Universidade de Lisboa

Faculdade de Medicina Dentária

HYDROGEN PEROXIDE DIFFUSION THROUGH DENTAL TISSUES – IN VITRO STUDY

Leonor Gonçalves da Silva Casqueiro

Orientador:

Professor Doutor Duarte Nuno da Silva Marques

Coorientador:

Professor Doutor João Miguel Lourenço Silveira

Dissertação

Mestrado Integrado em Medicina Dentária

2022

Dissertação formatada de acordo com as normas de publicação da revista

Materials MDPI

"Também as nuvens pretas logo depois desapareceram do céu e as estrelas brilharam, brilhou também a lua cheia. (...) E eles esqueceram que não eram iguais às demais crianças (...). Esqueceram tudo e foram iguais a todas as crianças, cavalgando os ginetes do carrossel, girando com as luzes."

Jorge Amado, Capitães da Areia, 18ª edição, Leya, 2019

Ao meu Avô Artur e ao meu Avô Júlio, que me viram começar esta etapa, mas não estão aqui para comemorar o fim desta viagem comigo. Obrigada por todas as lições, memórias felizes e carinhos, que vou levar comigo para a vida. Espero que estejam orgulhosos. Vivem para sempre no meu coração.

ACKNOWLEDGEMENTS

Ao meu orientador, Professor Doutor Duarte Marques, por me desafiar a ir sempre mais além, pelo seu rigor científico e por me ter incutido o gosto pela investigação.

Ao meu coorientador, Professor Doutor João Silveira pela preocupação que sempre demonstrou, pela sua boa disposição e acima de tudo por ter sempre uma palavra amiga, mesmo nas alturas mais difíceis.

Ao Grupo de Investigação de Biologia e Bioquímica Oral por todas as oportunidades que me proporcionou ao longo destes anos como colaboradora. Um agradecimento especial à Susana Dias e à Joana Dias pelo companheirismo e auxílio que me prestaram no desenvolvimento deste projeto.

Aos amigos que a faculdade me deu nestes últimos 5 anos, que marcaram esta etapa tão importante da minha vida e que me ajudaram a crescer, especialmente à Valeria Bostan, que partilhou comigo estes primeiros dois anos de prática clínica e aturou com toda a paciência as minhas teimosias.

Ao Jory por me apoiar e ajudar sempre, por ser o meu companheiro de todos os momentos, por todo o amor e dedicação e por estar na minha vida. És a minha maior aventura.

À minha família por todo o apoio e carinho. À minha Mãe por ser um exemplo de força de vontade e de determinação (e por me ter ajudado com a análise estatística deste projeto!… a paixão pelas matemáticas deve ter saltado uma geração…). Ao meu Pai e ao meu Irmão por alegrarem os meus dias, por darem os melhores abraços e por serem quem são.

ABSTRACT

Dental whitening products can contain hydrogen peroxide as active principle, which has a low enough molecular weight to penetrate through dental tissues, causing side effects. This in vitro study evaluates the hydrogen peroxide (HP) diffusion of two in-office whitening products with 6% and 40% HP in different types of teeth, and the influence of exposition area, dental tissues' thickness, and pulp chamber volume. Thirty-six intact human teeth were used in a positive pulpar pressure model. Each product was applied to 6 anterior teeth, 6 premolars, and 6 molars. 0.125 mL samples were collected every 10 minutes during product application and 30 minutes after protocol. Samples were analyzed through spectrophotometry by previously established methods and results expressed as mean and 95% confidence interval. Kolmogorov-Smirnov test, independent t-tests, Pearson Correlations, Kruskal Wallis and Linear Regression Models were used as appropriate, α=5%. There were statistically significant differences in the diffusion kinetics between whitening products for the premolar and molar groups. The HP concentrations obtained from the pulp chamber attained minimal cytotoxicity values. Although not statistically significant, area, thickness, and volume, presented a positive correlation with the diffused HP. Different whitening products and tooth types lead to various HP concentrations in the pulp chamber, which in some cases can lead to cytotoxicity.

Keywords: Teeth Whitening, Positive Pulpar Pressure, Hydrogen Peroxide Diffusion, Cytotoxicity.

RESUMO

O branqueamento dentário é o procedimento eletivo mais comum da Medicina Dentária, sendo minimamente invasivo e acessível, pelo que deve ser regulado. De acordo com a Diretiva da União Europeia, o branqueamento dentário pode ser realizado através de produtos de venda livre, técnicas em ambulatório e técnicas *in-office*. Os princípios ativos mais usados são o Peróxido de Hidrogénio (PH) e o Peróxido de Carbamida (PC).

O peróxido de hidrogénio é estruturalmente instável, apresentando uma hidrólise e ação mais rápidas, originando água e radicais livres de oxigénio. As partículas de hidrogénio e os radicais livres de oxigénio apresentam um peso molecular baixo o suficiente para que se verifique a sua difusão pelos tecidos dentários até à câmara pulpar, podendo causar efeitos secundários como sensibilidade, ulceração dos tecidos moles, dano no ADN dos odontoblastos e alterações no esmalte e dentina, assim como diminuição do metabolismo e viabilidade celulares.

Embora já existam estudos que tenham analisado a difusão do PH em dentes humanos intactos, os modelos *in vitro* utilizados não reproduzem a pressão pulpar positiva que se verifica *in vivo*. Assim, este estudo procura avaliar a difusão de PH para a câmara pulpar, usando um modelo de pressão pulpar positiva e recorrendo a dois produtos de branqueamento *in-office*: um sistema em verniz com 6% de PH - *VivaStyle Paint On Plus - Ivoclar - Vivadent®, Liechtenstein* (VS), e um sistema em gel com 40% de PH - *Opalescence Boost - Ultradent®, United States of America* (OP). Este estudo procurou ainda avaliar a influência do tipo de dente, da área de exposição, espessura da face vestibular e volume da câmara pulpar na difusão de PH.

36 dentes humanos hígidos foram selecionados como amostras, sendo seccionados 2-3 mm abaixo da junção amelo-cementária e tendo a sua câmara pulpar sido limpa com uma broca de turbina esférica. Cada produto de branqueamento foi aplicado em 6 dentes Anteriores (A), 6 dentes Pré-molares (PM) e 6 dentes Molares (M). Os grupos experimentais foram os seguintes: VS-A, VS-PM, VS-M, OP-A, OP-PM e OP-M. Nos grupos VS realizaram-se 6 aplicações de 10 minutos e nos grupos OP realizaram-se 3 aplicações de 20 minutos, de acordo com as instruções do fabricante.

Todas as faces do dente foram isoladas com verniz de unhas, exceto a face vestibular onde foi aplicado o produto correspondente. A área de exposição, a espessura da face vestibular e o volume da câmara pulpar foram registados para cada dente. As amostras foram adaptadas com cianoacrilato *(SuperCola 3®, Loctite, Henkel Adhesives, Germany)* a placas de policarbonato perfuradas, permitindo a comunicação com a câmara

pulpar. A estas perfurações foram adaptadas agulhas de 27G *(Luer Lock ®, B. Braun Melsungen, Germany)*, associadas a tubos de irrigação. Um destes encontrava-se associado a uma coluna de 14 cm de solução tampão de acetato de sódio (2M) (simulação da pressão pulpar), e o outro permitia a recolha de amostras.

Foram recolhidas amostras de 0.125 mL antes da aplicação de qualquer produto de branqueamento, e após a primeira aplicação de 10 em 10 minutos até perfazer 90 minutos totais, sendo que nos últimos 30 minutos, não se realizou a aplicação de produto. As amostras foram analisadas com recurso a espectrofotometria, por métodos previamente descritos segundo o método do leucocristal violeta, descrito por Mottola, H. em 1970. O limite citotóxico mínimo (LCM) foi considerado 4.70 µg/mL, de concentração de PH descrito por Almeida 2013.

A análise estatística foi realizada com recurso a IBM SPSS® (version 25) (*IBM Statistics, Inc. Chicago, IL, EUA*), recorrendo ao teste Kolmogorov-Smirnov, a t-tests de amostras independentes e variâncias desconhecidas, a Correlações de Pearson, ao teste Kruskal Wallis e a Modelos de Regressão Linear. Os resultados são apresentados como média e intervalo de confiança (IC) a 95%, com um nível de significância de α=5%.

A concentração dos produtos de branqueamento foi verificada através de um processo de três ensaios de titulação para cada produto. Os valores foram superiores aos declarados pelo fabricante, sendo que o VS registou uma média de 6.15% [5.93; 6.37] e OP registou uma média de 41.04% [37.59; 44.54]). No entanto, estas diferenças não foram estatisticamente significativas.

90 minutos após o início do protocolo de branqueamento, o grupo VS-A registou uma quantidade cumulativa na camara pulpar de 1.333 µg de PH [1.214; 1.452] e o grupo OP-A de 1.538 µg [1.457; 1.620]. Os grupos VS-PM e OP-PM registaram uma quantidade média de 1.208 µg [1.123; 1.291] e 3.628 µg [3.401; 3.855] de PH, respetivamente. Por fim, os grupos VS-M e OP-M registaram valores médios de 2.560 µg [2.297; 2.823] e 4.197 µg [3.997; 4.396], respetivamente. Detetaram-se diferenças estatisticamente significativas para o valor médio total entre produtos de branqueamento dentário para os grupos de pré-molares e de molares.

Para a aplicação do produto VS verificou-se uma cinética de difusão semelhante entre o grupo dos pré-molares e dos anteriores. No entanto, para a aplicação do produto OP verificou-se uma cinética de difusão semelhante entre o grupo dos pré-molares e dos molares.

Ao avaliar a influência da área de exposição, da espessura da face vestibular ou do volume da câmara pulpar na difusão de PH, as variáveis relacionam-se de forma positiva, com correlações de 0.077, 0.312 e 0.536, respetivamente. O volume da câmara pulpar apresenta a maior influência na difusão de PH. O teste Kruskal Wallis demonstrou que o tipo de dente influencia a mediana da massa total de PH difundido.

Desenvolveu-se um modelo de regressão linear com as variáveis em análise estatisticamente significativas que relaciona o tipo de dente *versus* o produto de branqueamento aplicado, o qual explica 72% da variabilidade de resultados verificada. Este modelo propõe que após a aplicação do mesmo produto de branqueamento, um dente pré-molar e um dente molar vão verificar, respetivamente, a difusão para a câmara pulpar de mais 0.482 μg e 1.943 μg de PH do que um dente anterior. É expectável que, para o mesmo tipo de dente, 90 minutos após o início do protocolo de aplicação de OP, se verifique a difusão de mais 1.421 μg de PH do que 90 minutos após o início do protocolo de aplicação de VS.

A maioria das amostras atingiu concentrações de PH abaixo do limite citotóxico mínimo (LCM) de 4.70 μg/mL, descrito por Almeida 2013. No entanto, quando comparados ao LCM de De Lima 2009 (2.22 μg/mL), todos os grupos ultrapassaram o limite citotóxico em algum dos tempos. A difusão do PH mesmo após a conclusão do protocolo de aplicação pode levar a uma citotoxicidade cumulativa, mas a concentração obtida não ultrapassou o valor de ID50 declarado por Hanks 1993.

Estudos clínicos futuros deverão avaliar qual o tipo de dente mais afetado por sensibilidade pós-branqueamento dentário. Além disso, os próximos estudos *in vitro* devem incluir diferentes produtos de branqueamento e grupos experimentais mais específicos (por exemplo, apenas primeiros pré-molares maxilares).

O presente estudo demonstra que os odontoblastos poderão ser expostos a PH e radicais livres de oxigénio em valores citotóxicos (sendo os Molares o tipo de dente de maior risco). Os valores alcançados dependem do produto de branqueamento.

Palavras-chave: Branqueamento Dentário, Pressão Pulpar Positiva, Difusão de Peróxido de Hidrogénio, Citotoxicidade.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

HP: Hydrogen Peroxide.

CP: Carbamide Peroxide.

DNA: Deoxyribonucleic Acid.

VS: VivaStyle Paint On Plus – Ivoclar Vivadent ®, Liechtenstein.

OP: Opalescence Boost – Ultradent ®, United States of America.

MCC: Minimum Cytotoxic Concentration.

ID50: 50% Inhibitory Dose of hydrogen peroxide to succinyl dehydrogenase activity in cultured cells.

95% CI: Confidence Interval considering $\alpha = 5\%$.

A: Anterior Teeth groups.

PM: Premolar Teeth groups.

M: Molar Teeth groups.

1. INTRODUCTION

In the last few decades, the demand for whiter teeth has increased, making teeth whitening the most popular elective dental procedure. [1-3] It is minimally invasive and easily accessible, resorting mainly to active principles such as hydrogen peroxide (HP) and carbamide peroxide (CP). [1-5] When associated with carbopol and glycerin, CP displays an increased structural stability and sustained action. Therefore, CP is usually used in ambulatory bleaching techniques, with concentrations between 10% - 16% CP [1, 2, 6]. When in contact with water, a solution of 10% CP will release oxygen, dividing itself into hydrogen peroxide (3.35%) and urea (6.66%). [1, 2] Instead, HP is more structurally unstable, has a swifter action and hydrolysis, breaking into water and free oxygen radicals [1, 2, 6], being the main active principle in in-office techniques, with concentrations ranging from 5% to 40%. [2, 6]

According to the European Union's Directive [7], teeth whitening can be performed through over-the-counter products (containing 0.1% HP or less), and through in-office and ambulatory techniques (containing 6% HP or equivalent, except if the products are marketed as medical devices).

Teeth whitening uses hydrogen particles and free oxygen radicals to remove chromogens from the tooth surface. However, due to a low enough molecular weight to penetrate through the dental tissues, these molecules can achieve the pulp chamber, thus causing cytotoxic effects.[6, 8, 9] Due to the toxic potential of free oxygen radicals, teeth whitening can cause clinical and histologic side effects, such as, dental sensitivity[8, 10-12], a decrease of adhesive properties of restorative dental materials[8, 10, 17-18], ulceration of soft tissues[19], DNA damage in odontoblasts[13-15], changes in enamel and dentin[8, 10, 16], and odontoblast alterations [20-22], decreasing cellular metabolism and viability.[19-21, 23-25]

There are two factors that decrease the molecular diffusion through the dental tissues into the pulp chamber: positive pulpar pressure and the whitening product's osmotic pressure. [19] Even in products with similar concentrations of HP, the diffusion and the biological effects are influenced by the product's brand and composition. [23]

Although some studies have tried to investigate the HP penetration through human dental intact tissues[26-32], there are no in vitro models that resemble in vivo conditions, including positive pulpar pressure, except for two pilot studies.[33-34] Due to blood flow, pulpar tissues apply a positive pressure on the remaining dental structure.[35] Ciucchi 1995 [35], simulated positive pulpar pressure on intact human teeth, measuring fluid movement through the dentin, according to the application of an exogenous pressure. Ciucchi

concluded that positive pulpar pressure is equivalent to the pressure generated by a 14 cm column of water. [35] Therefore, it would be important to evaluate the diffusion of HP present in different whitening products with different concentrations, while using a positive pulpar pressure model.

The present study aims to evaluate the diffusion of two in-office whitening products (with 6% and 40% HP) in different tooth types.

Null Hypothesis: There are no differences in the hydrogen peroxide diffusion kinetics between the two whitening products.

Secondary Aims: To evaluate the diffusion kinetics on different types of teeth, and the influence of area of exposure, pulp chamber volume and dental tissues thickness in the hydrogen peroxide diffusion kinetics of the two whitening products.

2. MATERIALS AND METHODS

Two in-office whitening products were used in the present study: a varnish system with 6% HP (*VivaStyle Paint On Plus* – VS - *Ivoclar - Vivadent®, Liechtenstein*) and a gel system with 40% HP (*Opalescence Boost* – OP - *Ultradent®, United States of America*). The concentration of HP was confirmed in both products through titration, resorting to the Cerium IV Sulfate (1M) technique described by Da Silva Marques 2012 and Cardoso 2015. [36, 33] Three measurements were performed for each whitening product. 500 mg of the product were dissolved in 225 mL of distilled water with 25 mL of a 1:5 dilute sulfuric acid solution being added. 5 mL of indicator solution of ferrocene were also added under agitation, turning the final solution orange. Each product sample was titrated until reaching the equivalence point, at which the solution became light blue. Resorting to the volume of Cerium IV Sulfate (1M) required to attain the equivalence point, it was possible to calculate the percentage of HP present in each sample. [37]

$$\%H_2O_2\ (w/w) = (VCeSO_4.4H_2O\ (mL) * 0.17) : \text{sample weight (g)}$$

EQUATION 1: Calculation of HP concentration in product formulation. $\%H_2O_2$ (w/w): HP concentration in % (w/w). $VCeSO_4.4H_2O$ (mL): volume of Cerium IV Sulfate (1M) required to reach the equivalence point.

2.1 Tooth Samples Preparation

Teeth were stored in a chloramine-based solution, in a cold and dry environment for no longer than 6 months. Thirty-six teeth in the absence of caries, restorations, or any development anomalies were used and distributed as described in Diagram 1. Teeth in each group (Anterior, Premolar and Molar) were randomly allocated to each whitening product with the use of an established software (*G*Power*® (version 3.1.9.7) – *Heinrich-Hein-University, Germany*).

Using a precision saw (*IsoMet 1000 Precision Saw – Buehler®, United States of America*), the sampled teeth were sectioned horizontally 2 to 3 mm bellow the cementoenamel junction. The pulpar tissue was removed with a round bur. The radicular portion was discarded.

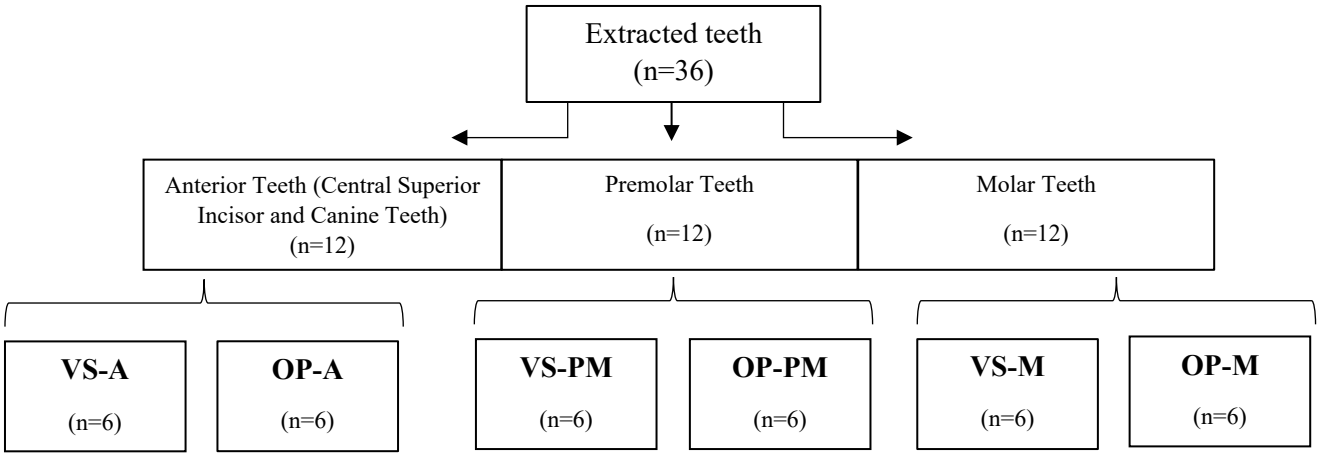
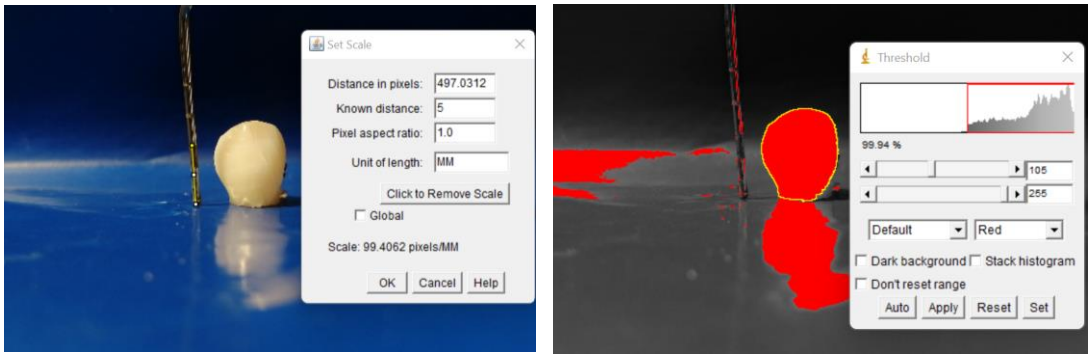


DIAGRAM 1: Sample Distribution. *VivaStyle Paint On Plus* varnish system with 6% HP **(VS) 6x 10-minute applications** at room temperature**.** *Opalescence Boost* gel system with 40% HP **(OP) 3x 20-minute applications** at room temperature.

The volume of the pulp chamber of each tooth sample was determined by subtracting the weight of the sample with an empty pulp chamber from the weight of the sample with their pulp chamber filled with distilled water. The thickness of the buccal surface of each sample was determined by resorting to a thickness gauge. Three measures were registered per sample: cervical, buccal, and incisal/occlusal. The area of the exposed surface was determined by a software that allowed for the calculation of irregular areas through a previously set scale *(ImageJ®, United States of America)*. Three calculations were registered per sample.



(a) **(b)**

FIGURE 1: (a) A periodontal probe was used to provide a known distance and to set the scale. (b) The photograph was transformed into an 8-bit file. The desired area was automatically selected before measuring.

Tooth samples were adhered to polycarbonate plates with cyanoacrylate *(SuperCola* 3®, *Loctite, Henkel Adhesives, Germany)*. The plates were drilled twice, allowing for the communication with the internal part of the pulp chamber. Every surface of the sample was isolated using nail polish *(Kiko Milano®, Italy),* except for the buccal surface, being exposed to the whitening product. 27G needles *(Luer Lock ®, B. Braun Melsungen, Germany)* were adapted to the polyacrylate plate. The entry tube was connected to a 14 cm tall column of sodium acetate (2M) buffer solution with a 4.5 pH, which allowed for the positive pulpar pressure. [35, 38-39] The exit tube was sealed with a needle holder during the application of the whitening product. Whenever necessary, the needle holder was removed to collect buffer samples.

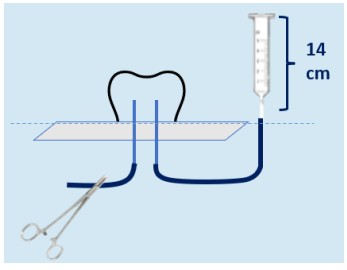


FIGURE 2: Representation of a Positive Pulpar Pressure Model.

2.1 Buffer Samples Collection

Samples (0.125 mL) were collected every 10 minutes during product application and for 30 minutes after the last application. Before product application, a buffer sample was collected through the system, to establish the basal value for each tooth sample. The whitening products were applied to the buccal surface of the tooth samples according to the indications of the manufacturer. The applied amount was determined by weighting the product container before and after every application. Before applying a new product layer, the teeth samples were cleaned with distilled water and a cotton pellet.

The diffused hydrogen peroxide concentration in the pulpar buffer was measured using a spectrophotometer at a wavelength of 596 nm at room temperature. As described by Mottola 1970 [40], the absorbance of 10 standard concentrations of hydrogen peroxide (ranging from 0 to 0.65 μg/mL) were measured to calibrate the spectrophotometer. For each collection time, a falcon tube was filled with a 0.125 mL extract from each tooth sample and mixed with 2 mL of sodium acetate buffer solution (2M, pH 4.5). 0.5 mL of leucocrystal violet solution (0.5 mg/mL; *Sigma-Aldrich Corporation, United States of America*), 0.25 mL of horseradish peroxidase solution (1 mg/mL; *Sigma-Aldrich Corporation, United States of America*) and 2.125 mL of distilled water were later added. The falcon tubes were vortexed, and the mixture transferred to microcuvettes to be measured in the spectrophotometer.

To infer on the toxic potential of HP, the minimum cytotoxic concentrations for odontoblasts (MCC) established by De Lima 2009[25] (2.22 μg/mL) and Almeida 2013[24] (4.70 μg/mL) were compared to results. The ID50 of HP for odontoblasts determined by Hanks 1993[19] (0.58 mmol/L which is equivalent to 19.73 μg/mL) was also considered.

The statistical analysis was performed with IBM SPSS® (version 25) (IBM Statistics, Inc. Chicago, IL, EUA), resorting to statistically significant differences were one sample t-test (to compare titration values to manufacturer's percentage of HP), the Kolmogorov-Smirnov test (to assess the normality of the distribution of each variable), t-tests with independent samples and unknown variances (to evaluate whether the mean value of HP diffusion for each time slot and tooth type is independent of the product used), Pearson Correlations (to determine the influence of area, volume and thickness in diffused HP weight), the Kruskal Wallis Test (to determine the influence of tooth type in diffused HP weight) and Linear Regression Models. Results were presented as mean values with

a confidence interval (CI) of 95%. Only p-values under 0.05 were considered statistically significant.

3. RESULTS

3.1 Titration

Three titration measurements were performed for each whitening product. For the VS varnish system, an average percentage of HP of 6.15% [5.93; 6.37] was determined, and for the OP gel system, an average percentage of HP of 41.04% [37.59; 44.51] was determined. Both were higher concentrations than the ones reported by the manufacturers. No statistically significant differences were detected between the measured values and the ones presented by the manufacturers.

3.2 HP Diffusion Kinetics

Following the manufacturer's instructions, in groups VS-A, VS-PM and VS-M the product was applied at 0', 10', 20', 30', 40', 50', and 60'; and in groups, OP-A, OP-PM, and OP-M the product was applied at 0', 20' and 40' minutes.

The cumulative total of HP after 90 minutes, was 1.333 μg [1.214; 1.452] for the VS-A group and 1.538 μg [1.457; 1.620] for the OP-A group. The VS-PM and the OP-PM registered a mean total retrieved amount of HP of 1.208 μg [1.123; 1.291] and 3.628 μg [3.401; 3.855], with statistically significant differences between the groups. The VS-M and OP-M groups registered values of 2.560 μg [2.297; 2.823] and 4.197 μg [3.997; 4.396], with statistically significant differences between the groups.

When comparing results between groups with the same whitening product, there were statistically significant differences in the diffusion kinetics between VS-A and VS-PM when compared to VS-M. On the contrary, in the OP group there were statistically significant differences between OP-PM and OP-M when compared to OP-A.

Regarding the Anterior Teeth Groups, it can be stated that the VS-A group registered the maximum value of HP diffusion (0.362 μg) at 60', whereas the OP-A group attained a maximum value of 0.325 μg at 50'. One can state that the VS-PM group registered the maximum value of HP diffusion at 60' (0.279 μg), whereas the OP-PM group reached the maximum value at 70' (0.488 μg), even though the diffusion values remained constant from 10' to 70'. Considering the analysis of the Molar Teeth groups, the VS-M group registered the maximum value of HP diffusion at 50' (0.625 μg), whereas

the OP-M group reached the maximum value at 60’ (0.865 μg), even though a peak of diffusion was also registered at 20’ (0.755 μg).

Time (min)	VS-A (n=6)	OP-A (n=6)	VS-PM (n=6)	OP-PM (n=6)	VS-M (n=6)	OP-M (n=6)	VS Overall (n=18)	OP Overall (n=18)
10’	0.081 [0.060; 0.102]	0.032 [0.028; 0.036]	0.071* [0.058; 0.084]	0.482* [0.423; 0.540]	0.062 [0.049; 0.075]	0.182 [0.130; 0.234]	0.071* [0.062; 0.080]	0.234* [0.189; 0.279]
20’	0.101 [0.081; 0.121]	0.085 [0.080; 0.090]	0.093* [0.078; 0.108]	0.432* [0.376; 0.487]	0.080* [0.053; 0.107]	0.755* [0.717; 0.793]	0.091* [0.079; 0.103]	0.424* [0.365; 0.483]
30’	0.086 [0.065; 0.107]	0.075 [0.058; 0.092]	0.095* [0.080; 0.110]	0.415* [0.391; 0.439]	0.158 [0.093; 0.223]	0.354 [0.315; 0.393]	0.113* [0.090; 0.136]	0.281* [0.248; 0.315]
40’	0.103 [0.075; 0.131]	0.176 [0.152; 0.200]	0.157* [0.137; 0.177]	0.438* [0.395; 0.481]	0.274 [0.227; 0.321]	0.432 [0.394; 0.470]	0.178* [0.154; 0.201]	0.349* [0.317; 0.380]
50’	0.321 [0.221; 0.421]	0.325 [0.283; 0.367]	0.167* [0.151; 0.183]	0.391* [0.340; 0.442]	0.625 [0.538; 0.712]	0.638 [0.554; 0.722]	0.371 [0.314; 0.428]	0.451 [0.408; 0.495]
60’	0.362 [0.279; 0.445]	0.319 [0.292; 0.346]	0.279* [0.260; 0.298]	0.470* [0.425; 0.515]	0.523 [0.420; 0.626]	0.865 [0.774; 0.956]	0.388 [0.340; 0.435]	0.551 [0.494; 0.609]
70’	0.164 [0.090; 0.238]	0.224 [0.177; 0.271]	0.197* [0.137; 0.257]	0.488* [0.450; 0.525]	0.405 [0.265; 0.545]	0.413 [0.385; 0.441]	0.255 [0.197; 0.314]	0.375 [0.344; 0.406]
80’	0.072* [0.061; 0.083]	0.178* [0.146; 0.210]	0.120* [0.081; 0.159]	0.389* [0.367; 0.411]	0.243 [0.197; 0.289]	0.336 [0.302; 0.370]	0.145* [0.121; 0.169]	0.301* [0.277; 0.326]
90’	0.045* [0.037; 0.053]	0.118* [0.103; 0.132]	0.030* [0.026; 0.034]	0.124* [0.091; 0.157]	0.189 [0.144; 0.234]	0.223 [0.196; 0.250]	0.088* [0.068; 0.109]	0.155* [0.138; 0.172]

TABLE 1: Mean Recovered HP Weight (μg) and 95% CI per Time Slot (min).

* The Null hypothesis is rejected.

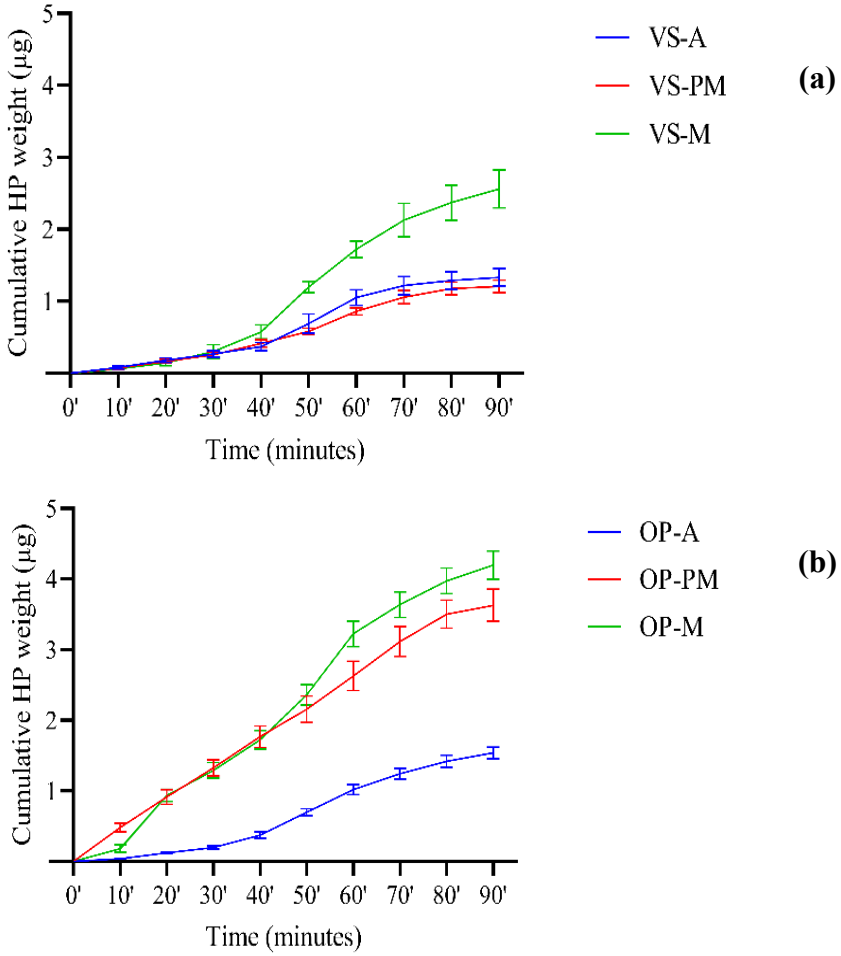


FIGURE 3: (a) Mean Cumulative Recovered HP Weight (µg) for VS application groups and 95% CI. (b) Mean Cumulative Recovered HP Weight (µg) for OP application groups and 95% CI.

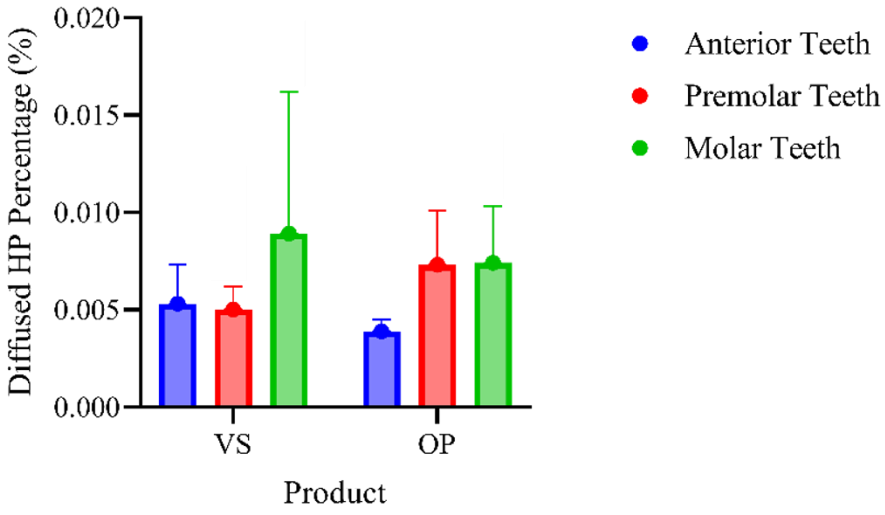


FIGURE 4: Mean Percentage of Recovered HP (%) and upper limit of the 95% CI.

90 minutes after the beginning of the whitening product application protocol, the VS groups registered retrieved percentages ranging from 0.00499% to 0.00887% of the applied HP content. Meanwhile, the OP groups registered the diffusion of 0.00386% to 0.00737% of the applied HP.

Pearson Correlations were determined between the diffused HP total weight and a second variable (area of exposure, pulp chamber volume and thickness of the buccal surface). It was possible to verify that the variables are correlated in a positive way, being the strongest correlation between diffused HP total weight and pulp chamber volume. The correlation between the area of exposure, pulp chamber volume and thickness of buccal surface were 0.077; 0.536, and 0.312, respectively.

The Kruskal Wallis Test demonstrated that the type of tooth significantly influences the median HP total weight obtained from the pulpar chamber.

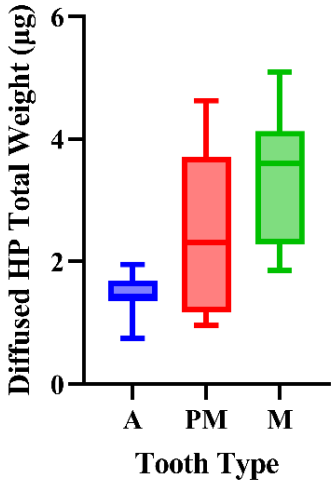


FIGURE 5: Box-plot representation of the influence of the tooth type on the Diffused HP Total Weight (µg)

To evaluate the correlation between the recovered HP weight (dependent variable), and the applied whitening product, the tooth type, and a fourth variable (area of exposure, pulp chamber volume, or thickness of the buccal surface), multiple linear regression models were developed.

Of the tested models, the model that evaluated tooth type versus whitening product presented statistically significant results for all variables. Taking this into account, the following equation was estimated, where PM?, M? and OP? are dummy variables representing whether or not the tooth is premolar, molar and whether or not the whitening product is OP, respectively.

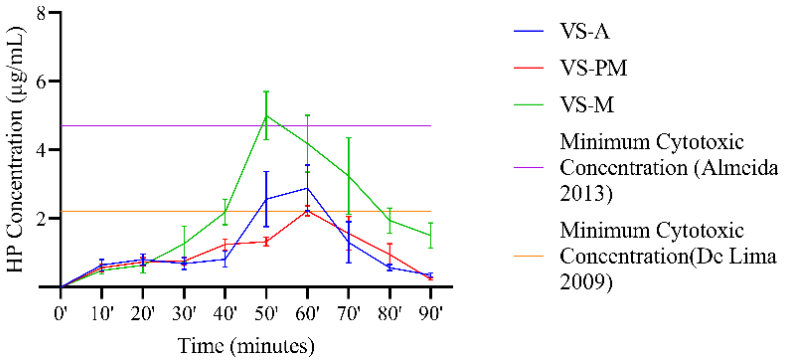
diffused HP total weight (µg) = 0.725 + 0.982(PM?) + 1.943(M?) + 1.4210(OP?)

EQUATION 2: Proposed model for the calculation of diffused HP total weight (µg) 90 minutes after the start of the application protocol with OP or VS in Anterior, Premolar, and Molar Teeth.

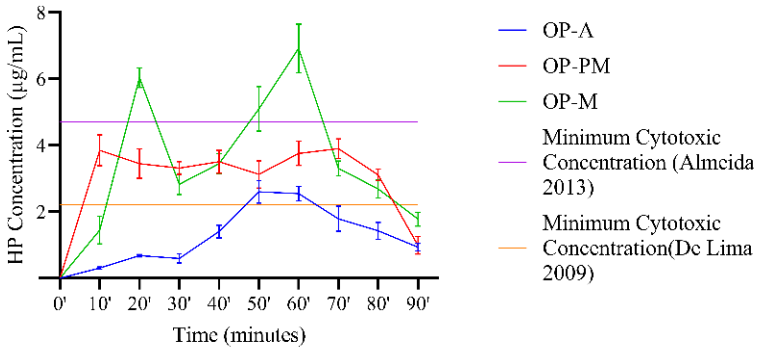
This model explains 72% of the variability verified in the diffused HP weight. This equation proposes that after the application of the whitening product, on average, in a Premolar Tooth and a Molar Tooth there will be diffusion of more 0.482 µg and 1.943 µg of HP to the pulpar chamber, respectively, when compared to an anterior tooth. It is also expected that the application protocol of the OP whitening product will lead to the diffusion of more 1.421 µg of HP when compared to the application protocol of the VS whitening product, independently of the tooth type.

3.3 HP Concentration in the Pulp Chamber

Considering the diffused HP weight (µg) and sample volume (mL) it was possible to determine the HP concentration (µg/mL) in the pulp chamber for each time slot (see figure 5).



(a)



(b)

FIGURE 6: (a) Mean HP Concentration (µg/mL) per tooth type for VS groups and 95% CI. (b) Mean HP Concentration (µg/mL) per tooth type for OP groups and 95% CI.

4. DISCUSSION

In this study, the in vitro hydrogen peroxide diffusion kinetics of two in-office whitening products was assessed, as well as the influence of different types of teeth, area of exposure, pulp chamber volume and dental tissues thickness on this diffusion. The results showed that there were statistically significant differences of hydrogen peroxide diffusion kinetics between the two whitening products.

In the Anterior Teeth groups (Table 1) significant differences in the diffusion were detected for later time slots (80' and 90') but not for the cumulative amount of retrieved HP. For Premolar Teeth groups, statistically significant differences were observed for all time slots and for the cumulative amount of diffused HP. Molar Teeth groups presented a statistically significant difference for an earlier time slot (20'), and for the cumulative HP weight.

The obtained different time patterns of statistically significant differences per tooth type could be due to differences in the area of exposure, the thickness of the buccal surface, and most probably pulp chamber volume (which has a higher correlation with the diffused HP weight). A larger sample is required to infer the exact influence of these variables on the diffused HP weight.

Studies that used intact human teeth and resorted to the violet leucocrystal and horseradish peroxidase spectrophotometry method to evaluate HP diffusion kinetics to the pulp chamber were assessed. Gokay 2005[29], and Bharti 2013[31], both resorted to central maxillary incisors and 30-minutes application protocols (with formulations ranging from 5.3% to 8.7% HP or equivalent to), retrieving HP weights ranging from 0.175 µg to 0.398 µg. Kwon 2012[26] and Cardoso 2015[33] used canine teeth and applied a 40% HP product for 60 minutes (three 20-minute applications), registering weights from 0.8 to 0.93 µg of diffused HP. Vieira 2018[32], used third molars and applied a product with 38% HP, registering a diffusion of 1.2056 µg after 45 minutes. For the same tooth types, and for the same application time, this study registered the diffusion of 0.268 µg, 1.012 µg and 1.723 µg HP, respectively, being in agreement with the existing literature.

Even though VS and OP have different concentrations of HP in the initial product formulation, the resulting diffused HP weight to the pulpar chamber is similar. A varnish system (such as VS) is composed of a cellulose matrix and an ethanolic base, and for the matrix to solidify, the ethanolic base must evaporate, allowing the HP to reach a higher superficial concentration, a higher concentration gradient, and a higher diffusion rate than expected.

Regarding cytotoxicity levels, most samples reached HP concentrations under the MCC of 4.70 μg/mL, as described by Almeida 2013.[24] However, when compared to the MCC determined by De Lima 2009[25], all groups surpassed the cytotoxic limit at some point in time: the VS-A group at 50' and 60', the VS-PM group at 60', the VS-M group from 40' until 80', the OP-A group at 50' and 60', the OP-PM group from 10' until 80', and the OP-M group from 20' until 80'. The diffusion of HP even after the conclusion of the application protocol can lead to cumulative cytotoxicity, however, none of the concentrations obtained surpassed the ID50 value stated by Hanks 1993. [19]

Further in-vitro studies should include different whitening products and HP concentrations and more specific sample groups (i.e., only central maxillary incisors, only mandibular premolars). Moreover, future clinical studies should assess which tooth type is more prone to post-whitening symptomatology.

5. CONCLUSION

The two products presented differences in the hydrogen peroxide diffusion kinetics to the pulp chamber for all types of teeth.

The proposed model for the calculation of diffused HP total weight states that OP is expected to lead to the diffusion into the pulpar chamber of more 1.421 μg of HP when compared to the application protocol of the VS whitening product, independently of the type of tooth.

The area of exposure, the thickness of the buccal surface, and the pulp chamber volume of the samples have a non-significant positive correlation with the final diffused hydrogen peroxide.

The present study demonstrates that odontoblasts may be exposed to HP and free oxygen radicals in cytotoxic concentrations because of teeth whitening (molars being the highest risk tooth type). The diffused HP values depend on the whitening product.

6. REFERENCES

1. Alkahtani R, Stone S, German M, Waterhouse P. A review on dental whitening. Journal of Dentistry. 2020;100:103423.

2. Kwon S, Wertz P. Review of the Mechanism of Tooth Whitening. Journal of Esthetic and Restorative Dentistry. 2015;27(5):240-257.

3. Maran B, Burey A, de Paris Matos T, Loguercio A, Reis A. In-office dental bleaching with light vs. without light: A systematic review and meta-analysis. Journal of Dentistry. 2018;70:1-13.

4. de Geus J, Wambier L, Kossatz S, Loguercio A, Reis A. At-home vs In-office Bleaching: A Systematic Review and Meta-analysis. Operative Dentistry. 2016;41(4):341-356.

5. Joiner A, Luo W. Tooth colour and whiteness: A review. Journal of Dentistry. 2017;67:S3-S10.

6. Demarco F, Meireles S, Masotti A. Over-the-counter whitening agents: a concise review. Brazilian Oral Research. 2009;23(suppl 1):64-70

7. DIRECTIVA 2011/84/UE DO CONSELHO Europeu, 20 de setembro de 2011. Jornal Oficial da União Europeia; 2011.

8. Rodríguez-Martínez J, Valiente M, Sánchez-Martín M. Tooth whitening: From the established treatments to novel approaches to prevent side effects. Journal of Esthetic and Restorative Dentistry. 2019;31(5):431-440.

9. Grazioli G, Valente LL, Isolan CP, Pinheiro HA, Duarte CG, Münchow EA. Bleaching and enamel surface interactions resulting from the use of highly-concentrated bleaching gels. Arch Oral Biol. 2018;87:157-162.

10. Goldberg M, Grootveld M, Lynch E. Undesirable and adverse effects of tooth-whitening products: a review. Clin Oral Investig. 2010;14: 1-10.

11. Rodd HD, Boissonade FM. Substance P expression in human tooth pulp in relation to caries and pain experience. Eur J Oral Sci. 2000; 108:467-474.

12. Bowles WR, Withrow JC, Lepinski AM, Hargreaves KM. Tissue levels of immunoreactive substance P are increased in patients with irreversible pulpitis. J Endod. 2003;29:265-267.

13. Sacono N. Efeito citotóxico de agentes clareadores à base de peróxido de hidrogénio a 20% e 38% sobre células odontoblastóides. Revista Odontológica do Brasil Central. 2010;19(48).

14. Bianchi L, Ribeiro A, de Oliveira Carrilho M, Pashley D, de Souza Costa C, Hebling J. Cytotoxicity of adhesive systems of different hydrophilicities on cultured odontoblast-like cells. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2013;101(8):1498-1507.

15. Dias Ribeiro A, Sacono N, Lessa F, Nogueira I, Coldebella C, 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 Endodontology. 2009;108(3):458-464.

16. Nilsson E, Bonte E, Bayet F, Lasfargues J. Management of Internal Root Resorption on permanent teeth Management of Internal Root Resorption on permanent teeth. Int J Dent. 2013;2014:1-7.

17. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations—a systematic review. Dent Mater. 2004;20:852-861

18. Xu Y, Zhou J, Tan J. Use of grape seed extract for improving the shear bond strength of total-etching adhesive to bleached enamel. Dent Mater J. 2018;37:325-331.

19. Hanks C, Fat J, Wataha J, Corcoran J. Cytotoxicity and Dentin Permeability of Carbamide Peroxide and Hydrogen Peroxide Vital Bleaching Materials, in vitro. Journal of Dental Research. 1993;72(5):931-938.

20. Huck C. Efeito Citotóxico Transdentinário do Peróxido de Hidrogénio em Diferentes Concentrações sobre Células de Linhagem Odontoblástica MDPC-23 [Pós-Graduação em Odontologia – Área de Dentística Restauradora]. Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista; 2005.

21. Soares D, Basso F, Hebling J, de Souza Costa C. Concentrations of and application protocols for hydrogen peroxide bleaching gels: Effects on pulp cell viability and whitening efficacy. Journal of Dentistry. 2014;42(2):185-198.

22. Coldebella C, Ribeiro A, Sacono N, Trindade F, Hebling J, Costa C. Indirect cytotoxicity of a 35% hydrogen peroxide bleaching gel on cultured odontoblast-like cells. Brazilian Dental Journal. 2009;20(4):267-274.

23. Llena C, Collado-González M, García-Bernal D, Oñate-Sánchez R, Martínez C, Moraleda J et al. Comparison of diffusion, cytotoxicity and tissue inflammatory reactions of four commercial bleaching products against human dental pulp stem cells. Scientific Reports. 2019;9(1).

24. Almeida L. Avaliação da alteração de cor, difusão de peróxido de hidrogênio e citotoxicidade trans-amelodentinária causadas por diferentes técnicas de clareamento dental. Universidade de São Paulo; 2013.

25. De Lima, A.F. et al., 2009. Cytotoxic effects of different concentrations of a carbamide peroxide bleaching gel on odontoblast-like cells MDPC-23. Journal of biomedical materials research. Part B, Applied biomaterials, 90(2), pp.907–12.

26. Kwon S, Yiming L, Oyoyo U, Aprecio R. Dynamic Model of Hydrogen Peroxide Diffusion Kinetics into the Pulp Cavity. The Journal of Contemporary Dental Practice. 2012;13(4):440-445.

27. Kwon S, Wertz P, Dawson D, Cobb D, Denehy G. The Relationship of Hydrogen Peroxide Exposure Protocol to Bleaching Efficacy. Operative Dentistry. 2013;38(2):177-185.

28. Gokay O, Mujdeci A, Algin E. Peroxide Penetration Into The Pulp From Whitening Strips. Australian Endodontic Journal. 2005;31(1):38-38.

29. Gokay O, Mujdeci A, Algin E. In vitro peroxide penetration into the pulp chamber from newer bleaching products. International Endodontic Journal. 2005;38(8):516-520.

30. Bowles W, Ugwuneri Z. Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. Journal of Endodontics. 1987;13(8):375-377.

31. Bharti R, Wadhwani W. Spectrophotometric evaluation of peroxide penetration into the pulp chamber from whitening strips and gel: An in vitro study. Journal of Conservative Dentistry. 2013;16(2):131.

32. Vieira L. Difusão do Peróxido de Hidrogénio de Agentes Branqueadores de Consultório com Diferentes Concentrações e Composições [Mestrado Integrado em Medicina Dentária]. Universidade Fernando Pessoa; 2018.

33. Cardoso C. Influência da pressão pulpar positiva na difusão de peróxido de hidrogénio de um produto de branqueamento através dos tecidos dentários in vitro – ensaio piloto [Mestrado Integrado]. Universidade de Lisboa; 2015.

34. Casqueiro L, Dias S, Pereira R, Silveira J, Mata A, Marques D. Difusão pulpar do peróxido de hidrogénio de um produto de branqueamento - estudo in vitro. Presentation presented at the 41st Congresso Anual SPEMD 2021.

35. Ciucchi B, Bouillaguet S, Holz J, Pashley D. Dentinal fluid dynamics in human teeth, in vivo. Journal of Endodontics. 1995;21(4):191-194.

36. Da Silva Marques, D.N. et al, Kinetic release of hydrogen peroxide from different whitening products. The European journal of esthetic dentistry: official journal of the European Academy of Esthetic Dentistry, 2012, 7(3), pp.344–52.

37. Vogel, A. I., & Jeffery, G. H., Vogel's Textbook of Quantitative Chemical Analysis. Wiley. 5th ed. /. Harlow Essex England; New York: Longman Scientific & Technical; Wiley; 1998.

38. Feitosa V, Gotti V, Grohmann C, Abuná G, Correr-Sobrinho L, Sinhoreti M et al. Two methods to simulate intrapulpal pressure: effects upon bonding performance of self-etch adhesives. International Endodontic Journal. 2014;47(9):819-826.

39. Sauro S, Pashley D, Montanari M, Chersoni S, Carvalho R, Toledano M et al. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dental Materials. 2007;23(6):705-713.

40. Mottola H, Simpson B, Gorin G. Absorptiometric determination of hydrogen peroxide in submicrogram amounts with leucocrystal violet and peroxidase as catalyst. Analytical Chemistry. 1970;42(3):410-411.