UNIVERSIDADE DE UBERABA

MESTRADO ACADÊMICO EM ODONTOLOGIA

MAIZA SEGATTO CURY

Avaliação da rugosidade superficial e da formação do biofilme na superfície da dentina tratada com diferentes agentes dessensibilizadores

UBERABA – MG

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AVALIAÇÃO DA RUGOSIDADE SUPERFICIAL E DA FORMAÇÃO DO BIOFILME NA SUPERFÍCIE DA DENTINA TRATADA COM DIFERENTES AGENTES DESSENSIBILIZADORES

Dissertação apresentada como requisito para obtenção do título de Mestre em Odontologia no Programa de Pós-Graduação em Odontologia da Universidade de Uberaba.

Área de concentração: Biomateriais
Orientador: Prof. Dr. Vinícius Rangel Geraldo-Martins

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Resumo

O objetivo deste estudo foi avaliar os efeitos dos diferentes métodos de tratamento da hipersensibilidade dentinária na rugosidade superficial e na formação do biofilme dentário sobre as superfícies tratadas. Após a avaliação inicial da rugosidade superficial (Sa), 50 fragmentos de raiz bovina receberam os seguintes tratamentos (n= 10): Grupo 1- Sem tratamento; G2- Verniz de fluoreto de sódio a 5%; G3- Aplicação profissional de um dentifrício dessensibilizante; G4- Escovação dentária com dentifrício dessensibilizante; G5- aplicação de laser Diodo (λ= 908 nm; 1,5 W, 20 s). Após os tratamentos, a Sa foi novamente avaliada. Depois, as amostras foram incubadas numa suspensão de *Streptococcus mutans* a 37 °C durante 24 horas. As unidades formadoras de colônias (UFC) foram contadas utilizando um estereoscópio, e os resultados foram expressos em UFC/mL. O teste ANOVA a 1 critério e o teste de Tukey compararam os dados de rugosidade e aqueles obtidos no teste de adesão bacteriana (α= 5%). O G2 (2,3 ± 1,67%) mostrou variação de Sa semelhante ao G1 (0,25 ± 0,41%) e G5 (5,69 ± 0,99%), porém diferente do grupo G3 (9,05 ± 2,39%). O G4 apresentou a maior variação de Sa (30,02 ± 3,83%, p <0,05). Não houve diferença estatística entre os G3 e G5. A adesão bacteriana foi maior no G4 (2208 ± 211,9). Concluiu-se que os tratamentos para hipersensibilidade dentinária alteram a Sa da dentina radicular, principalmente quando há atrito intenso na superfície do dente. Além disso, houve um aumento significativo na rugosidade da dentina proporcionando um maior acumulo de bactérias nessa superfície.

Palavras-chave: Dentina. Biofilme. Dentifrício. Laser. Hipersensibilidade dentinária.

Abstract

The aim of this study was to evaluate the surface roughness of root dentin after different treatments for dentin hypersensitivity (DH) and the biofilm formation on those surfaces. After initial surface roughness (Sa) assessment, fifty bovine root fragments received the following treatments (n= 10): Group 1- No treatment; G2- 5% sodium fluoride Varnish; G3- Professional application of a desensitizing dentifrice; G4- Tooth brushing with a desensitizing dentifrice and G5- Diode laser application (λ= 908 nm; 1.5 W, 20 s). Following, the Sa was evaluated again. Afterward, all samples were incubated in a suspension of *Streptococcus mutans* at 37 °C for 24 hours. The colony-forming units (CFU) were counted using a stereoscope, and the results were expressed in CFU/mL. The One-Way ANOVA and the Tukey´s tests compared the roughness data and the results obtained on the bacterial adhesion test (α= 5%). Group 2 (2.3 ± 1.67%) showed similar Sa variation than G1 (0.25 ± 0.41 %) and G5 (5.69 ± 0.99%), but different from group G3 (9.05 ± 2.39%). Group 4 showed the highest Sa variation (30.02 ±3.83%; $p<0.05$). No statistically differences were found between groups 3 and 5. Bacterial adhesion was higher in G4 (2208 ± 211.9), suggesting that bacterial growth is greater on rougher surfaces. Therefore the treatments for DH change the surface roughness of the root dentin, mainly when there is intense friction on the tooth surface. Also, a significant increase in the roughness of the dentin leads to a greater accumulation of bacteria on this surface.

Keyword: Dentin. Biofilms. Toothpastes. Lasers. Dentin Hypersensitivity.

Sumário

Introdução

1. Introdução

Ao longo dos anos a melhora da saúde bucal da população tem reduzido o número da doença cárie, o que contribuiu para uma maior longevidade dos dentes na cavidade bucal. Contudo alguns fatores como contatos oclusais prematuros, escovação mecânica com força excessiva, maus hábitos de higiene oral, presença de alimentos ácidos na dieta, doenças periodontais, entre outros, podem favorecer a exposição da dentina radicular, bem como as embocaduras dos túbulos dentinários para o meio bucal e com isso desencadear um quadro de dor denominado hipersensibilidade dentinária (HOLLAND et al., 1997; PINTO et al., 2012; DAVARI et al., 2013).

A hipersensibilidade dentinária é uma resposta dolorosa a estímulos térmicos, químicos, físicos, osmóticos, táteis e evaporativos (BRANNSTRÖM et al., 1967). Esse diagnóstico se diferencia de outras formas de doenças e patologias, e está relacionada com a perda de esmalte por abrasão, abfração e erosão, e a perda de cemento na porção radicular resultante da recessão gengival e/ou terapia periodontal. Essa sintomatologia dolorosa pode gerar incômodo por alterar funções vitais como a mastigação, deglutição, fonação e hábitos nutricionais (ASSIS et al., 2011) e, assim, desencadear problemas físicos e psicológicos nos pacientes acometidos ao gerar efeitos negativos na qualidade de vida, como por exemplo restrições alimentares (DAVARI et al., 2013).

A maior incidência é em pacientes do sexo feminino, entre a terceira e quarta década de vida (ASSIS et al., 2011; WEST et al., 2014). Os dentes mais acometidos são os caninos e pré molares, seguidos pelos incisivos e molares, nas faces vestibulares e especialmente, na região cervical (MÁRQUEZ et al., 2011).

A hipótese mais aceita para explicar a ocorrência da hipersensibilidade dentinária é a teoria da hidrodinâmica de Brannström. De acordo com os princípios dessa teoria, quando o dente é exposto a um estímulo, ocorre a saída do fluído contido nos túbulos dentinários, que se movimentam da polpa até as regiões amelodentinária e amelocementária. Essa movimentação altera a pressão intertubular que atinge as terminações nervosas e mecanorreceptores, causando a sensação dolorosa (BRANNSTRÖM et al., 1967).

Por isso identificar e tratar a causa da hipersensibilidade dentinária é fundamental, e o tratamento deve ser simples, eficaz e de resultado rápido (ORCHARDSON et al., 2006). O tratamento mais empregado para esta patologia é a oclusão das extremidades dos túbulos dentinários, que impedem a movimentação dos fluídos intratubulares (WEST et al., 2014). Outros mecanismos de tratamento existem, como a dessensibilização das terminações nervosas a fim de que não haja despolarização das fibras nervosas pela movimentação do fluido intratubular ou, também, o bloqueio dos túbulos através da formação de dentina terciária, um fator de resposta biológica resultante do uso dos lasers de baixa intensidade (SANGWAN et al., 2013). É importante considerar que, para cada paciente, o tipo de resposta será diferente devido ao limiar de dor de cada um.

O dentifrício é a opção de tratamento mais acessível para a população, pois apresenta boa relação custo/benefício, e se trata de um procedimento minimamente invasivo. Ela age por meio da oclusão dos túbulos dentinários e pode promover a precipitação de proteínas e cristais de cálcio e fosfato, sendo que o seu mecanismo de ação exato ainda é discutido (PINTO et al., 2012). Nos últimos anos foi reportada a redução significativa da sensibilidade dentinária com o emprego de dentifrícios, por um período de 4 a 12 semanas, com redução significativa da sensibilidade dolorosa (ACHARYA et al., 2013). Dentre os agentes ativos tem-se a arginina e carbonato de cálcio que atuam sobre a dentina, dissociando-se em carbonato e cálcio e, posteriormente, unem-se com as glicoproteínas salivares e causam obstrução física dos túbulos dentinários (BAE et al., 2015; MARQUEZ et al., 2011).

O nitrato de potássio é, o agente dessensibilizante mais encontrado nos dentifrícios, e seu mecanismo de ação se dá através do bloqueio da transmissão neural, esse agente reduz a sensibilidade de 8 a 12 semanas, sendo usado 2 vezes ao dia por 5 minutos, porém seu efeito não é duradouro (ACHARYA et al., 2013; DAVARI et al., 2013). Dentifrícios a base de estrôncio a 10% e citrato de potássio a 5,5% também estão sendo utilizados em estudos *in vitro* e *in vivo*, obtendo resultados satisfatórios em curto prazo devido à sua ação dessensibilizante combinada com agentes obliteradores. O citrato de potássio, assim como o nitrato de potássio, age pela interrupção da resposta neural, já os efeitos do estrôncio ainda estão sendo estudados (LIU et al., 2012; PINTO et al., 2012).

A tecnologia NovaMin, que foi desenvolvida a partir da década de 1990, é composta por fosfosilicato de cálcio e sódio, sendo 25% sódio, 25% cálcio, 6,8% fosfato e sílica. Os dentifrícios que contém essa tecnologia reagem com a superfície dentinária, quebrando suas partículas em tamanhos suficientemente pequenos para que possam penetrar e ocluir os túbulos dentinários. Isso, contribui para a formação da apatita hidroxicarbonata devido a liberação de íons fosfato e cálcio, que servem como uma barreira de proteção dos túbulos dentinários (ACHARYA et al., 2013).

O uso dos lasers tem crescido a cada dia na odontologia, sendo uma opção terapêutica adicional para o tratamento da sensibilidade dentinária (DANTAS et al., 2016). O laser de baixa potencia possui ação rápida, com efeito analgésico relatado em 91,29% dos dentes já tratados com essa técnica (GHOLAM et al., 2011). O laser também promove efeito antiinflamatório, cicatrizante e miorrelaxante devido, principalmente, à produção de dentina terciária, resultante do aumento da atividade metabólica dos odontoblastos. A esse processo se dá o nome de fotobiomodulação (LADALARDO et al., 2004).

O uso do laser propicia uma maior durabilidade e previsibilidade nos efeitos de dessensibilização, além de maiores taxas de resposta do paciente (HASHIM., 2014). Os lasers de diodo com comprimento de onda de 810 até 980 nm são considerados como um tratamento alternativo e possuem grande potencial para o tratamento da hipersensibilidade dentinária. Eles agem através da oclusão dos túbulos dentinários ou de um efeito de dessensibilização na diminuição do impulso nervoso e consequentemente, reduzindo o limiar de dor do nervo pulpar (JAIN et al., 2016; GHOLAM et al., 2011). Esta luz deve ser aplicada cuidadosamente devido seus efeitos térmicos sobre a polpa e estruturas adjacentes, uma vez que devido à geração de calor sobre a superfície, o laser altera a estrutura da dentina por meio de um rearranjo cristalino, gerando uma melhora de hipersensibilidade dentinaria (LIU et al., 2013).Verifica-se com seu uso uma diminuição da sensação de dor imediatamente após 15 minutos da primeira aplicação com descrição da melhora clínica pelos pacientes por até uma semana após a irradiação (HASHIM et al., 2014).

O verniz fluoretado 5% é o tratamento mais empregado, porém apresenta uma melhora da dor somente em um curto período de tempo, pois em longo prazo o fluxo salivar e fatores como escovação, alimentação, causam a remoção completa do produto da dentina. Atua formando cristais de fluoreto de cálcio à medida que o fluoreto de sódio reage com os íons cálcio presente nos túbulos dentinários obliterando parcialmente esses espaços (JAIN et al., 2016; DANTAS et al., 2016).

Apesar de terem sido propostos vários tratamentos para a hipersensibilidade dentinária, nenhum deles ainda é considerado totalmente eficaz. Assim, é necessário que se realizem novos estudos para avaliar outros tipos de tratamentos que sejam mais significativos para o controle dessa patologia.

Frente a esses tratamentos descritos para a hipersensibilidade dentinária é importante verificar a possibilidade de interferirem sobre a morfologia da dentina radicular no quesito rugosidade superficial, o que poderia torná-la mais susceptível a colonização bacteriana, especificamente *Streptococcus mutans*, causador da cárie dentária, uma vez que essa diferença na superfície influencia a formação do biofilme inicial (FU et al., 2013; PARK et al., 2012).

Objetivo

2. Objetivo

O objetivo deste estudo laboratorial foi avaliar os efeitos dos diferentes métodos de tratamento da hipersensibilidade dentinária na rugosidade superficial e na formação do biofilme dentário sobre as superfícies tratadas.

Artigo

Original Article

Surface roughness and bacterial adhesion on root dentin treated with different desensitizing agents

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Running Title: Effects of desensitizing treatments on the root dentin

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Original Article

Surface roughness and bacterial adhesion on root dentin treated with different desensitizing agents

Abstract

The treatments of dentin hypersensitivity (DH) may change the surface roughness of the root dentin, which can lead to the retention pigments and biofilm accumulation, increasing the risk of the occurrence of root caries. Objective: The aim was to compare the surface roughness of root dentin after different treatments of DH and the biofilm formation on those surfaces. Material and Methods: After initial surface roughness (Sa) assessment, fifty bovine root fragments received the following treatments (n= 10): Group 1- No treatment; G2- 5% sodium fluoride Varnish; G3- Professional application of a desensitizing dentifrice; G4- Tooth brushing with a desensitizing dentifrice and G5- Diode laser application (λ= 908 nm; 1.5 W, 20 s). After treatments, the Sa was evaluated again. Afterward, all samples were incubated in a suspension of S. mutans at 37 °C for 24 hours. The colony-forming units (CFU) were counted using a stereoscope, and the results were expressed in CFU/mL. The One-Way ANOVA and the Tukey´s tests compared the roughness data and the results obtained on the bacterial adhesion test (α= 5%). Results: G2 (2.3 ± 1.67%) showed similar Sa variation than G1 (0.25 ± 0.41 %) and G5 (5.69 ± 0.99%), but different from group G3 (9.05 ± 2.39%). Group 4 showed the highest Sa variation (30.02 ±3.83%; p<0.05). No statistically differences were found between groups 3 and 5. Bacterial adhesion was higher in G4 (2208 ± 211.9), suggesting that bacterial growth is greater on rougher surfaces. Conclusions: The treatments for DH change the surface roughness of the root dentin, mainly when there is intense friction on the tooth surface. Also, a significant increase in dentin roughness may lead to a higher accumulation of bacteria on that surface.

Keywords: Dentin, Biofilms, Toothpastes, Lasers, Dentin Hypersensitivity.

Introduction

The advances in dental treatment and the development of preventive therapies have promoted significant improvements in the oral health, which increased the permanency of the teeth in the oral cavity. However, periodontal treatment and oral hygiene habits, such as the use of acidic mouthwashes with abrasive slurries and mechanical brushing with excessive force, can expose the dentinal tubules to the oral environment[24].This condition may lead to the occurrence of an acute discomfort in teeth, known as cervical dentin hypersensitivity. Dentin hypersensitivity (DH) is a common, transient oral pain condition, caused by thermal, chemical or physical, stimuli on the exposed dentin[26]. Those stimuli promote fluid flow in the dentinal tubules and consequent nociceptor activation in the pulp/dentine border area, leading to pain. Pasts studies[12,25] reported a DH prevalence of 42% in clinically examined patients in European countries and, although DH occurs in adults of all ages and of both genders, the highest incidence is observed in female patients between 20 and 40 years old. The most affected teeth are the canines and pre molars, followed by the incisors and molars, being the cervical region of the buccal surfaces the most affected areas[12,25]. Dentin hypersensitivity may lead to physical and psychological problems in affected patients, causing negative effects on their quality of life, especially regarding the dietary restrictions.

The treatment for this disease involves, among other things, the use of desensitizing agents, the correct performance oral hygiene and the adoption of a diet with low acidic foods and drinks[1]. The aims of the treatments are to modify or the pulpal nerve response and to reduce the movements of fluids in the tubules. The main desensitizing agents used in the treatment of HD are fluoride varnishes, 8% arginine, calcium carbonate, calcium sodium phosphosilicate, casein derivatives, oxalates and low and high intensity laser[24].

Previous studies have demonstrated that the use of fluoride varnishes can provide immediate pain relief which lasts for several weeks[3,19]. This approach reduces the pain DH while the fluoride varnish stays on the tooth. The varnish application keeps fluoride in contact with the exposed dentin and forms calcium/phosphate precipitates, calcium fluoride and fluorapatite that can block dentin tubules[14]. Desensitizing agents, like arginine and calcium carbonate, present in toothpastes for professional use also demonstrated clinical efficacy in the treatment of HD[7]. The application of those products blocks the dentinal tubules with a plug of arginine, calcium, phosphate and carbonate. The effectiveness of this treatment is immediate and may last for several weeks[7].

Another alternative for reducing DH is the treatment with lasers. Previous studies have shown that high intensity lasers are able to occlude the dentinal tubules due to the melting and resolidification of dentin, since the aim tissue reaches high temperatures during irradiation[15,22]. Laser treatment is considered safe, fast, clean and painless for the patient[6]. An advantage over other methods of treatment would be the duration of treatment, as the laser changes the morphology of the dentin, this tissue would become more resistant to acid and abrasive challenges that would promote a new opening of dentinal tubules. Several studies have demonstrated the clinical effectiveness of Nd:YAG, Er,Cr:YSGG and diode lasers for the treatment of dentine hypersensitivity[10,17].

The several treatments of DH involve the product or light application directly to the affected tissue. In this way, it is probably that such treatments change the surface roughness of the root dentin[21]. The increase in surface roughness can lead the retention pigments and biofilm accumulation what, consequently, may increase the risk of the occurrence of root caries. In such way, studies that assess the morphological characteristics of dentine that received treatment for DH, as well as the biofilm retention on the treated surfaces are important. The objective of the present study was to compare the surface roughness of root dentin after different treatments of DH and the biofilm formation on those surfaces. The null hypothesis is that the dentin roughness, as well as the bacterial adhesion, are not changed after DH treatments.

Material and methods

Sample Preparation

Fifty freshly extracted bovine incisors were stored in a 0.1 percent thymol solution (pH 7.0) for up to one month. The crowns were separated from the roots using a water-cooled diamond disc (Isomet; 10.2 cm×0.3 mm, arbour size 1/2 in., series 15HC diamond; Buehler Ltd., Lake Bluff, IL, USA) mounted in a sectioning machine (Isomet 100, Buehler Ltd) roots were sectioned to obtain 50 buccal dentin blocks (5x5x3 mm), which were individually embedded in epoxy resin. Prior to the experiment, the specimens were cleaned and stored in distilled water. To standardize the root dentin substrate, the root dentin samples were ground flat and polished using water-cooled sandpaper (#600, 800, 1200, and 2400 grit, Saint-Gobain Abrasivos Ltda, Sao Paulo, SP, Brazil), using standardized conditions.

Surface Roughness Baseline

Prior to the experiments, the samples were cleaned with pumice and water, using a rubber cup in low speed. Two marks were made on each sample to make possible the final examination was conducted in the same position/area. Afterwards, a 24% EDTA (Ethylenediamine tetraacetic acid, Biodynamics Chemicals & Pharmaceuticals Ltda., Ibiporã, PR, Brazil) gel was applied on the exposed surface of all samples. After 1 minute, each sample was cleaned in distilled water. This procedure opened the dentinal tubules, simulating a pattern of exposed dentin. The surface roughness (Sa, µm) of each enamel sample was assessed by a 3D Laser Confocal Microscope (LEXT 4000, Olympus, Hamburg, Germany) at a magnification of 40×. All data were documented at a resolution of 1024x1024 pixels.

Experimental Groups

The samples were randomly divided in 5 groups (n= 10) and treated as follows:

Group 1 (G1) received no treatment (negative control). The samples of Group 2 (G2 – positive control) were dried with cotton and received the application of 9,0 mg of 5% Sodium Fluoride varnish (NaF; Duraphat, Colgate-Palmolive Ind. e Com. Ltda, Sao Paulo, SP, Brazil) during 10 seconds. After two minutes, the samples were placed in distilled water at 37 ^{o}C. After 24h, the varnish was removed with a gauze.

The samples of group 3 (G3) received a topical application of a desensitizing dentifrice (Elmex Sensitive Professional, GABA International Therwil, Switzerland), relative dentin abrasivity (RDA)= 30, composed of arginine (8%), sodium bicarbonate, 1450 ppm of sodium monofluorophosphate, calcium carbonate, water, sorbitol, sodium lauryl sulfate, sodium silicate, cellulose gum, titanium dioxide and sucralose. The dentifrice was rubbed on the dentin surface with the finger for one minute, following the manufacturer's instructions. At the end of the application, samples were washed with air/water spray to remove the excess dentifrice and immersed in distilled water at 37^{o}C. This procedure was performed once in each sample.

The samples of group 4 (G4) were brushed with an electric toothbrush (Oral-B Professional Care 5000, Procter and Gamble, Marktheidenfeld, Germany) and a toothpaste used for the treatment of DH (Elmex Sensitive Profesional, Gaba International). During the abrasive challenge, the electric toothbrush was fixed in a standardized fixed support. The brush head (Precision Clean, Procter and Gamble), has three sets of soft bristles with different shapes and positioned in different angles and heights. During brushing, the brush bristles came in contact with the surface of the dentin during 10 minutes with a force of 1.96 N at room temperature. Taking into consideration that a person brushes each tooth surface 3 times a day during 5 seconds on each brushing, the present abrasive protocol simulated a 120-day period of toothbrushing[16]. A solution (slurry) was obtained by mixing the dentifrice and distilled water in the ratio 1:2 by weight (200 mL of distilled water and 100 g of dentifrice - ISO Specification # 14569-1), respectively. This solution was prepared every day, 20 minutes before use. During the abrasive challenge, 1.0 ml of the slurry was injected laterally to the body of the test piece between the dentin sample and the toothbrush, every 30 seconds. The brush head was replaced after brushing 3 samples. At the end of the experiment, the excess of dentifrice was removed and the samples were cleaned in ultrasonic filled with distilled water for 3 minutes. Subsequently, samples were immersed in distilled water at 37^{o}C.

The samples from group 5 (G5) were irradiated with a high intensity diode laser (SoftLase, Zap Lasers, Pleasant Hills, CA, USA), with a 908 nm wavelength, using a power output of 1.5W (fluence= 1,194 J/cm^2) in a non-contacted continuous mode (1.0 mm far from the tissue). To deliver the light to the desired area, an optic fiber with a 400 µm diameter was used. The root dentin surface was scanned by the laser light for a period of 20 seconds (10 seconds horizontally and 10 seconds vertically). The optical fiber was cut to a new irradiation. After irradiation, specimens were placed in distilled water. The laser parameters were based on previous studies[15,17].

Analysis of final Surface Roughness

Quantitative changes on the dentin surface roughness (Sa, µm) were assessed by a 3D Laser Confocal Microscope (LEXT 4000, Olympus Co., Hamburg, Germany) at a magnification of 40×. All data were documented at a resolution of 1024x1024 pixels.

Bacterial Adhesion Test

First of all, the samples were individually placed into 24-well cell culture plates filled with deionized water and irradiated on a microwave (650 W/ 3 min). This method is effective for the complete disinfection of bovine dentin specimens without affecting the structure of the dental hard tissue[23]. For the bacterial adhesion test, *Streptococcus mutans* (ATCC 25175) grown repeatedly in sucrose medium was used. The dentin samples in the sterile cell culture plates were coated with 1.5 mL of Brain Heart Infusion broth supplemented with 5% sucrose (BHI-S; Difco, Sparks, MD, USA), inoculated overnight with standardized cultures (≅ 8.8x107 colony-forming units [CFU]/mL) in the same medium, and incubated for 24 hours at 37^{o}C. All incubations were carried out as previously described[5].

After incubation, the dentin fragments were removed from the wells with sterile tweezers, and washed in sterile distilled water to remove non-adhering micro-organisms. After washing, the samples were placed in tubes containing sterile distilled water and vortexed for 3 minutes. Subsequently, swab samples were taken from the dentin surface and then spread across the surface of the blood agar plates supplemented with sucrose. After 24 h of incubation at 37 ^{o}C, the colony-forming units were counted using a stereoscope, and the results were expressed in CFU/mL.

Data Analysis

The variation of the roughness observed in each group was transformed in percentage. The surface roughness results were submitted to the D´Agostino test to verify the normal distribution of the variables. The One-Way ANOVA and the Tukey´s test were performed to compare the averages. The data obtained in the bacterial adhesion test were submitted to the D´Agostino´s and to the Student´s tests. The Pearson correlation test was performed to evaluate the relationship between the surface roughness and bacterial adhesion. The level of significance adopted in all cases was 5%. All statistical analyses were performed using SPSS version 9.0 (SPSS Inc., Chicago, IL, USA).

Results

Figure 1 shows the percentage of the surface roughness variation (ΔSa) after each treatment, and positive values indicate that there was an increase in Sa. Group 2 (2.3 ± 1.67%) showed similar Sa variation than groups 1 (0.25 ± 0.41 %) and 5 (5.69 ± 0.99%), but different from group 3 (9.05 ± 2.39%). Group 4 showed the highest Sa variation (30.02 ±3.83%; $p<0.05$). No statistically differences were found between groups 3 and 5.

Table 1 shows the average CFU/mL obtained in all experimental groups. The samples of groups 1(1660 ± 197.9), G2 (1525 ± 166.4), G3 (1589 ± 82.2) and G5 (1648 ± 115.6) presented lower bacterial adhesion than group 4 (2208 ± 211.9). To verify whether the surface roughness had an influence on the bacterial adhesion on enamel surface, the Pearson correlation test was performed. There was a high positive correlation between surface roughness and bacterial adhesion (r=0.93; p=0.019).

Discussion

The null hypothesis that the dentin roughness, as well as the bacterial adhesion, are not changed after DH treatments was rejected, since the surface roughness of root dentin was changed when the DH treatments were performed. Also, bacterial adhesion was greater in G4, where samples were brushed with a desensitizing dentifrice.

As described before, DH is a common condition of difficult treatment that affects a large population. The proposed treatments are intended to promote the occlusion of the dentinal tubules to prevent the movement of fluids within the dentinal tubules, which consequently would inhibit of the activation of the nerve endings in the pulp tissue. Therefore, it is expected that some morphological change must occurs to promote the occlusion of the tubules.

One of the treatments proposed here and acted as a positive control was the use of 5% fluoride varnish (22,600 ppm of fluoride). It is indicated for the treatment of DH because of its immediate effect and easy application[3]. Fluoride delivered as a varnish is retained on root dentin by the varnish and forms calcium/phosphate precipitates, calcium fluoride and fluorapatite that can block dentine tubules[14]. Clinically, the varnish is gently applied on the root dentin surface with a disposable microbrush, and the dentin should not be actively cleaned or brushed for some hours to extend the contact of the varnish the tooth surface. In the present research, the varnish was applied in the same way described before and the varnish was completed removed from the samples with a gauze after 4 h. The results presented the surface roughness of the samples treated with varnish (G2) was similar to the found on non-treated samples (G1).

The samples of G3 were treated with a desensitizing dentifrice that, according to the manufacturer instructions, has to be manually rubbed on the dentin surface during 1 minute. The surface roughness of those samples were higher than the samples of the positive and negative controls, similar to the lased samples and lower than the brushed samples. The Elmex Sensitive Profesional presents the Pro-Argin® formula, which is the combination of arginine with calcium carbonate. It is a bioactive agent which has been developed as polishing paste and dentifrice for treating dentin hypersensitivity. Arginine and calcium carbonate work together to accelerate the natural mechanisms of occlusion by depositing a dentin-like material, containing calcium and phosphate, within the dentin tubules to form a plug and a protective layer on the dentin surface[2]. These plugs are stable and resistant to the erosive challenges, in addition to enabling the deposition of high levels of calcium, phosphorus, oxygen and carbonate on the surface of the dentin[13]. A recent study showed that the topical application of the Elmex Sensitive Professional was capable to reduce the root dentin permeability in almost 50% when compared to a non-treated dentin, due to an effective occlusion of the dentinal tubules[15].

Even with low abrasiveness (RDA= 30), the attrition due to topical application of dentifrice was able to increase the surface roughness of the G3 samples. This also produces smear layer, which promotes the occlusion of dentinal tubules[15].

The samples of G4 also received the application of the dentifrice, but with an electric brush. The electric brush used here brushed the samples with oscillating-rotating movements, with a brush head with three sets of soft bristles with different shapes and positioned in different angles and heights[8]. As described before, the force during brushing was standardized (1,96N) and a 120-day period of toothbrushing was simulated[16]. Past studies have reported that this method is more effective than the standard manual toothbrush to remove biofilm from dental surfaces[8,9]. However, the present study showed that this treatment of DH that produced the highest surface roughness, probably due to the friction of the bristles with the root dentin combined to the duration of the treatment. Although no studies that evaluated the surface roughness of the root dentin after electric toothbrushing were found, a previous published work stated that electric toothbrush produces higher wear on tooth structure than manual brushing. According to the authors, the higher dentin wear occurred due to the greater number of brushing movements produced by the electrical method, associated with the force used in during tooth cleaning[18]. The action of the bristles seems to be more significant than the abrasiveness of the dentifrice, since different dentifrices can promote the same amount of wear when a power toothbrush is used[11].

The G5 samples were treated with a diode laser, under a power output of 1.5 W during 20 s in a non-contacted mode. The present study showed that action of the diode laser increased the roughness of the root dentin, when compared to the negative control group, but the roughness was similar to that found on G3. In fact, the literature shows that the diode laser is able to increase the roughness of the root dentin when applied with an output power of less than 2.0W [4]. A recent study showed that this laser is effective in reducing dentinal permeability, due to the melting and recrystallization of the root dentin due to the heat produced on the dentin surface during irradiation[15]. Probably, these changes on root dentin have contributed to increase the roughness of the tissue in the present study.

The results obtained here showed that bacterial adhesion was higher in brushed samples. Although the samples from G3 and G5 presented greater surface roughness than group 1, this difference did not result in a greater accumulation of bacteria on root dentin of those groups. In fact, the statistical analysis showed a strong positive correlation between surface roughness and bacterial adhesion. Surface roughness is one of the properties of tooth that influence on the formation and on the accumulation of biofilm. In the rough and irregular surfaces the dental biofilm is formed in larger quantities and presents quicker maturation when compared to flat surfaces[20].

All the procedures performed in the present study were standardized, suggesting that these results could be even more significant in *in vivo* or *in vitro* studies, due to the patient's difficulty in controlling the strength and movements during toothbrushing. Thus, prior to the clinical indication, all the new treatments suggested for the reduction of DH should be evaluated because, as shown here, a treatment can, at the same time, reduce the dentin sensitivity and increase the risk of caries of that surface, due to the greater accumulation of biofilm on the root dentin.

Conclusions

In accordance to what was described here and under the conditions of the present study, it can be concluded that the treatments for DH change the surface roughness of the root dentin, mainly when there is intense friction on the tooth surface. Also, a significant increase in dentin roughness may lead to a higher accumulation of bacteria on that surface.

References

1. Bartlett DW, Fares J, Shirodaria S, Chiu K, Ahmad N, Sherriff M. The association of tooth wear, diet and dietary habits in adults aged 18-30 years old. J Dent. 2011;39:811-6.
2. Cummins D. Dentin hypersensitivity: from diagnosis to a breakthrough therapy for everyday sensitivity relief. J Clin Dent. 2009;20:1-9.
3. Dantas EM, Amorim FK, Nóbrega FJ, Dantas PM, Vasconcelos RG, Queiroz LM. Clinical Efficacy of Fluoride Varnish and Low-Level Laser

Radiation in Treating Dentin Hypersensitivity. Braz Dent J. 2016;27:79-82.

4. de Macedo HS, Colucci V, Messias DC, Rached-Júnior FJ, Fernandes FS, Silva-Sousa YT, Raucci-Neto W. Effect of Nd:YAG (1064-nm) and Diode Laser (980-nm) EDTA Agitation on Root Dentin Ultrastructure Properties. Photomed Laser Surg. 2015;33:349-56.
5. Geraldo-Martins V, Thome T, Mayer M, Marques M. The use of bur and laser for root caries treatment: a comparative study. Oper Dent 2013;38:290-298.
6. Hashim NT, Gasmalla BG, Sabahelkheir AH, Awooda AM. Effect of the clinical application of the diode laser (810 nm) in the treatment of dentine hypersensitivity. BMC Res Notes. 2014;13;7:31.
7. Kapferer I, Pflug C, Kisielewsky I, Giesinger J, Beier US, Dumfahrt H.Instant dentin hypersensitivity relief of a single topical application of an in-office desensitizing paste containing 8% arginine and calcium carbonate: a split-mouth, randomized-controlled study. Acta Odontol Scand. 2013;71:994-9.
8. Klukowska M, Grender JM, Conde E, Goyal CR. A 12-week clinical comparison of an oscillating-rotating power brush versus a marketed sonic brush with self-adjusting technology in reducing plaque and gingivitis. J Clin Dent. 2013;24:55-61.
9. Kurtz B, Reise M, Klukowska M, Grender JM, Timm H, Sigusch BW. A randomized clinical trial comparing plaque removal efficacy of an oscillating-rotating power toothbrush to a manual toothbrush by multiple examiners. Int J Dent Hyg. 2016;14:278-283.
10. Lee SY, Jung HI, Jung BY, Cho YS, Kwon HK, Kim BI. Desensitizing efficacy of nano-carbonate apatite dentifrice and Er,Cr:YSGG laser: a randomized clinical trial. Photomed Laser Surg. 2015;33:9-14.
11. Lopes RM, Turbino ML, Zezell DM, Scaramucci T, Aranha AC. The effect of desensitizing dentifrices on dentin wear and tubule occlusion. Am J Dent. 2015;28:297-302.
12. Mantzourani M, Sharma D. Dentine sensitivity: past, present and future. J Dent. 2013;41:S3-17.
13. Mello SV, Arvanitidou E, Vandeven M. The development of a new desensitising mouthwash containing arginine, PVM/MA copolymer,

pyrophosphates, and sodium fluoride--a hydraulic conductance study. J Dent. 2013;41:S20-5.

14. Petersson LG. The role of fluoride in the preventive management of dentin hypersensitivity and root caries. Clin Oral Investig. 2013;17:S63-71.
15. Rosa RR, Calazans FK, Nogueira RD, Lancellotti AC, Gonçalves LS, Geraldo-Martins VR. Effects of different desensitizing treatments on root dentin permeability. Braz Oral Res. 2016;30:e111.
16. Schmidt JC, Zaugg C, Weiger R, Walter C. Brushing without brushing?--a review of the efficacy of powered toothbrushes in noncontact biofilm removal. Clin Oral Investig. 2013;17:687-709.
17. Sgolastra F, Petrucci A, Severino M, Gatto R, Monaco A. Lasers for the treatment of dentin hypersensitivity: a meta-analysis. J Dent Res. 2013;92:492-9.
18. Sorensen JA, Nguyen HK. Evaluation of toothbrush-induced dentin substrate wear using an in vitro ridged-configuration model. Am J Dent. 2002;15:26B-32B.
19. Suri I, Singh P, Shakir QJ, Shetty A, Bapat R, Thakur R.A comparative evaluation to assess the efficacy of 5% sodium fluoride varnish and diode laser and their combined application in the treatment of dentin hypersensitivity. J Indian Soc Periodontol. 2016;20:307-14.
20. Tada K, Oda H, Inatomi M, Sato S. Characterization and Streptococcus mutans adhesion on air polishing dentin. Odontology. 2014;102:284-9.
21. Tawakoli PN, Sener B, Attin T. Mechanical effects of different Swiss market-leading dentifrices on dentin. Swiss Dent J. 2015;125:1210-9.
22. Umana M, Heysselaer D, Tielemans M, Compere P, Zeinoun T, Nammour S. Dentinal tubules sealing by means of diode lasers (810 and 980 nm): a preliminary in vitro study. Photomed Laser Surg. 2013;31:307-14.
23. Viana PS, Machado AL, Giampaolo ET, Pavarina AC, Vergani CE. Disinfection of bovine enamel by microwave irradiation: effect on the surface microhardness and demineralization/remineralization processes. Caries Res. 2010;44:349-57.
24. West N, Seong J, Davies M. Dentine hypersensitivity. Monogr Oral Sci. 2014;25:108-22.

25. West NX, Sanz M, Lussi A, Bartlett D, Bouchard P, Bourgeois D. Prevalence of dentine hypersensitivity and study of associated factors: a European population- based cross-sectional study. J Dent 2013;41: 841–851.
26. West NX, Seong J, Davies M. Management of dentine hypersensitivity: efficacy of professionally and self-administered agents. J Clin Periodontol. 2015;42:S256-302.

Figure

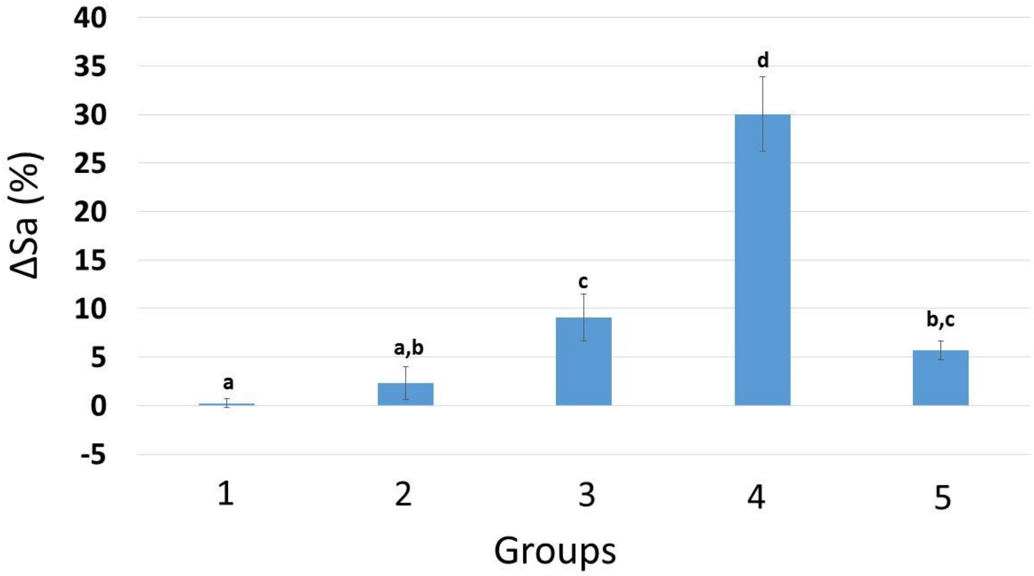

Figure 1- Variation of surface roughness (ΔSa) in the experimental groups. Different letters indicate the presence of statistically significant differences ($p<0.05$)

Table

Group	CFU/mL
1	1660 (±197.9)A
2	1525(±166.4)A
3	1589(±82.2)A
4	2208(±211.9)B
5	1648(±115.6)A

Table 1- Mean CFU/mL (± standard deviation) found in each experimental group. Different letters indicate the presence of differences statistically significant ($p<0.05$).

Conclusão

4- Conclusão

De acordo com o trabalho apresentando e as condições do presente estudo, conclui-se que os tratamentos empregados para diminuir e melhorar a hipersensibilidade dentinária alteram a rugosidade superficial da dentina, principalmente quando submetida a intensa fricção em sua superfície. Concomitantemente, o aumento da rugosidade superficial pode gerar um maior acúmulo de bactérias na superfície dentinária.

Referências

5- Referências

ACHARYA, A. B., et al. A clinical study of the effect of calcium sodium phosphosilicate on dentin hypersensitivity. **J Clin Exp Dent**, v. 5, n. 1, p. 18-22, 2013.

ASSIS, J. S., et al. Dentin hypersensitivity after treatment with desensitizing agents: a randomized, double-blind, split-mouth clinical trial. **Braz Dent J**, v. 22, n. 2, p. 157-61, 2011.

BAE, J. H., et al. Desensitizing toothpaste versus placebo for dentin hypersensitivity: a systematic review and meta-analysis**. J Clin Periodontol**, v. 42, n. 2, p. 131-41, 2015.

BARTLETT, D. W., et al. The association of tooth wear, diet and dietary habits in adults aged 18-30 years old. **J Dent**, v. 39, n. 12, p. 811-6, 2011.

BRANNSTROM, M., et al. The hydrodynamics of the dental tubule and of pulp fluid. A discussion of its significance in relation to dentinal sensitivity. **Caries Res**, v. 1, n. 4, p. 310-7, 1967.

CUMMINS, D. Dentin hypersensitivity: from diagnosis to a breakthrough therapy for everyday sensitivity relief. **J Clin Dent**, v. 20, n. 1, p. 1-9, 2009.

DANTAS, E. M., et al. Clinical Efficacy of Fluoride Varnish and Low-Level Laser Radiation in Treating Dentin Hypersensitivity. **Braz Dent J**, v. 27, n. 1, p. 79-82, 2016.

DAVARI, A., et al. Dentin hypersensitivity: etiology, diagnosis and treatment; a literature review. **J Dent (Shiraz)**, v. 14, n. 3, p. 136-45, 2013.

DE MACEDO, H. S., et al. Effect of Nd:YAG (1064-nm) and Diode Laser (980-nm) EDTA Agitation on Root Dentin Ultrastructure Properties. **Photomed Laser Surg**, v. 33, n. 7, p. 349-56, 2015.

GERALDO-MARTINS, V., et al. The use of bur and laser for root caries treatment: a comparative study. **Oper Dent**, v. 38, n. 3, p. 290-298, 2013.

FU, D., et al. Effect of desensitising paste containing 8% arginine and calcium carbonate on biofilm formation of Streptococcus mutans in vitro**. J Dent**, v. 41, n. 7, p. 619-27, 2013.

GHOLAMI, G. A., et al. An evaluation of the occluding effects of Er;Cr:YSGG, Nd:YAG, CO(2) and diode lasers on dentinal tubules: a scanning electron microscope in vitro study**. Photomed Laser Surg**, v. 29, n. 2, p. 115-21, 2011.

HASHIM, N. T., et al. Effect of the clinical application of the diode laser (810 nm) in the treatment of dentine hypersensitivity. **BMC Res Notes**, v. 7, p. 31-11, 2014.

HOLLAND, G. R., et al. Guidelines for the design and conduct of clinical trials on dentine hypersensitivity**. J Clin Periodontol**, v. 24, n. 11, p. 808-13, 1997.

JAIN, P. R., et al. Diode laser and fluoride varnish in the management of dentin hypersensitivity. **J Interdiscip Dentistry**, v. 5, n. 2, p. 71-4, 2015.

KAPFERER, I., et al. Instant dentin hypersensitivity relief of a single topical application of an in-office desensitizing paste containing 8% arginine and calcium carbonate: a split-mouth, randomized-controlled study. **Acta Odontol Scand**, v. 71, n. 3-4, p. 994-9, 2013.

KLUKOWSKA, M., et al. A 12-week clinical comparison of an oscillating-rotating power brush versus a marketed sonic brush with self-adjusting technology in reducing plaque and gingivitis. **J Clin Dent**, v. 24, n. 2, p. 55-61, 2013.

KURTZ, B., et al. A randomized clinical trial comparing plaque removal efficacy of an oscillating-rotating power toothbrush to a manual toothbrush by multiple examiners. **Int J Dent Hyg**, v. 14, n. 4, p.278-283, 2016.

LADALARDO, T. C., et al. Laser therapy in the treatment of dentine hypersensitivity. **Braz Dent J**, v. 15, n. 2, p. 144-50, 2004.

LEE, S. Y., et al. Desensitizing efficacy of nano-carbonate apatite dentifrice and Er,Cr:YSGG laser: a randomized clinical trial. **Photomed Laser Surg**, v. 33, n. 1, p. 9-14, 2015.

LIU, Y., et al. In Vitro Study of Dentin Hypersensitivity Treated by 980-nm Diode Laser**. J Lasers Med Sci**, v. 4, n. 3, p. 111-9, 2013.

LOPES, R. M., et al. The effect of desensitizing dentifrices on dentin wear and tubule occlusion. **Am J Dent**, v. 28, n. 5, p. 297-302, 2015.

MANTZOURANI, M., SHARMA, D. Dentine sensitivity: past, present and future. **J Dent**, vol. 41, p. S3-17, 2013.

MÁRQUEZ, M., et al. Efecto de la arginina 8%-carbonato de calcio y Del fluoruro de sodio al 5% em la reducción de la hipersensibilidad dentinaria post terapia periodontal: ensayo clínico **Rev. Clin. Periodoncia Implantol. Rehabil. Oral,** v. 4 n. 1, p. 22-3, 2011.

MELLO, S. V., et al. The development of a new desensitising mouthwash containing arginine, PVM/MA copolymer, pyrophosphates, and sodium fluoride--a hydraulic conductance study. **J Dent**, v. 41, p. S20-5, 2013.

ORCHARDSON, R.; GILLAM, D. G. Managing dentin hypersensitivity. **J Am Dent Assoc**, v. 137, n. 7, p. 990-8; 2006.

PARK, J. W., et al. The effects of surface roughness of composite resin on biofilm formation of Streptococcus mutans in the presence of saliva. **Oper Dent**, v. 37, n. 5, p. 532-9, 2012.

PETERSSON, L. G. The role of fluoride in the preventive management of dentin hypersensitivity and root caries. **Clin Oral Investig**, v. 17, p. S63-71, 2013.

PINTO, S. C., et al. A. Effect of desensitizing toothpastes on dentin. **Braz Oral Res**, v. 26, n. 5, p. 410-7, 2012.

ROSA, R. R., et al. Effects of different desensitizing treatments on root dentin permeability. **Braz Oral Res**, v. 30, n. 1, p e111, 2016.

SANGWAN, P., et al. Tertiary dentinogenesis with calcium hydroxide: a review of proposed mechanisms. **Int Endod J**, v. 46, n. 1, p. 3-19, 2013.

SCHMIDT, J. C., et al. Brushing without brushing?--a review of the efficacy of powered toothbrushes in noncontact biofilm removal. **Clin Oral Investig**, v. 17, n. 3, p. 687-709, 2013.

SGOLASTRA, F., et al. Lasers for the treatment of dentin hypersensitivity: a meta-analysis. **J Dent Res**, v. 92, n. 3, p. 492-9, 2013.

SORENSEN, J. A., NGUYEN, H. K. Evaluation of toothbrush-induced dentin substrate wear using an in vitro ridged-configuration model. **Am J Dent**, v. 15, n. 6, p. 26B-32B, 2012

SURI, I., et al. A comparative evaluation to assess the efficacy of 5% sodium fluoride varnish and diode laser and their combined application in the treatment of dentin hypersensitivity. **J Indian Soc Periodontol**, v. 20, n. 3, p. 307-14, 2016.

TADA, K., et al. Characterization and Streptococcus mutans adhesion on air polishing dentin. **Odontology**, v. 102, n. 2, p. 284-9, 2014.

TAWAKOLI, P. N., et al. Mechanical effects of different Swiss market-leading dentifrices on dentin. **Swiss Dent J**, v. 125, n. 11, p. 1210-9, 2015.

UMANA, M., et al. Dentinal tubules sealing by means of diode lasers (810 and 980 nm): a preliminary in vitro study. **Photomed Laser Surg**, v. 31, n. 7, p. 307-14, 2013.

VIANA, P. S., et al. Disinfection of bovine enamel by microwave irradiation: effect on the surface microhardness and demineralization/remineralization processes. **Caries Res**, v. 44, n. 4, p. 349-57, 2010.

WEST, N., et al. Dentine hypersensitivity. **Monogr Oral Sci** , v. 25, p. 108-22, 2014.

WEST, N. X., et al. Prevalence of dentine hypersensitivity and study of associated factors: a European population- based cross-sectional study. **J Dent**, v. 41, n. 10, p. 841–851, 2013.

WEST, N. X., et al. Management of dentine hypersensitivity: efficacy of professionally and self-administered agents. **J Clin Periodontol**, v. 42, p. S256-302, 2015.

6. Anexo

JOURNAL OF APPLIED ORAL SCIENCES

INSTRUCTIONS TO AUTHORS

Scope and policy

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Book chapter

Verbeeck RMH. Minerals in human enamel and dentin. In: Driessens FCM, Woltgens JHM, editors. Tooth development and caries. Boca Raton : CRC Press; 1986. p.95-152.

Papers published in journals

Wenzel A, Fejerskov O. Validity of diagnosis of questionable caries lesions in occlusal surfaces of extracted third molars. Caries Res. 1992;26:188-93.

Papers with more than 6 authors

The first 6 authors are cited, followed by the expression ", et al."

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood - leukemia in Europe after Chernobyl : 5 years follow-up. Br J Cancer. 1996;73:1006-12.

Papers without authors' names

Seeing nature through the lens of gender. Science. 1993;260:428-9.

Volume with supplement and/or Special Issue

Davisdson CL. Advances in glass-ionomer cements. J Appl Oral Sci. 2006;14(sp. Issue):3-9.

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Dental Update. Guildford 1991;18(1).

The authors are fully responsible for the correctness of the references.

3 ETHICAL PRINCIPLES AND REGISTRATION OF CLINICAL TRIALS

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The Journal of Applied Oral Science reassures the principles incorporated in the Helsinky Declaration and insists that all research involving human beings, in the event of publication in this journal, be conducted in conformity with such principles and others specified in the respective ethics committees of authors' institution. In the case of experiments with animals, such ethical principles must also be followed. When surgical procedures in animals were used, the authors should present, in the Material and Methods section, evidence that the dose of a proper substance was adequate to produce anesthesia during the entire surgical procedure. All experiments conducted in human or animals must accompany a description, in the Material and Methods section, that the study was approved by the respective Ethics Committee of authors' affiliation and provide the number of the protocol approval.

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