CRISTINA YOSHIE GARCIA TAKEUCHI

EFEITO DA FUMAÇA DE CIGARRO NAS PROPRIEDADES DE RESINAS COMPOSTAS E SUBSTRATOS DENTAIS

Tese apresentada ao Programa de Pós-Graduação em Dentística Restauradora, da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista, para obtenção do título de Doutor em Dentística Restauradora.

Orientador: *Prof. Dr. Welingtom Dinelli*
Co-orientadora: *Prof[a]. Dr[a]. Regina Guenka. Palma-Dibb*

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EFEITO DA FUMAÇA DE CIGARRO NAS PROPRIEDADES DE RESINAS COMPOSTAS E SUBSTRATOS DENTAIS

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Sumário

Takeuchi, CYG. Efeito da fumaça de cigarro nas propriedades de resinas compostas e substratos dentais [Tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2007.

RESUMO

O objetivo do presente estudo foi avaliar in vitro a microdureza, mudança de cor e presença de substâncias tóxicas após exposição à fumaça de cigarro na resina composta, dentina e esmalte dental e avaliar in situ a microdureza, rugosidade superficial e alteração de cor (ΔE) e luminosidade (ΔL) de resinas compostas frente à exposição à fumaça do cigarro associado à escovação. Para a fase in vitro foram confeccionados corpos-de-prova de resina composta (RC), esmalte (E) e dentina radicular bovina (DR) que foram planificados, polidos e expostos a fumaça de 10 cigarros/dia por 8 dias. Avaliou-se a microdureza Knoop e a alteração da cor (CIELAB) antes e depois do ato de fumar. Em seguida, as amostras foram preparadas para análise em microscopia eletrônica de varredura (MEV) e microanálise (EDS). Os dados da microdureza e da alteração de cor foram submetidos à ANOVA e Tukey (5%). A microdureza do E aumentou e da DR e RC diminuíram. Na alteração de cor a RC e o E apresentaram menor escurecimento do que a DR. Na fase in situ foram confeccionados corpos-de-prova com três tipos de resinas compostas que foram fixados em aparelho intrabucal palatino. Foram selecionados 22 voluntários, sendo 11 fumantes e 11 não-fumantes, que utilizaram o aparelho por 28 dias. A escovação dos espécimes foi realizada fora da boca, três vezes por dia somente na metade do dispositivo. Para os fumantes foi estipulado o consumo de 10 cigarros/dia. A mensuração da cor (CIELAB), da microdureza (KHN) e da rugosidade foram realizadas antes e após os 28 dias de exposição in situ. Os dados KHN foram analisados pela ANOVA e teste de Fisher's LSD. Os dados da rugosidade, ΔE e o ΔL foram analisados pelo método estatístico não-paramétrico Kruskal-Wallis, Wilcoxon e Friedman ($p<0.05$) que observaram que a fumaça de cigarro aumentou os valores de microdureza e de rugosidade para os fumantes com escovação. Contudo, pode-se concluir que na fase laboratorial a fumaça de cigarro interferiu na microdureza do E, DR e RC, sendo que todos apresentaram alteração de cor e o EDS identificou a presença de cádmio, arsênio e chumbo. Na fase in situ a fumaça de cigarro associada à escovação proporcionou melhores propriedades mecânicas em relação à microdureza, contudo afetou negativamente a lisura superficial das resinas compostas. Em relação a alteração de cor, a fumaça de cigarro aumentou o ΔE e o ΔL das resinas compostas avaliadas, entretanto a escovação foi capaz de limitar essa alteração.

Palavras chave: Resinas compostas; dureza, cor, tabaco.

Takeuchi, CYG. Effect of cigarette smoke on the properties of the composite resin and dental substrates [Tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2007.

ABSTRACT

The aim of this study was to assess in vitro the microhardness, color change and the presence of toxicant elements deriving from cigarette smoke in the composite resin, dentine and dental enamel and to evaluate in situ the microhardness, surface roughness and color change (ΔE) and lightness (ΔL) of composite resins upon cigarette smoke exposure associated with brushing. For the in vitro phase were fabricated specimens from composite resin (CR), enamel (E) and root dentin (RD) fragments that were wet ground and polished and exposed to the smoke of 10 cigarettes/day during 8 days. Knoop microhardness number (KHN) and the color was measured before and after exposure. After the samples were prepared for analysis with Scanning Electron Microscopy (SEM) and microanalysis (EDS). Data were submitted to ANOVA and Tukey's test ($p<0.05$). The microhardness of the E increased and the RD and CR decreased. The CR and E presented statistically significant less darkening and color change than RD. For the in situ phase were fabricated specimens three different composite resins that were attached to removable palatal appliance. Twenty-two volunteers took part in this study (11 smokers e 11 non-smokers) and received instructions to wear the appliances continuously by 28 days. For brushing of the specimens, the appliance was taken out the mouth and three times a daily was brushed. For the smokers, it was standardized consume of 10 cigarettes for day. The color changes (ΔE), microhardness (KHN) and surface roughness were measure before and after 28 days the exposure in situ. The microhardness data were analyzed by ANOVA and Fisher's LSD test. The roughness data, ΔE and the ΔL were analyzed by Kruskal-Wallis, Wilcoxon and Friedman ($p<0.05$). The cigarette smoke increased the microhardness and roughness values for smokers with brushing. Concluding that laboratory phase the cigarette smoke affected the microhardness of the E, RD and CR. All specimens presented color change and the EDS identified the presence of cadmium, arsenic and lead. In the in situ phase the cigarette smoke associated to brushing proportioned better mechanical properties in relation to microhardness; however it affected negatively the surface roughness of composite resins. With regard to the color change, the cigarette smoke increased the ΔE and ΔL of the composite resins tested, nevertheless the brushing was capable to limit this alteration.

Keywords: Composite resins, hardness, color, tobacco.

1. Introdução

Nos últimos 30 anos, de modo geral, o consumo de cigarro diminuiu, no entanto, vem aumentando entre as faixas etárias mais jovens ao longo dos anos[16]. A Organização Mundial de Saúde (OMS)[25] estima que 100 mil crianças e jovens se tornem fumantes regulares por dia no mundo. Estudos reportam que 90% dos fumantes iniciaram esse comportamento antes dos 19 anos, ou seja, na fase da adolescência. De acordo com a OMS[25], adolescente é o indivíduo que se encontra entre os dez e vinte anos de idade.

A preocupação dos adolescentes fumantes com a estética tem sido maior do que com a saúde. Esse dado, foi a conclusão do grupo de pesquisadores da Faculdade de Ciências Médicas da Santa Casa de São Paulo que realizaram pesquisa endossada pelo InCor (Instituto do Coração do Hospital das Clínicas de São Paulo) e pelo INCA (Instituto Nacional do Câncer), que constataram que os jovens estão mais preocupados com o escurecimento dos dentes do que com a possibilidade de adquirir câncer no pulmão após 20 ou 30 anos[8]. Em adição, na Finlândia, foi realizado um exercício de curta duração onde adolescentes fumantes tinham que identificar a presença de manchas provocadas pela fumaça de cigarro em seus dentes e perceberam que essa ocorrência contribuiu para redução do hábito de fumar[17].

Esses dados demonstram que a estética pode ter um papel fundamental em campanhas anti-tabagismo, particularmente na adolescência que constitui a fase mais vulnerável para iniciar, desenvolver e consolidar o hábito de fumar[6]. A Odontologia pode contribuir com essa campanha de forma a realizar mais estudos que avaliem o efeito da fumaça de cigarro nas estruturas dentais e nos materiais restauradores estéticos e divulgar os resultados, como recurso para estimularem adultos e principalmente jovens a pararem ou a não adquirir esse hábito.

Na literatura existem poucos estudos[2,11,20,21] sobre o efeito da fumaça de cigarro nas propriedades ópticas dos materiais restauradores estéticos e estruturas dentais, quando comparado aos inúmeros estudos[3,5,7,9,10,15,18,24] sobre o efeito do café, vinho tinto, chá e outros.

O manchamento das restaurações e dentes decorrentes do ato de fumar é a manifestação clínica mais imediata perceptível encontrada em pessoas fumantes[22] e tende a apresentar coloração castanho-escura, podendo chegar ao preto. A severidade da alteração de cor e sua distribuição são dependentes da quantidade e do tempo de exposição à fumaça de cigarro, além da técnica de higiene bucal[2, 22].

Outro fator relevante que pode influenciar a alteração de cor é possivelmente as transformações físicas resultantes principalmente das mudanças térmicas provocadas pela fumaça do cigarro. Pois, a fumaça produzida pelo ato de fumar é o resultado da combustão da matéria orgânica existente nos produtos derivados do tabaco, bem como do aporte de oxigênio e do grau da temperatura existente na ponta do cigarro, que pode chegar a aproximadamente 950ºC[23]. A fumaça de cigarro é composta por milhares de substâncias tóxicas como o monóxido de carbono, amônia, níquel, arsênio e metais pesados como chumbo e cádmio[19]. Quando essa fumaça entra em contato com a superfície de dentes e restaurações é possível que além de alterar a cor, ela possa alterar a lisura da superfície e a dureza, que são importantes propriedades mecânicas que contribuem com o sucesso clínico[13].

Neste contexto, é importante a avaliação do comportamento das resinas compostas e diferentes substratos dentais frente à exposição à fumaça de cigarro e verificar se há influência ou não nas propriedades físicas e mecânicas, a fim de apresentar novos resultados que venham corroborar com os estudos da área de saúde.

2. Proposição

O objetivo do presente estudo foi:

Avaliar *in vitro*:

1) A mudança de cor e microdureza de estruturas dentais e compósito após exposição à fumaça de cigarro;

2) A presença de substâncias tóxicas oriundas da fumaça de cigarro na resina composta, dentina e esmalte dental.

Avaliar *in situ*:

1) A microdureza e a rugosidade superficial de resinas compostas em fumantes;

2) A alteração de cor de resinas compostas em fumantes.

3.1. Capítulo 1 – Estudo *in vitro*

Color Change and Microhardness of Dental Structures and Composite after Exposure to Cigarette Smoking

Artigo submetido à publicação no periódico Operative Dentistry.

Color Change and Microhardness of Dental Structures and Composite after Exposure to Cigarette Smoking

Short title: Color change and microhardness after smoking simulation

Clinical relevance

Smoking can compromise the longevity of restorations and affect the physical properties of tooth structures.

SUMMARY

The purpose of this study was to evaluate the color change (ΔE_{ab}*) and microhardness of composite resin, bovine enamel and root dentin after exposure to cigarette smoke. Eight cylindrical specimens (6 mm diameter and 2 mm thickness) were fabricated from Filtek Supreme composite resin (CR), stored at 37°C for 24 hours and wet ground with abrasive paper. Eight bovine incisors were sectioned to provide 8 enamel (E) and 8 root dentin (RD) fragments that were wet ground and polished with abrasive paper to obtain sections with 6 mm in diameter and 2 mm thickness. All specimens were exposed to the smoke of 10 cigarettes/day during 8 days. Color was measured according to the CIELAB color space relative to CIE standard illuminant D65 at baseline and after exposure. Knoop microhardness number (KHN) (50 g load; 30 seconds) was measured before and after exposure. Data were submitted to ANOVA and Tukey's test ($p<0.05$). It was observed that cigarette smoke affected the color and the

microhardness of all evaluated specimens. CR and E presented statistically significant less darkening and color change (ΔE=6.12±1.18 and ΔE=6.69±3.05, respectively) than RD (ΔE=28.6±3.80). KHN means before (b) and after (a) exposure to cigarette exposure were: CR (b): 76.57, (a): 59.74; E (b): 229.63, (a) 302.13; RD (b): 41.89, (a): 34.04. Based on these results, it may be concluded that the cigarette smoke affected significantly the microhardness of the tested substrates, increasing enamel microhardness and decreasing composite resin and dentin microhardness. All specimens presented color change, especially the enamel.

INTRODUCTION

Adhesive esthetic dentistry has experienced a great development in recent years due to the improvement of adhesive systems and evolution of the physical and mechanical properties of the composites. Composite resin is the material of choice when esthetics is a primordial aspect and its longevity is directly related to color stability and capacity to withstand degradation in the oral environment.

Color stability is a critical factor to the long-term esthetics of composite restorations[1] and most anterior restorations are replaced due to unacceptable color match.[2] Discoloration has been widely investigate in *vitro* studies for a variety of staining agents derived from beverages,[3-7] foods[4,5,8,9] and tobacco.[10-12]

Color change has been assessed by different methods. The least subjective methods are spectrophotometry and colorimetric assay, which allow a quantitative analysis by comparison of the materials' color change while eliminating subjective interpretation bias[1,3]. Specimens analyzed in a spectrophotometer are enclosed in a compartment with standardized illumination, which avoids the influence of environmental light during testing. In this way, the results are reproducible and feasible to be expressed in different color systems, such as the universally adopted CIE (*Commission Internationale de I'Eclairage*) *L*a*b** system.[4-7,9,13,14]

Earlier studies[8,15] have also shown that consumption of certain beverages and foods may affect the esthetics as well as the physical properties (microhardness, surface roughness) of composite resins, undermining the quality and longevity of restorations. Badra and others[15] reported a decrease in Knoop microhardness of composite resin after coffee consumption. These authors suggested that prolonged immersion in a high-temperature solution may lead to significant alterations in certain properties of the composites[15]. Additionally, the exposure to cigarette smoke can result in surface damage because the combustion that occurs on the cigarette end leads to a temperature rise, creating cinder (maximum of 950ºC during a puff).[16] This heat is conduced through the inner part of the paper tube by aspired smoke and, the moment it enters the oral cavity, a temperature increase occurs.[17]

Therefore, the purpose of this study was to assess *in vitro* the influence of cigarette smoke on the color change and microhardness of composite resin, bovine enamel and root dentin.

MATERIALS AND METHODS

A flowchart of the study methodology is presented on Figure 1.

For the purposes of this study, 8 cylindrical specimens (6 mm de diameter and 2 mm thickness) were fabricated from the Filtek Supreme composite resin (3M/ESPE, St Paul, MN, USA; shade WB). The composite resin was inserted into a circular metal matrix in a single increment using a spatula. The matrix was covered with a polyester strip and a glass coverslip was placed over this set under a load of 0.5 kg in order to compact the resin and avoid bubble formation. After 30 seconds, the load and the coverslip were removed. The composite resin was polymerized through the polyester strip for 20 seconds, according to the manufacturers' instructions, using a halogen light-curing unit (Jetlite 4000, J. Morita, Japan) with light output of 1050 mW/cm^2 as measured with the curing radiometer coupled to the device.

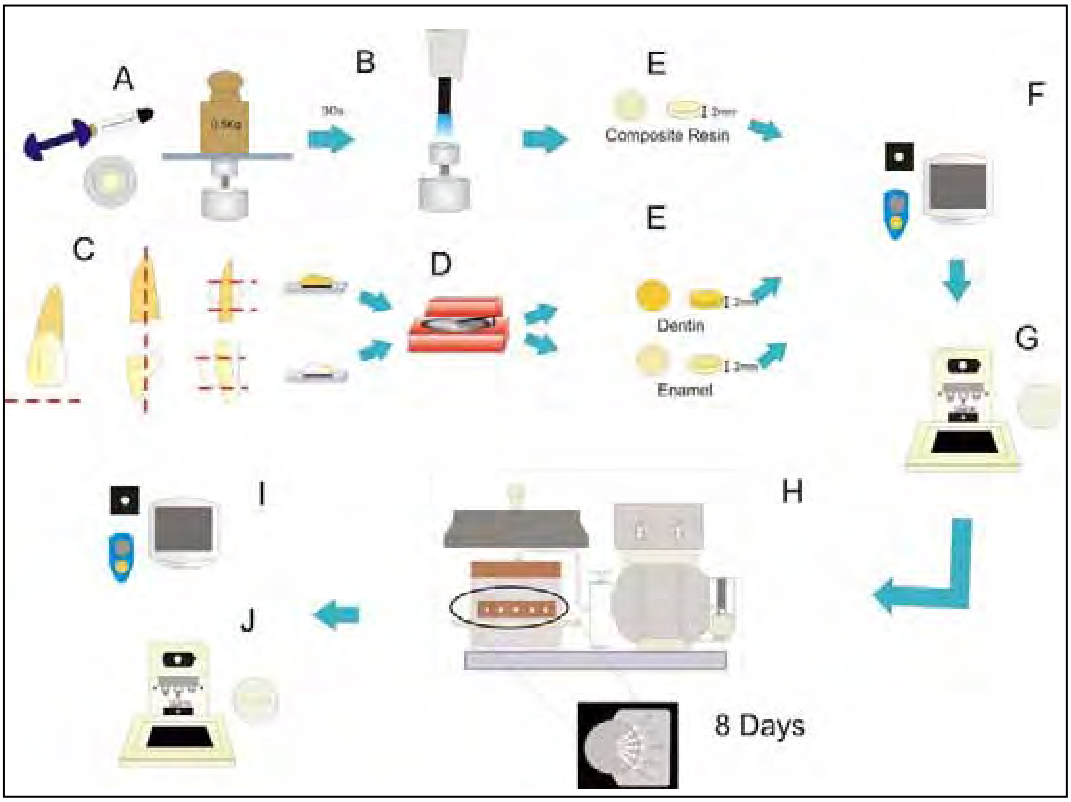

Figure 1. Flowchart of the study methodology. A) Insertion of Filtek Supreme composite resin in a single increment in the circular metal matrix, placement of a glass coverslip and 0.5 kg during 30 seconds. B) Removal of the load/coverslip set and light curing of the composite resin for 30 seconds C) Sectioning of bovine incisor to obtain enamel and root dentin fragments. D) Wet grinding and flattening of the specimens in a polishing machine. E) Dentin and enamel specimens with dimensions of 6 mm de diameter x 2 mm depth. F) Initial color reading in a spectrophotometer. G) Initial microhardness reading. H) Simulated smoking in a custom-made smoking machine. I) Final color reading. J) Final microhardness reading.

Eight bovine incisors were selected, cleaned of debris and tissue remnants immediately after extraction and stored in a 0.5% aqueous chloramine-T solution at 4°C. The teeth taken to a sectioning machine (Isomet, Buehler, Lake Bluff, IL, USA) and sectioned (transversally), thus producing 8 circular buccal enamel specimens and 8 root dentin specimens, all measuring 6 mm in diameter. The specimens were fixed in a clamping apparatus to hold them in such a way that the flat surface was positioned parallel to the horizontal plane. The 16 specimens had their surface wet ground and flattened with 400-grit abrasive paper in a polishing machine (DP-9U2, Panambra, Strues, A/S, Denmark) to obtain 2-mm-thick cylindrical specimens.

All specimens were individually stored in 100% relative humidity at 37°C for 24 hours, polished with 600-, 800- and 1200-grit aluminum oxide abrasive paper and kept in an ultrasonic bath (Sonic Clean, D.M.C. Equipamentos Ltda., São Carlos, SP, Brazil) in distilled deionized water for 10 minutes to remove polishing debris.

The specimens were gently dried with absorbent paper for 3s to maintain a surface condition similar to that of a clinical situation.[14] The color of the specimens was measured after blotting, according to CIELAB color space relative to the CIE standard illuminant D65 over a black background on a reflection spectrophotometer (Color guide 45/0, PCB 6807; BYK-Gardner, GmbH Gerestsried, Germany). A black background corresponds to the clinical condition of a class III or IV restoration, where the filling has the dark background of the oral cavity rather than a light-reflecting tooth substance. [18] Two measurements were made for each specimen: the first at baseline and the second after the smoking simulation. Color difference was calculated based on CIELAB as $\Delta E_{ab}* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{½}$. A value of $\Delta E*$ of up to 3.3 was considered clinically acceptable in the present study.[19]

Like color change, Knoop microhardness of the composite resin, enamel and root dentin surfaces was measured before and after smoking simulation using a microhardness tester (HMV-2000; Shimadzu Corporation, Kyoto, Japan; 50 g load, 30 seconds).[20] The specimens were individually fixed in the device in such a way that the test surface was perpendicular to micro-indenter tip. Three indentations equally spaced over a circle and not closer than 1 mm to the adjacent indentations or the margin of the specimen were taken and the average was calculated.

Smoking was simulated by using a smoking machine developed by Dinelli and others[12] at the Precision Workshop of the School of Dentistry of Araraquara, São Paulo State University, Brazil (Figure 2). This machine allows impregnation of the tooth structures and restorative materials by the pigments contained in cigarette smoke in order to simulate *in vitro* the clinical conditions of smokers' oral cavity. The smoking machine works by aspiration, directing the cigarette smoke through the cigarette holders in such a way that the pigments from the circulating smoke are deposited the on the specimen. This cycle is programmed within a time interval that simulates a smoker's usual smoke inhaling with duration of two seconds. A timer is set to allow the aspiration of environmental air every 12 seconds in order to simulate exhaustion and subsequent smoke elimination.

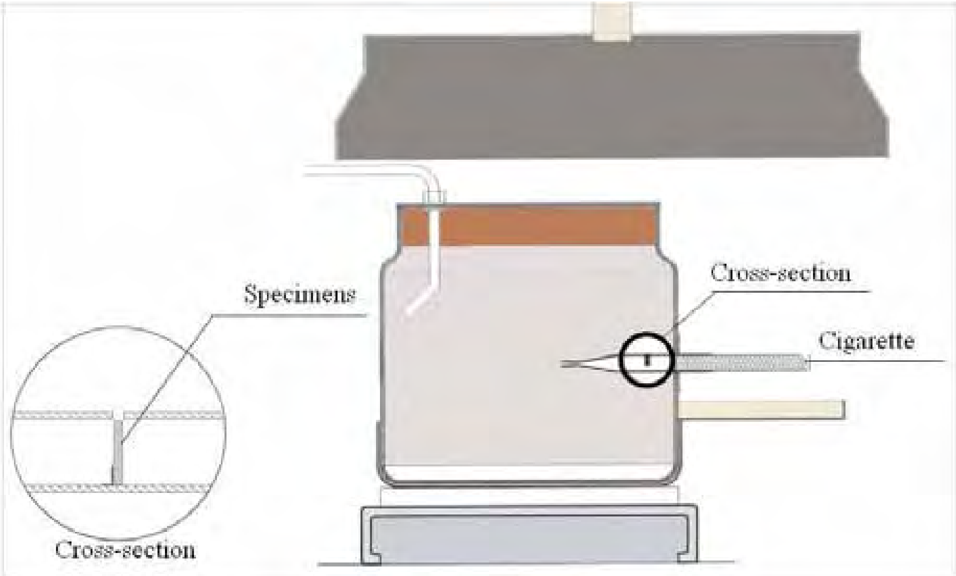

Figure 2. Cross-sectional view of the smoking machine, showing a specimen positioned and a cigarette in the holder.

For each specimen, simulated smoking of 10 cigarettes/day of the commercial brand Marlboro (Philip Morris Brazil Ind. e Com. Ltda, Santa Cruz do Sul, RS, Brazil) was standardized during a period of 8 days. Between the smoking simulations, the specimens were placed in individual vials with 2 mL of artificial saliva at 37ºC. Every 24 hours, the specimens were washed with 250 mL of distilled water and immersed in fresh artificial saliva to avoid sedimentation.

Data collected from the color change and microhardness tests were analyzed for homogeneity and normality and were subjected to ANOVA and Tukey's test at a 5% significance level ($\alpha = 0.05$).

RESULTS

Color Change Analysis

Data analysis revealed statistically significant difference ($p<0.05$) between the measurements at baseline and after exposure to cigarette smoke. Table 1 displays the means and standard deviations of color change. The composite resin and enamel presented statistically significant less darkening and color alteration than dentin.

Table 1. ΔE means and standard deviation obtained for the composite and dental tissues.

	Color change (ΔE)
Composite Resin	6.12* (± 1.18)
Enamel	6.69* (± 3.05)
Dentin	28.60*[a] (± 3.80)

* Indicates unacceptable color change values ($\Delta E^* \geq 3.3$).
[a] Statistically significant difference at 5%.

Microhardness Analysis

Table 2 exhibits the Knoop microhardness means (KHN) (and standard deviations) and statistical comparisons. Analysis of variance showed significant differences between the microhardness means at baseline and after exposure to cigarette smoking ($p<0.05$). Enamel presented a statistically significant increase of microhardness while dentin and composite resin showed significant decrease of microhardness.

Table 2. Knoop microhardness (KHN) means and standard deviation obtained for the composite and dental tissues.

	Initial (baseline)	Final (after cigarette smoke exposure)
Composite Resin	76.57 (± 7.76)a	59.74 (± 7.12)b
Enamel	229.63 (± 73.33)A	302.13 (± 85.35)B
Dentin	41.89 (± 6.87)^{a}	34.04 (± 9.36)^{b}

* Different letters indicate statistically significant difference at 5%.

DISCUSSION

The CIE $L^*a^*b^*$ color system utilized in this study represents a uniform color space with equal distances corresponding to equally perceived color differences.[13] In this three-dimensional color space, the three axes are L^*, a^* and b^*. The L^* value is a measure of the lightness of an object and is quantified on a scale in which perfect black has a zero L^* value and a perfect reflecting diffuser has a 100 L^* value. The a^* and b^* values are on the chromatic scale and correspond to red as $+a^*$ and to green as $-a^*$. Yellow corresponds to $+b^*$ and blue to $-b^*$.[21] Several authors have reported that ΔE^* values in the range of 2-3 are just perceptible and that ΔE^* of 3.3 is the critical value for visual perception.[21] In the present study, exposure to cigarette smoke promoted a remarkable staining of the composite resin and tooth structures, resulting in a clinically unacceptable color change (ΔE between 6.12 and 28.60).

Discoloration of restorations and teeth are very common in heavy smokers and may interfere with esthetics. The susceptibility to staining of a composite may be attributed to its organic matrix or filler type. Filtek Supreme is a nano-hybrid composite with primary 20-nm silica filler and loosely bonded cluster zirconium/silica particle size ranging from 0.6 to 1.4 µm.[7] The resin system consists of three major components: Bis-GMA, UDMA and Bis-EMA. According to the manufacturer, most part of TEGDMA, a somewhat hydrophilic monomer, has been replaced with a blend of UDMA and Bis-EMA, which provides better hydrophobia and lesser atmospheric humidity changes.

The susceptibility of tooth structure to staining may be attributed to defects on enamel and dentin surface[22], because the pigments that penetrate into the dentinal tubules incorporate to their organic contents.[22] These results corroborate those of Raptis and others[10] and Roulet-Mehrens and Roulet[11], who reported that cigarette smoke increases staining. Vogel[22] reported that the color and distribution of the stain is dependent on the type, quantity and length of exposure.

Possible causes for the staining of enamel and dentin include pigmented foods or drinks, chlorhexidine therapy, caries, presence of metals, such as iron, and high temperature.[3-9,23-25] In addition, the teeth of smokers tend to develop tobacco yellow, brown, dark brown or even black stains.[26] Thousands of chemical substances and gases are found in tobacco smoke, among which ammonia, arsenic hydrogen cyanide, lead, nicotine, pesticides, radioactive polonium and other.[27,28] It is possible that many of the substances that stain the teeth and restorations of smokers might have caused the changes in microhardness observed in the present study.

In this study, exposure to cigarette smoke increased microhardness of enamel (Table 2). Zhou and Hsiung[29] reported that the surface roughness could also interfere with this measurement, but in the very early stage because the uniform alignment of enamel prisms is more important than the relative orientation between prism axis and the indenter.[29] It is assumed that this fact may have occurred due to a possible temperature rise on enamel, which, in turn, might have altered the space disposition of hydroxyapatite crystals within the enamel or even modified them. Palamara and others[25] reported that heat is able to modify the crystal morphology.

On the other hand, there was a decrease in dentin microhardness, which might have been due to possible interferences of the heat and chemical reaction originating from the cigarette smoke. Dentin is composed of tubules of about 0.8-2.5-µm inner diameter, filled with odontoblast cytoplasmatic fluid[30] and the tubule lumens are delimited by peritubular dentin, which is more mineralized than the intertubular dentin, which contains only 30% in volume of mineralized collagen[30]. It is possible that the heat of cigarette smoke caused either the collapse or structural loss of the organic portion of the substrate, weakening the tooth structure, or perhaps structural alteration in the inorganic portion of dentin. Therefore, it may be speculated that dentin morphology and composition favored the occurrence of this phenomenon.

Cigarette smoke and other pigments in foods and beverages may produce alterations on the surface and subsurface of the teeth and restorative materials, possibly leading to changes in their physical properties[8,15,31]. However, due to the differences in the physicochemical properties of teeth compared to restorative materials, staining of dental tissues and dental materials occur in different ways and the alterations in their properties are also distinct. Further research is required to clarify the mechanism of these processes, and mainly to verify the actual influence of heat on tooth structure and dental materials on a long-term basis and under clinical conditions.

CONCLUSIONS

Within the limitations of this study, the following conclusions may be drawn:

(1) Cigarette smoke influenced the color change of all specimens, the bovine root dentin being more significantly affected than bovine enamel and composite resin;

(2) Cigarette smoke increased the microhardness of bovine enamel and decreased the microhardness of composite resin and bovine root dentin.

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3.2. Capítulo 2 – Estudo *in vitro*

Detection of lead and cadmium released by cigarette smoke in dental structures and resin composite

Artigo submetido à publicação no periódico Journal Public Health Dentistry.

Brief Communication

Detection of lead and cadmium released by cigarette smoke in dental structures and resin composite

Detection of lead and cadmium in dental structures and resin composite

Abstract

The aim of this study was to investigate *in vitro* the presence of toxicant elements deriving from cigarette smoke in the resin composite, dentine and dental enamel. 8 specimens of resin composite, 8 of dentine and 8 of enamel of bovine teeth were made and exposed to the cigarette smoke. After the simulation of the tobacco the specimens were examined with scanning electron microscopy (SEM) and the energy dispersive X-ray analysis (EDS). The microanalysis identified the presence of cadmium, arsenic and lead in the different specimens.

Key Words: smoking, lead, cadmium, resin composite.

Introduction

The smoking habit is certainly a problem of public health. It is estimated that one-third of the adult population on the planet today, number which corresponds to 1 billion and 200, are smokers, according to the Word Health Organization (1). Smoking is the main avoidable cause of death all over the world, once the total amount of deaths due to cigarette usage surpasses 4 million victims annually, that corresponds to 10 thousand deaths a day.

These are alarming data, since it is well-known that the smoking habit is a risk factor for several diseases. That means that a smoker has more chances of contracting several diseases than a non-smoker. When a person inhales the smoke of a cigarette, a mixture of gases and particles stemming from burning tobacco together with thousands of toxic substances, among these, several carcinogenic ones are also being inhaled (2).

Many of these substances proceed from tobacco planting or are produced during its burning. In such a way that, in cigarette smoke, ammonia, lead, formaldehyde, terebentine, arsenics, cetonics, radioactive polonium, carbon monoxide, among many others (3). Smoking can cause tumors not only in the lungs, but also in the mouth, larynx, pharynx, esophagus, pancreas, kidneys and bladder (3).

There are many studies in literature which evaluate smoking and its consequences, rare, however, are those which concentrate its analyses on the detection of the harmful elements to teeth or to restorative materials. In this context, the purpose of the present study was to investigate *in vitro* the presence of toxic substances stemming from cigarette smoke in the resin composite, dentin and dental enamel.

Methods

Preparation of specimens

Eight cast cylinder specimens of nanocomposite resin (Filtek Supreme -3M/ESPE) were made having 6 mm in diameter x 2mm in depth. Resin was inserted into only one increment in the matrix aperture of circular metal; next, it was covered with polyester strip and photopolymerized with halogenous light for 20 seconds, in accordance with manufacturer's instructions.

The enamel and dentine specimens were obtained from bovine incisors, that were coupled to a precision sectioning instrument (Isomet, Buehler, Lake Bluff, IL, USA), so that 8 circular fragments of dental enamel from the vestibular surface and 8 dentine fragments of 6 mm in diameter each were obtained. The 16 fragment surfaces were planified with a sanding machine (DP9 U2, Panambra/Struers, A/S) using sandpaper of granulation 400 under constant refrigeration, in order that cast cylinder specimens 2.0 mm thick were obtained.

Afterwards, the specimens were stored for 24 hours at 37ºC with 100% relative humidity, to allow the polishing procedures with aluminum oxide abrasive sandpaper of granulation 600, 800 and 1200 to be performed.

Simulation of the act of smoking

The smoking machine was developed by Dinelli *et al*. (4) (1996) and permits impregnation of pigments contained in cigarette smoke in the specimens of dental structures and restoration materials, in order to reproduce *in vitro* the smokers mouth cavity conditions. The machine works as it aspires and conducts smoke through glass cannulas aiming to allow it to circulate and deposit the chemical products on the specimen. This cycle is programmed within a span of time that simulates smoke aspiration normally performed by a smoker with the duration of two seconds. The temporizer permits the ambient air to be inhaled every 12 seconds, simulating smoke exhaustion and afterwards, smoke elimination.

It was standardized the use of one pack of Marlboro (Philip Morris Brazil Ind. e Com. Ltda., Santa Cruz do Sul, RS, Brazil) cigarettes a day for each specimen during the period of 8 days. In the interval between one simulation and the other the specimens were kept in artificial saliva at a constant temperature of 37ºC. Every 24 hours the specimens were washed with 250 ml of distilled water and re-immersed in fresh solution of artificial saliva, to prevent sedimentation.

After simulation of the act of smoking, the samples were prepared for analysis with Scanning Electron Microscopy (SEM) and microanalysis (EDS).

SEM and Microanalysis

Samples were first sectioned in the middle and then immerged in a solution of glutaraldeyd (2.5%) tamponated with sodium cacodylate (0.1 M) with a pH of 7.4, for twelve hours at 4ºC. Next, they were washed with 10 ml of sodium cacodylate buffer (0.1 M) with a pH of 7.4, for one hour, and again washed with distilled water for one minute. Dehydration was made in an increasing series of ethanol 25, 50 and 75% for 20 minutes each, 95% for 30 minutes and 100% for 60 minutes. Samples were fixed in stubs with the treated surfaces turned to the superior face using appropriate tape and metallized with gold. It was used the Scanning Electron Microscope Philips, model XL30FEG Philips, with a resolution of 2nm to 30KV, to which it was coupled an energy dispersive x-ray detector (EDS, Oxford, mod. LINK ISIS 300, operated with a resolution of 65eV) equipment, that allows to determine which chemical elements are present at the selected place and identify which mineral is being observed. The lower diameter of the bundle permits the determination of the mineral composition in small -size samples (<5μm), thus allowing almost punctual analysis.

Statistical analysis

Photomicrographic analysis in SEM and microanalysis were qualitative.

Results

SEM and Microanalysis

In the photomicrographic analysis in SEM, no morphological alterations were found; however, with the help of microanalysis it was possible to observe the presence of chemical elements in the dental structure and resin composite proceeding from cigarette smoke, elements such as cadmium, arsenic and lead (Figure 1: A, B and C).

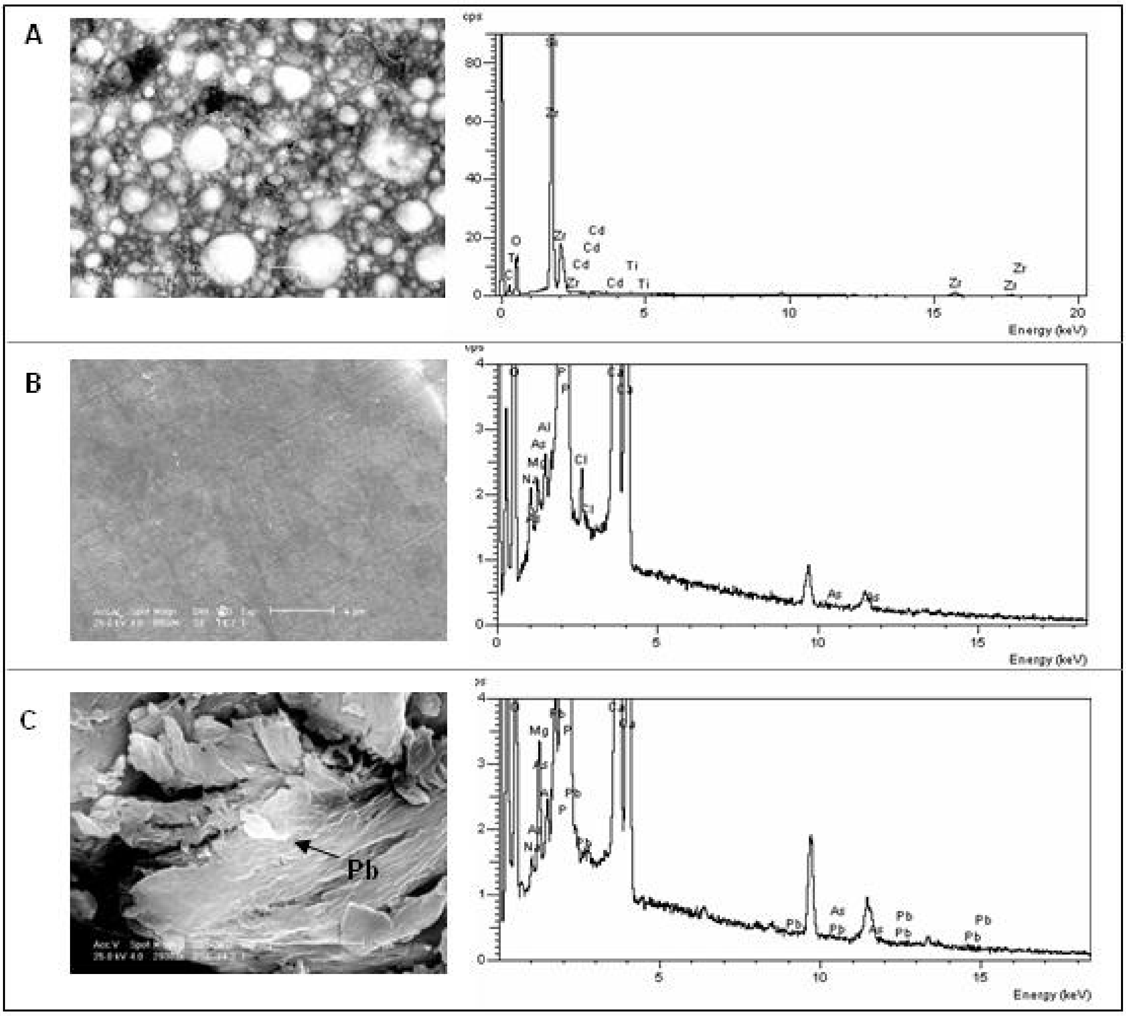

Figure 1 – Materials analyzed in SEM and its respective microanalysis. No morphological alterations were observed in the evaluated material. A) *Resin composite:* microanalysis evincing presence of heavy metal cadmium. B) *Enamel:* microanalysis evincing presence of arsenics. C) *Dentine:* microanalysis evincing presence of heavy metal lead.

Discussion

In the microanalysis made in this short duration study it could be detected the presence of heavy metals and a semi-metal resulting from cigarette smoke. These results reinforce Malara et al. (5), who also observed the presence of two heavy metals, lead and cadmium in deciduous teeth of children who live together with smokers.

The smoke of one cigarette contains an average of 1-2µg of cadmium, whose inhalation is estimated to be 0.1-0.2 µg/cigarette (2). Cadmium, as well as other heavy metals, is not naturally found in the human organism, thus, any existing concentration can be hazardous to health. This element functions as a cancerous agent, teratogenic, and besides, it can harm the reproductive system.

Besides cadmium, a significant amount of lead was found. The concentration of lead in a cigarette can vary from 3-12 µg (2). Transfer of this element to smoke is of about 2%, which means daily inhalation of 1.2-4.8 µg for a person who smokes 20 cigarettes per day (2). Lead toxic effects can occur as time passes if urgent corrective steps are not taken. That element can cause neurologic disturbances such as headaches, seizures, deliriums and muscular tremors well as gastrointestinal and renal disturbances.

Therefore, results indicate the need for further studies to determine the actual effects of those elements in dental structures and in the human body in the long term. From the clinical point of view, it is our opinion that it is the duty of the dental surgeon, as well as of all professional of the health area to perform their duties as health agents to guide their patients as to the infinite damage provoked by cigarette and stimulates them to reduce or even stop the habit of smoking.

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3.3. Capítulo 3 – Estudo *in situ*

***In situ* evaluation of microhardness and surface roughness of composite resins in smokers**

In situ evaluation of microhardness and surface roughness of composite resins in smokers

ABSTRACT

Objectives. The aim of this *in situ* study was to investigate the influence of cigarette smoke on the microhardness and the roughness of composite resins associated with brushing.

Methods. A total of 132 disc-shaped specimens (6 x 2 mm) were fabricated with Filtek Z250, Charisma and Filtek Supreme (n=22), polished and attached to removable palatal appliance. The appliance had three individual retentions on either side of the midline, which accommodated one specimen of each group facing into the oral cavity. Twenty-two volunteers took part in this study (11 smokers e 11 non-smokers) and received instructions to wear the appliances continuously by 28 days, but to remove them during meals (3 times a day). For brushing of the specimens, the appliance was taken out the mouth and one side (right or left, as determined by randomization) three times a daily was brushed. For the smokers, it was standardized consume of 10 cigarettes for day. The microhardness data were analyzed by three-way ANOVA and the means were compared by Fisher's LSD test. The roughness data were analyzed by using Kruskal-Wallis, Wilcoxon and Friedman (p<0.05).

Results. The cigarette smoke increased the values of microhardness for the smokers with brushing and any influence in the roughness values.

Significance. The cigarette smoke associated with brushing proportioned better mechanical properties in relation to microhardness, yet it affects negatively the surface smoothness of composite resin.

Keywords: In situ models, Composite resins, Cigarette smoke, Microhardness, Roughness

1. Introduction

Ideally, *in situ* models, should serve as bridges between the natural uncontrolled clinical conditions and the highly controlled laboratory conditions [1,2]. Therefore, *in situ* studies can be employed to evaluate some of the factors present in the oral environment that are not accurately evaluated in *in vitro* studies and can be used as a predictive method to evaluate clinical situations [3]. In addition, they have the advantage of exposing restorative materials to saliva, pH changes, foods, beverages, stains and thermal variances within the oral environment [1,4], under clinically controlled conditions, however, without causing any damage to the study subjects [5]. This methodology is also suitable for evaluation of other phenomena that occur in the oral cavity, such as: influence the dentifrices [5-7], effect caused by soft drinks [8], effect of at-home bleaching [9] and influence of oral environmental on composite resin properties [10].

In situ studies can evaluate the composite resins properties in individuals with harmful habits, as cigarette and to correlate with health patients. This type of the study allows to verify if the heat allied to the substances originating from the cigarette smoke might cause a physicochemical alteration on the composite surface and possibly interfere directly with the quality, longevity and clinical performance of the composite resin, not only with respect to its esthetics, but also regarding its resistance to degradation in the oral environment.

Therefore, the aim of this study was to investigate *in situ* the influence of cigarette smoke on the microhardness and the roughness of composite resins associated with brushing.

2. Materials and methods

Ethical Aspects and Experimental Design

The research proposal was reviewed by the Ethics in Research Committee of the School of Dentistry of Ribeirão Preto (University of São Paulo, Brazil) and the study design was approved.

The study population consisted of 22 periodontally healthy volunteers, over 21 years of age, with absence of signs of the disease caries and not using any medication, comprising 11 smokers (7 females and 4 males) and 11 non-smokers (7 females and 4 males). All participants were fully informed about the study purposes and signed a written informed consent form, in addition to receiving oral hygiene instructions, scaling and root planning, and jet prophylaxis with sodium bicarbonate particle stream prior to the beginning of the study.

The following factors were studied: cigarette/brushing in four levels (GI - smokers/with brushing, GII - smokers/without brushing, GIII - non-smokers/with brushing and GIV - non-smokers/without brushing); composite resin in three levels (Filtek Z250 - Z, Charisma - C and Filtek Supreme - S); and time in two levels (before and after 28 days of intraoral exposure). The response variables were microhardness and surface roughness, which were repeatedly measured on the same experimental units over time.

Preparation of the Specimens

Two conventional micro-hybrid composite resins (Filtek Z250 and Charisma) and one nano-hybrid composite resin (Filtek Supreme) were used. The materials' main composition, shade, batch number and manufacturer are listed in Table 1.

Table 1 – Composite Resins investigated in this study

Brand name	Main Composition	Shade	Batch number	Manufacturer
Filtek Z250 (Z)	Combination of aggregated Zirconia/silica cluster filler, bisGMA, UDMA and Bis-EMA	A1	5UR	3M ESPE, St. Paul, MN, USA
Charisma (C)	Barium aluminum fluoride glass, Bis-GMA, TEGDMA	A1	010203	Heraeus Kulzer GmbH, Wehrheim/TS, Germany
Filtek Supreme (S)	Combination of aggregated Zirconia/silica cluster filler, bisGMA, UDMA and Bis-EMA	WB	5BA	3M ESPE, St. Paul, MN, USA

bisGMA: bisphenol A diglycidil ether dimethacrylate; UDMA: urethane dimethacrylate; bis-EMA: bisphenol A polyethylene glycol diether dimethacrylate; TEGDMA: triethylene glycol dimethacrylate.

Forty-four specimens of each composite resin (6 mm de diameter and 2 mm thickness) were fabricated using a metal circular matrix. The composite resin was inserted into the matrix in a single increment using a spatula. Thereafter, the matrix was covered with a polyester strip and a glass coverslip was placed over this set under a load of 0.5 kg in order to compact the resin and avoid bubble formation. After 30 seconds, the load and the coverslip were removed. The composite resin was polymerized through the polyester strip for 20 seconds, according to the manufacturers' instructions, using a halogen light-curing unit (Jetlite 4000, J. Morita, Japan) with light output of 1050 mW/cm^2 as measured with the curing radiometer coupled to the device.

Next, the specimens were stored in 100% de relative humidity at 37ºC for 24 hours. After this period, the surfaces were polished with 2000-grit aluminum oxide abrasive paper and 0.3-µm alumina paste for 30 seconds each.

The specimens were then kept in an ultrasonic bath (Sonic Clean, D.M.C. Equipamentos Ltda., São Carlos, SP, Brazil) with distilled deionized water for 10 minutes to remove polishing debris.

Fabrication of the Intraoral Appliances

Upper and lower dental arch impressions were made with alginate (Jeltrate Chromatic; Dentsply/Caulk, Milford, DE, USA) and poured with stone plaster gesso (Herodent, Vigodent Ind e Com., Rio de Janeiro, RJ, Brazil). For each volunteer, an intraoral removable palatal appliance was constructed in acrylic resin, half colorless and half with glitter (Glitter Ind e Com Ltda, São Paulo SP, Brazil). The appliances had three individual retentions on either side of the midline, which accommodated one specimen of each composite resin facing the oral cavity. The specimens were randomly attached to the appliances with carving wax. Care was taken to guarantee that no wax would cover the specimens.

Intraoral Phases

All volunteers were instructed to use the intraoral palatal appliance continuously during 28 consecutive days, removing only during meals and toothbrushing. Each volunteer received a kit containing: 1 dentifrice (Colgate MFP - Colgate-Palmolive; Divisão da Kolynos do Brasil Ltda., Osasco, SP, Brazil), 1 toothbrush (Oral B Indicator 38 - Gillette do Brasil Ltda., Manaus, AM, Brazil), 1 plastic case to keep the appliance, sterile gauze and written instructions. For brushing of the specimens, the appliance was taken out of the mouth. A pea size amount of dentifrice was applied onto the toothbrush bristles and 40 strokes (back and forth) [11,12] were performed only on the side of the appliance with glitter, three times daily. The rationale for choosing the appliance's half with glitter was to better distinguish the side brushed by the volunteers. The participants were instructed to keep the appliance wrapped in moist gauze inside the case whenever the appliance was taken from the mouth.

The smoker volunteers were instructed to smoke as most as 10 cigarettes/day of the commercial brand Marlboro (Philip Morris Brazil Ind. e Com. Ltda., Santa Cruz do Sul, RS, Brazil). After 28 days of intraoral exposure, the specimens were retrieved from the appliances by carefully removing the wax with a plastic instrument and were ultrasonically cleaned for 5 minutes.

Surface Microhardness Analysis

Knoop microhardness on composite resin surface was measured using a microhardness tester (HMV-2000; Shimadzu Corporation, Kyoto, Japan; 50 g load, 30 seconds). The specimens were individually fixed in the device in such a way that the composite resin test surface was perpendicular to micro-indenter tip. Three indentations equally spaced over a circle and not closer than 1 mm to the adjacent indentations or the margin of the specimen were taken and the average was calculated.

Surface Roughness Analysis

Surface roughness was assessed by using a contact profilometer (Surfcorder SE 1700; Kosaka Corp., Tokyo, Japan) equipped with a 2-µm-radius diamond stylus, which traversed the surface at a constant speed of 0.05 mm/s with a force of 0.7 m*N*. Each specimen was individually fixed in a clamping apparatus and scanned in three locations. Three additional measurements were accomplished by rotating the specimen 90º and the average (*Ra*) was obtained from the six values. The scans were 1.25 mm in length, and the data were filtered with a cutoff of 0.08 mm.

Statistical Analysis

Sample distribution and homogeneity were analyzed. Since a normal, homogeneous distribution was observed for microhardness data were submitted to three-way ANOVA parametric test. Multiple comparisons were done by Fisher’s LSD test at 0.05 significance level. The roughness data were found not to be normally distributed, so non-parametric statistics were used for analysis. All sets of data were subjected to a Kruskal-Wallis, Wilcoxon and Friedman test were used to identify statistically significance differences to a 95% confidence level.

3. Results

Microhardness

Microhardness means expressed in KHN (± standard deviations) (Figure 1). For the factor smokers/brushing, GI-smokers/brushing (81.70 ± 15.64) presented statistically significant higher microhardness (p<0.05) than the other groups, followed by GII-smokers/without brushing (75.36 ± 13.33), GIII-non-smokers/with brushing (69.00 ± 13.11) and GIV-non-smokers/without brushing (65.05 ± 11.88), all these groups differing significantly to each other (p<0.05).

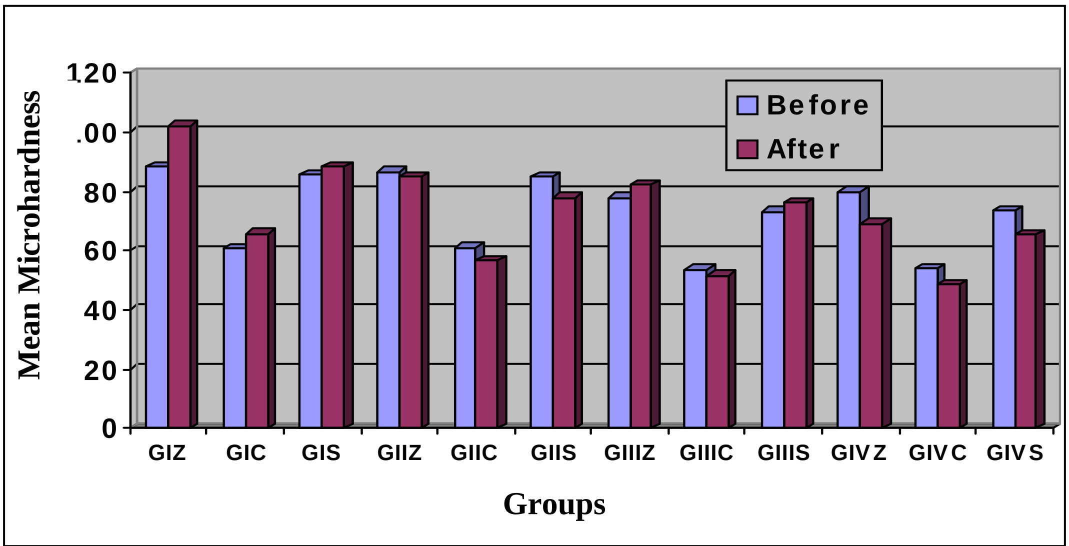

Figure 1. Mean microhardness (KHN) of composite resins

For the factor composite resin, there was statistically significant difference (p<0.05) among all materials, Z250 (Z) (83.88 ± 1.02) presenting significantly higher microhardness than Supreme (S) (78.07 ± 1.70) and Charisma (C) (56.38 ± 1.02). For the factor time, there was statistically significant difference (p<0.05) between microhardness means recorded before (73.23 ± 5.36) and after 28 days of intraoral exposure (72.32 ± 10.05).

Analyzing the interaction composite resin x time, there was statistically significant difference (*p*<0.05) among the materials, Z250 exhibiting significantly higher means for both time points. Analyzing the interaction smokers/brushing as a function of time, it was observed that only GI (smokers/with brushing) presented a statistically significant (*p*<0.05) increase in microhardness after 28 days of exposure to the oral environment (Table 2). For GII (smokers/without brushing) and GIV (non-smokers/without brushing), there was a statistically significant difference decrease (*p*<0.05) in final microhardness.

Table 2. Microhardness means expressed in KHN (± standard deviations) for the interaction smokers/brushing as a function of time.

Groups	Before	After	Regardless of time
GI - Smokers/with brushing	78.14(±15.27)b	85.26(±18.43)a	81.70(± 15.64)A
GII - Smokers/without brushing	77.57(±14.51)b	73.15(±14.79)c	75.36(± 13.33)B
GIII - Non-Smokers/with brushing	68.10(±12.72)c	69.90(±16.29)c	69.00(± 13.11)C
GIV - Non-Smokers/without brushing	69.12(±13.48)c	60.97(±11.02)d	65.05(± 11.88)D

Different letters indicate statistically significant difference. Critical value = 1.96.

Surface Roughness

Roughness data (median values) in expressed in µm (Figure 2). The ranking for surface roughness from most to lest were as follows: GI-smokers/with brushing (0.09) = GIII-non-smokers/with brushing (0.09) > GII- smokers/without brushing (0.08) = GIV-non-smokers/without brushing (0.07).

For the factor composite resin, the Z250 (0.09) presenting significantly higher roughness than Supreme (0.07) and Charisma (0.07) (p<0.05), which did not differ from one another. For the factor time, there was statistically significant difference (*p*<0.05) between median recorded before (0.07) and after 28 days of intraoral exposure (0.08).

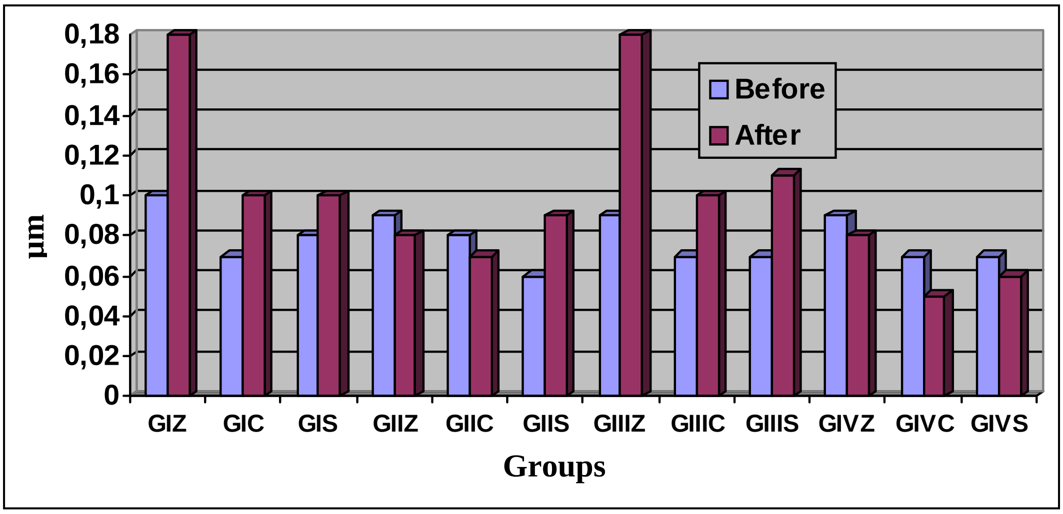

Figure 2. Medians Surface roughness (µm) of composite resins

Analyzing the interaction smokers/brushing as a function of time, it was observed that GI (smokers/with brushing) and GIII (non-smokers/with brushing) presenting significantly higher roughness after 28 days of exposure to the oral environment (Table 3). For GII (smokers/without brushing) and GIV (non-smokers/without brushing), no statistically significant difference was observed among them.

Table 3. Roughness medians expressed in µm for the interaction smokers/brushing as a function of time.

Group	Before	After	Regardless of time
GI - Smokers/with brushing	0.08b	0.13a	0.10A
GII - Smokers/without brushing	0.08b	0.08b	0.08B
GIII - Non-Smokers/with brushing	0.08b	0.13a	0.11A
GIV - Non-Smokers/without brushing	0.08b	0.06b	0.07B

Different letters indicate statistically significant difference.

4. Discussion

The intraoral models are considered superior to *in vitro* investigations since specimens are exposed to oral conditions [13]. In this study, the intraoral model used permitted to assess, in more controlled way the effects of cigarette smoke, saliva and brush abrasion on the microhardness and surface roughness of resin composites.

The findings of the conducted research showed that the cigarette smoke influenced positively the microhardness values of resin composites do GI (smokers/with brushing). In the literature, none study assessed the influence of cigarette smoke in the properties in dental materials. Nevertheless, investigations [14,15] reported the resin matrix is susceptible to thermal changes could alter their mechanical properties. Therefore, it may be speculate that, in this *in situ* experiment that the obtained result can be due to a thermal alteration caused by heat from cigarette smoke. Michailesco [16] observed temperature change (36.9 to 48.3˚C) corresponded to smoking cigarette on surface of silver amalgam *in vivo* study. However, previous studies [16,17] demonstrate big range of intra-oral temperatures that occur on dental restorative and tooth surfaces at various sites in the oral cavity.

In the present study, the specimens were random fixed in palatal intra-oral appliance, making the contact of composite resins with the cigarette smoke more possible, suffering more temperature variation. The combustion that happens in the cigarette extremity, promotes a temperature increase, creating cinder (maximum of 950ºC during a puff).[35] The heat temperature in the composite resin can increase the degree of conversion as well as alter the physical properties [18,19].

From the data presented, it can be observed that the Z250 was superior to Supreme, followed by Charisma. This difference the microhardness between composites may be attributed to its inorganic filler content or filler type [20]. Previous studies shown are a positive correlation between the hardness and inorganic filler content [20] and harder filler particles of composites [21]. Z250 is a micro-hybrid resin composite with 60 vol% inorganic filler and the high microhardness value may be related to the presence, distribution and size of the particles of zirconia/silica (0.01 to 3.5 µm). Whereas the Supreme is a nanocomposite with 59.5 vol% inorganic filler and a primary 20-nm silica filler and loosely bonded cluster zirconia/silica particle size ranging from 0.6 to 1.4 µm [22]. Charisma probably has a lower microhardness the all resin composites due to absence the zirconia and silica despite to have the highest inorganic filler content (64 vol%).

With regard to the interaction between the two factors (smokers/brushing and time) the only for GI (smokers/with brushing) occurred increase significant the microhardness. This result demonstrated that when the smoking group brushed the microhardness was increased. This fact may be occurred because the composite resins are susceptible to softening caused by organic acid produced in dental plaque [23] and the brushing procedure can remove the softer surface because this softened layer is vulnerable to mechanical forces, resulting in exposure of a harder composite surface.

For GIII (non-smokers/with brushing) the brushing avoided the decrease of microhardness because the abrasion could also eliminate the softer surface and keep the microhardness value [4]. On the other hand, for the GII (smokers/without brushing) and GIV (non-smokers/without brushing) the microhardness values decreased as time passed probably because of the softness caused by dental plaque. The other factor that could have influenced is saliva: studies have investigated the process of biodegradation on resin composites in the presence of salivary enzymes [24,25], which have been associated with promoting biodegradation of surface [26], leading to softening [27]. Human saliva is a complex mixture of substances whose major constituent are proteins which include immunoglobulins, statherins, acidic proline rich proteins and enzymes like esterases [26,28]. Lee et al. [22] reported which enzymes in human saliva are capable of softening the surface of dimethacrylate polymers, probably by inducing a hydrolysis of methacrylate ester bonds.

Beyond the microhardness other important characteristic of dental restorative materials is surface roughness. Materials with roughened surfaces enhance bacterial adhesion, having a smaller free surface energy, could influence biofilm adherence, periodontal disease, recurrent decay and staining of the composite resin [29-31]. In the present study, the results showed that in smoking and non-smoking groups that brushed, the composite resins surface roughness increased. These results corroborate with *in vitro* work of [29, 32] that reported the roughness increase on the surface specimens after toothbrush and dentifrice abrasion.

With regard to the composite resin the roughness of Z250 was superior to Supreme and Charisma that were similar. Several studies [33-35] shown that dental material submitted to abrasion may have its roughness increased or reduced, depending on the proportion organic matrix/inorganic fillers, particle size in its composition, their size and content of filler particles, degree of conversion of the polymer matrix and silane coupler. Heath and Wilson [11] reported that roughness of composites also may be influenced by different reasons, such as the amplitude of the traveled course of the toothbrush, weight applied on the toothbrush head, dentifrice abrading degree and temperature. Nevertheless, it is important to emphasize that in this study, the same brand of dentifrice and toothbrush were employed by all the volunteers. For the time factor had difference between before and after because the brushing abrasion that it got to elevate the roughness values.

Thus, it was observed the effect of the cigarette smoke on the mechanical properties of composite resins. Moreover, future *in situ* and *in vivo* studies are needed to determine the effects of cigarette smoke on the other properties of dental restorative materials and on dental structures. Because, this effects of cigarette smoke on composites may differ depending on many others factors found in the oral environment.

5. Conclusions

On the basis of the results of this study, it can be concluded that:

(1) the cigarette smoke/brushing increased the microhardness of composite resins, yet it proportioned smaller surface smoothness, therefore, more roughness;

(2) the groups GII (smokers/without brushing) and GIV (non-smokers/without brushing) which no brushing presented smaller values of microhardness and any influence in the surface roughness.

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3.4. Capítulo 4 – Estudo *in situ*

Avaliação *in situ* da alteração de cor de resinas compostas em fumantes

Resumo: O objetivo deste estudo foi avaliar *in situ* a alteração de cor de resinas compostas frente à exposição à fumaça do cigarro e verificar a influência da escovação. Cento e trinta e dois corpos-de-prova cilíndricos (6 x 2 mm) foram confeccionados com Filtek Z250, Charisma e Filtek Supreme (n=22), polidos e fixados em aparelho intrabucal palatino. No dispositivo foram preparadas 6 cavidades, sendo 3 para cada metade, que recebeu um espécime de cada material. Foram selecionados 22 voluntários, sendo 11 fumantes e 11 não-fumantes, os quais foram instruídos a utilizar o aparelho continuamente por 28 dias consecutivos, retirando-o somente para alimentação e escovação dental. A escovação (Colgate MFP e Escova Indicator/Oral B) dos espécimes foi realizada fora da boca, três vezes por dia somente em uma metade do dispositivo. Para os fumantes foi estipulado o consumo de 10 cigarros/dia. A mensuração da cor foi realizada de acordo com a escala de cor CIELAB. O ΔE_{ab} foi calculado pela equação $\Delta E_{ab}* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{½}$ e o $\Delta L* = L*(t) - L*(0)$. Os dados foram analisados pelo método estatístico não-paramétrico Kruskal-Wallis e Wilcoxon a nível de 5% de significância. Foi observado que os fumantes apresentaram alteração de cor e de luminosidade mais intensa do que os não fumantes e que as resinas compostas escovadas foram diferentes estatisticamente das não escovadas. Pode-se concluir que a fumaça de cigarro aumentou a alteração de cor e de luminosidade das resinas compostas avaliadas e a escovação foi capaz de diminuir essa alteração.

Palavras-chave: *Resinas compostas, manchamento, escovação.*

Abstract: The aim of this *in situ* study was to assess the color change of dental resin composites to exposure by cigarette smoke and evaluated the influence of brushing. A total of 132 disc-shaped specimens (6 x 2 mm) were fabricated with Filtek Z250, Charisma and Filtek Supreme (n=22), polished and attached to removable palatal appliance. The appliance had three individual retentions on either side of the midline, which accommodated one specimen of each group facing into the oral cavity. Twenty-two volunteers took part in this study (11 smokers e 11 non-smokers) and received instructions to wear the appliances continuously by 28 days, but to remove them during meals (3 times a day) For brushing (Colgate MFP and toothbrush Indicator/Oral B) of the specimens, the appliance was taken out the mouth and one side (right or left, as determined by randomization) three times a daily was brushed. For the smokers, it was standardized consume of 10 cigarettes for day. Color was measured according to the CIELAB color scale. ΔE_{ab} after in situ exposure was calculated by the equation $\Delta E_{ab}* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{½}$ and the $\Delta L* = L*(t) - L*(0)$. The data were analyzed by Kruskal-Wallis, Wilcoxon and Friedman ($p<0.05$). The smokers presented change color and lightness more intense than non-smokers and the composite resins brushed were different than did not brush. Concluding that the cigarette smoke increased the ΔE and ΔL of the composite resins tested and the brushing was capable to reduce this alteration.

Keywords: *resin composite, staining, brushing.*

Introdução

O hábito de fumar constitui fator de risco para inúmeras doenças no ser humano, principalmente na cavidade bucal, pois o resultante da combustão pode promover o desenvolvimento e progressão de doença periodontal, câncer bucal, falhas de implante, diminuição ou perda temporária das papilas linguais e manchamento dos dentes e restaurações[10, 13, 17].

A alteração de cor dos materiais restauradores estéticos e estruturas dentais é a manifestação mais visível e imediata encontrada em tabagistas. Essa característica representa um impacto negativo para os fumantes que identificaram a presença de manchas com níveis de pigmentações que variavam de moderado a severo em seus dentes e restaurações provocadas pela fumaça de cigarro e perceberam que isso os desagradavam, tornando-os insatisfeitos com seu próprio sorriso[1].

As pigmentações ou manchas decorrentes da exposição à fumaça de cigarro tendem a apresentar cor castanho-escuro[16]. A severidade e a distribuição do manchamento em resinas compostas são dependentes principalmente da duração e freqüência do hábito[16], além da localização da restauração[15] e da técnica de higiene bucal que dependendo, pode aumentar a susceptibilidade ao manchamento dos materiais estéticos, devido ao efeito do amolecimento da matriz por ácidos orgânicos provenientes do biofilme bacteriano[2].

Neste contexto, o objetivo deste estudo foi avaliar a alteração de cor de resinas compostas frente à exposição à fumaça do cigarro e verificar a influência da escovação num modelo experimental *in situ*.

Material e método

Aspectos éticos e Delineamento Experimental

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia de Ribeirão Preto-USP. Foram selecionados 24 voluntários (sem doença periodontal, com ausência de sinais da doença cárie e não fazendo uso de medicamentos), maiores de 21 anos, sendo 11 fumantes (7 mulheres e 4 homens) e 11 não-fumantes (7 mulheres e 4 homens). Todos os participantes foram informados por escrito sobre os objetivos da pesquisa e assinaram o termo de consentimento, além de receberem instruções de higiene bucal e profilaxia com jato de bicarbonato mais raspagem coronária, antes de iniciarem a pesquisa.

Os fatores examinados foram: fumante em dois níveis (GI - fumante, GII - não-fumante), escovação em dois níveis (a - com escovação e b - sem escovação) e resina composta em três níveis (Z - Filtek Z250, C - Charisma e S - Filtek Supreme).

Preparação dos espécimes

Neste estudo, foram utilizadas duas resinas compostas micro-híbridas e uma nano-híbrida. A composição principal, cor, lote e fabricante estão listados na Tabela 1.

Tabela 1. Resinas compostas investigadas neste estudo

Nome comercial	Composição principal	Cor	Lote	Fabricante
Filtek Z250	combinação de agregados de Zircônia/sílica, bis-GMA, UDMA e bis-EMA	A1	5UR	3M ESPE, St. Paul, MN, USA
Charisma	bis-GMA, TEGDMA	A1	010203	Heraeus Kulzer GmbH, Wehrheim/TS, Germany
Filtek Supreme	combinação de agregados de Zircônia/sílica, bis-GMA, UDMA e bis-EMA	WB	5BA	3M ESPE, St. Paul, MN, USA

bisGMA: bisfenol A doglicidil éter dimetacrilato; UDMA: uretano dimetacrilato; bis-EMA:bisfenol A polietileno glycol dieter dimetacrilato; TEGDMA: trietileno glycol dimetacrilato.

Quarenta e oito corpos-de-prova de cada resina composta (6 mm de diâmetro e 2 mm de espessura) foram confeccionados com matriz circular de metal. A resina composta foi inserida com auxílio de espátula em um único incremento na matriz. Em seguida, o conjunto foi coberto com tira de poliéster e sobre este foi aplicada uma lamínula de vidro e um peso de ½Kg com finalidade de compactar a resina e evitar a formação de possíveis bolhas. Após 30 segundos o peso e a lamínula de vidro foram removidos e a fotopolimerização com luz halógena por 20 segundos foi realizada conforme instruções dos fabricantes. A densidade de potência de 1050mW/cm^2 foi verificada através do radiômetro acoplado ao aparelho fotopolimerizador (Jetlite 4000, J.Morita, Japão).

Em seguida, os espécimes foram armazenados por 24 horas a 37ºC em 100% de umidade relativa. Após esse período foi realizado o polimento das superfícies com lixa d'água de óxido de alumínio de granulação 2000 e alumina 0,3µ por 30 segundos cada. Os espécimes foram colocados em água deionizada em ultrasson (Sonic Clean, D.M.C. Equipamentos Ltda., São Carlos, SP, Brasil) por 10 minutos, com intuito de remover resíduos provenientes do procedimento de polimento.

Confecção do aparelho intrabucal

Moldes do arco superior e inferior foram obtidos com alginato (Jeltrate Chromatic, Dentsply /Caulk, Milford, DE, USA) e vazados com gesso pedra (Herodent, Vigodent Ind e Com., Rio de Janeiro, RJ, Brasil). Cada voluntário recebeu um aparelho intrabucal palatino removível confeccionado com resina acrílica. De um lado (esquerdo) foi mantida a resina acrílica incolor e do outro lado (direito) foi aplicado glitter. Cada aparelho possuía seis cavidades, três no lado direito e três no lado esquerdo, que acomodaram um espécime de cada resina composta.

Fase intrabucal

Todos os voluntários foram instruídos a utilizar o aparelho intrabucal palatino continuamente por 28 dias consecutivos, retirando–o somente para alimentação e escovação dental. Cada voluntário recebeu um kit contendo: 1 dentifrício, 1 escova dental, 1 estojo para guardar o aparelho, gaze estéril e as instruções por escrito. O dentifrício utilizado foi o Colgate MFP (Colgate-Palmolive – Divisão da Kolynos do Brasil Ltda., Osasco, SP, Brasil) de média abrasividade que possui o carbonato de cálcio como agente abrasivo e a escova dental de cerdas macia (Oral B - Gillette do Brasil Ltda., Manaus, AM, Brasil). A escovação dos espécimes foi realizada fora da boca três vezes ao dia. O dentifrício foi aplicado com o tamanho de uma “ervilha” na escova dental e 40 movimentos (ida e volta) foram realizados pelo voluntário somente na metade do aparelho contendo glitter. Quando o aparelho não estivesse na boca este deveria ficar envolto por uma gaze levemente umedecida com água dentro do estojo.

Os voluntários fumantes foram orientados a fumar somente 10 cigarros/dia da marca Marlboro (Philip Morris Brasil Ind. e Com. Ltda., Santa Cruz do Sul, RS, Brasil). Após os 28 dias de exposição intrabucal, os espécimes foram retirados do dispositivo intrabucal e lavados em ultra-som por 5 minutos.

Avaliação da cor

A cor dos espécimes foi medida de acordo com o sistema CIELAB, com iluminação padrão D65 sobre fundo branco com o espectrofotômetro de colorimetria (Color guide 45/0, PCB 6807 BYK-Gardner GmbH Gerestsried, Alemanha,). O espaço CIELAB corresponde a um sistema de coordenadas, onde o valor de L^* é a medida de luminosidade de um objeto e é quantificado com uma escala que varia do preto (zero) ao branco (100). Os valores de a^* e b^* são medidas de cromaticidade, onde a^* designa o eixo verde-vermelho. Isso significa que, quando o valor for negativo mostrará desvio para tonalidade verde e quando for positivo mostrará o desvio no sentido do vermelho. O valor b* denomina o eixo azul-amarelo e quando este apresentar valor negativo o desvio será no sentido da tonalidade azul e quando for positivo mostrará o desvio no sentido do amarelo. Neste estudo, foram realizadas duas leituras em cada espécime, uma antes (baseline) e a outra após 28 dias de exposição *in situ*. A diferença de cor foi obtida pelo cálculo $\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. O valor de ΔE^* de 3,3 foi considerado clinicamente aceitável no presente estudo[5]. A diferença de luminosidade (ΔL^*) também foi calculada pela fórmula $\Delta L^* = L^*(t) - L^*(0)$, onde (t) corresponde ao tempo e (0) corresponde ao *baseline*.

Análise estatística

Os dados foram analisados quanto a sua normalidade e homogeneidade, como não foi possível provar a normalidade de variância das medidas optou-se pelo método estatístico não-paramétrico Kruskal-Wallis e Wilcoxon a nível de 5% de significância.

Resultado

Análise de cor

Na tabela 2 estão dispostas as medianas da diferença de cor (ΔE) dos espécimes, sendo possível observar que o grupo fumante apresentou alteração de cor mais intensa e estatisticamente diferente do grupo não-fumante ($p<0.05$).

Tabela 2. Mediana dos valores de ΔE e ΔL para fumantes e não fumantes		
	Diferença de Cor $(\Delta E)^{+t}$	Diferença de Luminosidade $(\Delta L)^{+t}$
Fumante	0,99*a	-0,44a
Não-fumante	0,58*b	-0,01b

* Valor indicativo ($\Delta E^*<3,3$) mudança de cor aceitável
Letras diferentes indicam diferença estatisticamente significante.

Pode-se observar na tabela 3 que as resinas compostas submetidas à escovação apresentaram menor alteração de cor e diferença estatisticamente significativamente do que as não submetidas à escovação ($p<0.05$).

Tabela 3. Mediana dos valores de ΔE e ΔL dos materiais escovados e não escovados.		
	Diferença de Cor $(\Delta E)^{+t}$	Diferença de Luminosidade $(\Delta L)^{+t}$
Com escovação	0,67* b	0,09 b
Sem escovação	0,90* a	-0,39 a

* Valor indicativo ($\Delta E^*<3,3$) mudança de cor aceitável
Letras diferentes indicam diferença estatisticamente significante.

Para o fator resina composta, não foi observada diferença entre as diversas resinas compostas testadas (Tabela 4).

Tabela 4. Mediana dos valores de ΔE e ΔL para os materiais testados		
	Diferença de Cor $(\Delta E)^{+t}$	Diferença de Luminosidade $(\Delta L)^{+t}$
Filtek Z250 (Z)	0.75*a	- 0.02a
Charisma (C)	0.72*a	- 0.30a
Filtek Supreme (S)	0.90*a	- 0.23a

* Valor indicativo ($\Delta E^*<3,3$) mudança de cor aceitável
Letras diferentes indicam diferença estatisticamente significante.

Análise de luminosidade

Na análise de luminosidade (ΔL) dos espécimes, pode-se observar comportamento semelhante à alteração de cor, ou seja, o grupo fumante apresentou alteração de luminosidade mais intensa e estatisticamente diferente do grupo não-fumante ($p<0.05$) (Tabela 2). Na tabela 3, pode-se observar que as resinas compostas submetidas à escovação apresentaram menor alteração de luminosidade e diferença estatisticamente significativamente das não submetidas à escovação ($p<0.05$). Para o fator resina composta, não foi observada diferença de luminosidade entre as diferentes resinas avaliadas (Tabela 4).

Discussão

As resinas compostas vêm sendo amplamente utilizadas desde sua introdução devido as suas propriedades estéticas[3]. Porém ao mesmo tempo em que a estética é o fator de escolha para seu uso, também pode ser fator preocupante uma vez que a mudança de cor é o maior responsável pela substituição das restaurações de resina composta[8]. Esse fato se agrava principalmente em pessoas fumantes, que estão sujeitos a pigmentos extrínsecos de cor castanho-escuros depositados pelo tabaco[16].

Os resultados deste estudo revelam que as resinas compostas dos fumantes apresentaram maior alteração de cor e de luminosidade do que as dos não fumantes. Esse dado corrobora com vários estudos[2, 6, 12, 14, 18] que reportam aumento do manchamento provocado pela fumaça de cigarro. Esse fato se deve provavelmente a complexa composição da fumaça de cigarro com milhares de substâncias como a nicotina, monóxido de carbono, alcatrão, entre outras[10], que em contato com a superfície da resina composta e subseqüente deposição, seriam responsáveis pela mudança de cor e de luminosidade. Entretanto, apesar das resinas compostas dos fumantes terem apresentado maior manchamento, o ΔE no valor de 0,99 resultou numa mudança de cor clinicamente aceitável (ΔE <3,3)[5], sendo que a severidade do manchamento está diretamente relacionada com o tempo de exposição e a freqüência do hábito[16]. Neste estudo, o tempo de exposição foi de apenas 28 dias. Provavelmente o curto período e a quantidade de cigarros deste estudo não foram suficientes para formação de manchas mais pronunciadas, como reportado por Asmussen e Hansen[2] que verificaram manchamento mais evidente para as restaurações em fumantes (mais de 10 cigarros/dia) no período de 36 meses num estudo *in vivo*.

O modelo *in situ* utilizado neste estudo, de acordo com Zero[19], pode servir como uma "ponte" entre situações clínicas não controladas naturais (*in vivo*) e uma situação laboratorial altamente controlada (*in vitro*). Uma das grandes vantagens desse tipo de modelo é a exposição dos espécimes na boca humana, onde é possível simular a dinâmica da cavidade bucal sob condições clinicamente controladas e principalmente sem trazer prejuízos aos indivíduos. As amostras são fixadas em dispositivos intrabucais removíveis, que permitem ser retirados da boca a qualquer momento facilitando a inserção ou remoção dos espécimes para realização dos testes laboratoriais[11]. O modelo *in situ* pode trazer novas oportunidades de medir e analisar outros mecanismos como o de formação de manchas extrínsecas, pois são importantes por permitir interações da amostra entre bactéria, saliva e tecidos duros da cavidade bucal, junto com a sensibilidade das análises laboratoriais[7].

Existem muitos fatores envolvidos no manchamento dos materiais restauradores estéticos diretos. Dentre esses fatores, Luce e Campbell[9] relatam o tempo decorrido entre a confecção da restauração e o procedimento de acabamento/polimento, grau de polimerização, porcentagem e tamanho das partículas de carga, e tipo de matriz resinosa. No presente estudo, o tempo decorrido entre a confecção do espécime e o polimento, assim como o grau de polimerização foram os mesmos para todas as resinas compostas avaliadas (Z, C e S), o que justificaria a similaridade do manchamento entre elas, apesar de possuírem diferentes tipos de matriz resinosa, porcentagem e tamanho de carga.

Em relação ao procedimento de escovação observou-se menor alteração de cor e de luminosidade nas resinas compostas submetidas à escovação do que as não submetidas à escovação. Esse resultado corrobora com os trabalhos de Collins et al.[4], van Dijken[15], Vogel[16] que verificaram a ação dos dentifrícios na remoção de pigmentos extrínsecos depositados pelo tabaco. A técnica de higiene bucal e a localização da restauração também influem na prevenção e/ou remoção de manchas. De acordo com Asmussen e Hansen[2] o procedimento de escovação insatisfatório pode influenciar o acúmulo de pigmentos pelo amolecimento da matriz por ácidos orgânicos provenientes do biofilme bacteriano, aumentando dessa forma, a susceptibilidade ao manchamento dos materiais estéticos. A localização das restaurações também é um fator importante na alteração de cor como pôde ser observado em pacientes fumantes que apresentaram manchamento nas superfícies proximais e linguais, enquanto que o mesmo não ocorreu na superfície vestibular[15]. Entretanto, no presente estudo, avaliou-se somente a ação do dentifrício na remoção de pigmentos, pois todos os voluntários utilizaram o mesmo modelo de dispositivo intrabucal e o escovaram com o mesmo modelo de escova dental, dentifrício e com o mesmo número de movimentos.

Conclusão

Diante dos resultados obtidos neste estudo podemos concluir que:

(1) a fumaça de cigarro aumentou a alteração de cor e de luminosidade das resinas compostas avaliadas; e

(2) a escovação foi capaz de limitar a alteração de cor e de luminosidade.

Agradecimento

Este artigo é parte da tese submetida pelo primeiro autor à Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista (UNESP) como parte dos requisitos para obtenção do título de Doutor em Dentística Restauradora. Os autores expressam enorme gratidão aos voluntários que fizeram parte desta pesquisa. Este estudo foi suportado pelo CNPQ (Processo 470654/2006-6).

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4. Considerações finais

Diante dos resultados apresentados é possível observar que a fumaça de cigarro foi capaz de alterar além das propriedades ópticas as propriedades mecânicas das estruturas dentais e materiais restauradores estéticos. Esse dado é de grande importância, porque tanto a rugosidade como a dureza são propriedades mecânicas que avaliam as alterações superficiais de forma quantitativa desses elementos[1,4], podendo servir como parâmetro para predizer o desempenho clínico do material[1]. De forma que a qualidade, a longevidade e a performance clínica satisfatória do material restaurador estético dependem além do fator estético da sua resistência à degradação no meio bucal.

O efeito da fumaça de cigarro nas estruturas dentais e resinas compostas dependem de muitos fatores como tipo, quantidade e tempo de exposição[22]. No presente estudo, a diferença de comportamento da resina composta no estudo *in vitro* com o estudo *in situ* pode ter várias justificativas que se encontram no ambiente bucal que não pôde ser replicado *in vitro*.

Os estudos *in situ* geralmente são empregados por analisar muitos fenômenos que se encontram no ambiente bucal e que não são precisamente controlados nos estudos *in vitro*, pelo fato destes não refletir efetivamente o que ocorre no ambiente bucal sob ação da saliva, mudanças de pH, dieta e escovação[4]. Por esses motivos, optamos por realizar esses dois modelos de estudo a fim de verificar a interação da resina composta com a fumaça de cigarro. Entretanto, o modelo *in situ* é dispendioso, consome muito tempo e requer atenção da influência de fatores para reduzir as variáveis que podem interferir na pesquisa[1,2].

Desta forma considero de grande importância à realização de mais estudos *in situ* que possam suportar ou não, os resultados dos trabalhos *in vitro* e investigar os vários fenômenos responsáveis pelas alterações das propriedades físicas e mecânicas de estruturas dentais e resinas compostas.

5. Conclusão

De acordo com a metodologia empregada e os resultados obtidos, julgamos lícito concluir que:

Estudos *in vitro*

1) A fumaça de cigarro influenciou a alteração de cor de todos os espécimes, sendo que a dentina radicular bovina foi mais afetada do que o esmalte bovino e a resina composta;
2) A fumaça de cigarro aumentou a microdureza do esmalte bovino e diminuiu a microdureza da resina composta e da dentina radicular bovina;
3) A microanálise (EDS) detectou a presença de elementos químicos na estrutura dental e resina composta provenientes da fumaça do cigarro, sendo estes: cádmio, arsênio e chumbo.

Estudos *in situ*

1) A fumaça de cigarro/escovação aumentou os valores de microdureza das resinas compostas, contudo proporcionou menor lisura de superfície, ou seja, maior rugosidade;
2) Os fumantes/sem escovação e os não-fumantes/sem escovação apresentaram menores valores de microdureza e nenhuma influência na rugosidade superficial;
3) A fumaça de cigarro aumentou a alteração de cor e de luminosidade das resinas compostas avaliadas e a escovação limitou essa alteração de cor e de luminosidade.

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Anexos

UNIVERSIDADE DE SÃO PAULO
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COMITÊ DE ÉTICA EM PESQUISA
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OF.CEP/046/FORP/28022007

Prezado(a) Professor(a),

Ref.: Processo n. 2005.1.1132.58.6

De ordem da Senhora Coordenadora do Comitê de Ética em Pesquisa desta Faculdade, informamos que o referido Comitê, em sua 75ª Sessão realizada no dia 28 de fevereiro de 2007, **aprovou o Relatório Parcial, a alteração no Projeto e a prorrogação de 12 (doze) meses** para entrega do Relatório Final do Projeto de Pesquisa envolvendo seres humanos intitulado: **"Efeito da fumaça de cigarro nas propriedades de resinas compostas *in situ*"**, desenvolvido por Vossa Senhoria, na Faculdade de Odontologia de Ribeirão Preto, devendo o atestado para publicação final ser expedido pelo Comitê de Ética em Pesquisa, após a apreciação do Relatório Final pelo referido Comitê.

Na oportunidade, esclarecemos que o **Relatório Final** deverá ser encaminhado à Secretaria do Comitê de Ética em Pesquisa até o dia **28 de fevereiro de 2008**, conforme modelo que se encontra no *site* da FORP/USP (*link*: Colegiados e Comissões - Comitê de Ética em Pesquisa – Formulários do Pesquisador para entrega dos Relatórios Parcial ou Final).

Atenciosamente,

Luci Rose Nassif Menezes
Secretária *"ad-hoc"* do Comitê de Ética em Pesquisa

Ilma. Sra.
Profa. Dra. REGINA GUENKA PALMA DIBB
Professora Associada do Departamento de Odontologia Restauradora - FORP/USP

LRNM/bgsp

TERMO DE CONSENTIMENTO LIVRE ESCLARECIDO

Pelo presente instrumento, declaro para os devidos fins éticos e legais que o senhor(a) _____________________, nacionalidade ____________, portador(a) da cédula de identidade número ___________, residente na ___________________ _________________________na cidade de ___________________________, estado de ________________ concordo(a) em participar voluntariamente da pesquisa intitulada "Efeito da fumaça de cigarro nas propriedades de resinas compostas *in situ* " após leitura detalhada e minuciosa da CARTA DE INFORMAÇÃO AO PACIENTE, devidamente explicada verbalmente (oral) pelos pesquisadores responsáveis em seus mínimos detalhes, avisado dos serviços e procedimentos aos quais será submetido, não restando quaisquer dúvidas a respeito do lido e explicado, assina seu CONSENTIMENTO LIVRE E ESCLARECIDO em concordância em participar da pesquisa proposta por estar em pleno acordo com o teor do mesmo, conforme a CARTA DE INFORMAÇÃO AO PACIENTE.

Fica claro que o paciente pode, a qualquer momento, retirar seu CONSENTIMENTO LIVRE E ESCLARECIDO e deixar de participar do estudo alvo da pesquisa e ciente que todo trabalho realizado torna-se informação confidencial guardada por força do sigilo profissional (Art. 9º do código de ética Odontológica). Declaro ter recebido uma cópia deste Termo de Consentimento.

Por estar entendido e de acordo, assino o presente termo.

Ribeirão Preto, ___ de ______________ de 2006.

____
Assinatura do voluntário

____
Profª. Drª. Regina Guenka Palma-Dibb
Pesquisadora responsável

____
Prof. Dr. Welingtom Dinelli
Pesquisador responsável

____
Cristina Yoshie Garcia Takeuchi
Pesquisadora responsável

Araraquara, 30 de agosto de 2007.

Cristina Yoshie Garcia Takeuchi