UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

VANESSA SCHEFFER DE MATTOS

EFEITOS DOS AGENTES DESINFETANTES SOBRE CONES DE GUTA-PERCHA: ANÁLISE ATRAVÉS DE ESPECTROSCOPIA RAMAN.

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Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Cirurgião-Dentista.

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RESUMO

MATTOS, Vanessa Scheffer. **Alterações estruturais em cones de guta-percha após imersão em agentes irrigantes com agentes irrigantes**: análise através de espectroscopia Raman. 2013. 34f. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Faculdade de Odontologia – Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

Este estudo buscou descrever o efeito de substâncias auxiliares na composição química superficial de cones de guta percha após a imersão em agentes irrigantes com hipoclorito de sódio (NaOCl) em diferentes concentrações e com clorexidina (CHX) 2% líquida ou gel. Os cones de guta-percha foram imersos em soluções de NaOCl 0,5%, 1,0%, 2,5% ou 5,25%, CHX gel 2% ou CHX líquida 2% por 1, 5, 10, 20 e 30 minutos. Após a imersão em agentes irrigantes, os mesmos foram lavados em água bidestilada e deionizada, e então secos com filtro de papel absorvente. Um outro grupo idêntico não sofreu o processo de lavagem. Logo após, a análise da composição química superficial foi realizada através de espectroscopia Raman. Constataram-se alterações crescentes entre os grupos de NaOCl conforme aumento das concentrações utilizadas, independentemente do tempo, e a formação de cristais de NaOCl a partir da concentração de 2,5%. Grupos em que se utilizou CHX, tanto na forma de solução quanto na forma de gel, demonstraram alterações menos evidentes. Foi concluído que a utilização de NaOCl a baixas concentrações e CHX a 2% solução ou gel apresentaram alterações superficiais baixas e semelhantes entre os grupos, independentes do tempo utilizado para os agentes irrigantes.

Palavras-chave: Imersão em agentes irrigantes. Endodontia. Guta-percha. Raman.

ABSTRACT

MATTOS, Vanessa Scheffer. **Structural changes in gutta-percha after immersion in agent irrigators**: analysis by Raman spectroscopy. 2013. 34f. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2012.

This study attempts to describe the effect of auxiliary substances in surface chemical composition of gutta-percha after immersion in sodium hypochlorite (NaOCl) in different concentrations and with chlorhexidine (CHX) 2% solution and gel. Gutta-percha were immersed in solutions of NaOCl, 0.5%, 1.0%, 2.5% or 5.25%, or 2% CHX gel 2% CHX solution for 1, 5, 10, 20 30 minutes. After immersion, they were washed in double-distilled and deionized water, and then were dried with a paper towel. Another group did not have the washing process. Then, the analysis of the surface chemical composition was performed by Raman spectroscopy. Increasing changes were observed among the groups immersed in NaOCl according to the increase of the concentrations used regardless of time. Besides, there was the formation of crystals o NaOCl at the concentration of 2.5%. CHX groups had less significant changes, in solution and also for gel forms. It was concluded that the use of NaOCl low concentrations and CHX 2% solution or gel forms exhibited surface changes low and similar among groups, independent of the time.

Keywords: Disinfection. Endodontics. Gutta-percha. Raman.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

CHX	Digluconato de clorexidina
GP	Guta percha
NaOCl	Hipoclorito de Sódio

SUMÁRIO

1 INTRODUÇÃO

Cones de guta percha (GP) são empregados para o preenchimento de canais radiculares em associação com os cimentos endodônticos. Sobre sua manufatura, podemos dizer que os cones são produzidos sob condições assépticas e são comercializados em embalagens estéreis e seladas. Entretanto estes cones podem se tornar contaminados através do manuseio, por aerosóis e por outras fontes durante o processo de armazenamento. A imersão em agentes irrigantes dos cones de guta percha tem sido recomendada antes do processo de obturação do canal (1, 2), porém devido suas características termoplásticas, os cones de guta percha não podem ser esterilizados de maneira convencional. A utilização de autoclave ou calor seco é capaz de alterar a estrutura dos cones e produzir alterações dimensionais no produto (3, 4).

Alterações na superfície dos cones de guta percha após a imersão em agentes irrigantes têm sido intensamente investigadas através dos métodos de microscopia de força atômica (AFM) e da microscopia de varredura (SEM) (9, 13-15). A análise através da microscopia de varedura não mostrou efeitos do NaOCl ou da CHX sobre os cones de GP (9, 16). Já a formação de cristais de cloro é frequentemente observada quando os cones de GP são expostos ao NaOCl (13, 15) e estes remanescentes são observados sobre a superfície, especialmente nos casos onde não houve lavagem dos cones após sua imersão em agentes irrigantes (15). Não há um concenso na literatura sobre os reais efeitos da imersão em agentes irrigantes química sobre os cones de GP. Valois et al (14) observaram que quando os cones são expostos a concentrações de NaOCl 5,25% em solução por 1 minuto, alterações elásticas são observadas na superfície dos cones de GP quando analisadas através da microscopia de força atômica.

A espectroscopia Raman fornece informações estruturais em macromoléculas como polímeros e sistemas biológicos, envolvendo uma íntima interação entre posições atômicas, distribuição de elétrons e forças intermoleculares (17). Os cones de guta percha são formados por uma porção orgânica (18-26%) e uma outra inorgânica (73-82%) (18). O polímero da guta percha é representado pela porção *trans*1,4-poliisopreno. De acordo com Cornell & Koenig (19), a porção *trans*1,4-poliisopreno representa uma banda característica da GP em que no Raman

corresponde ao pico 1662 cm^{-1} . Este pico corresponde a vibração da ligação dupla entre carbonos $_{v}$(C=C) para os polibutadienos, durante a determinação do que contém a estrutura da GP. A degradação do polímero poliisopreno é associada com processos oxidativos (20) que podem ser induzidos pela exposição a luz, calor ou pela presença de oxigênio. O grupamento C=C pode absorver a luz e pode também reagir com o oxigênio produzindo um radical carboxila (-C=O) (21). Silva et al (20) and Maniglia-Ferreira et al (22) relataram que o processo de degradação *in vivo* que afeta o poliisopreno envolve oxidação, indicando decréscimo da massa molar do polímero e produzindo grupos carboxila (C=O) e hidroxila (-OH).

Embora sejam relatadas alterações superficiais em cones de guta-percha após o processo de imersão em agentes irrigantes, não se sabe se soluções desinfetantes são capazes de alterar a estrutura química do polímero de guta-percha, promovendo sua degradação. Este estudo teve por objetivo avaliar, através da espectroscopia Raman, as alterações superficiais nos cones de guta percha expostos a diferentes concentrações de NaOCl e a CHX 2%, solução e gel, por diferentes períodos, com e sem posterior lavagem.

2 ARTIGO CIENTÍFICO

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Effect of disinfecting agents over gutta percha points: a RAMAN spectroscopy analysis

Vanessa Scheffer de Mattos – DDS Student, Federal University of Rio Grande do Sul – Porto Alegre, RS, Brazil.

Alessandra Tolfo Ledur Vaucher – DDS, MSc Student, Federal University of Rio Grande do Sul – Porto Alegre, RS, Brazil.

Vicente Castelo Branco Leitune – DDS, MSc, PhD, Adjunct Professor, Dental Materials Devision, Department of Conservative Dentistry, Federal University of Rio Grande do Sul – Porto Alegre, RS, Brazil.

Fabrício Mezzomo Collares – DDS, MSc, PhD, Adjunct Professor, Dental Materials Devision, Department of Conservative Dentistry, Federal University of Rio Grande do Sul – Porto Alegre, RS, Brazil.

Francisco Montagner – DDS, MSc, PhD, Adjunct Professor, Endodontic Division, Department of Conservative Dentistry, Federal University of Rio Grande do Sul – Porto Alegre, RS, Brazil.

Correspondence Author

Dr Francisco Montagner
Federal University of Rio Grande do Sul
Department of Conservative Dentistry - Endodontic Division
Rua Ramiro Barcellos, 2492. – Bairro Santana.
Porto Alegre, RS – Brazil.
90035-003
E-mail: francisco.montagner@ufrgs.br
Phone: +55 51 3308 5430

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Abstract

Introduction: This study attempts to describe the effect of auxiliary substances in surface chemical composition of gutta-percha after immersion in sodium hypochlorite in different concentrations and with chlorhexidine 2% solution and gel through the Raman spectroscopy. **Methods:** Gutta-percha cones were immersed in solutions of sodium hypochlorite, 0.5%, 1.0%, 2.5% or 5.25%, or 2% chlorhexidine gel 2% chlorhexidine solution for 1, 5, 10, 20 or 30 minutes. After immersion, half of the samples were washed in double-distilled and deionized water and then dried with a paper towel. The remaining immersed gutta-percha cones were not washed. The micro-Raman spectroscopy was employed to analyze alteration in the gutta-percha polymer. The presence of the 1662 cm^{-1} peak and its intensity represented the chemical group of $_{\nu}$(C=C) for the *trans*1,4-polyisoprene. **Results:** Changes in the intensity of 1662 cm-1 peak were observed in samples immersed in sodium hypochlorite, specifically when there was an increase in the solution concentration, despite the time of exposure. Besides, there was the formation of crystals of sodium hypochlorite at the concentration of 2.5%. Chlorhexidine digluconate groups had less significant changes, for both solution or gel forms. **Conclusions:** It was concluded that the use of sodium hypochlorite low concentrations and chlorhexidine digluconate 2% solution or gel forms promoted low surface changes in gutta-percha points, independent of the time of exposure.

Keywords: Disinfection. Endodontics. Gutta-percha. Raman.

Introduction

Gutta-percha (GP) cones are employed to fill root canals in association with endodontic sealers. According to the manufacturer, they are produced under aseptic conditions. GP cones are sold in sterile and sealed packages. However, they can become contaminated during handling, by aerosols, and by physical sources during the storage process. The disinfection of GP cones has been recommended before canal filling procedures (1, 2). The thermoplastic characteristics of GP cones do not allow their sterilization with conventional methods. Autoclaving or dry heat exposure may change the GP structure and produce dimensional alteration (3, 4).

The superficial landscape of GP cones has been extensively assessed through atomic force microscope (AFM) and the scanning electron microscope (SEM) methods (9, 13-15). Scanning electron microscopy analysis showed no effect of NaOCl and CHX over this material (9, 16). Chloride crystal formation is frequently observed after NaOCl exposure (13, 15) and MTAD remainings are also observed over GP surface, especially if a final rinse is not performed in the cones after disinfection (15). There is no consensus in the current literature regarding the effects of chemical disinfection over the GP. Valois et al (14) observed that when GP was exposed to 5.25% NaOCl solution for 1 minute, elastic alterations were detected in the superficial area through atomic force microscope.

Raman spectroscopy provides structural insights into macromolecules like polymers and biological systems. It involves an intimate interplay between atomic positions, electron distribution and intermolecular forces (17). Gutta-percha points are formed by organic (18-26%) and inorganic fractions (73-82%) (18). The gutta-percha polymer is the *trans*1,4-polyisoprene. According to Cornell et al (19), the *trans*1,4-polyisoprenes showed a fingerprint band in Raman that corresponds to the 1662 cm^{-1} wavelength. It represented the carbon-carbon double band $_{\nu}$(C=C) vibration in the Raman spectra for polybutadienes, during the determination of the structure content. Polyisoprene degradation is associated with oxidative process (20). It can be induced by light exposure, heat or by the presence of oxygen. The C=C groups can absorb light and can also react with oxygen, producing the carboxyl radicals (-C=O) (21). Silva et al (20) and Maniglia-Ferreira et al (22) reported that *in*

vivo aging process of polyisoprenes involves oxidation, indicating decrease of polymer molar mass and production of carboxyl (C=O) and hydroxyl (-OH) groups.

Therefore, the aim of the present study was to evaluate by RAMAN espectroscopy the alterations on the surface of gutta-percha cones exposed to several concentrations of NaOCl, 2% CHX solution or 2% CHX gel in different time periods, with or without washing.

Methods

The present research was approved by the Research Board, Dental School, Federal University of Rio Grande do Sul (Porto Alegre, RS, Brazil). A total of 61 gutta-percha cones (Dentsply Ind & Com, Petropolis, RJ, Brazil), were selected for the present study. The samples were divided into groups, according to the type of substance to be tested (0.5%, 1%, 2.5% and 5.25% NaOCl solution, 2% CHX gel and 2% CHX solution), the time of exposure to each substance and if there was any washing or not. Groups are described as follows:

- Control group (n=1): one gutta-percha cone was evaluated without any exposure to the chemical auxiliary substances or saline;
- Group 1 (n=10): Cones were immersed in 1 ml of 0.5% NaOCl solution for 1, 5, 10, 15 or 20 minutes, followed or not by a final rinse;
- Group 2 (n=10): Cones were immersed in 1 ml of 1.0% NaOCl solution for 1, 5, 10, 15 or 20 minutes, followed or not by a final rinse;
- Group 3 (n=10): Cones were immersed in 1 ml of 2.5% NaOCl solution for 1, 5, 10, 15 or 20 minutes, followed or not by a final rinse;
- Group 4 (n=10): Cones were immersed in 1 ml of 5.25% NaOCl solution for 1, 5, 10, 15 or 20 minutes, followed or not by a final rinse;
- Group 5 (n=10): Cones were immersed in 1 ml of 2.0% CHX gel for 1, 5, 10, 15 or 20 minutes, followed or not by a final rinse; and

- Group 6 (n=10): Cones were immersed in 1 ml of 2.0% CHX solution for 1, 5, 10, 15 or 20 minutes, followed or not by a final rinse.

Small volumes (30ml) of each chemical substance were prepared 24 hours before the beginning of the experiment. All substances were prepared by the same manufacturer (Farmácia Marcela, Farmácia de Manipulação, Porto Alegre, RS, Brazil). After removal from the test solutions, the groups with washing were individually transferred to tubes containing 3 ml of saline solution and vortexed for 1 min. The samples belonging to groups without washing were placed over an absorbent paper and left on the bench for 20 minutes. The procedure was repeated three times in order to rinse the auxiliary chemical substances out of the cones. Then, the cones were dried with absorbent paper.

Chemical alteration in the surface of gutta-percha cones were detected through micro-Raman spectroscopy (Bruker Optics, SENTERRA model). The chemical group of $_{\nu}$(C=C) for the *trans*1,4-polyisoprene was identified , corresponding to the 1662 cm^{-1} wavelength (Cornell; Koenig, 1969). The range of the spectrum Raman analysis is 80–2700 cm^{-1}. The representative peak for each test group was depicted in a graph, according to the disinfecting protocol. Different peak intensities in the 1662 cm^{-1} wavelenght represented the $_{\nu}$(C=C) bound content for each sample, as measured through peak intensity

Results

The Raman shifts for samples immersed by NaOCl in different concentrations, periods of time and according to the adoption of a rinsing protocol were showed in **Figure 1**. GP immersed by 0.5% and 1% NaOCl solutions were similar, despite the time of immersion and the final rinse, suggesting a low degradation of C=C. Samples immersed in 2.5% and 5.25% NaOCl solutions had a decrease in the C=C peak area, especially in non rinsed GP cones (**Fig. 1C** and **Fig. 1D**).

Figure 2 represented GP cones after immersion in 2% CHX in the liquid or gel forms. Small alterations in the peaks for C=C were observed in the samples, despite the time of immersion and the final rinse.

Sodium hypochlorite crystals were observed over gutta-percha points after immersion, in samples not exposed to a final rinse with saline (x40 magnification). After the final rinse, there was a reduction in the crystal size, and they were not completely removed from the GP cones (**Fig. 3**).

Figure 1. Peak intensity for gutta-percha samples after immersion in NaOCl in different concentration and specific periods of time, with or without the final rinsing.

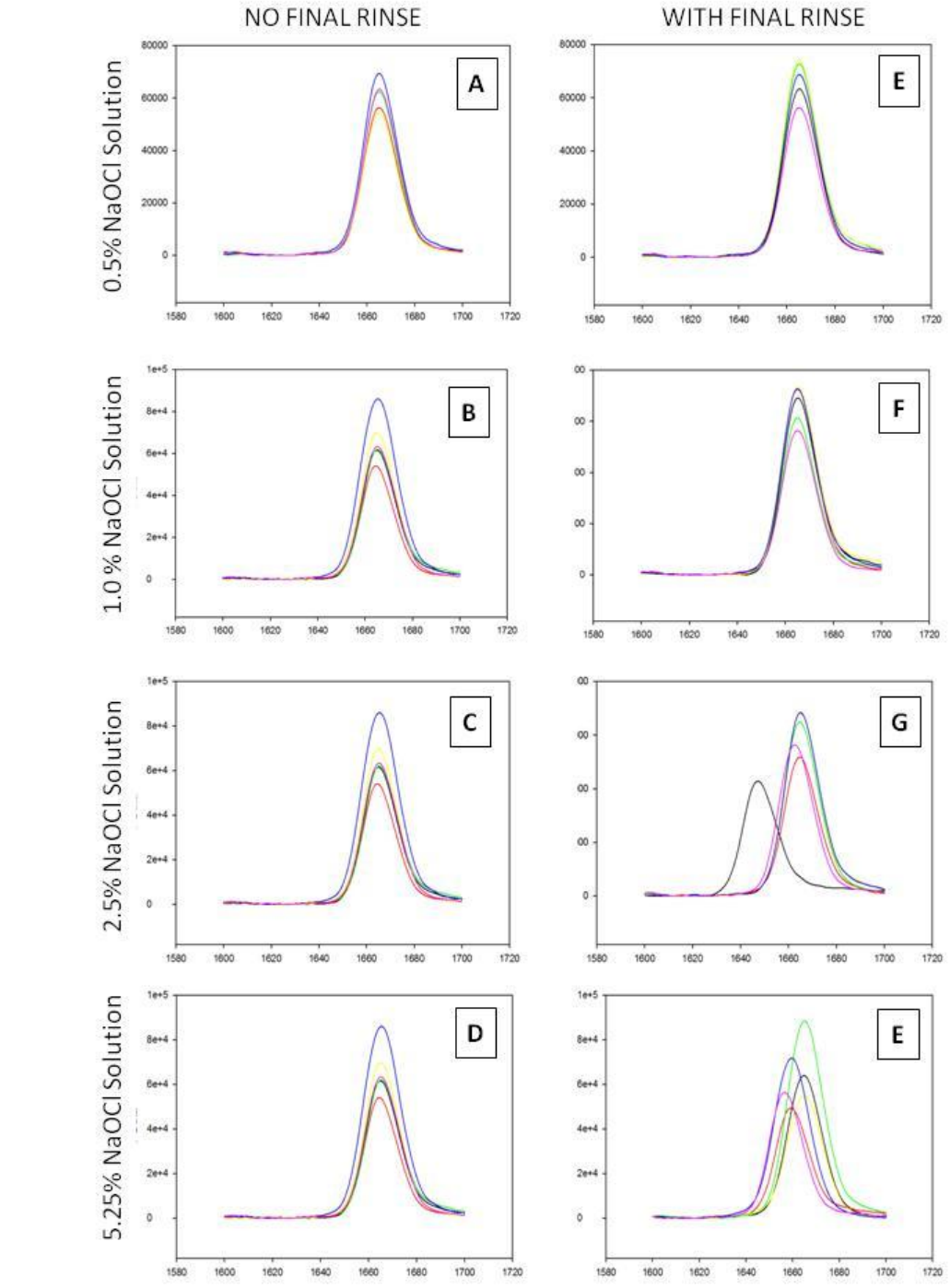

Figure 1. Raman spectrum for gutta-percha cones immersed in NaOCl: A) 0,5% NaOCl Solution, no final rinse; B) 1% NaOCl Solution, no final rinse; C) 2,5% NaOCl Solution, no final rinse; D) 5,25% NaOCl Solution, no final rinse; E) 0,5% NaOCl Solution, with final rinse; F) 1% NaOCl, Solution, with final rinse; G) 2,5% NaOCl Solution, with final rinse; H) 5,25% NaOCl Solution, with final rinse.

Figure 2. Peak intensity for gutta-percha samples after immersion in 2% CHX liquid or gel, with or without the final rinsing.

NO FINAL RINSE WITH FINAL RINSE

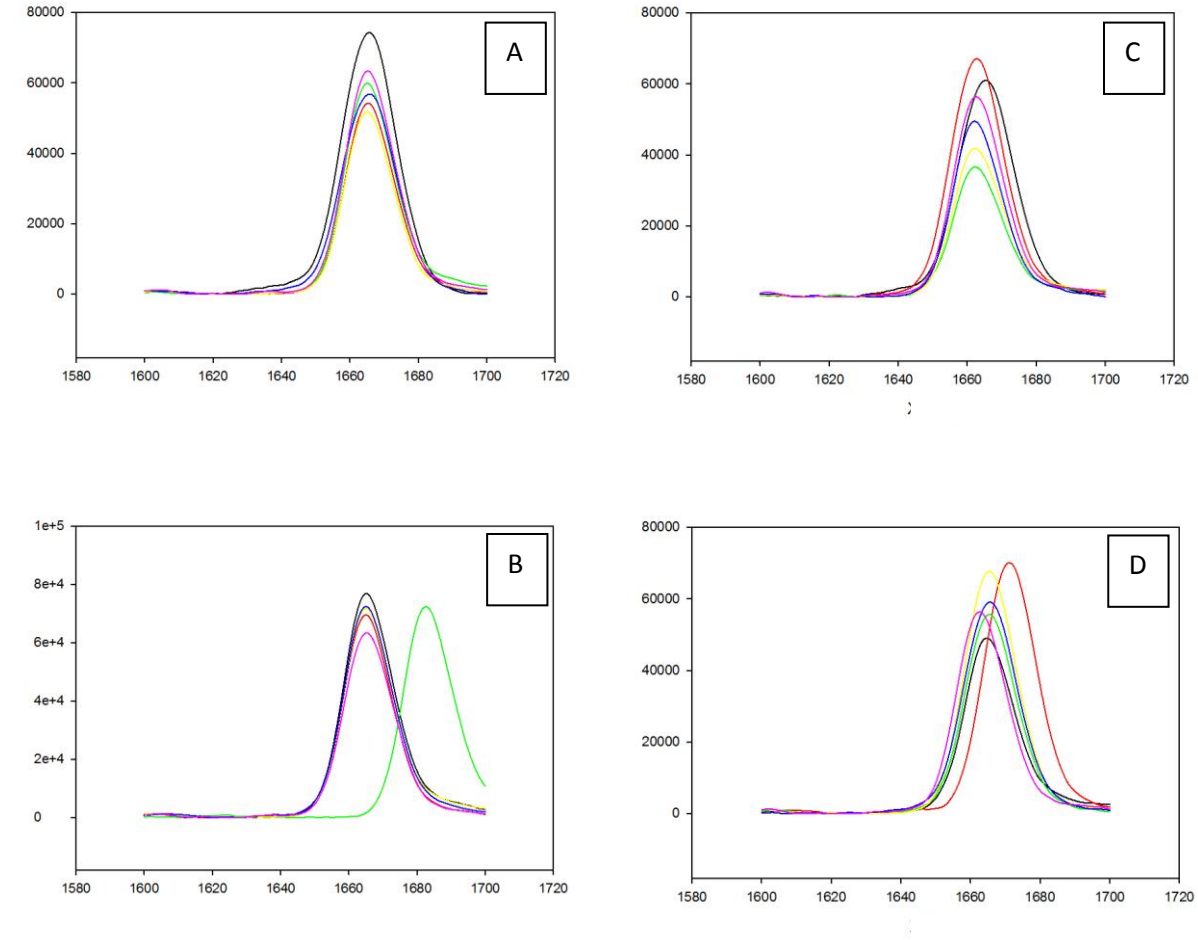

Figure 2. Raman spectrum for gutta-percha cones immersed in 2% CHX: A) 2% CHX Solution, no final rinse; B) 2% CHX Gel, no final rinse; C) 2% CHX Solution, with final rinse; D) 2% CHX Gel, with final rinse.

Figure 3. Sodium hypochlorite crystals over gutta-percha points after immersion, without the final rinse (20x).

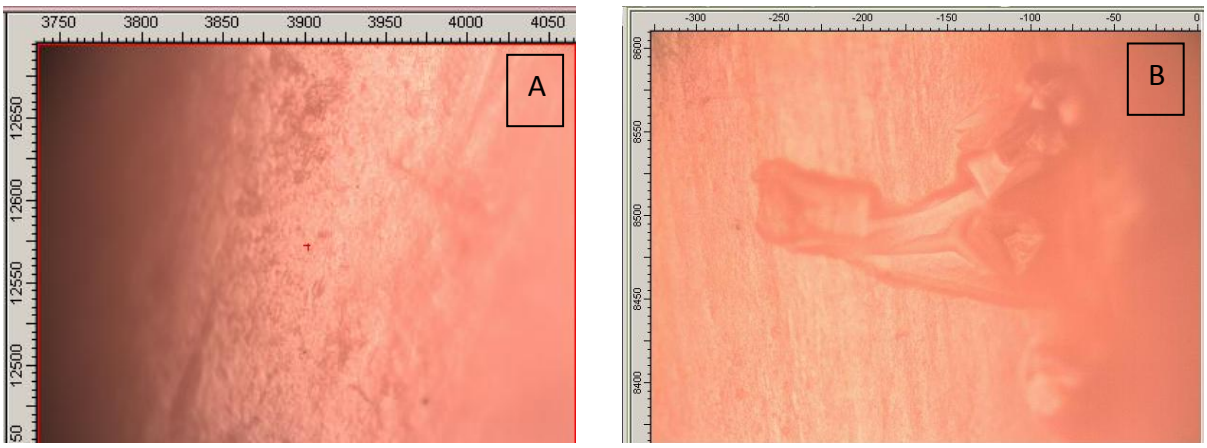

Figure 3. A) Image of gutta-percha cone without immersion in irrigators agents or saline solution; B) Sodium hypochlorite chrystal over gutta-percha points after immersion, without the final rinse; (magnification 20x)

Discussion

Although it is known that the GP cones landscape could not be affected by exposure to disinfectant agents, there is little evidence on the changes that can occur in its chemical composition. Using a qualitative/quantitative microscopic approach allowed comparisons to be made of the effect of NaOCl and 2% CHX in different periods of time of the chemical composition in gutta-percha cones.

Gutta-percha points consist of both organic and inorganic compounds. The specific percentages of each component vary according to the manufacturer and seem to affect the physical properties (22, 23). Maniglia-Ferreira et al (18) reported that Brazilian gutta-percha points have different contents of gutta-percha and zinc oxide, ranging from 15.2% to 21.6% and from 69.8% to 81.9%, respectively. Gutta-percha is a trans-1,4-polyisoprene, obtained from the coagulation of latex produced by trees of the Sapotaceae family (24). Additional organic compounds such as wax/resins were also detected (18). Inorganic compounds such as zinc oxide and metal sulfates act as antimicrobial agents and radiopacifiers. It has been shown that

exposition to heating and aging may change gutta-percha points chemical structure, compromising their stability and properties (13, 18, 22, 25), scanning electron microscopy with energy-dispersive X-ray spectrometry (SEM-EDX) (10, 16, 18) and, atomic force microscopy (AFM) (14, 26). The Raman spectroscopic technique enables to obtain vibrational spectra of different substrates by analyzing scattered light caused by visible or near-visible monochromatic laser excitation (27). This method has been used to evaluate the conversion degree of resin compounds, the detection of calcium fluoride in enamel (27), and the composition of dental calculus (28). The method allows simple sample preparation and easy spectral/band analysis because the sample is positioned under an optical microscope and it is scanned with a lateral resolution (28). The peak corresponding to the 1662 cm^{-1} in the Raman spectrum was chosen to describe the C=C that represents the *trans*1,4-polyisoprene (27) Horizontal deviation in the peak position (**Figs. 1G, 1H, 2B, 2C** and **2D**) provides feasible values for data analysis and they are associated with the confocal distance and laser temperature. Therefore, this method depicted the composition and allowed direct visualization of the gutta-percha cones, before and after immerse in NaOCl and CHX. Furthermore it has not produced heating during sample analysis that could lead to gutta-percha melting, changing its superficial features.

Sodium hypochlorite has a disinfecting action on contaminated gutta-percha cones (8, 29), and its effect depends on the concentration and time of exposure (29). NaOCl is a non-selective oxidative organic solvent, and presents the ability to dissolve pulp tissue (30, 31). In the present study, highly concentrated solutions in long periods of time (especially 20 min) produced a change in the C=C related raman peaks, suggesting a polymer degradation. Valois et al (14) reported aggressive deteriorative effects on gutta-percha cone elasticity and topographic changes after exposure to 5.25% NaOCl solution, after a 5-minute period. The peaks for C=C in samples immersed in NaOCl solutions were more similar after final rinse when compared to samples before rinsing, especially for the 2.5% and 5.25% solutions. The final rinse may remove the superficial components that were affected by the oxidative process. The deposition of chloride crystals on the surface of the GP cones was observed, despite time of exposure and NaOCl concentration, as also reported by Short et al (13) and Prado et al (10). A final rising with saline promoted crystal dissolution but they were not completely removed, as previously reported by

Prado et al (10) and Gomes et al (9). Further studies should be conducted to determine the influence of the superficial chemical alterations on C=C bounds and polymer stability and degradation over time.

Chlorhexidine digluconate has been employed as an endodontic irrigant (32, 33), intracanal medicament and as a fast disinfecting agent for GP cones (5, 9). CHX has the ability to dissociate into positively charged ions that are able to interact on the cell membrane of bacteria, interfering with both active and passive transport mechanisms (34). Therefore, its efficacy depends on the interaction between CHX positively charged molecule and the negatively charged phosphate groups on the microbial cell walls, disrupting the osmotic equilibrium (34). The bactericidal action of highly concenctrated CHX solution and gel forms relies on the precipitation of the citoplasmatic content, resulting in cell death (34). In the present study, no chemical composition alterations were observed on the surface of GP cones. Previous studies reported no changes in the superficial landscape of GP cones after CHX disinfection (5, 9, 14). It could be associated with CHX's mechanism of action, because it is not able to dissolve organic tissues (31). It was possible to observe a mild effect of CHX in both liquid and gel forms in the superficial composition of the samples after immerse, as represented by small variation in raman peaks associated with the C=C bound. Lian Cheng et al et al (35) reported that a 1610cm^{-1} peak was associated with the presence of CHX. There was no CHX related peak increase for the analyzed spectra, suggesting that residual components may not remain over GP cones, despite the presence or absence of a final rinsing. Furthermore, debris that can be found over GP cones might overcome any CHX content. It should be emphasized that GP cones exposed to CHX gel were left on the bench for additional drying before RAMAN analysis. A possible interaction between Resilon components and CHX was previously reported, especially after 20-30 minutes of exposure, inhibiting *E. faecalis* growth (8). As GP cones have no mineral phase, this interaction may not occur, as shown by the RAMAN analysis.

According to the results of the present study, the action of low concentrated sodium hypochlorite solutions and CHX in both liquid and gel forms possibly promoted some alteration in the superficial GP polyisoprene.

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3 CONCLUSÃO

A grande preocupação do Cirurgião-dentista está em garantir o sucesso do tratamento endodôntico através de uma eficiente limpeza dos canais radiculares e uma obturação de qualidade. Para isso, é preciso valer-se de materiais que possiblitem um selamento adequado e com o mínimo de contaminação para a não proliferação de microrganismos que persistem após os procedimentos de imersão em agentes irrigantes do sistema de canais radiculares.

Dessa forma, é importante que a guta-percha, material amplamente utilizado para obturação de canais radiculares, seja testada frente às alterações durante a sua imersão em agentes irrigantes. De acordo com os resultados do presente estudo, a ação oxidativa causada pelas soluções de hipoclorito de sódio a baixas concentrações e pela CHX em ambas as formas, gel e solução, possivelmente promoveram alguma alteração nos picos C=C na superficie da guta percha poliisopreno.

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ANEXO – Documento de aprovação do Projeto de Pesquisa na Comissão de Pesquisa em Odontologia, da Faculdade de Odontologia, da Universidade Federal do Rio Grande do Sul.

Pesquisador:

Sair

Dados do Projeto de Pesquisa

Projeto Nº: 22643
Título: Características químicas superficiais de cones de guta-percha após a desinfecção - análise através de espectroscopia RAMAN

Área do Conhecimento: Endodontia

Início: 15/04/2012
Previsão de conclusão: 20/12/2012
Término: 31/01/2013

Situação: projeto desativado
Justificativa: Projeto com previsão de conclusão até 31/12/2012 ou não informada não atualizado até 31/01/2013.

Origem: Faculdade de Odontologia
Departamento de Odontologia Conservadora
Projeto Isolado com linha temática NULL

Objetivo: O objetivo geral do estudo será descrever o efeito de substâncias químicas auxiliares na composição superficial de cones de guta percha após a desinfecção.

Palavras-Chave
Desinfecção
Endodontia
Guta-percha
Raman