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JOANA YUMI TERUYA UCHIMURA

ANÁLISE DA VARIAÇÃO DE COR E DA ESTRUTURA DOS DENTES SUBMETIDOS AO CLAREAMENTO INTERNO

Orientadora: Profª. Dra. Mírian Marubayashi Hidalgo (DOD)

Coorientadora: Profª. Dra. Francielle Sato (DFI)

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Dissertação apresentada ao Programa de Pós-graduação em Odontologia Integrada, da Universidade Estadual de Maringá, como parte dos requisitos para a obtenção do título de Mestre.

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Dedico este trabalho
aos meus pais, Taqueco e Nelson;
a minha irmã, Liza;
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disciplina, trabalho árduo e determinação.
São duas pernas que devem caminhar juntas”

Augusto Cury

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LISTA DE ABREVIATURAS E SÍMBOLOS

*a**	Eixo que vai do vermelho ao verde
AFM	Microscopia de Força Atômica
*b**	Eixo que vai do amarelo ao azul
*C**	Croma
CDD	Dispositivo com Carga Acoplada
CEJ	Junção Amelocementária
C-H	Ligação de carbono com hidrogênio
C-O-H	Ligação do oxigênio com carbono e hidrogênio
CH2	Metileno
CIE	Comissão Internacional de Iluminação
CIE 1976	Comissão Internacional de Iluminação, criou em 1976 a equação da diferença de cor que relaciona uma medida para um valor *L**, *a**, *b**
CIE 94	Redefinição em 1994 da CIE 76 mantendo o *L * a * b ** como espaço de cor, com introdução de pesos específicos
CIEDE 2000	Redefinição em 2000 da CIE 94, adicionando cinco correções
CIELAB	Espaço de cor uniforme *L**, *a**, *b**
cm	Centímetro
cm^{-1}	Número de onda
CO_2	Dióxido de carbono
CO_3^{-2}	Íon Carbonato
C–O	Monóxido de Carbono
CR400	Modelo do colorímetro Konica Minolta
D65	Iluminante padrão para Luz do dia com temperatura de cor de 6500K definido pela Comissão Internacional de Iluminação
EDTA	Ácido etilenodiamino tetra-acético

et al	E colaboradores
FESEM	Microscopia de Emissão de Campo Eletrônica de Varredura
FTIR	Espectroscopia no Infravermelho via Transformada de Fourier
FTIR-PAS	Espectroscopia Fotoacústica no Infravermelho via Transformada de Fourier
FT-Raman	Espectroscopia Raman via Transformada de Fourier
GmbH	Sociedade com Responsabilidade Limitada da Alemanha
h	Hora
h°	Tonalidade ou Matiz
H_2O	Água
HP	Peróxido de hidrogênio
k	Fator paramétrico que representam condições experimentais, sendo igual a 1.
KBr	Brometo de potássio
k_C	Fator paramétrico para a variação em condições experimentais por Croma
k_H	Fator paramétrico para a variação em condições experimentais por Matiz
k_L	Fator paramétrico para a variação em condições experimentais de Luminosidade
L^*	Luminosidade
mL	Mililitro
MLE	Maximum-likelihood Estimation test
Micro-Raman/ MRS	Espectroscopia Raman via Microscópio
min	Minuto
mm	Milímetro
mW	Megawatt

n	Tamanho da amostra
n°	Número
NBS	National Bureau of Standards
NBS1	Alteração extremamente leve
NBS2	Alteração pequena
NBS3	Alteração perceptível
NBS4	Alteração marcante
NBS5	Alteração extremamente acentuada
NBS6	Alteração de cor
nm	Nanômetro
O–C=O	Dióxido de carbono
p	Nível de significância estatística
ph	Potencial hidrogeniônico, que indica a acidez, neutralidade ou alcalinidade
PO_4^{-3}	Íon fosfato
RT	Rotação de Matiz
s	Segundo
SAS	Statistical Analysis System
S_C	Função de ponderação de Compensação por Croma
SD	Desvio Padrão
S_H	Função de ponderação de Compensação por Matiz
S_L	Função de ponderação de Compensação de Luminosidade
SP	Perborato de sódio
tN	Natural
tD	Escurecido
t7	7 dias após a primeira sessão de clareamento

t14	7 dias após a segunda sessão de clareamento
µm	Micrometro
UNESP	Universidade Estadual Paulista
v_1	Modo vibracional 1 e seus graus de degenerescência
v_2	Modo vibracional 2 e seus graus de degenerescência
v_3	Modo vibracional 3 e seus graus de degenerescência
v_4	Modo vibracional 4 e seus graus de degenerescência
ν_{as}	Modo vibracional de estiramento assimétrico
VERTEX 70	Modelo do FTIR
ν_s	Modo vibracional de estiramento simétrico
$\bar{x}$	Média
x	por
1	Medida de referência da amostra padrão
¼	Um quarto ou 0,25
2	Ultima medida realizada com amostra alterada
δ	Deformação
%	Porcentagem
®	Marca Registrada de produto ou de empresa
°	Grau
°C	Graus Celsius
20x	Vinte vezes
Δa^*_{ab}	diferença dos valores a^* entre as amostras 1 e 2, CIE 1976
Δb^*_{ab}	diferença dos valores b^* entre as amostras 1 e 2, CIE 1976
ΔC^*_{ab}	Diferença de Croma de a^*, b^* entre as amostras 1 e 2, CIE 1976
$\Delta C'$	Diferença de Croma transformado entre as amostras 1 e 2

ΔE^*_{ab}	Diferença de cor entre as amostras 1 e 2, CIE 1976
ΔE^*_{94}	Diferença da distância métrica de cor, CIE 1994, com $L^* C^* h^*$ espaço de cores com as diferenças de Luminosidade, Croma e Matiz calculados a partir de $L^* a^* b^*$ coordenadas.
ΔE_{00}	Diferença da distância métrica de cor, CIE 2000, com correções no termo de rotação de matiz (R_T), compensação por cores neutras ($L^* C^* h^*$), compensação de luminosidade (S_L), compensação por croma (S_C) e compensação por matiz (S_H)
ΔH^*_{ab}	Diferença métrica de Matiz da CIE a^*, b^* entre as amostras 1 e 2, CIE 1976
$\Delta H'$	Diferença de Matiz transformado entre as amostras 1 e 2
ΔL^*_{ab}	diferença L^* entre as amostras 1 e 2, CIE 1976
$\Delta L'$	Diferença de Luminosidade transformada entre as amostras 1 e 2

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1. CONTEXTUALIZAÇÃO

A aparência com os dentes sempre se apresentou como um fator preocupante para muitas pessoas, mas isso tem sido potencializado na atualidade, mais precisamente no que se refere à cor. Neste contexto, a procura por tratamentos clareadores nos consultórios odontológicos tem aumentado e, consequentemente, a variedade de produtos clareadores oferecidos pelo mercado. Assim, o conhecimento dos cirurgiões-dentistas sobre a efetividade dos produtos oferecidos, protocolos de utilização e consequências, se apresentam como fatores de extrema importância, tanto quanto o correto diagnóstico para o sucesso do tratamento (ADEYEMI et al., 2006).

Existem diversas causas que levam ao escurecimento dentário e estas podem ser classificadas em extrínsecas, intrínsecas ou combinadas. As causas extrínsecas são ocasionadas por fatores externos como o tipo de dieta alimentar, vinho, café, chá, cigarros, entre outros. As causas intrínsecas podem ser sistêmicas relacionadas a drogas como a tetraciclina; metabólicas (calcificação distrófica, fluorose, etc); genéticas; ou, locais como a necrose pulpar, hemorragia intrapulpar, manutenção de remanescentes de tecido pulpar pós-tratamento endodôntico, cimentos endodônticos, materiais de preenchimento coronário, entre outras (WATTS; ADDY, 2001; PLOTINO et al., 2008).

Nos casos em que o clareamento interno é indicado, entre as quatro causas locais mais frequentemente observadas, o trauma dentário corresponde a 84%, com rompimento dos vasos sanguíneos causando hemorragia na câmara pulpar e a consequente difusão de hemácias, seguida por hemólise. A hemoglobina liberada se combina com o tecido pulpar putrefato e com os sulfatos de hidrogênio produzidos pelas bactérias, originando o sulfato ferroso, responsável pela pigmentação dentária. A cárie avançada é responsável por 12%, com comprometimento pulpar e formação de produtos na forma de proteínas pigmentadas que penetram nos túbulos e escurecem a dentina ao redor. Necroses decorrentes do tratamento ortodôntico pela aplicação de forças inapropriadas que causam pequeno rompimento vascular e da impactação dentária de um dente vizinho, ocasionam compressão dos vasos sanguíneos e nervos representando, igualmente, 2% dos casos (AMATO et al., 2006; PLOTINO et al., 2008; UYSAL et al., 2009).

O clareamento interno é um tratamento simples, conservador, pois mantém a estrutura dentária remanescente em relação a outros tipos de tratamento; é também de menor custo, se comparado àqueles mais radicais que optam pela cobertura total do dente, sejam facetas de resina composta ou porcelana. Pode ser ainda um tratamento suplementar quando há o planejamento de prótese *metal-free*, em que apenas o componente protético não consegue encobrir o escurecimento (ARI; UNGÖR, 2002; PLOTINO et al., 2008; ABBOTT; HEAH, 2009; CARDOSO et al., 2013).

O clareamento interno ocorre basicamente por uma reação de oxidação na qual os radicais livres penetram nos túbulos dentinários e quebram as cadeias moleculares tornando o dente mais claro (PLOTINO et al., 2008; CARDOSO et al., 2013). Assim, a efetividade e o sucesso do clareamento interno estão diretamente relacionados com a habilidade do agente em penetrar nos túbulos dentinários (CARRASCO et al., 2003; CARDOSO et al., 2013).

Os agentes clareadores mais utilizados tanto na forma individual quanto na combinada são o perborato de sódio e o peróxido de hidrogênio (ARI; UNGÖR, 2002; PLOTINO et al., 2008) e por isso, escolhidos por nós para o desenvolvimento deste trabalho.

O perborato de sódio tem sido utilizado como agente clareador desde 1907, sendo disponível em diferentes composições: mono, tri e tetrahidratado (PLOTINO et al., 2008). Comparando-se a efetividade dessas diferentes formas de perborato de sódio preparadas com água destilada, observou-se que apresentaram resultados semelhantes (ARI; UNGÖR, 2002). Foi Spasser (1961) apud ARI; UNGÖR (2002), o precursor do uso conjunto do perborato de sódio com a água, e em 1963, Nutting; Poe apud ARI; UNGÖR, 2002, sugeriram a substituição da água por peróxido de hidrogênio, como forma de agilizar o processo e o resultado do clareamento, sendo esse uso posteriormente ratificado pela preocupação com a ocorrência de reabsorção radicular (ARI; UNGÖR, 2002). Dentre as várias técnicas indicadas para o seu uso, o protocolo mais utilizado é o *walking bleach*, realizado pelo preenchimento da câmara pulpar com uma pasta composta por perborato de sódio misturado com água ou peróxido de hidrogênio, deixada na forma de curativo por um período de três (ARI; UNGÖR, 2002; CAVALLI et al., 2009) a sete dias (ARI; UNGÖR, 2002; CHNG et al., 2002; LIM et al., 2004; CARDOSO et al., 2013).

O uso do peróxido de hidrogênio na concentração aproximada de 30% é antiga, desde 1960, associada ou não ao perborato de sódio (AMATO et al., 2006). A maioria dos produtos oferecidos para clareamento interno e utilizados nos consultórios odontológicos no Brasil apresentam a concentração de 35%, como a Whitness HP®, Whitness HP Maxx®,

Opalescence Endo®, Opalescence Xtra®, e tem sido amplamente estudados (CARRASCO-GUERISOLI et al., 2009; CARDOSO et al., 2013;). O protocolo do seu uso para o clareamento imediato consiste em três aplicações de 10 a 15min por sessão (CARRASCO-GUERISOLI et al., 2009; CARDOSO et al., 2013;). Há indicação na literatura de utilização de menor concentração, quando se realiza o *walking bleach* (CHNG et al., 2002).

O estudo de Cavalli e colaboradores (2009) utilizando diferentes agentes clareadores: perborato de sódio com água, peróxido de carbamida a 35%, peróxido de hidrogênio a 25% e peróxido de hidrogênio a 35%, com o uso da técnica *walking bleach* durante três dias, chamou nossa atenção ao demonstrar, por meio da microscopia eletrônica de transmissão, imagens de desmineralização em algumas regiões e em outras, mineralização circundada por fibras colágenas. Nos questionamos se haveria alteração estrutural molecular da dentina com o uso de agentes clareadores comumente utilizados internamente.

Para pesquisar os agentes clareadores in vitro, idealmente, se faz necessário o escurecimento das coroas e Freccia; Peters (1982) propuseram que os dentes fossem imersos em tubos individuais com sangue contendo o mínimo de soro. Para a hemólise, ou seja, a ruptura das hemácias e a penetração nos túbulos dentinários, indicaram duas centrifugações por dia, durante três dias e, após retirar o dente, adicionar água destilada e novamente centrifugar. O dente deveria ser recolocado nesse hemolisado (com a hemoglobina), centrifugado diariamente e ao final do período de três dias, lavado em água corrente e seco para remoção do excesso de sangue. Vários outros trabalhos utilizaram este método como referência, com algumas modificações como a remoção do plasma, diferentes velocidades, tempos e quantidades de centrifugação ou variação na quantidade de dias em que os dentes foram mantidos no sangue (YUI et al., 2008; CARDOSO et al., 2013). Para simular a dinâmica real do escurecimento, causado a partir da polpa para o exterior, optamos por utilizar o sangue lisado no interior da câmara pulpar desde o inicio, acelerando o processo de degradação da hemoglobina com o uso do laser ultravioleta azul.

Considerando o nosso objetivo de estudar a alteração dentinária coronária, acompanhamos o método proposto por Cavalli e colaboradores (2009) na qual se realizou o acesso endodôntico e a limpeza da cavidade de modo convencional, sem a realização do tratamento endodôntico e iniciando o experimento com o seccionamento radicular a 5mm da junção amelocementária e a colocação de um tampão de cimento de ionômero de vidro.

As cores dos dentes são determinadas pelas diferentes propriedades ópticas das três camadas interligadas, o esmalte, a dentina e a polpa. Clinicamente, a alteração de cor é

determinada por diversos métodos utilizados individualmente ou de forma combinada, como a análise pelo observador, comparação com uma escala de cores e/ou exame de fotografias. As referências utilizadas para comparação, normalmente são obtidas por uma escala de cor padrão ou dentes que não foram submetidos ao clareamento. No entanto, esta análise visual é subjetiva e ainda pode aumentar sua variabilidade dentre os diferentes observadores, ambientes e incidência de luz (ADEYEMI et al., 2006). Para reduzir a margem de erro e aumentar a precisão destas comparações e afirmações, tem sido amplamente utilizados para a pesquisa científica, aparelhos específicos para a mensuração da alteração de cor, como os colorímetros (ADEYEMI et al., 2006).

Os colorímetros utilizam o sistema de avaliação de cores CIELAB, que é um sistema uniforme e tridimensional do espaço de cores. Possuí três eixos com distâncias iguais, correspondentes às diferenças de cores percebidas. O *L** é a luminosidade, variando de 0 (preto) ao 100 (branco); *a** representa o eixo que vai do vermelho ao verde; e, *b** do amarelo ao azul. Os eixos *a** e *b** são utilizados para se obter o croma e a matiz. Quando se possuí dois objetos em pontos distintos deste sistema e se quer obter a diferença colorimétrica entre eles, realiza-se o cálculo do ΔE. Existem várias fórmulas para o cálculo da diferença de cor, como a CIE 1976: $\Delta E^*_{ab} = [(\Delta L^*_{ab})^2 + (\Delta a^*_{ab})^2 + (\Delta b^*_{ab})^2]^{1/2}$); CIE 94: $\Delta E^*_{94} = [(\Delta L^*_{ab} / k_L S_L)^2 + (\Delta C^*_{ab} / k_C S_C)^2 + (\Delta H^*_{ab} / k_H S_H)^2]^{1/2})$; e, a CIEDE 2000: $\Delta E_{00} = ((\Delta L' / k_L S_L)^2 + (\Delta C' / k_C S_C)^2 + (\Delta H' / k_H S_H)^2 + R_T (\Delta C' / k_C S_C) (\Delta H' k_H S_H))^{1/2}$, considerada a mais precisa (SHARMA et al., 2005; BROWNING et al., 2009; WAN et al., 2009) e utilizada em nosso trabalho.

Recentes estudos têm utilizado a espectroscopia vibracional para análise da interação química entre materiais nas áreas biológicas, inclusive na Odontologia (PARK et al., 2004; UBALDINI et al., 2012; LEANI et al., 2013; UBALDINI et al., 2013). As técnicas mais utilizadas são a espectroscopia Raman e no Infravermelho via Transformada de Fourier e suas variações como espectroscopia Raman via Transformada de Fourier (FT-Raman), espectroscopia Raman via Microscópio (Micro-Raman) e espectroscopia Fotoacústica no Infravermelho via Transformada de Fourier (FTIR-PAS).

Pela espectroscopia Micro-Raman foi possível, por exemplo, caracterizar as estruturas do esmalte bovino durante o processo de clareamento e observar que houve uma redução de intensidade dos componentes orgânicos e inorgânicos, o que foi interpretado como ocorrência de uma dissolução deles após o tratamento com peróxido de hidrogênio a 30%, sem, no entanto, resultar em alterações na sua morfologia (PARK et al., 2004). Pesquisa realizada em

2013, nesta Universidade Estadual de Maringá, com a utilização das Espectroscopias Micro-Raman e FTIR-PAS verificou que a dinâmica da difusão do peróxido de hidrogênio nos tecidos dentários é determinada pela afinidade química do agente clareador com a porção orgânica. No esmalte, onde a porção orgânica representava 2%, os sinais passaram inalterados; na dentina, com 38% de compostos orgânicos, houve grande interação e 63% do peróxido de hidrogênio permaneceu ligado às suas moléculas (UBALDINI et al., 2013). No referido trabalho foi realizada a avaliação da permeação do agente clareador no esmalte dentário, após uso da técnica de clareamento externo executado no consultório odontológico, enquanto no presente estudo será analisada alteração estrutural molecular das matrizes orgânica e inorgânica da dentina, após aplicação do agente clareador pela técnica do clareamento interno.

Foi neste contexto que foram concebidos e desenvolvidos os dois artigos aqui apresentados. O primeiro será enviado para o Journal of Endodontics e o segundo, para o Journal of Dental Research (ANEXOS A e B).

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2. ARTIGO 1

Colorimetric Analysis Comparing the Effectiveness of Bleaching Agents: a Clinical Reproduction *in vitro* Study

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Abstract

Introduction: There is a lack of studies comparing *in vitro* bleaching agents in a dynamic process simulating a clinical situation over time. The objectives were to compare the effectiveness of hydrogen peroxide and sodium perborate assessing whether the final color of bleached teeth reached the previous natural color and to certify the time period considered sufficient to reach the whitening. **Methods**: Twenty extracted bovine central incisors were artificially stained with human blood from the inside. Standard endodontic cavity access and cervical sealing with glass ionomer were made. Discolored specimens were divided in groups: HP- 35% hydrogen peroxide and SP- sodium perborate with bidistilled water, with an experimental period of 14 days: 2 sessions with a 7 days interval. Color measurement was performed by a colorimeter and CIEDE2000 color-difference formula was calculated based on the CIELAB color coordinates. **Results:** Analysis of Maximum-likelihood Estimates demonstrated that the time, color, and type of treatment were significant factors for color analysis of the internal bleaching. Analysis of Parameter Estimates demonstrated that 14-day period had significantly higher values of ΔE_{00} than 7-day period. Treatment with perborate achieved significantly higher levels than peroxide bleaching. **Conclusion:** The final color of bleached teeth with 35% hydrogen peroxide and sodium perborate were equal or lighter than the previous natural color, although sodium perborate was more effective at 7- and 14-day intervals. The period of time considered sufficient to achieve the whitening was 7 days.

Key Words: Tooth discoloration, Tooth bleaching, Color perception

Nowadays, the need and demand for aesthetics increased, therefore having white teeth and beautiful smile have become extremely important (1). In cases of non-vital discolored teeth, which are very related to Endodontics, the intracoronal bleaching has been considered a simpler and less invasive procedure than others treatments, when color change is present and there is sufficient coronal structure to accomplish the filling of bleaching agent in the pulp chamber (2, 3).

There are several studies that compared the effectiveness of hydrogen peroxide or sodium perborate with different mixtures and concentrations (4-14). However, to our knowledge, only few studies in the literature have compared different *in vitro* bleaching agents in a dynamic process (4, 5, 14). In those studies, the artificial darkness of the teeth was not done from the inside, such as promoted by the pulp necrosis and hemoglobin degradation, neither the same specimens underwent all steps of the clinical protocol: natural, discolored, and bleached.

Researches using methods to simulate the coronal darkening were reported in the literature (4–6, 10, 14, 15), in which teeth were immersed in blood to achieve the darkness by the permeation from the outside. Others, did not use the artificial darkening techniques and compared the final color of teeth only with the natural control (6-8, 12, 13, 16). Few authors used the same specimens during all the experimental time (1,13-14, 17) such as the same natural tooth subjected to discoloration, and analyzed after bleaching with their respective material and exposure times. Some studies used a control group different than the bleaching agents groups (5, 6).

The objectives of this study were to compare *in vitro* effectiveness of 35% hydrogen peroxide and sodium perborate to assess whether the final color of bleached teeth reached the previous natural color and to certify the period of time considered sufficient to reach the whitening.

Material and Methods

Preparation of Specimens

The studied samples were 20 extracted bovine central incisors, donated by a regulated and certified slaughter house, belonging to animals from the same herd. They were thoroughly cleaned; calculus and remnants of periodontal ligament were removed, brushed, rinsed and

stored in a physiologic solution at 4°C. After that, they were submitted to visual inspection to assure that there were no cracks. Specimens were individually positioned by the incisal part with the height of 1/4 of the crown in blocks of 1.5 x 1.5cm made of acrylic resin (JET, Clássico, São Paulo, Brazil) to be base and support during the experiment (Ver APÊNDICE A). They were positioned in a sectioning machine with cooled water (ISOMET, São Paulo, Brazil). Roots were sectioned at 5mm from the cementoenamel junction (CEJ), dental pulp was removed, and irrigation with physiologic solution was performed.

Artificial Staining

Specimens were artificially stained with human blood using a modified procedure based on usual techniques (6, 15), however with the permeation from the inside. The blood was obtained from a healthy donor and centrifuged to obtain a biphasic solution: plasma which was removed, and precipitate which distilled water was added (20mL H_2O/30mL blood). The hemoglobin degradation process was accelerated by using a laser (442nm) for 2h, with homogenization every 30min. This blood was injected into the teeth by the region of the anatomical neck until the fulfilling of the pulp chamber. Afterwards the sealing was performed with light-cured glass ionomer core buildup (Vitro Fill LC®, DFL, Rio de Janeiro, Brazil) and kept for 5 weeks at 37°C, immersed in Artificial Saliva (Botica Ouro Preto, Maringá, Brazil) preconized by Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista (UNESP, ANEXO C), with daily replacement. At the first 2 days, vials contained specimens were shaken on a Vortex for 30min every 12h (6, 13).

Endodontic Access

A standard endodontic access cavity was made in specimens using spherical diamond bur in a high-speed handpiece and inactive point, 3081 (KG Sorensen, Cotia, Brazil). The cavity was cleaned using 1% sodium hypochlorite and 17% trisodium EDTA (ethylenediamine tetraacetic acid, Biodinâmica, Ibiporã, Brazil). Following the clinical protocol, a cervical sealing was conducted with light-cured glass ionomer liner (Vitrebond®, 3M, Sumaré, Brazil) at the height of CEJ.

Bleaching Procedure

Specimens were randomly divided into two groups of equal size (n=10) according to the bleaching agent used: Group HP- 35% hydrogen peroxide (Whitness HP®, FGM, Joinville, Brazil), with the ratio of 3 drops of peroxide for 1 drop of thickener, applied within

the pulp chamber for 15min, 3 times per session, and after that the chamber was washed, dried and a cotton pellet was introduced; and, Group SP- sodium perborate (Whitness perborate®, FGM, Joinville, Brazil), mixed with bidistilled water with a consistency of wet sand, fulfilling the pulp chamber (7).

A piece of absorbent paper (Melitta filter paper, Mellita Brasil, São Paulo, Brazil) was placed on both groups, adapted in the coronal access (10), and then, sealed with light-cured glass ionomer core buildup (Vitro Fill LC®, DFL, Rio de Janeiro, Brazil). Bleaching agents were used for 14 days: hydrogen peroxide was used into 2 sessions with an interval of 7 days, and perborate was refilled in the pulp chamber at day 7, both based on the literature (5, 10, 13, 14).

Color Assessment

The following color parameters were measured: lightness (L^*), a^*, b^*, Chroma (C^*) and Hue (h°). L^* is the shade alteration in black and white ranging from 0 to 100 with higher numbers being brighter, a^* is the change in saturation from red to green, whereas b^* is from blue to yellow. The axes a^* and b^* were used to obtain C^* and h°. The hue e-specific color was measured from 0° to 360°, where 0° is red, 90° yellow, 180° green and 270° blue (18). The color of the specimens was measured using a Konica Minolta CR-400 reflectance colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), in the illuminant D65, which provides the International Commission on Illumination L^*, a^*, b^* color system (CIELAB) (19). The specimens were removed from the artificial saliva and were gently dried with absorbent paper to remove the excess of moisture. Right after, light-cured glass ionomer core buildup was withdrawn to remove the bleaching agent, irrigation with physiologic solution was performed, and sealed again. The samples were placed over a black background support and under a black mold, with 5mm diameter, built for standardization of size and experimental region, aiming not to disperse or reflect the light at regions that were not the specimen (Fig. 1).

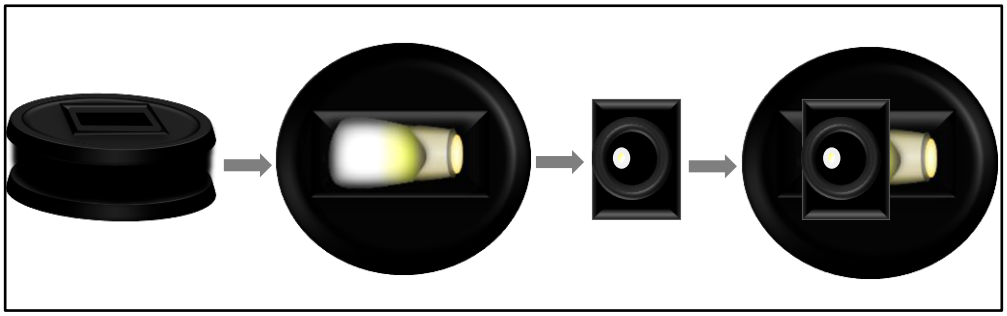

Figure 1. Black background support and the black mold·

Afterwards, the measurement was performed, always observing the perfect fitting, according to the periods: tN (Natural), tD (Discolored), t7 (7 days after 1st session of bleaching), t14 (7 days after 2nd session of bleaching). Three measurements were taken for each specimen, the average was calculated, and CIEDE2000 color-difference formula was computed based on the CIELAB (L^*, a^*, b^*) color coordinates, $\Delta E_{00} = ((\Delta L'/k_L S_L)^2 + (\Delta C'/k_C S_C)^2 + (\Delta H'/k_H S_H)^2 + R_T (\Delta C'/k_C S_C) (\Delta H' k_H S_H))^{1/2}$. This formula accounts the difference between the latest measure L_2^*, a_2^*, b_2^* and first or reference measure L_1^*, a_1^*, b_1^* (20, APÊNDICES B e C). Different references were used depending on the analysis performed, Natural or Discolored. The ΔE_{00} values were classified according to the National Bureau of Standards (NBS), where NBS1 (0.0 to 0.5) values represents an extremely slight change, NBS2 (0.5 to 1.5): slight change, NBS3 (1.5 to 3.0): perceivable change, NBS4 (3.0 to 6.0): marked change, NBS5 (6.0 to 12.0): extremely marked change, and, NBS6 (12.0 or more): change to another color (21).

Results analysis and ethical issues

Data were analyzed using Statistical Analysis System (SAS® 9.3 Institute, Cary NC) with a multinomial logistic regression and repeated measures analysis, using the Maximum-likelihood Estimation tests (MLE) and Analysis of Parameter Estimates, once the values were classified in categorical levels according to the literature for color difference. A 95% level of confidence was considered to be significant.

This study was reviewed and approved by the Local Ethics Committee (14382313.9.0000.0104, ANEXOS D e E).

Results

The CIELAB color space mean and standard deviation values for each stage of the dynamic process of intracoronal bleaching are listed in Table 1.

TABLE 1. CIELAB color coordinates of specimens during the dynamic process of intracoronal bleaching using 35% Hydrogen Peroxide and Sodium Perborate.

Stage of process	L^* $\bar{x}$ ± SD	a^* $\bar{x}$ (±SD)	b^* $\bar{x}$ (±SD)	C^* $\bar{x}$ (±SD)	h° $\bar{x}$ (±SD)
Natural	51.14 ± 1.39	-0.30 ±0.18	3.00 ±1.41	3.03 ±1.41	-1.30 ±0.67
Discolored	44.17 ±1.62	1.07 ±0.47	1.20 ±2.00	2.25 ±1.29	0.44 ±0.87
HP(10)					
t7	51.26 ±1.48	0.92 ±0.39	3.30 ±1.69	3.53 ±1.48	1.18 ±0.35
t14	51.26 ±1.48	-0.01 ±0.48	2.19 ±1.18	2.30 ±1.03	-0.14 ±1.38
SP (10)					
t7	51.02 ±1.36	0.43 ±0.36	2.95 ±0.98	3.01 ±0.97	1.09 ±0.94
t14	51.02 ±1.36	-0.40 ±0.39	2.32 ±0.77	2.38 ±0.77	-0.77 ±1.23

L^* (lightness, ranging from zero to 100 with higher number being brighter); a^* (green-red coordinate); b^* (blue-yellow coordinate); C^* (chroma, lower number means lower chroma); h° (transformed value of hue, specific color measured from zero to 360°) $\bar{x}$- mean; SD- standard deviation

The mean and standard deviation of CIEDE2000 color differences of L_2^*, a_2^*, b_2^* and L_1^*, a_1^*, b_1^* and color coordinate differences are in Table 2.

TABLE 2. CIEDE2000 color differences and color coordinates differences after use of 35% Hydrogen Peroxide and Sodium Perborate with Discolored and Natural teeth.

Stage of process	ΔE_{00} $\bar{x}$ (±SD)	$\Delta L'$ $\bar{x}$ (±SD)	$\Delta C'$ $\bar{x}$ (±SD)	$\Delta H'$ $\bar{x}$ (±SD)
Discolored				
HP (10)				
t7	5.89 ±1.56	5.39 ±1.66	1.09 ±1.44	-0.89 ±2.30
t14	9.78 ±1.11	9.58 ±1.25	-0.27 ±1.24	-0.05 ±2.36
SP (10)				
t7	6.94 ±1.61	6.36 ±1.94	0.44 ±1.43	0.66 ±2.75
t14	13.20 ±1.63	12.80 ±1.64	-0.17 ±1.35	0.98 ±3.28
Natural				
HP (10)				
t7	3.11 ±1.28	-1.84 ±1.44	0.95 ±1.42	-1.96 ±0.65
t14	2.80 ±1.18	2.34 ±1.35	-0.40 ±1.20	0.34 ±1.06
SP(10)				
t7	2.24 ±1.03	-0.33 ±1.95	-0.24 ±1.48	-0.93 ±0.42
t14	6.07 ±1.38	6.09 ±1.40	-0.86 ±1.43	0.45 ±0.79

ΔE_{00} is the CIEDE2000 color differences; $\Delta L'$ is the difference in processing of lightness between measures; $\Delta C'$ is the difference in processing of chroma between measures; $\Delta H'$ is the difference between the processing of hue in measures; $\bar{x}$- mean; SD- standard deviation

Distributions of the ΔE_{00} values classified according to the National Bureau of Standards (NBS) are shown in Table 3, with the results of groups HP and SP. Successful bleaching was accomplished when classification was above NBS4 for Discolored teeth.

Bleached teeth had a more pronounced whiteness than the Natural teeth, observed in both bleaching agents, at 7- and 14-day period (100%). The ΔE_{00} values of perborate were higher than peroxide at 7- and 14-day period compared to discolored, demonstrating major bleaching, and when compared to Natural they were higher only for 14-day period, achieving greater whiteness.

TABLE 3. Changes of color shades during intracoronal bleaching using 35% Hydrogen Peroxide and Sodium Perborate with Discolored and Natural teeth as control, according to NBS parameters.

Stage of process	NBS1	NBS2	NBS3	NBS4 (%)	NBS5 (%)	NBS6 (%)
Discolored						
HP (10)						
t7	—	—	—	50	50	—
t14	—	—	—	—	100	
SP (10)						
t7	—	—	—	30	70	—
t14	—	—	—	—	20	80
Natural						
HP (10)						
t7	—	10	40	50	—	—
t14	—	20	20	60	—	—
SP (10)						
t7	—	30	40	30	—	—
t14	—	—	—	50	50	—

NBS- National Bureau of Standards

Analysis of Maximum-likelihood Estimates (MLE) demonstrated that the time ($p<0.0001$), color ($p<0.0001$), and type of treatment ($p=0.0035$) were significant factors for color analysis of the internal *in vitro* bleaching. Analysis of Parameter Estimates demonstrated that 14-day period had significantly higher values of ΔE_{00} than 7-day period ($p<0.0001$), however it was observed that at 7-day period all teeth had greater or equal whitening of Natural, achieving treatment success. For this *in vitro* study, treatment with perborate achieved significantly higher levels than peroxide bleaching ($p<0.0001$).

Discussion

In this study all specimens after being artificially discolored were subjected to bleaching using HP or SP and showed to achieve the desired whiteness at 7-day period. At the end of experimental time (14 days) they were equal or lighter than the natural color. The bleaching agent SP was more effective at 7- and 14-day periods in these *in vitro* experiments, which simulated the dynamic of intracoronal bleaching.

There are few *in vitro* studies comparing the efficacy of 35% hydrogen peroxide in the immediate technique with sodium perborate in the *walking bleach* technique in the scientific literature reports. A study conducted by Ari & Ungor (5) comparing different types of sodium perborate mixed with distilled water or with 30% hydrogen peroxide using the *walking bleach* technique showed no statistically significant differences between treatment groups. Thus, they indicated the use of sodium perborate with distilled water instead of hydrogen peroxide to minimize the possible occurrence of external root resorption. However, a differential in relation to our study would be that in their study SP was present in all groups. Hydrogen peroxide in the immediate internal bleaching technique has been rarely reported in the literature, there is a study using this technique and showed no significant difference between the ΔE_{ab}^{*} of the groups of 35% hydrogen peroxide and sodium perborate with distilled water (14). We calculated the color difference of bleached teeth using the CIEDE2000 formula which is currently considered more sophisticated than their predecessors, CIELAB ΔE_{ab}^{*} or CIE94 ΔE_{94} (20).

Hydrogen peroxide was not used following all the manufacturer's instructions which suggest performing an intracoronal bleaching and following up with an external bleaching. We used only intracoronal bleaching to analyze its action, without any other interference, supported by results of Cardoso et al. (14) who did not use the HP following all the manufacturer's instructions. They compared HP and SP which had a similar performance concerning the final color after bleaching. The difference between their results and ours could have occurred because they have used the ΔE_{ab}^{*} average instead of using CIEDE2000 formula.

To the best of our knowledge there are no studies that have used the natural color as the baseline for the calculation of color difference. The authors performed the comparison only with artificially discolored tooth (5, 14, 22). The comparison with natural color is important in a clinical procedure because the bleached tooth must be whiter than its neighbors

once its whiteness may reduce right after the treatment and then it will be similar and harmonious to the adjacent teeth (2).

Some studies *in vitro* have demonstrated that 7 days were not enough to achieve success in internal bleaching, and yet even after 21 days not all teeth were able to reach its original color, demonstrating statistical significance between times (5). Another study (22) compared the effectiveness of sodium perborate with distilled water and hydrogen peroxide, both using the *walking bleach* technique, and showed that differences between them were significant at 7 days, but not at 14 days. Our study demonstrated 100% success in whitening teeth at 7-day period and in the treatment with sodium perborate the final color right after the procedure was lighter than the natural.

An *in vitro* study may differ from a clinical procedure. It must be taken into consideration that as tooth bleaching was achieved artificially, the adhesion of the pigment may be lower than a clinical reality, where we have the presence of bacteria and its products. The time that the tooth remains clinically discolored also may influence the chance of a successful treatment (5, 23). Finally, the use of bovine teeth takes a shorter time than human teeth to reach whiteness once the former shows greater diameter of dentin tubules (24). However the difficulty of finding same group of human extracted teeth, especially anterior, without cavities, fractures or cracks justified our choice.

In conclusion, the final color of bleached teeth with 35% peroxide and sodium perborate were equal or lighter than the natural color, although sodium perborate was more effective at 7- and 14-day periods in these *in vitro* experiments which simulated the dynamic of intracoronal bleaching. The period of time considered sufficient to achieve the whitening was 7 days.

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3. ARTIGO 2

Vibrational Spectroscopy of the Dynamic Process of Intracoronal Bleaching

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ABSTRACT

The aim of this *in vitro* study was to analyze by vibrational spectroscopy the tooth dentin molecular structure after staining and bleaching using 35% hydrogen peroxide (HP) and sodium perborate (SP) simulating a clinical situation over time. Twenty extracted bovine incisors were artificially stained with human blood from the inside. Discolored specimens were divided in Groups: HP and SP, in an experimental period of 14 days: 2 sessions with a 7-day interval. Vibrational spectroscopy was measured by Micro-Raman Spectroscopy (MRS) and Fourier Transform Infrared (FTIR). MRS spectra were performed at the labial surface, and dentin powder samples for FTIR measurements were obtained from lingual surface. Measurements were taken in 4 periods: when teeth were Natural, Discolored, after 7 and 14 days of bleaching. MRS and FTIR data were processed using the OPUS® *software.* Raman spectra, between 1710-1620 cm^{-1} and 1360-1300 cm^{-1}, of Natural teeth were different from Discolored, and similar to Bleached in the 2 groups in both periods of time. FTIR analysis did not show significant changes. Discoloration causes interaction between artificial staining and tooth dentin, which returns to natural condition by using bleaching procedures.

KEY WORDS: Dentin, Bleaching agents, Spectrum analysis, Tooth bleaching.

INTRODUCTION

Intracoronal bleaching is a highly suggested treatment for discolored teeth because it is effective and conservative. The effects, however, that intracoronal bleaching causes to tooth structure are still under study (Jiang *et al*., 2007).

There is a vast literature demonstrating the effects of bleaching agents *in vitro* (Benetti *et al*., 2004; Park *et al*., 2004; Chng *et al*., 2005; Bistey *et al*., 2007; Camps *et al*., 2007; Jiang *et al*., 2007; Jiang *et al*., 2008; Toledano *et al*., 2011; Ubaldini *et al*., 2013). To our knowledge, however, no study has analyzed pre- and post-treatment structural change in a dynamic process simulating a clinical situation over time after the use of different intracoronal bleaching products in non-vital teeth. Specially studies regarding hydrogen peroxide using immediate technique, and sodium perborate using the *walking bleach* technique.

The method ordinarily used to simulate the coronal darkening is to immerse tooth in blood to achieve the darkness by the permeation from the outside (Freccia and Peters, 1982; Ari and Ungor, 2002; Carrasco *et al.*, 2003; Yui *et al*., 2008; Cardoso *et al*., 2013), not done from the inside, such as promoted by the pulp necrosis.

Researches (Chng *et al.*, 2005; Bistey *et al*., 2007; Jiang *et al*., 2007; Toledano *et al*., 2011; Ubaldini *et al.*, 2013) have shown structural alterations caused by bleaching in dentin and enamel, using 10%, 20%, 25% or 30% hydrogen peroxide. The changes reported were: high collagen degradation (Toledano *et al*., 2011), destruction of organic components and changes in the mineral components of dentin that were mostly caused by its oxidizing ability and acidity, respectively (Jiang *et al.*, 2007), and changes in the surfaces of intertubular dentin (Chng *et al.*, 2005).

The aim of this *in vitro* study was to analyze the dentin structure in the dynamic process of tooth artificially stained from the inside and bleached using 35% hydrogen peroxide and sodium perborate simulating a clinical situation over time.

MATERIALS & METHODS

Preparation of specimens

The studied samples were 20 extracted bovine central incisors, donated by a regulated and certified slaughter house, belonging to animals from the same herd. They were thoroughly cleaned; calculus and remnants of periodontal ligament were removed, brushed, rinsed and

stored in a physiologic solution at 4°C. After that, they were submitted to visual inspection to assure that there were no cracks. Fig. 1 shows schematic representation of the methodological procedures. Specimens were individually positioned by the incisal part with the height of 1/4 of the crown in blocks of 1.5 x 1.5cm made of acrylic resin (JET, Clássico, São Paulo, Brazil) to be base and support during the experiment (Ver também APÊNDICE A). They were positioned in a sectioning machine with cooled water (ISOMET, São Paulo, Brazil). Roots were sectioned at 5mm from the cementoenamel junction (CEJ), dental pulp was removed, and irrigation with physiologic solution was performed. The remainder was also sectioned, at 3mm from the CEJ in the coronary portion, to allow the future spectrophotometers reading. The incisal border was attached to a resin block. To make a complete sealing of it, saddle was done with the aid of a microbrush with cyanoacrylate (Scotch, 3M, Sumaré, Brasil) on the outside.

Artificial staining

Specimens were artificially stained with human blood using a modified procedure based on usual techniques (Freccia and Peters, 1982; Carrasco *et al.*, 2003), however with the permeation from the inside. The blood was obtained from a healthy donor and centrifuged to obtain a biphasic solution: plasma which was removed, and precipitate in which distilled water was added (20mL H_2O/30mL blood). The hemoglobin degradation process was accelerated by using a laser (442nm) for 2h, with homogenization every 30min. This blood was injected into the teeth by the region of the anatomical neck until the fulfilling of the pulp chamber. Afterwards, the sealing was performed with light-cured glass ionomer core buildup (Vitro Fill LC®, DFL, Rio de Janeiro, Brazil) and kept for 5 weeks at 37°C, immersed in Artificial Saliva (Botica Ouro Preto, Maringá, Brazil) preconized by Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista (UNESP, ANEXO C), with daily replacement. At the first 2 days, vials contained specimens were shaken on a Vortex for 30min every 12h (Carrasco *et al.*, 2003; Martin-Biedma *et al.*, 2010).

Endodontic access

A standard endodontic access cavity was made in specimens using spherical diamond burs in a high-speed handpiece and 3081 inactive points (KG Sorensen, Cotia, Brazil). The cavity was cleaned using 1% sodium hypochlorite and 17% trisodium EDTA (ethylenediamine tetraacetic acid, Biodinâmica, Ibiporã, Brazil). Following the clinical protocol a cervical

sealing was conducted with light-cured glass ionomer liner (Vitrebond®, 3M, Sumaré, Brazil) at the height of CEJ.

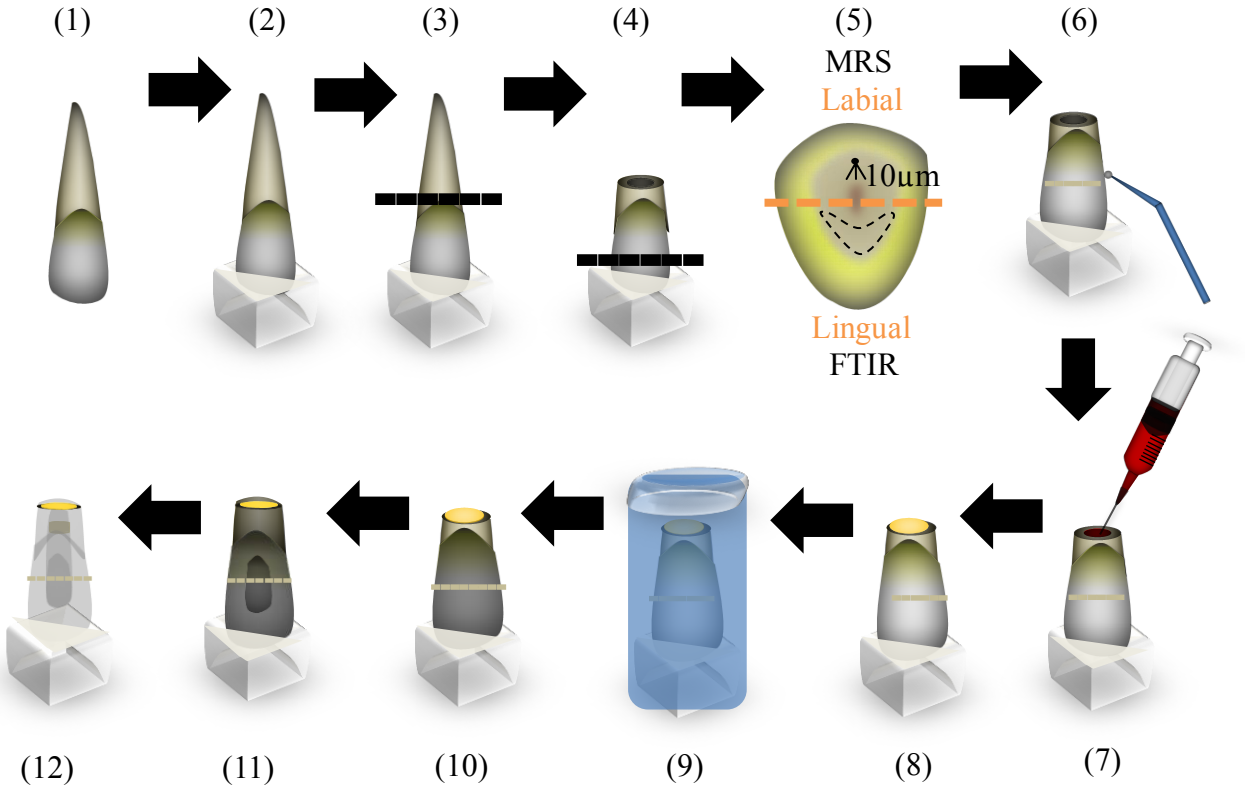

Figure 1. Schematic representation of the methodological procedures. (1) Bovine central incisor (2) positioned by the incisal part with the height of 1/4 of the crown in blocks of 1.5 x 1.5cm. (3) Root sectioned from 5mm from the CEJ and discarded. (4) The remainder was sectioned from 3mm of the CEJ in the coronary portion. (5) Specimen was divided by an imaginary line according to the subsequent reading: labial performed Micro-Raman at the center of the teeth, in a distance of 10µm from pulp cavity, and lingual where the powder samples for FTIR measurements were obtained. (6) Saddle with the aid of a microbrush with cyanoacrylate. (7) Blood injected into the tooth by the anatomical neck until the fulfilling the pulp chamber. (8) Sealing performed with light-cured glass ionomer core buildup. (9) The set was immersed in Artificial Saliva for 5 weeks at 37°C. At the first 2 days, vials were shaken on a Vortex for 30min every 12h. (10) Labial aspect of discolored tooth. (11) Standard endodontic access cavity at Lingual. (12) Cervical sealing was conducted with light-cured glass ionomer liner, 2mm thick, at the height of CEJ. After that, specimens were divided into two groups (HP, SP) and intracoronal bleaching was performed.

Bleaching procedure and color assessment

Specimens were randomly divided into two groups of equal size (n=10) according to the bleaching agent used: Group HP- 35% hydrogen peroxide (Whitness HP®, FGM, Joinville, Brazil), with the ratio of 3 drops of peroxide for 1 drop of thickener, applied within the pulp chamber for 15min, 3 times per session, and after that, the chamber was washed, dried and a cotton pellet was introduced; and, Group SP- sodium perborate (Whitness perborate®, FGM, Joinville, Brazil), mixed with bidistilled water with a consistency of wet sand, fulfilling the pulp chamber (Chng *et al*., 2002).

A piece of absorbent paper (Melitta filter paper, Mellita Brasil, São Paulo, Brazil) was placed on both groups, adapted in the coronal access (Yui *et al.*, 2008) and then, sealed with light-cured glass ionomer core buildup (Vitro Fill LC®, DFL, Rio de Janeiro, Brazil). Bleaching agents were used for 14 days: hydrogen peroxide was used into 2 sessions with an interval of 7 days, and perborate was refilled in the pulp chamber at day 7, both based on the literature (Ari and Ungör, 2002; Yui *et al*., 2008; Martin-Biedma *et al.*, 2010; Cardoso *et al*., 2013).

The color of the specimens was measured using a Konica Minolta CR-400 reflectance colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) in the illuminant D65, which provides the International Commission on Illumination L^*, a^*, b^* color system (CIELAB) (Browning *et al*., 2009). The final color of bleached teeth using 35% HP and SP were equal or lighter than the previous natural color, although SP was more effective at 7- and 14-day interval. For more detail see Uchimura *et al*. (2014).

Preparation to Spectroscopic measurement

The specimens were removed from the individual vials with artificial saliva and gently dried with absorbent paper to remove the excess of moisture. The light-cured glass ionomer core buildup was withdrawn to remove the bleaching agent, then irrigation with physiologic solution was performed, and the cavity was sealed again. The region where the cross-sectioning was made, at coronary 3mm from CEJ, was opened and divided by an imaginary line according to the subsequent reading: labial to MRS, and lingual to FTIR spectrometers measurements.

The measurements were taken during the dynamic process, according to the experimental periods: Natural, Discolored, 7- and 14-day interval after the beginning of the intracoronal bleaching.

Micro-Raman Spectroscopy

The Raman spectra were performed at the center of the teeth at the labial surface of the dentin, in a distance of 10μm from pulp cavity. Measurements were taken using a Bruker Senterra dispersive Raman microscope spectrometer (Bruker Optik GmbH, Ettingen, Germany) which the detector is a CCD (Charge Coupled Device) camera cooled at temperature of -90ºC. The laser excitation wavelength was 785nm with 100mW of power and focused on the sample by 20x objective. All the Raman spectra are an average of 50 scans collected in 50-3600cm^{-1} spectral range with spatial resolution between 3–5cm^{-1} and integration time of 3s.

Fourier Transform Infrared Spectroscopy

The dentin powder samples for FTIR measurements were obtained from the lingual surface of teeth which were removed using a low-speed handpiece and a diamond burs n°1 (KG Sorensen, Cotia, Brazil). The powder samples were diluted in potassium bromide (KBr), homogenized and compressed to make a pellet. The spectra were collected from Vertex 70 vacuum FTIR spectrometer (Bruker, Billerica, USA) minimizing spectral features caused by water vapor or CO_2 absorptions, where the pellet perfectly fitted. The final spectrum of each measurement was a result of 128 scans; spectral resolution was 4cm^{-1} obtained between 4000-400cm^{-1}.

Results analysis and ethical issues

MRS and FTIR data were processed using the OPUS® *software* (Bruker Opitik GmbH). Raman-peaks intensity were normalized in relation to v_1 PO_4^{-3} (961cm^{-1}), with baseline correction. Gaussian deconvolution was performed to analyze Raman spectra.

This study was reviewed and approved by the Local Ethics Committee (14382313.9.0000.0104).

RESULTS

Micro-Raman Spectroscopy

Table 1 shows the main peaks present in the Raman spectra and their assignments.

Table 1. Main Raman peaks of Natural tooth dentin and their assignments.

Peak number	Peak position (cm^{-1})	Band assignments
1	2985; 2940	ν_{as}C-H asymmetric; ν_{s}C-H symmetric
2	1667	Amide I
3	1423	δCH_2
4	1341; 1319	δCH_2 and δ CH_3
5	1270; 1246	Amide III
6	1103; 1070;1037; 1002	$\nu_{1s} PO_4^{-3}$; CO_3^{-2} B-type; $\nu_{3s}PO_4^{-3}$; Phenylalanine
7	959,5	$\nu_{1s} PO_4^{-3}$
8	610; 585	$\nu_{4s} PO_4^{-3}$
9	449; 428	$\nu_{2s}PO_4^{-3}$

ν_{as}: assymetric streching; ν_{s}: symmetric streching; δ: bending

The Natural and Discolored tooth dentin spectra are shown in the Fig. 2A. The numbers indicate the peaks of Natural tooth dentin which position and assignments are in Table 1. The insets (B and C) indicate the region between 1710–1620cm^{-1} and 1360–1300cm^{-1}, respectively, where the analyses were performed.

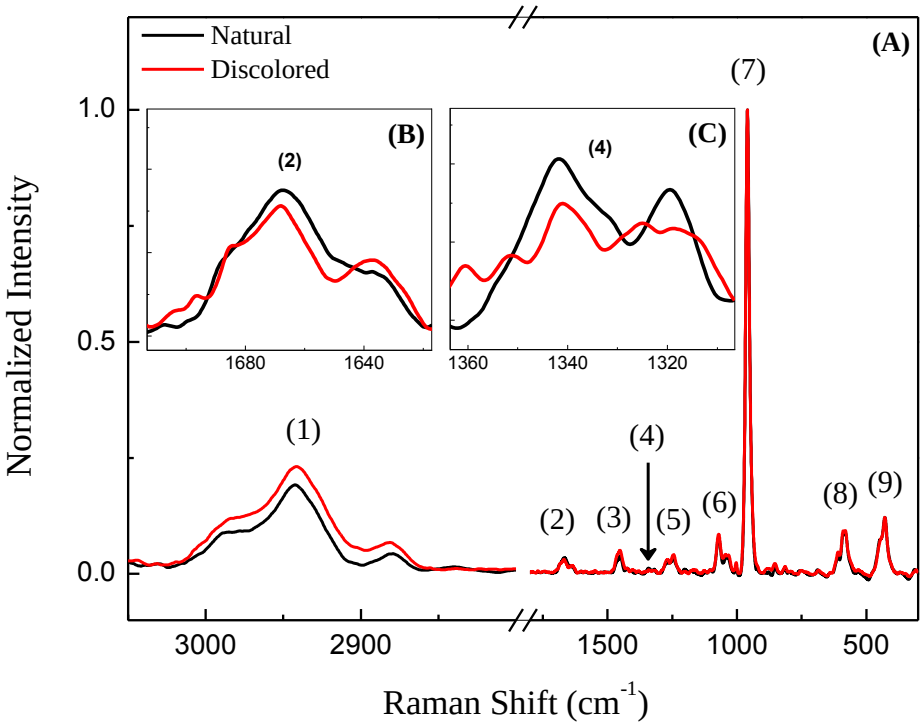

Figure 2. (A) Comparison of Raman spectra of Natural and Discolored tooth dentin (n = 20); (B and C) insets of main spectra where the analyses were performed because there were differences between them.

Fig. 3 shows, respectively, the Raman spectra mean of group HP (n=10) and group SP (n=10) for Natural tooth dentin, after artificial staining (Discolored), and 7- and 14-day period after the beginning of the intracoronal bleaching. The amide I region between 1710cm^{-1} and 1620cm^{-1} for both groups is represented by Fig. 3A and Fig. 3C, and the region of 1360 to 1300cm^{-1} is in the Fig. 3B and 3D.

The mean spectra for Natural, Discolored and bleached with HP and SP tooth dentin were deconvoluted by Gaussian functions to obtain peaks before and after treatments. In the 1710-1620cm^{-1} (amide I) spectral range, all specimens presented Gaussians centered at 1689cm^{-1}, 1683cm^{-1}, 1667cm^{-1}, 1645cm^{-1} and 1632cm^{-1} features of secondary protein structures. For SP bleached group the 1689cm^{-1} peak and the 1683cm^{-1} absent from the Gaussian fitting were overlapping. At the spectral range of 1360 to 1300cm^{-1}, the peak center was at 1350cm^{-1} [$_{\delta}$(C–H) + $_{\delta}$(C–O-H)], 1342cm^{-1} and 1319cm^{-1} ($_{\delta}CH_2$ and $_{\delta}CH_3$) and 1334cm^{-1} [$_{vs}$(C–O) + $_{\delta}$(O–C=O)]. Discolored tooth dentin spectra showed additional peak at 1325cm^{-1}. No additional peaks were observed after the bleaching process.

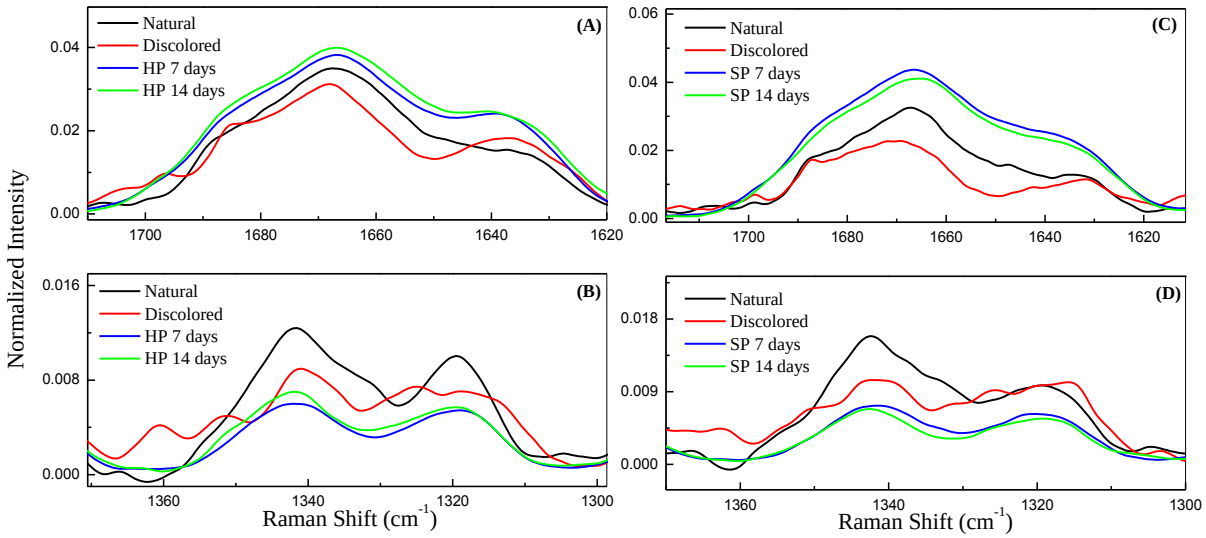

Figure 3. Raman spectra of Natural, Discolored, and bleached tooth dentin using (A and B) 35% hydrogen peroxide (HP, n=10) and (C and D) sodium perborate (SP, n=10) after 7-day (7 days) and 14-day (14 days) period. (A and C) Amide I region between 1710cm^{-1} and 1620cm^{-1}, and (B and D) region of 1360cm^{-1} to 1300cm^{-1}.

Fig. 4 shows the Gaussian fit of Group SP in the 1710-1620cm^{-1} spectral region before and after the treatments. For the changed spectral range, Gaussian fitting was performed for all the averaged spectra of both groups, taking into account the numbers of peaks necessary to

the best fit of the experimental data and the Raman literature for tooth dentin structure. Only Group SP results were presented because they are similar from those of Group HP.

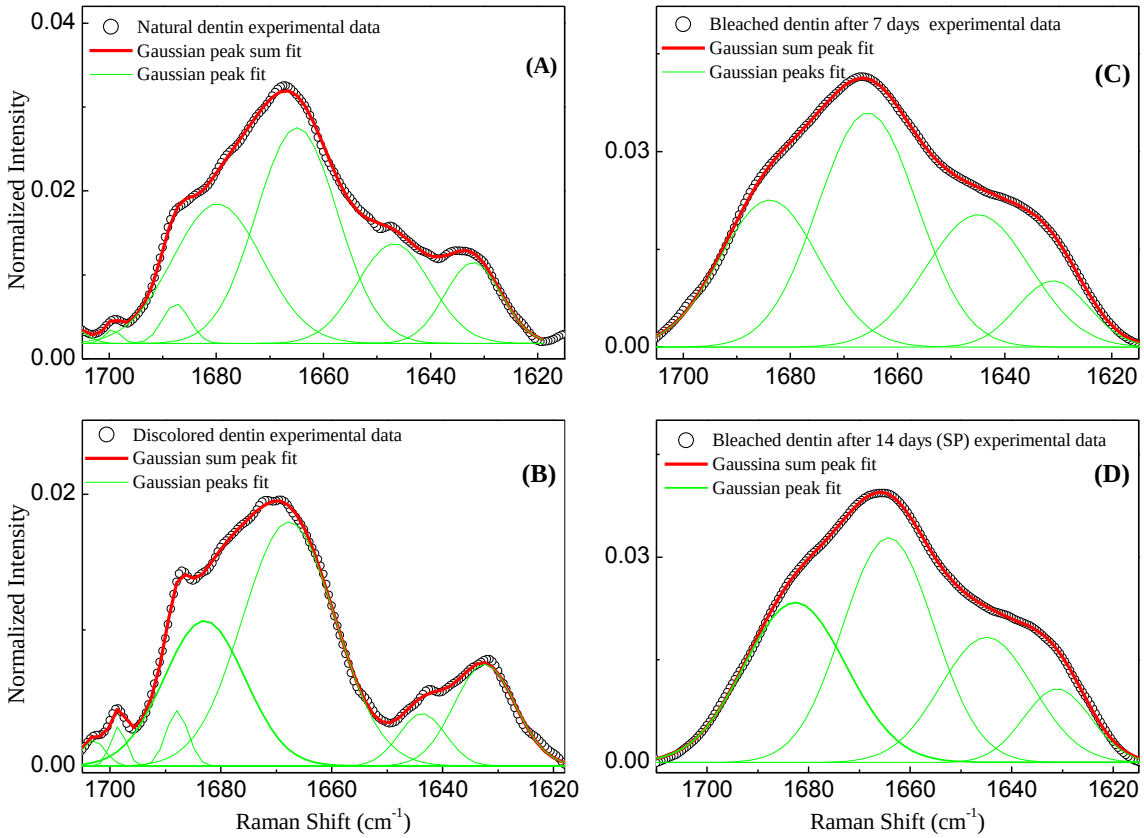

Figure 4. Gaussian fitting of Raman spectra between 1710-1620cm^{-1} of Natural, Discolored and bleached tooth dentin using sodium perborate after 7-day (SP 7 days) and 14-day (SP 14 days) period. Open circles represents experimental Raman data, green lines are the Gaussian peaks of dentin structure and the red line is the sum of green Gaussian peaks.

Fig. 5 presents the area of peaks obtained by Gaussian fitting treatments at 1325, 1645 and 1683cm^{-1}, and the width of the peak centered at 1319cm^{-1}. All peaks showed similar behavior independently of the bleaching agent used.

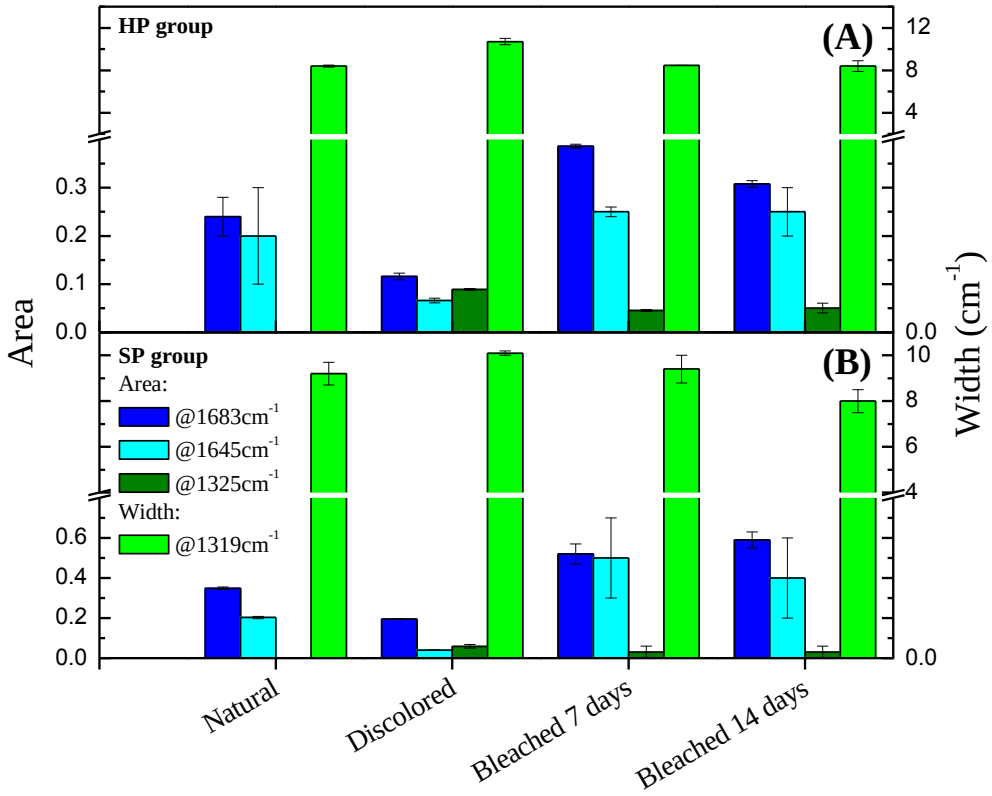

Figure 5. Peak areas before and after treatments centered at 1325, 1645 and 1683cm^{-1} (left axis) and peak width at 1319cm^{-1} (right axis) of groups (A) HP and (B) SP.

FTIR Spectroscopy

We also performed FTIR spectroscopy in the tooth dentin in groups HP and SP comparing Natural, Discolored and bleached after 7- and 14-day period. No significant spectral change was observed.

DISCUSSION

To the best of our knowledge this is the first study to examine the structure of tooth dentin in a dynamic process simulating a clinical situation over time, *i.e.* pre- and post- *in vitro* intracoronal bleaching using different techniques, such as HP at the immediate technique, and SP using the *walking bleach* one. Our results demonstrated conformational change of functional groups in the tooth dentin by MRS analysis of Discolored teeth. The interaction between blood and dentin in the deformation of C-H region showed the appearance of a peak at 1325cm^{-1} and a small broadening at 1319cm^{1} (Fig. 5). Furthermore,

there was a narrowing at 1683cm^{-1} and a decrease in the peak area at 1645cm^{-1}. Our results suggest that the organic region of tooth dentin was more susceptible to interaction with blood, and bleaching with 35% HP and SP did not show structural changes in dentin. The FTIR analysis demonstrated to have lower sensitivity for detection of changes in this type of study.

We showed, by MRS analyses, that blood possibly interacted with dentin in a physical or chemical form, which is not specifically denoted, in protein regions of 1710cm^{-1} to 1620cm^{-1} and of 1370cm^{-1} to 1300cm^{-1}. A conformational alteration of proteins may be occurred when the tooth comes in contact with the blood. The results indicate the restoration to Natural aspect of dentin spectra after the action of bleaching agent, which probably paralyzed the change process which was just beginning (Fig. 5). The 1683cm^{-1} peak maximum area was observed after 7-day period of bleaching process in the group HP. For this period and after, the 14-day period, in the group SP this peak stabilized in relation to Natural, while for group HP we observed a decrease of the area at 14-day period. This may indicate that the SP process reached the bleaching faster than the group HP. The appearance of a peak at 1325cm^{-1} and its decrease after bleaching process for both bleach agents suggest that the remaining blood into the dentin was partially removed since the peak still appears in the bleach spectra. Blood interaction with tooth showed a decrease of 1645cm^{-1} peak area followed by an increase of the area after bleaching process with SP and HP. However the observed increase was larger than the area of the same peak in relation to Natural spectra; this behavior may be due to the increase of a neighbor peak at 1667cm^{-1} after bleaching process with SP and HP, which area (not plotted) is larger than the 1645cm^{-1} peak area. The amide I conformation might have been modified during the HP and SP bleaching process resulting in an increase of the peak intensities. The discoloration of teeth by pulpar hemorrhage is due to the combination between degraded hemoglobin and hydrogen sulfide (Freccia and Peters, 1982), but we could not analyze this reaction in this study.

Others authors (Park *et al*., 2004; Cesar *et a*l., 2009) reported that bleaching teeth using HP for a longer time have not shown structural changes by FT-Raman Spectroscopy either. Ubaldini et al. (2013), demonstrating alteration in dentin structure after simulating in-office dental bleaching with HP, showed the chemical affinity of the 25% HP with the organic portion of dentin after 60min of uninterrupted treatment by Micro-Raman and FTIR-PAS analyses. Using different techniques of spectroscopy, Carrasco-Guerisoli *et al*. (2009) performed topographic analysis with Scanning electron microscope and explained that structural change in dentin was influenced by the low pH and by oxidation of HP; Chng *et al.*

(2004) using Atomic Force Microscopy (AFM) showed that exposure of 30% HP during a 24h period resulted on surface changes in the intertubular dentin.

Performing structural teeth analysis for the intracoronal *walking bleach* technique with SP mixed with distilled water, Martin-Biedma *et al*. (2010) also found no changes by emission scanning electron microscopy (FESEM).

FTIR Spectroscopy did not show differences in tooth structure compared to others kind of analyses (Jiang *et al.,* 2007; Sato *et al*., 2013). In our study, Micro-Raman Spectroscopy showed changes in spectra of tooth dentin after discoloration with a return-to-natural condition after bleaching procedures, while FTIR was not able to detect these changes. However, Bistey *et al*. (2007) using FTIR showed changes in the enamel after the use of bleaching agents observing that longer period of use and greater concentration of HP resulted in more pronounced changes.

The use of the spectroscopy to identify tooth tissues structural changes is still new in Dentistry, specifically in techniques for intracoronal bleaching and *in vitro* studies engaged in a dynamic process of the clinical protocol. Our study analyzes the alterations in dentin in a clinical reality, which the same specimen was used in all test steps, the blood was used to discolor the specimen, and the bleaching agents were put from the inside at the pulp chamber. Measures using vibrational spectroscopy, Micro Raman and FTIR, were performed at time intervals of 7 days with no bleaching agent used during the measurement.

In the studied dynamic process, discoloration causes interaction of artificial staining with tooth dentin, which return to their natural condition after intracoronal bleaching techniques using 35% HP or SP. It is presumed that an aggregation of blood in the structure has resulted in a slight masking of the structural conformation. Thus, in our study conditions molecular structural changes in dentin after use of the bleaching agents were not observed and Micro-Raman Spectroscopy was indicates to use for this type of analysis.

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ANEXO A

Normas para publicação de artigos no JOE

Writing an effective article is a challenging assignment. The following guidelines are provided to assist authors in submitting manuscripts.

The *JOE* publishes original and review articles related to the scientific and applied aspects of endodontics. Moreover, the *JOE* has a diverse readership that includes full-time clinicians, full-time academicians, residents, students and scientists. Effective communication with this diverse readership requires careful attention to writing style.

General Points on Composition

1. Authors are strongly encouraged to analyze their final draft with both software (*e.g.*, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery since their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision, or have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the *JOE*:

2. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section “c” below), or sentences with little to no transition within a paragraph.

3. Keep to the point. The subject of the sentence should support the subject of the paragraph. For example, the introduction of authors’ names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, “In 1983, Langeland et al., reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals” can be edited to: “Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al., 1983)." In this example, the paragraph’s subject is sodium hypochlorite and sentences should focus on this subject.

4. Sentences are stronger when written in the active voice, *i.e.*, the subject performs the action. Passive sentences are identified by the use of passive verbs such as “was,” “were,” “could,” etc. For example: “Dexamethasone was found in this study to be a factor that was associated with reduced inflammation,” can be edited to: “Our results demonstrated that dexamethasone reduced inflammation.” Sentences written in a

direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

5. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)

6. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.

7. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."

8. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.

9. Authors should be aware that the *JOE* uses iThenticate, plagiarism detection software, to assure originality and integrity of material published in the *Journal*. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by new manuscript author's own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected, or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

Organization of Original Research Manuscripts

Please Note: *All abstracts should be organized into sections that start with a one-word title (in bold),* i.e.*, Introduction, Methods, Results, Conclusions, etc., and should not exceed more than 250 words in length.*

1. **Title Page:** The title should describe the major emphasis of the paper. It should be as short as possible without loss of clarity. Remember that the title is your advertising

billboard—it represents your major opportunity to solicit readers to spend the time to read your paper. It is best not to use abbreviations in the title since this may lead to imprecise coding by electronic citation programs such as PubMed (*e.g.*, use "sodium hypochlorite" rather than NaOCl). The author list must conform to published standards on authorship (see authorship criteria in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals at *www.icmje.org*). The manuscript title, name and address (including email) of one author designated as the corresponding author. This author will be responsible for editing proofs and ordering reprints when applicable. The contribution of each author should also be highlighted in the cover letter.

2. **Abstract:** The abstract should concisely describe the purpose of the study, the hypothesis, methods, major findings and conclusions. The abstract should describe the new contributions made by this study. The word limitations (250 words) and the wide distribution of the abstract (*e.g.*, PubMed) make this section challenging to write clearly. This section often is written last by many authors since they can draw on the rest of the manuscript. Write the abstract in past tense since the study has been completed. Three to ten keywords should be listed below the abstract.

3. **Introduction:** The introduction should briefly review the pertinent literature in order to identify the gap in knowledge that the study is intended to address and the limitations of previous studies in the area. The purpose of the study, the tested hypothesis and its scope should be clearly described. Authors should realize that this section of the paper is their primary opportunity to establish communication with the diverse readership of the *JOE*. Readers who are not expert in the topic of the manuscript are likely to skip the paper if the introduction fails to succinctly summarize the gap in knowledge that the study addresses. It is important to note that many successful manuscripts require no more than a few paragraphs to accomplish these goals. Therefore, authors should refrain from performing extensive review or the literature, and discussing the results of the study in this section.

4. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components to this section are the detailed description of the materials used and their components, the experimental design, the procedures employed, and the statistical tests used to analyze the results. The vast majority of manuscripts should cite prior studies using similar methods and succinctly describe the essential aspects used in the present study. Thus, the reader should still be able to understand the method used in the experimental approach and concentration of the main reagents (*e.g.*, antibodies, drugs, etc.) even when citing a previously published method. The inclusion of a "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then the authors should carefully describe the method and include validation experiments. If the study utilized a **commercial product**, the manuscript must state that they either followed manufacturer's protocol *or* specify any changes made to the protocol. If the study used an ***in***

***vitro* model** to simulate a clinical outcome, the authors must describe experiments made to validate the model, or previous literature that proved the clinical relevance of the model. Studies on **humans** must conform to the Helsinki Declaration of 1975 and state that the institutional IRB/equivalent committee(s) approved the protocol and that informed consent was obtained after the risks and benefits of participation were described to the subjects or patients recruited. Studies involving **animals** must state that the institutional animal care and use committee approved the protocol. The statistical analysis section should describe which tests were used to analyze which dependent measures; p-values should be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, drop-outs from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.

5. **Results:** Only experimental results are appropriate in this section (*i.e.*, neither methods, discussion, nor conclusions should be in this section). Include only those data that are critical for the study, as defined by the aim(s). Do not include all available data without justification; any repetitive findings will be rejected from publication. All Figures, Charts and Tables should be described in their order of numbering with a brief description of the major findings. Author may consider the use of supplemental figures, tables or video clips that will be published online. Supplemental material is often used to provide additional information or control experiments that support the results section (*e.g.*, microarray data).

6. **Figures:** There are two general types of figures. The first type of figures includes photographs, radiographs or micrographs. Include only essential figures, and even if essential, the use of composite figures containing several panels of photographs is encouraged. For example, most photo-, radio- or micrographs take up one column-width, or about 185 mm wide X 185 mm tall. If instead, you construct a two columns-width figure (*i.e.*, about 175 mm wide X 125 mm high when published in the *JOE*), you would be able to place about 12 panels of photomicrographs (or radiographs, etc.) as an array of four columns across and three rows down (with each panel about 40 X 40 mm). This will require some editing to emphasize the most important feature of each photomicrograph, but it greatly increases the total number of illustrations that you can present in your paper. Remember that each panel must be clearly identified with a letter (*e.g.*, "A," "B," etc.), in order for the reader to understand each individual panel. Several nice examples of composite figures are seen in recent articles by Jeger et al (J Endod 2012;38:884–888); Olivieri et al., (J Endod 2012;38:1007 1011); Tsai et al (J Endod 2012;38:965–970). Please note that color figures may be published at no cost to the authors and authors are encouraged to use color to enhance the value of the illustration. Please note that a multipanel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for maximum number of allowable figures).
   The second type of figures are graphs (*i.e.*, line drawings including bar graphs) that plot a dependent measure (on the Y axis) as a function of an independent measure

(usually plotted on the X axis). Examples include a graph depicting pain scores over time, etc. Graphs should be used when the overall trend of the results are more important than the exact numerical values of the results. For example, a graph is a convenient way of reporting that an ibuprofen-treated group reported less pain than a placebo group over the first 24 hours, but was the same as the placebo group for the next 96 hours. In this case, the trend of the results is the primary finding; the actual pain scores are not as critical as the relative differences between the NSAID and placebo groups.

7. **Tables:** Tables are appropriate when it is critical to present exact numerical values. However, not all results need be placed in either a table or figure. Instead, the results could simply state that there was no inhibition of growth from 0.001-0.03% NaOCl, and a 100% inhibition of growth from 0.03-3% NaOCl (N=5/group). Similarly, if the results are not significant, then it is probably not necessary to include the results in either a table or as a figure. These and many other suggestions on figure and table construction are described in additional detail in Day (1998).

8. **Discussion:** This section should be used to interpret and explain the results. Both the strengths and weaknesses of the observations should be discussed. How do these findings compare to the published literature? What are the clinical implications? Although this last section might be tentative given the nature of a particular study, the authors should realize that even preliminary clinical implications might have value for the clinical readership. Ideally, a review of the potential clinical significance is the last section of the discussion. What are the major conclusions of the study? How does the data support these conclusions

9. **Acknowledgments:** All authors must affirm that they have no financial affiliation (*e.g.*, employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest should be disclosed. Any author for whom this statement is not true must append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Likewise the sources and correct attributions of all other grants, contracts or donations that funded the study must be disclosed

10. **References:** The reference style follows Index Medicus and can be easily learned from reading past issues of the *JOE*. The *JOE* uses the Vancouver reference style, which can be found in most citation management software products. Citations are placed in parentheses at the end of a sentence or at the end of a clause that requires a literature citation. Do not use superscript for references. Original reports are limited to 35 references. There are no limits in the number of references for review articles.

Manuscripts Category Classifications and Requirements

Manuscripts submitted to the *JOE* must fall into one of the following categories. The abstracts for all these categories would have a maximum word count of 250 words:

1. CONSORT Randomized Clinical Trial-Manuscripts in this category must strictly adhere to the Consolidated Standards of Reporting Trials-CONSORT- minimum guidelines for the publication of randomized clinical trials. These guidelines can be found at *www.consort-statement.org/*. These manuscripts have a limit of 3,500 words, [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

2. Review Article-Manuscripts in this category are either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by extensive review of the literature will should be categorized as "Case Report/Clinical Technique". These manuscripts have a limit of 3,500 words, [including abstract, introduction, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

3. Clinical Research (*e.g.*, prospective or retrospective studies on patients or patient records, or research on biopsies, excluding the use of human teeth for technique studies). These manuscripts have a limit of 3,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

4. Basic Research Biology (animal or culture studies on biological research on physiology, development, stem cell differentiation, inflammation or pathology). Manuscripts that have a primary focus on biology should be submitted in this category while manuscripts that have a primary focus on materials should be submitted in the Basic Research Technology category. For example, a study on cytotoxicity of a material should be submitted in the Basic Research Technology category, even if it was performed in animals with histological analyses. These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or 4 tables*.

5. Basic Research Technology (Manuscripts submitted in this category focus primarily on research related to techniques and materials used, or with potential clinical use, in endodontics). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 3 figures and tables *.

6. Case Report/Clinical Technique (*e.g.*, report of an unusual clinical case or the use of cutting-edge technology in a clinical case). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or tables*.

* Figures, if submitted as multipanel figures must not exceed 1 page length. Manuscripts submitted with more than the allowed number of figures or tables will require approval of the *JOE* Editor or associate editors. If you are not sure whether your manuscript falls within one of the categories above, or would like to request preapproval for submission of additional figures please contact the Editor by email at *jendodontics@uthscsa.edu*.

Importantly, adhering to the general writing methods described in these guidelines (and in the resources listed below) will help to reduce the size of the manuscript while maintaining its focus and significance. Authors are encouraged to focus on only the essential aspects of the study and to avoid inclusion of extraneous text and figures. The Editor may reject manuscripts that exceed these limitations.

ANEXO B

Normas para publicação de artigos no JDR

The *Journal of Dental Research* (*JDR*) is a peer-reviewed scientific journal dedicated to the dissemination of new knowledge and information on all science relevant to dentistry and to the oral cavity and associated structures in health and disease. The*Journal of Dental Research's* primary readership consists of oral, dental and craniofacial researchers, clinical scientists, hard-tissue scientists, dentists, dental educators, and oral and dental policy-makers. The *Journal* is published monthly, allowing for frequent dissemination of its leading content. The *Journal of Dental Research* also offers OnlineFirst, by which forthcoming articles are published online before they are scheduled to appear in print.

Authors of all types of articles should be aware of the following guidelines when submitting to *JDR*.

ONLINE SUBMISSION
Submissions to the *Journal of Dental Research* are only accepted for consideration via the SAGETrack online manuscript submission site at http://mc.manuscriptcentral.com/jdr.
Authors who do not have an active account within the system are required to create a new account by clicking, "Create Account," on the log-in page. The system will prompt the authors through a step by step process to create their account. Once created authors can submit their manuscripts by entering their "Author Center" and clicking the button by "Click Here to Submit a New Manuscript."

If any difficulty is encountered at any time during the account creation or submission process, authors are encouraged to contact the *Journal of Dental Research*Publications Coordinator, Kourtney Skinner, at kskinner@iadr.org.

MANUSCRIPT REQUIREMENTS BY TYPE
The *Journal of Dental Research* accepts the following types of manuscripts for consideration:
Original Research Reports: These manuscripts are based on clinical, biological, and biomaterials and bioengineering subject matter. Manuscripts submitted as research reports have a limit of 2,700 words (including abstract, introduction, materials, methods results, discussion and acknowledgments; excluding figure legends and references); a total of 4 figures or tables; 30 references; and must contain a 200 word abstract.

Letters to the Editor*: Letters must include evidence to support a position about the scientific or editorial content of the *JDR*. Manuscripts submitted as a letter to editor have a limit of 250 words. No figures or tables are permitted. Letters on published articles must be submitted within 3 months of the article's print publication date.

Guest Editorials*: A clear and substantiated position on issues of interest to the readership community can be considered for this manuscript type. Guest Editorials are limited to 1,000 words. No figures or tables are permitted.

Discovery!: Essays that explore seminal events and creative advances in the development of dental research are considered for the "Discovery!" section of the*Journal*. Manuscripts submitted for "Discovery!" have a limit of 2,500 words and a total of 2 figures or tables. Manuscripts are to be submitted by invitation only. Questions regarding "Discovery!" should be directed to Dr. Marty Taubman, at mtaubman@forsyth.org.

***Critical Reviews in Oral Biology & Medicine*:** These manuscripts should summarize information that is well known and emphasize recent developments over the last three years with a prominent focus on critical issues and concepts that add a sense of excitement to the topic being discussed. Manuscripts are to be submitted by invitation only. Authors interested in submitting to this section must contact the Editor of *Critical Reviews in Oral Biology & Medicine*, Dr. Dana Graves, at gravesdt@umdnj.edu for submission approval and instructions. Manuscripts submitted as Critical Reviews have a limit of 4,000 words; a total of 6 figures or tables; 60 references; and must contain a 200 word abstract.

Additional Instructions for Critical Reviews:

-It is important to include several illustrations or diagrams to enhance clarity. Manuscripts that lack figures or diagrams typically receive a low priority score.

-Summarize important concepts in tables or flow charts or show critical data in the form of figures. NOTE: authors will need to obtain permission to reproduce a previously published figure or table.

-Due to the broad readership, abbreviations commonly recognized in one field may not be readily apparent to those in a different field. Keep abbreviation use to a minimum.

-The cover page, abstract, text, summary, figure legends, and tables should be combined into a single Word document. Figures should be submitted as a separate document.

-To view examples of recent Critical Reviews in the *Journal*, please click the following links: http://jdr.iadrjournals.org/cgi/content/full/86/9/800 orhttp://jdr.iadrjournals.org/cgi/content/full/85/7/584

***Brief responses to Letters to the Editor or Guest Editorials will be solicited for concurrent publication.**

Clinical Reviews (formerly Concise Reviews): These manuscripts are generally systematic reviews of topics of high clinical relevance to oral, dental and craniofacial research. Meta-analyses should be considered only when sufficient numbers of studies are available. Manuscripts that include investigations of limited study quality of under-studied areas are typically not acceptable as topics for a clinical review. Although some systematic reviews may be well done, those that receive highest scientific priority will only be considered given the very limited space allowed for these reviews in the *Journal*. Pre-submission inquiries for clinical reviews must contact the Editor-in-Chief, Prof. William Giannobile, william.giannobile@umich.edu for submission approval and instructions. Manuscripts submitted as Clinical Reviews have a strict limit of 4,000 words (including abstract, and the main text of the manuscript including acknowledgments; excluding figure legends and references); a total of 6 figures or tables; up to a maximum of 60 references; and

must contain a 200 word abstract. Manuscripts above the 4,000 word/6 figure or table limit may use supplemental appendices for other supporting information that would be available online only.

Additional Instructions for Clinical Reviews:
-It is important to include illustrations or diagrams to enhance clarity. Manuscripts that lack figures or diagrams typically receive a low priority score.

-Summarize important concepts in tables or flow charts or show critical data in the form of figures. NOTE: authors will need to obtain permission to reproduce a previously published figure or table.

-Due to the broad readership, abbreviations commonly recognized in one field may not be readily apparent to those in a different field. Keep abbreviation use to a minimum.

-The cover page, abstract, text, summary, figure legends, and table(s) should be combined into a single Word document. Figures should be submitted as a separate document.

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All submissions must include a title page and be accompanied by a cover letter and list of suggested reviewers. Cover letters should certify the research is original, not under publication consideration elsewhere, and free of conflict of interest. Title pages should include: abstract word count, total word count (Abstract to Acknowledgements), total number of tables/figures, number of references, and a minimum of 6 keywords. Keywords cannot be words that have been included in the manuscript title. Key words should be included selected from Medical Subject Headings (MeSH) to be used for indexing of articles. See: http://www.nlm.nih.gov/mesh/MBrowser.html for information on the selection of key words.

Please submit the names and email addresses of four preferred reviewers when prompted by the SAGETrack system. Preferred reviewers cannot be colleagues at the contributors' institution or present or former collaborators.

TITLES
Titles can consist of a maximum of 75 characters (including spaces). Titles do not normally include numbers, acronyms, abbreviations or punctuation. The title should include sufficient detail for indexing purposes but be general enough for readers outside the field to appreciate what the paper is about.

ACKNOWLEDGEMENTS
Authors are required to report all sources of support for their project or study, including but not limited to: grant funds, commercial sources, funds from a contributors' institution. Do not refer to a study being "partially funded by the cited sources." Consultancies and funds paid directly to investigators must also be listed. Authors are required to specify during the submission process if their paper received funding from NIH, NIDCR, or any other NIH Institute or Center and provide the grant number. To comply with the NIH Public Access

Mandate, for qualifying NIH-funded papers, the *Journal of Dental Research* will deposit the final, copyedited paper to PubMed Central on behalf of the authors.
Any perceived or actual conflicts of interest need to be identified in the acknowledgments section. The *JDR* abides by the International Committee of Medical Journal Editors guidelines for the Ethical Considerations in the Conduct and Report of Research (http://www.icmje.org/ethical_4conflicts.html). Authors are requested to include this information in the acknowledgments section and the corresponding author must confirm that all co-authors have reported any potential conflicts.

FIGURE AND TABLE REQUIREMENTS
These guidelines are intended to aid authors in providing figures that will reproduce well in both print and online media. Submitting digital image files that conform to these guidelines will prevent delays in the review and publication processes, and maximize the published quality of your figures.

Figure Types
JDR figures can fall into one of three categories: **Continuous-tone images, Line-art images**, and **Combination images**. Each image type has specific requirements in terms of the resolution needed for publication and the file types best suited for the figure. See the following panels for examples and requirements.

Resolution
In order for a figure to be used in publication, its Digital Image File must have the required resolution when it is created. The resolution cannot be raised after the original image is made. Attempting to do so (for example, with Adobe Photoshop's© *"Image Size"* command) results in the addition of artificial pixels that distort the image and lower its sharpness. The figures on the right show an example of this reduced sharpness.

Fonts
Limit fonts used in any figure to Times, Times New Roman, Arial, Frutiger, and Sabon. Other fonts cannot be guaranteed to reproduce properly.
Files containing figures and tables should be clearly labeled to indicate their placement in the text or appendix. Tables should be viewable in a portrait view. Tables that are created in a landscape view are more suitable for an appendix.
If the online version is in color and the printed version in black and white, please submit separate files for each version. Figures should be identical except in color or grayscale. The cost of color figures in the print version will be borne by the authors. Rates for color reproduction are $300 per initial page of color and $150 for each additional page of color. However, there are no charges for figures and diagrams printed in black and white. Color figures many be included in the online version of *JDR*with no extra charges.

REFERENCES
Citations should be arranged in alphabetical order by last name of the first author without numbering. When citing a reference in the text, provide attribution for the subject under discussion. "Et al" should be used when the cited work is by six or more contributors. When the cited work is by two contributors, use both surnames cited in the following manner: Last Name1, First Name1, Last Name2, First Name2. When citing multiple references by the same author(s) in the same year, use "a," "b," etc. (e.g., Jones, 1980b). Multiple references should

be listed in chronological order of publication, separated by semi-colons. Avoid using abstracts as references.
When citing a Website, list the authors and title if known, then the URL, include the date it was accessed in parentheses. Include among the reference papers accepted but not yet published; designate the journal and add "in press." Information from manuscripts submitted but not yet accepted should be cited in the text as "unpublished observations" in parentheses. The references must be verified by the author(s) against the original documents and checked for correspondence between references cited in the text and listed in the "References" section. All items should be listed alphabetically by the author's last name. For multiple entries by the same author/authors, they should be cited as follows:
1. One author: chronologically by year of publication
2. Two authors: alphabetical by last names
3. Three or more authors: chronologically by year of publication
4. Same author, same publication, chronologically by date of publication, using a), b), etc., to designate order

"Unpublished observations" and "personal communications" may be inserted into and cited in the text with written permission from the correspondents, but are not to be used as references.

SUPPLEMENTAL FILES
Additional supporting data may be referenced as a supplemental appendix for publication online only. All supplemental appendix files must be submitted with the manuscript for review. Supplemental files may include additional figures or tables that exceed the *Journal's* limit. Material intended for the supplemental appendix must have "supplemental" or "appendix" in the file name upon upload.
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MANUSCRIPT COMPONENTS
Manuscripts submitted to the Journal of Dental Research should be arranged in the following order:

1) Title Page
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2) Abstract
The abstract should be a self-standing summary of the text and not exceed 200 words. Concisely describe the background and rationale, hypothesis or study objective, design and

key methods, essential results, and conclusions. Avoid abbreviations. The abstract will be re-published separately by information retrieval services.

3) Introduction

Briefly and clearly describe the background and rationale for the stated hypothesis to be tested or objective to be studied. Sufficient detail must be provided to permit the interdisciplinary reader to evaluate the results without review of earlier publications. Describe and cite only the most relevant earlier studies; avoid presentation of an exhaustive review of the field. Do not include a summary of the results presented in the manuscript.

4) Materials & Methods

To provide sufficient technical information so that the experiments can be repeated, the experimental or study design, specific procedures, type of statistical analysis must be described clearly and carefully. Use section subheadings in a logical order to title each category or method. Previously published methods should be named and cited (e.g., "ultrasonic treatment" rather than mention of the cited contributors' names). New methods must be described completely. Present the data that validate the new method. Present descriptive information about large numbers of experimental reagents,microbes,
test materials, primer sequences, in tabular form with a brief explanation in the text. Proprietary names and sources of supply of all commercial products must be given in parentheses in the text (name and model of product, company, city, and state orcountry). Report generic names and terms wherever possible. For protocols involving the use of human subjects or specimens, indicate succinctly that subjects' rights have been protected by an appropriate institutional review board and informed consent was granted. When laboratory animals are used, indicate the level of institutional review and assurance that the protocol ensures humane practices. A method used for only part of one experiment may be described briefly in the "Results" section, table footnote, or figure legend.

5) Results

This section serves to introduce data in the text, tables and figures, and to call attention to their significant parts. Report results concisely, using tables and figures to present important differences or similarities that cannot otherwise be presented or summarized in the text. The rationale and design of experiments should be made clear in theprevious sections of the manuscript. Number tables and figures in the order in which they are described and cited in the text. All tabular data should identify and report either standard deviation values or standard errors of the means, the number of replicate determinations or human or animal subjects, and probability values and name(s) of statistical test(s) for reported differences. Restrict presentation of photo- and electron micrographs to those essential to the results. If essential to the results, color can be published at the discretion of the Editor.

6) Discussion

Explain and interpret the results with a scientifically critical view of the previously published work in the field. Highlight the advances made by the new data. Indicate the limitations of the findings. State the conclusions of the report, and explain why they are merited by the data. Subjective comments, interpretation, or reference to the previous literature is appropriate for this section.

7) Acknowledgments

Recognize individuals who provided assistance to the project. Report all sources of grant and other support for the project or study, including funds received from contributors' institutions and commercial sources. Do not refer to a study being only partially funded by the cited sources. Consultancies and funds paid directly to investigators must also be listed, with statements such as "This investigation was supported in part by USPHS Research Grant DE-0000-00 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892".

8) Tables

Limit one table per page. In the order of mention in the text, number each table consecutively with Arabic numerals in the heading. In the heading, follow the table number with a brief descriptive title, generally highlighting the key result. Design tables to highlight key results and comparisons. Make every effort to make the presentation of data clear, simple, and uncluttered. As column headings, use accurate descriptors instead of symbols, acronyms, or abbreviations. To avoid overlong titles and cumbersome tables, use explanatory footnotes whenever possible. In the table or title, indicate the order of footnotes with lower-case letters in superscript. If needed in footnotes, cite the short form of references in parentheses. In tabular columns and the text, decimals less than one must have the decimal point preceded by a zero. To ensure that the presentation is clear, report only the number of significant digits appropriate to the sensitivity and discrimination of the measure and the differences to be illustrated. Column headings should be simple and clear so that tables will be understandable without consultation of the text. Generally, column headings identify dependent variables, while independent variables are identified by row descriptors on the left.

9) Figures

Figures are illustrative materials, including photomicrographs, radiographs, charts, and graphs. Digitized figures must be certified by the contributors to be an accurate representation of the original data and not electronically edited. Figures must be discussed thoroughly in the text. Authors should present their figures as black & white images unless they specifically confirm that they are willing to fund color reproduction. Below each figure, oriented upright, label with contributors' names and figure number (and letter) in sequence corresponding to its mention in the text. Graphs should be labeled briefly and clearly at the abscissa and ordinate, including the units of measure. All figures must be labeled to allow for easy readability and visualization if reduced by 50% or more. Ideally, all figures should be provided at the optimum size for publication. The title and other identification may appear in the legend. Label figures clearly. Each figure label must indicate the number corresponding to the citation in the text, an arrow indicating the top, and contributors' abbreviated names

- Legends

Legends for all figures, including charts and graphs, must be typed together on a separate page and should be understandable without reference to the text. Include a title highlighting the key result and a key for any symbols or abbreviations used in the figure.

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Manuscripts submitted to the *Journal of Dental Research* are accepted for consideration giving the understanding that it contains original material that has not been submitted for publication or has been previously published elsewhere. Any form of publication other than an abstract only constitutes prior publication.

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CONSORT 2010 CHECKLIST COMPLETION RANDOMIZED CLINICAL TRIALS POLICY

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The CONSORT checklist can be downloaded from:
http://mc.manuscriptcentral.com/societyimages/jdr/CONSORT+2010+checklist%5b1%5d.doc.

The ARRIVE guidelines can be found here:
www.nc3rs.org.uk/downloaddoc.asp?id=1206&page=1357&skin=0.

The STROBE checklists can be found here:
www.strobe-statement.org/index.php?id=strobe-home

The *Journal of Dental Research* encourages authors to register their clinical trials in a public trials registry. Authors of manuscripts describing such studies are asked to submit the name of the registry and the study registration number prior to publication. In accordance with the aforementioned "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," clinical trials will only be considered for publication if they are registered.

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For protocols involving the use of human subjects, authors should indicate in their Methods section that subjects' rights have been protected by an appropriate Institutional Review Board and written informed consent was granted from all subjects. When laboratory animals are used, indicate the level of institutional review and assurance that the protocol ensured humane practices.

PUBLIC GENE DATA

Prior to submission, the *Journal of Dental Research* asks that novel gene sequences be deposited in a public database and the accession number provided to the *Journal*. Authors may want to use the following *Journal* approved databases:

GenBank: http://www.ncbi.nlm.nih.gov/Genbank/submit.html
EMBL: http://www.ebi.ac.uk/embl/Submission/index.html
DDBJ: http://www.ddbj.nig.ac.jp/sub-e/html

Manuscript submissions including microarray data should include the information recommended by the MIAME guidelines in their submission, and/or identify the submission details for the experiments details to one of the publicly available databases such as ArrayExpress or GEO.

OPEN ACCESS COMPLIANCE STATEMENT

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Authors are required to specify during the submission process if their paper received funding from NIH or NIDCR and provide the grant number.

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All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chairperson who provided only general support. Editors should ask corresponding authors to declare whether they had assistance with study design, data collection, data analysis, or manuscript preparation. If such assistance was available, the authors should disclose the identity of the individuals who provided this assistance and the entity that supported it in the published article. Financial and material support should also be acknowledged.

Groups of persons who have contributed materially to the paper but whose contributions do not justify authorship may be listed under such headings as “clinical investigators” or “participating investigators,” and their function or contribution should be described—for example, “served as scientific advisors,” “critically reviewed the study proposal,” “collected data,” or “provided and cared for study patients.” Because readers may infer their endorsement of the data and conclusions, these persons must give written permission to be acknowledged.

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Please note that the *Journal of Dental Research* secures completed contributor forms electronically via the SAGETrack online submission and review system. It is important to provide a valid email address for all co-authors listed at the time of submission. In the instance of manuscript acceptance for publication in the *Journal*, these email addresses will be used to distribute the contributor forms to co-authors.

Without the completion of the contributor forms for all co-authors listed, accepted manuscripts cannot continue into production, delaying publication.

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ANEXO C

Fórmula da saliva artificial elaborada pela Faculdade de Ciências Farmacêuticas, da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP

Fórmula da Saliva Artificial utilizada para armazenamento dos dentes a 37˚C, durante o experimento.

Componentes	**Quantidade**
Cloreto de potássio	0,96 g
Cloreto de sódio	0,67 g
Cloreto de Magnésio	0,04 g
Fosfato de Potássio	0,27 g
Cloreto de Cálcio	0,12 g
Nipagin	0,01 g
Nipasol	0,1 g
Carboxil Metil Celulose	8 g
Sorbitol	24 g
Água purificada q.s.p	1000 mL

Fonte: UNESP- Universidade Estadual Paulista

ANEXO D

UNIVERSIDADE ESTADUAL DE MARINGÁ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ANÁLISE DA ALTERAÇÃO ESTRUTURAL E VARIAÇÃO DA COR DOS DENTES SUBMETIDOS AO CLAREAMENTO DENTAL INTERNO

Pesquisador: Mirian Marubayashi Hidalgo
Área Temática:
Versão: 3
CAAE: 14382313.9.0000.0104
Instituição Proponente: Universidade Estadual de Maringá
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 316.965
Data da Relatoria: 17/06/2013

Apresentação do Projeto:
Trata-se de protocolo de pesquisa proposto por pesquisadora vinculada ao Departamento de Odontologia da Universidade Estadual de Maringá.

Objetivo da Pesquisa:
Avaliar os efeitos e a efetividade in vitro do clareamento interno com peróxido de hidrogênio a 35% ao longo do tempo.

Avaliação dos Riscos e Benefícios:
Avalia-se que os possíveis riscos a que estarão submetidos os sujeitos da pesquisa serão suportados pelos benefícios apontados.

Comentários e Considerações sobre a Pesquisa:
As variáveis a serem estudadas para verificar a alteração da estrutura e de cor dos dentes submetidos ao clareamento dentário serão: alteração estrutural, de cor, luminescência dos dentes submetidos ao tratamento e os períodos de tempo. Os espécimes serão subdivididos em 2 grupos experimentais. O número de amostras para cada condição será de 10, totalizando 80 espécimes, sendo 40 para cada técnica de clareamento e 40 controle. O tempo do experimento será no total de 30 dias, e as leituras serão realizadas no seguintes momentos: T0,TE,T1,T2. O aparelho de

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Bairro: Jardim Universitário **CEP:** 87.020-900
UF: PR **Município:** MARINGA
Telefone: (44)3011-4444 **Fax:** (44)3011-4518 **E-mail:** copep@uem.br

UNIVERSIDADE ESTADUAL DE MARINGÁ

Continuação do Parecer: 316.965

leitura de cores a ser utilizado será o colorímetro Konica Minolta CR400 e para medir a luminescência o espectrofotômetro ultravioleta visível Cintra 20. O aparelho utilizado para verificar alteração na estrutura inorgância será o microscópio dispersivo Raman, e para a orgânica será o FTIR vertex 70. Para verificar a influência dos períodos de tempo e ação dos das diferentes técnicas, se utilizará a Análise de Variância de Medidas Repetidas (ANOVA), (p 0.05). Posteriormente, de forma a verificar a similaridade entre os grupos, será realizado o teste de Tukey HDS post hoc test (p0.05). Para a análise de espectroscopia ultravioleta visível, Raman e FTIR será utilizada a análise qualitativa.

O protocolo de pesquisa restou pendente junto a este Comitê para que fosse esclarecida a necessidade do uso de sangue humano no experimento. Em resposta ao Comitê a pesquisadora esclarece que ante a possibilidade de certificação por exames recentes, o uso imediato de material recém coletado e o pequeno volume necessário, optou-se pela utilização de sangue humano de doador saudável.

Considerações sobre os Termos de apresentação obrigatória:
Acostou todos os termos de apresentação obrigatória.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:
O Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Maringá é de parecer favorável à aprovação do protocolo de pesquisa apresentado.

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:
Face ao exposto e considerando a normativa ética vigente, este Comitê se manifesta pela aprovação do protocolo de pesquisa em tela.

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UNIVERSIDADE ESTADUAL DE MARINGÁ

Continuação do Parecer: 316.965

MARINGA, 26 de Junho de 2013

Assinador por:
Ricardo Cesar Gardiolo
(Coordenador)

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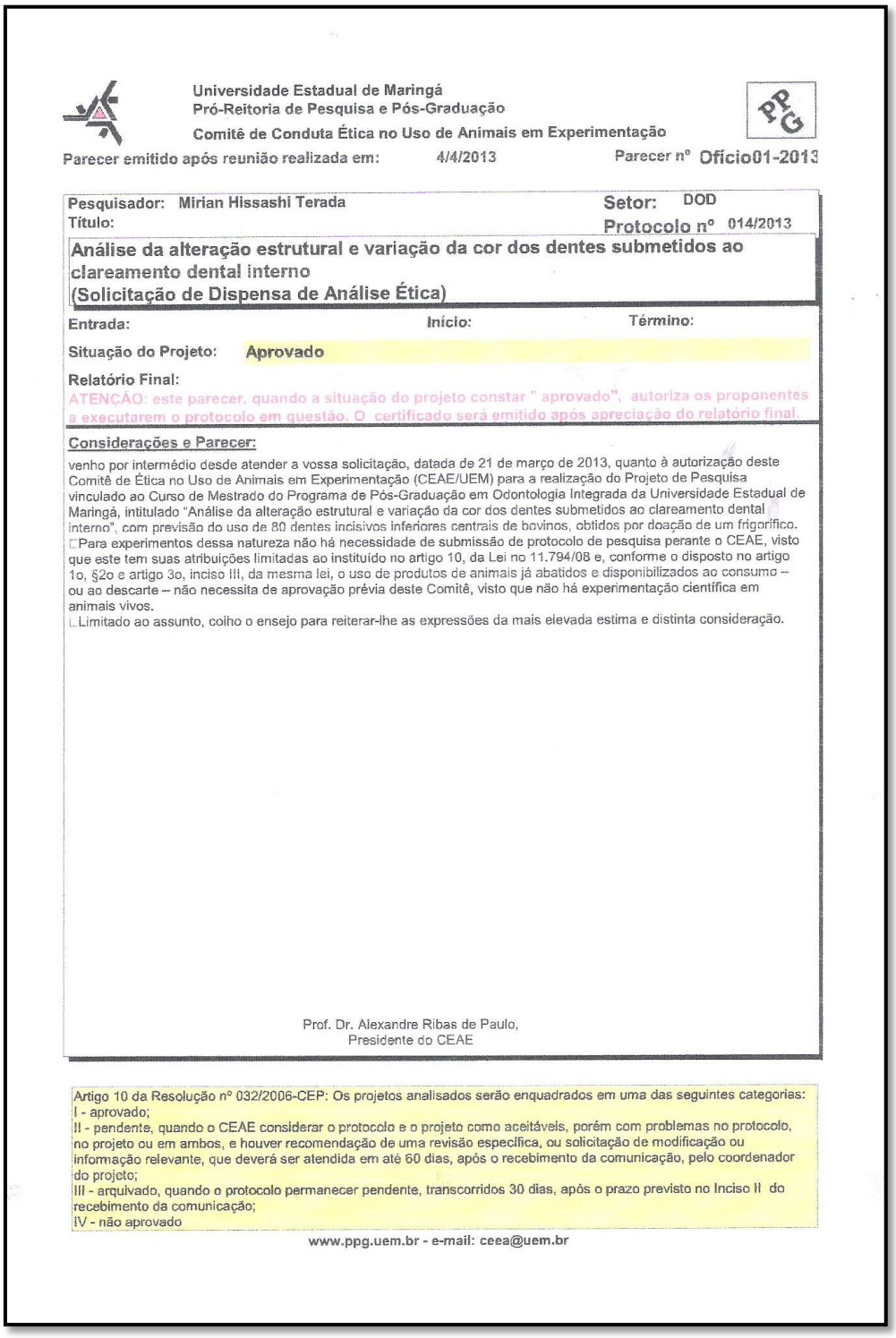
ANEXO E



Universidade Estadual de Maringá
Pró-Reitoria de Pesquisa e Pós-Graduação
Comitê de Conduta Ética no Uso de Animais em Experimentação

Parecer emitido após reunião realizada em: 4/4/2013 Parecer nº Ofício01-2013

Pesquisador: Mirian Hissashi Terada Setor: DOD
Título: Protocolo nº 014/2013

Análise da alteração estrutural e variação da cor dos dentes submetidos ao clareamento dental interno (Solicitação de Dispensa de Análise Ética)

Entrada: Início: Término:

Situação do Projeto: Aprovado

Relatório Final:

ATENÇÃO: este parecer, quando a situação do projeto constar " aprovado", autoriza os proponentes a executarem o protocolo em questão. O certificado será emitido após apreciação do relatório final.

Considerações e Parecer:

venho por intermédio desde atender a vossa solicitação, datada de 21 de março de 2013, quanto à autorização deste Comitê de Ética no Uso de Animais em Experimentação (CEAE/UEM) para a realização do Projeto de Pesquisa vinculado ao Curso de Mestrado do Programa de Pós-Graduação em Odontologia Integrada da Universidade Estadual de Maringá, intitulado "Análise da alteração estrutural e variação da cor dos dentes submetidos ao clareamento dental interno", com previsão do uso de 80 dentes incisivos inferiores centrais de bovinos, obtidos por doação de um frigorífico.
Para experimentos dessa natureza não há necessidade de submissão de protocolo de pesquisa perante o CEAE, visto que este tem suas atribuições limitadas ao instituído no artigo 10, da Lei no 11.794/08 e, conforme o disposto no artigo 1o, §2o e artigo 3o, inciso III, da mesma lei, o uso de produtos de animais já abatidos e disponibilizados ao consumo – ou ao descarte – não necessita de aprovação prévia deste Comitê, visto que não há experimentação científica em animais vivos.
Limitado ao assunto, colho o ensejo para reiterar-lhe as expressões da mais elevada estima e distinta consideração.

Prof. Dr. Alexandre Ribas de Paulo,
Presidente do CEAE

Artigo 10 da Resolução nº 032/2006-CEP: Os projetos analisados serão enquadrados em uma das seguintes categorias:
I - aprovado;
II - pendente, quando o CEAE considerar o protocolo e o projeto como aceitáveis, porém com problemas no protocolo, no projeto ou em ambos, e houver recomendação de uma revisão específica, ou solicitação de modificação ou informação relevante, que deverá ser atendida em até 60 dias, após o recebimento da comunicação, pelo coordenador do projeto;
III - arquivado, quando o protocolo permanecer pendente, transcorridos 30 dias, após o prazo previsto no Inciso II do recebimento da comunicação;
IV - não aprovado

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APÊNDICE A

Metodologia Utilizada - Artigo1 e 2

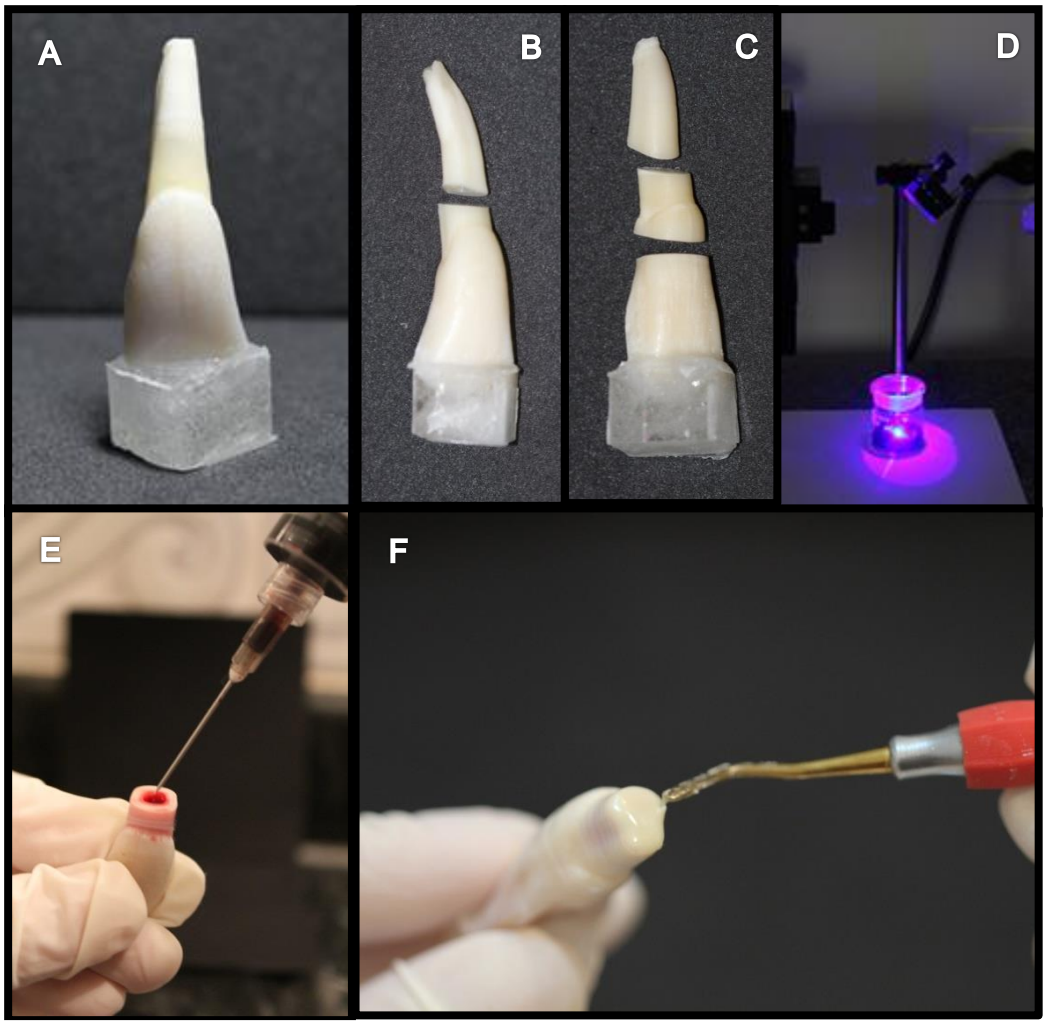

Figura 1. (*A*) Base de resina acrílica. (*B*) Dente após seccionamento 5mm acima da JAC. (*C*) Dente após seccionamento 3mm abaixo da JAC na porção coronária. *(D)* Sangue sob laser ultravioleta azul. *(D)* Inserção do sangue pelo colo anatômico. *(E)* Selamento com cimento de ionômero de vidro fotoativado Vitro Fill LC®.

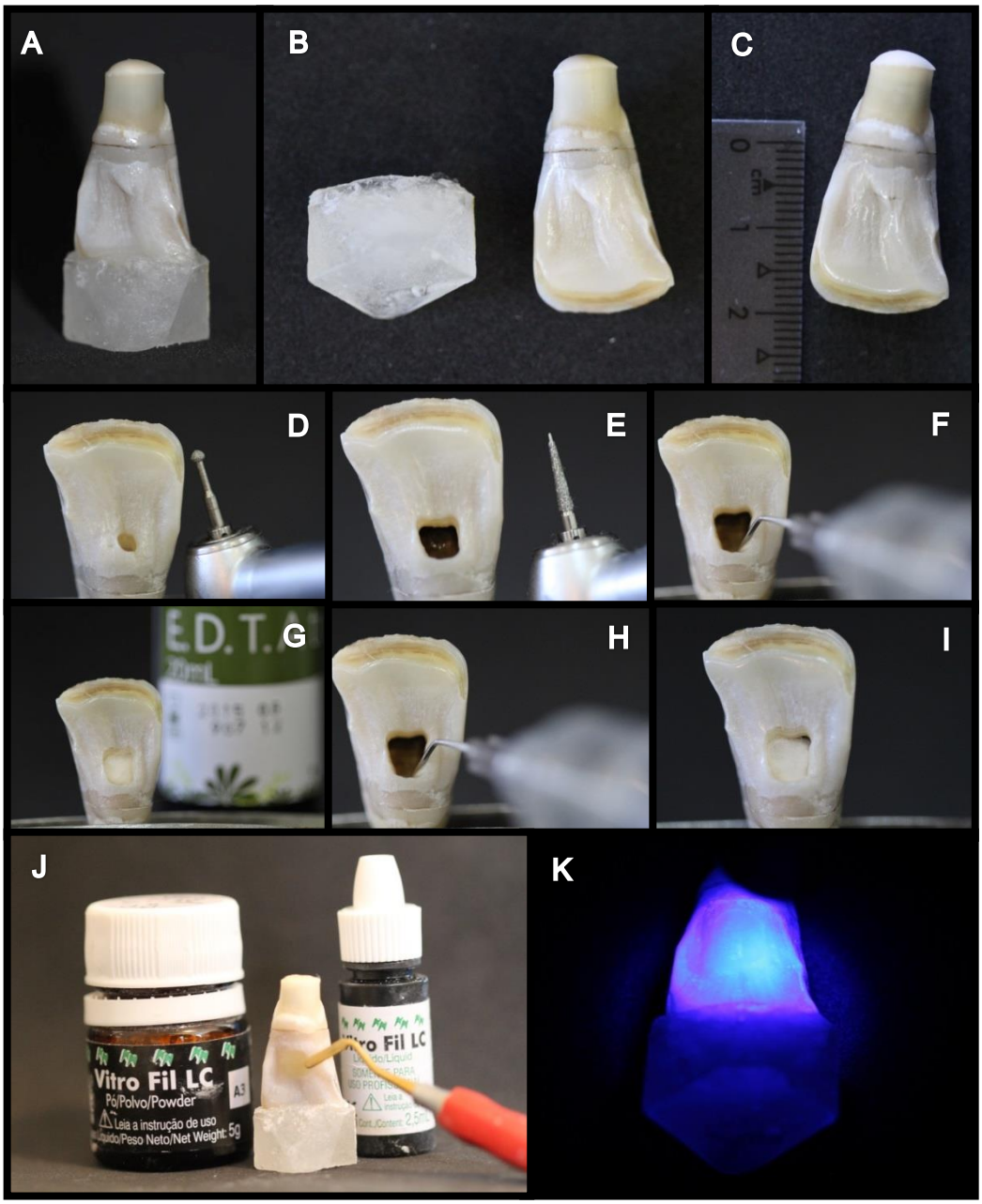

Figura 2. (*A*) e (*B*) Dente retirado da base de resina acrílica. *(C)* Ponto de eleição para a abertura coronária - face lingual a 5mm da JAC. *(D)* Abertura realizada com broca esférica. *(E)* Utilização da ponta inativa. *(F)* Irrigação com Hipoclorito de sódio 1%. *(G)* Limpeza da câmara pulpar com EDTA trissódico 17% por 3min. *(H)* Neutralização do EDTA com Hipoclorito de sódio 1%. *(I)* Inserção de bolinha de algodão. *(J)* Selamento com cimento de ionômero de vidro fotoativado Vitro Fill LC®. *(K)* Fotoativação por 20s.

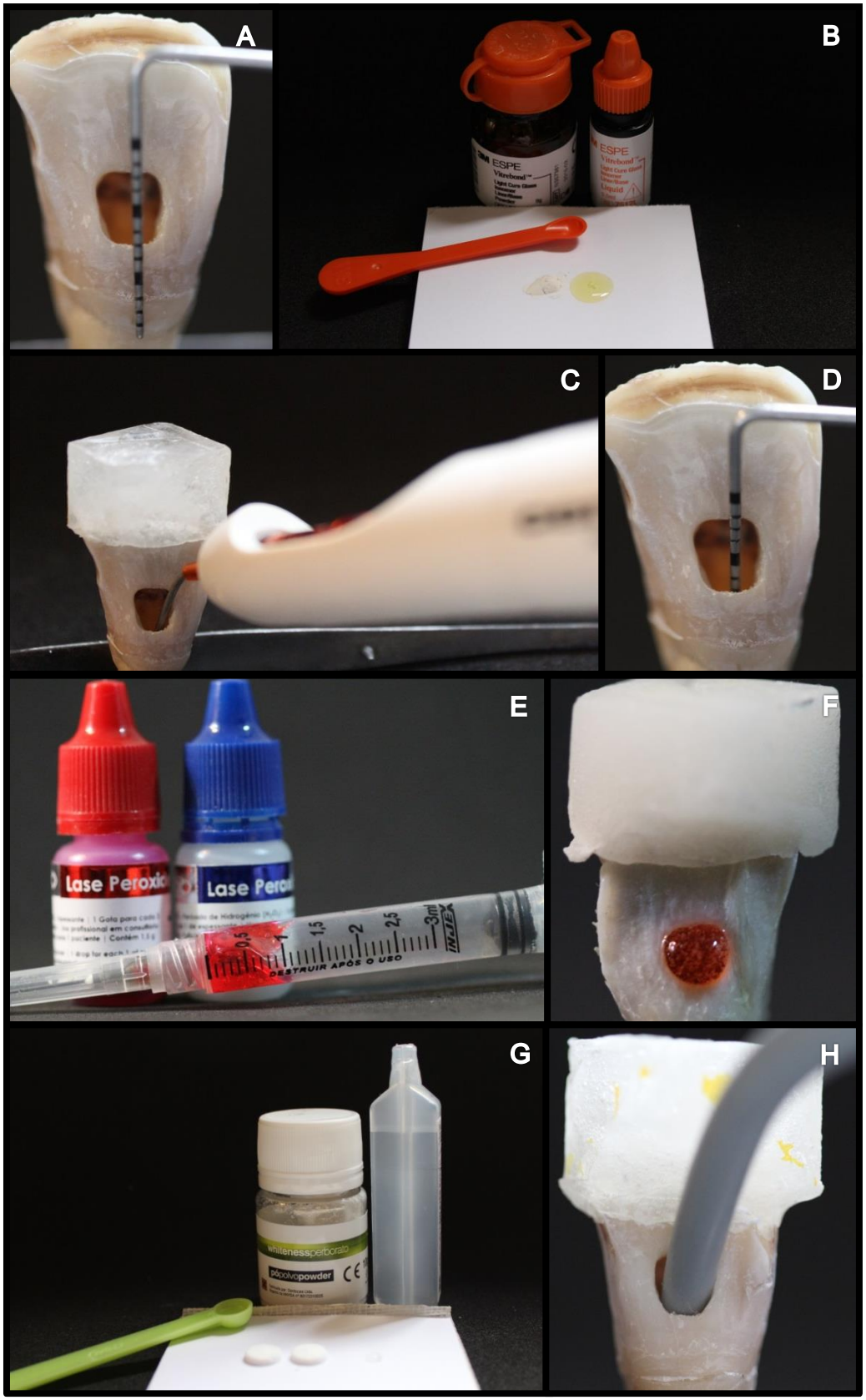

Figura 3. (*A*), *(B), (C)* e *(D)* Tampão cervical com Vitrebond® abaixo da JAC com 2 mm de espessura. (*E*) e *(F)* Clareamento interno com peróxido de hidrogênio a 35% - Lase Peroxide Sensy®. *(G)* e *(H)* Clareamento interno com perborato de sódio - Whitness perborato®.

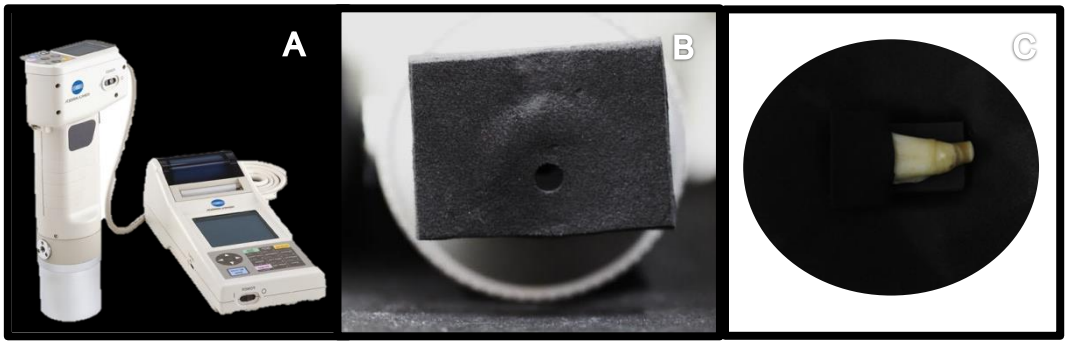

Figura 4. (*A*) Colorímetro - Konica Minolta CR-400®. (*B*) Molde preto para realização das medidas de forma padronizada, com abertura de 5mm de diâmetro. *(C)* Base do molde preto.

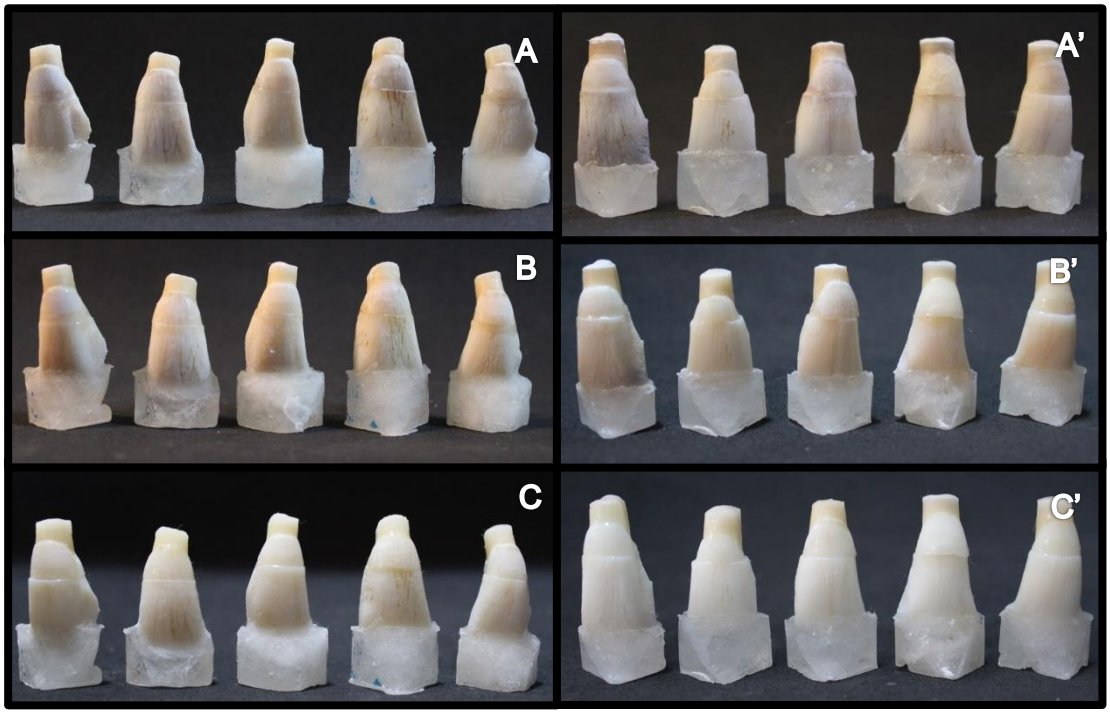

Figura 5. *(A) e (A')* Espécimes escurecidos artificialmente. (*B*) e (*B'*) 7 dias após o inicio do clareamento. *(C) e (C)* 14 dias após o inicio do clareamento. (*B*) e (*C*) Clareados internamente com peróxido de hidrogênio a 35% Lase Peroxide Sensy®. *(B')* e *(C')* Clareados internamente com perborato de sódio - Whitness perborato®.

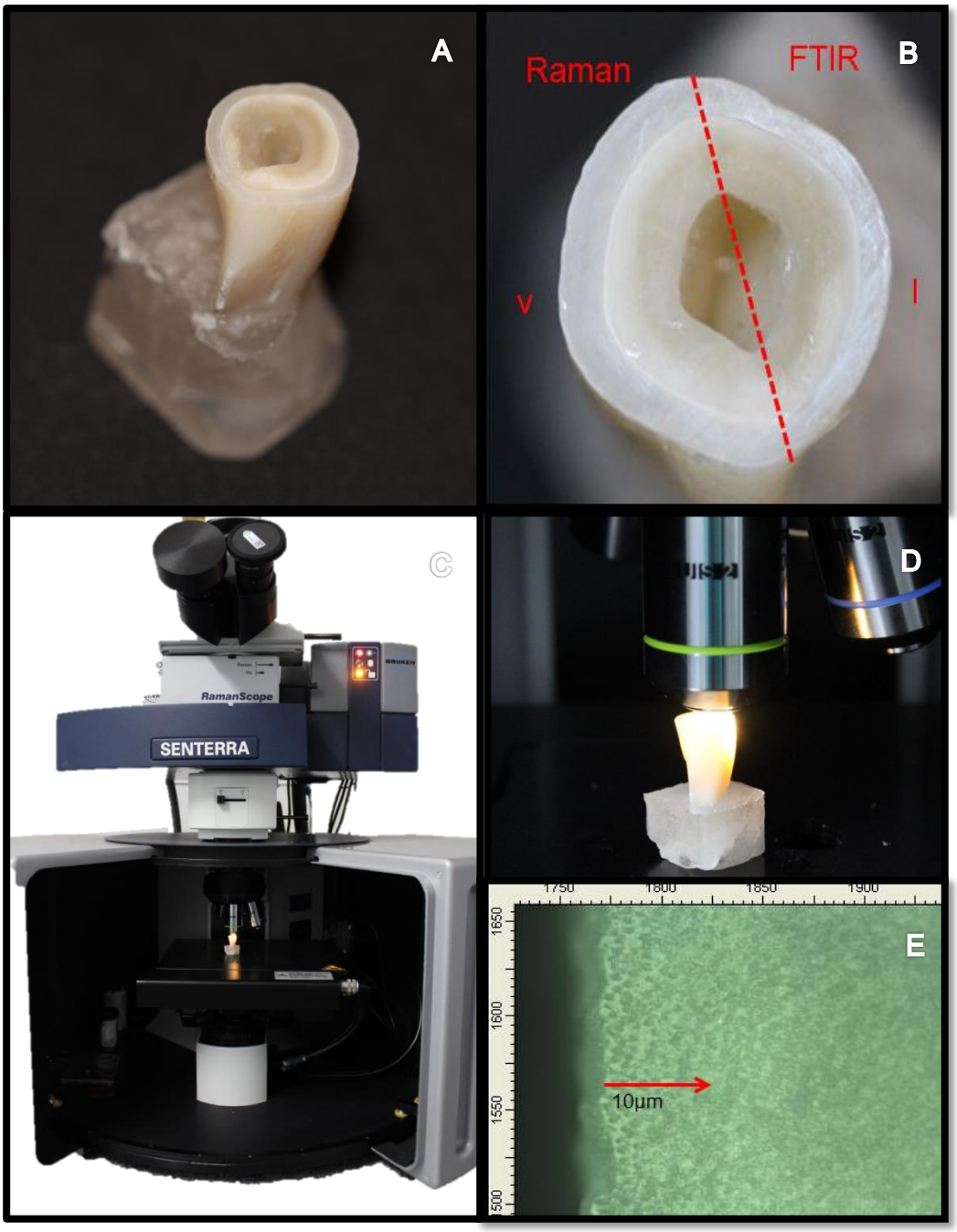

Figura 6. *(A)* Região seccionada a 3mm abaixo CEJ na porção coronária para leitura Raman e retirada de amostras para FTIR. (*B*) Linha imaginária dividindo o espécime em sua porção vestibular para leitura Raman e lingual para FTIR. *(C)* Bruker Senterra microscópio dispersivo Raman (*D*) Leitura com lente objetiva de 20x. *(E)* Leitura realizada no centro da superfície vestibular a 10µm da cavidade pulpar.

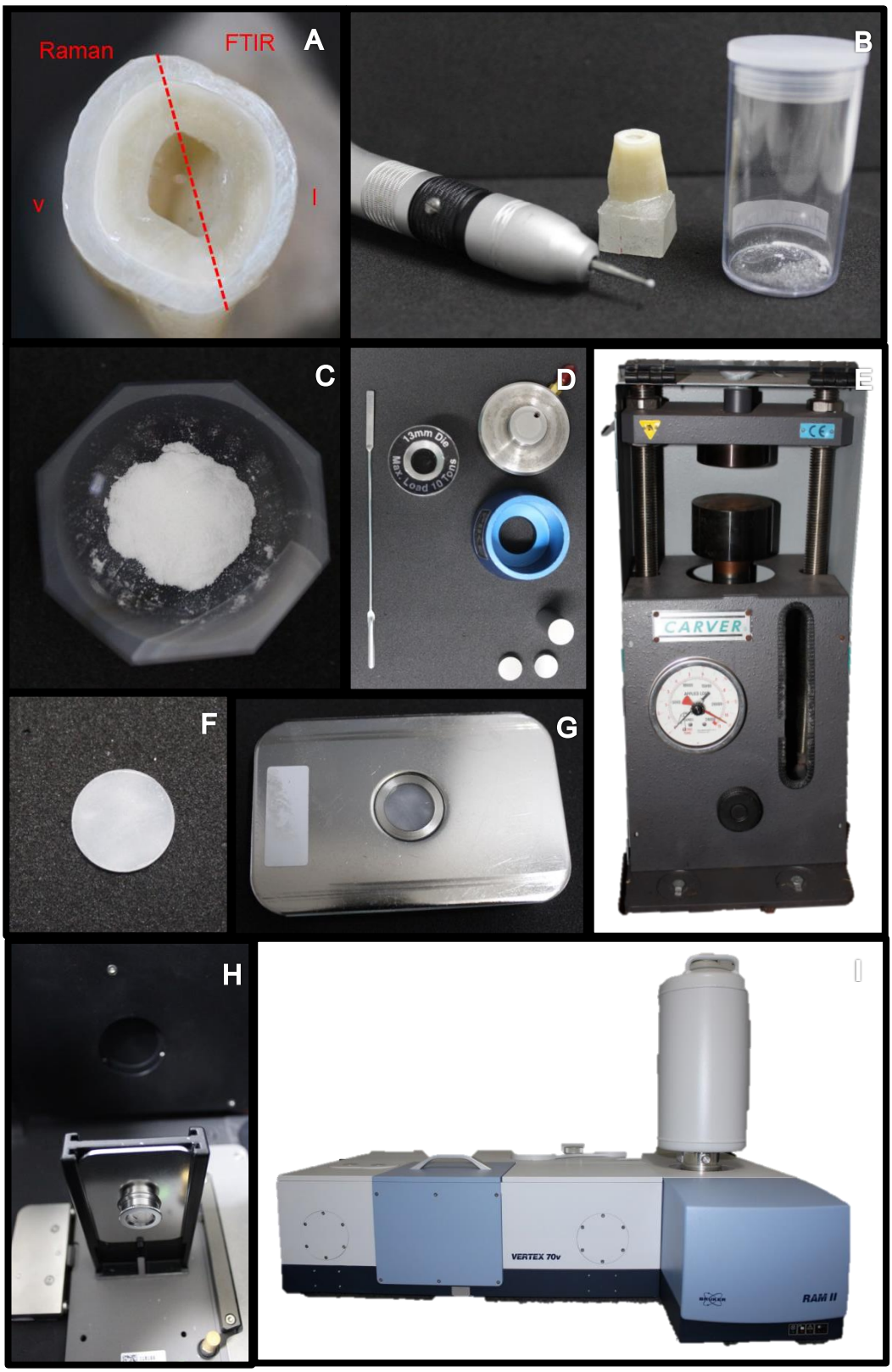

Figura 7. *(A)*Linha imaginária dividindo o espécime em sua porção vestibular para leitura Raman e lingual para FTIR. *(B)* Retirada do pó de dentina da região lingual com uma peça reta em baixa rotação , utilizando uma broca diamantada nº 1. (*C*) Homogenização do pó da dentina com o KBr (0.002g dentina/1.998gKBr). (*D*) Materiais utilizados para confecção da pastilha para leitura no FTIR. *(E)* Prensa utilizada para confecção da pastilha. (*F)*Pastilha de dentina com KBr. *(G) e (H)* Adaptação da pastilha para leitura no FTIR. *(I)* Espectrômetro FTIR a vácuo Vertex 70.

APÊNDICE B

Cálculo utilizado para CIEDE2000 - Artigo1

$\Delta E_{00} = ((\Delta L'/k_L S_L)^2 + (\Delta C'/k_C S_C)^2 + (\Delta H'/k_H S_H)^2 + R_T (\Delta C'/k_C S_C)\ (\Delta H'\ k_H S_H))^{1/2}$

$\Delta L' = L_2^* - L_1^*, \Delta C' = C_2' - C_1', \Delta H' = 2(C_1'\ C_2')^{½} \sin(\Delta h'/2)$

C_1', C_2' foi encontrado utilizando a expressão $C_1' = (a_1^{2'} + b_1^{2*})^{½}$, $C_2' = (a_2^{2'} + b_2^{2*})^{½}$, $a_1' = a_1(1+G)$ $a_2' = a_2(1+G)$, $G = 0.5(1-(\dot{C}^*_{ab}{}^7 / \dot{C}^*_{ab}{}^7 + 25^7)^{1/2})$, $\dot{C}^*_{ab}{}^7 = \dot{C}^*_{1ab} + \dot{C}^*_{2ab}/2$, onde $\dot{C}^*_{1ab} = (a_1^*)^2 + (b_1^*)^2$, $\dot{C}^*_{2ab} = (a_2^*)^2 + (b_2^*)^2$

$\Delta h' = 0$ quando $C_1'C_2' = 0$ ou

$\Delta h' = h_2' - h_1'$ quando $C_1'C_2' \neq 0$ $|h_2' - h_1'| \leq 180^\circ$ ou

$\Delta h' = (h_2' - h_1') - 360$ quando $C_1'C_2' \neq 0$ $|h_2' - h_1'| > 180^\circ$ ou

$\Delta h' = (h_2' - h_1') + 360$ quando $C_1'C_2' \neq 0$ $(h_2' - h_1') < -180^\circ$, onde

$h_1' = 0$ quando $b_1^* = a_1' = 0$, $b_2^* = a_2' = 0$ ou $h_1' = \tan^{-1}(b_1^* / a_1')$, $h_2' = \tan^{-1}(b_2^* / a_2')$

The $S_L = 1 + 0.015(\bar{L}' - 50)^2 / (20 + (\bar{L}' - 50)^2$, onde $\bar{L}' = (L_1^* + L_2^*)/2$, $S_C = 1 + 0.045\ \dot{C}'$ onde $\dot{C}' = (C_1' + C_2')/2$, $S_h = 1 + 0.015\ \dot{C}'\ T$ onde $T = 1 - 0.17\cos(\ddot{h}' - 30^\circ) + 0.24\cos(2\ddot{h}') + 0.32\cos(3\ddot{h}' + 6^\circ - 020\cos(4\ddot{h}' - 63^\circ)$

$R_T = -\sin(2\Delta\theta)\ R_C$ onde $\Delta\theta = 30\exp(-(\ddot{h}' - 275^\circ/25)^2)$ e

$\ddot{h}' = h_1' + h_2'/2$ quando $|h_1' - h_2'| \leq 180^\circ$; $C_1'C_2' \neq 0$ ou

$\ddot{h}' = h_1' + h_2' + 360/2$ quando $|h_1' - h_2'| > 180^\circ = (h_1' + h_2') < 360$; $C_1'C_2' \neq 0$ ou

$\ddot{h}' = h_1' + h_2' + 360/2$ quando $|h_1' - h_2'| > 180^\circ = (h_1' + h_2') \geq 360$; $C_1'C_2' \neq 0$ ou

$\ddot{h}' = (h_1' + h_2')$ quando $C_1'C_2' \neq 0$

APÊNDICE C

Transformação das coordenadas do CIELAB em CIEDE2000 - Artigo1

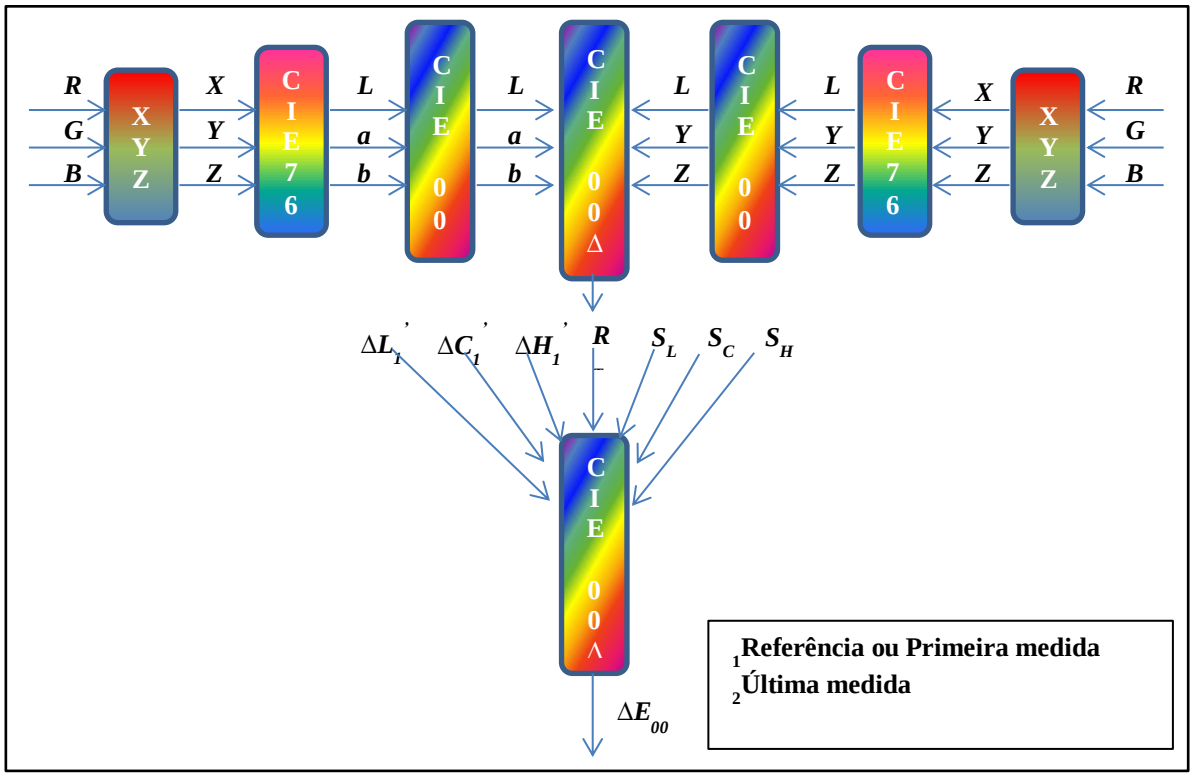

Figura 1. Representa a transformação dos parâmetros de CIELAB em CIEDE2000.