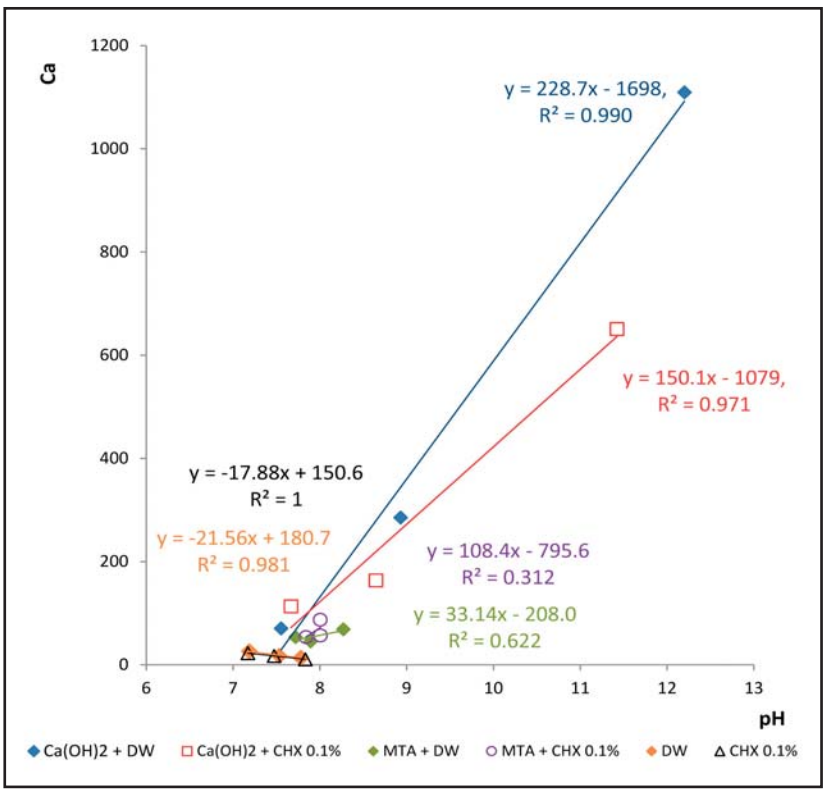

Fig. 2: Correlation between pH and calcium ion diffusion.

Table 2: Mean values and standard deviations for calcium ions (mg/l) for all treatments and periods.

Groups	7 days	30 days	60 days
I. $Ca(OH)_2$ + DW	70.47 ± 24.64^{b}	285.27 ± 82.47^{c}	1109.33 ± 79.41^{c}
II. $Ca(OH)_2$ + 0.1% CHX	113.27 ± 11.97^{c}	163.47 ± 63.22^{b}	650.67 ±157.60^{b}
III. MTA + DW	53.47 ± 8.81^{ab}	45.40 ± 12.94^{a}	68.67 ± 26.31^{a}
IV. MTA + 0.1% CHX	53.70 ± 13.46^{ab}	57.03 ± 4.35^{ab}	87.33 ± 11.15^{a}
V. DW	26.20 ± 0.36^{a}	13.57 ± 3.21^{a}	17.33 ± 3.21^{a}
VI. 0.1% CHX	22.47 ± 2.67^{a}	10.60 ± 1.37^{a}	17.00 ± 5.57^{a}

DW: Distilled water; Ca(OH)2: calcium hydroxide; CHX: chlorhexidine; MTA, mineral trioxide aggregate. Different letters in the column indicate statistically significant differences between treatments in the same time period ($p \leq 0.05$).

$Ca(OH)_2$ treatment may reduce dentin fracture strength[7,8]. MTA could be a treatment option in cases with external inflammatory root resorption, particularly when root fracture could be a risk[20].

The diffusion of $Ca(OH)_2$ through dentinal tubules has been evaluated in several studies[22, 27-29]. In this study, calcium ion release from MTA and $Ca(OH)_2$ was evaluated over two months. The accuracy of the technique used is important for precise assessment of the amount of calcium ion released. Several methods such as atomic absorption spectrometry, ultraviolet spectrophotometer, fluorometry, flame photometry and complexometric titration with EDTA have been used[30]. The present study used an atomic absorption spectrometer.

In the present study, all the experimental groups showed an increase in pH over time. This is in agreement with Duarte et al.[31], who found that all pastes behaved similarly in terms of pH and calcium ion release for the different study periods.

The vehicles employed in calcium hydroxide pastes and MTA influence their diffusion capacity. Estrela et al.[3] explained that distilled water allows the fastest and most significant dissociation. This is consistent with our study, where the highest levels of calcium ion release were obtained when distilled water was used as a vehicle. The highest pH values were also observed with distilled water at 60 days.

Hansen et al.[32] found different pH values at different levels of the root, possibly related to the number and direction of dentinal tubules at each level. Perez et al.[33] showed that the pH of dentin depends on the type, location and duration of $Ca(OH)_2$ application. In agreement with this, our study found higher pH and calcium ion values at 60 days.

Evans et al.[34] reported that *Enterococcus faecalis* was resistant to $Ca(OH)_2$ at pH 11.1, but at pH 11.5, 99.9% were killed. In a previous study, we found that the pH values of the experimental groups were not alkaline enough to eliminate *E. faecalis* from the root canal in the lapse of a week or even a month[35]. However, in the present study, $Ca(OH)_2$ with DW at 60 days had a pH value (12.2 ± 0.26) high enough to kill *E. faecalis*.

Due to the antimicrobial effectiveness of CHX, it has been suggested as a vehicle for $Ca(OH)_2$ pastes. The antimicrobial activity of the association of $Ca(OH)_2$ and CHX has already been evaluated, revealing effectiveness against several bacterial populations[17, 36]. Stowe et al.[15] observed that adding CHX to MTA could enhance antimicrobial activity. According to our results, CHX would be an efficient vehicle for $Ca(OH)_2$ allowing the diffusion of calcium ions. Moreover, for MTA pastes using CHX as vehicle, calcium ion diffusion was similar or higher than for MTA with distilled water.

Mori et al.[37] concluded that the concentration of CHX influenced calcium diffusion. They demonstrated that 2% CHX allowed higher diffusion than 0.2% CHX. In contrast, the present study found higher calcium ion diffusion with 0.1% chlorhexidine than the results reported by Mori et al.[37]. Another interesting factor to consider is the higher viscosity and easier preparation of the pastes with CHX compared to those prepared with distilled water.

This study showed that at 30 days there was no difference in the pH values between $Ca(OH)_2$ pastes and MTA pastes. However, Heward et al.[20] found that after four weeks, intracanal placement of MTA resulted in significantly higher pH than did $Ca(OH)_2$. Differences in methodologies might explain this apparent disparity.

Ozdemir et al.[38] showed diffusion of calcium through the defects in the dentin in MTA-filled roots with a significant increase in concentration over time. Each drug presents different diffusion characteristics, which are directly related to its interaction with the tooth structure[39]. In the present study, MTA pastes present the lowest calcium ion release values at all experimental times. In contrast, Tonomaru-Filho[40] reported that MTA and Sealer 26 presented the highest values of calcium ion release after 28 days, out of six sealer materials evaluated. Similarly to our results, Tonomaru-Filho[40] also reported that control group released calcium ions, even though the teeth were unfilled, because the tooth itself can release calcium ions from its structure.

In this *in vitro* study, all the pastes analyzed increased the pH values over time, although calcium hydroxide and distilled water pastes showed the highest pH values and calcium ion diffusion. The greatest diffusion of ions took place between 30 and 60 days. Calcium hydroxide paste groups showed better diffusion capacity than MTA. Nevertheless, further studies should be undertaken to determine whether MTA could be a good option for teeth at risk of fracture.

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Root surface temperature variation during mechanical removal of root canal filling material. An *in vitro* study

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ABSTRACT

The aim of this study was to analyze in vitro temperature changes on the outer surface of the dental root during mechanical filling removal procedures.

Thirty recently extracted single-rooted lower premolars were cut transversally at 16 mm from the apex in order to standardize sample length. Endodontic treatment was performed on them. The filling material was subsequently removed using Gates Glidden (G1, G2, G3); Peeso (P1, P2, P3) and PostecPlus FRC (FRC) reamers while temperatures were measured on the outer surface using a digital device with thermocouple at 0, 2, 4, 6, 8, 10 and 15 seconds. Temperatures were compared using repeated measures ANOVA followed by pairwise comparison with Tukey's test.

All reamers caused significant temperature variation between different times ($p<0.05$). Pairwise comparisons indicated that temperature increased with time for all reamers ($p<0.05$). Significant differences in temperature were found between different reamers after 0, 2, 4, 6, 8, 10 and 15 seconds ($p<0.05$). Temperature at the root surface increased considerably. Values higher than 50ºC were recorded, the greatest increase from baseline being 16ºC. Accordingly, if the procedure were begun at 37ºC (physiological temperature), the temperature in the surrounding tissues - cementum, periodontium and bone - would rise to 53ºC. An increase in 10ºC above body temperature at the root surface may cause lesions in surrounding tissues. While removing filling material, it is essential to cool, control action time and use instruments in perfect condition, all of which may contribute to reducing the heat generated and transmitted to the outer root surface.

Key words: *Root canal preparation; transition temperature; endodontic.*

Variaciones térmicas en la superficie radicular durante la desobturación mecánica del conducto. Estudio *in vitro*

RESUMEN

El objetivo del presente trabajo fue estudiar los cambios térmicos in vitro en la superficie externa de la raíz del diente, generados durante los procedimientos de desobturación mecánica.

Se utilizaron 30 premolares inferiores unirradiculares recientemente extraídos, que fueron seccionados transversalmente a 16 mm del ápice para estandarizar la longitud de las muestras. Se realizó luego su tratamiento endodóntico. Las mediciones de temperatura fueron realizadas mediante un dispositivo digital con termocupla, en la superficie externa a diferentes tiempos (t = 0, 2, 4, 6, 8, 10 y 15 segundos), durante la desobturación con fresas de Gates Glidden (G1, G2, G3); de Peeso (P1, P2, P3) y la correspondiente a PostecPlus FRC (FRC). Los registros de temperatura fueron comparados mediante pruebas de ANOVA de medidas repetidas, seguidas por comparaciones entre pares mediante la prueba de Tukey.

Con todas las fresas se encontró una variación significativa de la temperatura entre los diferentes tiempos ($p<0.05$). Las comparaciones entre pares indicaron que la temperatura se incrementó con el tiempo en todas las fresas ($p<0.05$). Se detectaron diferencias significativas de temperatura entre diferentes fresas después de 0, 2, 4, 6, 8, 10 y 15 segundos ($p<0.05$).

El aumento de la temperatura de la superficie radicular fue importante ya que los valores registrados superan los 50ºC, teniendo en cuenta que el aumento de temperatura mayor fue de 16ºC. Si partimos de los 37ºC (temperatura fisiológica), la temperatura presente en los tejidos circundantes; cemento, periodonto y hueso; alcanzaría los 53ºC. Un aumento de 10ºC por encima de la temperatura corporal en la superficie radicular podría causar lesiones en los tejidos circundantes. La utilización de refrigeración, el control del tiempo de acción y el uso de un instrumental en estado óptimo son parámetros ineludibles debido a que los mismos pueden contribuir a disminuir el calor generado y trasmitido hacia la superficie externa de la raíz durante la desobturación.

Palabras clave: *Preparación del conducto radicular; temperatura de transición; endodoncia.*

INTRODUCTION

In teeth with endodontic treatment, crown and root structures are weakened by loss of tissue as a result of previous restorations, caries and preparation for endodontic access. It is therefore important to note that rehabilitating an endodontically treated tooth involves working on a structure that has been diminished both mechanically and biologically. The main reinforcement in an endodontically treated tooth is constituted by its own tissues and anatomical structures, so as a general principle; the selected restoration procedures should preserve as much tissue as possible[1]. When much of the clinical crown has been lost, the remaining dentin often does not provide sufficient anchorage for a restoration. Such cases call for intraradicular restoration using materials such as posts made from organically based materials and bonded with resin cements to the remaining tooth. Their mechanical behavior is similar to that of dental tissues and thereby improves the distribution of forces [2].

Filling material has to be removed from the canal to provide a smooth bonding surface between wall and anchor, at the same time preserving tooth anatomy. This is done using reamers. It is important to handle and control rotary instrument speed adequately in order to avoid increasing the temperature at the root surface.

Bone tissue is sensitive to temperatures over 47º C (10° higher than body temperature), which may damage microcirculation and connective tissue, cementim, periodontium and alveolar bone as well as causing dentine resorption and chronic immflamation of the periodontim and adjacent bone tissue [3-6]. Damage may be reversible if it is limited a and temperature does not exceed 53°C, (alkaline phosphatase denaturing point); however, higher temperatures may cause irreversible bone damage. The mechanisms of such damage are not fully understood. Despite the low thermal conductivity of dentine, it can still transmite heat to the outer surface of the root and tooth-supporting tissues when rotary systems are used during endodontic preparation [7-12].

The aim of this study is to analyze temperature changes on the outer surface of the root caused by mechanical procedures for removing filling from a root canal.

MATERIALS AND METHODS

This *in vitro* study used an experimental design to simulate usual endodontic clinical procedures on 30 single-rooted lower premolars which had been recently extracted by orthodontic indication. Sex, age and reason for extraction were not considered as study variables. Extracted teeth were stored in 0.5% chloramine-T solution at 4 ºC.

Inclusion criteria were:

- Straight, single-rooted teeth.
- Conical roots with circumferential diameter 15.5 ± 2.0 mm.

Root length was standardized at 16 mm as measured from apex to crown, at which level it was cut transversally using a diamond disc (KG Sorensen, Brazil) with plentiful cooling. Preoperative periapical radiographs were taken of each tooth for 0.7 second with Skydent Speed E film and New Life Radiology 65KV 8mA Denimed X-ray equipment by paralleling technique with focus-to-object distance 10 cm, to obtain an image of the longitudinal axis of the tooth.

One specialist performed endodontic treatment on all teeth using the ProTaper Universal system (Densply-Maillefer. Ballaigues, Switzerland). Catheterization was performed using a K 10 file, followed by preparation of access using K 10-15-20 files (Dentsply Maillefer, Switzerland) and Protaper system S1-S2-Sx files, which were only used on the coronal and middle thirds. Rinses with 10 ml 2.5% sodium hypochlorite were applied between files and canal apical patency was maintained with a No. 10 patency file. Working length was determined by measuring the canal with a K N 15 file. Mechanical preparation was done with ProTaper F1-F2-F3 files for the apical third and ProTaper S1, S2, F1, F2 and F3 files for the middle and coronal thirds. Finally, 17% EDTAC was allowed to act for 5 minutes, simulating the time for which dentin remains in contact with endodontic irrigants, and then rinsed with 10 ml 2.5% sodium hypochlorite [13,14].

Root canals were dried with standardized absorbent paper points (Dentsply) and filled using hybrid technique with size 30 gutta-percha points (Dentsply-Maillefer), Sealer 26 (Dentsply-Maillefer) and size 15 accessory points, which were thermoplasticized using a size 30 gutta-condensor (Dentsply-Maillefer). Canal openings were sealed with glass ionomer (Vitrebond - 3M. Seefeld, Germany). Samples were stored for 7 days at 37ºC and 100% humidity in an oven (Biomerican, model bs615).

Canal preparation and filling removal were performed by one standardized operator in order to

reduce bias. Filling material was removed from the canal using Gates Glidden No. 1, 2 and 3 (Dentsply Maillefer, Switzerland), Peeso No. 1, 2 and 3 (Dentsply Maillefer, Switzerland) and Postec Plus FRC 3 system reamers (tapered reamers with 1.3 mm diameter at cervical level and 0.6mm diameter at apical level for size 1, 1.5mm/0.8mm for size 2 and 2mm/1mm for size 3) (Ivoclar Vivadent, Liechtenstein). Each reamer was used to prepare five beds, as intended by the commercial kit. No cooling was used during the procedure.

Temperature was measured at baseline (time 0) and at 2, 4, 6, 8, 10 and 15 seconds after the start of filling removal. Temperature variations were measured with a digital device with thermocouple (M890G Temperature Meter, Fig. 1). The sensor was firmly attached to the outer surface of the middle third of the root (Fig. 2).

Statistical analysis

Results were analyzed statistically. Thirty temperature measurements were recorded for each combination of time and reamer. Mean and standard deviation (SD) were reported for temperatures for each of these groups. Temperature was compared among times for each reamer and among reamers for each time. These comparisons were performed by repeated measures ANOVA, which is appropriate for pairwise data, as in this experimental design[15]. When ANOVA provided a significant result, pairwise comparisons were performed between groups using Tukey's test. For all tests, results were considered significant when $p<0.05$. Analyses were performed using the software Infostat version 2013[16].

Fig. 1: Digital device with thermocouple.

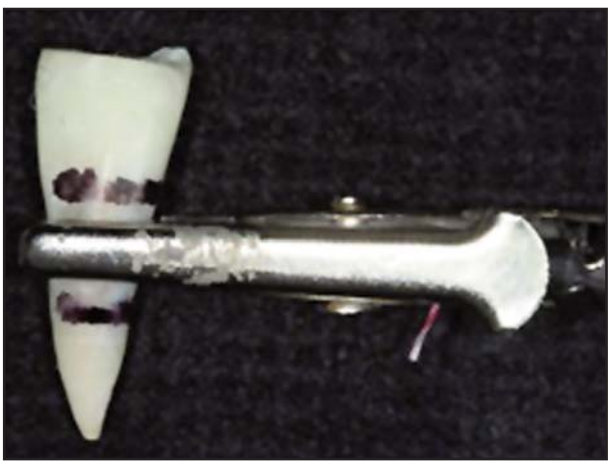

Fig. 2: Measuring samples.

RESULTS

Table 1 summarizes the results. Temperature change over time was similar for the different reamers. Repeated measurements ANOVA provided a significant global result ($p<0.05$). Pairwise comparison with Tukey's test showed that temperature differed significantly among different times, with higher temperatures as time increased. There was only one exception: for Gates 1, pair wise comparisons showed no significant difference between baseline and 2 seconds.

Temperatures were not compared between reamers for baseline (time 0) because all values were equal (23ºC). For each subsequent time, significant differences in temperature were found between reamers using repeated measures ANOVA. Specifically, pairwise comparison using Tukey's test showed the following:

- At all times, temperatures with Gates 1 were lower than the rest.

- At two seconds, there was no significant difference among Gates 2, Gates 2, Largo 1, Largo 2 and Largo 3. Temperature with FRC 3 were higher than the rest.
- At four seconds, temperature with Largo 1 was lower than temperature with Gates 2, Gates 3, Largo 2, Largo 3 and FRC 3. Temperature with Largo 3 and FRC 3 was higher than with Gates 2, Gates 3 and Largo 2.
- The results at 6, 8, 10 and 15 seconds were similar. The highest temperature was reached with Largo 3. Temperatures with Gates 3, Largo 2 and FRC 3 were higher than with Gates 2 and Largo 1.

Fig. 3 shows results for baseline and at 8, 10 and 15 seconds.

Table 1: Comparison of temperatures among different reamers and times, with repeated measures ANOVA followed by post hoc pairwise comparisons (Tukey's test).

Time (s)	Temperature (ºC) (Mean ± SD, n=30)							ANOVA between *reamers
	Gates 1	*Gates 2*	*Gates 3*	*Largo 1*	*Largo 2*	*Largo 3*	*FRC 3*	
0	23 ± 0 (a)	23 ± 0 (a)	23 ± 0 (a)	23 ± 0 (a)	23 ± 0 (a)	23 ± 0 (a)	23 ± 0 (a)	-
2	23 ± 0 (a,C)	24 ± 1 (b,B)	24 ± 0 (b,B)	24 ± 1 (b,B)	24 ± 1 (b,B)	24 ± 1 (b,B)	25 ± 1 (b,A)	$F_{6,203}$=16 P <0.0001
4	24 ± 1 (b,D)	26 ± 1 (c,B)	26 ± 1 (c,B)	25 ± 1 (c,C)	26 ± 1 (c,B)	27 ± 1 (c,A)	27 ± 1 (c,A)	$F_{6,203}$=97 P <0.0001
6	25 ± 0 (c,D)	27 ± 1 (d,C)	28 ± 1 (d,B)	27 ± 1 (d,C)	28 ± 1 (d,B)	31 ± 1 (d,A)	28 ± 1 (d,B)	$F_{6,203}$=207 P <0.0001
8	26 ± 0 (d,D)	28 ± 1 (e,C)	30 ± 1 (e,B)	28 ± 1 (e,C)	30 ± 2 (e,B)	35 ± 1 (e,A)	30 ± 1 (e,B)	$F_{6,203}$=262 P <0.0001
10	28 ± 0 (e,D)	30 ± 1 (f,C)	32 ± 1 (f,B)	30 ± 1 (f,C)	32 ± 1 (f,B)	37 ± 1 (f,A)	32 ± 1 (f,B)	$F_{6,203}$=482 P <0.0001
15	29 ± 0 (f,D)	33 ± 1 (g,C)	34 ± 1 (g,B)	33 ± 1 (g,C)	34 ± 1 (g,B)	39 ± 1 (g,A)	34 ± 1 (g,B)	$F_{6,203}$=604 P <0.0001
ANOVA between times	$F_{6,174}$=967 P <0.0001	$F_{6,174}$=1280 P <0.0001	$F_{6,174}$=1686 P <0.0001	$F_{6,174}$=1320 P <0.0001	$F_{6,174}$=694 P <0.0001	$F_{6,174}$=4923 P <0.0001	$F_{6,174}$=2059 P <0.0001	

Letters indicate results of post hoc pairwise comparisons by Tukey's test. Different lowercase letters indicate significant differences (P <0.05) between times for the same reamer. Different uppercase letters indicate significant differences (P <0.05) between reamers for the same time.

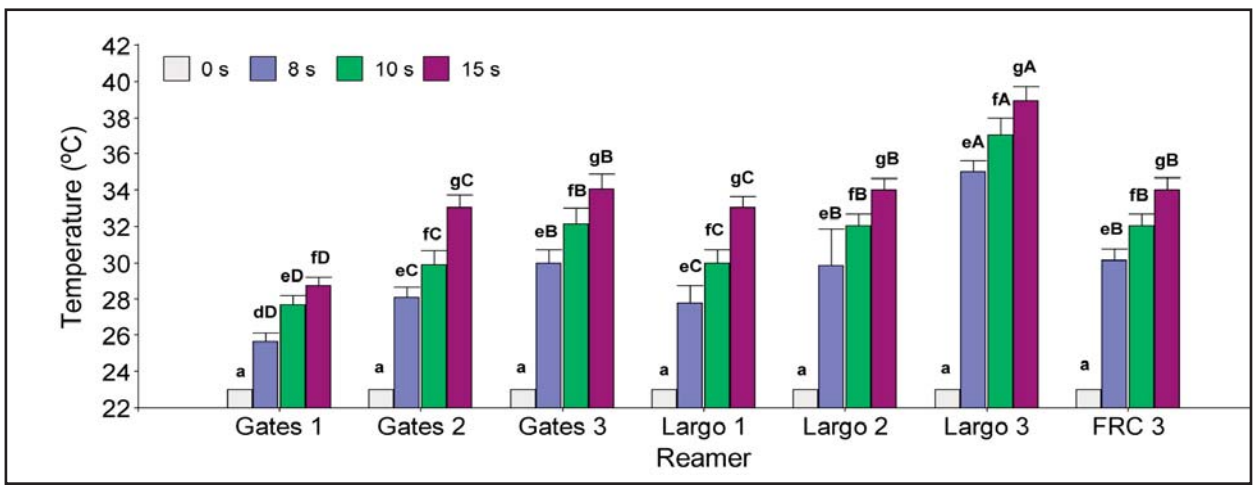

Fig. 3: Temperature reached at different times (0, 8, 10 and 15 seconds) for each reamer (mean + SE). Different lowercase letters indicate significant differences (P< 0.05) between times for the same reamer. Different uppercase letters indicate significant differences (P< 0.05) between reamers for the same time.

DISCUSSION

The harmful effects of heat on the outer root surface during filling removal procedures are clinically important because an increase greater than 10°C above physiological temperature (37ºC) can alter the viability of supporting tissues and cause bone necrosis, cell apoptosis and sometimes, in more severe cases, tooth ankylosis[9]. Heat generation while removing filling from the canal system can be modified in various ways, including type of instrument used (acuity, sharpness and size), condition of instrument cutting edges, rotation, cutting pressure applied and contact time with tooth structure.

This study found temperature increases of up to approximately 16ºC at 15 seconds during canal preparation. Wider reamers were associated to greater heat generation. Manufacturers' protocols for filling removal say that working time should be shorter than 1 minute. Based on our results, we suggest that this time limit should be considerably lower.

Another factor to consider during filling removal is how thick the remaining dentin is in the canal walls. Stripping dentin tissue excessively from the canal walls during preparation with the aim of increasing anchorage and achieving better fit of the element to be bonded is contraindicated. It will not only weaken the walls, but also increase heat transmission outward. Excessive removal of root dentin is known to compromise the root, and preserving root dentin is directly related to root strength [17,18]. Thus, knowledge of the internal tooth anatomy contributes to dental practice which is more conservative of tissues, and avoids causing excessive damage to teeth and tooth supporting tissues during preparation. Customizing posts prior to bonding may contribute to reducing the occurrence of irreversible injury.

Lubieniecka *et al.*[19] replicated the clinical situation of removing filling from canals during post space preparation and analyzed it with a thermal imaging camera. The effect of cooling was clear in the cervical region of the tooth, where temperature was very close to initial temperature reading. The highest temperature on the surface of the root corresponded to the greatest depth that the drill reached. The periapical zone experienced very little or no temperature increase[19]. Weller *et al.*[20] recorded the highest temperature increase at the most coronal part of the root, which is closely related to larger width of reamers, more gutta-percha in this part of the tooth and thinner dentin walls. Other authors such as Lima Machado and Antoniazzi[21] reported higher temperatures for instrumentation of the cervical third than for the middle and apical thirds.

Some authors suggest that clinicians should take into account that dentin is considered to be a good thermal insulator, and the thicker it is, the less heat will be transferred to the outer surface of the root[22-24]. However, depending on their anatomy, not all teeth have a thick layer of dentin, e.g. lower incisors have very thin walls. Care must therefore be exercised to avoid damaging tooth support tissue when remaining dentin is less than 1 mm thick[22].

Further studies could consider working length, type and size of reamer used and cooling while preparing root canal. The use of anatomic posts could also be considered, since they are more respectful of tooth anatomy, requiring less thinning of dentin walls and consequently providing treatment that is more conservative of remaining dentin structures.

CONCLUSION

Within the limitations of the present study, the increase in temperature at the root surface during mechanical removal of filling was important, since the values recorded at the outer root surface were high enough to damage tissues surrounding the tooth. Even though temperatures increased to critical values for surrounding tissues, the absolute results of this *in vitro* study are not directly transferable to real clinical situations, since they would be influenced by periodontal tissues, periodontal blood circulation and the oral environment.

These results suggest the need for further studies to enable current protocols to be adapted.

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Methodological aspects in the study of periodontal breakdown in rats: influence of the presence and time of ligature

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ABSTRACT

The aim of the present work was to evaluate the effect of different times on alveolar bone loss (ABL) and whether the presence of ligature on one side affects ABL on the contralateral site.

This is a secondary analysis of databases from studies conducted at the Federal University of Rio Grande do Sul. Included studies used ligature-induced periodontal disease in rats. In order to be included, the studies were required to have a control group without any ligatures and an intra-group control. Three studies were included, which used different time periods: 2 weeks with ligature and 8 weeks without ligature; 5 weeks with ligature and 17 weeks without ligature; 22 weeks with and without ligature. Animals were raised similarly and sacrificed by decapitation. Maxillae were defleshed with 9% sodium hypochlorite. Pictures were taken and five measurements were obtained from each image.

The presence of ligature generated significantly greater ABL compared to sides without ligature. Comparing sides with ligature, ABL was lower at 2 weeks than at 5 and 22 weeks. Sides without ligature showed no significant difference between 8 and 17 weeks for spontaneous periodontitis. However, after 22 weeks, animals exhibited significantly greater ABL when compared to other periods. The presence of ligature on one side did not influence ABL on the contralateral side.

Two weeks of ligature-induced periodontal disease seems to be sufficient to demonstrate significant ABL. Teeth without ligature contralateral to teeth with ligature may be considered sound controls, thereby reducing the amount of animals needed in periodontal research.

Key words: *Periodontal diseases; periodontitis; rats.*

Aspectos metodológicos no estudo da destruição periodontal em ratos: influência da presença e tempo de ligadura

RESUMO

Objetivo: avaliar o efeito de diferentes períodos experimentais e se a presença de ligadura em um dos lados afeta a perda óssea alveolar (POA) no lado contralateral.

O presente estudo trata-se de uma análise secundária dos bancos de dados de estudos realizados na Universidade Federal do Rio Grande do Sul. Os estudos incluídos utilizaram o modelo de indução de doença periodontal por ligadura em ratos. Os estudos necessitavam possuir grupo controle sem ligadura, assim como controle intra-grupo. Foram incluídos 3 estudos, com diferentes períodos de análise: 2 semanas com ligadura e 8 semanas sem ligadura; 5 semanas com ligadura e 17 semanas sem ligadura; 22 semanas com e sem ligadura. Os ratos foram criados nas mesmas condições, sacrificados por decapitação, as maxilas retiradas e os tecidos moles removidos com hipoclorito de sódio 9%. Tomadas fotográficas foram realizadas e cinco mensurações foram obtidas de cada imagem. A presença de ligadura gerou uma perda óssea alveolar significativamente maior quando comparado ao lado sem ligadura. Nos lados com ligadura um período de 2 semanas mostra menor perda óssea alveolar que 5 e 22 semanas. Lados sem ligadura foram avaliados e não observou-se diferença significativa entre 8 e 17 semanas para periodontite espontânea. No entanto a partir de 22 semanas os animais exibiram significativamente maior perda óssea alveolar quando comparado aos demais tempos experimentais. A presença de ligadura em um dos lados não influenciou a perda óssea do lado contralateral. Duas semanas de doença periodontal induzida por ligadura parece ser suficiente para demonstrar perda óssea significativa e a utilização de lados contralaterais de dentes com ligadura é possível de ser considerada como controles saudáveis, reduzindo o número de animais em pesquisa.

Palavras-chave: *Doenças periodontais; periodontite; ratos.*

INTRODUCTION

Periodontitis is highly prevalent worldwide and is a major cause of tooth loss in adults[1, 2]. It affects the underlying supporting structures of the teeth, resulting in loss of connective tissue and bone support[3, 4].

Animal models contribute to the body of evidence, with increasing translational potential[5]. Experimental periodontitis models have been used to understand the etiopathogenic processes involved in periodontal disease, and to study new therapeutic agents and other factors associated with periodontitis[6-10]. Rats are often used in studies of experimental periodontitis because their anatomy in the molar region is very similar to that of humans[11-15].

Periodontitis and bone loss in rats may be spontaneous, as described in some recent studies,[16,17] or induced. There is clear evidence in the literature demonstrating bone loss in rats induced by the injection of lipopolysaccharides (LPS) from different bacterial strains including *P.gingivalis* or the use of ligatures in the gingival sulcus around molars[6,12]. The latter is based on the creation of a bacterial retention factor created by ligature placement, mimicking what happens in humans faster and more intensely. The use of ligatures as a periodontal disease induction model has been suggested by some authors as a representative model for studying the pathogenesis of periodontal disease[13]. However, issues such as the possible trauma generated by the presence of ligature and potential loss of ligatures during the trial period should be taken into account when the study is planned. The "split mouth" design uses ligatures, for example, on one side but not on the contralateral site, which serves as a control. If the "split mouth" design is used, the possibility of a crossover effect should be considered, since the presence of an irritant on one side may affect the contralateral site. Regarding time of periodontal disease induction, there is no consensus in the literature. Some authors report larger alveolar bone loss levels in the first 7-15 days after placement of the ligature[14], though periods of 4 or more weeks are widely used[15].

There are different methods proposed in the literature to measure alveolar bone loss in rats: histometric, morphometric, radiographic measurements and computed tomography. They are all widely used, accurate and capable of detecting alveolar bone loss in rats[18]. To date, no single method is considered the gold standard for the measurement of periodontal disease in rats, so the method should be chosen according to the purpose of the study.

The aim of the present study is to evaluate alveolar bone loss at different time points and to ascertain whether the presence of ligature on one side affects alveolar bone loss on the contralateral site in these time periods. The hypothesis to be tested is that the placement of a ligature on one side of the animal does not increase spontaneous alveolar bone loss on the contralateral side. In addition, a hypothesis is proposed suggesting that time does not substantially increase bone loss beyond 2 weeks of ligature placement.

MATERIALS AND METHODS

Study Design

This is a secondary analysis of a database of studies in which periodontal breakdown was induced by ligature in Wistar rats. Data were retrieved from eligible studies of periodontal disease models induced by ligature in rats conducted by the periodontology research group at Federal University of Rio Grande do Sul. To be eligible for the analysis, a study was required to include control groups that did not undergo periodontal disease induction and intra-group controls, i.e. sites contralateral to those in which periodontal disease was induced by ligature placement. The search in databases revealed 19 eligible studies, of which 3 met the inclusion criteria. These three studies used different experimental periods, as follows: Study 1: 2 weeks of ligature and 8 weeks of spontaneous periodontal breakdown; Study 2: 5 weeks of ligature and 17 weeks of periodontal breakdown, and Study 3: 22 weeks for both spontaneous and induced periodontal breakdown.

Animals

All studies included utilized 45 to 60 day-old male Wistar rats (weighing 250-350g). Animals were housed in groups of 4-5 under a 12-hour light/dark cycle at room temperature (22°C ± 2°C) with free access to water and standardized rat chow (Nuvilab CR-1, NUVITAL®, Curitiba, PR, Brazil). The animals remained throughout the experimental periods at two different locations, according to the study, with the same routines. A total 88 rats were included in the analysis.

The studies included in this analysis followed important aspects of methodological care, as provided in the ARRIVE Guidelines[19]. For example, randomization, blinding, calibration of examiners, reproducibility and care in the handling of animals, especially in the reduction of pain and discomfort, were observed in all included.

Periodontal disease model

Periodontal disease was induced by placing a silk ligature (Ethicon, Johnson & Johnson, São Paulo, Brazil) on the right upper second molar with the knot tied on the buccal side[20-23]. The contralateral second molar was considered the intra-group control. Ligature placement was performed under general anesthesia with inhaled 5V% isoflurane (Isoforine™ Cristália, SP, Brazil), vaporized in 100% oxygen by facemask or by intraperitoneal injection of 5% ketamine/2% xylazine (10 mg/kg—1:1). A veterinarian performed all anesthetic procedures. All animals were sacrificed by decapitation.

Specimen preparation

Maxillae were removed, sectioned, and defleshed in 9% sodium hypochlorite for 2 hours and the remaining soft tissue was removed mechanically, after which the specimens were washed and dried. For better visualization of the cemento-enamel junction, maxillae were stained with 1% methylene blue, following Fernandes et al[24].

Morphometric analysis

Morphometric analysis was performed by standard digital photographs. Pictures were taken using a 6.1 megapixel digital camera (Nikon™ Coolpix, Ayutthaya, Thailand) attached to a tripod and equipped with 100mm macrolens with minimal focal distance. Specimens were fixed to an endodontic ruler, parallel to the ground. Photographs were taken of the buccal and palatal aspects of right and left hemimaxillae.

Measurements were made linearly from the cemento-enamel junction to the bone crest, using Adobe Photoshop™ CS4 software (Adobe Systems Inc., San Jose, CA, USA). Five measurements were performed on each surface of the second molar, both buccally and palatally (two on the distal root, two on the mesial root and one on the furcation). The measurements in pixels were converted into millimeters using as reference the markings of the endodontic ruler to which the hemimaxillae were attached. Fig. 1 shows buccal aspects of specimens without (Fig. 1A) and with (Fig. 1B) periodontal disease inductionused in one of the three studies.

For all included studies, specimen preparation, photographs and morphometric analysis were performed at the Laboratory of Periodontology of the Federal University of Rio Grande do Sul, following the methods proposed by Fernandes et al[24].

Statistical analysis

The normality of data was checked by Shapiro-Wilk test and the data were found to have normal distribution. Mean and standard deviations of Alveolar Bone Loss (ABL) at different time points were generated and compared by one-way ANOVA followed by Bonferroni multiple comparisons test. Contralateral sites in animals with and without ligature were compared by independent sample t-test. All analyses were performed on Stata 10.1 for Macintosh (Stata™, College Station, TX). The level of significance was set at .05.

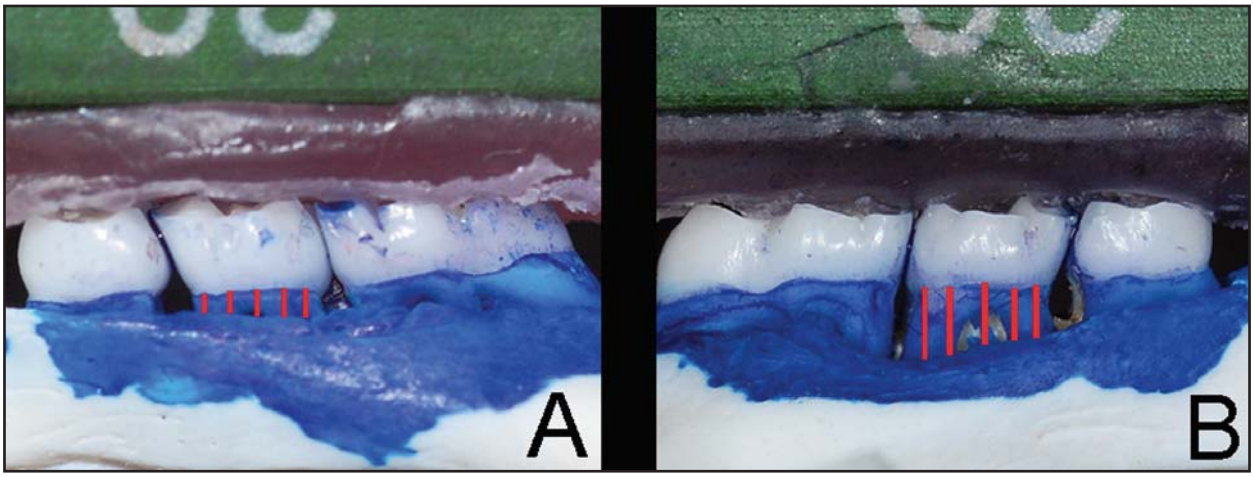

Fig. 1: Representative photograph of a specimen illustrating morphometric analysis in maxillae without (A) and with (B) ligature-induced periodontal breakdown.

RESULTS

Sites with ligature exhibited significantly higher ABL than sites in which periodontal disease was not induced (characterized as spontaneous ABL), showing that the model was effective in reproducing alveolar bone loss, which isone of the most important signs of periodontal disease (Figs. 2 and 3). The difference between ABL values was 40%, 54% and 57% for Studies 1, 2 and 3, respectively, depending on the experimental period (the longer the experimental period, the greater the periodontal destruction). Figs. 2 and 3 show mean ABL for sites with and without ligature, according to experimental period, respectively. For sites with ligature, ABL was significantly lower at 2 weeks of periodontal breakdown than at 5 and 22 weeks. However, no significant difference was observed between 5 and 22 weeks, showing that a 5-week period is sufficient to produce signs of periodontal disease (Fig. 2).

Sites without ligature showed no significant difference in ABL between 8 and 17 weeks. However, at 22 weeks, a statistically significant difference was detected, showing that longer periods are needed to produce spontaneous ABL in Wistar rats (Fig. 3).

Fig. 4 shows the comparison between control sites in animals submitted or not submitted to ligature-induced periodontal disease. No statistically significant difference was observed between 22 and 5 weeks, suggesting that the presence of ligature on one side does not affect mean alveolar bone loss at the contralateral site.

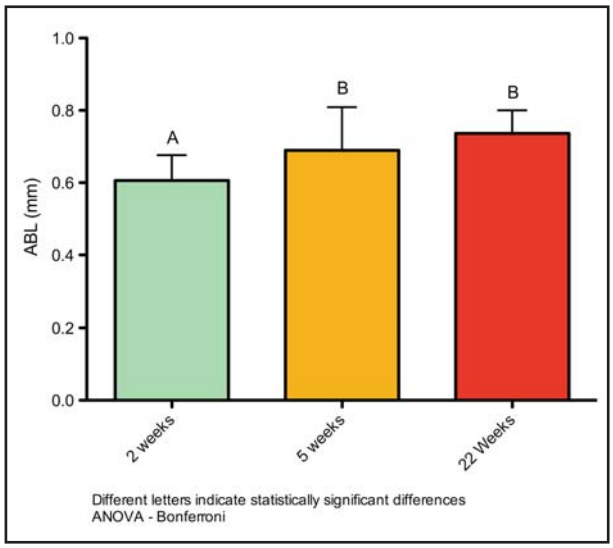

Fig. 2: Mean Alveolar Bone Loss (ABL) for sites with ligature according to experimental period.

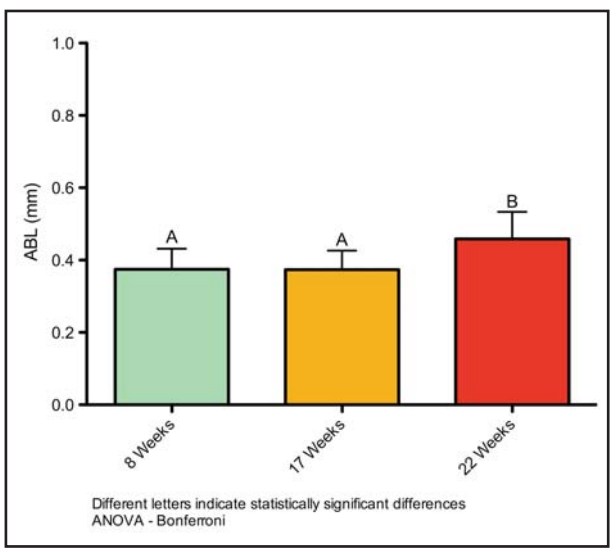

Fig. 3: Mean Alveolar Bone Loss (ABL) for sites without ligature according to experimental period.

DISCUSSION

This is a methodological study, the aim of which was to achieve better understanding of the effect of induction time and of a potential crossover effect of the presence of ligature on the contralateral side in studies using ligature-induced periodontal breakdown in rats.

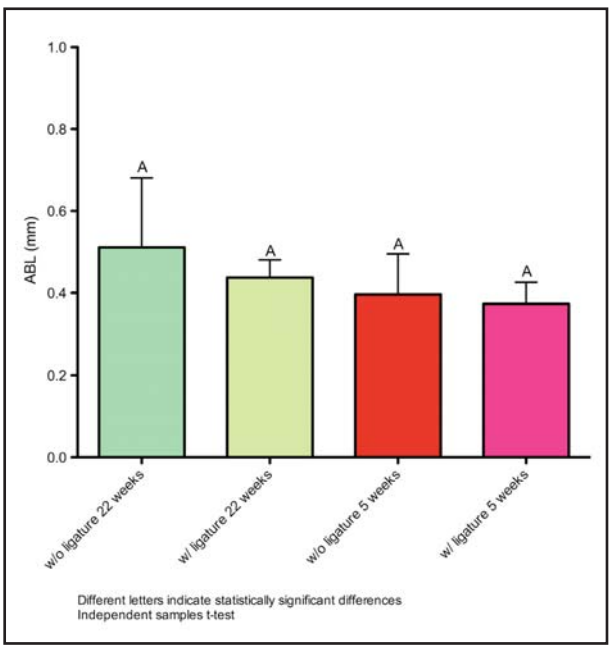

Fig. 4: Comparison of Alveolar Bone Loss (ABL) at control sites for animals with and without ligature.

Studies utilizing animal models are essential to understanding etiopathogenic aspects of periodontal diseases. Several studies have been performed using different animal strains[25,8]. Wistar rats are one of the most widely used species in studies on pathogenesis of periodontal diseases.They are non-isogenic rats that present variability in immune response that is similar to humans[26], as well as having similar anatomical characteristics to humans[11].

In periodontal research in animals, discussions have arisen regarding which the best models of induction are, which the best methods of analysis are, and whether it is really necessary to induce the disease[14]. Some studies have thus looked at naturally occurring periodontal disease, which could be an interesting way of demonstrating the effect of different exposures without the high-intensity challenge[16, 17].

The present study utilized morphometric analyses for periodontal breakdown. Different methods have been used, including histology, morphometry and tomography. They lead to different approaches, but are considered reproducible and capable of demonstrating periodontal breakdown[18]. In addition, the sites of analysis (area, proximal, furcation, buccal, etc.) have also been studied, and have all been shown to be reproducible and capable of detecting occurrence of alveolar bone loss[27,28].

In animal research there has been much discussion of the "3Rs" recommendation: reduce, refine and replace[29]. In this regard, a "split-mouth" design (in which ligatures, for example, are placed on one side and the contralateral tooth serves as a control) enables the number of animals to be reduced, since the use of a totally non-manipulated/exposed control is unnecessary. However, to the best of the authors' knowledge, the literature has not yet addressed this point. Thus, the novelty of the present study resides in further enabling evidence-based choices of using contralateral sides as controls, thereby reducing the number of animals used in periodontal research.

An interesting point in periodontal research using animals is the time of induction of periodontal disease. There is no consensus in the literature demonstrating that any one time is better than another in terms of occurrence of periodontal breakdown. Studies have used different time intervals ranging from 1 week to months[30,14]. If it is possible to effectively establish a minimum induction time for periodontal breakdown, the principle of refining the method can be contemplated.

Considering the points raised, and that we are a research group with experience in studies of periodontal pathogenesis in rats, we decided to analyze data from our database to address these issues. In order to be included in the present study, the experiment was required to have a total control group, with no exposure either to an external agent or to periodontal disease induction, and a control group not exposed to an external agent, with ligatures on one side but not on the other. From a database of 19 experiments performed with similar protocols, three studies fulfilled these criteria and were included in the present analysis.

It should be emphasized that all experiments used similar housing, temperature, food and liquid intake, and manipulation strategies, enabling direct inter-study comparisons. In addition, the laboratory procedures and analyses, including randomization, blinding and reproducibility, were performed identically. All these research principles support a consistent level of internal validity[31]. None of the included studies used the same induction time, therefore the analysis does not merge groups.

With regard to induction time, the results of the present study indicate that the amount of alveolar bone loss in ligature-induced models is time-dependent, i.e. at 2 weeks there is less alveolar bone loss than at 5 and 22 weeks. However, the comparison between 5 and 22 weeks does not demonstrate any additional breakdown.

This should be considered from different perspectives. One important point is that the studies that demonstrate sufficient effect after shorter periods (2 weeks, for example) were performed on isogenic rats[14]. On the other hand, it should be emphasized that the study included in this analysis with 2-week induction found statistically significant differences as compared to controls, indicating that periodontal breakdown was actually achieved. In one study, no statistically significant difference was observed between 29, 43 and 57 days of ligatures[32]. The benefit of using less experimental time relates to the cost-effectiveness of research.

It should also be noted that groups with ligatures always present significantly higher degrees of periodontal breakdown than groups without ligatures. However, some studies have shown some effects only at sites without ligatures, suggesting a

potential increased challenge that may mask the effect of the presence of naturally occurring biofilm[33]. This is not supported by the mechanical effect of the presence of the ligature, since germ-free animals exposed to ligature-induced periodontal disease did not present significant periodontal breakdown[34]. Thus, a 2-week period seems to be sufficient for ligature-induced periodontal breakdown; however, depending on the exposure variable to be tested, additional time may be necessary, and after 5 weeks the breakdown seems to leveloff.

In animals with spontaneous alveolar bone loss, periodontal breakdown takes longer. Moreover, the studies use different times, since the whole experimental time frame is considered, not only the induction time.

The present analysis also studied the crossover effect with the aim of better understanding one of the supposed biases of split-mouth designs. This bias is considered "supposed" because no published paper has provided support to this hypothesis. The present study endeavors to shed some light on this discussion, restricted to animal studies, which could nevertheless be further investigated in human clinical studies. The main basis for the hypothesis is that no drug or event with a known systemic effect should be part of the experiment.

The analysis in the present study demonstrates that the presence of a silk ligature on one molar has no statistically significant effect on the contralateral side. This is supported by the fact that the values of periodontal breakdown encountered at sites without ligature from animals exposed to ligature on the contralateral side does not differ from the mean values of spontaneous periodontal bone loss in animals not exposed to any additional manipulation. The present results thus suggest that total control groups are unnecessary in periodontal disease studies in rats.

To conclude, two weeks of ligature-induced periodontal disease seem to be sufficient to demonstrate significant bone loss, and teeth without ligature contralateral to teeth with ligature may be considered sound controls, thereby reducing the amount of animals needed in periodontal research.

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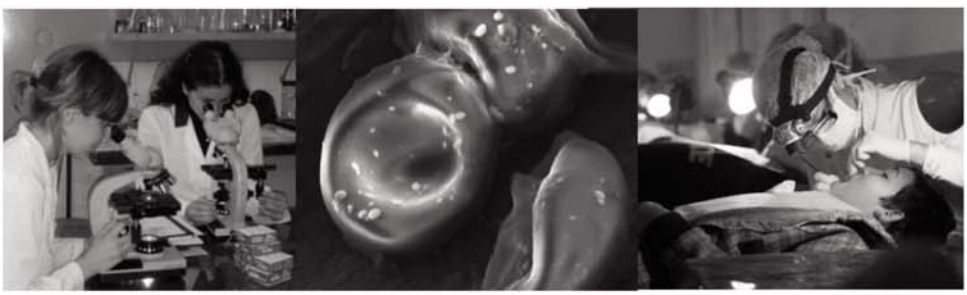

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