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**ESTRATÉGIAS PARA A REMOÇÃO DE PIGMENTOS DEPOSITADOS
SOBRE BRAQUETES ESTÉTICOS**

Santa Maria, RS
2016

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**ESTRATÉGIAS PARA A REMOÇÃO DE PIGMENTOS DEPOSITADOS SOBRE
BRÁQUETES ESTÉTICOS**

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, área de concentração Odontologia, ênfase em Prótese Dentária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Odontológicas**.

Orientadora: Prof^ª Dr^ª. Liliana Gressler May
Co-Orientadora: Prof^ª Dr^ª. Letícia Borges Jacques

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RESUMO

ESTRATÉGIAS PARA A REMOÇÃO DE PIGMENTOS DEPOSITADOS SOBRE BRÁQUETES ESTÉTICOS

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O objetivo desse estudo foi avaliar a eficiência dos métodos de limpeza na remoção de pigmentos depositados sobre bráquetes estéticos, após imersão em café. Foram selecionados 160 bráquetes (80 monocristalinos e 80 policristalinos) de pré-molares superiores. Os bráquetes foram fixados em uma lâmina de vidro e logo depois a mensuração de cor foi realizada com um espectrofotômetro (T0). Cada meio de imersão (café ou saliva) continha 80 bráquetes (40 para cada tipo de cerâmica). Logo após a coloração (30 dias de imersão) novas aferições foram feitas (T1). Posteriormente, os espécimes foram divididos randomicamente em 4 grupos: Grupo 1 – Escovação; Grupo 2 – Clareamento; Grupo 3 – Jateamento com bicarbonato de sódio; Grupo 4 – Jateamento com bicarbonato de sódio seguido de Clareamento. Novas aferições foram feitas depois de cada método de limpeza (T2). A variação da cor dos bráquetes foi avaliada de acordo com 2 fórmulas: CIELAB E CIEDE2000. As diferenças entre as médias de ΔE de acordo com o tratamento, braquete e solução foram avaliadas através de análise de regressão linear. O café promoveu maior manchamento. A estrutura cristalina não influenciou na alteração de cor. Os métodos com menores médias de ΔE , no café, foram o jateamento com posterior clareamento e o clareamento sozinho, seguido dos grupos que receberam jateamento e escovação (tanto para CIELAB quanto para CIEDE2000). A fórmula CIEDE2000 foi mais sensível em detectar diferenças de cor não-aceitáveis e perceptíveis quando comparado à fórmula CIELAB. Conclui-se que os métodos mais eficazes na remoção de pigmentos depositados sobre bráquetes estéticos são o jateamento com bicarbonato de sódio com posterior clareamento e o clareamento sozinho.

Palavras-chave: Bráquetes Ortodônticos. Corantes. Bicarbonato de Sódio. Clareadores.

Espectrofotômetros. Cor.

ABSTRACT

STRATEGIES FOR REMOVAL OF PIGMENTS DEPOSITED ON AESTHETIC BRACKETS

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The aim of this study was to evaluate the efficiency of cleaning methods in removing pigment deposited on aesthetic brackets, after immersion in coffee. We selected 160 premolar brackets (80 monocrystalline and polycrystalline 80). The brackets were fixed on a glass blade and soon, thereafter, color measurement was performed using a spectrophotometer (T0). For each means of immersion, 80 brackets were selected (40 for each type of ceramic). Soon after staining (30 days of immersion), new color measurements were made (T1). Subsequently, the specimens were divided randomly into four groups for brushing, bleaching, blasting with sodium bicarbonate jet and sodium bicarbonate jet followed by bleaching. New color measurements were made after each cleaning method was used (T2). The color change of the brackets was evaluated according to two formulae, i.e., CIELAB and CIEDE2000. The differences between the average ΔE according to treatment, bracket, and solution were evaluated by linear regression analysis. The greatest staining was caused by coffee. The crystalline structure did not influence the color change. The methods with lower averages of ΔE for coffee were sodium bicarbonate jet followed by bleaching and bleaching, followed by the group that received brushing and sodium bicarbonate jet (both CIELAB as to CIEDE2000). The CIEDE2000 formula was more sensitive in detecting non-acceptable and perceptible differences in color when compared with the CIELAB formula. It was concluded that the most effective methods to remove pigment deposited on aesthetic brackets are sodium bicarbonate jet followed by bleaching and bleaching alone.

Key words: Orthodontic Brackets. Coloring Agents. Sodium Bicarbonate. Bleaching. Spectrophotometers. Color.

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1 INTRODUÇÃO

O amplo acesso a serviços de Ortodontia nos dias atuais requer a necessidade de cada vez mais o Cirurgião-Dentista primar pela estética, padrão que é exigido pela maioria dos pacientes que procuram o consultório odontológico. E isso abrange não somente o tipo de bráquete a ser utilizado, mas também suas características ao longo do tempo (como por exemplo, a estabilidade de cor) frente a alterações que podem ocorrer no ambiente bucal.

A utilização dos bráquetes estéticos tem indicação no tratamento ortodôntico principalmente em pacientes adultos, que refutam a colocação de aparelho ortodôntico pela aparência indesejável dos bráquetes metálicos (JADADD *et.al*, 2011). Segundo Khan e Horrocks (1991), os principais fatores desmotivadores do uso de aparelhos ortodônticos por parte dos adultos são o tempo prolongado de tratamento e a aparência anti-estética dos bráquetes.

O primeiro bráquete estético, apresentado por Newman em 1969, era um composto a base de policarbonato que, apesar de inicialmente apresentar algumas vantagens, ao longo do tempo foram apontadas inúmeras desvantagens, como por exemplo: descoloração quando exposto a alimentos e líquidos, devido sua alta capacidade de absorção de água (FERNANDEZ, L.; CANUT, J.A., 1999; OLSEN *et al.*, 1997), desgaste excessivo do bráquete e pobreza de torque (DOBRIN, 1961).

Em 1986 surgiram os primeiros bráquetes cerâmicos com a intenção de eliminar as desvantagens dos bráquetes de policarbonato. O material empregado é o óxido de alumínio (Al_2O_3), o qual apresenta características como alta dureza e resistência a altas temperaturas (WINCHESTER, 1992). Além desses fatores, também é passível de sofrer degradação química e é friável, resultando em propagação de falhas por imperfeições ou impurezas. Devido a essa friabilidade, a cerâmica não seria recomendada para a confecção de um bráquete, pois este apresenta ângulos vivos e secções transversais reduzidas, aumentando assim sua fragilidade. Essa limitação é contornada aumentando-se o tamanho dos bráquetes cerâmicos, tornando-os mais volumosos que os metálicos. Embora a cerâmica apresente maior resistência mecânica, melhor estética e menor desgaste e menor pigmentação em relação ao policarbonato, a fricção com os fios ortodônticos é alta. Para se compensar esta desvantagem, canaletas metálicas foram incorporadas, proporcionando maior lisura e menor atrito com o fio (SAUNDERS e KUSY, 1994; TSELEPIS *et al.*, 1994).

O processo de fabricação pode ocorrer de duas formas, resultando em tipos de bráquetes cerâmicos diferentes: cerâmica policristalina e cerâmica monocristalina. Os bráquetes de

cerâmica policristalina são cristais de óxido de alumínio sinterizados a altas temperaturas (próximo à 1950°C), de forma que possam ser moldados e posteriormente cortados até a conformação do formato final do bráquete. Logo depois, aplica-se um tratamento térmico para a remoção de imperfeições das superfícies e liberação de estresses estruturais criados durante a realização dos cortes. São os mais comuns e populares, principalmente devido à relativa facilidade de produção quando comparados aos bráquetes de cerâmica monocristalina (GHAFARI, 1992; MALTAGLIATI, 2006).

O processo de confecção dos suportes monocristalinos consiste na sinterização de uma massa formada de partículas de alumina com um aglutinador a altas temperaturas (2100 °C). Essa massa é resfriada vagarosamente, permitindo assim um cuidado maior na cristalização. Posteriormente, esse material é recortado no tamanho dos bráquetes. Técnicas de corte ultrassônico, diamantado ou a combinação das duas são utilizadas para a sua formatação. Após o corte, os bráquetes de safira, como são chamados, são aquecidos para remover as imperfeições na superfície e para aliviar o estresse produzido nos procedimentos de corte. Esse processo de produção confere à peça menor opacidade, tornando-a extremamente estética e com alta resistência a tensões (MALTAGLIATI, 2006; OMANA, 1992). Assim, suportes monocristalinos são mais translúcidos que os policristalinos (JENA *et al.*, 2007).

Os bráquetes cerâmicos monocristalinos e policristalinos são resistentes a penetração de pigmentos e descoloração provenientes de substâncias químicas encontrada na cavidade bucal (SWARTZ, 1988), porém os pigmentos oriundos do vinho, café e chá (BISHARA, 1997) podem afetar os bráquetes no ambiente oral.

Apesar da estética ser a principal vantagem dos bráquetes estéticos em relação aos suportes metálicos, suas propriedades ópticas são pouco estudadas. Os bráquetes estéticos podem sofrer alterações em suas propriedades ópticas no ambiente oral devido ao manchamento por substâncias corantes presentes em alimentos e bebidas (GHAFARI, 1992; BISHARA; FEHR, 1997; KARAMOUZOS *et al.*, 1997; FERNANDEZ; CANUT, 1999; BISHARA, 2003). Essa questão torna-se de grande interesse para o ortodontista na medida em que os pacientes exigem aparelhos ortodônticos cada vez menos aparentes e mais estáveis em relação à cor.

Em estudos específicos de alteração de cor de bráquetes cerâmicos em diferentes meios e tempos, a literatura parece indicar, dentre as substâncias corantes, o café como sendo a de maior potencial pigmentante (OLIVEIRA *et.al.*, 2013; MENDONÇA *et al.*, 2011).

O mecanismo de manchamento de bráquetes cerâmicos ainda não está totalmente esclarecido na literatura. Sabe-se que em compósitos resinosos, a descoloração por chá, por

exemplo, ocorre por meio da adsorção de corantes polares aos componentes orgânicos das resinas, sendo essa ligação frágil, a ponto do corante ser removido com a escovação. Por outro lado, o manchamento por café se processa por absorção e adsorção dos corantes polares, provavelmente, devido à compatibilidade da fase orgânica do polímero com esse corante específico (UM e RUYTER, 1991). Diferentes dos compósitos resinosos, a composição dos bráquetes cerâmicos é, principalmente, inorgânica. Por esse motivo, torna-se difícil a explicação de como se processa a interação entre os corantes das soluções e os componentes desses materiais. Para entender melhor esse mecanismo, pesquisas nessas áreas devem ser desenvolvidas.

O tempo de um tratamento ortodôntico é considerado longo e durante esse período o bráquete pode sofrer algumas consequências negativas, tais como fratura e alterações de cor. A fratura, decorrente da alta friabilidade do material (KARAMOUZOS, 1997), pode acontecer em dois momentos: durante o curso do tratamento ou no momento da descolagem do bráquete (LINDAUER, 1994; ARICI, 1997). Essa fratura acontece, principalmente, nas aletas, e o restante da cerâmica que permanece aderida ao dente deve ser removida com brocas de alta rotação, aumentando as possibilidades de injúria ao paciente e gerando dificuldades adicionais ao ortodontista (SOBREIRA *et al.*, 2007). De acordo com Ryf *et al.*, 2011, após a descolagem do bráquete, podem permanecer remanescentes do adesivo utilizado para a colagem e sua remoção completa sem danificar o esmalte dentário é difícil de se conseguir. A alteração de cor causa incômodo em muitos pacientes, mas pode ser minimizada através do emprego de métodos de limpeza para a remoção dos pigmentos. Isto também possibilita uma diminuição do custo, pois a troca frequente do bráquete devido à pigmentação pode elevar o custo do tratamento ortodôntico. Oliveira *et al.* em 2014 estudaram o efeito da escovação dentária no manchamento de bráquetes estéticos cerâmicos e concluíram que esse método reduziu o grau de pigmentação desses bráquetes.

Um dos problemas enfrentados pelos ortodontistas na clínica diária é a falta de comprometimento do paciente no que diz respeito a uma higiene mais eficaz. Além disso, pacientes que possuem dietas ricas em bebidas e alimentos potencialmente corantes, em que apenas a escovação pode não ser eficiente para remover pigmentos.

Dentre os tratamentos comumente utilizados na prática clínica para a remoção de pigmentos superficiais em estruturas presentes na cavidade bucal, destacam-se o uso de géis clareadores e o uso de jato de bicarbonato.

O clareamento consiste na remoção de pigmentos da estrutura através da quebra de moléculas instáveis ao calor e umidade (Ex. H₂O₂) em radicais livres capazes de interagir

quimicamente com as moléculas dos pigmentos, por meio de uma reação de oxi-redução, quebrando-as até serem eliminadas total ou parcialmente (AYAD, 2009). Os clareadores empregados na atualidade são o peróxido de hidrogênio, o perborato de sódio (usados em dentes desvitalizados) e o peróxido de carbamida, em diferentes concentrações. Podem ser veiculados de diversas formas, tais como géis de uso caseiro ou profissional, dentifrícios, tiras e vernizes (DAHL, 2003). Existem duas técnicas de clareamento dental: a técnica caseira e a técnica de consultório. A técnica de consultório parece ser a mais adequada para o uso em pacientes ortodônticos. Isto porque a técnica caseira utiliza moldeiras e, na presença do aparelho ortodôntico, isso torna-se inviável. A técnica de consultório consiste na aplicação do peróxido de hidrogênio de 35% a 38%, por até 45 minutos. Em geral, a técnica original preconiza utilizar três trocas do gel em intervalos de 15 minutos (FRANCCI, 2010).

Além do uso de géis clareadores, a literatura indica o uso do jato de bicarbonato de sódio como um meio eficaz de remoção de manchas extrínsecas e placa bacteriana das superfícies dos dentes. Esse método profilático exige menos tempo que os métodos tradicionais de polimento e remove manchas três vezes mais rápido quando comparado ao uso de curetas (CHRISTENSEN R., 1981; BERKSTEIN S. *et al*, 1987; DESPAIN B. *et al*, 1988). Também tem sido relatado que o jato de bicarbonato de sódio não torna a superfície do esmalte dentário rugosa, portanto não causa danos ao mesmo (GALLOWAY e PASHLEY, 1987). Samra *et al.* em 2012 estudaram o efeito da profilaxia dental que utiliza jato de bicarbonato de sódio em resinas compostas submetidas ao desafio corante com café e encontraram uma redução significativa na coloração de todos os materiais testados. No entanto, não há estudos que avaliaram o efeito do jato de bicarbonato na estabilidade de cor de bráquetes ortodônticos estéticos.

Na Odontologia existem técnicas instrumentais para a mensuração da cor, que são medidas objetivas obtidas a partir de aparelhos como espectrofotômetros, colorímetros e técnicas computadorizadas de análises de imagem (JOINER, 2004; ISHIKAWA-NAGAI *et al.*, 2005; CAL; GUNERE; KOSE, 2006). A percepção instrumental das propriedades ópticas tem sido preferida sobre a visual, porque torna esse processo objetivo, quantificável e rápido (MEIRELLES *et al.*, 2008). Os espectrofotômetros estão entre os instrumentos mais precisos, úteis e de fácil utilização para mensuração de cor em Odontologia e são capazes de medir os comprimentos de onda da reflectância ou transmitância de um objeto (JOINER 2004; ISHIKAWA-NAGAI *et al.*, 2005; CHU; TRUSHKOWSKY; PARAVINA, 2010).

Tendo em vista a necessidade de se quantificar a cor por meio de instrumentos, foram desenvolvidos sistemas para a identificação numérica da cor. Dentre eles, o sistema de

avaliação colorimétrica de acordo com a Comissão Internationale de l'Eclairage – CIE é muito utilizado. Esse sistema utiliza os parâmetros L^* , a^* e b^* para a definição de cor, em que L^* indica a variação de luminosidade e as variáveis a^* e b^* são as coordenadas relativas à cor nas axiais vermelho–verde e amarelo–azul, respectivamente (JOINER, 2004; CIE, 2004).

Para a verificação de alteração de cor (ΔE), as leituras iniciais e finais dos parâmetros de cor obtidos através do espectrofotômetro podem ser aplicadas em equações recomendadas pela CIE e fornecem uma representação quantitativa de diferença de cor. A equação largamente utilizada nos estudos de variação de cor em Odontologia é a CIELAB, que inclui apenas os valores de luminosidade e croma no seu cálculo. Mais recentemente, a fórmula CIEDE2000 foi desenvolvida, incluindo não somente luminosidade e croma, mas também funções de ponderações de luminosidade, croma e matiz e um termo interativo entre as diferenças de croma e matiz, proporcionando uma melhor percepção para pequenas diferenças de cor, o que não é visto na fórmula CIELAB (LUO; CUI; RIGG, 2006; CUI *et al.*, 2002). A equação do CIEDE2000 é consideravelmente mais sofisticada do que suas antecessoras CIELAB e CIE94 (SHARMA; WU; DALAL, 2005).

A partir da possibilidade de que bráquetes cerâmicos podem ser manchados por substâncias ingeridas na dieta e das evidências de que a remoção desses bráquetes pode causar danos ao esmalte justifica-se o estudo de um método eficaz na eliminação de pigmentos de bráquetes estéticos durante o tratamento ortodôntico. Portanto, o objetivo desse estudo foi avaliar a eficiência de métodos de limpeza na remoção de pigmentos depositados sobre bráquetes estéticos (cerâmica monocristalina e cerâmica policristalina) submetidos ao desafio corante (café). As hipóteses testadas foram: 1) bráquetes de estrutura cristalina diferente iriam se comportar de maneira diferente 2) os métodos de limpeza seriam capazes de remover os pigmentos e 3) A fórmula CIEDE2000 seria mais eficaz na detecção de pequenas diferenças de cor.

2 ARTIGO – STRATEGIES FOR STAINING REMOVAL ON AESTHETIC BRACKETS

Este artigo será submetido à publicação no periódico Journal of Dentistry. As normas para a publicação estão descritas no Anexo A.

STRATEGIES FOR STAINING REMOVAL ON AESTHETIC BRACKETS

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ABSTRACT

STRATEGIES FOR STAINING REMOVAL ON AESTHETIC BRACKETS

OBJECTIVES: The aim of this study was to evaluate the efficiency of cleaning methods in removing pigment deposited on aesthetic brackets, after immersion in coffee. **METHODS:** We selected 160 premolar brackets (80 monocrystalline and polycrystalline 80). The brackets were fixed on a glass blade and soon, thereafter, color measurement was performed using a spectrophotometer (T0). For each means of immersion, 80 brackets were selected (40 for each type of ceramic). Soon after staining (30 days of immersion), new color measurements were made (T1). Subsequently, the specimens were divided randomly into four groups for brushing, bleaching, sodium bicarbonate jet and sodium bicarbonate jet followed by bleaching. New color measurements were made after each cleaning method was used (T2). The color change of the brackets was evaluated according to two formulae, i.e., CIELAB and CIEDE2000. The differences between the average ΔE according to treatment, bracket, and solution were evaluated by linear regression analysis. **RESULTS:** The greatest staining was caused by coffee. The crystalline structure did not influence the color change. The methods with lower ΔE averages for coffee were sodium bicarbonate jet followed by bleaching and bleaching, followed by the group that received sodium bicarbonate jet and brushing (both CIELAB as to CIEDE2000). The CIEDE2000 formula was more sensitive in detecting non-acceptable and perceptible differences in color when compared with the CIELAB formula. It was concluded that the most effective methods to remove pigments deposited on aesthetic brackets are sodium bicarbonate jet followed by bleaching and bleaching alone. **CLINICAL SIGNIFICANCE:** Sodium bicarbonate jet followed by bleaching and bleaching alone can be a good alternative to maintain color stability during orthodontic treatment. These methods prevent damage to the enamel that can be caused by exchange of brackets and reduces the cost to the dentist in the clinic.

Key words: Orthodontic Brackets. Coloring Agents. Sodium Bicarbonate. Bleaching. Spectrophotometers. Color.

1. INTRODUCTION

The demand for orthodontic treatment has increased in recent years and with it the emergence of new brackets with higher esthetic properties. The use of these devices is indicated in orthodontic treatment mainly in adult patients who refuse the placement of an orthodontic appliance because of the undesirable appearance of the metal brackets¹. According to Khan and Horrocks² (1991), there are two main demotivating factors affecting the use of orthodontic appliances by adults: prolonged treatment and the poor esthetic appearance of the brackets. The first esthetic bracket, devised by Newman in 1969, was a polycarbonate base compound. Although it had some advantages, over time numerous disadvantages were identified, including discoloration when exposed to food and liquid, excessive wear of the bracket, and deficient torque³.

The first ceramic brackets were developed in 1986 with the intention of eliminating the drawbacks of polycarbonate brackets. The material used was aluminum oxide (Al_2O_3), which has desirable features including high hardness and resistance to high temperatures⁴. The manufacturing process may be performed in two ways, thereby providing two different types of ceramic, i.e., ceramic polycrystalline and monocrystalline ceramic. The polycrystalline ceramic brackets are crystals of aluminum oxide sintered at high temperatures (near 1950°C), so they can be modeled and subsequently cut to form the final shape of the bracket. Soon after, a heat treatment is applied to remove imperfections in the surfaces and release the structural stresses created during the course of the cuts. These brackets are the most common and popular, mainly because of their relative ease of manufacture when compared with monocrystalline ceramic brackets^{5,6}. The manufacturing process of brackets monocrystalline involve sintering of a mass formed by alumina particles with a binder at high temperatures (2100°C). This mass is cooled slowly, thereby allowing greater care in crystallization. Subsequently, the material is cut to the size of the brackets. Ultrasonic cutting techniques, diamond, or a combination of both are used for formatting. After cutting, the sapphire brackets, as they are called, are heated to remove surface imperfections and to relieve the stress produced by the cutting procedures. This manufacturing process provides the piece with a lower opacity, making it both highly esthetic and resistant to stress^{6,7}. Thereby, monocrystalline brackets are more translucent than polycrystalline brackets⁸.

Ceramic structures are resistant to penetration of extrinsic pigment molecules⁹. However, similarly to other structures in the oral cavity, surface adsorption of pigments will occur in patients with a diet rich in staining agents. The pigments derived from wine, coffee and tea¹⁰ may affect the brackets in the oral environment. In specific studies of color change in ceramic brackets in different ways and at different times, the literature seems to indicate that, of all the potential colorants, coffee is the substance with the greatest potential for pigmentation^{11,12}.

Orthodontic treatment is considered a long-term process and during this time, the bracket may undergo negative events, such as color changes and fractures. Fractures, which happen because of the high friability of the material¹³, can occur in two stages, i.e., during the course of treatment or at the time when the bracket is taken off (since ceramic brackets present problems related to excessive bond strength)^{14,15}. According to Ryf *et al.*¹⁶, 2011, after taking off the bracket, remnants of the adhesive used for bonding may remain, so it is difficult to remove the bracket without damaging the tooth enamel. The color change causes discomfort in many patients, but can be minimized by using cleaning methods for removing the pigments. This also enables a reduction in cost because the frequent exchange of bracket due to the pigmentation may raise the cost of orthodontic treatment. Oliveira *et al.*¹¹, in 2014, studied the effect of tooth brushing on stained esthetic ceramic brackets, and concluded that this method reduced the amount of pigment present on the surfaces of the brackets.

One of the main problems faced by orthodontists in daily practice is the lack of commitment by patients, because of the difficulty of implementing a more detailed hygiene regimen. In addition, there are patients with diets containing high levels of food pigments, wherein the brushing alone may be ineffective.

The treatments that are most commonly used in clinical practice for removal of pigments from surface structures present in the oral cavity are bleaching agents and the bicarbonate jet. There are two dental whitening techniques, one home-based and the other office-based. The office-based technique involves application of hydrogen peroxide 35%–38% for up to 45 minutes¹⁷ and the bicarbonate jet removes stains three times faster than cures^{18,19,20}.

Among the formulae for color change evaluation (ΔE), stand out CIELAB and CIEDE2000. The equation of CIEDE2000 is considerably more sophisticated than their predecessors CIELAB and CIE94²¹.

From the possibility that ceramic brackets can be stained by substances ingested in the diet and the evidence that the removal of these brackets can cause damage to the enamel justified the study of an effective method in eliminating aesthetic brackets pigments during orthodontic treatment. In this context, the aim of this study was to evaluate the efficiency of cleaning methods in removing pigment deposited on aesthetic brackets subjected to staining challenge (coffee). The hypotheses tested were that 1) brackets of different crystalline structure would behave in different ways 2) the cleaning methods chosen would remove the pigments and 3) CIEDE2000 formula would be more sensitive in detecting small color differences

2. MATERIALS AND METHODS

2.1 SAMPLE SELECTION AND DIVISION OF GROUPS

A preliminary pilot study was performed to calculate the minimum sample size required to verify a difference in ΔE between blasting with sodium bicarbonate and bleaching, using a level of significance of 5% and a statistical power of 80%. It was observed that $n=9$ would be sufficient to demonstrate statistically significant differences between groups. Therefore, 80 upper premolar brackets of the brand Eurodonto (Curitiba, PR, Brazil) were selected for each group (80 monocrystalline ceramic and 80 polycrystalline ceramic), giving a total of 160 brackets (see Table 1).

2.2 PREPARATION OF SPECIMENS

To facilitate both the cleaning and color evaluation procedures, the brackets were fixed with resin composite (Filtek Z250 XT; 3M-ESPE, St Paul, MN, USA), color A1, on a glass blade, which was made in a square shape (2.5 cm \times 2.5 cm) and sandblasted, thus improving the adhesion of the bracket to the glass blade. The slides were then submitted to acid etching for 20 seconds, with subsequent washing and drying for 10 seconds. Next, Adper Single Bond 2 adhesive (3M-ESPE) was applied following the manufacturer's instructions and light-cured for 20 seconds. Finally, the bracket was fixed in the center of the blade with a single increment of resin composite and light-cured for 20 seconds.

2.3 COLOR PARAMETERS EVALUATION

The color of each bracket was measured before pigmentation (T0) after being fixed in the glass blade.

The color parameters were measured with the spectrophotometer (SP60, X-Rite, Grandville, MI, USA) in analysis mode, using D65 illuminant, 10° observer angle and CIE $L^* a^* b^*$ color system (Commission International L'Eclairage) in the readings. This color measurement system quantitatively determines color by using three parameters (L^* , a^* and b^*). In this system, L^* is the luminosity axis with values varying from zero (black) to one hundred (white), and a^* and b^* are the color coordinates on green-red axis and in blue-yellow axis, respectively. The color parameters were measured over a neutral gray background (CIE- $L^*= 50,30$, $a^*= -1,41$, $b^*= -2,37$) (Mennon gray cards, Mennon Photographic and Technical Co., Beijing, China). The position of the brackets for reading was always in the center of the spectrophotometer, with the lingual side toward the equipment center and the labial side faced up, and the color reading was made on the different axes ($L^* a^* b^*$). Before each reading, the spectrophotometer was calibrated according to the manufacturer's recommendations. For each specimen, the readings of L^* , a^* , and b^* coordinates were repeated three times and the median of those readings was used for the statistical analysis.

2.4 PIGMENTATION OF BRACKETS

For pigmentation, this study followed the methodology of work realized by Erta *et al.*²², in 2006, which performs a calculation for estimating the time of immersion, where, according to the coffee makers, the average hours of consumption for a cup is 15 minutes and the average consumption of this drink per day is 3,2 cups. Thus, 24 hours of immersion in this solution represents a month of consumption of this drink. Therefore, following this calculation, 30 days of immersion is equivalent to 2 years and 6 months of consumption of this drink, simulating, approximately, the time of an orthodontic treatment. Like this, 80 brackets (40 polycrystalline ceramic and 40 monocrystalline ceramic) used for each pigmentation solution were immersed in coffee or artificial saliva (control group) for 30 days (Table 2). To avoid degradation, the solutions were changed every 3 days and pH was controlled using a pHmeter (400 – QA, QUIMIS® /Diadema, São Paulo, Brazil). The brackets were placed in plastic bowls and in an

incubator at 37°C. Before and after each solution change, the brackets were washed in water for 30 seconds and dried with paper towels.

2.5 COLOR EVALUATION AFTER PIGMENTATION

After pigmentation, the color of each group was checked (T1). Before each color reading, the brackets were rinsed with water and dried with absorbent paper to remove any dye residue deposited on the brackets. After the color was measured, the brackets soaked in each solution were randomly divided into four groups (n=10 each) according to the type of treatment received, i.e., brushing (positive control), bleaching, sodium bicarbonate jet or sodium bicarbonate jet and subsequent bleaching. During the cleaning methods, the brackets were kept in artificial saliva

2.6 CLEANING METHODS

2.6.1 Brushing

After 30 days of pigmentation, daily brushing was performed twice a day for 30 days by using an electric toothbrush (Oral-B Professional Care 500 Floss Action, Cincinnati, OH, USA) at a distance of 1 cm from the specimen for 5 seconds in each bracket. In a recent study²³, which uses electric toothbrushes, the authors consider two minutes sufficient time for brushing. If brushing time is calculated according to a dentition having 28, the result of brushing time for each tooth is 5 seconds. Immediately after brushing, the brackets were rinsed for 10 seconds and dried with an air jet (10 seconds) and gauze.

2.6.2 Bleaching

The brackets were submitted to the bleaching procedure, with hydrogen peroxide 35% (Whiteness HP Maxx, Joinville, Santa Catarina, Brazil), using the office technique, since the home technique becomes infeasible in patients using orthodontic appliances to require the use of trays.

The hydrogen peroxide gel was applied on the surface of the bracket with a microbrush, always seeking to use the same amount of gel to each specimen (at an

approximate thickness of 1 mm). After correct application of the gel, samples were placed in a plastic bowl containing wet gauze, which was then sealed and placed in an incubator at 37°C for the duration of the bleaching process. The gel was left in contact with the brackets for 15 minutes and removed with water for 10 seconds. The brackets were subsequently dried with gauze, and new applications of the gel were made, totaling three applications per session. In total, three sessions were performed with an interval of 7 days between each session. At the end of these bleaching sessions, new color measurements were made (T2, evaluation of color after bleaching).

2.6.3 Sodium Bicarbonate Jet

To facilitate sodium bicarbonate jet (PROPHYflex 3 (KaVo Dental, Charlotte, NC, USA), the specimens were placed with the labial surface facing upwards. Each specimen was contained at a distance of 1 cm from the bicarbonate jet, which was used for approximately 3 seconds, moving the jet from the cervical face to the incisal face. The blasting procedure was then repeated from the mesial face to the distal face for 3 seconds. At the end of the sodium bicarbonate jet sessions, a further color evaluation was performed (T2, color evaluation after blasting).

2.6.4 Bicarbonate jet and Bleaching

For the third group, the sodium bicarbonate jet was applied as described above. The specimens were then placed in distilled water for 24 hours and after this period the application of the bleaching agent (as discussed above) was performed. At the end of this process, a further color measurement was performed (T2, color evaluation after sodium bicarbonate jet and bleaching).

2.7 COLOR CHANGE EVALUATION (ΔE) AFTER THE CLEANING PROCEDURES

For the evaluation of change color (ΔE), the initial and final readings of the color parameters obtained from a spectrophotometer can be applied in equations recommended by the Commission International L'Eclairage (CIE)²⁴ and provide a quantitative representation of color difference. In this study, two formulas were used to

compare the differences between two color measurements. The first is that recommended for the CIE L* a* b* method in which the color difference (ΔE) is calculated as follows:

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$$

Where ΔE is the color change, $\Delta L = L1 - L0$ (final reading – initial reading), $\Delta a = a1 - a0$ (final reading – initial reading), and $\Delta b = b1 - b0$ (final reading – initial reading).

The second equation is the method recommended by CIEDE2000, wherein the color difference (ΔE) is calculated as follows:

$$\Delta E' = \left[\left(\frac{\Delta L'}{K_L S_L} \right)^2 + \left(\frac{\Delta C'}{K_C S_C} \right)^2 + \left(\frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C} \right) \left(\frac{\Delta H'}{K_H S_H} \right) \right]^{1/2}$$

Where ΔE is the color change, ΔL , ΔC e ΔH are the differences in brightness, chroma, and hue for a pair of samples in CIEDE2000 and RT is the rotation function, which represents the interaction between differences in chroma and hue in the blue region. The weighting functions S_L , S_C e S_H adjust the overall color difference according to variation in the position of the color difference of the coordinates L' , a' and b' . The parametric factors K_L , K_C e K_H are correction terms for experimental conditions.

In both color evaluation formulas, the following ΔE were considered:

- $\Delta E1$: difference of color between the values after pigmentation and the initial values of the brackets;
- $\Delta E2$: difference of color between values after cleaning methods and the initial values of the brackets.

The values proposed by Paravina et al., in 2015, were considered as clinical limits, which the thresholds of perceptibility and acceptability from the ΔE are shown in Table 3.

2.8 DATA ANALYSIS

The data were analyzed using STATA version 13.0 for Windows (Stata Corporation, College Station, TX, USA). Normality was verified using the Shapiro-Wilk test. The differences between the average ΔE values according to the type of treatment, type of bracket used, and each solution were evaluated by linear regression analysis. A 5% significance level was adopted for all analyses.

3. RESULTS

3.1 CIELAB FORMULA GROUPS (ΔE_{ab})

The average ΔE_{ab} found according to the type of bracket, cleaning method, and type of solution (coffee or saliva) that were submitted to the CIELAB equation are described in Table 4. In general, the color changes were greater for specimens immersed in coffee than those immersed in saliva. There was no significant differences in color change according to type of bracket (polycrystalline or monocrystalline) in specimens that were immersed in coffee (ΔE_1 and ΔE_2). Considering the ΔE_2 , the crystal structure influenced the pigmentation of immersed specimens in saliva. In brackets immersed in coffee, it can be seen that a greater average (ΔE_2) color change was found in the groups submitted to sodium bicarbonate jet and brushing, when compared to the groups submitted to sodium bicarbonate jet followed by bleaching and bleaching. There was no difference between the specimens that received sodium bicarbonate jet followed by bleaching and the specimens that received only bleaching, and these treatments showed greater power to remove pigments when compared to the other groups. Between the group that received brushing and the group that received sodium bicarbonate jet there was no statistical difference. In the brackets immersed in saliva, bleaching had a lower average ΔE than the group treated by sodium bicarbonate jet and the group that received blasting followed by bleaching ($p < 0.05$). Lastly, no statistically significant difference was found between brushing and blasting.

3.2 CIEDE2000 FORMULA GROUPS (ΔE_{00})

The average ΔE_{00} found according to type of bracket, cleaning method, and type of solution (coffee or saliva) that were submitted to the CIEDE2000 equation are described in Table 5. In general, color changes were greater for specimens immersed in coffee than for those immersed in saliva. There were no significant differences in color change according to type of bracket (polycrystalline or monocrystalline) for specimens immersed in saliva and those immersed in coffee. In brackets immersed in coffee, it can be seen that a greater average (ΔE_2) color change was found in the groups submitted to sodium bicarbonate jet and brushing, when compared to the groups submitted to sodium bicarbonate jet followed by bleaching and bleaching. There was no difference between the specimens that received sodium bicarbonate jet followed by bleaching and the specimens that received only bleaching, and these treatments showed greater power to remove pigments when compared to the other groups. Between the group that received brushing and the group that received sodium bicarbonate jet there was no statistical difference. In brackets immersed in saliva, sodium bicarbonate jet followed by bleaching had a lower average ΔE when compared with the group treated with bleaching and the group that received brushing ($p < 0.05$), but the difference between the group that received sodium bicarbonate jet followed by bleaching and the group that received only sodium bicarbonate jet was statistically significant (ΔE was lower for the group that was treated by sodium bicarbonate jet followed by bleaching). There were no statistically significant differences between the groups that received brushing or bleaching.

Table 6 presents the frequency of brackets classified according to thresholds of acceptability and perceptibility proposed by Paravina *et al.* It can be seen that the CIEDE2000 formula was more sensitive in detecting differences in non-acceptable and perceptible color when compared with the CIELAB formula (Chi-Square test, $p < 0.001$).

4. DISCUSSION

The first hypothesis of this present study was rejected, since the crystalline structure of the brackets did not influence the color change. The action of pigmentation on the brackets ($\Delta E1$) as well as the action of the cleaning treatment on pigmented brackets ($\Delta E2$) was similar for both monocrystalline and polycrystalline ceramic brackets. Our finding is in accordance with that of Lee²⁵, in 2008, who reported finding no relationship between pigmentation resistance and crystalline structure of aesthetic brackets.

We can also conclude that both monocrystalline ceramic brackets as the brackets of polycrystalline ceramic pigmented considerably after being immersed in coffee. In the literature, there are a few studies about color changes of different esthetic ceramic brackets after immersion in dye solutions. According to them^{26,27}, the color of ceramic brackets changes over time when exposed to potentially dye solutions commonly present in peoples's diet.

This pigmentation can also be related to the composite resin used in this study for fixing the brackets on the glass blade (Filtek Z250 XT). This composite has in its composition inorganic filler particles (zirconia and silica) and organic matrix (BIS-GMA, UDMA, BIS-EMA, PEGDMA and TEGDMA). According to some authors, the factors most responsible for color change are the amount and specific type of pigment²⁸. Coffee, for example, contains a huge amount of yellow dye (with high polarity), and discoloration of composite resins by coffee occurs, primarily, by adsorption and absorption of polar dyes into the organic component of the resin (i.e., the organic polymer phase is compatible with the yellow dye)²⁹. We believe that in our study, this interaction of the organic matrix of the resin composite with the yellow dye present in coffee influenced the color measurement and the final ΔE result of the aesthetic brackets.

Another aspect to consider is regarding the translucency / opacity of ceramic brackets. For monocrystalline ceramic brackets the light will pass more easily (due to the single crystalline structure), and interact with the pigmented resin mentioned above, increasing the ΔE values for these brackets. Of note, Lee³⁰, in 2011, reported that translucency of these brackets alone does not lead to better esthetic performance. On the other hand, polycrystalline ceramic brackets are opaque, because they contain in their structure boundaries of union between numerous crystals and impurities incorporated during the manufacturing process, which hinders the passage of light⁸, avoiding the

contact with pigmented resin. These structural imperfections present in the grain boundary region can make polycrystalline brackets more susceptible to pigmentation, which would explain the high values of ΔE_1 for these brackets. Some studies found that the slots of ceramic brackets have greater roughness value than conventional metal brackets^{31,32}, making ceramic brackets more susceptible to pigmentation. Sanders *et al.*³³, said that this roughness is significantly higher for polycrystalline brackets. However, more studies should be done to better understand the mechanism of staining of ceramic brackets.

Paravina *et al.*³⁴, in 2015, conducted a multicenter prospective study that aimed to determine the thresholds of acceptability and perceptibility of color differences in dentistry. The initial hypothesis were that there would be no difference in the thresholds of acceptability and perceptibility between two formulas used to calculate the color difference (CIELAB and CIEDE2000). However, at the end of the study, this hypothesis was discarded, and the perceptibility value found for CIELAB was $\Delta E_{ab} = 1,2$ and acceptability was $\Delta E_{ab} = 2,7$. As for CIEDE2000, the perceptibility value was $\Delta E_{00} = 0,8$ and the acceptability value was $\Delta E_{00} = 1,8$. In this study, these parameters were used to analyze the color differences that occurred in each solution. Regarding the solutions used, after a month of immersion coffee had the highest ΔE , which is in agreement with previous studies reporting coffee as a solution with a higher power of pigmentation in esthetic brackets^{9,10}. The color changes generated by saliva, which exceeded the limit of acceptability in two formulas analyzed, probably due to the fact that at T0 color coordinates were evaluated with dehydrated brackets, while in others times (T1 and T2) the brackets were read after immersion. This difference in the pigmentation power of these solutions is probably because the type (yellow) and amount of pigment present in coffee. Additionally, coffee has greater power in pigmentation because its pigment has an affinity with the organic components of the resin used in fixation, making the ΔE values higher for coffee.

The second hypothesis of this study was confirmed, since the ΔE values reduced after the specimens are subjected to cleaning methods. When the brackets were submitted to a coffee colorant challenge, the most effective methods for the removal of pigments were sodium bicarbonate jet followed by bleaching and bleaching (in both formulas used for evaluating the color change). The action of bleaching agents in composites has been tested by some authors^{35,36}, which concluded that bleaching agents were effective in reducing the discoloration in these materials. In our study, the

bleaching agent may have reduced staining of resin used for fixing the bracket, thus decreasing the ΔE values for bleaching. In addition, sodium bicarbonate jet may also have helped to reduce this pigmentation, by removing the pigments that were on the composite resin and on the surface of the bracket. Samra *et al.*³⁷, in 2012, examined the ability of a bicarbonate jet to reverse discoloration of a series of resin-based and ceramic lithium disilicate materials and concluded that the sodium bicarbonate jet reduced staining in all materials tested, being more evident for resin-based materials.

Considering all these aspects mentioned, the composite resin used for fixing the brackets can be considered a limitation of our study, since the pigmentation occurred, mainly, due to interaction of the organic matrix of the resin composite with the yellow dye present in coffee. However, the color change of the fixing agent also occurs clinically, following the pigmentation of the bracket and, as explained above, the same cleaning methods used in this study have stain removal effect in composites.

Given the subjective nature of color, quantification methods that permit the numeric expression of color are preferable^{38,39}. The color change in dental materials can be evaluated visually and by instrumental techniques such as spectrophotometry and colorimetry⁴⁰. Spectrophotometers are used to measure the intensity of each wavelength, decomposing the light reflected by the sample when illuminated by the polychromatic and diffuse light emitted by the device⁴¹. Spectrophotometers is more accurate for color measurement, because it minimizes the loss of light from the edges of samples and maximizes the collection of light reflected in all directions⁴². Color stability studies have generally used the CIE L * a * b * system. In this system, L* is a measure of brightness of an object. It is quantified on a scale in which black has a L* value of zero; and light, which is totally reflected, has a L* value of 100. On the same scale, a* accounts for the amount of red (+a*) and green (-a*) whereas b* accounts for the amount of yellow (+b*) and blue (-b*)^{24,43}.

For the evaluation of color (ΔE), the initial and final readings of the color parameters obtained from a spectrophotometer can be applied in equations recommended by the Commission International L`Eclairage (CIE). The equation widely used in color variation studies is the CIELAB, but this is increasingly losing ground to the CIEDE2000 equation, which is based on the CIELAB formula and includes not only chroma brightness and hue weighting functions, but also an interactive term between chroma and hue differences to improve the performance of blue color. The CIEDE2000 equation gives much better fits for experimental data based on small color differences

than does the CIELAB formula, which includes only lightness and chroma^{44,45}. Our study confirms this observation because the CIEDE2000 equation was more sensitive for detecting non-acceptable and perceivable differences in color when compared with measurements performed by CIELAB.

We know that an *in vitro* study has methodological limitations and reproducibility of the conditions presents in the oral cavity becomes difficult, since this has a complex flora and their by-products, and also have plaque buildup in the material studied. Nevertheless, through this study, we can concluded that cleaning methods can be a good alternative for maintaining color stability in esthetic brackets during orthodontic treatment and the crystalline structure is not the most important factor that we must consider in choosing the bracket in our office, but the features that conjunct (bracket + composite) will present over time.

5. CONCLUSION

Within the limitations of an *in vitro* study, it may be concluded that:

- 1) The crystalline structure does not affect the color stability of ceramic brackets;
- 2) The pigments generated by coffee may be removed by cleaning methods and sodium bicarbonate jet followed by bleaching and bleaching are the most effective;
- 3) CIEDE2000 equation is the most sensitive for detecting small differences in color.

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TABLES

Table 1. Brands, manufacturers and type of bracket.

Brands	Manufacturer	Type of bracket
Zetta	Eurodonto Produtos Ortodônticos, Curitiba, PR, Brazil	Monocrystalline ceramic
Maia	Eurodonto Produtos Ortodônticos, Curitiba, PR, Brazil	Polycrystalline ceramic

Table 2. Solutions, brands and preparation methods.

Solution	Brand	Preparation
Coffe	Nescafé Tradição(Nestlé, Brasil Ltda, Brazil)	Solution prepared with 50g of instant coffe added to 200ml of boiling distilled water
Artificial Saliva	Saliform (Fórmula e Ação, São Paulo, Brazil)	Prepared solution neutral pH,tasteless and odorless

Table 3. Thresholds of perceptibility and acceptability according to Paravina *et al.*, 2015.

	PERCEPTIBILITY	ACCEPTABILITY
CIELAB (ΔE_{ab})	1,2	2,7
CIEDE2000(ΔE_{00})	0,8	1,8

Table 4. ΔE averages according to cleaning methods and type of bracket for CIELAB.

VARIABLE	AVERAGES OF COLOR CHANGE		AVERAGES OF COLOR CHANGE	
	<i>Coffee</i>		<i>Saliva</i>	
	$\Delta 1$	$\Delta 2$	$\Delta 1$	$\Delta 2$
Cleaning Methods:				
1) Brushing	-	4,22(1,52) ^a	-	3,69 (1,41) ^a
2) Bleaching	-	3,01(1,10) ^b	-	3,15(1,84) ^b
3) Sodium Bicarb. Jet	-	5,07(1,63) ^a	-	4,55(1,53) ^{a,b}
4) Sodium Bicarb. Jet + Bleaching	-	2,86(1,27) ^{c,b}	-	3,32(2,10) ^{c,b}
Type of bracket:				
1) MONOCRYSTALLINE	9,08 (3,13)	3,99(1,72)	2,71(1,92)	3,37(1,77)
2) POLYCRYSTALLINE	7,61 (1,06)	3,62(1,55)	1,84(0,65)	2,98(1,62)
Total	8,34(2,40)	3,81(1,64)	2,28(1,46)	3,68(1,82)

*Different letters indicate statistically significant differences between treatments ($p \leq 0.05$) - Linear Regression Model.

#Statistically significant difference between the types of brackets ($p \leq 0.05$) - Linear Regression Model.

$\Delta E1$: difference of color between the values after pigmentation and the initial values of the brackets.

$\Delta E2$: difference of color between values after treatment and the initial values of the brackets.

Table 5. ΔE averages according to cleaning methods and type of bracket for CIEDE2000.

VARIABLE	AVERAGES OF COLOR CHANGE		AVERAGES OF COLOR CHANGE	
	<i>Coffee</i>		<i>Saliva</i>	
	$\Delta 1$	$\Delta 2$	$\Delta 1$	$\Delta 2$
Cleaning Methods:				
1) Brushing	-	4,02(1,39) ^a	-	3,18(2,01) ^{a,c}
2) Bleaching	-	2,77(0,99) ^b	-	2,35(1,72) ^{b,c}
3) Sodium Bicarb. Jet	-	4,73(1,48) ^a	-	3,76(0,97) ^a
4) Sodium Bicarb. Jet + Bleaching	-	2,60(1,13) ^{c,b}	-	2,14(1,10) ^c
Type of Bracket:				
1) MONOCRYSTALLINE	7,94(2,47)	3,71(1,60)	2,44(1,74)	2,65(1,73)
2) POLYCRYSTALLINE	7,18(1,21)	3,34(1,45)	1,62(0,56)	2,42(1,48)
Total	7,56(1,94)	3,53(1,53)	2,03(1,33)	2,53(1,61)

*Different letters indicate statistically significant differences between treatments ($p \leq 0.05$) - Linear Regression Model.

#Statistically significant difference between the types of brackets ($p \leq 0.05$) - Linear Regression Model.

$\Delta E1$: difference of color between the values after pigmentation and the initial values of the brackets.

$\Delta E2$: difference of color between values after treatment and the initial values of the brackets.

Table 6. Classification of the brackets according to the thresholds of acceptability and perceptibility (Paravina *et al.*, 2015)

THRESHOLDS OF ACCEPTABILITY AND PERCEPTIBILITY		
TYPE MEASUREMENT		
	CIELAB n(%)	CIEDE2000 n(%)
ACCEPTABILITY		
ACCEPTABLE	227 (31,53)	130 (18,06)
NON-ACCEPTABLE	493 (68,47)	590 (81,94)
PERCEPTIBILITY		
NON-PERCEIVABLE	72(10,00)	3,6 (5,00)
PERCEIVABLE	648 (90,00)	684 (95,00)

3 CONCLUSÃO

Apesar das limitações de um estudo *in vitro*, os resultados desse presente estudo mostram que o jateamento com bicarbonato de sódio com posterior clareamento e o clareamento usado sozinho são os métodos mais eficazes para a remoção de pigmentos depositados sobre bráquetes estéticos, protelando, assim, a necessidade de remoção desses bráquetes.

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The introduction must be presented in a structured format, covering the following subjects, although not under subheadings: succinct statements of the issue in question, and the essence of existing knowledge and understanding pertinent to the issue. In keeping with the house style of Journal of Dentistry, the final paragraph of the introduction should clearly state the aims and/or objective of the work being reported. Prospective authors may find the following form of words to be helpful: "The aim of this paper is to ..." Where appropriate, a hypothesis (e.g. null or a priori) should then be stated.

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Wilson NHF, Mjör I. The teaching of class I and class II direct composite restorations in European dental schools. *Journal of Dentistry* 2000; **28**: 15-21.

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[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, *J. Sci. Commun.* 163 (2010) 51–59.

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[2] W. Strunk Jr., E.B. White, *The Elements of Style*, fourth ed., Longman, New York, 2000.

Reference to a chapter in an edited book:

[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), *Introduction to the Electronic Age, E-Publishing Inc.*, New York, 2009, pp. 281–304.

Reference to a website:

[4] Cancer Research UK, *Cancer statistics reports for the UK*. <http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/>, 2003 (accessed 13.03.03).

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