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AVALIAÇÃO "IN VITRO" DE DIFERENTES TÉCNICAS DE CLAREAMENTO DE DENTES ESCURECIDOS POR SANGUE

Santa Maria, RS
2019

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Dentística, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Odontológicas**.

Orientador: Prof. Dra. Letícia Brandão Durand

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Aprovado em 11 de Julho de 2019:

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Santa Maria, RS
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RESUMO

AVALIAÇÃO "IN VITRO" DE DIFERENTES TÉCNICAS DE CLAREAMENTO DE DENTES ESCURECIDOS POR SANGUE

AUTOR: Bruna Gaidarji
ORIENTADOR: Prof. Dra. Letícia Brandão Durand

Neste trabalho será apresentado um estudo que avaliou a efetividade de três técnicas de clareamento em dentes manchados por sangue, simulando um escurecimento dental pós hemorragia pulpar. Trata-se de um estudo laboratorial, in vitro, em que dentes humanos provenientes do Banco de Dentes Permanentes Humanos do Curso de Odontologia da Universidade Federal de Santa Maria, foram selecionados e submetidos a um protocolo de manchamento por sangue humano. Após o manchamento, os dentes foram randomizados e separados em grupos (n=10), de acordo com uma das três técnicas clareadoras. O primeiro grupo foi submetido à técnica Walking Bleach, utilizando perborato de sódio associado a peróxido de hidrogênio líquido a 20%, aplicando a mistura na câmara pulpar. O segundo grupo foi submetido à técnica Inside/Ouside, utilizando peróxido de hidrogênio a 7,5% interna e externamente. O terceiro grupo foi submetido à técnica de clareamento de Consultório, utilizando peróxido de hidrogênio a 35% interna e externamente. A leitura da cor dos dentes foi realizada em cinco tempos: após o protocolo de manchamento (T0 – baseline), após uma semana de clareamento (T1), após duas semanas de clareamento (T2), após três semanas de clareamento - conclusão do tratamento (T3) e após uma semana de acompanhamento (T4), por meio de um espectrofotômetro clínico (Easyshade Advance 4.0, VITA), que apresenta a mensuração da cor em números, onde as coordenadas L* a* b* da cor foram anotadas. As coordenadas foram inseridas nas fórmulas CIELAB e CIEDE2000, para calcular a diferença de cor. A diferença de cor (ΔE) foi calculada entre T1-T0 (1 semana de clareamento), T2-T0 (2 semanas de clareamento), T3-T0 (finalização do clareamento), T4-T0 (acompanhamento de uma semana). A distribuição normal dos dados foi verificada pelo teste Kolmogorov-Smirnov, e os valores médios de ΔE_{ab}, ΔE_{00}, ΔL*, Δa* e Δb* foram submetidos aos testes ANOVA e Tukey PostHoc. A análise estatística utilizou $\alpha=0.05$ como significância. Os resultados demonstraram semelhante efetividade de clareamento entre as técnicas no período de acompanhamento de 1 semana. A técnica de clareamento em Consultório apresentou mudança de cor expressiva desde a primeira semana de tratamento, enquanto as técnicas Walking Bleach e Inside/Outside, demonstram maior efetividade no clareamento a partir da segunda semana de tratamento.

Palavras-chave: Clareamento dental. Descoloração de dente. Manchas de sangue. Perborato de sódio. Permeabilidade dentária. Peróxido de hidrogênio.

ABSTRACT

"IN VITRO" EVALUATION OF DIFFERENT BLEACHING TECHNIQUES ON BLOOD-STAINED TEETH

AUTOR: Bruna Gaidarji
ORIENTADOR: Prof. Dra. Letícia Brandão Durand

This research will present a study that evaluated the effectiveness of three bleaching techniques on blood stained teeth, simulating a dental discoloration after pulpal hemorrhage. It is an *in vitro* study, in which human teeth were selected from the Human Bank of teeth of the Dentistry School of the Federal University of Santa Maria, and submitted to a human blood staining protocol. After staining, the teeth were randomized and separated into groups (n=10) according to one of three whitening techniques. The first group was submitted to the Walking Bleach technique, using sodium perborate associated with liquid hydrogen peroxide at 20%, applying the mixture to the pulp chamber. The second group was submitted to the Inside/Outside technique, using hydrogen peroxide at 7.5% internally and externally. The third group was submitted to the In-Office bleaching technique, using hydrogen peroxide at 35% internally and externally. The color measurements were performed at five time points: after the staining protocol (T0 - baseline), after one week of bleaching (T1), after two weeks of bleaching (T2), after completion of treatments (T3) and one-week follow-up (T4), using a clinical spectrophotometer (Easyshade Advance 4.0, VITA), which shows the color measurement in numbers, where the coordinates L * a * b * of the color were annotated. The coordinates were inserted into CIELAB and CIEDE2000 the formulas, to calculate the color difference (ΔE^*_{ab} and ΔE_{00}, respectively). The color difference (ΔE) was calculated between T1-T0 (1 week of bleaching), T2-T0 (2 weeks of bleaching), T3-T0 (completion of treatments) and T4-T0 (one-week follow-up). The normal distribution of the data was verified by the Kolmogorov-Smirnov test, and the mean values of ΔE^*_{ab}, ΔE_{00}, ΔL^*, Δa^* and Δb^* were submitted to ANOVA and Tukey's PostHoc tests. Statistical analysis used $\alpha=0.05$ as significance. The results demonstrated that all bleaching techniques presented similar effectiveness at 1-week follow-up. The In-Office bleaching technique, presented expressive color change since the first application, as for Walking bleach and Inside/Outside techniques, demonstrated greater bleaching effectiveness from the second week onwards of treatment.

Keywords: Blood stains. Hydrogen peroxide. Sodium Perborate. Tooth bleaching. Tooth discoloration. Tooth permeability.

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1 INTRODUÇÃO

O traumatismo dental constitui a principal causa das alterações de cor nos dentes (GUPTA e SAXENA, 2014). O escurecimento pode ocorrer principalmente pelo extravasamento de sangue na porção coronária e pela presença de restos de tecido pulpar provenientes do tratamento endodôntico. Os produtos da degradação do sangue liberam ferro durante a hemólise que, combinado com sulfeto de hidrogênio, pigmenta a estrutura dental (GLOCKNER et al., 1999).

Dentes anteriores escurecidos comprometem a estética do sorriso e geram uma grande demanda por tratamentos estéticos(ALMOHAREB, 2017; GBADEBO e AJAYI, 2015; GUPTA e SAXENA, 2014). O correto diagnóstico da etiologia do escurecimento é importante na determinação do tratamento apropriado para cada situação. Além disso, deve-se considerar a expectativa e a percepção de estética do paciente na indicação do tratamento estético (NIXON et al., 2007).

Abordagens minimamente invasivas devem ser priorizadas no tratamento de dentes escurecidos (NIXON et al., 2007). Facetas de resina composta, de cerâmica ou coroas cerâmicas são procedimentos que demandam desgaste da estrutura dental, possuem custo mais elevado e são mais invasivos quando comparados às técnicas de clareamento (GLOCKNER et al., 1999). O clareamento, apresenta muitas vantagens, pois além de permitir a conservação da estrutura dental, é mais econômico (ALMOHAREB, 2017). Quando os resultados são efetivos, pode-se observar efeitos positivos na percepção estética e nas relações sociais (BERSEZIO et al., 2018).

O processo clareador do dente escurecido acontece por meio da liberação do oxigênio do agente clareador (BERSEZIO et al., 2018). O mecanismo basicamente consiste na divisão do peróxido em água e oxigênio, que se difunde através da estrutura dental, causando oxidação e redução de pigmentos orgânicos (DEMARCO et al., 2013).

Uma variedade de técnicas e materiais estão disponíveis para clareamento vital e não vital (NIXON et al., 2007). Para o tratamento de dentes escurecidos com tratamento endodôntico pode-se utilizar abordagens intracoronárias, extracoronárias ou uma combinação de procedimentos internos e externos (DIETSCHI, 2006; JOINER e LUO, 2017; PLOTINO et al., 2008). A técnica Walking Bleach é um tratamento intracoronário que utiliza uma mistura de perborato de sódio com peróxido de hidrogênio líquido ou água destilada na câmara pulpar do dente escurecido, sendo trocada em intervalos regulares até a obtenção da cor desejada (SULIEMAN, 2017).

Outra opção de tratamento de dentes escurecidos com endodontia é o clareamento realizado no consultório, em que peroxido de hidrogênio entre 30-37% é aplicado, por um determinado período, internamente na câmara pulpar e/ou externamente da face vestibular e lingual/palatina do dente a ser clareado (SULIEMAN, 2017). O clareamento também pode ser realizado pela técnica Inside/Outside, na qual utiliza-se o peróxido de carbamida ou peróxido de hidrogênio em baixas concentrações. Essa técnica é uma combinação do clareamento caseiro supervisionado e do clareamento interno, pois após o selamento cervical ser realizado, o dente permanece aberto e o paciente utiliza uma moldeira customizada para depositar o gel clareador no dente e na câmara pulpar, poucas horas por dia, de acordo com o protocolo estabelecido pelo profissional, (SULIEMAN, 2017).

Embora existam diferentes produtos, protocolos e técnicas disponíveis, a literatura é escassa em estudos comparativos entre as técnicas e materiais (BERSEZIO e LEDEZMA et al., 2018; DIETSCHI, 2006; LISE et al., 2018). Não há um consenso sobre a efetividade e os benefícios distintos de cada técnica (POYSER et al., 2004) e poucas evidências científicas, relacionadas à técnica de clareamento interno e externo, estão disponíveis. Ademais, o clareamento de dentes escurecidos por hemorragia ainda é considerado um desafio. Assim, a importância do presente estudo que teve por objetivo avaliar efetividade de três técnicas de clareamento dental para dentes escurecidos por sangue. As técnicas avaliadas foram o clareamento interno (Walking Bleach - perborato de sódio), o clareamento de consultório (peróxido de hidrogênio 35%) e o clareamento interno e externo supervisionado (Inside/Outside - peróxido de hidrogênio 7,5%).

2 ARTIGO – Effectiveness of three bleaching techniques on blood-stained teeth - an in vitro study

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Effectiveness of three bleaching techniques on blood-stained teeth - an in vitro study

Running tittle: In vitro effectiveness of bleaching techniques

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Abstract

Effectiveness of three bleaching techniques on blood-stained teeth - an in vitro study

Summary: The aim of this in vitro study was to evaluate the bleaching effectiveness of the walking bleach, the inside/outside and the in-office techniques on blood-stained teeth. Thirty extracted third molars were selected from a tooth bank, prepared and stained with human blood. After randomization, the teeth were allocated to three groups (n=10), according to the bleaching agents and techniques to be tested: walking bleach with sodium perborate (WB-SP), inside/outside bleaching with 7.5% hydrogen peroxide (I/O-HP7.5), and in-office bleaching with 35% hydrogen peroxide (OF-HP35). Color measurements were performed in triplicate with a clinical spectrophotometer. An individual silicone guide with a central orifice was used to standardize the exact location of each color measurement. The color measurements were conducted at baseline (T0) and weekly (T1, T2 and T3), until treatment was completed, and again at one-week follow-up (T4). Color difference was calculated by CIELab and CIEDE2000 formulas from mean L*, a*, b* values, according to each evaluation period and baseline. The mean values for ΔE^*_{ab}, ΔE_{00}, ΔL^*, Δa^* and Δb^* were submitted to ANOVA and Tukey's post-hoc tests. Significant differences were observed between bleaching techniques (p=0.00) and evaluation timepoints (p=0.03) for Δb^*, and among evaluation timepoints for ΔE_{00}, ΔE^*_{ab} and ΔL^* (p=0.00). Conversely, regarding Δa^*, no significant differences were observed between the techniques (p=0.24) and evaluation timepoints (p=0.06). All techniques and bleaching agents presented color variation within the perceptibility and acceptability interpretation thresholds adopted for whitening-dependent color differences for ΔE^*_{ab} and ΔE_{00} ($\Delta E_{00}>5.4$ and $\Delta E^*_{ab}>8.1$). All the techniques presented similar bleaching effectiveness at the one-week follow-up evaluation, with no color regression, thereby indicating treatment maintenance and stability.

Clinical Relevance: This in vitro study showed that the walking bleach with sodium perborate, the inside/outside bleaching with 7.5% hydrogen peroxide, and the in-office bleaching with 35% hydrogen peroxide techniques presented similar effectiveness and stability for bleaching of blood-stained teeth.

Keywords: Tooth Bleaching; Nonvital Tooth Bleaching; Intracoronal Bleaching; Discoloration, Tooth Permeability; Blood Stain; Color; Hydrogen Peroxide; Sodium Perborate

Introduction

Dental discoloration leads to esthetic problems that compromise self-esteem, especially if the anterior zone is affected[1–3]. A psychophysical study on color perception and whiteness of teeth indicates that whiter teeth are associated with positive personality traits, such as greater competence, intellectual capacity and satisfaction in relationships[4]. After bleaching treatments, patients commonly report a positive effect regarding esthetic perception and social life, as well as improvement of interpersonal relations[5].

Discoloration of non-vital teeth may occur due to pulp necrosis, filling materials, hemorrhage after trauma, and pulp removal[2, 3, 6, 7]. The major cause of discoloration of non-vital anterior teeth is pulp necrosis associated with dental trauma[3]. Dental trauma causes rupture of blood vessels, and consequent penetration of erythrocytes into dentinal tubules. The erythrocytes undergo hemolysis and release hemoglobin, which breaks down and releases iron, responsible for tooth pigmentation[6, 7].

Discoloration is common among young adults[1–3], and has prompted a greater demand for esthetic treatment[4]. Treatment options for cosmetic improvement of discolored teeth include bleaching, adhesive restorations, veneers, and ceramic crowns[3, 8]. Bleaching is the most conservative choice, since it presents positive esthetic outcomes[9], and is less invasive than prosthetic treatments [3, 8, 10–12]. Bleaching of nonvital teeth is minimally invasive and effective, and presents considerable stability over time[5, 13]. In addition, it is a faster and less costly treatment than prosthetic rehabilitation [14].

Different protocols have been proposed for bleaching of non-vital discolored teeth, including the walking bleach, the in-office bleaching and the inside/outside bleaching techniques[10]. These methods may be applied either intracoronally or extracoronally, or else both intra- and extracoronally [4, 10, 12]. The bleaching agents may vary in concentration, composition and instructions for use, according to the technique. The bleaching agents currently used are hydrogen peroxide and carbamide peroxide, at concentrations ranging from 4% to 40%, as well as sodium perborate used alone or in association with hydrogen peroxide[8, 12, 15–17]. Although many products, protocols and techniques have been tested, there is no consensus regarding the effectiveness of these bleaching options on non-vital teeth[9, 18, 19].

The aim of this study was to evaluate the effectiveness of three bleaching techniques (walking bleach, inside/outside bleaching and in-office-bleaching) on discolored teeth stained by blood. The bleaching agents varied in composition, concentration and instructions for use. In addition, the color change was evaluated at different timepoints during treatment (T1, T2,

and T3), as well as at one-week follow-up (T4). The null hypothesis was that there would be no difference in color change among the bleaching techniques, at each evaluation timepoint during treatment and at the follow-up evaluation.

Methods and Materials

Study design and ethics

This in vitro study was reviewed and approved by the research ethics committee of the participating institution (protocol number 2.576.646). The effectiveness of three non-vital bleaching techniques was evaluated on extracted human teeth stained with blood. The color change was measured by a clinical spectrophotometer (Easyshade Advance 4.0, VITA), and calculated by the CIELab and CIEDE 2000 formulas at baseline (after staining) and after bleaching, at different time intervals and at one-week follow-up.

Tooth preparation and staining

Human third molars with intact crowns were selected from a tooth bank. After visual inspection, the teeth with excessive wear, fissures, fractures and discolorations were excluded. Thirty teeth were included in the study. They were cleaned with an ultrasonic scaler to remove all calculus and remnants of the periodontal ligament, polished with a rubber cup and pumice, washed, and stored in distilled water.

The teeth were sectioned transversely 2 mm below the cemento-enamel junction with a water-cooled diamond disc, and the pulp tissue was removed. Artificial staining of the specimens was performed with the same methodology proposed by Frecia & Peters (1982)[6], in order to simulate discoloration by intrapulpal hemorrhage, and standardize the color shade before bleaching procedures. The human blood samples were obtained by donation of blood bags that had an expired shelf life, or that would not be used by the university hospital hemotherapy service of the participating institution.

The specimens were completely immersed in test tubes containing 10 mL of human blood. A high speed centrifuge (Centrifuge 5804R, Eppendorf, São Paulo, SP, Brazil) was used at 10,000 rpm for ten minutes at 37°C twice daily for three consecutive days for centrifuging

of samples, hemolysis of red cells, and penetration of degradation products into the dentin tubules.

Afterwards, the teeth were removed from the test tubes, and 20 mL of distilled water was added to the blood samples and centrifuged again to hemolysate the red blood cells, resulting in two layers: a precipitate containing the cell membranes, and a hemolysate containing the hemoglobin protein. The hemolysate was collected with a pipette, placed back into the test tubes containing the teeth, and centrifuged for another three days as described previously. Next, the teeth were washed with distilled water to remove excess blood pigment.

Bleaching protocols

The specimens were randomized and assigned to groups according to the bleaching techniques: walking bleach with sodium perborate – WB-SP (n=10), inside/outside bleaching with 7.5% hydrogen peroxide – I/O-HP7.5 (n=10), and in-office bleaching with 35% hydrogen peroxide – OF-HP35 (n=10). The bleaching agents ranged in concentration and instructions for use. The staining procedure, bleaching treatments and color measurement were carried out by the same operator. Information regarding the bleaching techniques, bleaching agent composition, manufacturer and batch number are presented in Table 1, and the group division and methodology flowchart are presented in Figure 1.

Sodium perborate (Whiteness Perborato, FGM, Joinville, SC, Brazil) was used in the walking bleach protocol (WB-SP). A homogenous mixture of sodium perborate with 20% hydrogen was applied to the pulp chamber and sealed with a temporary restorer (Bioplic, Biodinâmica, Ibiporã, PR, Brazil). A fresh portion of perborate was applied and sealed with a temporary material at 7 and 14 days. The product was completely removed 7 days after the last application. The complete treatment consisted of three applications of the sodium perborate mixture and twenty-one full days of treatment.

The inside/outside protocol (I/O-HP7.5) was performed with 7.5% hydrogen peroxide (HP7.5 - White Class, FGM, Joinville, SC, Brazil). The bleaching gel was applied to the pulp chamber using a needle-tipped syringe, and also to the dental surface, forming a 1-mm-thick layer. The application time was two hours per day for twenty-one consecutive days. After removal of the gel, the specimens were cleaned with distilled water.

The in-office protocol (OF-HP35) was carried out with 35% hydrogen peroxide (HP35 - Whiteness HP, FGM, Joinville, SC, Brazil). The gel was applied weekly for four weeks,

according to the manufacturer's instructions (3 drops of hydrogen peroxide to 1 drop of thickener, mixed homogenously and applied to both the dental surface and internally in the pulp chamber, in sessions of 3 applications lasting 15 minutes each). The total treatment time was 4 sessions lasting 45 minutes each, with a 7-day interval between each session.

A compress with deionized water was placed under each tooth during the I/O-HP7.5 and OF-HP35 bleaching processes to avoid dehydration. The WB-SP and I/O-HP7.5 treatments were discontinued after 21 days, whereas the OF-HP35 treatment was discontinued after four applications of the bleaching protocol. The final color measurement was made after completing the treatments (T3), and at one-week follow-up, (T4) to allow release of the residual oxygen, enable rehydration of the specimens, and evaluate stability of the treatments.

Color measurement

After staining the specimens, the color of each one was measured with a clinical spectrophotometer (Easyshade Advance 4.0, VITA) at baseline and weekly, for 28 days: T0 - baseline, T1 - 7 days, T2 - 14 days, T3 - 21 days (completion of treatments), T4 - 28 days (one-week follow-up).

An individual silicone (Zetalabor, Zhermack, Labordental, São Paulo, SP, Brazil) guide with a central orifice was used to standardize the exact location of each color measurement. The orifice was made in the silicone guide with a 6-mm cylinder cutting edge positioned on the middle third of the largest surface of each tooth (Figures 2 and 3).

Three color measurements per specimen were made with a clinical spectrophotometer (Easyshade Advance 4.0, VITA) by a single trained operator, and the mean L*, a*, b* values were calculated for each tooth, at each timepoint.

The effectiveness of the treatments was evaluated by ΔE_{ab}^* and ΔE_{00}; the color difference was calculated by the CIELab and CIEDE2000 equations, and by ΔL^*, Δa^* and Δb^*, at baseline (T0) and at the different timepoints (T1, T2, T3 and T4).

The CIELab color difference (ΔE^*_{ab}) was calculated as follows[20]:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b)^2]^{1/2}$$

where ΔL* corresponds to lightness, Δa* refers to the green-red and Δb* to the blue-yellow axis differences at T0, and to T1, T2, T3 and T4, respectively.

The CIEDE2000 color difference (ΔE_{00}) calculation was[20]:

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

where ΔL', ΔC' and ΔH' refer to lightness, chroma, and hue differences among the color measurements. KL, KC and KH are the parametric factors for the conditions and illuminating influence, set at 1 in this study[21]. RT (rotation function) accounts for the interaction of hue and chroma differences in the blue region. SL, SC and SH are the weighting functions for the color difference adjustment, considering the location variation of L*, a* and b* coordinates.

Statistical analysis

The normal distribution was verified by the Kolmogorov-Smirnov test, and the mean values of ΔE^*_{ab}, ΔE_{00}, ΔL*, Δa* and Δb* were submitted to ANOVA and Tukey's post-hoc tests. Statistical analysis was performed at $\alpha=0.05$ using OriginPro 2015 software (OriginLab Co; Northampton, MA, USA).

The effectiveness of the bleaching technique and the interpretation of whitening-dependent color differences were based on perceptibility thresholds and interpretation ratings reported by Paravina, Pérez & Ghinea (2019)[22]. The whitening outcome with color differences exceeding $\Delta E_{00}>5.4$ and $\Delta E^*_{ab}>8.1$ were assumed to result in excellent bleaching effectiveness.

Results

All the techniques and bleaching agents presented color variation within the perceptibility and acceptability interpretation thresholds adopted for whitening-dependent color differences for ΔE^*_{ab} and ΔE_{00} ($\Delta E_{00}>5.4$ and $\Delta E^*_{ab}>8.1$)[22], indicating excellent effectiveness of the bleaching treatments.

Significant differences were observed between the bleaching techniques (p=0.00) and the evaluation timepoints (p=0.03). For Δb* the I/O-HP7.5 technique showed greater reduction in Δb* than WB-SP, at 14 days (T0-T2) and after completion of the bleaching treatments (T0-T3).

As for ΔE_{00}, ΔE^*_{ab} and ΔL*, significant differences were observed only among evaluation timepoints (p=0.00). The WB-SP technique showed higher ΔE_{00} and ΔL* values at T2, T3 and T4, and higher ΔE^*_{ab} values at T3 and T4. As for the I/O-HP7.5 technique, significant differences were observed only for ΔL*, where higher ΔL* was found after T3 and T4. As for Δa*, no significant differences were observed among the techniques (p=0.24) and evaluation timepoints (p=0.06). Regarding the OF-HP35 technique, no significant differences were observed for any of the color parameter changes at the evaluation timepoints (T0-T1; T0-T2; T0-T3 and T0-T4).

Graph 1 shows a tendency of continuous increase in luminosity in all the experimental groups during the treatments. As for the red-green and the yellow-blue axes, there was an abrupt decrease in values, especially after the first evaluation. This behavior tends to be repeated in subsequent evaluations for the a* (red-green axis) coordinate. However, a slight recurrence was observed for the b* coordinate after the second evaluation, and a further reduction was seen in the values after completion of the treatment and at one-week follow-up.

Discussion

The present study evaluated the effectiveness of different bleaching techniques and products on teeth stained by blood. The staining method reported by Freccia & Peters, 1982[6], was used to generate standardized staining of the samples, compatible with clinical situations where there is severe discoloration. After the staining protocol was completed, color measurements were performed with a clinical spectrophotometer, resulting in L, a*, b* values in the range of the C3, C4 and B4 shades of the Vita Lumin Classic Shade Guide[23]. The baseline point indicated that the blood staining method produced sufficient staining for evaluation of the bleaching agents[7], thus representing a challenging clinical situation[24–26].

The null hypothesis was partially accepted, since no difference was observed in the whitening-dependent color change among the bleaching techniques at follow-up. All the techniques showed adequate bleaching, as well as maintenance and stability of the treatments over time. However, the number of applications influenced the final color change. Regarding

the walking bleach and the inside/outside techniques, color change increased after the second and third sessions of treatment. As for the in-office technique, expressive color variation was observed as of the first application, indicating that this treatment produces color change more rapidly[13].

Bleaching is considered effective when a change of at least 5 units of ΔE is achieved[27]. In the present study, a color change in ΔE^*_{ab} of approximately 30 units for walking bleach, 29 units for inside/outside bleaching and 25 units for in-office bleaching was obtained. As for ΔE_{00}, a color change of 20 units, 17 units, and 16 units was achieved for walking bleach, inside/outside bleaching and in-office bleaching, respectively.

Traditionally the CIELab formula is used to calculate color difference; however, the CIEDE2000 formula is a more recent method, and presents important adjustments that improve assessment of visual data in dentistry[22, 28, 29]. Thus, the use of the CIEDE2000 formula is strongly suggested for color assessment research, inasmuch as it provides a better representation of visual perception[30–33]. The present study provided data on color difference using both formulas, that presented similar results. The whiteness index is recommended for complementary information in dentistry [22, 34], since it is a reliable tool for predicting and quantifying tooth whiteness[35].

When the L*a*b* parameters were separately analyzed it was found that all bleachjng treatments were able to produce an increase of L* and a reduction of a* and b* values. This behavior is expected after bleaching, and is consistent with studies that have evaluated the effectiveness of bleaching agents and techniques[16, 17, 36, 37]. The threshold for discrimination of whiteness ranges from 1.10-1.15 units for L* and b*, and is 3.24 for a*[38]. The bleaching technique evaluated in the present study showed an increase in L* and a decrease in a* and b* coordinates exceeding the thresholds for whiteness perception. These features resulted in yellowness reduction and consequently in lighter and more luminous teeth. [16, 17, 39, 40].

Research results must take into account not only the analytic statistics, but also the perceptibility and acceptability thresholds, which provide relevant information commensurate with real life situations and clinical outcome[22, 34]. In the present study, the interpretation of bleaching technique effectiveness was based on perceptibility threshold ratings reported by Paravina, Pérez & Ghinea (2019)[22]. All the test groups obtained whitening results that exceeded $\Delta E_{00}>5.4$ and $\Delta E^*_{ab}>8.1$, considered excellent bleaching effectiveness[22]. The clinical outcome should consider not only perceptual thresholds, but also the patient's individual esthetic standards and expectation[34].

Comparison of our data with that of other studies was difficult due to variations in techniques, formulations and concentrations of the bleaching agents, as well as methodological differences. Yui et al (2008)[7] evaluated the effectiveness of the walking bleach technique on blood stained teeth. The staining technique, instrumental color measurement, evaluation periods and color change researched in their study were similar to ours. However, they conducted no follow-up, and used different formulations for the walking bleach technique. Valera et al (2009)[41] evaluated the effectiveness of walking bleach with sodium perborate, and sodium perborate associated with 16% carbamide peroxide on teeth stained by rabbit blood. The color was analyzed at the same evaluation periods; however, follow-up was not performed, and color change was evaluated by subjective analysis with Vita shade guide.

The present in vitro study evaluated the different timepoints during the treatments, and a 1-week follow-up. Longitudinal clinical trials are needed to investigate the long-term side effects, risks, and treatment recurrence and stability[11, 19, 42]. Berzebio et al, 2017[9] and 2018[13], evaluated the walking bleach technique in vivo with 35% hydrogen peroxide and 37% carbamide peroxide. Follow-up results were recorded after 1 week, 1 month [9], and 6 months of treatment[13]. Both treatments were highly effective and stable, with color change values similar to those of our study. Lise et al (2018)[19] compared the efficacy of walking bleach with sodium perborate, and inside-outside bleaching with 10% carbamide peroxide in patients. Both treatments presented adequate bleaching, which remained stable for 1 year, with mean ΔE^*_{ab} values consistent with those of our results.

Although several studies have assessed the walking bleach[7–9, 19, 43], the in-office bleaching[10, 17, 32, 44–46] and the inside-outside techniques, previous research has assessed inside-outside technique only with carbamide peroxide[19, 47–49]. We believe this is the first in vitro study that evaluates the effectiveness of the inside-outside technique with a low concentration of hydrogen peroxide (7.5%). The results of the present study revealed that this combination could be an effective and reliable option for the bleaching of non-vital discolored teeth.

Considering that all the techniques and bleaching agents resulted in a similar bleaching effect at the follow-up period, any of the modalities studied could be applied successfully. When patients expectation a rapid bleaching effect, and longer chair time is not an issue, in-office treatment with 35% hydrogen peroxide should be the first treatment option[16]. On the other hand, when time and number of appointments are not determining factors, the inside/outside and the walking bleach techniques are other options that should be considered.

The appropriate choice should take into account the peculiarities and characteristics of each technique. Certain risks associated with the inside/outside technique should be borne in

mind before indicating this modality. During the inside/outside treatment, the access remains open, thus allowing bacteria and stains to penetrate into dentin[12]. Additionally, teeth with extensive loss of structure may present increased risk of fracture, thus making closed access techniques more indicated[12, 47].

Another point regarding the treatment choice is patient compliance. The success of the inside/outside technique is directly associated with patient cooperation and commitment[19]. Therefore, this technique should be indicated with caution for patients with limited motor ability, low motivation or poor collaborative skills. In-office bleaching or walking bleach techniques are more indicated in these cases, because they do not require patient collaboration regarding product use[19, 47].

Information regarding efficacy of bleaching treatments, number of appointments, chair time, and the need for patient collaboration and compliance, as well as possible recurrence of former tooth color should be provided to the patients beforehand[8, 17]. Well-informed patients tend to present high overall satisfaction with the treatment[8]. Additionally, selection of the technique and bleaching agents should be based on proper diagnosis and understanding of bleaching mechanisms and biological factors associated with successful outcomes[8, 12, 24].

Conclusion

All the techniques studied presented similar efficacy in the bleaching of blood-stained teeth at the one-week follow-up evaluation. Regarding the in-office technique, optimal color change was observed as of the first application. As for the walking bleach and inside/outside techniques, greater color change was observed from the second week of treatment onwards.

References

1. Xiao J, Zhou XD, Zhu WC, Zhang B, Li JY, & Xu X (2007) The prevalence of tooth discolouration and the self-satisfaction with tooth colour in a Chinese urban population *Journal of Oral Rehabilitation* **34(5)** 351–360, https://doi.org/10.1111/j.1365-2842.2007.01729.x.
2. Hasan MM, Nademi Q, Askari Z, Zaman S, & Sharbatti S Al (2014) Prevalence of Self-Reported Tooth Discoloration among the students of Gulf Medical University , Ajman , UAE In: *Proceedings of the 6th Annual Scientific Meeting of Gulf Medical University* 70–80.
3. Gbadebo S, & Ajayi D (2015) Self Reported Tooth Discolorations Among Patients Seen At Dental Center University College Hospital Ibadan Decolorations Dentaires

Auto-Rapportees Chez Les Patients Vus Au Centre Dentaire Du Centre Hospitalier Universitaire D ' Ibadan *Journal of the West African College of Surgeons* **5(3)** 66–77.
4. Joiner A, & Luo W (2017) Tooth colour and whiteness: A review *Journal of Dentistry* **67(June)** S3–S10, https://doi.org/10.1016/j.jdent.2017.09.006.
5. Bersezio C, Martín J, Mayer C, Rivera O, Estay J, Vernal R, Haidar ZS, Angel P, Oliveira OB, & Fernández E (2018) Quality of life and stability of tooth color change at three months after dental bleaching *Quality of Life Research* **27(12)** 3199–3207, https://doi.org/10.1007/s11136-018-1972-7.
6. Freccia WF, & Peters DD (1982) A technique for staining extracted teeth: a research and teaching aid for bleaching *Journal of Endodontics* **8(2)** 67–69, https://doi.org/10.1016/S0099-2399(82)80260-4.
7. Yui KCK, Rodrigues JR, Mancini MNG, Balducci I, & Gonçalves SEP (2008) Ex vivo evaluation of the effectiveness of bleaching agents on the shade alteration of blood-stained teeth *International Endodontic Journal* **41(6)** 485–492, https://doi.org/10.1111/j.1365-2591.2008.01379.x.
8. Gupta SK, & Saxena P (2014) Evaluation of patient satisfaction after non-vital bleaching in traumatized discolored intact anterior teeth *Dental Traumatology* **30(5)** 396–399, https://doi.org/10.1111/edt.12109.
9. Bersezio C, Martin J, Peña F, Rubio M, Estay J, Vernal R, Junior OO, & Fernández E (2017) Effectiveness and Impact of the Walking Bleach Technique on Esthetic Self-perception and Psychosocial Factors: A Randomized Double-blind Clinical Trial *Operative Dentistry* **42(6)** 596–605, https://doi.org/10.2341/16-133-C.
10. Dietschi D, Rossier S, & Krejci I (2006) In vitro colorimetric evaluation of the efficacy of various bleaching methods and products. *Quintessence international (Berlin, Germany : 1985)* **37(7)** 515–26.
11. Amato M, Serena Scaravilli M, Farella M, & Riccitiello F (2006) Bleaching teeth treated endodontically: Long-term evaluation of a case series *Journal of Endodontics* **32(4)** 376–378, https://doi.org/10.1016/j.joen.2005.08.018.
12. Plotino G, Buono L, Grande NM, Pameijer CH, & Somma F (2008) Nonvital Tooth Bleaching: A Review of the Literature and Clinical Procedures *Journal of Endodontics* **34(4)** 394–407, https://doi.org/10.1016/j.joen.2007.12.020.
13. Bersezio C, Ledezma P, Mayer C, Rivera O, Junior OBO, & Fernández E (2018) Effectiveness and effect of non-vital bleaching on the quality of life of patients up to 6 months post-treatment: a randomized clinical trial *Clinical Oral Investigations* **22(9)** 3013–3019, https://doi.org/10.1007/s00784-018-2389-y.
14. Almohareb T (2017) Management of Discolored Endodontically Treated Tooth Using Sodium Perborate *Journal of International Oral Health* **9(3)** 133–5, https://doi.org/10.4103/jioh.jioh.
15. Gökay O, Ziraman F, Çali Asal A, & Saka OM (2008) Radicular peroxide penetration from carbamide peroxide gels during intracoronal bleaching *International Endodontic Journal* **41(7)** 556–560, https://doi.org/10.1111/j.1365-2591.2008.01384.x.
16. Borges A, Zanatta R, Barros A, Silva L, Pucci C, & Torres C (2014) Effect of Hydrogen Peroxide Concentration on Enamel Color and Microhardness *Operative Dentistry* **40(1)** 96–101, https://doi.org/10.2341/13-371-l.
17. Lee SS, Kwon SR, Ward M, Jenkins W, Souza S, & Li Y (2018) A 3 months clinical evaluation comparing two professional bleaching systems of 25% and 40% hydrogen peroxide and extended treatment outcome using a power versus a manual toothbrush *Journal of Esthetic and Restorative Dentistry* **(September 2017)** 124–131, https://doi.org/10.1111/jerd.12440.
18. Dietschi D (2006) Nonvital bleaching: general considerations and report of two failure

cases. *The European journal of esthetic dentistry* **1(1)** 52–61.
19. Lise DP, Siedschlag G, Bernardon JK, & Baratieri LN (2018) Randomized clinical trial of 2 nonvital tooth bleaching techniques: A 1-year follow-up *Journal of Prosthetic Dentistry* **119(1)** 53–59, https://doi.org/10.1016/j.prosdent.2017.03.004.
20. Cie (2004) CIE technical report *Colorimetry, 3rd Edition* **552** 24, https://doi.org/ISBN 3 901 906 33 9.
21. Kamishima N, Ikeda T, & Sano H (2005) Color and Translucency of Resin Composites for Layering Techniques *Dental Materials* **24(3)** 428–432.
22. Paravina RD, Pérez MM, & Ghinea R (2019) Acceptability and perceptibility thresholds in dentistry: A comprehensive review of clinical and research applications *Journal of Esthetic and Restorative Dentistry* **31(2)** 103–112, https://doi.org/10.1111/jerd.12465.
23. Park JH, Lee YK, & Lim BS (2006) Influence of illuminants on the color distribution of shade guides *Journal of Prosthetic Dentistry* **96(6)** 402–411, https://doi.org/10.1016/j.prosdent.2006.10.007.
24. Chu SJ, & Mieleszko AJ (2015) Color matching strategies for non-vital discolored teeth: Part 2. In-vivo bleaching options for discolored teeth preparations *Journal of Esthetic and Restorative Dentistry* **27(S1)** S18–S23, https://doi.org/10.1111/jerd.12153.
25. Freire A, & Archegas LRP (2010) Porcelain laminate veneer on a highly discoloured tooth: a case report. *Journal (Canadian Dental Association)* **76(a)** 126.
26. Todd M, Brackett W, & Romero M (2017) Correction of a Single Discolored Anterior Tooth Due to Internal Resorption: A Clinical Report. *Compendium of continuing education in dentistry (Jamesburg, NJ : 1995)* **38(5)** e13–e16.
27. Paravina R (2008) New shade guide for tooth whitening monitoring: Visual assessment *The Journal of Prosthetic Dentistry* **99(3)** 178–184.
28. Ghinea R, Pérez MM, Herrera LJ, Rivas MJ, Yebra A, & Paravina RD (2010) Color difference thresholds in dental ceramics In: *Journal of Dentistry* Elsevier, e57–e64, https://doi.org/10.1016/j.jdent.2010.07.008.
29. Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, Sakai M, Takahashi H, Tashkandi E, & Del Mar Perez M (2015) Color difference thresholds in dentistry *Journal of Esthetic and Restorative Dentistry* **27(S1)** S1–S9, https://doi.org/10.1111/jerd.12149.
30. Pecho OE, Ghinea R, Alessandretti R, Pérez MM, & Della Bona A (2016) Visual and instrumental shade matching using CIELAB and CIEDE2000 color difference formulas *Dental Materials* **32(1)** 82–92, https://doi.org/10.1016/j.dental.2015.10.015.
31. Pecho OE, Ghinea R, Perez MM, & Della Bona A (2017) Influence of Gender on Visual Shade Matching in Dentistry *Journal of Esthetic and Restorative Dentistry* **29(2)** E15–E23, https://doi.org/10.1111/jerd.12292.
32. Zlatarić KD, Žagar M, & Illeš D (2019) A clinical study assessing the short-term efficacy of combined in-office/at-home whitening treatment *Journal of Esthetic and Restorative Dentistry* **31(2)** 140–146, https://doi.org/10.1111/jerd.12467.
33. Salas M, Lucena C, Herrera LJ, Yebra A, Della Bona A, & Pérez MM (2018) Translucency thresholds for dental materials *Dental Materials* **34(8)** 1168–1174, https://doi.org/10.1016/j.dental.2018.05.001.
34. Pérez MM, Herrera LJ, Carrillo F, Pecho OE, Dudea D, Gasparik C, Ghinea R, & Bona A Della (2018) Whiteness difference thresholds in dentistry *Dental Materials* **35(2)** 292–297, https://doi.org/10.1016/j.dental.2018.11.022.
35. Luo W, Westland S, Ellwood R, Pretty I, & Cheung V (2009) Development of a whiteness index for dentistry *Journal of Dentistry* **37(SUPPL. 1)** 21–26, https://doi.org/10.1016/j.jdent.2009.05.011.

36. Kirchhoff AL, Raldi DP, Salles AC, Cunha RS, & Mello I (2015) Tooth discolouration and internal bleaching after the use of triple antibiotic paste *International Endodontic Journal* **48(12)** 1181–1187, https://doi.org/10.1111/iej.12423.
37. Meireles SS, Fontes ST, Coimbra LAA, Bona Á Della, & Demarco FF (2012) Effectiveness of different carbamide peroxide concentrations used for tooth bleaching: an in vitro study *Journal of Applied Oral Science* **20(2)** 186–191, https://doi.org/10.1590/s1678-77572012000200011.
38. Westland S, Luo W, Li Y, Pan Q, & Joiner A (2017) Investigation of the perceptual thresholds of tooth whiteness *Journal of Dentistry* **67(September)** S11–S14, https://doi.org/10.1016/j.jdent.2017.09.013.
39. Khashayar G, Dozic A, Kleverlaan C, & Feilzer A (2012) Data Comparison Between Two Dental Spectrophotometers *Operative Dentistry* **37(1)** 12–20, https://doi.org/10.2341/11-161-c.
40. Shaheen MAEM, Elkateb MA, Bakry NS, & Meligy OAESEM (2017) Efficacy of 10 percent carbamidee peroxide as an intracoronal bleachiing agent in nonvital discolored primary teeth *Journal of Dentistry for Children* **84(1)** 22–29.
41. Valera MC, Camargo CHR, Carvalho CAT, Oliveira LD de, Camargo SEA, & Rodrigues CM (2009) Effectiveness of carbamide peroxide and sodium perborate in non-vital discolored teeth *Journal of Applied Oral Science* **17(3)** 254–261, https://doi.org/10.1590/s1678-77572009000300024.
42. Meireles SS, Heckmann SS, Leida FL, Santos IS, Bona Ád, & Demarco FF (2008) Efficacy and Safety of 10% and 16% Carbamide Peroxide Tooth-whitening Gels: A Randomized Clinical Trial *Operative Dentistry* **33(6)** 606–612, https://doi.org/10.2341/07-150.
43. Bersezio C, Vildósola P, Sáez M, Sánchez F, Vernal R, Oliveira O, Jorquera G, Basualdo J, Loguercio A, & Fernández E (2018) Does the Use of a "Walking Bleaching" Technique Increase Bone Resorption Markers? *Operative Dentistry* **43(3)** 250–260, https://doi.org/10.2341/16-334-c.
44. Gurgan S, Cakir FY, & Yazici E (2010) Different light-activated in-office bleaching systems: A clinical evaluation *Lasers in Medical Science* **25(6)** 817–822, https://doi.org/10.1007/s10103-009-0688-x.
45. Basting R, Amaral F, França F, & Flório F (2012) Clinical Comparative Study of the Effectiveness of and Tooth Sensitivity to 10% and 20% Carbamide Peroxide Home-use and 35% and 38% Hydrogen Peroxide In-office Bleaching Materials Containing Desensitizing Agents *Operative Dentistry* **37(5)** 464–473, https://doi.org/10.2341/11-337-c.
46. Bizhang M, Chun Y-HP, Damerau K, Singh P, Raab WH-M, & Zimmer S (2009) Comparative Clinical Study of the Effectiveness of Three Different Bleaching Methods *Operative Dentistry* **34(6)** 635–641, https://doi.org/10.2341/08-069-c.
47. Bizhang M, Heiden A, Blunck U, Zimmer S, Seemann R, & Roulet JF (2003) Intracoronal bleaching of discolored non-vital teeth *Operative Dentistry* **28(3)** 334–340.
48. Poyser NJ, Kelleher MGD, & Briggs PFA (2004) Managing discoloured non-vital teeth: the inside/outside bleaching technique. *Dental update* **31(4)** 204–10, 213–4, https://doi.org/10.12968/denu.2004.31.4.204.
49. Myers ML, Romero MF, Susin LF, & Babb CS (2019) Treatment and esthetic management of traumatized maxillary central incisors with endodontic therapy and the inside/outside bleaching technique: A clinical report *Journal of Prosthetic Dentistry* 1–5, https://doi.org/10.1016/j.prosdent.2018.10.020.

Tables

Table 1. Composition and information regarding the bleaching agents and bleaching technique.

Bleaching Agent/ Manufacturer Technique	Composition	Batch Number
Whiteness Perborato (FGM, Joinville, Santa Catarina, Brazil) Walking bleach	Powder phase: sodium perborate and thickener. Liquid phase: 20% hydrogen peroxide.	220118
White Class 7.5% (FGM, Joinville, Santa Catarina, Brazil) Inside/Outside bleaching	7.5% hydrogen peroxide gel, Carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water and surfactant.	280218
Whiteness HP 35% (FGM, Joinville, Santa Catarina, Brazil) In-Office bleaching	Hydrogen peroxide at 30% - 35%, thickener, red dye, glycol, and water.	210318

Table 2. Means and standard deviations of color and parameter changes for each technique and evaluation time.

Technique	T0-T1 (7 days)	T0-T2 (14 days)	T0-T3 (21 days) Completion of treatments	T0-T4 (28 days) 1-week follow-up
		ΔE_{00} values		
WB-SP	10.53 (2.59)^{b}	15.89 (3.99)^{a}	18.03 (4.14)^{a}	20.02 (3.64)^{a}
I/O-HP7.5	14.14 (3.56)^{a}	15.97 (3.24)^{a}	17.75 (3.06)^{a}	17.74 (3.43)^{a}
OF-HP35	12.92 (2.72)^{a}	13.88 (2.67)^{a}	15.67 (3.55)^{a}	16.59 (3.75)^{a}
		ΔE^*_{ab} values		
WB-SP	18.03 (3.03)^{b}	23.15 (4.90)^{ab}	26.71 (5.23)^{a}	30.64 (4.56)^{a}
I/O-HP7.5	24.89 (5.97)^{a}	26.32 (5.63)^{a}	29.49 (5.80)^{a}	29.66 (6.17)^{a}
OF-HP35	21.53 (3.67)^{a}	21.76 (3.91)^{a}	24.05 (6.54)^{a}	25.81 (7.30)^{a}
		ΔL^* values		
WB-SP	6.38 (5.62)^{b}	15.82 (5.57)^{a}	19.13 (6.02)^{a}	20.25 (6.18)^{a}
I/O-HP7.5	8.33 (6.77)^{b}	12.79 (6.16)^{ab}	15.08 (5.45)^{a}	14.54 (6.65)^{a}
OF-HP35	6.47 (4.90)^{a}	9.31 (6.06)^{a}	12.49 (7.19)^{a}	14.68 (5.53)^{a}
		Δa^* values		
WB-SP	-12.01 (2.02)	-14.76 (2.44)	-15.85 (2.37)	-17.95 (2.24)
I/O-HP7.5	-15.46 (3.54)	-16.10 (3.06)	-16.92 (3.32)	-16.99 (3.33)
OF-HP35	-15.01 (3.26)	-15.23 (3.03)	-16.08 (3.68)	-16.56 (3.60)
		Δb^* values		
WB-SP	-9.56 (5.22)^{a}	-4.61 (5.18)^{aB}	-6.24 (7.02)^{aB}	-11.62 (7.56)^{a}
I/O-HP7.5	-16.56 (6.02)^{a}	-15.13 (5.69)^{aA}	-18.05 (5.32)^{aA}	-18.50 (5.46)^{a}
OF-HP35	-13.11 (2.45)^{a}	-10.97 (2.65)^{aAB}	-11.37 (4.02)^{aAB}	-12.64 (5.34)^{a}

WB-SP, walking bleach sodium perborate; I/O-HP7.5, Inside/Outside hydrogen peroxide 7.5%; OF-HP35, In-office hydrogen peroxide 35%. Means with identical superscript lowercase letters in same line are not significantly different ($p>.05$). Means with identical superscript uppercase letters for each columns are not significantly different ($p>.05$).

Figures

Figure 1. Division of groups, and methodology flowchart

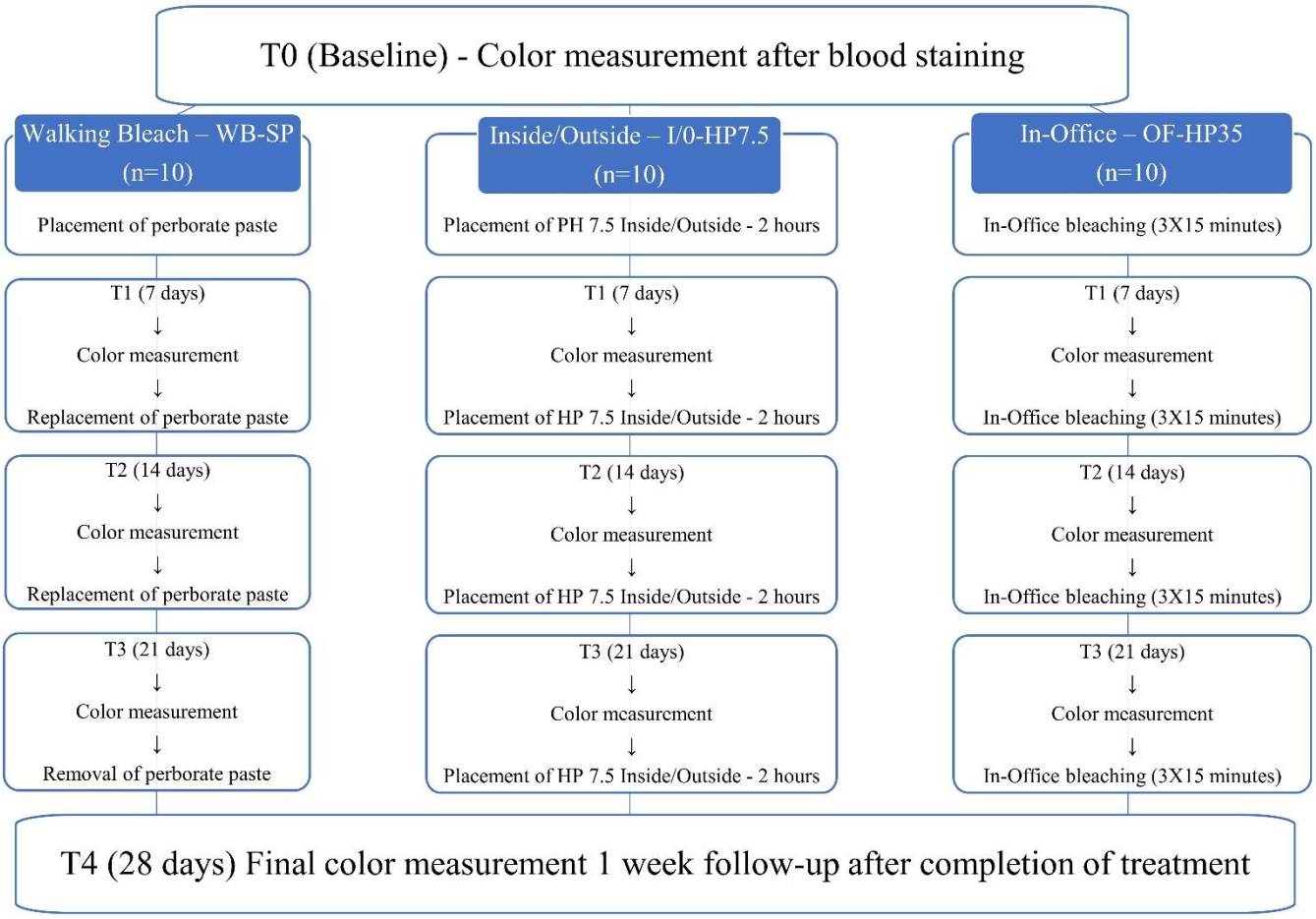


Figure 2. Silicone guide used for standardization of the location of the color measurements

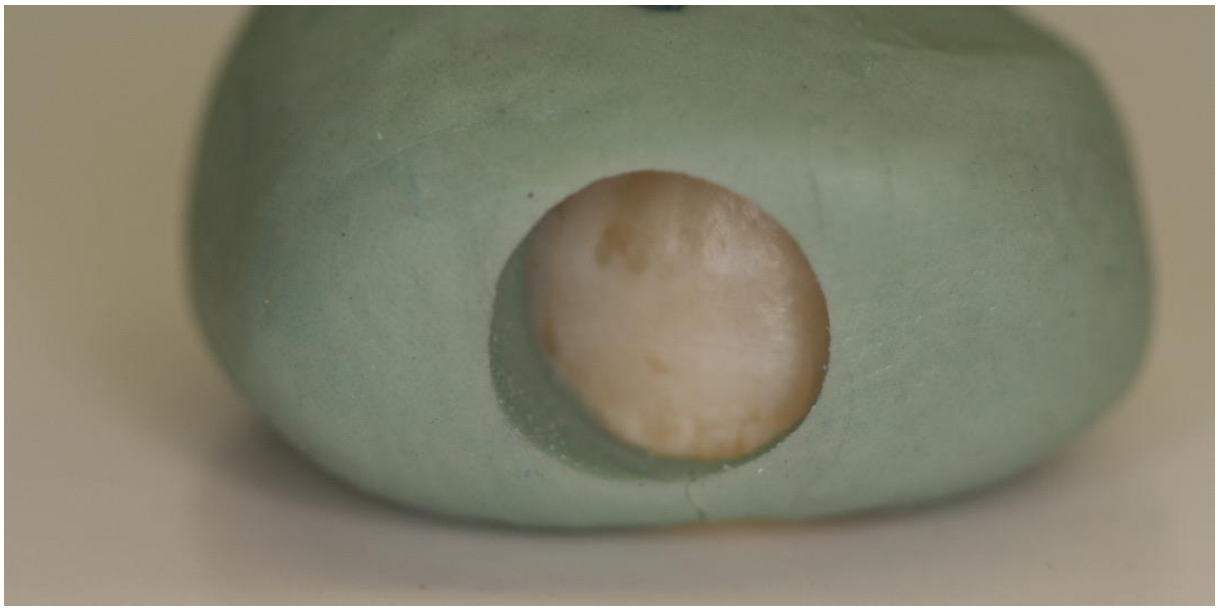
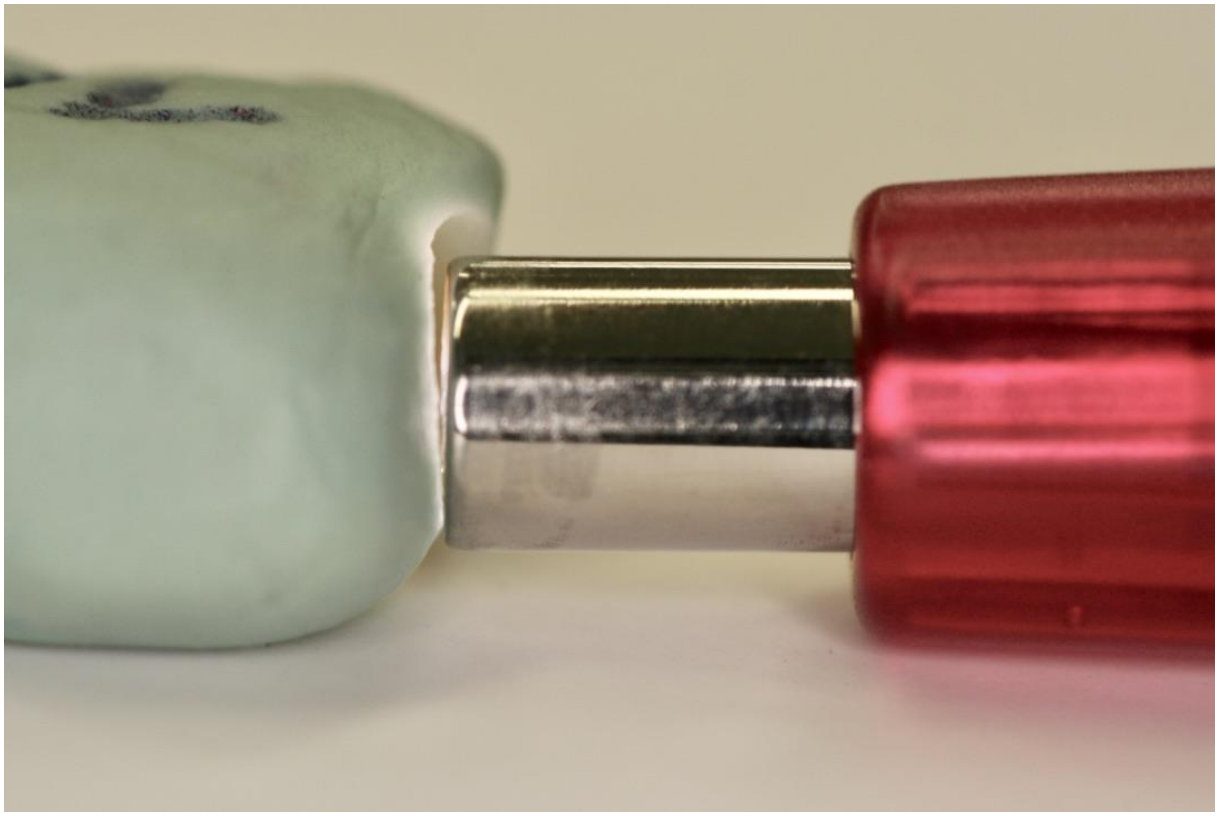
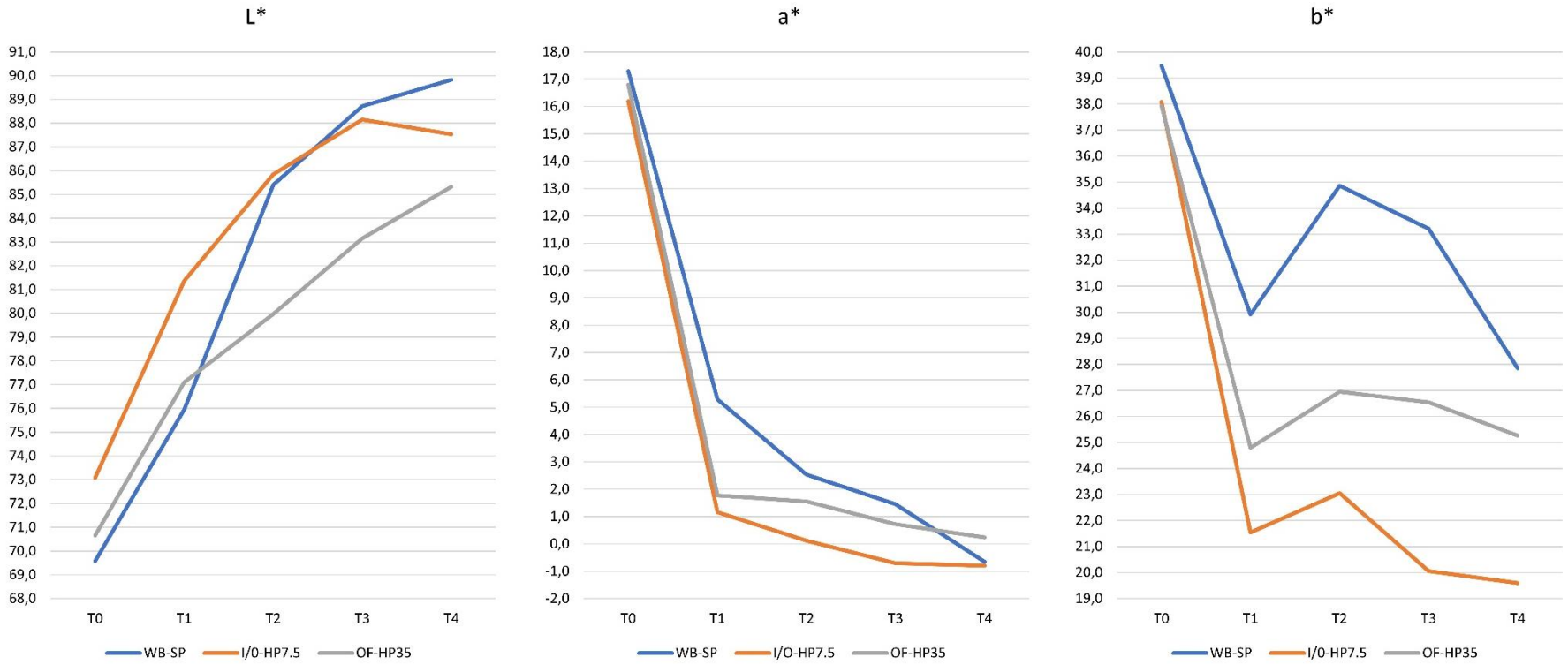


Figure 3. Demonstration of the color measurement performed on middle third of largest surface of each tooth



Graph

Graph 1. Color parameters L* a* b* at T0 (baseline), T1, T2, T3 (completion of the treatment) and T4 (1-week follow-up) for each group (WB-PB, I/O-PH7.5 and OF-PH35)



3 CONSIDERAÇÕES FINAIS

Com base nos achados do artigo que compõe a presente dissertação, pode-se concluir que as três técnicas de clareamento testadas para dentes manchados com sangue apresentaram efetividade semelhante e se mostraram estáveis no acompanhamento de uma semana após a conclusão dos tratamentos. Para técnica de clareamento em Consultório foi observada mudança de cor expressiva desde a primeira aplicação do protocolo clareador, já para as técnicas Walking Bleach e Inside/Outside, foi observada maior efetividade no clareamento a partir da segunda semana de tratamento.

Como as técnicas clareadoras mostraram efetividade semelhante no período de acompanhamento de 1 semana, a escolha do tratamento deve levar em conta as características e peculiaridades de cada técnica. Fatores como a fragilidade da estrutura dental, colaboração do paciente e tempo disponível para o tratamento devem ser levados em consideração.

Em situações em que é necessário a obtenção de resultados rápidos, a técnica mais indicada é o clareamento em Consultório, que demonstrou resultados expressivos após uma aplicação do protocolo clareador.

Quando o fator tempo não é um determinante e o paciente apresenta dificuldades de colaboração e adesão ao tratamento, a técnica Walking Bleach é indicada, pois além de produzir resultados satisfatórios e estáveis após a segunda semana de tratamento, a técnica é realizada exclusivamente pelo profissional, sem a necessidade de colaboração do paciente.

Em situações em que o paciente tem um tempo maior para se dedicar ao clareamento, é comprometido e colaborador, pode-se escolher a técnica Inside/Outside, em que o resultado depende do uso do gel clareador conforme as instruções do profissional. Nesse caso, deve-se levar em consideração a fragilidade do remanescente dental, já que nessa técnica o dente permanece aberto durante o tratamento.

Referências

ALMOHAREB, T. **Management of Discolored Endodontically Treated Tooth Using Sodium Perborate**. Journal of International Oral Health, v. 9, n. 3, p. 133–5, 2017.

BERSEZIO, Cristian e LEDEZMA, Paulina e colab. **Effectiveness and effect of non-vital bleaching on the quality of life of patients up to 6 months post-treatment: a randomized clinical trial**. Clinical Oral Investigations, v. 22, n. 9, p. 3013–3019, 1 Dez 2018.

BERSEZIO, Cristian e MARTÍN, Javier e colab. **Quality of life and stability of tooth color change at three months after dental bleaching**. Quality of Life Research, v. 27, n. 12, p. 3199–3207, 2018.

DEMARCO, Flávio Fernando e colab. **Preferences on Vital and Nonvital Tooth Bleaching: A Survey Among Dentists from a City of Southern Brazil**. Brazilian Dental Journal, v. 24, n. 5, p. 527–531, Out 2013.

DIETSCHI, Didier. **Nonvital bleaching: general considerations and report of two failure cases.** The European journal of esthetic dentistry, v. 1, n. 1, p. 52–61, 2006.

GBADEBO, So e AJAYI, DM. **Self Reported Tooth Discolorations Among Patients Seen At Dental Center University College Hospital Ibadan Decolorations Dentaires Auto-Rapportees Chez Les Patients Vus Au Centre Dentaire Du Centre Hospitalier Universitaire D ' Ibadan**. Journal of the West African College of Surgeons, v. 5, n. 3, p. 66–77, 2015.

GLOCKNER, K e colab. **Five-year follow-up of internal bleaching.** Brazilian dental journal, v. 10, n. 2, p. 105–10, 1999.

GUPTA, Saurabh K. e SAXENA, Payal. **Evaluation of patient satisfaction after non-vital bleaching in traumatized discolored intact anterior teeth**. Dental Traumatology, v. 30, n. 5, p. 396–399, Out 2014.

JOINER, Andrew e LUO, Wen. **Tooth colour and whiteness: A review**. Journal of Dentistry, v. 67, n. June, p. S3–S10, 2017.

LISE, Diogo Pedrollo e colab. **Randomized clinical trial of 2 nonvital tooth bleaching techniques: A 1-year follow-up**. Journal of Prosthetic Dentistry, v. 119, n. 1, p. 53–59, 2018.

NIXON, Peter J e colab. **Conservative Aesthetic Techniques for Discoloured Teeth: 1. The Use of Bleaching**. Dental Update, v. 34, n. 2, p. 98–107, Jul 2007.

PLOTINO, Gianluca e colab. **Nonvital Tooth Bleaching: A Review of the Literature and Clinical Procedures**. Journal of Endodontics, v. 34, n. 4, p. 394–407, 2008.

POYSER, Neil J e KELLEHER, Martin G D e BRIGGS, Peter F A. **Managing discoloured non-vital teeth: the inside/outside bleaching technique.** Dental update, v. 31, n. 4, p. 204–10, 213–4, 2004.

SULIEMAN, M. **An Overview of Bleaching Techniques: 2. Night Guard Vital Bleaching and Non-Vital Bleaching**. Dental Update, v. 32, n. 1, p. 39–46, 2017.

ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *OPERATIVE DENTISTRY*

Manuscript Submission (Author Guidelines)

General Requirements

Operative Dentistry requires electronic submission of all manuscripts. All submissions must be sent to Operative Dentistry using the Allen Track upload site. A mandatory and nonrefundable $50.00 fee is required at submission. Your manuscript will only be considered officially submitted after it has been approved through our initial quality control check, and any quality problems have been resolved. You will have 6 days from when you start the process to submit and approve the manuscript. After the 6 day limit, if you have not finished the submission, your submission may be removed from the server. You are still able to submit the manuscript, but you must start from the beginning. Be prepared to submit the following manuscript files in your upload:

- A Laboratory or Clinical Research Manuscript file must include: o a title

  o a running (short) title
  o a clinical relevance statement
  o a concise summary (abstract)
  o introduction, methods & materials, results, discussion and conclusion
  o references (see Below)

- The manuscript body **MUST NOT** include any:

  o Author identifying information such as:

  ▪ Authors names or titles

  - ▪ Acknowledgements
  - ▪ Correspondence information
  - ▪ Response to reviewer files should also NOT include any author identifying information, such as a signature at the end, etc.

    o Figures

    o Graphs

    o Tables

- An acknowledgement, disclaimer and/or recognition of support (if applicable) must be uploaded as a separate file and uploaded as

miscellaneous material.

- Appendix material that you would like us to publish electronically

with your article, but not as part of your printed manuscript (such as indices, supplemental tables, etc.), should be submitted as ***supplemental material***. It will not be typeset, and will appear exactly as you provide to Operative Dentistry. References submitted as part of supplemental material should appear in our preferred reference format. Supplemental material is viewable by the reviewers, and so SHOULD NOT contain any author identifiable information.

- All figures, illustrations, graphs and tables must also be provided as individual files. Figures should be submitted without figure letters or numbers within the image itself, these designations will be added by the journal staff as needed. All Figures should be high-resolution images, which are used by the editor in the actual typesetting of your manuscript. Please refer to the instructions below for acceptable formats and sizes.
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- ALL studies using human tissue must have an accompanying Institutional Review Board (IRB) statement – it must indicate that either the board has approved the study, or that the study is exempted from approval. There are no exceptions to this policy.
- All studies using animal tissue must have an accompanying approval from the appropriate ethics board.
- All manuscripts reporting on a Clinical Trial must indicate that the trial information was submitted to a public Clinical Trial Registry. A URL of where the trial appears in a registry is required to be submitted with the manuscript.

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- All materials submitted for publication must be submitted exclusively to Operative Dentistry.
- The editor reserves the right to make literary corrections.
- Currently, color will be provided at no cost to the author if the editor deems it essential to the manuscript. However, we reserve the right to convert to gray scale if color does not contribute significantly to the quality and/or information content of the paper.
- The author(s) retain(s) the right to formally withdraw the paper from consideration and/or publication for any reason up to the submission of the final paper to our press vendor for publication.
- International authors whose native language is not English must have their work reviewed by a native English speaker prior to submission.

o Manuscripts that are rejected before peer-review for English correction should be entered as a new manuscript upon resubmission. In the manuscript comments box the comment, “this is a resubmission of manuscript number XX-XXX” should be noted.

o Manuscripts that are rejected after peer-review are not eligible for resubmission.

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- Spelling must conform to the American Heritage Dictionary of the English Language, and SI units for scientific measurement are preferred.
- While we do not currently have limitations on the length of manuscripts, we expect papers to be concise; authors are also encouraged to be selective in their use of figures and tables, using only those that contribute significantly to the understanding of the research.
- Acknowledgement of receipt is sent automatically upon acceptance through quality control. This may take up to 7 days. If you do not receive such an acknowledgement, please check your author homepage at http://jopdent.allentrack.net if the paper does not appear there please resend your paper.

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CORRESPONDING AUTHOR must provide a WORKING / VALID e-mail address which will be used for all communication with the journal. ***NOTE:** Corresponding authors MUST update their profile if their e-mail or postal address changes. If we cannot contact authors within seven days, their manuscript will be removed from our publication queue.

AUTHOR INFORMATION must include: • full name of all authors

• complete mailing address for each author
• valid email address for each author
• degrees (e.g. DDS, DMD, PhD)
• affiliation (e.g. Department of Dental Materials, School of Dentistry, University of Michigan)

MENTION OF COMMERCIAL PRODUCTS/EQUIPMENT must include:

• full name of product
• full name of manufacturer
• city, state and country of manufacturer

MANUSCRIPTS must be provided as Word for Windows files. Files with the .doc and .docx extensions are accepted.

TABLES may be submitted as either Word (.doc and .docx) or Excel (.xls and

.xlsx) files. All tables must be legible, with fonts being no smaller than 7 points. Tables have the following size limitations: In profile view a table must be no larger than 7 x 9 inches; landscape tables should be no wider than 7 inches. It is the Editor's preference that tables not need to be rotated in order to be printed, as it interrupts the reader's flow.

ILLUSTRATIONS, GRAPHS AND FIGURES must be provided as **TIFF** or high resolution **JPEG** files with the following parameters:
• **line art** (and tables that are submitted as a graphic) must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 600 dpi and a maximum resolution of 1200 dpi. This means the shortest side should be no smaller than 3000 pixels.

• **gray scale/black & white figures** must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means the shortest side should be no smaller than 1500 pixels.

• **color figures and photographs** must be sized with the short edge being no shorter than 3.5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means that the shortest side should be no smaller than 1050 pixels.

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• a running (short) title
• purpose
• description of technique
• list of materials used
• potential problems
• summary of advantages and disadvantages • references (see below)

LITERATURE AND BOOK REVIEW MANUSCRIPTS must include as part of the narrative:

• a running (short) title

• a clinical relevance statement based on the conclusions of the review • conclusions based on the literature review...without this, the review is just an exercise and will not be published • references (see below)

References

REFERENCES must be numbered (superscripted numbers) consecutively as they appear in the text and, where applicable, they should appear after punctuation.
The reference list should be arranged in numeric sequence at the end of the manuscript and should include:

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2. Full article title.

3. Full journal name in italics (**no** abbreviations), volume and issue numbers and first and last page numbers complete (i.e. 163-168 NOT attenuated 163-68).

4. Abstracts should be avoided when possible but, if used, must include the above plus the abstract number and page number.

5. Book chapters must include chapter title, book title in italics, editors' names (if appropriate), name of publisher and publishing address.

6. Websites may be used as references, but must include the date (day, month and year) accessed for the information.

7. Papers in the course of publication should only be entered in the references if they have been accepted for publication by a journal and then given in the standard manner with "In press" following the journal name.

8. **DO NOT** include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

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- Journal article-multiple authors: Eick JD, Gwinnett AJ, Pashley DH & Robinson SJ (1997) Current concepts on adhesion to dentin *Critical Review of Oral and Biological Medicine* **8(3)** 306-335.
- Journal article: special issue/supplement: Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **(Supplement 6)** 119-144.
- Abstract: Yoshida Y, Van Meerbeek B, Okazaki M, Shintani H & Suzuki K (2003) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **82(Special Issue B)** Abstract #0051 p B-19.
- Corporate publication: ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters *Geneve: International Organization for Standardization* **1st edition** 1-25.
- Book-single author: Mount GJ (1990) *An Atlas of Glass-ionomer Cements* Martin Duntz Ltd, London.
- Book-two authors: Nakabayashi N & Pashley DH (1998) *Hybridization of Dental Hard Tissues* Quintessence Publishing, Tokyo.
- Book-chapter: Hilton TJ (1996) Direct posterior composite restorations In: Schwarts RS, Summitt JB, Robbins JW (eds) *Fundamentals of Operative Dentistry* Quintessence, Chicago 207-228.
- Website-single author: Carlson L (2003) Web site evolution; Retrieved online July 23, 2003 from: http://www.d.umn.edu/~lcarlson/cms/evolution.html

- Website-corporate publication: National Association of Social Workers (2000) NASW Practice research survey 2000. NASW Practice Research Network, 1. 3. Retrieved online September 8, 2003 from: http://www.socialworkers.org/naswprn/default

• Journal Article with DOI: SA Feierabend, J Matt & B Klaiber (2011) A Comparison of Conventional and New Rubber Dam Systems in Dental Practice. *Operative Dentistry* **36(3)** 243-250, http://dx.doi.org/10.2341/09-283-C

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