UNIVERSIDADE ESTADUAL DE PONTA GROSSA
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA – DOUTORADO
ÁREA DE CONCENTRAÇÃO: DENTÍSTICA RESTAURADORA

SIBELLI OLIVIERI PARREIRAS

PENETRAÇÃO DO PERÓXIDO DE HIDROGÊNIO EM DIFERENTES CONCENTRAÇÕES NA CÂMARA PULPAR E O MANEJO DA SENSIBILIDADE PÓS-OPERATÓRIA EM CLAREAMENTO DENTAL

PONTA GROSSA
2017

SIBELLI OLIVIERI PARREIRAS

PENETRAÇÃO DO PERÓXIDO DE HIDROGÊNIO EM DIFERENTES CONCENTRAÇÕES NA CÂMARA PULPAR E O MANEJO DA SENSIBILIDADE PÓS-OPERATÓRIA EM CLAREAMENTO DENTAL

Tese apresentada como pré-requisito para a obtenção do título de doutor na Universidade Estadual de Ponta Grossa no Curso de Doutorado em Odontologia – Área de concentração: Dentística Restauradora. Linha de pesquisa: Propriedades físico-químicas e biológicas dos materiais.

Orientadora: Profª. Drª. Alessandra Reis.
Co-orientador: Profº. Drº. Alessandro Dourado Loguercio.

PONTA GROSSA
2017

Ficha Catalográfica
Elaborada pelo Setor de Tratamento da Informação BICEN/UEPG

Parreiras, Sibelli Olivieri
P227p Penetração do peróxido de hidrogênio em diferentes concentrações na câmara pulpar e o manejo da sensibilidade pós-operatória em clareamento dental/ Sibelli Olivieri Parreiras. Ponta Grossa, 2017.
107f.

Tese (Doutorado em Odontologia - Área de Concentração: Dentística Restauradora), Universidade Estadual de Ponta Grossa.
Orientadora: Profª Drª Alessandra Reis.
Coorientador: Prof. Dr. Alessandro Dourado Loguercio.

1.Clareamento dental. 2.Peróxido de hidrogênio. 3.Sensibilidade da dentina. 4.Permeabilidade do esmalte dentário. I.Reis, Alessandra. II. Loguercio, Alessandro Dourado. III. Universidade Estadual de Ponta Grossa. Doutorado em Odontologia. IV. T.

CDD: 617.67

SIBELLI OLIVIERI PARREIRAS

Penetração do peróxido de hidrogênio em diferentes concentrações na camara pulpar e o manejo da sensibilidade pós-operatória em clareamento dental.

Tese apresentada ao Programa de Pós-graduação Stricto sensu em Odontologia da Universidade Estadual de Ponta Grossa, como requisito parcial à obtenção do título de Doutora em Odontologia, área de concentração em Dentística Restauradora, linha de pesquisa de Propriedades Físico-Químicas e Biológicas dos Materiais.

Ponta Grossa, 22 de Setembro de 2017.

Prof. Dr. Marcos Hahlbohm D'Oliveira Schroeder
Universidade Federal do Rio de Janeiro

Profª. Drª. Thays Regina Ferreira da Costa
Universidade Federal do Paraná

Profª. Drª. Márcia Fernanda de Rezende Siqueira
Universidade Estadual de Ponta Grossa

Profª. Drª. Giovana Mongruel Gomes
Universidade Estadual de Ponta Grossa

Profª. Drª. Alessandra Reis Silva Loguercio
Universidade Estadual de Ponta Grossa

Dedico este trabalho

*A **Deus,***
que permitiu que tudo pudesse ser realizado.

*A minha mãe, **Marisa,***
pelo apoio incondicional em todos os momentos.

*Ao meu pai, **Hélio,***
pela compreensão e incentivo ao longo de minha trajetória.

AGRADECIMENTOS

À minha orientadora, **Profª. Drª Alessandra Reis**. Sou muito agradecida por Deus ter me permitido ser orientada por alguém que me ensinou tanto. Sabe que além de sabedoria e competência, mas também uma grande determinação e carisma, que a torna uma pessoa muito especial e faz com que todos queiram estar a sua volta. Meu sincero obrigada e admiração para sempre!

À **Universidade Estadual de Ponta Grossa**, ao Magnífico Reitor **Profº. Dr. Carlos Luciano Sant´Ana Vargas.** Ao **Profº. Dr. João Carlos Gomes** e a **Profª. Dra. Osnara Maria Mongruel Gomes** pela oportunidade, apoio e compreensão em todos os momentos. Vocês são exemplos de dedicação na Odontologia.

Aos coordenadores da Pós, **Profª. Drª. Denise Stadler Wambier**, por toda ajuda necessária para a realização deste trabalho **e Profº. Drº. Alessandro Loguercio**, muito obrigada por todo o conhecimento transmitido, por toda a ajuda em trabalhos desde a minha graduação.

A todos os **professores** da UEPG, que fizeram parte da minha formação acadêmica por compartilhar seus conhecimentos e experiências.

À minha **família**, pelo amor e apoio incondicional nos momentos alegres e tristes. A família é o amor de Deus nos oferecendo um pouquinho do céu aqui na Terra.

Ao meu namorado **Andres Felipe Cartagena**, pelo companheirismo, carinho e ajuda em todo momento. Obrigada por tudo!

Aos meus **amigos,** em especial aqueles que acompanharam de perto a minha trajetória, pelo carinho, incentivo e amizade. Vocês são bênçãos na minha vida.

À banca de qualificação, **Profª Drª Viviane Hass** e **Profª Drª Marcia Fernanda de Rezende Siqueira,** e à banca de defesa, **Profº Drº Marcos Hahlbohm D´ Oliveira Schroeder, Profª Drª Thays Regina Ferreira da Costa, Profª Drª Giovana Mongruel Gomes** e **Profª Drª Marcia Fernanda de Rezende Siqueira,** pelas valorosas contribuições que enriqueceram este trabalho.

A todos os **funcionários da UEPG** que sempre estiveram dispostos a nos ajudar com muito amor e competência.

E a todos que diretamente ou indiretamente contribuíram para a realização deste trabalho.

DADOS CURRICULARES

SIBELLI OLIVIERI PARREIRAS

NASCIMENTO 27.01.1987	Cornélio Procópio, Paraná - Brasil
FILIAÇÃO	Marisa Olivieri Parreiras Hélio Luis Parreiras
2007 – 2011	Curso de Graduação em Odontologia pela Universidade Estadual de Ponta Grossa (UEPG). Ponta Grossa – PR, Brasil.
2012 – 2013	Curso de Pós-Graduação em Odontologia. Universidade Estadual de Ponta Grossa (UEPG) nível de Mestrado em Odontologia – Área de Concentração em Dentística Restauradora. Ponta Grossa – PR, Brasil.
2014 – 2017	Curso de Pós-Graduação em Odontologia. Universidade Estadual de Ponta Grossa (UEPG) nível de Doutorado em Odontologia – Área de Concentração em Dentística Restauradora. Ponta Grossa – PR, Brasil.

RESUMO

Parreiras, SO. **Penetração do peróxido de hidrogênio em diferentes concentrações na câmara pulpar e o manejo da sensibilidade pós-operatória em clareamento dental**. [Tese – Doutorado em Odontologia – Área de Concentração: Dentística Restauradora - Universidade Estadual de Ponta Grossa; 2017].

Os objetivos deste estudo foram 1) quantificar a concentração do peróxido de hidrogênio (PH) de géis clareadores com diferentes concentrações do mesmo fabricante, 2) quantificar a concentração de PH na câmara pulpar em dentes com aplicação prévia de agentes dessensibilizantes ao clareamento de consultório e 3) avaliar o efeito de um gel dessensibilizante experimental na sensibilidade pós-operatória de pacientes submetidos a clareamento de consultório. Para os estudos 1 e 2, a quantificação de PH na câmara pulpar foi realizada por meio do método espectrofotométrico com a enzima *horseradish peroxidase*. No estudo 1, foram divididos aleatoriamente 90 dentes em nove grupos (n = 10) e submetidos a diferentes concentrações de gel clareador de mesmo fabricante, simulando o procedimento clareador caseiro e de consultório. No estudo 2, 50 dentes foram selecionados e os diferentes agentes dessensibilizantes: Desensibilize KF 2%, Mi Paste e Desensibilize Nano-P foram aplicados previamente ao clareamento de consultório com PH 35%. No estudo 3, 42 pacientes com os caninos com cor A2 ou mais escuros foram selecionados e divididos aleatoriamente em dois grupos: placebo (controle negativo) e tratamento. Os pacientes do grupo tratamento foram submetidos ao gel experimental a base de nitrato de potássio 5% e glutaraldeído 5% por 10 min previamente ao clareamento de consultório com PH 35%. A sensibilidade dental foi avaliada imediatamente, após 1 h, 24 h e 48 h do clareamento e registradas pelos próprios pacientes por meio das escalas numéricas de 5 pontos e visual analógica. A cor foi avaliada através da escala Vita classical, Vita Bleachedguide e espectrofotômetro Vita Easyshade (Vita Zahnfabrik, Bad Sackingen, Alemanha), inicialmente, 1 semana após cada sessão e um mês depois do término do procedimento. Os dados dos experimentos 1 e 2 foram avaliados por análise de variância (ANOVA) e teste de tukey para o contraste das médias (α = 0,05). Para a análise do risco de sensibilidade dental no estudo 3, os dados foram analisados por meio do teste Mc Nemar e a mudança de cor por teste t de Student (α = 0,05). Foi observado que a quantidade de PH que alcançou a câmara pulpar no grupo clareamento de consultório foi significativamente maior em comparação aos clareamentos caseiro (p = 0,65). Enquanto que os protocolos caseiros apresentaram resultados similares. O uso de agentes dessensibilizantes previamente ao clareamento de consultório reduziu a passagem de PH para a câmara pulpar, de forma que os dessensibilizantes Mi Paste e Nano-P tiveram menor concentração de PH na câmara pulpar (p < 0,001). Foi também possível observar que o risco de sensibilidade dentária do grupo gel dessensibilizante [31,7% (95% IC 19,6 – 46,9)] e para o grupo controle [70,7 (95% IC 55,5 – 82,3%) foram estatisticamente significante (p < 0,0001). Pode-se concluir que fatores como concentração do gel clareador e o uso prévio de agentes dessensibilizantes interferem na difusão do PH, porém a utilização do gel experimental à base de nitrato de potássio 5% e glutaraldeído 5% foi eficaz na redução da sensibilidade pós-operatória em pacientes submetidos a clareamento de consultório.

Palavras-chave: Clareamento Dental. Peróxido de hidrogênio. Sensibilidade da dentina. Permeabilidade do esmalte dentário.

ABSTRACT

Parreiras, SO. **Penetration of hydrogen peroxide from different concentrations bleaching products into pulp chamber and management of postoperative sensitivity tooth bleaching.** [Tese – Doutorado em Odontologia – Área de Concentração: Dentística Restauradora - Universidade Estadual de Ponta Grossa; 2017].

The objectives of this study were: 1) to evaluate the diffusion of hydrogen peroxide (HP) of bleaching gels with different concentrations from the same manufacturer, 2) quantify the HP concentration in the pulp chamber in teeth with prior application of desensitizing agents and 3) evaluate the effect of an experimental desensitizing gel on the postoperative sensitivity of patients undergoing in-office tooth bleaching. For studies 1 and 2, the quantification of HP in the pulp chamber was performed using the spectrophotometric method with the horseradish peroxidase enzyme. In study 1, 90 teeth were randomly divided into nine groups (n = 10) and submitted to different concentrations of whitening gel from the same manufacturer, simulating the home and in-office whitening procedure. In study 2, 50 teeth were selected at the different desensitizing agents: Desensibilize KF 2%, Mi Paste and Desensibilize Nano-P were applied prior to the office bleaching. In study 3, 42 patients with canines with A2 or darker color were selected and randomly divided into two groups: placebo (negative control) and treatment. Patients in the treatment group were submitted to 5% potassium nitrate and 5% glutaraldehyde gel for 10 min prior to bleaching with 35% hydrogen peroxide. The dental sensitivity was evaluated immediately, after 1 h, 24 h and 48 h of the bleaching and registered by the patients themselves through the numerical scales of 5 points and visual analog. The color was evaluated through the Vita classical, Vita Bleachedguide and Vita Easyshade spectrophotometer (Vita Zahnfabrik, Bad Sackingen, Germany), initially, 1 week after each session and one month after the end of the procedure. Data from experiments 1 and 2 were evaluated by analysis of variance (ANOVA) and tukey test for the means contrast (α = 0.05). For the analysis of the risk of dental sensitivity in study 3, the data were analyzed using the Mc Nemar test and the color change by Student's t-test (α = 0.05). It was observed that the amount of HP that reached the pulp chamber in the office bleaching group was significantly higher compared to home bleaching (p = 0.65). The home protocols presented similar results. The use of desensitizing agents prior to the office bleaching reduced the passage of HP to the pulp chamber, so that the desensitizers Mi Paste and Nano-P had lower HP concentrations in the pulp chamber (p < 0.001). It was also possible to observe that the risk of tooth sensitivity of the desensitizing gel group [31.7% (95% CI 19.6 - 46.9)] and for the control group [70.7 (95% CI 55.5 - 82.3%) were statistically significant (p < 0.0001). It can be concluded that factors such as concentration of the bleaching gel and the previous use of desensitizing agents interfere in the diffusion of PH, but the use of the experimental gel based on 5% potassium nitrate and 5% glutaraldehyde was effective in reducing post-operative in patients undergoing in-office tooth bleaching.

Keywords: Tooth Bleaching. Hydrogen peroxide. Dentin Sensitivity. Dental Enamel Permeability.

LISTA DE ILUSTRAÇÕES

Figura 1 Preparo dos dentes. A) Delimitação da área; B) Acesso à câmara pulpar..... 18

Figura 2 Avaliação de cor inicial do canino superior com a escala Vita Classical (Vita Zahnfabrik)..... 24

Figura 3 Aplicação do gel dessensibilizante previamente ao clareamento de consultório..... 25

Figura 4 Avaliação da cor por meio do espectrofotômetro Vita Easyshade (Vita Zahnfabrik)..... 26

Quadro 1 Diluição para obtenção da curva de calibração..... 18

LISTA DE TABELAS

Tabela 1 Composição, número do lote e modo de aplicação dos géis clareadores de acordo com as recomendações dos fabricantes..... 19

Tabela 2 Número de lote, composição e modo de aplicação dos produtos usados no estudo..... 22

LISTA DE ABREVIATURAS E SIGLAS

a *	Eixo cromático vermelho-verde
ADA	American Dental Association
ANOVA	Análise de Variância
b*	Eixo cromático azul-amarelo
COEP	Comissão de Ética em Pesquisa
ΔE	Variação de cor
ΔUEV	Variação de Unidades na Escala Vita
DNA	Ácido desoxirribonucleico
g	Grama (s)
h	Hora (s)
ISO	Organização Internacional para Padronização
Kg	Quilograma (s)
L*	Luminosidade
min	Minuto (s)
mL	Mililitro (s)
mm	Milímetro (s)
mM	Milimol
mol	Mol
mW	Miliwatt (s)
nm	Nanômetro (s)
pH	Potencial hidrogeniônico
s	Segundo (s)
TCLE	Termo de Consentimento Livre e Esclarecido
UEPG	Universidade Estadual de Ponta Grossa
µg	Micrograma (s)

LISTA DE SÍMBOLOS

%	Porcentagem
±	Mais ou menos
α	Alpha
p	Probabilidade de significância
µ	Micro
$^{-}$OH	Íon hidroxila
°C	Graus Celsius
<	Menor
>	Maior
H_0	Hipótese nula

SUMÁRIO

1 INTRODUÇÃO..... **13**

2 PROPOSIÇÃO..... 15
2.1 ESTUDO 1..... 15
2.2 ESTUDO 2..... 15
2.3 ESTUDO 3..... 16

3 MATERIAL E MÉTODOS..... 17

3.1 ESTUDO 1..... 17
3.1.1 Preparo das soluções e obtenção da curva analítica..... 17
3.1.2 Preparo dos dentes..... 18
3.1.3 Protocolo experimental..... 19
3.1.4 Quantificação da concentração inicial de peróxido de hidrogênio dos produtos clareadores..... 20
3.1.5 Análise estatística..... 21

3.2 ESTUDO 2..... 21
3.2.1 Preparo das soluções e obtenção da curva analítica..... 21
3.2.2 Preparo dos dentes..... 22
3.2.3 Protocolo experimental..... 22
3.2.4 Análise estatística..... 23

3.3 ESTUDO 3..... 23
3.1.1 Seleção dos pacientes..... 23
3.1.2 Protocolo experimental / intervenção..... 24
3.1.3 Avaliação da cor e sensibilidade..... 25
3.1.4 Análise estatística..... 26

4 ARTIGOS..... 27

4.1 ARTIGO 1..... 28
4.2 ARTIGO 2..... 44
4.3 ARTIGO 3..... 61

5 DISCUSSÃO..... 81

6 CONCLUSÃO..... 84

REFERÊNCIAS..... 85

ANEXO A – APROVAÇÃO DO ESTUDO *in vitro* PELA COMISSÃO DE ÉTICA EM PESQUISA DA UNIVERSIDADE ESTADUAL DE PONTA

GROSSA 88

ANEXO B – APROVAÇÃO DO ESTUDO *in vitro* PELA COMISSÃO DE ÉTICA EM PESQUISA DA UNIVERSIDADE ESTADUAL DE PONTA GROSSA 92

ANEXO C – APROVAÇÃO DO ESTUDO CLÍNICO PELA COMISSÃO DE ÉTICA EM PESQUISA DA UNIVERSIDADE ESTADUAL DE PONTA GROSSA..... 96

ANEXO D – REGISTRO NO REBEC..... 100

1 INTRODUÇÃO

A introdução da técnica de clareamento dental mudou as atitudes e percepções individuais na Odontologia. Nos últimos anos, a demanda por produtos clareadores dentais aumentou e em alguns países, cirurgiões-dentistas têm relatado um aumento de até 77,8% na busca pelo tratamento (Theobald et al.[1] 2006). No entanto, apesar do clareamento dental ser comprovadamente um tratamento conservador para remoção dos pigmentos dentais, aproximadamente 36 a 100% dos pacientes em algum momento do tratamento relatam sensibilidade dental (Kielbassa et al.[2] 2015, Rezende et al.[3] 2016, Loguercio et al.[4] 2017, Bonafe et al.[5] 2013).

Apesar da etiologia dessa sensibilidade pós-operatória ainda ser questionável, a hipótese teórica mais aceita parece estar relacionada à quantidade de peróxido de hidrogênio que reage potencialmente na polpa, causando estresse oxidativo, que leva a danos celulares (Dias Ribeiro et al.[6] 2009, Trindade et al.[7] 2009, Lima et al.[8] 2013, Cooper et al.[9] 1992, Caviedes-Bucheli et al. 2008[10], Fugaro et al. 2004[11]) e pode inibir a atividade enzimática, resultando em um processo inflamatório. Além disso, o peróxido de hidrogênio pode ativar fibras nervosas e também deflagrar o estímulo de dor (Wang et al. 2015[12]).

Com o objetivo de conseguir alívio para a sensibilidade, muitos autores têm buscado entender a etiologia e o manejo da dor após o clareamento. Uma das formas de redução da dor, por exemplo, está relacionado com a escolha da técnica clareadora pelo cirurgião dentista e o paciente, visto que estão disponíveis diversas formas de abordagem para o clareamento, desde concentrações mais altas (20 – 38%) e baixas (3 – 10%) de peróxido de hidrogênio ou de seu precursor, o peróxido de carbamida 10 a 22% (ADA[13] 2009). Diante dessa variedade, algumas técnicas clínicas como a diminuição da concentração de peróxido de hidrogênio (Bortolatto et al.[14] 2016), diminuição no tempo e frequência de aplicação do gel clareador podem ser utilizadas visando a redução desses efeitos adversos através da redução da taxa de penetração do peróxido de hidrogênio na câmara pulpar (Cooper et al.[9] 1992, Bowles e Ugwuneri[15] 1987). Porém, não se sabe ainda qual a correlação entre a concentração de peróxido de hidrogênio inicial do gel clareador e a quantidade do mesmo que alcança a câmara pulpar.

Uma outra abordagem refere-se à utilização de agentes dessensibilizantes previamente ao clareamento dental. A aplicação tópica de nitrato de potássio e fluoreto de sódio, por exemplo, tem mostrado resultados promissores (Wang et al.[12] 2015). O uso de fluoreto de sódio e nitrato de potássio por 10 min é capaz de reduzir pela metade a sensibilidade dental, bem como a intensidade com que a mesma ocorre (Tay et al.[16] 2009). Fosfopeptídeos de caseína/fosfato de cálcio amorfo e nanofosfato de cálcio são outras duas substâncias citadas na literatura como agentes dessensibilizantes capazes de reduzir a sensibilidade durante o clareamento de consultório (Giniger et al.[17] 2005, Giniger et al. 2005[18], Borges et al.[19] 2012, Vano et al.[20] 2015). Porém, apesar da literatura relatar a eficácia desses materiais, nenhum estudo de quantificação de peróxido de hidrogênio na câmara pulpar após a utilização de agentes dessensibilizantes previamente foi encontrado.

Um outro produto promissor composto por glutaraldeído e 2-hidroxietil metacrilato (HEMA), conhecido comercialmente como Gluma Desensitizer (Heraeus Kulzer, Hanau, Alemanha) mostrou redução significativa no risco da sensibilidade dental durante e após o clareamento dental (Mehta et al.[21] 2002, Ibrahim et al. 2011[22]). Enquanto o glutaraldeído tem efeito reticulador no colágeno da dentina e o nitrato de potássio diminui a capacidade de repolarização das fibras nervosa na polpa dental após sofrerem despolarização devido ao impulso de dor (Markowitz et al.[23] 1991). A associação dos materiais com diferentes modos de ação em uma única aplicação pode ter efeito sinérgico na redução da sensibilidade induzida pelo clareamento dental. No entanto, nenhum estudo avaliou o efeito da associação destes dois agentes em um só produto na sensibilidade dental, em pacientes submetidos ao clareamento de consultório.

Portanto, o objetivo deste estudo foi quantificar a concentração do peróxido de hidrogênio na câmara pulpar de dentes submetidos a diferentes protocolos clareadores e também após aplicação de agentes dessensibilizantes na superfície dental. Adicionalmente, através de um estudo clínico randomizado, avaliou-se o efeito de um gel dessensibilizante experimental na redução da sensibilidade pós-operatória.

2 PROPOSIÇÃO

2.1 ESTUDO 1

2.1.1 Proposição geral

O propósito do presente estudo foi avaliar a concentração de peróxido de hidrogênio que alcança a câmara pulpar de dentes submetidos a diferentes protocolos de clareamento caseiro e de consultório.

2.1.2 Proposição específica

1. Quantificar a concentração de peróxido de hidrogênio na câmara pulpar após clareamento caseiro com peróxido de carbamida (10%, 16% e 22%) e peróxido de hidrogênio (4%, 6%, 7,5%, 10%) e clareamento de consultório com peróxido de hidrogênio 35%.

2. Quantificar a concentração de peróxido de hidrogênio nas bisnagas dos mesmos produtos acima e avaliar sua correlação com a concentração de peróxido de hidrogênio encontrada na câmara pulpar.

2.2. ESTUDO 2

2.2.1 Proposição geral

O propósito do presente estudo foi avaliar a concentração de peróxido de hidrogênio na câmara pulpar de dentes submetidos a agentes dessensibilizantes previamente ao clareamento de consultório.

2.2.2 Proposição específica

1. Quantificar a concentração de peróxido de hidrogênio na câmara pulpar de dentes submetidos ao clareamento de consultório com peróxido de hidrogênio 35% após aplicação de agentes dessensibilizantes a base de nitrato de potássio 5% e fluoreto de sódio 2% (Desensibilize KF2%®); fosfopeptídeos de caseína/fosfato de cálcio amorfo e nanofosfato de cálcio (Mi Paste®) e nanohidroxiapatita (Nano-P®).

2.3 ESTUDO 3

2.3.1 Proposição geral

O propósito deste ensaio clínico randomizado triplo-cego foi avaliar o efeito da associação de glutaraldeído 5% e nitrato de potássio 5% utilizado previamente ao clareamento de consultório na sensibilidade dental e efetividade do clareamento.

2.3.2 Proposição específica

1. Avaliar o risco e a intensidade da sensibilidade dental após clareamento de consultório realizado após aplicação prévia de um gel experimental à base de glutaraldeído 5% e nitrato de potássio 5% utilizando as escalas numéricas de 5 pontos e visual analógica (0 – 10).

2. Avaliar a efetividade do clareamento de consultório nas mesmas condições descritas acima após 1 mês do clareamento utilizando as escalas Vita classical e Vita Bleachedguide e o espectrofotômetro Vita Easyshade.

3 MATERIAL E MÉTODOS

Nesta sessão será descrita a metodologia de forma resumida de cada estudo. As informações detalhadas deste item podem ser encontradas nos artigos referentes a cada estudo.

3.1 ESTUDO 1

O projeto deste estudo *in vitro* foi aprovado pela Comissão de Ética em Pesquisa (COEP) da Universidade Estadual de Ponta Grossa, através do parecer no 1.614.755 (Anexo A). A metodologia detalhada deste experimento está descrita no ARTIGO 1 (página 28).

3.1.1 Preparo das soluções e obtenção da curva analítica

Para este estudo, foram utilizados reagentes de grau analítico sem purificação anterior e foram preparadas com água deionizada de um sistema Milli-Q Millipore (MS2000, Gehaka, São Paulo, SP, Brasil). O peróxido de hidrogênio 34 a 36%, foi adquirido da Labsynth (Labsynth, Diadema, SP, Brasil). Uma solução de 5.000 μg/mL deste peróxido de hidrogênio foi preparada em uma solução tampão de acetato (pH 4,5) resultando em uma solução padrão de peróxido de hidrogênio. Esta solução foi então titulada com uma solução padrão de permanganato de potássio (Mendham e Afonso[24] 2002) para determinação da concentração de peróxido de hidrogênio da mesma. Alíquotas desta solução padrão foram diluídas volumetricamente a fim de obter soluções padrão de 0,032 – 0,397 μg/mL para obtenção da curva analítica (Tabela 1).

Quadro 1 – Diluição para obtenção da curva de calibração

Massa de PH (µg)	Concentração de PH (µg/mL)	Solução Tampão (µL)	Solução Padrão (µL)	Peroxidase (µL)	Violeta Leucocristal (µL)	Água Deionizada (µL)
1.192	0.397	75	25	50	100	2750
0.953	0.318	80	20	50	100	2750
0.715	0.238	85	15	50	100	2750
0.477	0.159	90	10	50	100	2750
0.381	0.127	92	8	50	100	2750
0.191	0.064	96	4	50	100	2750
0.095	0.032	98	2	50	100	2750
0.000	0.000	100	0	50	100	2750

3.1.2 Preparo dos dentes

Raízes de 90 pré-molares foram cortados aproximadamente 3 mm apicalmente a junção cemento - esmalte. O acesso à câmara foi ampliada cuidadosamente com uma broca esférica (#1014, KG Sorensen, São Paulo, SP, Brasil) para permitir a introdução de uma micropipeta (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland) no interior da câmara pulpar (Figura 1). Todos os dentes foram fixados verticalmente em uma placa de cera e a superfície vestibular de cada dente foi isolada pela aplicação de uma barreira de resina fotoativada (Top Dam, FGM Produtos Dentais, Joinvile, SC, Brasil).

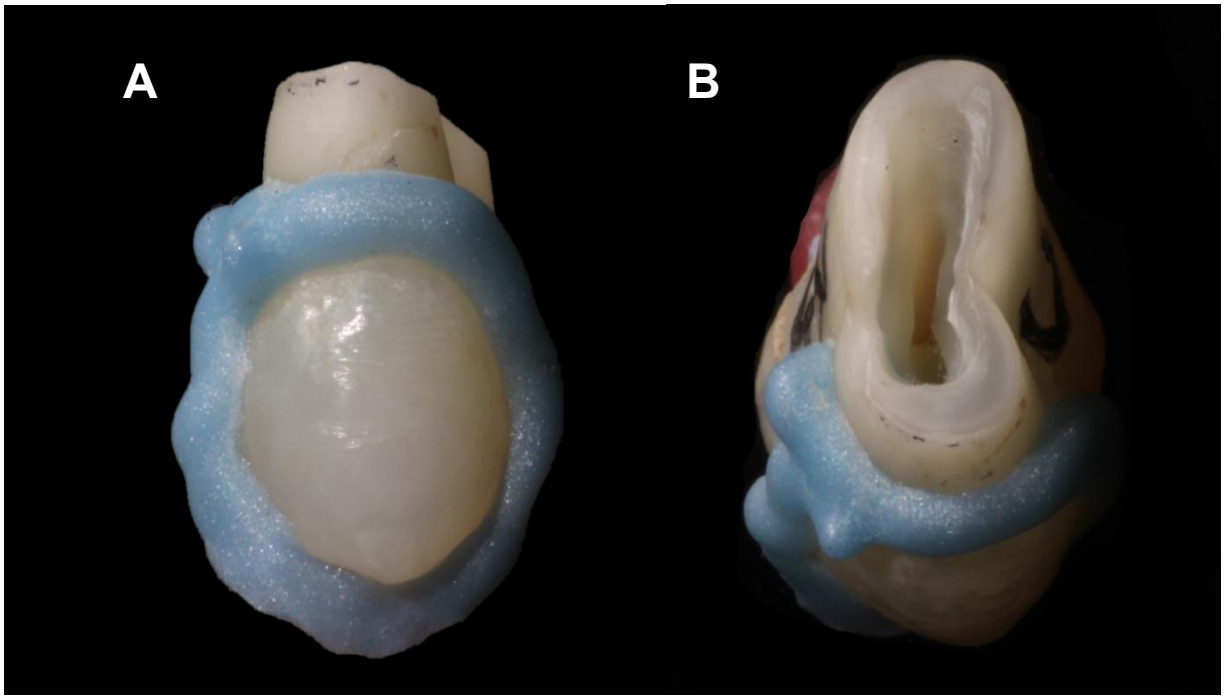


Figura 1 – Preparo dos dentes. A) Delimitação da área; B) Acesso à câmara pulpar

3.1.3 Protocolo experimental

Uma alíquota de 25 µL de tampão de acetato (pH 4,5) foi colocada no interior da câmara pulpar de cada dente. Os espécimes foram então submetidos a diferentes protocolos de clareamento: 1) controle (sem tratamento clareador) ; 2) peróxido de carbamida 10% (Whiteness Perfect 10%®, FGM); 3) peróxido de carbamida 16% (Whiteness Perfect 16%®, FGM); 4) peróxido de carbamida 22% (Whiteness Perfect 22%®, FGM); 5) peróxido de hidrogênio 4% (Whiteness Class 4%®, FGM); 6) peróxido de hidrogênio 6% (Whiteness Class 6%®, FGM); 7) peróxido de hidrogênio 7,5% (Whiteness Class 7,5%®, FGM); 8) peróxido de hidrogênio 10% (Whiteness Class 10%®, FGM); 9) peróxido de hidrogênio 35% (Whiteness HPMaxx 35%®, FGM). Os géis clareadores foram aplicados de acordo com as recomendações dos fabricantes (Tabela 2).

Tabela 1 – Composição, número do lote e modo de aplicação dos géis clareadores de acordo com as recomendações dos fabricantes

Grupos	***Composição***	***Número do lote***	***Modo de aplicação***
Peróxido de Carbamida 10%	Peróxido de Carbamida 10%, Carbopol, Nitrato de potássio, fluoreto de sódio, umectante (Glicol), Água deionizada.	02.04.14 21.09.2016	**3 h por dia**
Peróxido de Carbamida 16%	Peróxido de Carbamida 16%, Carbopol, Nitrato de potássio, fluoreto de sódio, umectante (Glicol), Água deionizada.	18.08.2016 19.10.2016	**3 h por dia**
Peróxido de Carbamida 22%	Peróxido de Carbamida 22%, Carbopol, Nitrato de potássio, fluoreto de sódio, umectante (Glicol), Água deionizada.	12.11.2014 03.10.2016	**1 h por dia**
Peróxido de hidrogênio 4%	Peróxido de hidrogênio 4%. Carbopol, nitrato de potássio, fluoreto de sódio, gluconato de cálcio, estabilizadores, água deionizada e surfactante.	13.11.2013 26.07.2016	**2 h por dia**
Peróxido de hidrogênio 6%	Peróxido de hidrogênio 6%. Carbopol, nitrato de potássio, fluoreto de sódio, gluconato de cálcio, estabilizadores, água deionizada e surfactante.	25.09.2014 25.09.2014	**1 h 30min por dia**

Peróxido de hidrogênio 7,5%	Peróxido de hidrogênio 7,5%. Carbopol, nitrato de potássio, fluoreto de sódio, gluconato de cálcio, estabilizadores, água deionizada e surfactante.	10.12.2014 26.09.2016	**1 h por dia**
Peróxido de hidrogênio 10%	Peróxido de hidrogênio 10%. Carbopol, nitrato de potássio, fluoreto de sódio, gluconato de cálcio, estabilizadores, água deionizada e surfactante.	23.06.2015 29.09.2016	**30 min por dia**
Peróxido de hidrogênio 35%	Peróxido de hidrogênio 30% - 35%, espessantes, glicol, carga inorgânica e água deionizada.	26.05.2014 02.08.2016	**3 sessões de 15 min cada**

Ao final do tratamento clareador, a solução de tampão de acetato foi removida da câmara pulpar de cada dente com uma micropipeta e transferida a uma cubeta de plástico. A câmara pulpar de cada dente foi lavada por quatro vezes com 25 µL de tampão de acetato, no qual foi colocado na cubeta de vidro. Após, 2.725 µL de água destilada foi adicionada a cubeta junto com 100 µL de 0,5 mg mL^{-1} de leucocristal violeta (Aldrich, Sigma-Aldrich Chemie GmbH, Steinheim, Alemanha) e 50 µL de 1 mg mL^{-1} de enzima peroxidase (Peroxidase Type VI-A, Sigma Chemial Co., St Louis, Missouri, EUA).

A absorbância da resultante cor violeta foi medida no espectrofotômetro Cary 50 UV-Vis a 596 nm (Varian, Palo Alto, California, EUA). De acordo com Beer´s law, a absorbância é diretamente proporcional a concentração. Dessa forma, a concentração de peróxido de hidrogênio foi determinada em (µg/mL) pela comparação da curva de calibração previamente obtida.

3.1.4 Quantificação da concentração inicial de peróxido de hidrogênio dos produtos clareadores

Posteriormente, foi utilizado permanganato de potássio como agente oxidante para descrever a concentração inicial de peróxido de hidrogênio em cada gel clareador utilizado no estudo. Uma quantidade de aproximadamente 0,2 mg de gel clareador foi coletada e diluída em 50 mL de água destilada e 10 mL de 1 $mol.L^{-1}$ de ácido sulfúrico. A solução de permanganato de potássio (0,06 $mol.L^{-1}$) foi então adicionada a esta solução (Mendham e Afonso[24] 2002, Marson et al.[25] 2015),

resultando em uma solução de cor violeta. Esta mudança de cor indica o ponto de equivalência, isto é, o momento em que todo peróxido de hidrogênio presente foi consumido. Foram realizadas três titulações para cada sistema clareador. A concentração média de peróxido de hidrogênio foi calculada. Além disso, foi calculada a quantidade de peróxido de hidrogênio disponível (%PH) por tempo de utilização (h).

3.1.5 Análise estatística

Os dados foram avaliados através da análise de variância um fator e teste de Tukey para contraste das médias com um nível de significância de 5%. A correlação de Pearson foi utilizada para avaliar a média entre a concentração de peróxido de hidrogênio encontrado na câmara pulpar e a concentração inicial dos géis.

3.2 ESTUDO 2

O projeto deste estudo *in vitro* foi aprovado pela Comissão de Ética em Pesquisa (COEP) da Universidade Estadual de Ponta Grossa através do parecer no 808.118 (Anexo B). A metodologia detalhada deste experimento está descrita no ARTIGO 2 (página 44).

3.2.1 Preparo das Soluções e obtenção da curva analítica

Para este estudo, foram utilizados reagentes de grau analítico sem purificação anterior e foram preparadas com água deionizada de um sistema Milli-Q Millipore (MS2000, Gehaka, São Paulo, SP, Brasil). O peróxido de hidrogênio 34 a 36%, foi adquirido da Labsynth (Labsynth, Diadema, SP, Brasil). Uma solução de 5.000 μg/mL deste peróxido de hidrogênio foi preparada em uma solução tampão de acetato (pH 4,5) resultando em uma solução padrão de peróxido de hidrogênio. Esta solução foi então titulada com uma solução padrão de permanganato de potássio (Mendham e Afonso[24] 2002) para determinação da concentração de peróxido de hidrogênio da mesma. Alíquotas desta solução padrão foram diluídas

volumetricamente a fim de obter soluções padrão de 0,032 – 0,397 µg/mL para obtenção da curva analítica (Tabela 1 – página 18).

3.2.2 Preparo dos Dentes

Raízes de 50 pré-molares foram cortados aproximadamente 3 mm apicalmente a junção cemento - esmalte como descrito na seção acima no - *3.1.2 Figura 1* (página 18). O acesso à câmara foi ampliada cuidadosamente com uma broca esférica (#1014, KG Sorensen, São Paulo, SP, Brasil) para permitir a introdução de uma micropipeta (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland) no interior da câmara pulpar. Todos os dentes foram fixados verticalmente em uma placa de cera e a superfície vestibular de cada dente foi isolada pela aplicação de uma barreira de resina fotopolimerizável (Top Dam, FGM Produtos Dentais, Joinvile, SC, Brasil).

3.2.3 Protocolo Experimental

Uma alíquota de 25 µL de tampão de acetato (pH 4,5) foi colocada no interior da câmara pulpar de cada dente. Os agentes dessensibilizantes foram aplicados de acordo com as recomendações dos fabricantes (Tabela 3).

Tabela 2. Número de lote, composição e modo de aplicação dos produtos usados no estudo.

Grupos experimentais [fabricante]	***Composição***	***Número do lote***	***Modo de aplicação***
Dessensibilize KF2%® [FGM, Brasil]	Nitrato de potássio a 5% e fluoreto de sódio 2%, água deionizada, glicerina e espessantes.	09.09.2014	**Uma aplicação simples de 10 min**
Mi Paste® [GC Corporation, Japão]	Fosfopeptídeo de caseína (CPP) e fosfato de cálcio amorfo (ACP).	13.07.2008	**Uma aplicação simples de 5 min**
Nano-P® [FGM, Brasil]	9000 ppm de fluoreto de sódio, nitrato de potássio 5%, e fosfato de nanocálcio.	09.09.2014	**Uma aplicação simples de 5 min**
Whiteness HP Maxx® [FGM, Brasil]	35% PH, espessantes, glicol, carga inorgânica e água deionizada.	20.08.2014	**Três aplicações de 15 min cada**

Após a remoção dos mesmos, o gel clareador a base de peróxido de hidrogênio 35% (Whiteness HP Maxx, FGM Produtos Dentais, Joinvile, SC, Brasil) foi aplicado por 3 vezes de 15 min cada. Ao final do tratamento clareador, a solução tampão de cada dente foi removida e transferida para um tubo de vidro, o qual foi adicionado água deionizada, enzima e corante. A solução resultante violeta foi avaliada em espectrofotômetro UV-VIS a absorbância de 596 nm.

3.2.4 Análise Estatística

Os dados foram avaliados através da análise de variância um fator e teste de Tukey para contraste das médias com um nível de significância de 5%.

3.3 ESTUDO 3

O projeto deste ensaio clínico randomizado triplo-cego foi aprovado pela Comissão de Ética em Pesquisa (COEP) da Universidade Estadual de Ponta Grossa através do parecer nº 843.554 (Anexo C). O estudo foi registrado no ensaiosclinicos.gov.br sob o número de identificação RBR-7B7CMN (Anexo D). A metodologia detalhada destes experimentos está descrita nos ARTIGO 3 (página 60).

3.1.1 Seleção dos pacientes

Quarenta e dois voluntários que tiveram interesse em realizar o clareamento dental e que se enquadraram nos critérios de inclusão e exclusão do estudo foram selecionados. Os caninos superiores deveriam ser classificados como cor A2 ou mais escuro, de acordo com a escala Vita Classical (VITA Zahnfabrik, Bad Sackingen, Alemanha), Figura 2.

Figura 2 – Avaliação da cor inicial dos caninos superiores com a escala Vita Classical (Vita Zahnfabrik)

3.1.2 Protocolo experimental / intervenção

O gel dessensibilizante foi manipulado pela adição de nitrato de potássio 5% (Merck, São Paulo, SP, Brasil) e glutaraldeído 5% (Vetec, Rio de Janeiro, RJ, Brasil) em um gel de natrosol (hidroxietil-celulose). O gel placebo possuía a mesma formulação, porém sem a adição dos agentes dessensibilizantes.

Cada paciente atuou como seu próprio controle usando a técnica de boca dividida. Dessa forma, através da randomização, um dos lados do arco superior da maxila recebia o gel dessensibilizante, enquanto o outro lado recebia o placebo. O gel permaneceu em contato com os dentes por 10 min, sendo agitado com escova Robinson acoplada em baixa rotação por 10 s e removido com ajuda de uma cânula de sucção e lavagem com água. O procedimento clareador foi realizado com peróxido de hidrogênio 35% (Whiteness HP Maxx, FGM Produtos Dentais, Joinvile, SC, Brasil) por 3 vezes de 15 min cada. Duas sessões de clareamento foram realizadas com 1 semana de intervalo.

Figura 3 – Aplicação do gel dessensibilizante previamente ao clareamento de consultório.

3.1.3 Avaliação da cor e sensibilidade

A sensibilidade foi avaliada durante o clareamento, 1 h, 24 h e 48 h após o clareamento. Os pacientes avaliaram numa escala de 0 a 4 na qual 0 = “nenhuma dor” e 4 indicando a “pior dor” e na escala visual analógica (VAS).

A cor foi avaliada em quatro períodos: anteriormente ao tratamento, após uma semana da 1ª e 2ª sessão, e um mês depois do término do tratamento. Para a análise da cor subjetiva foram utilizadas escalas de cor Vita classical e Vita Bleachedguide. Para análise objetiva foi utilizado o espectrofotômetro Vita Easyshade (Vita Zahnfabrik), de acordo com o sistema Vita e CIEL*a*b* (Figura 4).

Figura 4 – Avaliação da cor por meio do espectrofotômetro Vita Easyshade (Vita Zahnfabrik).

3.1.4 Análise estatística

Os dados para risco absoluto de sensibilidade dental para ambos os grupos foram avaliados por meio do teste McNemar (α = 0,05). Para a comparação da intensidade da dor, os dados foram analisados por meio do teste Wilcoxon (α = 0,05). Para a avaliação de cor, empregou-se o teste t de Student (α = 0,05).

4 ARTIGOS

4.1 Baseline and after bleaching hydrogen peroxide concentration

4.2 Effect of preliminary application of desensitizing agent on penetration of hydrogen peroxide from in-office bleaching gel into the pulp chamber

4.3 Effect of preliminary application of glutaraldehyde/potassium nitrate gel on reduction of bleaching-induced tooth sensitivity: a triple-blind randomized clinical trial

ARTIGO 1

TÍTULO: Baseline and after bleaching hydrogen peroxide concentration

STATUS: em correção/não submetido

REVISTA: a ser definida

TITLE PAGE

Baseline and after bleaching hydrogen peroxide concentration

Sibelli Olivieri Parreiras [DDS, MSc, PhD student][1], Michael Favoreto [undergraduate student][1], Gustavo Cruz [undergraduate student][1], Anderson Gomes [undergraduate student][2] Christiane Philippini Ferreira Borges [DDS, PhD][2], Alessandro Loguercio [DDS, MSc, PhD][3], Alessandra Reis [DDS, PhD][3]

[1]School of Dentistry. Department of Restorative Dentistry. State University of Ponta Grossa, PR, Brazil.

[2] School of Chemistry. Department of Chemistry. State University of Ponta Grossa, PR, Brazil.

[3] Associate Professors. School of Dentistry. State University of Ponta Grossa, PR, Brazil.

Corresponding author:

Prof. Alessandra Reis. Universidade Estadual de Ponta Grossa. Departamento de Odontologia. Rua Carlos Cavalcanti, 4748 – Bloco M – sala 64. CEP - 84030-900; e-mail: reis_ale@hotmail.com.

Baseline and after bleaching hydrogen peroxide concentration

ABSTRACT

This in vitro study aimed to quantify the hydrogen peroxide (HP) penetration into the pulp chamber of teeth submitted to different protocols bleaching gels. Ninety pre-molars were randomly divided by lottery into nine groups according to the bleaching agent used and its concentration (n = 10): control (no bleaching) [C], carbamide peroxide 10 % [CP 10%], carbamide peroxide 16% [CP 16%], carbamide peroxide 22% [CP 22%], hydrogen peroxide 4% [HP 4%], hydrogen peroxide 6% [HP 6%], hydrogen peroxide 7.5% [HP 7.5%], hydrogen peroxide 10% [HP 10%] and hydrogen peroxide 35% [HP35%]. Teeth were sectioned 3 mm from the cementoenamel junction and acetate buffer was placed in the pulp chamber. Bleaching gels were applied according to manufacturer's´ recommendations in a single clinical session. Finished the bleaching procedure, the acetate buffer solution was removed and leucocrystal violet and peroxide enzyme solutions were added. The absorbance of the resulting solution was determined in a spectrophotometer, and converted into concentration (μg/mL) equivalent of HP. Data were subjected to ANOVA and Tukey´s test for pairwise comparison (α = 0.05). HP 35% had higher amounts of HP in the pulp chamber (p = 0.28). While CP 10%, CP 16%, CP 22%, HP 6%, HP 7.5% and HP 10% showed similar concentrations. It follows that the amount of HP that reaches the pulp chamber is not proportional to the concentration of whitening gels.

Keywords: Tooth bleaching. Dental enamel permeability. At-home bleaching. In-office bleaching.

Introduction

The demand for dental bleaching products has increased significantly over the past year. In some countries, the demand for tooth bleaching has increased approximately 77.8% [1]. Hydrogen peroxide (HP) and carbamide peroxide (CP) are the most common dentist-supervised products used in dental clinics for bleaching [2]. For at-home bleaching, hydrogen peroxide ranging from 3% to 10% and carbamide peroxide ranging from 10% to 22% are the products employed. On the other hand, in-office bleaching employs highly concentrated hydrogen peroxide gels - typically in the range of 20 to 38% [3-5].

All these bleaching products have shown effective color changes both at the immediate and in middle-term follow-ups [6, 7]. However, more than 50% of the patients submitted to at-home and in-office bleaching experience bleaching-induced tooth sensitivity. The risk and severity of tooth sensitivity depends on: 1) the bleaching technique [8], which are directly related to the hydrogen peroxide concentration and usage time [9-12] and 2) patient-related factors, such as baseline tooth color and age [8].

In regard to the bleaching technique, Rezende et al. (2016)[8] reported that at--home bleaching protocols have lower tooth sensitivity than in-office bleaching. In a 0 to 4 pain scale, the mean tooth sensitivity of at-home bleaching was 0.5 ± 0.9, while in-office bleaching was 2.8 ± 2.9. Perhaps, such differences may be related to the amount of hydrogen peroxide that reaches the pulp chamber. Hydrogen peroxide from carbamide peroxide products, for instance, appears to penetrate less than the hydrogen peroxide of equivalent concentration [13]. Tooth exposure with 15% carbamide peroxide gel (equivalent to 5.3% hydrogen peroxide) resulted in a mean pulp cavity concentration of peroxide that was less than half caused by exposure to pure 5% hydrogen peroxide [13].

The penetration of hydrogen peroxide within the dental structures, that occurs in only 15 minutes [14], seems to be dose dependent [14]. In a clinical study using similar protocols, a higher risk of tooth sensitivity was experienced by the patients who used the 20% CP (71.4%) than the ones who used 10% CP (36.8%) [9].

Variations in the amount of the hydrogen peroxide that reaches the pulp chamber using different bleaching protocols are of clinical interest such it may allow clinicians to choose the best protocol for highly sensitive patients. In face of that the aim of this study was to quantify and correlate the initial amount of hydrogen peroxide of bleaching products with the amount of hydrogen peroxide that reaches the pulp chamber using different bleaching protocols with varied concentrations.

Material and Methods

The local Ethics Committee from the State University of Ponta Grossa, Paraná, Brazil approved this study under protocol number 1.614.755. A total of ninety sound premolars without fractures and enamel defects were randomly divided by lottery into nine groups according to dental bleaching system to be used: 1) control (exposure to distilled water); 2) carbamide peroxide 10% (CP 10%); 3) carbamide peroxide 16% (CP 16%); 4) carbamide peroxide 22% (CP 22%); 5) hydrogen peroxide 4% (HP 4%); 6) hydrogen peroxide 6% (HP 6%); 7) hydrogen peroxide 7.5% (HP 7.5%); 8) hydrogen peroxide 10% (HP 10%); 9) hydrogen peroxide 35% (HP 35%). All dental bleaching systems used in the present study are from the same company (FGM Dental products, Joinville, SC, Brazil). Ten teeth were used in each group.

Quantification of the HP concentration in the pulp chamber

The roots of all teeth were cut approximately 3 mm apical to the cemento-enamel junction. Then the pulp tissue was removed and pulp chamber washed with distilled water. The entrance to the pulp cavities was widened carefully with a round bur (#1014; KG Sorensen, Barueri, SP, Brazil) to allow the introduction of a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland) inside the pulp chamber.

Throughout this study, analytical-grade chemicals without previous purification were used; they were prepared with deionized water from a Millipore Milli-Q system (MS2000, Gehaka, São Paulo, SP, Brazil). HP was purchased from LABSYNTH (34%- 36%, Diadema, SP, Brazil). A 5000 μg/mL stock solution was prepared in acetate buffer solution (pH 4) and standardized by conventional methods. The

solution was titrated with potassium permanganate standard solution [15]. Aliquots of the stock solution of HP were diluted volumetrically to obtain working standard solutions of 0.032-0.397 μg/mL [16] (Figure 1).

All teeth were fixed vertically to a wax plaque, and the labial surface of each tooth was isolated by applying a light-cured resin dam (Top Dam, FGM Dental Products). A 25 μL aliquot of acetate buffer (pH 4.5) was placed into the pulp chamber of each tooth to absorb and stabilize any peroxide that might penetrate into the pulp chamber.

The bleaching gels were applied over the enamel surface according to the manufacturer's recommendations (Table 1) to reproduce the clinical condition. A single-day session was performed for each material. After the exposure period, the acetate buffer solutions in the pulp chamber of each tooth were removed by means of a mechanical micropipette (LABMATE Soft; HTL Lab Solutions) and transferred to a glass tube. The pulp chamber of each tooth was rinsed four times with 25 μL of acetate buffer, and this solution was removed from the pulp chamber and placed into the same glass tube. Next, more deionized water (2.725 μL) was added to the glass tube along with 100 μL of 0.5 mg/mL of leuco-crystal violet (Aldrich; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 50 μL of 1 mg/mL enzyme horseradish peroxidase (Peroxidase Type VI-A; Sigma Chemical Co, St Louis, MO, USA). This procedure was repeated separately for each tooth.

The absorbance at 596 nm of the resultant violet color in the tubes was measured in a Cary 50 UV-Vis spectrophotometer (Varian, Palo Alto, CA, USA). According to Beer's Law, absorbance is directly proportional to the concentration. Therefore, the concentration of HP (μg/mL) was determined by comparing it to the calibration curve previously obtained (Figure 1). By knowing the concentration (μg/mL) and volume of the solution, the HP mass (μg) can be calculated [16].

Quantification of the initial HP concentration in the bleaching products

Potassium permanganate was used as an oxidizing agent to describe the initial concentration of hydrogen peroxide in each bleaching product. Hydrogen peroxide reduces the permanganate to a colorless product based on a reduction-

oxidation reaction, according to the following formula [15, 17]:

$$2KMnO_4 + 5H_2O_2 + 4H_2SO_2 = 2KHSO_4 + 2MnSO_4 + 5O_2 + 8H_2O.$$

The bleaching agents, whenever required, were mixed in accordance with manufacturer´s instructions and were analytically weighted. A sample of bleaching product (approximately 0.2 mg) was collected and diluted in 50 mL of distilled water and 10 mL of 1 $mol.L^{-1}$ sulfuric acid. Then, the potassium permanganate solution (0.06 $mol.L^{-1}$) was added to this solution [15, 17], resulting in a violet color solution. This color change indicated the equivalence point, *i.e.*, the moment when all of the H_2O_2 have been consumed. Three titrations for each bleaching gel system were performed.

The HP mean concentration was calculated by the following formula:

% H_2O_2 (by weight) = (((((V_{KMnO4}. C $_{KMnO4}$)/1000).5)/2).34).100)/m_{H202} (grams of H_2O_2 of the sample used). We also calculated the amount of available hydrogen peroxide product (%HP) per usage time (h).

Statistical analysis

The data related to the concentration of HP that reached the pulp chamber were subjected to one-way analysis of variance (ANOVA) and Tukey's tests for pairwise comparisons (alpha = 0.05). The Pearson correlation between the means of the HP concentration that reached the pulp and the initial HP concentration in each product was calculated.

Results

Different amounts of hydrogen peroxide were found in the pulp chamber of the different bleaching protocols (Figure 2). The amount of HP from the HP 35% was significantly higher than all at-home protocols evaluated ($p < 0.0001$). No statistically significant differences between at-home protocols were observed ($p = 0.649$).

The HP concentrations claimed by the manufacturer and the values obtained from permanganate potassium titration are shown in Figure 3. Titration values showed that the concentration of the products was similar to that claimed by the manufacturer.

A strong and positive correlation ($r = 0.98$; $p = 0.0026$) was found between the titrated concentration of all products and the concentration of peroxide in the pulp chamber (Figure 4). However, when the same correlation was run only for titrated concentration of the at-home products (excluding the in-office bleaching), a non-significant correlation was observed ($r = 0.52$; $p = 0.22$; Figure 4).

Discussion

In the present study, a higher penetration of hydrogen peroxide in extracted human teeth were observed for the in-office tooth bleaching product than the at-home bleaching agents. These results are in agreement with several previous studies that demonstrated that significant hydrogen peroxide amounts can be diffused through dentin after bleaching agent application with in-office protocols [13, 14, 18]. Additionally, some authors report that the use of higher concentrations of hydrogen peroxide creates micropores on enamel surface, which affects surface and inner structure of enamel and may facilitates the passage of the HP [19].

In the present study, the concentration of hydrogen peroxide found in the pulp chamber for 35% HP was not similar, but lower than other published studies [10, 16, 20-22]. For instance, when 35% HP was applied *in vitro* on the dental structure, an amount of HP ranging from 1.22 to 6.22 μg was observed in the pulp chamber of teeth from other studies [10, 16, 20-22], while in the present study an average of 0.27 μg was detected for HP 35%.

The comparison of these studies is difficult, since the type of dental substrate used (human or bovine), composition of the gels, experimental protocol employed, hydrogen peroxide delivery method and differences of cavity preparation are likely responsible for such differences. However, this does not reduce the internal validity of all studies as the same conditions were applied for the different treatments within

the study.

In regard to at-home bleaching protocols, no statistical significant difference was observed in the amount of hydrogen peroxide that reached the pulp chamber. This was expected for systems that have the amount of active hydrogen peroxide. For instance, 10% carbamide peroxide gel, yields a maximum of 3.5% hydrogen peroxide, and therefore we anticipated the similar hydrogen peroxide diffusion of CP 10% and HP 4%. The same phenomenon was observed to 22% CP and 10% HP, which presents similar concentrations of active hydrogen peroxide.

However, the non-significant differences between systems with different initial hydrogen peroxide concentration was, at first glance, surprising, as previous studies suggested that the amount of hydrogen peroxide that penetrates the dental structure is influenced by its original concentration in the bleaching agent [13, 14, 18].

In agreement with such findings, clinical studies do not demonstrate significant differences between at-home bleaching products of different active hydrogen peroxide concentration both in terms of whitening efficacy and tooth sensitivity. For instance, a clinical situation, in which different concentrations of CP (10%, 15%, 17%) were compared, the whitening results were similar [23-26]. Likewise, a similar whitening degree was observed when lower concentration of hydrogen peroxide were also compared with 20% CP [27-29]. In regard to tooth sensitivity, some authors affirm that 16% CP provokes more sensitivity than does 10% CP [24], whereas in another studies, there were no significant differences between these concentrations [3, 29].

In all clinical studies cited above, the products were employed according to the manufacturer's instructions, and this may be the key for understanding why similar hydrogen peroxide concentrations was observed for at-home products. In the present study, we also followed the manufacturer´s recommendations of all products in a way to simulate the clinical condition, as we wanted to compare bleaching protocols rather than bleaching product concentrations.

Bleaching protocols vary in the mode and duration of product application. Products with low active hydrogen peroxide were applied for longer periods of times than highly concentrated products. Therefore, the low concentration of hydrogen

peroxide might have been compensated for the longer exposure time they were left on the enamel surface (Table 2). This means that not only concentration, but also application time is responsible for the amount of product that reaches the pulp chamber. This explains the lack of correlation between initial hydrogen peroxide concentration and the percentage of hydrogen peroxide that reached the pulp chamber for at-home bleaching products. Had the products been used for the same time, which is not representative of a clinical condition, a significant correlation might have been observed.

This correlation was, however, significant only when the 35% hydrogen peroxide; the product with the highest hydrogen peroxide concentration, was included in the data. The titrated concentration of hydrogen peroxide multiplied by the usage time (h) give us the amount of product per hour available for penetration. This relation was highest for the 35% HP product (23% HP per hour) in comparison with all other at-home products, which ranged from 5 to 19 %HP/h). The only product that showed a different behavior was CP 16% and deserver further investigations.

Therefore, we can conclude from this *in vitro* study that the at-home products herein tested and used by the time recommended by the manufacturer led to similar hydrogen peroxide concentration in the pulp chamber of bleached teeth.

References:

1. Theobald AH, Wong BK, Quick AN & Thomson WM (2006) The impact of the popular media on cosmetic dentistry *N Z Dent J* **102(3)** 58-63.
2. Joiner A (2007) Review of the effects of peroxide on enamel and dentine properties *J Dent* **35(12)** 889-896, 10.1016/j.jdent.2007.09.008.
3. Mokhlis GR, Matis BA, Cochran MA & Eckert GJ (2000) A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use *J Am Dent Assoc* **131(9)** 1269-1277.
4. Haywood VB (2005) Treating sensitivity during tooth whitening *Compend Contin Educ Dent* **26(9 Suppl 3)** 11-20.
5. (ADA) ADA (2009) Tooth whitening/bleaching: treatment considerations for dentists and their patients. ADA Council on Scientific Affairs Chicago.
6. Boushell LW, Ritter AV, Garland GE, Tiwana KK, Smith LR, Broome A & Leonard RH (2012) Nightguard vital bleaching: side effects and patient satisfaction 10 to 17 years post-treatment *J Esthet Restor Dent* **24(3)** 211-219, 10.1111/j.1708-8240.2011.00479.x.
7. Tay LY, Kose C, Herrera DR, Reis A & Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: a 2-year double-blind randomized clinical trial *Am J Dent* **25(4)** 199-204.

8. Rezende M, Loguercio AD, Kossatz S & Reis A (2016) Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: A multi regression and logistic analysis *J Dent* **45** 1-6, 10.1016/j.jdent.2015.11.003.
9. Basting RT, Amaral FL, Franca FM & Florio FM (2012) Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents *Oper Dent* **37(5)** 464-473, 10.2341/11-337-c.
10. Briso AL, Goncalves RS, Costa FB, Gallinari Mde O, Cintra LT & Santos PH (2015) Demineralization and hydrogen peroxide penetration in teeth with incipient lesions *Braz Dent J* **26(2)** 135-140, 10.1590/0103-6440201300225.
11. Parreiras S, Mena-Serrano A, Moreira CG, Otuki M, Loguercio D & Reis A (2014) Penetration and cytotoxicity of a bleaching gel activated by LED/laser in restored teeth *Am J Dent* **27(6)** 301-306.
12. Soares DG, Basso FG, Pontes EC, Garcia Lda F, Hebling J & de Souza Costa CA (2014) Effective tooth-bleaching protocols capable of reducing H(2)O(2) diffusion through enamel and dentine *J Dent* **42(3)** 351-358, 10.1016/j.jdent.2013.09.001.
13. Cooper JS, Bokmeyer TJ & Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents *J Endod* **18(7)** 315-317, 10.1016/s0099-2399(06)80479-6.
14. Bowles WH & Ugwuneri Z (1987) Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures *J Endod* **13(8)** 375-377, 10.1016/s0099-2399(87)80196-6.
15. Mendham J & Afonso JC (2002) *Vogel: Quantitative chemical analysis.* Livros Tecnicos e Científicos Editora S.A. Rio de Janeiro.
16. Mena-Serrano AP, Parreiras SO, do Nascimento EM, Borges CP, Berger SB, Loguercio AD & Reis A (2015) Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber *Oper Dent* **40(2)** E76-82, 10.2341/13-352-l.
17. Marson FC, Goncalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH & Briso AL (2015) Penetration of hydrogen peroxide and degradation rate of different bleaching products *Oper Dent* **40(1)** 72-79, 10.2341/13-270-l.
18. Gokay O, Mujdeci A & Algin E (2005) In vitro peroxide penetration into the pulp chamber from newer bleaching products *Int Endod J* **38(8)** 516-520, 10.1111/j.1365-2591.2005.00979.x.
19. Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G & Jenei A (1999) An atomic force microscopy study on the effect of bleaching agents on enamel surface *J Dent* **27(7)** 509-515.
20. de Almeida LC, Soares DG, Gallinari MO, de Souza Costa CA, Dos Santos PH & Briso AL (2015) Color alteration, hydrogen peroxide diffusion, and cytotoxicity caused by in-office bleaching protocols *Clin Oral Investig* **19(3)** 673-680, 10.1007/s00784-014-1285-3.
21. Camargo SE, Cardoso PE, Valera MC, de Araujo MA & Kojima AN (2009) Penetration of 35% hydrogen peroxide into the pulp chamber in bovine teeth after LED or Nd:YAG laser activation *Eur J Esthet Dent* **4(1)** 82-88.
22. Omrani LR, Taher A, Albujeer A, Parvin M, Daryakenari G, Gorgani-Fi S, Kermanshah H & N. C (2016) Penetration of hydrogen peroxide into the pulp chambre after conventional and laser-assisted bleaching *SADJ* **71(2)** 58-61.
23. Kihn PW, Barnes DM, Romberg E & Peterson K (2000) A clinical evaluation of 10 percent vs. 15 percent carbamide peroxide tooth-whitening agents *J Am Dent Assoc* **131(10)** 1478-1484.

24. Meireles SS, Heckmann SS, Leida FL, dos Santos Ida S, Della Bona A & Demarco FF (2008) Efficacy and safety of 10% and 16% carbamide peroxide tooth-whitening gels: a randomized clinical trial *Oper Dent* **33(6)** 606-612, 10.2341/07-150.
25. Braun A, Jepsen S & Krause F (2007) Spectrophotometric and visual evaluation of vital tooth bleaching employing different carbamide peroxide concentrations *Dent Mater* **23(2)** 165-169, 10.1016/j.dental.2006.01.017.
26. Krause F, Jepsen S & Braun A (2008) Subjective intensities of pain and contentment with treatment outcomes during tray bleaching of vital teeth employing different carbamide peroxide concentrations *Quintessence Int* **39(3)** 203-209.
27. Delgado E, Hernandez-Cott PL, Stewart B, Collins M & De Vizio W (2007) Tooth-whitening efficacy of custom tray-delivered 9% hydrogen peroxide and 20% carbamide peroxide during daytime use: a 14-day clinical trial *P R Health Sci J* **26(4)** 367-372.
28. Ziebolz D, Helms K, Hannig C & Attin T (2007) Efficacy and oral side effects of two highly concentrated tray-based bleaching systems *Clin Oral Investig* **11(3)** 267-275, 10.1007/s00784-007-0108-1.
29. Alonso de la Pena V & Lopez Raton M (2014) Randomized clinical trial on the efficacy and safety of four professional at-home tooth whitening gels *Oper Dent* **39(2)** 136-143, 10.2341/12-402-c.

TABLES

Table 1. Batch number, composition and mode of application of the products used in this study

Groups	*Main components*	*Batch number*	*Mode of application*
10% Carbamide Peroxide	10% Carbamide Peroxide, Neutralized Carbopol, Potassium Nitrate, Sodium Fluoride, Humectant (Glycol), Deionized Water.	02.04.14 21.09.2016	**3 h a day**
16% Carbamide Peroxide	16% Carbamide Peroxide, Neutralized Carbopol, Potassium Nitrate, Sodium Fluoride, Humectant (Glycol), Deionized Water.	18.08.2016 19.10.2016	**3 h a day**
22% Carbamide Peroxide	22% Carbamide Peroxide, Neutralized Carbopol, Potassium Nitrate, Sodium Fluoride, Humectant (Glycol), Deionized Water.	12.11.2014 03.10.2016	**1 h a day**
4% Hydrogen Peroxide	Available in 4% hydrogen peroxide gel. Neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water and surfactant.	13.11.2013 26.07.2016	**2 h a day**
6% Hydrogen Peroxide	Available in 6% hydrogen peroxide gel. Neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water and sur factant.	25.09.2014 25.09.2014	**1 h 30min a day**
7.5% Hydrogen Peroxide	Available in 7.5% hydrogen peroxide gel. Neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water and sur factant.	10.12.2014 26.09.2016	**1 h a day**
10% Hydrogen Peroxide:	Available in 10% hydrogen peroxide gel. Neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water and sur factant.	23.06.2015 29.09.2016	**30 min a day**
35% Hydrogen Peroxide	hydrogen peroxide at 30% - 35%, thickeners, dye mixture, glycol, inorganic load and deionized water.	26.05.2014 02.08.2016	**3 sessions of 15 min each**

* Whiteness Perfect 10% (CP 10%); Whiteness Perfect 16% (CP 16%); Whiteness Perfect 22% (CP 22%); Whiteness Class 4% (HP 4%); Whiteness Class 6% (HP 6%); Whiteness Class 7.5%: (HP 7.5%); Whiteness Class 10% (HP 10%); Whiteness HPMaxx (HP 35%)
* all products used in this study are the same manufacturer`s (FGM Dental Products, Joinville, SC, Brazil)

FIGURES

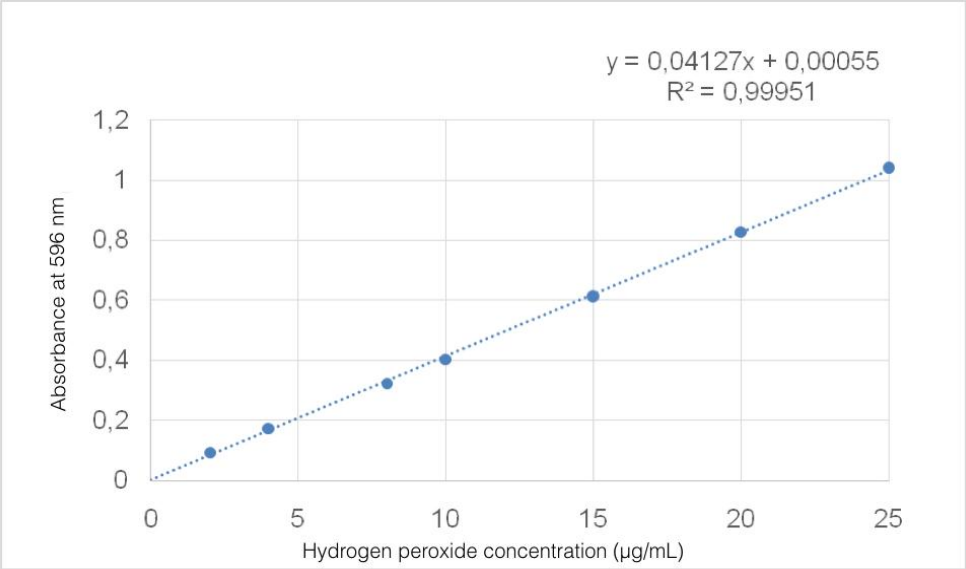


Figure 1. Spectrophotometric calibration curve used in this study, R = 0.99951

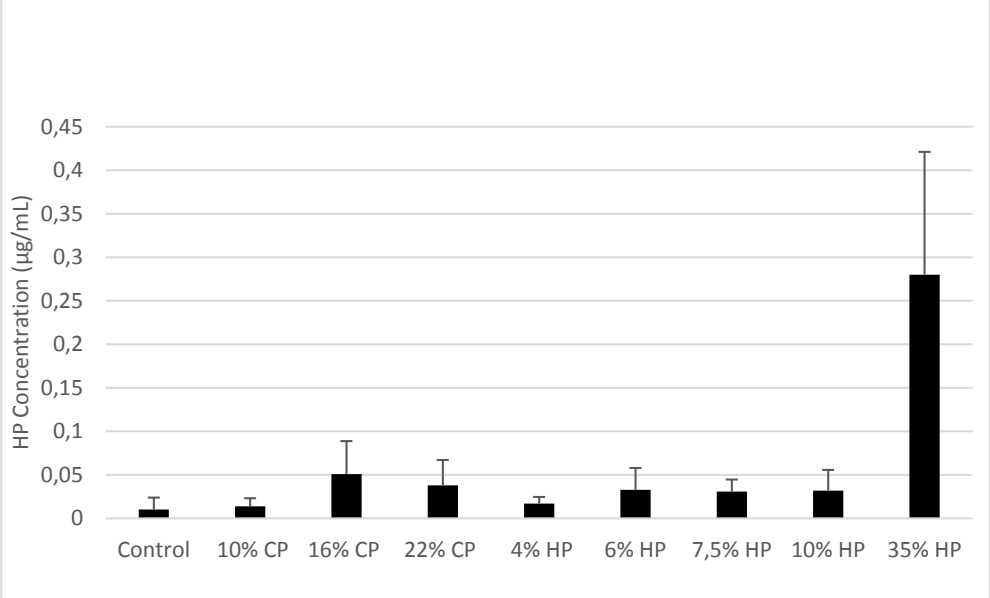


Figure 2. Concentration of hydrogen peroxide found in the pulp chamber.

Bleaching gel	Concentration given by the manufacturer	Concentration of HP found in the product	Amount of available titrated hydrogen peroxide product per hour (%HP.h)
Perfect 10%	10% CP	3.91%	11.7
Perfect 16%	16% CP	6.63%	19.8
Perfect 22%	22% CP	8.97%	9
Class 4%	4% HP	4.40%	8.8
Class 6%	6% HP	5.15%	7.8
Class 7,5%	7,5% HP	7.93%	7.9
Class 10 %	10% HP	10.22%	5
HP Maxx 35%	35% HP	31%	23.2

Figure 3. Concentration of hydrogen peroxide found in the comercial products along with the amount of available product per hour.

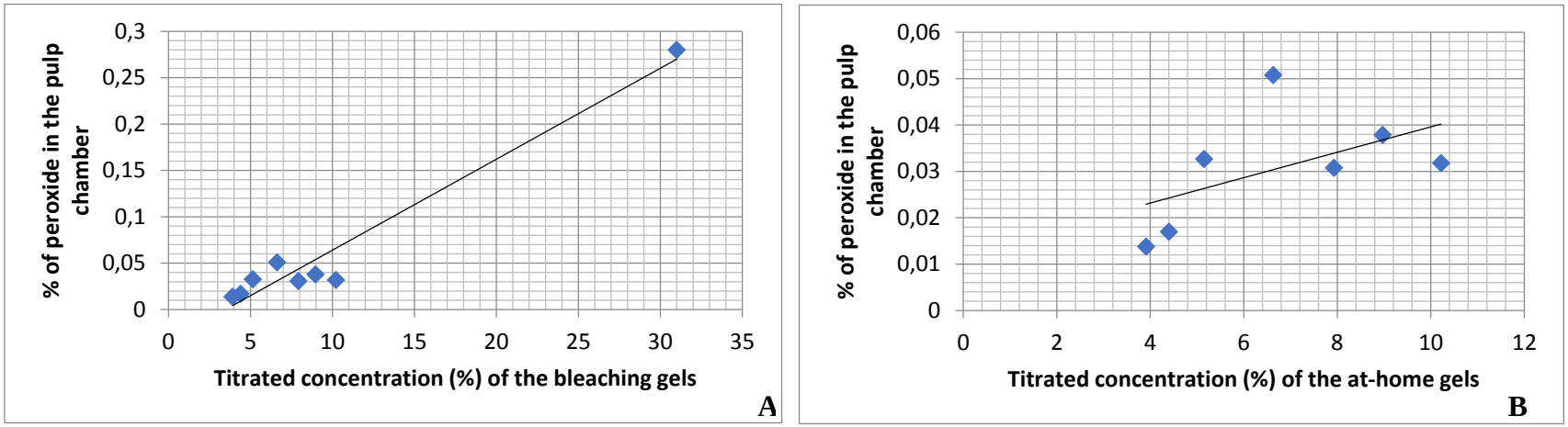


Figure 4. Correlation of concentration of peroxide in the pulp chamber in all products (A) and at-home (B) tooth bleaching systems.

ARTIGO 2

TÍTULO: Effect of preliminary application of desensitizing agent on penetration of hydrogen peroxide from in-office bleaching gel into the pulp chamber

STATUS: submetido para avaliação

REVISTA: Brazilian Oral Research

TITLE PAGE

Effect of preliminary application of desensitizing agent on penetration of hydrogen peroxide from in-office bleaching gel into the pulp chamber

Preliminary application of desensitizing agent on penetration of hydrogen peroxide

Sibelli Olivieri Parreiras [DDS, MSc][1], Rubia Elisa Lenz [undergraduate student][2], Michael Favoreto [undergraduate student][2], Maria Eduarda Serra [undergraduate student][3] Christiane Philippini Ferreira Borges [DDS, PhD][4], Alessandro D. Loguercio [DDS, MSc, PhD][5], Alessandra Reis [DDS, PhD][5]

[1] PhD student. School of Dentistry. Department of Restorative Dentistry. State University of Ponta Grossa, PR, Brazil.

[2] School of Dentistry. Department of Restorative Dentistry. State University of Ponta Grossa, PR, Brazil.

[3] School of Chemistry. Department of Chemistry. State University of Ponta Grossa, PR, Brazil.

[4] Associate Professor. School of Chemistry. Department of Chemistry. State University of Ponta Grossa, PR, Brazil.

[5] Associate Professor. School of Dentistry. State University of Ponta Grossa, PR, Brazil.

Corresponding author:

Prof. Alessandra Reis, State University of Ponta Grossa, Dental Post-Graduate Program, Rua Carlos Cavalcanti, 4748, Bloco M - Uvaranas, Ponta Grossa, Paraná – Brazil. E-mail: reis_ale@hotmail.com.

ABSTRACT

Purpose: This *in vitro* study aimed to quantify the hydrogen peroxide (HP) penetration into the pulp chamber, after application of desensitizing agents prior to in-office bleaching. Methods: fifty pre- molars were sectioned 3 mm from the cementoenamel junction and acetate buffer was placed in the pulp chamber. The teeth were randomly divided by lottery into five groups. In the positive control group, only the in-office bleaching gel was used and in the negative control group no treatment was used. Three different active agents were applied according to manufacturers' recommendations before to in-office bleaching: Desensibilize KF2% group (a potassium nitrate desensitizing gel); Mi Paste group (a casein phophopeptides and amorphous calcium phosphate based paste); and Desensibilize Nano-P group (a nanohydroxyapatite paste). The bleaching procedure was carried out with 35%HP in a single clinical session with three 15-min applications. After bleaching, the acetate buffer solution was removed and leucocrystal violet and peroxide enzyme solutions were added. The absorbance of the resulting solution was determined in a spectrophotometer, and converted into concentration (μg/mL) equivalent of HP. Data were subjected to ANOVA and Tukey´s test for pairwise comparison ($\alpha = 0.05$). Results: the use of all products reduced the penetration of HP in the pulp chamber compared to the positive group ($p < 0.001$). Mi Paste and Nano P were the products that yielded the lowest HP penetration, which was similar to the negative control group. The use of desensitizing agents before tooth bleaching seems to be an alternative to reduce the amount of HP that reaches the pulp chamber of vital teeth.

Clinical Significance: The use of desensitizing agents before tooth bleaching should be considered. It seems to be an alternative to reduce the amount of HP that produce cells damage and tooth sensitivity.

Introduction

Bleaching agents are commercially available to professional or home long-

term use products. All these agents and components that contain peroxide break down into hydrogen peroxide (HP), which then, through an oxidation process, penetrates the tooth structure and oxidizes pigment molecules.[1]

Unfortunately, the penetration of the HP in the pulp chamber may result in DNA and cell damage[2-5] and may inhibit pulpal enzyme activity,[6] resulting in a reversible inflammatory process. Furthermore, HP can activate A-δ nerve fiber,[7-8] which may be responsible for the high risk of tooth sensitivity (TS) experienced by patients after in-office dental bleaching.[9-11]

In an attempt to minimize this tooth sensitivity, manufacturers have added different compounds in bleaching products and clinicians have attempted to apply desensitizing agents prior to the bleaching session.[12-16] These desensitizing agents include active components such as potassium nitrate and fluorides, casein phosphopeptides, amorphous calcium phosphate (CPP-ACP), and nano-calcium phosphate. Indeed there are some clinical trials that point out that the preliminary application of desensitizing agents on the enamel surface before in-office bleaching substantially reduces the bleaching-induced TS.[12-17]

Authors have claimed that these products act either by reducing the excitability of the intradental nerve endings or by producing dentin tubule obliteration.[18-21] They can also minimize bleaching-induced TS by lowering the amount of HP that reaches the pulp chamber and consequently the pulp damage caused by the HP. To the extent of the author's knowledge, no study has attempted to investigate if desensitizing agents applied previously to in-office bleaching with 35% HP can affect the amount of HP that reaches the pulp chamber. Therefore, the present study aimed to quantify the concentration of HP in the pulp chamber after the application of desensitizing agents administered before an in-office bleaching.

Material and methods

This study was approved by the local Ethics Committee of the State University of Ponta Grossa, Paraná, Brazil under protocol number 808.118. A total of fifty sound premolar teeth without visible fractures and enamel defects were randomly divided by lottery into five groups according to desensitizing agents to be used: 1) positive

control group (no desensitizing agent); 2) Desensibilize KF 2% (FGM, Joinvile, SC, Brazil); 3) Mi Paste (Corporation, Tokyo, Japan); 4) Desensibilize Nano-P (FGM). An additional negative control group, in which no bleaching treatment was performed, was added to the experimental design. Ten teeth were used in each group.

The roots of all teeth were cut approximately 3 mm apical to the cemento-enamel junction, and the pulp tissue was removed and washed with distilled water. The entrance to the pulp cavities was widened carefully with a round bur #1014 (KG Sorensen, SP, Brazil) to allow the introduction of a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland) inside the pulp chamber.

Throughout this study, analytical-grade chemicals without previous purification were used; they were prepared with deionized water from a Millipore Milli-Q system (MS2000) (Gehaka, SP, Brazil). Hydrogen peroxide (HP) was purchased from local company (34%-36%) (LABSYNTH, SP, Brazil). A 5000 μg/mL stock solution was prepared in acetate buffer solution (pH 4) and standardized by conventional methods. The solution was titrated with potassium permanganate standard solution.[22] Aliquots of the stock solution of HP were diluted volumetrically to obtain working standard solutions of 0.032-0.397 μg/mL (Table 1; Figure 1).

All teeth were fixed vertically to a wax plaque, and the labial surface of each tooth was isolated by applying a light-cured resin dam (Top Dam) (FGM). A 25 μL aliquot of acetate buffer (pH 4.5) was placed into the pulp chamber of each tooth to absorb and stabilize any peroxide that might penetrate into the pulp chamber.

The desensitizing agents were performed according to manufacturer's recommendations (Table 2). After that, the bleaching gels were applied over the enamel surface by 3 applications of 15 min each. After the exposure period, the acetate buffer solutions in the pulp chamber of each tooth were removed by means of a mechanical micropipette (LABMATE Soft) and transferred to a glass tube. The pulp chamber of each tooth was rinsed four times with 25 μL of acetate buffer, and this solution was removed from the pulp chamber and placed into the same glass tube. Next, more deionized water (2.725 μL) was added to the glass tube along with 100 μL of 0.5 mg/mL of leucocrystal violet (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 50 μL of 1 mg/ mL enzyme horseradish peroxidase (Peroxidase Type

VI-A) (Sigma-Aldrich Chemie GmbH).

The absorbance at 596 nm of the resultant violet color in the tubes was measured in a Cary 50 UV- Vis spectrophotometer (Varian, Palo Alto, CA, USA). According to Beer's Law, absorbance is directly proportional to the concentration. Therefore, the concentration of HP (μg/mL) was determined by comparing it to the calibration curve previously obtained (Figure 1). The data related to HP concentration and mass were subjected to one-way analysis of variance (ANOVA) and Tukey's tests for pairwise comparisons (alpha = 0.05).

Results

The HP concentration that reached the pulp chamber in each group is depicted in Figure 2. One-way ANOVA revealed statistically significant differences among the groups ($p < 0.001$). No HP penetration was found in the negative control group in which no HP peroxide was applied. In-office tooth bleaching without desensitizing agents (positive control group) caused the highest HP diffusion when compared to all the desensitizing agents. The statistically significant and lowest HP penetration value was obtained in the Mi Paste and Nano-P groups in comparison with KF2% ($p < 0.001$).

Discussion

Several types of techniques for peroxide concentration have been used by authors, such as catalase bioassay,[23] spectrophotometric analysis,[24, 25] and electrochemical analysis.[26] However, most of these methods showed limitations, including a lack of sensitivity, the susceptibility to interference by other substances in samples, or long times of analysis.[27] Within this context, the method herein selected was the colorimetric method using the leucocrystal violet and horseradish peroxidase enzyme. This is a convenient, sensitive and selective method with high rates of accuracy.[28] Furthermore, this method has been used repeatedly to quantify hydrogen peroxide inside the pulp chamber of teeth after dental bleaching.[29-32]

The results of the present study confirmed the ability of hydrogen peroxide to penetrate the tooth structure and reach the pulp chamber in a single in-office session. This finding has been also observed by several studies using the same

methodology.[29, 31-34] However, we also observed that a lower amount of hydrogen peroxide was found when desensitizing agents were used prior to in-office dental bleaching.

The application of 5% potassium nitrate and 2% sodium fluoride before the in-office bleaching reduced the hydrogen peroxide concentration in the pulp chamber by almost half compared to the positive control group. These results suggested that the application of fluoride agents is a proven approach that strengthens the enamel structure, making it less susceptible to demineralization by the formation of stronger fluorapatite crystals and enhancing enamel remineralization.[35-37] For this, the use of fluorides could decrease the susceptibility of enamel to erosion promoted by bleaching treatments,[38, 39] which might be one of the hypotheses for the reduction in the penetration of hydrogen peroxide into the pulp chamber and the lower rates of bleaching-induced TS observed when this type of desensitizing agent was applied before in-office bleaching in clinical trials.[16, 40]

The potassium nitrate included in desensitizers, dentifrices, and whitening materials when applied to the external surface of the tooth can penetrate the enamel and dentin within 30 minutes and reach the pulp chamber,[41] exerting an important role on the reduction of bleaching-induced TS. This effect is probably attributed to its depolarizing effect of the dental nerve fibers and not by a blockage of hydrogen peroxide passage. The level of potassium nitrate penetration is influenced by concentration and may also be partly affected by the viscosity of the material as well as other constituents of proprietary preparations.[41]

Therefore, one may hypothesize that the reduction of HP within the pulp chamber in this group could be due to the resistance of the enamel caused by the presence of fluoride or by the physical presence of these desensitizing agents within the dental structure that may prevent the easy passage of hydrogen peroxide to the pulp chamber. Future studies need to be conducted to test this hypothesis.

Not surprisingly, in the CPP-ACP group, the level of the hydrogen peroxide concentration found in the pulp chamber was very low and similar to the negative control group, which was not submitted to any bleaching treatment. CPP-ACP compounds have been shown to be superior to fluoride for remineralization of

enamel.[42] In addition, the use of CPP-ACP associated with fluoride was more effective in increasing the acid resistance of primary teeth enamel than other fluoride varnishes.[43-45] An *in situ* remineralization study demonstrated that the addition of 2% CPP-ACP to the 1100 ppm-F dentifrice increased enamel subsurface remineralization by 156% relative to that produced by the 1100 ppm-F dentifrice alone.[46]

The explanation for this superior performance is based on the technology and composition of CPP-ACP materials. The CPP have been shown to stabilize calcium and phosphate, preserving them in an amorphous or soluble form termed as amorphous complexes.[47] CPP - which contains the active sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu—has a remarkable ability to stabilize calcium and phosphate as nanoclusters of ions[48] and ACPs exhibit a very high solubility and are readily converted to hydroxyapatite, which makes them suitable mineralizing agents.[49]

Furthermore, CCP-ACP could compensate for any loss of flexural strength of the enamel and dentine complex during bleaching.[50] In this context, we hypothesized that CPP-ACP can impose an important resistance in hard tissues to the penetration of the hydrogen peroxide to the pulp chamber and, consequently, reduce the TS observed in some clinical studies.[13, 18-20]

With respect to the nano-calcium phosphate paste, a lower amount of hydrogen peroxide was observed in the pulp chamber, which was statistically similar to the negative control group and CPP-ACP group. It is important to emphasize that the Nano-P paste used in this study is composed of nano-calcium phosphate (in the form of hydroxyapatite), 9000 ppm of fluoride, and 5% potassium nitrate. Therefore the reduction of the passage of hydrogen peroxide may be attributed to several factors working together. As mentioned before, fluoride can strengthen the enamel structure, making it less susceptible to demineralization and may be one of the reasons for the observed results. According the manufacturer´s details, the hydroxyapatite, by being nanometric in size, can penetrate more easily inside the dentin tubules and enamel microcracks, also producing their occlusion.[51]

Additionally, calcium phosphates serve as a source of calcium and phosphate ions that interact with the substrate, thus increasing fluoride incorporation during the

remineralization process.[52] This may explain the ability of the product to reduce the concentration of hydrogen peroxide in the pulp chamber.

Unfortunately, few studies in the literature have evaluated the effects of nano-calcium phosphate before dental bleaching, and those available reported conflicting results. Some authors demonstrated that the use of a nano-hydroxyapatite product in conjunction with dental bleaching did not reduce the risk and intensity of tooth sensitivity,[52,53] which goes against the findings of the present investigation. In contrast, another study found a reduction of risk of TS sensitivity during the bleaching treatment with 6% HP gel containing 2% nano-hydroxyapatite.[54] This controversy between the laboratory results and the clinical results highlights the need for further studies on this topic.

In summary, the desensitizing agents are able to reduce the amount of hydrogen peroxide that reaches the pulp chamber. However, Mi Paste and Nano-P were the products that yielded the highest reduction, similar to the control group that received no bleaching treatment.

References:

1. Haywood VB (1992) History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique *Quintessence Int* **23(7)** 471-488.
2. Dias Ribeiro AP, Sacono NT, Lessa FC, Nogueira I, Coldebella CR, Hebling J & de Souza Costa CA (2009) Cytotoxic effect of a 35% hydrogen peroxide bleaching gel on odontoblast-like MDPC-23 cells *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **108(3)** 458-464, 10.1016/j.tripleo.2009.05.006.
3. Lima AF, Ribeiro AP, Soares DG, Sacono NT, Hebling J & de Souza Costa CA (2013) Toxic effects of daily applications of 10% carbamide peroxide on odontoblast-like MDPC-23 cells *Acta Odontol Scand* **71(5)** 1319-1325, 10.3109/00016357.2012.762992.
4. Soares DG, Ribeiro AP, da Silveira Vargas F, Hebling J & de Souza Costa CA (2013) Efficacy and cytotoxicity of a bleaching gel after short application times on dental enamel *Clin Oral Investig* **17(8)** 1901-1909, 10.1007/s00784-012-0883-1.
5. Trindade FZ, Ribeiro AP, Sacono NT, Oliveira CF, Lessa FC, Hebling J & Costa CA (2009) Trans-enamel and trans-dentinal cytotoxic effects of a 35% H2O2 bleaching gel on cultured odontoblast cell lines after consecutive applications *Int Endod J* **42(6)** 516-524, 10.1111/j.1365-2591.2009.01544.x.

6. Bowles WH & Ugwuneri Z (1987) Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures *J Endod* **13(8)** 375-377, 10.1016/s0099-2399(87)80196-6.
7. Browning WD, Chan DC, Myers ML, Brackett WW, Brackett MG & Pashley DH (2008) Comparison of traditional and low sensitivity whiteners *Oper Dent* **33(4)** 379-385, 10.2341/07-134.
8. Cooper JS, Bokmeyer TJ & Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents *J Endod* **18(7)** 315-317, 10.1016/s0099-2399(06)80479-6.
9. Basting RT, Amaral FL, Franca FM & Florio FM (2012) Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents *Oper Dent* **37(5)** 464-473, 10.2341/11-337-c.
10. Martin J, Fernandez E, Bahamondes V, Werner A, Elphick K, Oliveira OB, Jr. & Moncada G (2013) Dentin hypersensitivity after teeth bleaching with in-office systems. Randomized clinical trial *Am J Dent* **26(1)** 10-14.
11. Tay LY, Kose C, Herrera DR, Reis A & Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: a 2-year double-blind randomized clinical trial *Am J Dent* **25(4)** 199-204.
12. Bonafe E, Loguercio AD, Reis A & Kossatz S (2014) Effectiveness of a desensitizing agent before in-office tooth bleaching in restored teeth *Clin Oral Investig* **18(3)** 839-845, 10.1007/s00784-013-1055-7.
13. Borges BC, Pinheiro MH, Feitosa DA, Correia TC, Braz R, Montes MA & Pinheiro IV (2012) Preliminary study of a novel in-office bleaching therapy modified with a casein phosphopeptide-amorphous calcium phosphate *Microsc Res Tech* **75(11)** 1571-1575, 10.1002/jemt.22102.
14. Navarra CO, Reda B, Diolosa M, Casula I, Di Lenarda R, Breschi L & Cadenaro M (2014) The effects of two 10% carbamide peroxide nightguard bleaching agents, with and without desensitizer, on enamel and sensitivity: an in vivo study *Int J Dent Hyg* **12(2)** 115-120, 10.1111/idh.12054.
15. Pale M, Mayoral JR, Llopis J, Valles M, Basilio J & Roig M (2014) Evaluation of the effectiveness of an in-office bleaching system and the effect of potassium nitrate as a desensitizing agent *Odontology* **102(2)** 203-210, 10.1007/s10266-013-0132-3.
16. Tay LY, Kose C, Loguercio AD & Reis A (2009) Assessing the effect of a desensitizing agent used before in-office tooth bleaching *J Am Dent Assoc* **140(10)** 1245-1251.
17. Mehta D, Venkata S, Naganath M, LingaReddy U, Ishihata H & Finger WJ (2013) Clinical trial of tooth desensitization prior to in-office bleaching *Eur J Oral Sci* **121(5)** 477-481, 10.1111/eos.12067.
18. Giniger M, Macdonald J, Ziemba S & Felix H (2005) The clinical performance of professionally dispensed bleaching gel with added amorphous calcium phosphate *J Am Dent Assoc* **136(3)** 383-392.
19. Giniger M, Spaid M, MacDonald J & Felix H (2005) A 180-day clinical investigation of the tooth whitening efficacy of a bleaching gel with added amorphous calcium phosphate *J Clin Dent* **16(1)** 11-16.
20. Grobler SR, Majeed A, Moola MH, Rossouw RJ & van Wyk Kotze T (2011) In vivo Spectrophotometric Assessment of the Tooth Whitening Effectiveness of Nite White 10% with Amorphous Calcium Phosphate, Potassium Nitrate and Fluoride, Over a 6-month Period *Open Dent J* **5** 18-23, 10.2174/1874210601105010018.

21. Haywood VB, Caughman WF, Frazier KB & Myers ML (2001) Tray delivery of potassium nitrate-fluoride to reduce bleaching sensitivity *Quintessence Int* **32(2)** 105-109.
22. Mendham J & Afonso JC (2002) *Vogel: Quantitative chemical analysis.* Livros Tecnicos e Científicos Editora S.A. Rio de Janeiro.
23. Danish (2009) Development of an Analysis Method to Determine Peroxides in or Released From Teeth- Whitening/Dental-Bleaching Products Environmental Protection Agency.
24. Rao MS, Mohan Rao AR, Ramana KV & Sagi SR (1991) Thallimetric oxidations-VI Titrimetric and spectrophotometric methods for the determination of phosphite and analysis of binary mixtures of phosphite and oxalate *Talanta* **38(8)** 937-940.
25. da Silva Marques DN, Silveira JM, Marques JR, Amaral JA, Guilherme NM & da Mata AD (2012) Kinetic release of hydrogen peroxide from different whitening products *Eur J Esthet Dent* **7(3)** 344-352.
26. Campuzano S, Pedrero M & Pingarron JM (2005) A peroxidase-tetrathiafulvalene biosensor based on self-assembled monolayer modified Au electrodes for the flow-injection determination of hydrogen peroxide *Talanta* **66(5)** 1310-1319, 10.1016/j.talanta.2005.01.048.
27. Nakabayashi Y & Yoshikawa H (2000) Amperometric Biosensors for Sensing of Hydrogen Peroxide Based on Electron Transfer between Horseradish Peroxidase and Ferrocene as a Mediator *Analytical Sciences* **16(6)** 609-613, 10.2116/analsci.16.609.
28. Mottola HA, Simpson BE & Gorin G (1970) Absorptiometric determination of hydrogen peroxide in submicrogram amounts with leuco crystal violet and peroxidase as catalyst *Analytical Chemistry* **42(3)** 410-411, 10.1021/ac60285a017.
29. Briso AL, Lima AP, Goncalves RS, Gallinari MO & dos Santos PH (2014) Transenamel and transdentinal penetration of hydrogen peroxide applied to cracked or microabrasioned enamel *Oper Dent* **39(2)** 166-173, 10.2341/13-014-l.
30. Camargo SE, Cardoso PE, Valera MC, de Araujo MA & Kojima AN (2009) Penetration of 35% hydrogen peroxide into the pulp chamber in bovine teeth after LED or Nd:YAG laser activation *Eur J Esthet Dent* **4(1)** 82-88.
31. Parreiras S, Mena-Serrano A, Moreira CG, Otuki M, Loguercio D & Reis A (2014) Penetration and cytotoxicity of a bleaching gel activated by LED/laser in restored teeth *Am J Dent* **27(6)** 301-306.
32. Mena-Serrano AP, Parreiras SO, do Nascimento EM, Borges CP, Berger SB, Loguercio AD & Reis A (2015) Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber *Oper Dent* **40(2)** E76-82, 10.2341/13-352-l.
33. de Almeida LC, Soares DG, Azevedo FA, Gallinari Mde O, Costa CA, dos Santos PH & Briso AL (2015) At-Home Bleaching: Color Alteration, Hydrogen Peroxide Diffusion and Cytotoxicity *Braz Dent J* **26(4)** 378-383, 10.1590/0103-6440201300380.
34. Goncalves RS, Costa CA, Soares DG, dos Santos PH, Cintra LT & Briso AL (2016) Effect of Different Light Sources and Enamel Preconditioning on Color Change, H2O2 Penetration, and Cytotoxicity in Bleached Teeth *Oper Dent* **41(1)** 83-92, 10.2341/14-364-l.
35. Featherstone JD, Glena R, Shariati M & Shields CP (1990) Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration *J Dent Res* **69 Spec No** 620-625; discussion 634-626.

36. Santos Lde M, Reis JI, Medeiros MP, Ramos SM & Araujo JM (2009) In vitro evaluation of fluoride products in the development of carious lesions in deciduous teeth *Braz Oral Res* **23(3)** 296-301.
37. Chu CH, Mei ML & Lo EC (2010) Use of fluorides in dental caries management *Gen Dent* **58(1)** 37-43; quiz 44-35, 79-80.
38. Jiang T, Ma X, Wang Y, Tong H, Shen X, Hu Y & Hu J (2008) Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering and laser-induced fluorescence *J Biomed Opt* **13(1)** 014019, 10.1117/1.2870114.
39. Ushigome T, Takemoto S, Hattori M, Yoshinari M, Kawada E & Oda Y (2009) Influence of peroxide treatment on bovine enamel surface--cross-sectional analysis *Dent Mater J* **28(3)** 315-323.
40. Wang Z, Ma X, Jiang T, Wang Y, Feng Y & Li R (2015) The dentin tubule occlusion effects of desensitizing agents and the stability against acids and brushing challenges *Am J Dent* **28(3)** 128-132.
41. Kwon SR, Dawson DV, Schenck DM, Fiegel J & Wertz PW (2015) Spectrophotometric Evaluation of Potassium Nitrate Penetration Into the Pulp Cavity *Oper Dent* **40(6)** 614-621, 10.2341/14-214-l.
42. Hay KD & Thomson WM (2002) A clinical trial of the anticaries efficacy of casein derivatives complexed with calcium phosphate in patients with salivary gland dysfunction *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **93(3)** 271-275.
43. Tuloglu N, Bayrak S, Tunc ES & Ozer F (2016) Effect of fluoride varnish with added casein phosphopeptide-amorphous calcium phosphate on the acid resistance of the primary enamel *BMC Oral Health* **16(1)** 103, 10.1186/s12903-016-0299-4.
44. Cochrane NJ, Shen P, Yuan Y & Reynolds EC (2014) Ion release from calcium and fluoride containing dental varnishes *Aust Dent J* **59(1)** 100-105, 10.1111/adj.12144.
45. Schemehorn BR, Wood GD, McHale W & Winston AE (2011) Comparison of fluoride uptake into tooth enamel from two fluoride varnishes containing different calcium phosphate sources *J Clin Dent* **22(2)** 51-54.
46. Reynolds EC (2009) Casein phosphopeptide-amorphous calcium phosphate: the scientific evidence *Adv Dent Res* **21(1)** 25-29, 10.1177/0895937409335619.
47. Cross KJ, Huq NL, Stanton DP, Sum M & Reynolds EC (2004) NMR studies of a novel calcium, phosphate and fluoride delivery vehicle-alpha(S1)-casein(59-79) by stabilized amorphous calcium fluoride phosphate nanocomplexes *Biomaterials* **25(20)** 5061-5069, 10.1016/j.biomaterials.2004.01.045.
48. Cochrane NJ, Saranathan S, Cai F, Cross KJ & Reynolds EC (2008) Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride *Caries Res* **42(2)** 88-97, 10.1159/000113161.
49. Gurunathan D, Somasundaram S & Kumar S (2012) Casein phosphopeptide-amorphous calcium phosphate: a remineralizing agent of enamel *Aust Dent J* **57(4)** 404-408, 10.1111/adj.12006.
50. Khoroushi M, Mazaheri H & Manoochehri A (2011) Effect of CPP-ACP application on flexural strength of bleached enamel and dentin complex *Oper Dent* **36(4)** 372-379, 10.2341/10-280-l.
51. Meier M, Cardoso V, Mittelstadt FG, Santos R, Odebrecht CM, Ferri L & Lippmann B (2010) Nanostructured Calcium Phosphate (Nano P) Technology: Innovation on Dental Desensitisation and Remineralization *J Dent Res* **4584(89 (Spec Iss B))**.
52. Loguercio AD, Tay LY, Herrera DR, Bauer J & Reis A (2015) Effectiveness of nano-calcium phosphate paste on sensitivity during and after bleaching: a

randomized clinical trial *Braz Oral Res* **29** 1-7, 10.1590/1807-3107BOR-2015.vol29.0099.
53. Browning WD, Cho SD & Deschepper EJ (2012) Effect of a nano-hydroxyapatite paste on bleaching-related tooth sensitivity *J Esthet Restor Dent* **24(4)** 268-276, 10.1111/j.1708-8240.2011.00437.x.
54. Vano M, Derchi G, Barone A, Genovesi A & Covani U (2015) Tooth bleaching with hydrogen peroxide and nano-hydroxyapatite: a 9-month follow-up randomized clinical trial *Int J Dent Hyg* **13(4)** 301-307, 10.1111/idh.12123.

TABLES

Table 1 – Data points for the calibration curve

H_2O_2 Data For Each Point		**Solutions required to obtain 3000μL for Each Point for the Calibration Line**				
H_2O_2 Weight, μg	H_2O_2 Concentration, μg/mL	Acetate Buffer Solution, μL	47.67 μg/mL H_2O_2 Solution, μL	Peroxidase, μL	Leuco-crystal violet, μL	Deionized Wate μL
1.192	0.397	75	25	50	100	2750
0.953	0.318	80	20	50	100	2750
0.715	0.238	85	15	50	100	2750
0.477	0.159	90	10	50	100	2750
0.381	0.127	92	8	50	100	2750
0.191	0.064	96	4	50	100	2750
0.095	0.032	98	2	50	100	2750
0.000	0.000	100	0	50	100	2750

Table 2. Batch number, composition and mode of application of the products used in this study

Material [manufacturers/batch number]	***Composition***	***Mode of Application***
Dessensibilize KF2% [FGM, Brazil/09.09.2014]	Potassium nitrate 5% and sodium fluoride 2%, deionised water, glicerin, and thickeners.	A single 10-min application
Mi Paste [GC Corporation, Japan/13.07.2008]	Casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP).	A single 5-min application
Nano-P [FGM, Brazil/09.09.2014]	9000 ppm fluoride sodium, 5% potassium nitrate, and nano-calcium phosphate.	A single 5-min application
Whiteness HP Maxx [FGM, Brazil/20.08.2014]	35% HP, thickeners, dye mixture, glycol, inorganic load, and deionized water.	3 application of 15 min each

FIGURES

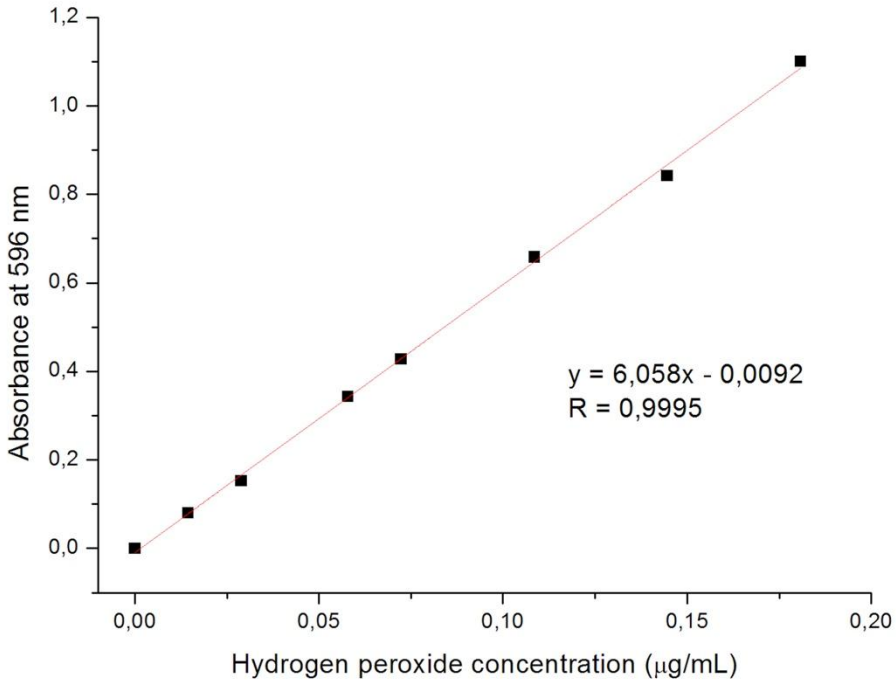


Figure 1. Spectrophotometric calibration curve used in this study, R = 0.9995

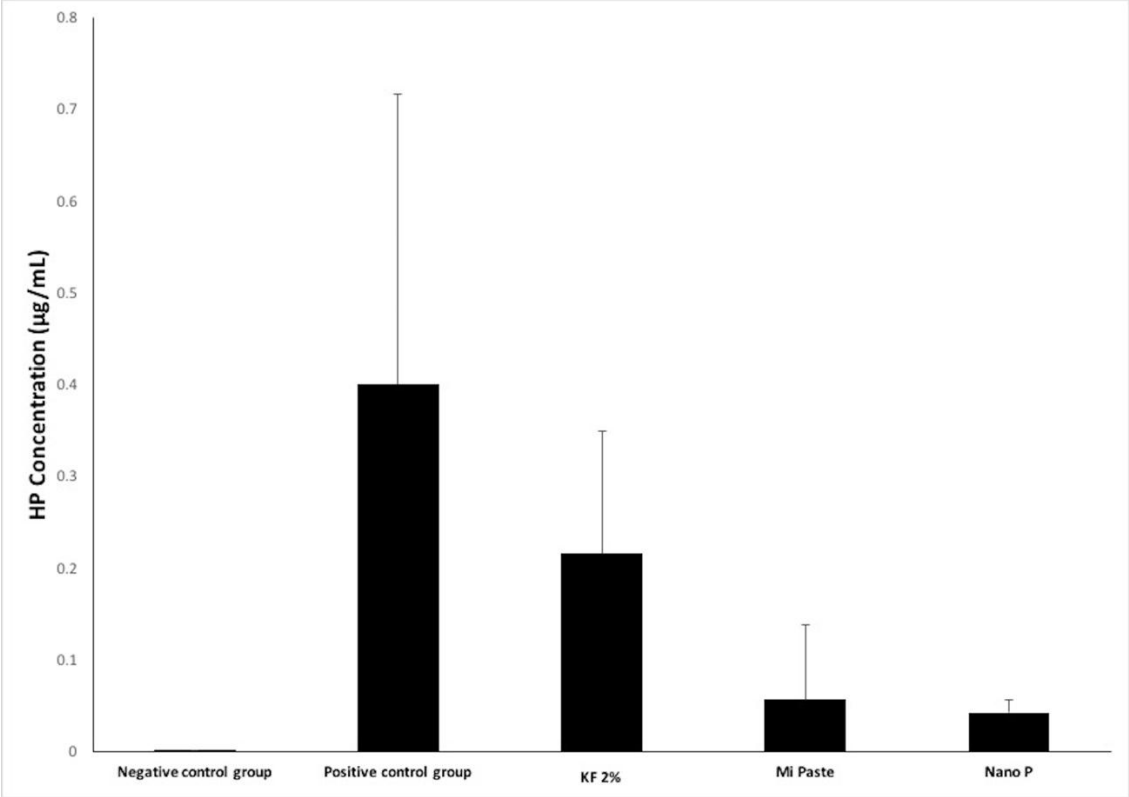


Figure 2. Concentration of hydrogen peroxide found in the pulp chamber

ARTIGO 3

TÍTULO: Effect of preliminary application of glutaraldehyde/potassium nitrate gel on reduction of bleaching-induced tooth sensitivity: a triple-blind randomized clinical trial

STATUS: submetido para avaliação

REVISTA: Journal of American Dental Association

TITLE PAGE

Effect of preliminary application of glutaraldehyde/potassium nitrate gel on reduction of bleaching-induced tooth sensitivity: a triple-blind randomized clinical trial

Short title: Effect of an experimental dessensitizing agent on bleaching-induced tooth sensitivity

Sibelli Olivieri Parreiras[1] [DDS, MSc, PhD student]; Anna Luiza Szesz[1] [DDS, MSc, PhD student]; Fabiana Madalozzo Coppla[1] [DDS, MSc, PhD student]; Eveline Claudia Martini[1] [DDS, MSc, PhD student]; Paulo Vitor Farago[2] [MS, PhD]; Alessandro Loguercio[3] [DDS, MSc, PhD]; Alessandra Reis[3] [DDS, PhD]

[1] PhD student. School of Dentistry. Department of Restorative Dentistry. State University of Ponta Grossa, PR, Brazil.

[2] Postgraduate Program in Pharmaceutical Sciences, Department of Pharmaceutical Sciences, State University of Ponta Grossa, Brazil.

[3] Associate Professors. School of Dentistry. Department of Restorative Dentistry. State University of Ponta Grossa, PR, Brazil.

Corresponding author:

Professora Alessandra Reis. Universidade Estadual de Ponta Grossa, Rua Carlos Cavalcanti, 4748, Bloco M, Sala 64A– Uvaranas, Ponta Grossa, Paraná – CEP 84030–900, Brazil; e- mail: reis_ale@hotmail.com

Abstract:

Objectives: This randomized study, split-mouth, triple-blind evaluated the efficacy of a desensitizing gel based 5% potasium nitrate and 5% glutaraldehyde applied before in-office bleaching with 35% hydrogen peroxide (HP). Methods: We selected 42 patients who received both treatments: the desensitizing gel and the placebo gel (control). Before tooth whitening with HP (3 applications of 15 min each), the desensitizing gels were applied and maintaned in contact with the tooth enamel for 10 min. Two bleaching sessions with a one-week interval between them were performed. Pain intensity was assessed using a numerical scale (NRS) and visual analogue scale (VAS). The color was evaluated by digital spectrophotometer (VITA) and the value-oriented shade guide VITA Classical and VITA Bleachedguide 3D-MASTER (VITAPAN, VITA). The risk of tooth sensitivity was evaluated by the McNemar test and the color change by paired Student t test (α = 5%). Results: The color change on both sides of the arch was statistically similar ($p > 0.05$). The risk of tooth sensitivity of the desensitizing gel group [31.7% (95% CI 19.6 – 46.9)] and the control group [70.7 (95% CI 55.5 – 82.3%) was statistically significant ($p < 0.0001$) as well as the intensitiy of pain in the first 24 hours ($p < 0.001$).

Conclusions: It was concluded that the prior application of desensitizing gel based on 5% potassium nitrate and 5% glutaraldehyde before whitening, reduced the risk and severity of dental sensitivity, without altering the effectiveness of whitening.

Keywords: Tooth bleaching. Dentin desensitizing agents. Randomized clinical trial.

Introduction

Bleaching-induced tooth sensitivity (TS) is the most common side clinical effect of tooth bleaching procedure. There is a wide range in the reported risk of tooth sensitivity, however the highest risk is usually seen for in-office tooth bleaching (reported range between 60 – 98%)[1-7]. This could be explained by the higher concentration of hydrogen peroxide in the in-office bleaching agent.

Although pain and discomfort caused by bleaching-induced TS is generally mild and transient, it can occasionally be severe and irritating, leading to patient´s withdrawal from the bleaching treatment. Investigators have hypothesized that bleaching-induced TS has been associated with the passage of hydrogen peroxide, through enamel and dentin, to the pulp where it produces an inflammatory reaction[8-9] and it may also directly activate nerves that cause pain[10-11].

Some attempts have been employed to prevent this side effect. The administration of selective anti-inflammatory drugs[3], ibuprofen[2,12], ascorbic acid[13] and dexamethasone[14] was not capable to reduce the risk and intensity of bleaching-induced TS[15].

Topical application of potassium nitrate and fluoride as desensitizers has shown promising results for at-home and in-office bleaching[6]. The previous application of a gel composed of 5% potassium nitrate and 2% sodium fluoride for 10 min reduced the risk of in-office bleaching-induced TS by half as well as the intensity of TS in a clinical trial[1]. Potassium nitrate (KNO_3) is the primary agent for at-home desensitizing toothpastes that works by preventing nerve transmission that leads to sensations of pain[16].

Additionally, two clinical trial showed that the previous desensitization with Gluma desensitizer liquid or gel (Heraeus Kulzer, Hanau, Germany), composed of 5 wt% glutaraldehyde and 35% weight% HEMA[17-18], significantly reduced in-office bleaching-induced TS during and after whitening compared with a placebo pre-treatment [4,19]. Glutaraldehyde, have been added to some desensitizing gels[20] and adhesives systems[21] in order to reduce TS. Glutaraldehyde is a low molecular weight aldehyde and is also used as a cross-linking material[22], an ingredient used in cosmetic and toiletry industries and in chemical specialty products. Additionally, it is an effective fixative or flocculating agent and forms a physiological seal by coagulating the plasma proteins within the dentinal tubules[23].

Perhaps the formulation of a desensitizing gel composed of two active agents could reduce even more the risk of bleaching-induced TS. To the best of our knowledge, there is no report about the effect of a desensitizing gel composed of potassium nitrate and glutaraldehyde. Considering the high risk of bleaching-induced

TS reported by patients submitted to in-office bleaching[7], the aim of this randomized clinical study was to evaluate the risk and intensity of bleaching-induced TS after in-office bleaching following topical application of an experimental desensitizing gel containing potassium nitrate and glutaraldehyde.

Material and Methods

Study design and protocol registration

This was a randomized, split-mouth and placebo-controlled clinical trial with an equal allocation ratio. The clinical investigation was approved (protocol number 843.554) by the scientific review committee and by the committee for the protection of human participants of the local university.

This research protocol was registered in the Brazilian clinical trials registry under the identification number RBR-7B7CMN. We prepared this article using the protocol established by the Consolidated Standards of Reporting Trials statement[24]. Two weeks before the bleaching procedures, all volunteer participants received a dental prophylaxis with pumice and water in a rubber cup and signed an informed consent form.

Eligibility criteria

Participants included in the clinical trial were at least 18 years old, had good general and oral health, and did not report any type of tooth sensitivity (TS). The participants were required to have six caries-free maxillary anterior teeth without restorations. The participants should be free of periodontal disease, and be willing to review and sign the informed consent form. The canine tooth had to be shade A2 or darker as judged by comparison with a value-oriented shade guide (VITA Classical, VITA Zahnfabrik). Two calibrated investigators performed the color evaluations with the shade guide, independently. The two examiners were required to have an agreement of at least 85% (k statistic) before beginning the study evaluation.

Participants with anterior restorations or dental prosthesis, with orthodontics apparatus, with severe internal tooth discoloration (tetracycline stains, fluorosis, pulpless teeth) were not included in the study. In addition, pregnant and lactating

women, participants with any other pathology that could cause sensitivity (such as recession, dentinal exposure, visible cracks in teeth), taking anti-inflammatory or analgesic drugs, smoking, bruxing, or participants who had undergonetooth-whitening procedures were excluded.

Settings and locations

Based on pre-established criteria, we selected 42 volunteers for this study. The study was performed from November 13, 2014, to December 20, 2015, in the School of Dentistry from the local University.

Sample size calculation

The primary outcome of this study was the absolute risk of bleaching-induced tooth senstivity (TS). The absolute risk of TS (that is, the number of patients [percent] who reported pain at some period during dental bleaching) was reported to be approximately 87%[1,3] for the bleaching product Whiteness HP Maxx (FGM Dental Products, SC, Brazil). Thus, a minimum sample size of 42 participants was required to have a 80% chance of detecting, as significant at the 2-sided 5% level, a decrease in the primary outcome measure from 86% in the control group to 50% in the experimental group.

Random sequence generation and allocation concealment

We used a simple randomization scheme. The random sequence was generated by a software freely available online (www.sealed.envelope.com) by an investigator who was not involved in the implementation of the study. The identification of the group to be implemented in the right side of the patient's upper arch were kept concealed until implementation in opaque, sealed and consecutively numbered envelopes, which were only opened by the operator immediately after the beggining of the intervention. The treatment to be implemented in the other side of the patient's arch was the group not revealed in the envelope.

Blinding

This was a triple-masked clinical trial, in which the patient, operator, and evaluator were masked to the group assignment. A third researcher, not involved in

the evaluation process, was responsible for the randomization process, delivery and guidance on the administration of the gels.

The desensitizing gel was synthesized by adding 5% potassium nitrate (Merck, São Paulo, SP Brazil) and 5% glutaraldehyde (Vetec, Rio de Janeiro, RJ, Brazil) in hydroxyethylcelulose gel. The placebo had the same formulation, except the desensitizing agents. The dessensitizing gels were transparent and identical in consistency. They were placed in identical syringes. The syringes were marked only with numbered codes so that neither the operators nor the patients could identify them. All procedures were done in the Pharmaceutical Laboratory of the local University.

Study intervention

All patients received the same bleaching treatment. Two operators performed the bleaching protocol in all patients. The clinicians isolated the gingival tissue of the teeth to be bleached using a light-cured resin dam (Top Dam, FGM Dental Products), and each tooth was light-cured for 10 s (Radii-cal, SDI). Additionally, the light-cured gengival barrier was placed between the central incisors in order to prevent the contact between the different desensitizing gels.

After placement of a lip retractor (Arcflex, FGM Dental Products), the operators placed the experimental gel composed by 5% glutaraldehyde and 5% potassium nitrate (Local University, Pharmaceutical Laboratory) in one of the sides of the maxillary upper arch of the patient while the other side received the placebo gel. The side of the product application was defined by the randomization scheme.

The desensitizing or placebo gel was left undisturbed for 10 min. Then, the gel was slightly agitated on each dental surface with a rubber cup mounted in a slow-speed handpiece for 10 s. The gels were removed with an aspirator tip and water rinsing and both sides of the maxillary arch of the patient's were bleached with a 35% hydrogen peroxide gel (Whiteness HP Maxx, FGM) in three 15-min applications in accordance with the manufacturer's directions. The researcher refreshed the in-office bleaching agent every 15 min during the 45-min application period. Two bleaching sessions were performed 1 week apart. After the bleaching therapy and data analysis for this study, at-home was performed in the inferior arch for 14 days with 16%

carbamide peroxide.

Bleaching-induced tooth sensitivity (TS) evaluation

The researcher evaluated the TS during bleaching, and up to 1 h, 24 h, and 48 h post-bleaching. The patients were asked to indicate the numeric value that best described their degree of TS in each one of the periods described above using a 5-point numeric rating scale (NRS) in which 0 = none,1 = mild, 2 = moderate, 3 = considerable, and 4 = severe[1,2,25-27] or a visual analog scale (VAS)[2-4,28,29].

The VAS scale is a 10-centimeter horizontal line with scores of 0 and 10 at their ends, in which 0 = no sensitivity, and 10 = severe sensitivity. The patient should mark with a vertical line across the horizontal line of the scale the region that best representes the TS intensity that he had during each time assessment. Then, the distance in millimeters from the zero end was measured with the aid of a millimeter ruler.

We merged the data from both bleaching sessions. The worst score (NRS scale) or the highest numeric value (VAS scale) obtained from both bleaching sessions at each assessment point was taken for statistical purposes and for the determination of the overall risk and intensity of bleaching-induced TS.

If the participant scored 0 (no sensitivity) in all time assessments from both bleaching sessions, this participant was considered to be insensitive to the bleaching protocol. In all other circumstances, the participants were considered to have bleaching-induced TS. This dichotomization allowed us to calculate the absolute risk of TS, which represented the percentage of patients who reported TS at least once during treatment. We also calculated the overall TS intensity for each time assessment.

Color evaluation

We recorded color at baseline, 1 week after the first bleaching session, 1 week after the second bleaching, and 1 month after the end of the bleaching treatment. We never performed color evaluation immediately after each bleaching session so that the effect of dehydration and demineralization on color measures never influenced the color evaluation. We performed color evaluation using the shade guides VITA

Classical and the VITA Bleachedguide 3D-MASTER. In addition, we performed an objective color evaluation with the spectrophotometer VITA Easyshade (VITA Zahnfabrik).

For the subjective examination, we arranged the VITA Classical shade guide's 16 tabs from highest (B1) to lowest (C4) value. Although this scale is not linear in the truest sense, we treated the changes as representing a continuous and approximately linear ranking for the purpose of analysis, as already performed in several published studies[4,13,25-28,30-32]. The VITA Bleachedguide 3D-MASTER contains lighter shade tabs and it is already organized from highest (0M1) to lowest (5M3) value[14,31,33]. The measurement area of interest for shade matching was the middle one-third of the facial surface of the canine.

Two examiners, masked to the allocation assignment, scheduled the participants for bleaching and evaluated their teeth against the shade guide at the different time assessments. Color changes were calculated from the beginning of the active phase up to and including the individual recall times by calculating the change in the number of shade guide units (ΔSGU for both scales), which occurred toward the lighter end of the value-oriented list of shade tabs. In the event of disagreements between the examiners during shade evaluation, a consensus was reached through discussion.

For the objective examination, before the spectrophotometer measurement, the examiner took an impression of the maxillary arch with a dense silicone paste (Coltoflax and Perfil Cub Kit, Vigodent). The impression was extended to the maxillary canine and served as a standard color measurement guide for the spectrophotometer. For each dental component to be evaluated, we created a window on the labial surface of the molded silicone guide using a metal device with a radius of 6 millimeters and well-formed borders[25].

We determined the shade using the parameters of the spectrophotometer on which these values were indicated: L*, a*, and b*, in which L* represents the luminosity (value from 0 [black] to 100 [white]), and a* and b* represent the color shade, in which a* is the measurement along the red-green axis and b* is the measurement along the yellow-blue axis, respectively. The color comparison before

and after treatment is given by differences between the 2 colors, measured with the spectrophotometer (ΔE), which is calculated using the formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. [34]

Statistical analysis

We performed the analysis following the intention-to-treat protocol, and we involved all participants who were randomly assigned[24]. In cases of missing data, the last observation was carried forward. The statistician was masked to the study groups. We compared the absolute risk of TS for both groups using the McNemar's test (α = 0.05). We calculated the relative risk, the confidence interval for the effect size and the number needed to treat (NNT).

We performed the comparison of the bleaching-induced TS intensity (NRS data) of the 2 groups at each assessment points using the Wilcoxon Signed Rank Test. For the TS intensity obtained in the VAS scale we also employed the Wilcoxon Signed Rank Test, as data did not show a normal distribution.

We compared the color changes between groups (ΔSGU for both scales and ΔE between baseline vs. 1 month post-bleaching) using Student t-test. In all statistical tests, the significance level was 0.05. We performed all analyses by using the software SigmaPlot version 11.0 (Systat Software).

Results

Characteristics of included participants

A total of 76 participants were examined in a dental chair to check if they meet the inclusion and exclusion criteria (Fig. 1). The baseline color of the participants was similar (placebo: 7.8 ± 2.2; experimental: 7.9 ± 2.2) and the mean age (years) between participants of this study were 22.1 ± 5.8 ranging from 18 to 52 years.

Adherence to the protocol and drop-outs

One patient discontinued intervention in this clinical investigation, because she decided to travel during treatment. It was performed only the first bleaching session. All participants attended the recall visits one-month post-bleaching. For these

participants, the last observation was carried forward for statistical purposes to keep the intention-to-treat analysis[24] Figure 1 depicts the participant flow diagram in the different phases of the study design.

Tooth sensitivity

In regard to the absolute risk of TS, significant difference was observed between groups as seen in Table 1 ($p < 0.0001$). In regard to TS intensity (Tables 2 and 3), the groups differed statistically under the two pain scales used in this study during bleaching and up to 1 h ($p < 0.008$). It is also clear that bleaching-induced TS did not last longer than 24 h after the bleaching protocol ($p > 0.05$; Tables 2 and 3).

Color evaluation

Significant whitening was observed in both study groups under the subjective and objective evaluation methods ($p < 0.005$). At the end of the bleaching protocol, a whitening of 4.7 to 6.0 shade guide units (for both scales) was detected for both groups and the ΔE varied by 5.6 to 5.9 units (Table 4). The results of the subjective (Vita Classical: $p = 0.77$; Vita Bleachedguide: $p = 0.16$) and the objective evaluation with the spectrophotometer: $p = 0.28$) matched the hypothesis of equality between the groups after bleaching. The effect size and the confidence interval for the overall mean difference is also presented in Table 4 and is evidence of no statistical difference between groups.

Adverse effects

Only in two cases from the desensitizing group that the gel inadvertly touched the patients's gingival tissue and this was consider an adverse effect related to the desensitizer group.

Discussion

The present clinical trial showed that pre-bleaching desensitization with an desensitizing gel containing glutaraldehyde and 5% potassium nitrate, in a single formulation, for 10 min significantly reduced bleaching-induced TS compared to teeth submitted to a placebo treatment.

As reported in the introduction section, the benefits of a glutaraldehyde product for reduction of bleaching-induced TS was reported in two clinical studies that performed in-office bleaching with 38 – 40% hydrogen peroxide[4,19]. Similarly, the effect of potassium nitrate for reduction of bleaching-induced TS was also reported in other clinical trials[1,6,35].

In these studies, the effects of the potassium nitrate and glutaraldehyde were evaluated isolated differently from the present study. In the study of Tay et al. the risk of bleaching-induced TS in the group of participants that received potassium nitrate desensitizing gel was 46.7%, while in the present study the risk of the experimental group was 31.7%, which represents a reduction of approximately 32%. This suggests that perhaps the association of these two agents showed a synergistic effect in reducing the undesirable side effect of TS by working in two different mechanisms for pain reduction.

One of the active compounds of the desensitizing gel tested – glutaraldehyde – might have reduced TS by cross-linking with enamel matrix proteins (amelogenin, enamelin, and ameloblastin) and with proteins in the dentin tubular liquid, thus reducing easy passage of the peroxide radicals[4]. However, this is a hypothesis yet to be confirmed by basic studies. The mechanism of action of potassium nitrate remains unknown, but it seems to work by reducing the dentinal sensory nerve activity due to the depolarizing activity of the k+ ion[36-37]. In other words, on the application of the gel, potassium ions flow through the networks of dentinal tubules and the odontoblastic processes into the pulp. It results in an increased external concentration of K ions around the nerves and interruptions of neuron functions. This is caused by the actual bathing of nerve tissue with a high grade of potassium because of which the nerve enters an absolute refractory period allowing no stimulus to excite the nerve and thus anesthetizes the tooth.

The preliminary effect of the desensitising gel based on glutaraldehyde (molar mass 100 g mol^{-1}) and potassium nitrate (molar mass 101 g mol^{-1}) can be possible due to the low molecular size of the active compound so that they might penetrate through enamel and dentin along the same pathway as the peroxide radicals[4,38].

We have selected the concentration of 5% for potassium nitrate based on the

fact that the American Dental Association Council on Dental Therapeutics has granted the Seal of Acceptance to dentifrices containing 5% potassium nitrate[39]. Adittionaly, previous studies that employed 5% potassium nitrate as desensitizing agents showed effective results when compared to a placebo gel[1,6,40].

One of the concerns that may arise about the desensitizing gel is the likely cytotoxicity of glutaraldehyde, which is highly discussed in the literature. Nevertheless, the cytotoxicity of glutaraldehyde is dependent on the concentration used, and up to 8% glutaraldehyde was shown to be non-cytotoxic[41]. In an in vitro study, it was observed that MDPC-23 cells maintained their viability when 2.5% and 5% glutaraldehyde solutions were applied on the etched dentin. At 10%, glutaraldehyde slightly reduced MDPC-23 viability; however, no detrimental effect was seen for total protein synthesis, alkaline phosphatase activity and deposition of mineralized nodules[42]. Additionally, in the present study, the glutaraldehyde-containing desensitizing gel was applied on enamel, not in dentin, which may decrease even further the likely cytotoxicity of glutaraldehyde on the pulp chamber. So, it is plausible to conclude that the glutaraldehyde at 5% concentration used in this study might be safe when applied on dental surfaces before tooth bleaching.

We have not added fluoride in the experimental desensitizing gel because the mechanism of fluoride to prevent dentin sensitivity is based on dentin tubule oclusion, which reduces the fluid flow movement and prevents the stimulation of nerve cells [36-37]. As teeth submitted to dental bleaching are not supposed to have exposed dentin, fluoride would not pose an important role on the reduction of bleaching-induced TS.

However, as for the hydrogen peroxide, we should avoid contact of the glutaraldehyde-containing desensitizer gel with soft tissues. We observed a slight clinical burn of the marginal gingival tissues when the desensitizing gel inadvertily touched them. For this purpose, the placement of a gingival barrier before application of the experimental gel is highly recommended and agitation of the gel should be done manually or under low speed.

With respect to color change, the results of this study indicate that pre-bleaching application of the desensitizing agent with active compounds did not jeopardize the whitening effect. An equivalent and significant tooth color

enhancement was observed in both study groups. The degree of whitening obtained in this study is similar to that reported in other clinical trials[4-5,7,30] and also in a recent literature review[6].

It worth to mentioning that in the present investigation color changes were evaluated in the canines instead of the incisors, as commonly done in bleaching studies. This procedure was adopted, mainly because the color change was not a primary outcome in the present study. However, this procedure was already done in other studies in the literature[43-45]. A significantly stronger overall increase in whitening was observed in canines than in incisors[43,45] which was 1.4 to 1.6 times more pronounced than incisors, indicating that the use of canines instead of incisors is an interesting option for bleaching studies.

Finally, the limitations of this study should be reported. Most of the participants in this study were young adults with teeth of a lighter color at baseline, which affects the generalizability of the findings of the investigation to the overall population.

Conclusion

We should conclude that the experimental desensitizing gel composed of 5% potassium nitrate and 5% glutaraldehyde was effective to reduce the risk and intensity of bleaching-induced TS without jeopardizing the whitening results.

References:

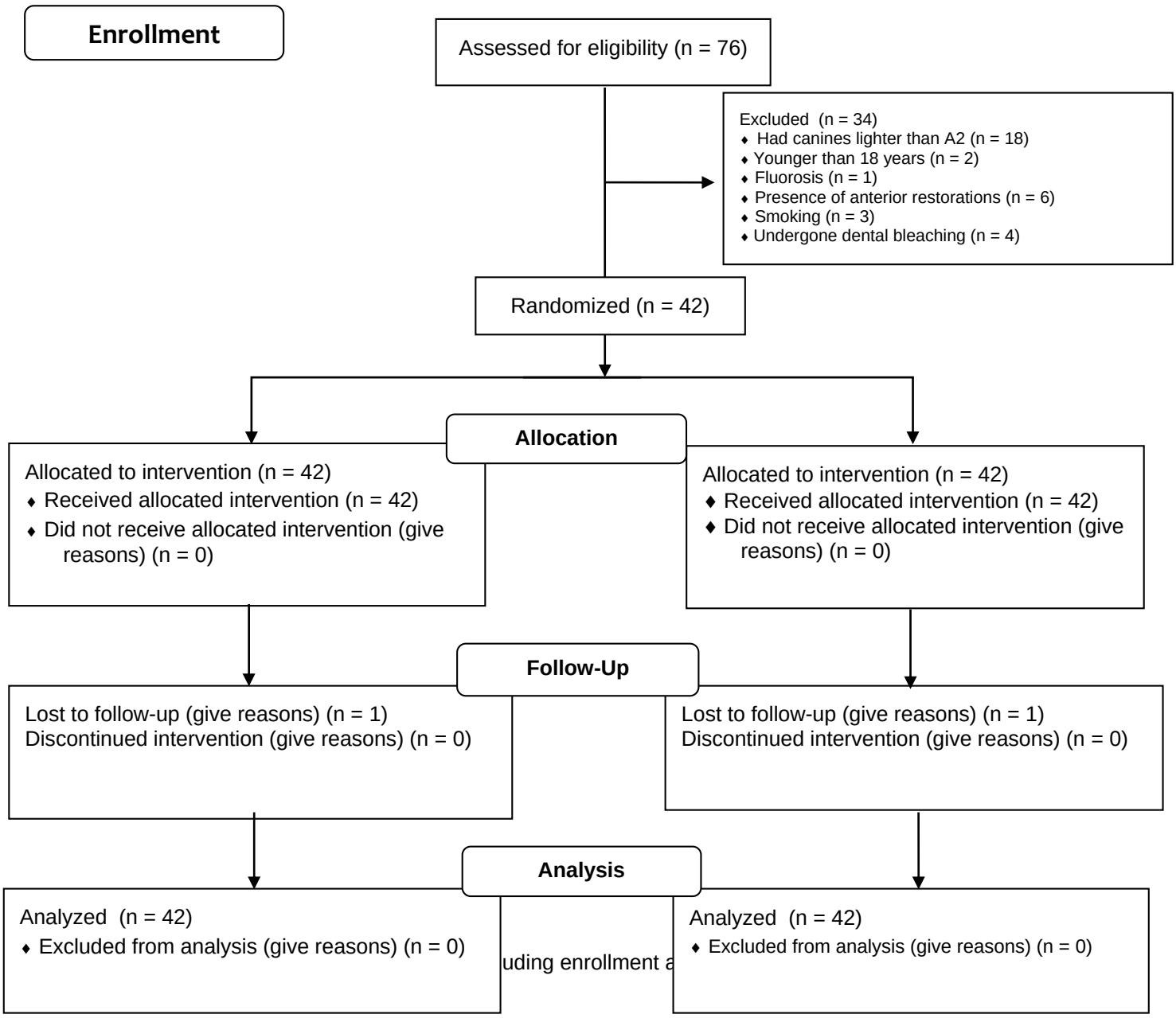
1. Tay LY, Kose C, Loguercio AD, Reis A. Assessing the effect of a desensitizing agent used before in-office tooth bleaching. *J Am Dent Assoc* 2009;**140**(10):1245-51.
2. Paula E, Kossatz S, Fernandes D, Loguercio A, Reis A. The effect of perioperative Ibuprofen use on tooth sensitivity caused by in-office bleaching. *Oper Dent* 2013;**38**(6):601-8.
3. de Paula EA, Loguercio AD, Fernandes D, Kossatz S, Reis A. Peri- operative use of an anti-inflammatory drug on tooth sensitivity caused by in-office bleaching: a randomized, triple-blind clinical trial. *Clin Oral Investig* 2013;**17**(9):2091-7.
4. Mehta D, Venkata S, Naganath M, LingaReddy U, Ishihata H, Finger WJ. Clinical trial of tooth desensitization prior to in-office bleaching. *Eur J Oral Sci* 2013;**121**(5):477-81.
5. Kugel G, Papathanasiou A, Williams AJ 3rd, Anderson C, Ferreira S. Clinical evaluation of chemical and light-activated tooth whitening systems. *Compend Contin Educ Dent* 2006;**27**(1):54-62.
6. Wang Y, Gao J, Jiang T, Liang S, Zhou Y, Matis BA. Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth

bleaching treatment—A systematic review and meta-analysis. *J Dent* 2015;**43**(8):913-23.
7. Rezende M, Loguercio AD, Kossatz S, Reis A. Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: A multi regression and logistic analysis. *J Dent* 2016;**45**:1-6.
8. Soares DG, Basso FG, Hebling J, de Souza Costa CA. Concentrations of and application protocols for hydrogen peroxide bleaching gels: effects on pulp cell viability and whitening efficacy. *J Dent* 2014;**42**(2):185-98.
9. Soares DG, Basso FG, Pontes EC, Garcia Lda F, Hebling J, de Souza Costa CA. Effective tooth-bleaching protocols capable of reducing H(2)O(2) diffusion through enamel and dentine. *J Dent* 2014;**42**(3):351-8.
10. Cooper JS, Bokmeyer TJ, Bowles WH. Penetration of the pulp chamber by carbamide peroxide bleaching agents. *J Endod* 1992;**18**(7):315-7.
11. Browning WD, Chan DC, Myers ML, Brackett WW, Brackett MG, Pashley DH. Comparison of traditional and low sensitivity whiteners. *Oper Dent* 2008;**33**(4):379-85.
12. Charakorn P, Cabanilla LL, Wagner WC, Foong WC, Shaheen J, Pregitzer R, Schneider D. The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching. *Oper Dent* 2009;**34**(2):131-5.
13. de Paula EA, Kossatz S, Fernandes D, Loguercio AD, Reis A. Administration of ascorbic acid to prevent bleaching-induced tooth sensitivity: a randomized triple-blind clinical trial. *Oper Dent* 2014;**39**(2):128-35.
14. Rezende M, Bonafé E, Vochikovski L, Farago PV, Loguercio AD, Reis A, Kossatz S. Pre- and postoperative dexamethasone does not reduce bleaching-induced tooth sensitivity: A randomized, triple-masked clinical trial. *J Am Dent Assoc* 2016;**147**(1):41-9.
15. Faria-E-Silva AL, Nahsan FP, Fernandes MT, Martins-Filho PR. Effect of preventive use of nonsteroidal anti-inflammatory drugs on sensitivity after dental bleaching: a systematic review and meta-analysis. *J Am Dent Assoc* 2015;**146**(2):87-93.
16. Haywood VB. Considerations for managing bleaching sensitivity. Dentin Hypersensitivity: Consensus-Based Recommendations for the Diagnosis and Management of Dentin Hypersensitivity (a Supplement to Inside Dentistry) 2008;**4**(9):25-31.
17. Baba N, Taira Y, Matsumura H, Atsuta M. Surface treatment of dentin with GLUMA and iron compounds for bonding indirect restorations. *J Oral Rehabil* 2002;**29**(11):1052-8.
18. Qin C, Xu J, Zhang Y. Spectroscopic investigation of the function of aqueous 2-hydroxyethylmethacrylate/glutaraldehyde solution as a dentin desensitizer. *Eur J Oral Sci* 2006;**114**(4):354-9.
19. Ibrahim Ma, El Banna M. Evaluation of the sustainability of different desensitizing agents after in-office bleaching. *Life Sci J* 2011;**8**:164–8.
20. Samuel SR, Khatri SG, Acharya S, Patil ST. Evaluation of instant desensitization after a single topical application over 30 days: a randomized trial. *Aust Dent J* 2015;**60**(3):336-42.
21. Patil SA, Naik BD, Suma R. Evaluation of three different agents for in-office treatment of dentinal hypersensitivity: a controlled clinical study. *Indian J Dent Res* 2015;**26**(1):38-42.
22. Reddy N, Reddy R, Jiang Q. Crosslinking biopolymers for biomedical applications. *Trends Biotechnol* 2015;**33**(6):362-9.

23. Arrais CA, Chan DC, Giannini M. Effects of desensitizing agents on dentinal tubule occlusion. *J Appl Oral Sci* 2004;**12**(2):144-8.
24. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2011;**9**(8):672-7.
25. Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, Baratieri LN. Clinical performance of vital bleaching techniques. *Oper Dent* 2010;**35**(1):3-10.
26. Meireles SS, Heckmann SS, Leida FL, dos Santos Ida S, Della Bona A, Demarco FF. Efficacy and safety of 10% and 16% carbamide peroxide tooth-whitening gels: a randomized clinical trial. *Oper Dent* 2008;**33**(6): 606-12.
27. Reis A, Kossatz S, Martins GC, Loguercio AD. Efficacy of and effect on tooth sensitivity of in-office bleaching gel concentrations: a randomized clinical trial. *Oper Dent* 2013;**38**(4):386-93.
28. Gurgan S, Cakir FY, Yazici E. Different light-activated in-office bleaching systems: a clinical evaluation. *Lasers Med Sci* 2010;**25**(6):817-22.
29. He LB, Shao MY, Tan K, Xu X, Li JY. The effects of light on bleaching and tooth sensitivity during in-office vital bleaching: a systematic review and meta-analysis. *J Dent* 2012;**40**(8):644-53.
30. Kossatz S, Martins G, Loguercio AD, Reis A. Tooth sensitivity and bleaching effectiveness associated with use of a calcium-containing in-office bleaching gel. *JADA* 2012;**143**(12):e81-e87.
31. Paravina RD, Johnston WM, Powers JM. New shade guide for evaluation of tooth whitening—colorimetric study. *J Esthet Restor Dent* 2007;**19**(5):276-83.
32. Paravina RD. New shade guide for tooth whitening monitoring: vi- sual assessment. *J Prosthet Dent* 2008;**99**(3):178-84.
33. de Geus JL, de Lara MB, Hanzen TA, Fernández E, Loguercio AD, Kossatz S, Reis A. One-year follow-up of at-home bleaching in smokers before and after dental prophylaxis. *J Dent* 2015;**43**(11):1346-51.
34. Commission Internationale de l'Eclairage. Recommendations on Uniform Color Spaces, Color Difference Equations, Psychometric Color Terms. Supplement 2 to CIE publication 15 (E2-31.1)1971 (TC-1.3)1978. Paris, France: Bureau Central de la CIE; 1978.
35. Haywood VB, Cordero R, Wright K, Gendreau L, Rupp R, Kotler M, Littlejohn S, Fabyanski J, Smith S. Brushing with a potassium nitrate dentifrice to reduce bleaching sensitivity. *J Clin Dent* 2005;**16**(1):17-22.
36. Bartold PM. Dentinal hypersensitivity: a review. *Aust Dent J* 2006;**51**(3):212-8; quiz 276.
37. Markowitz K, Kim S. Hypersensitive teeth. Experimental studies of dentinal desensitizing agents. *Dent Clin North Am* 1990;**34**(3):491-501.
38. Kwon SR, Dawson DV, Schenck DM, Fiegel J, Wertz PW. Spectrophotometric Evaluation of Potassium Nitrate Penetration Into the Pulp Cavity. *Oper Dent* 2015;**40**(6):614-21.
39. Recommendations for evaluating agents for the reduction of dentinal hypersensitivity. Ad Hoc Advisory Committee on Dentinal Hypersensitivity Council on Dental Therapeutics. *J Am Dent Assoc* 1986;**112**:709–10.
40. Bonafé E, Loguercio AD, Reis A, Kossatz S. Effectiveness of a desensitizing agent before in-office tooth bleaching in restored teeth. *Clin Oral Investig* 2014;**18**(3):839-45.
41. Umashankar PR, Mohanan PV, Kumari TV. Glutaraldehyde treatment elicits toxic response compared to decellularization in bovine pericardium. *Toxicol Int*

2012;**19**(1):51-8.
42. Scheffel DL, Soares DG, Basso FG, de Souza Costa CA, Pashley D, Hebling J. Transdentinal cytotoxicity of glutaraldehyde on odontoblast-like cells. *J Dent* 2015;**43**(8):997-1006.
43. Wetter NU, Branco EP, Deana AM, & Pelino JE. Color differences of canines and incisors in a comparative long-term clinical trial of three bleaching systems. *Lasers Medl Sc* 2009;**24(6):**941-7.
44. Mena-Serrano AP, Garcia E, Luque-Martinez I, Grande R, Loguercio AD, Reis A. A Single-Blind Randomized Trial About the Effect of Hydrogen Peroxide Concentration on Light-Activated Bleaching. *Oper Dent* 2016 Jun 28. [Epub ahead of print]
45. Ontiveros JC, Eldiwany MS, & Paravina R. Clinical effectiveness and sensitivity with overnight use of 22% carbamide peroxide gel. *Journal of Dentistry* 2012;**40 (Supplement 2)** e17-e24.

FIGURES


Enrollment
Assessed for eligibility (n = 76)
Excluded (n = 34)
♦ Had canines lighter than A2 (n = 18)
♦ Younger than 18 years (n = 2)
♦ Fluorosis (n = 1)
♦ Presence of anterior restorations (n = 6)
♦ Smoking (n = 3)
♦ Undergone dental bleaching (n = 4)
Randomized (n = 42)
Allocation
Allocated to intervention (n = 42)
♦ Received allocated intervention (n = 42)
♦ Did not receive allocated intervention (give reasons) (n = 0)
Allocated to intervention (n = 42)
♦ Received allocated intervention (n = 42)
♦ Did not receive allocated intervention (give reasons) (n = 0)
Follow-Up
Lost to follow-up (give reasons) (n = 1)
Discontinued intervention (give reasons) (n = 0)
Lost to follow-up (give reasons) (n = 1)
Discontinued intervention (give reasons) (n = 0)
Analysis
Analyzed (n = 42)
♦ Excluded from analysis (give reasons) (n = 0)
uding enrollment a
Analyzed (n = 42)
♦ Excluded from analysis (give reasons) (n = 0)

TABLES

Table 1 – Comparison of the number of patients who experienced TS during the bleaching regimen in both groups along with absolute and the risk ratio (*) and the number needed to treat (NNT).

Gel	Absolute risk (95% CI)	Relative Risk (95% CI)	NNT
Desensitizing	31.7 (19.6 – 46.9)	0.45 (0.27 – 0.73)	3 (6 – 2)
Placebo	70.7 (55.5 – 82.3)		

(*) McNemar's test (p < 0.0001).

Table 2 – Medians and interquartile ranges of the TS intensity at different assessment points using the NRS scale.

Assessment times	Experimental group	Placebo	p-value*
During bleaching	0 (0 – 1)	1 (0 – 2)	< 0.001
Up to1 h	0 (0 – 0)	1 (0 – 2)	< 0.001
Up to 24 h	0 (0 – 0)	0 (0 – 0)	0.063
Up to 48 h	0 (0 – 0)	0 (0 – 0)	0.50

**Wilcoxon Signed Rank test.*

Table 3 – Means and standard deviations of the bleaching-induced TS intensity at the different assessment points using VAS scales.

Assessment times	Experimental	Placebo	p-value*
During bleaching	0.5 ± 1.0	2.0 ± 2.4	< 0.001
Up to1 h	0.4 ± 0.9	2.2 ± 2.4	< 0.001
Up to 24 h	0.1 ± 0.5	0.5 ± 1.1	0.008
Up to 48 h	0.0 ± 0.3	0.2 ± 0.6	0.250

**Wilcoxon Signed Rank test.*

Table 4 – Color change in ΔSGU (Vita Classical and Vita Bleachedguide) and ΔE between baseline vs. 1-month post bleaching, along with the p-value of the pairwise comparison as well as the effect size (95% confidence interval). Values are means ± standard deviation.

Color evaluation tool	Groups		p-value*	Mean difference (95% CI)
	Experimental	Placebo		
Vita Classical	6.0 ± 2.3	5.9 ± 2.3	0.77	- 0.1 (- 1.11 to 0.91)
Vita Bleached	4.9 ± 1.5	4.7 ± 1.4	0.16	- 0.2 (- 0.84 to 0.44)
ΔE	5.9 ± 2.3	5.6 ± 4.0	0.28	- 0.3 (- 1.74 to 1.13)

** Student t-test*

5 DISCUSSÃO

O fato de o peróxido de hidrogênio possuir baixo peso molecular facilita sua difusão através do esmalte e dentina, promovendo assim, o clareamento dental (Basting et al.[26] 2012, Alonso de la Pena e Lopez Raton[27] 2014). Porém o mesmo alcança a câmara pulpar e produz a liberação de mediadores inflamatórios na polpa dental (Costa et al.[28] 2010) que pode ser a causa da sensibilidade pós-operatória experimentada pelos pacientes.

Alguns estudos já publicados na literatura relatam maior quantidade de peróxido de hidrogênio na câmara pulpar em dentes submetidos ao clareamento de consultório quando comparado aos protocolos de clareamento caseiro (Cooper et al.[9] 1992, Bowles e Ugwuneri[15] 1987, Gokay et al.[29] 2005). Em um estudo recente foi observado que maior concentração de PH e maior tempo de contato do gel pode levar a fortes estresses oxidativos em associação com uma intensa redução da viabilidade celular (Soares et al.[30] 2014). Estas observações nos permitem afirmar que a redução da concentração de peróxido de hidrogênio nos géis clareadores parece ser uma opção viável a fim de se obter menor citotoxicidade (Soares et al.[30] 2014). Porém, quando comparados os produtos disponíveis para clareamento caseiro, não houve diferença estatisticamente significante na concentração de peróxido de hidrogênio na câmara pulpar, como observado no estudo 1.

Uma das explicações para essa similaridade se refere ao modo e tempo de aplicação destes produtos, pois produtos com menores concentrações são aplicados na superfície do dente por maiores períodos. O que também pode explicar a não correlação observada entre a concentração inicial de peróxido de hidrogênio e a quantidade de peróxido de hidrogênio que alcança a câmara pulpar.

Surpreendentemente, a literatura tem relatado que em situações clínicas um grau similar de clareamento é obtido com clareamento caseiro mesmo em diferentes concentrações (Braun et al.[31] 2007, Krause et al.[32] 2008, de Geus et al.[33] 2016). Infelizmente, nestes estudos clínicos os produtos foram utilizados de acordo com as instruções do fabricante e isto pode ser o fator para o entendimento da similar concentração de PH observada nos produtos caseiros. Por isso, no presente estudo seguimos as recomendações do fabricante de todos os produtos de forma a fornecer uma melhor resposta para a extrapolação clínica.

Porém, o clareamento caseiro pode levar a desvantagens, como irritação gengival, deglutição do PH e um maior tempo para se alcançar a mudança de cor, o que indica-se, nestes casos, do clareamento de consultório. Apesar da evolução dos materiais dentários, ainda não técnica clareadora sem o indesejável efeito da sensibilidade dental (Kielbassa et al.[2] 2015). Entretanto, o clareamento de consultório está relacionado com as mais altas taxas de sensibilidade (Kielbassa et al.[2] 2015, Rezende et al.[3] 2016, Loguercio et al.[4] 2017, Bonafe et al.[5] 2013).

Um dos manejos é utilizar agentes dessensibilizantes previamente ao clareamento. No estudo 2 podemos observar que a aplicação de nitrato de potássio 5% e fluoreto de sódio 2% reduziram pela metade a concentração de peróxido de hidrogênio na câmara pulpar. E esta redução pode ser explicada pelo aumento da resistência do esmalte dental causada pela presença do fluoreto de sódio ou pela sua presença dentro da estrutura dental (Featherstone et al.[34] 1990, Santos L de et al.[35] 2009, Chu et al.[36] 2010, Tal et al.[37] 1976) prevenindo a passagem do peróxido de hidrogênio para a câmara pulpar.

Além disso, o presente estudo mostrou resultados promissores com o uso de fosfopeptídeos de caseína/fosfato de cálcio amorfo (CPP-ACP) e nanofosfato de cálcio. Com o uso desses agentes dessensibilizantes, a difusão do PH foi tão baixa quanto o grupo que não houve tratamento clareador. A literatura tem reportado desempenho superior do CPP-ACP quando comparado aos fluoretos na remineralização do esmalte e no aumento da resistência do mesmo à desmineralização (Tuloglu et al.[38] 2016, Cochrane et al.[39] 2014, Schemehorn et al.[40] 2011). Da mesma forma, produtos como nanofosfato de cálcio possuem em sua formulação partículas de tamanho nanométrico em forma de hidroxiapatita que penetram mais facilmente nas microfraturas de esmalte não visíveis (Meier et al.[41] 2010) possivelmente produzindo sua obliteração e dificultando a difusão do peróxido de hidrogênio.

O glutaraldeído também tem sido utilizada com o objetivo de reduzir a dor dos pacientes causado pela sensibilidade pós clareamento (Mehta et al.[21] 2013, Ibrahim et al.[22] 2011). No ensaio clínico randomizado triplo-cego (estudo número 3) foi possível observar uma redução do risco de 39% com o uso de um gel experimental contendo glutaraldeído 5% e nitrato de potássio 5% em uma única formulação por 10 min previamente ao clareamento de consultório. De acordo com Mehta et al.

2013[21], é possível que este efeito benéfico seja advindo da ação do glutaraldeído em produzir reticulação das proteínas dos túbulos dentinários, reduzindo assim a passagem do peróxido de hidrogênio e da ação do nitrato de potássio em reduzir a transmissão do estímulo nervoso e consequentemente, a sensibilidade dental (Wang et al.[12] 2015, Tay et al.[16] 2009).

6 CONCLUSÃO

O gel clareador com alta concentração de peróxido de hidrogênio resultou em uma maior quantidade de peróxido de hidrogênio na câmara pulpar; no entanto não houve diferença significante entre os géis clareadores de uso caseiro quando usados de acordo com as recomendações do fabricante.

A utilização de agentes dessensibilizantes a base de nitrato de potássio e fluoreto de sódio, fosfopeptídeos de caseína/fosfato de cálcio amorfo e nanofosfato de cálcio e nanohidroxiapatita reduziram a concentração do peróxido de hidrogênio na câmara pulpar, sendo estes dois últimos produtos os que apresentaram menor concentração de PH na polpa.

No estudo 3, a aplicação prévia de um gel experimental a base de nitrato de potássio 5% e glutaraldeído 5% reduziram o risco e a intensidade da sensibilidade pós-operatória em pacientes submetidos ao clareamento de consultório sem afetar o efeito clareador.

***REFERÊNCIAS**

1. Theobald AH, Wong BK, Quick AN, Thomson WM. The impact of the popular media on cosmetic dentistry. The New Zealand dental journal. 2006 Sep;102(3):58-63.
2. Kielbassa AM, Maier M, Gieren AK, Eliav E. Tooth sensitivity during and after vital tooth bleaching: A systematic review on an unsolved problem. Quintessence international. 2015 Nov-Dec;46(10):881-97.
3. Rezende M, Loguercio AD, Kossatz S, Reis A. Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: A multi regression and logistic analysis. Journal of dentistry. 2016 Feb;45:1-6.
4. Loguercio AD, Servat F, Stanislawczuk R, Mena-Serrano A, Rezende M, Prieto MV, et al. Effect of acidity of in-office bleaching gels on tooth sensitivity and whitening: a two-center double-blind randomized clinical trial. Clinical oral investigations. 2017 Mar 17.
5. Bonafe E, Bacovis CL, Iensen S, Loguercio AD, Reis A, Kossatz S. Tooth sensitivity and efficacy of in-office bleaching in restored teeth. Journal of dentistry. 2013 Apr;41(4):363-9.
6. Dias Ribeiro AP, Sacono NT, Lessa FC, Nogueira I, Coldebella CR, Hebling J, et al. Cytotoxic effect of a 35% hydrogen peroxide bleaching gel on odontoblast-like MDPC-23 cells. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2009 Sep;108(3):458-64.
7. Trindade FZ, Ribeiro AP, Sacono NT, Oliveira CF, Lessa FC, Hebling J, et al. Trans-enamel and trans-dentinal cytotoxic effects of a 35% H2O2 bleaching gel on cultured odontoblast cell lines after consecutive applications. International endodontic journal. 2009 Jun;42(6):516-24.
8. Lima AF, Ribeiro AP, Soares DG, Sacono NT, Hebling J, de Souza Costa CA. Toxic effects of daily applications of 10% carbamide peroxide on odontoblast-like MDPC-23 cells. Acta odontologica Scandinavica. 2013 Sep;71(5):1319-25.
9. Cooper JS, Bokmeyer TJ, Bowles WH. Penetration of the pulp chamber by carbamide peroxide bleaching agents. Journal of endodontics. 1992 Jul;18(7):315-7.
10. Caviedes-Bucheli J, Ariza-García G, Restrepo-Méndez S, Ríos-Osorio N, Lombana N, Muñoz HR. The effect of tooth bleaching on substance P expression in human dental pulp. Journal of Endodontics. 2008 Dec;34(12):1462-5.
11. Fugaro JO, Nordahl I, Fugaro OJ, Matis BA, Mjör IA. Pulp reaction to vital bleaching. Operative dentistry. 2004 Jul-Aug;29(4):363-8.
12. Wang Y, Gao J, Jiang T, Liang S, Zhou Y, Matis BA. Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth bleaching treatment-A systematic review and meta-analysis. Journal of dentistry. 2015 Aug;43(8):913-23.
13. Tooth whitening/bleaching: treatment considerations for dentists and their patients., (2009).
14. Bortolatto JF, Trevisan TC, Bernardi PS, Fernandez E, Dovigo LN, Loguercio AD, et al. A novel approach for in-office tooth bleaching with 6% H2O2/TiO_N and LED/laser system-a controlled, triple-blinded, randomized clinical trial. Lasers in medical science. 2016 Apr;31(3):437-44.
15. Bowles WH, Ugwuneri Z. Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. Journal of endodontics. 1987 Aug;13(8):375-7.

*De acordo com as normas do PPGO-UEPG.

16. Tay LY, Kose C, Loguercio AD, Reis A. Assessing the effect of a desensitizing agent used before in-office tooth bleaching. Journal of the American Dental Association (1939). 2009 Oct;140(10):1245-51.
17. Giniger M, Spaid M, MacDonald J, Felix H. A 180-day clinical investigation of the tooth whitening efficacy of a bleaching gel with added amorphous calcium phosphate. The Journal of clinical dentistry. 2005;16(1):11-6.
18. Giniger M, Macdonald J, Ziemba S, Felix H. The clinical performance of professionally dispensed bleaching gel with added amorphous calcium phosphate. Journal of American Dental Association. 2005 Mar;136(3):383-92.
19. Borges BC, Pinheiro MH, Feitosa DA, Correia TC, Braz R, Montes MA, et al. Preliminary study of a novel in-office bleaching therapy modified with a casein phosphopeptide-amorphous calcium phosphate. Microscopy research and technique. 2012 Nov;75(11):1571-5.
20. Vano M, Derchi G, Barone A, Genovesi A, Covani U. Tooth bleaching with hydrogen peroxide and nano-hydroxyapatite: a 9-month follow-up randomized clinical trial. Int J Dent Hyg. 2015 Nov;13(4):301-7.
21. Mehta D, Venkata S, Naganath M, LingaReddy U, Ishihata H, Finger WJ. Clinical trial of tooth desensitization prior to in-office bleaching. European journal of oral sciences. 2013 Oct;121(5):477-81.
22. Ibrahim MA, Banna ME. Evaluation of the sustainability of Different Desensitizing Agents after in-Office Bleaching. Life Science Journal. 2011;8(1):164-8.
23. Markowitz K, Bilotto G, Kim S. Decreasing intradental nerve activity in the cat with potassium and divalent cations. Archives of oral biology. 1991;36(1):1-7.
24. Mendham J, Afonso JC. Vogel: Quantitative chemical analysis. Rio de Janeiro: Livros Tecnicos e Científicos Editora S.A.; 2002.
25. Marson FC, Goncalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH, et al. Penetration of hydrogen peroxide and degradation rate of different bleaching products. Operative dentistry. 2015 Jan-Feb;40(1):72-9.
26. Basting RT, Amaral FL, Franca FM, Florio FM. Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents. Operative dentistry. 2012 Sep-Oct;37(5):464-73.
27. Alonso de la Pena V, Lopez Raton M. Randomized clinical trial on the efficacy and safety of four professional at-home tooth whitening gels. Operative dentistry. 2014 Mar-Apr;39(2):136-43.
28. Costa CA, Riehl H, Kina JF, Sacono NT, Hebling J. Human pulp responses to in-office tooth bleaching. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2010 Apr;109(4):e59-64.
29. Gokay O, Mujdeci A, Algin E. In vitro peroxide penetration into the pulp chamber from newer bleaching products. International endodontic journal. 2005 Aug;38(8):516-20.
30. Soares DG, Basso FG, Hebling J, de Souza Costa CA. Concentrations of and application protocols for hydrogen peroxide bleaching gels: effects on pulp cell viability and whitening efficacy. Journal of dentistry. 2014 Feb;42(2):185-98.
31. Braun A, Jepsen S, Krause F. Spectrophotometric and visual evaluation of vital tooth bleaching employing different carbamide peroxide concentrations. Dental

materials : official publication of the Academy of Dental Materials. 2007 Feb;23(2):165-9.
32. Krause F, Jepsen S, Braun A. Subjective intensities of pain and contentment with treatment outcomes during tray bleaching of vital teeth employing different carbamide peroxide concentrations. Quintessence international. 2008 Mar;39(3):203-9.
33. de Geus JL, Wambier LM, Kossatz S, Loguercio AD, Reis A. At-home vs In-office Bleaching: A Systematic Review and Meta-analysis. Operative dentistry. 2016 Jul-Aug;41(4):341-56.
34. Featherstone JD, Glena R, Shariati M, Shields CP. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. Journal of dental research. 1990 Feb;69 Spec No(620-5; discussion 34-6.
35. Santos Lde M, Reis JI, Medeiros MP, Ramos SM, Araujo JM. In vitro evaluation of fluoride products in the development of carious lesions in deciduous teeth. Brazilian oral research. 2009 Jul-Sep;23(3):296-301.
36. Chu CH, Mei ML, Lo EC. Use of fluorides in dental caries management. General dentistry. 2010 Jan-Feb;58(1):37-43; quiz 44-5, 79-80.
37. Tal M, Oron M, Gedalia I, Ehrlich J. X-ray diffraction and scanning electron microscope investigations of fluoride-treated dentine in man. Arch Oral Biol. 1976;21(5):285-90.
38. Tuloglu N, Bayrak S, Tnc ES, Ozer F. Effect of fluoride varnish with added casein phosphopeptide-amorphous calcium phosphate on the acid resistance of the primary enamel. BMC oral health. 2016 Sep 26;16(1):103.
39. Cochrane NJ, Shen P, Yuan Y, Reynolds EC. Ion release from calcium and fluoride containing dental varnishes. Australian dental journal. 2014 Mar;59(1):100-5.
40. Schemehorn BR, Wood GD, McHale W, Winston AE. Comparison of fluoride uptake into tooth enamel from two fluoride varnishes containing different calcium phosphate sources. The Journal of clinical dentistry. 2011; 22(2):51-4.
41. Meier M, Cardoso V, Mittelstadt FG, Santos R, Odebrecht CM, Ferri L, et al. Nanostructured Calcium Phosphate (Nano P) Technology: Innovation on Dental Desensitisation and Remineralization. Journal of dental research. 2010 4584(89 (Spec Iss B)).

*De acordo com as normas do PPGO-UEPG.

ANEXO A

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Quantificação de peróxido de hidrogênio na câmara pulpar de dentes submetidos a diferentes protocolos de clareamento

Pesquisador: Alessandra Reis

Área Temática:

Versão: 2

CAAE: 54401716.6.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.614.755

Apresentação do Projeto:

Projeto que visa a analise de Oitenta pré-molares hígidos do Banco de Dentes da UEPG sem fraturas e defeitos de esmalte serão aleatoriamente divididos em oito grupos de
acordo com o tratamento: Controle (sem tratamento clareador) [C], Peróxido de Carbamida 10% [PC10%], Peróxido de Carbamida 16% [PC16%],
de Peróxido de Carbamida 22% [PC22%], Peróxido de Hidrogênio 4% [PH4%], Peróxido de Hidrogênio 7 ½% [PH7 ½%] e Peróxido de Hidrogênio 10%
[PH10%], Peróxido de Hidrogênio 35% [PH35%].

Objetivo da Pesquisa:

O objetivo do trabalho é medir a concentração e quantificar a penetração de peróxido de hidrogênio na câmara pulpar com diferentes protocolos de
tratamento: clareamento caseiro - PC nas concentrações 10%, 16%, 22% e PH 4%, 7 ½% e 10%; e clareamento de consultório - PH 35%, todos de
mesma marca comercial.

Avaliação dos Riscos e Benefícios:

Riscos:

No presente estudo os pesquisadores poderão ter como riscos, manipulação com materiais perfurocortantes e contato com soluções. No entanto,
todos os riscos poderão ser evitados com o uso de Equipamentos de Proteção Individual (EPI).
Benefícios:
Contribuir com o conhecimento sobre a permeabilidade do esmalte frente à comparação de vários géis clareadores com diferentes concentrações
sob mesmas condições experimentais.

Comentários e Considerações sobre a Pesquisa:

Espera-se, com os resultados desta pesquisa, contribuir com o conhecimento sobre a permeabilidade do esmalte frente à comparação de vários
géis clareadores com diferentes concentrações sob mesmas condições experimentais. Além disso, este trabalho visa à produção de um artigo
científico ao final do projeto e a formação profissional e científica do aluno, motivando-o a desenvolver trabalhos de pesquisa na área.

Considerações sobre os Termos de apresentação obrigatória:

Em anexo e de acordo com as normas da resolução 466

Recomendações:

Ao final do projeto de pesquisa solicita-se seja informada a Propesp e a Coep via plataforma brasil o término do referido projeto.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Considerações Finais a critério do CEP:

Ao final do projeto de pesquisa solicita-se seja informada a Propesp e a Coep via plataforma brasil o término do referido projeto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_642986.pdf	17/06/2016 09:40:07		Aceito
Outros	AsTZ4sBk3sEZPaGmmiInQWlePIG29BxjuR9rkze8b7X2.jpg	17/06/2016 09:39:40	Alessandra Reis	Aceito

Folha de Rosto	IMG_1580.pdf	21/03/2016 09:16:29	Alessandra Reis	Aceito
Projeto Detalhado / Brochura Investigador	projetoestruturado.doc	26/02/2016 12:24:55	Alessandra Reis	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PONTA GROSSA, 30 de Junho de 2016

Assinado por:
ULISSES COELHO
(Coordenador)

ANEXO B

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da aplicação de agentes dessensibilizantes na permeabilidade de peróxido de hidrogênio a 35% na câmara pulpar

Pesquisador: Alessandra Reis

Área Temática:

Versão: 1

CAAE: 36571914.3.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 808.118

Data da Relatoria: 25/09/2014

Apresentação do Projeto:

Este estudo in vitro tem o objetivo de quantificar a penetração de peróxido de hidrogênio na câmara pulpar, após a aplicação de agentes dessensibilizantes previamente ao clareamento de consultório. Será desenvolvido com a utilização de 50 pré-molares Espera-se, com os resultados desta pesquisa, contribuir com o conhecimento sobre a permeabilidade do esmalte frente a aplicação prévia de agentes dessensibilizantes do clareamento de consultório.

Objetivo da Pesquisa:

Quantificar a penetração de peróxido de hidrogênio na câmara pulpar, após a aplicação de agentes dessensibilizantes previamente ao clareamento de consultório.

Avaliação dos Riscos e Benefícios:

Os autores apresentam que os riscos podem ser minimizados com o uso de equipamentos de proteção individual, como óculos, luvas e máscaras. O uso de EPIs evitará o contato com substâncias químicas que podem causar queimaduras leves.

Como benefícios do desenvolvimento desta pesquisa, os autores relatam a contribuição com a ciência sobre o efeito da aplicação de agentes dessensibilizantes e a permeabilidade do esmalte dentário quando submetido ao clareamento de consultório.

Comentários e Considerações sobre a Pesquisa:

Cinquenta pré-molares rígidos do Banco de Dentes da UEPG serão aleatoriamente divididos em cinco grupos de acordo com os agentes dessensibilizantes utilizados: Peróxido de hidrogênio (PH) 35%, KF2%® + PH 35%, GLUMA Desensitizer Power Gel® + PH 35%, Recaldent® + PH 35%. Um grupo controle composto por dentes sem nenhum tratamento também será utilizado, resultando em um total de 10 dentes para cada grupo. Para avaliar a penetração de peróxido de hidrogênio na câmara pulpar, os dentes serão seccionados 3 mm da junção cemento-esmalte, o tecido pulpar removido e um tampão de acetato será colocado na câmara pulpar para absorver e estabilizar o peróxido que penetrar. Os agentes dessensibilizantes serão aplicados de acordo com as recomendações dos fabricantes. Após a remoção destes produtos, o procedimento clareador será realizado com peróxido de hidrogênio a 35 % em três aplicações de 15 min. Posteriormente, a solução de tampão de acetato na câmara pulpar de cada dente será removida e misturada com 2 mL da solução reagente. A densidade óptica da solução resultante rosa será determinada espectrofotometricamente, e convertida em microgramas equivalente do peróxido de hidrogênio.

Considerações sobre os Termos de apresentação obrigatória:

Apresenta Folha de Rosto devidamente preenchida e assinada.

Apresenta aceite do Banco de Dentes para o fornecimento dos mesmos.

Descreve a metodologia utilizada adequadamente, assim como riscos e benefícios da mesma.

Recomendações:

não se aplica.

Conclusões ou Pendências e Lista de Inadequações:

Proposta de projeto adequada à aprovação pelo COEP.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PONTA GROSSA, 26 de Setembro de 2014

Assinado por:
ULISSES COELHO
(Coordenador)

Endereço: Av. Gen. Carlos Cavalcanti, nº 4748. UEPG, Campus Uvararanas, Bloco M, Sala 100.
Bairro: Uvaranas **CEP:** 84.030-900
UF: PR **Município:** PONTA GROSSA
Telefone: (42)3220-3108 **E-mail:** coep@uepg.br

ANEXO C

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação clinica do efeito da associação do Nitrato de Potássio a 5% e agente dessensibilizante à base de glutaraldeído na prevalência da sensibilidade pós-clareamento dental

Pesquisador: Alessandro Dourado Loguercio

Área Temática:

Versão: 1

CAAE: 37675414.4.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 843.554

Data da Relatoria: 22/10/2014

Apresentação do Projeto:

A associação do nitrato de potássio a 5% com um agente dessensibilizante à base de glutaraldeido diminui o percentual de pacientes com
sensibilidade após o clareamento dental;A associação do nitrato de potássio a 5% com um agente dessensibilizante à base de glutaraldeido diminui
a intensidade da dor após clareamento dental;A associação do nitrato de potássio a 5% com um agente dessensibilizante à base de glutaraldeido
não influencia na eficácia do clareamento dental. A Descrição deste estudo será realizado de acordo com a normativa do CONSORT (Consolidated Standards of Reporting Trials statement). Com
base em critérios pré-estabelecidos, serão selecionados 42 voluntários da cidade de Ponta Grossa, PR, Brasil, para este estudo. Duas semanas
antes dos procedimentos de clareamento, todos os voluntários passarão por uma triagem, profilaxia com pedra-pomes e água com uma taça de
borracha e assinarão um termo de consentimento livre e esclarecido.Procedimento Dessensibilizante e ClareadorOs participantes serão
aleatoriamente divididos em dois grupos: placebo e experimental.

Objetivo da Pesquisa:

Assim o objetivo deste estudo será avaliar o efeito dessensibilizante da associação do nitrato de potássio a 5% com um agente dessensibilizante à
base de glutaraldeído aplicado topicamente antes do clareamento dental com peróxido de hidrogênio a 35%, comparado a um placebo, em
pacientes adultos

Avaliação dos Riscos e Benefícios:

Critério de Inclusão:

1-Os candidatos devem possuir todos os dentes de canino a canino superior e inferior, sem manifestações de cárie, restaurações, dentina exposta e
sensibilidade dentária espontânea.2-Aptos a assinar um termo de consentimento livre e esclarecido3-Maiores de 18 anos.4-Comprometer-se a
comparecer para exames periódicos.5- Ter boas condições de saúde oral e geral.6-Incisivos centrais com cor A2 ou mais escuros, comparando com
a escala de cor (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany)

Critério de Exclusão:

1.Pacientes que tenham realizado algum procedimento clareador anteriormente.2-Grávidas ou lactantes.3-Fumantes4-Pacientes que apresentam
manchas por tetraciclina (grau I,II,III ou IV)2, fluorose ou tratamento endodôntico.5-Pacientes bruxômanos e com periodontite severa.6-Uso contínuo
de algum medicamento com ação anti-inflamatória ou antioxidante.

Riscos:

Não há riscos nesse estudo, devido ser um tratamento já bastante realizado e fundamentado na literatura e clinicamente.

Benefícios:

Diminuição da sensibilidade dental após clareamento dental.

Comentários e Considerações sobre a Pesquisa:

A efetividade do clareamento nos dois grupos serão avaliados utilizando uma via de medida repetida ANOVA.Os níveis de sensibilidade dentária nos
diferentes períodos de tempo para cada grupo serão comparadas utilizando o teste de Friedman e para analisar a intensidade da sensibilidade
dentária em cada período de tempo entre os grupos será utilizado o teste de Wilcoxon.

Desfecho Primário:

Diminuição do percentual de pacientes com sensibilidade após o clareamento dental

Desfecho Secundário:

Intensidade da dor e eficacia do clareamento

Considerações sobre os Termos de apresentação obrigatória:

Documentação pertinente anexada adequadamente dentro das normas

Recomendações:

Não se aplica

Conclusões ou Pendências e Lista de Inadequações:

Projeto de pesquisa bem delineado cientificamente e do ponto de vista da bioética

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PONTA GROSSA, 23 de Outubro de 2014

Assinado por:
ULISSES COELHO
(Coordenador)

ANEXO D

USUÁRIO SENHA
ENTRAR Esqueceu a senha? Registrar-se

Ensaios Clínicos

PT | ES | EN

NOTÍCIAS | SOBRE | AJUDA | CONTATO

Buscar ensaios
BUSCA AVANÇADA

HOME / ENSAIOS REGISTRADOS /

RBR-7b7cmn
Avaliação clinica do efeito da associação do Nitrato de Potássio a 5% e agente dessensibilizante à base de glutaraldeído na prevalência da sensibilidade pós- clareamento dental

Data de registro: 6 de Ago. de 2014 às 15:37
Last Update: 28 de Maio de 2016 às 16:03

Tipo do estudo:

Intervenções

Título científico:

PT-BR
Avaliação clinica do efeito da associação do Nitrato de Potássio a 5% e agente dessensibilizante à base de glutaraldeído na prevalência da sensibilidade pós-clareamento dental

EN
Clinical evaluation of the effect of the combination of Potassium Nitrate 5% and desensitizing agent-based glutaraldehyde in the prevalence of post-bleaching sensitivity

Identificação do ensaio

Número do UTN: U1111-1160-0918

Título público:

PT-BR
Avaliação clinica do efeito da associação do Nitrato de Potássio a 5% e agente dessensibilizante à base de glutaraldeído na prevalência da sensibilidade pós-clareamento dental

EN
Clinical evaluation of the effect of the combination of Potassium Nitrate 5% and desensitizing agent-based glutaraldehyde in the prevalence of post-bleaching sensitivity

Acrônimo científico:

Acrônimo público:

Identificadores secundários:

843.554
Órgão emissor: Comitê de Ética em Pesquisa da Universidade Estadual de Ponta Grossa I

37675414.4.0000.0105
Órgão emissor: Plataforma Brasil

Patrocinadores

Patrocinador primário: Universidade Estadual de Ponta Grossa

Patrocinadores secundários:

Instituição: Universidade Estadual de Ponta Grossa

Fontes de apoio financeiro ou material:

Instituição: Universidade Estadual de Ponta Grossa

Condições de saúde

Condições de saúde ou problemas:

PT-BR	EN
Sensibilidade da dentina, Clareamento Dental; prevenção	Dentin Sensitivity, Dental Bleaching, Prevention.

Descritores gerais para as condições de saúde:

PT-BR	ES	EN
C07: Doenças estomatognáticas	**C07:** Enfermedades estomatognáticas	**C07:** Stomatognathic diseases
C23: Condições patológicas, sinais e sintomas	**C23:** Condiciones patológicas, signos y síntomas	**C23:** Pathological conditions, signs and symptoms
C25: Distúrbios induzidos quimicamente	**C25:** Trastornos químicamente inducidos	**C25:** Chemically-induced disorders

Descritores específicos para as condições de saúde:

PT-BR	ES	EN
E06.420.750: Clareamento Dental	**E06.420.750:** Blanqueamiento de Dientes	**E06.420.750:** Tooth Bleaching
C07.793.266: Sensibilidade da Dentina	**C07.793.266:** Sensibilidad de la Dentina	**C07.793.266:** Dentin Sensitivity

Intervenções

Categorias das intervenções

Other

Intervenções:

PT-BR	EN
Dois operadores com experiência clínica, e que não participaram do processo de triagem	Two operators with clinical experience, and not involved in the screening and

e aleatorização serão responsáveis pela implementação do estudo. Neste estudo do tipo paralelo, boca-dividida, participou 42 pacientes, de modo que cada paciente recebeu o gel placebo e o gel contendo dessensibilizante. Cada paciente terá a proteção dos lábios, língua e tecido peribucal da área de clareamento realizada através de um afastador labial (Arc Flex, FGM, Joinville, SC, Brasil) e os tecidos gengivais dos dentes a serem clareados serão isolados do agente clareador através da confecção de uma barreira de resina fotopolimerizável (Top Dam, FGM, Joinville, SC, Brasil). Será aplicado um dessensibilizante a base de glutaraldeído combinado com nitrato de potássio 5% o qual será manipulado em uma farmácia especializada ou placebo por 10 min, em seguida esfregados com o auxilio de uma taça de borracha acoplada a um contra-ângulo por 20 segundos em cada dente. O procedimento clareador será realizado com peróxido de hidrogênio 35% (Whiteness HP Maxx 35% FGM, Joinville, SC, Brasil) em duas sessões, com intervalo de uma semana entre elas onde em cada uma serão realizadas 3 aplicações de 15 minutos totalizando 45 min, conforme recomendações do fabricante.

randomization process will be responsible for implementing the study. In this study the parallel type, split-mouth, involved 42 patients, so that each patient received the placebo gel and the gel containing desensitizing. For this, each patient will have the protection of the lips, tongue and perioral tissue whitening area held by a lip retractor (Arc Flex, FGM, Joinville, Brazil) and the gingival tissues of the teeth to be whitened are isolated agent whitener by making a barrier of light-cured resin (Top Dam, FGM, Joinville, Brazil). A desensitizing glutaraldehyde-based combined with potassium nitrate 5% will be applied which will be handled by a specialized chemist or placebo for 10 min, then rubbed with the aid of a rubber cup attached to a handpiece for 20 seconds each tooth. The bleaching procedure will be performed with 35% hydrogen peroxide (Whiteness HP Maxx 35% FGM, Joinville, Brazil) in two sessions, with an interval of one week between them where each one will be held 3 applications 15 minutes totaling 45 min , according to the manufacturer's recommendations.

Descritores para as intervenções:

PT-BR

D27.505.696.663.850.014.640:
Dessensibilizantes Dentinários

ES

D27.505.696.663.850.014.640:
Desensibilizantes Dentinarios

Recrutamento

Situação de recrutamento: Recruiting

País de recrutamento

Brazil

Data prevista do primeiro recrutamento: 2014-08-13

Data prevista do último recrutamento: 2016-05-15

Tamanho da amostra alvo:	Gênero para inclusão:	Idade mínima para inclusão:	Idade máxima para inclusão:
42	-	18 Y	50 Y

Critérios de inclusão:

PT-BR

Os candidatos devem possuir todos os dentes de canino a canino superior e inferior; sem manifestações de cárie e restaurações; dentina exposta e sensibilidade dentária espontânea; aptos a assinar um termo de consentimento livre e esclarecido; maiores de 18 anos; comprometer-se a comparecer para exames periódicos; ter boas condições de saúde oral e geral; incisivos centrais com cor A2 ou mais escuros.

EN

Candidates must have all the teeth of the upper and lower canine; canine without manifestations of caries and/or restorations; exposed dentin and spontaneous tooth sensitivity; able to sign a consent form; over 18 years; Commit to attend for regular examinations; having good conditions of oral and general health; central incisors to A2 or darker color.

Critérios de exclusão:

PT-BR

Pacientes que tenham realizado algum procedimento clareador anteriormente; grávidas ou lactantes; fumantes; pacientes que apresentam manchas por tetraciclina, fluorose ou tratamento endodôntico; pacientes bruxômanos e com periodontite severa; uso contínuo de algum medicamento com ação anti-inflamatória ou antioxidante.

EN

Pacientes who have previously done some bleaching procedure; pregnant or lactating; smokers, patients with tetracycline stains, fluorosis or endodontic treatment; patients with severe periodontitis and bruxists; use of any medication with anti-inflammatory or antioxidant action.

Tipo do estudo

Desenho do estudo:

PT-BR

Ensaio clínico de prevenção, randomizado-controlado, paralelo, triplo-cego, com dois braços.

EN

Clinical trial for prevention, randomized controlled, parallel, triple-blind, two-arm.

Programa de acesso expandido	Enfoque do estudo	Desenho da intervenção	Número de braços	Tipo de mascaramento	Tipo de alocação	Fase do estudo
Nenhum	Prevention	Parallel	2	Triple-blind	Randomized-controlled	N/A

Desfechos

Desfechos primários:

PT-BR

Verificar o número de pacientes que relatam dor espontânea em algum momento durante a técnica de clareamento dental e até 48 horas após o procedimento, através de auto-relato. Este número será transformado em percentual de pacientes com dor e será denominado de risco absoluto de sensibilidade dental.

EN

Check the number of patients that report spontaneous pain in any moment of the bleaching protocol and up to 48 h post-bleaching, through self-report. This number will be presented as percentage of patients with tooth sensitivity and will be named absolute risk of tooth sensitivity

Desfechos secundários:

PT-BR

A intensidade da sensibilidade dental será avaliada através da escala numérica de 5 pontos (NRS 0-4) e da escala visual analógica VAS (0-10) e será apresentada como média e desvio padrão em diferentes períodos (durante o clareamento dental, até 1 h, de 1 h a 24 h e de 24 a 48 h pós-clareamento).

EN

The intensity of tooth sensitivity will be assessed using the numeric 5-point scale (NRS 0-4) and the visual analogue scale (VAS 0-10) and will be presented as means and standard deviations in different periods (during dental bleaching, up to 1 h, 1 h to 24 h and 24 h to 48 h post-bleaching).

PT-BR

A diferença de cor entre a condição inicial e 1 mês após clareamento será avaliada através das escalas de cor Vita Classical e Vita Bleachedguide e também com o espectrofotômetro Vita Easyshade. Os dados serão apresentados na forma de médias e desvios padrões.

EN

The difference in color between the baseline condition and 1 month post-bleaching will be assessed by Vita Classical and Bleachedguide Vita shade guide as well as with the Vita Easyshade spectrophotometer. The data will be presented as means and standard deviations

Contatos

Contatos para questões públicas

Nome completo: Alessandro Loguercio
Endereço: Av. General Carlos Cavalcanti, 4748
Cidade: Ponta Grossa / Brazil
CEP: 84030-900
Fone: (42) 3220-3000
E-mail: aloguercio@hotmail.com
Filiação: Universidade Estadual de Ponta Grossa

Nome completo: Alessandro Dourado Loguercio
Endereço: Av. General Carlos Cavalcanti, 4748
Cidade: Ponta Grossa / Brazil
CEP: 84030-900
Fone: +55(42)3220-3000
E-mail: aloguercio@hotmail.com
Filiação: Universidade Estadual de Ponta Grossa

Contatos para questões científicas

Nome completo: Alessandro Loguercio
Endereço: Av. General Carlos Cavalcanti, 4748
Cidade: Ponta Grossa / Brazil
CEP: 84030-900
Fone: (42) 3220-3000
E-mail: aloguercio@hotmail.com
Filiação: Universidade Estadual de Ponta Grossa

Nome completo: Alessandro Dourado Loguercio
Endereço: Av. General Carlos Cavalcanti, 4748
Cidade: Ponta Grossa / Brazil
CEP: 84030-900
Fone: +55(42)3220-3000
E-mail: aloguercio@hotmail.com
Filiação: Universidade Estadual de Ponta Grossa

Contatos para informação sobre os centros de pesquisa

Nome completo: Alessandro Loguercio	**Nome completo:** Alessandro Dourado Loguercio
Endereço: Av. General Carlos Cavalcanti, 4748	**Endereço:** Av. General Carlos Cavalcanti, 4748
Cidade: Ponta Grossa / Brazil	**Cidade:** Ponta Grossa / Brazil
CEP: 84030-900	**CEP:** 84030-900
Fone: (42) 3220-3000	**Fone:** +55(42)3220-3000
E-mail: aloguercio@hotmail.com	**E-mail:** aloguercio@hotmail.com
Filiação: Universidade Estadual de Ponta Grossa	**Filiação:** Universidade Estadual de Ponta Grossa

Links adicionais:

Download no formato ICTRP

Download no formato XML OpenTrials

OpenTrials v1.2