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VERUSKA LIMA MOURA BRASIL

AVALIAÇÃO DO EFEITO DO PERÓXIDO DE HIDROGÊNIO EM ALTAS CONCENTRAÇÕES SOBRE A POLPA DENTÁRIA DE RATOS: ESTUDO HISTOLÓGICO

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Tese apresentada ao Colegiado do Programa de Pós-Graduação em Odontologia do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, como requisito parcial para obtenção do grau de doutor em Odontologia, área de concentração em Clínica Odontológica Integrada.

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Orientador:

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BANCA EXAMINADORA

_____

Profa. Dra. ELAINE JUDITE DE AMORIM CARVALHO (Examinador Externo)
Universidade Federal de Pernambuco

_____

Prof. Dr. ROBINSOM VIEGAS MONTENEGRO (Examinador Externo)
Universidade Federal da Paraíba

_____

Prof. Dr. GUSTAVO PINA GODOY (Examinador Interno)
Universidade Federal de Pernambuco

Profa. Dra. ANDREA CRUZ CÂMARA (Examinador Interno)
Universidade Federal de Pernambuco

Prof. Dr. DANYEL ELIAS CRUZ PEREZ (Presidente)
Universidade Federal de Pernambuco

DEDICATÓRIA

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“Você não está atrasado, nem adiantado. Você está exatamente na hora certa. ”

(SHANKAR, 1988, p. 33)

RESUMO

A demanda por procedimentos conservadores estéticos na Odontologia fez o clareamento de consultório ser requisitado de maneira ascendente. Além do efeito clareador, o peróxido de hidrogênio (PH) usado no tratamento clareador também causa sensibilidade dentária e injúrias ao tecido pulpar, fazendo com que seja necessário manter as técnicas em constante estudo. O objetivo do estudo foi avaliar *in vivo* os efeitos agudos em polpas dentárias vitais de molares de ratos submetidos a altas concentrações de géis clareadores a base de PH em diferentes períodos de análise. Para tanto, 120 molares maxilares e mandibulares de 30 ratos Wistar foram distribuídos em grupos de acordo com o agente clareador: 1–35% PH, 2–38% PH, 3-40% PH, 4-sem tratamento (controle) e com o período de análise: A-sessão única (3x15 minutos) com análise imediata, B-duas sessões (3x15 minutos) com análise imediata e C- duas sessões (3x15 minutos) com análise após 72 horas (n=12/grupo). Os animais foram eutanasiados imediatamente ou 72 horas após o clareamento e os maxilares foram submetidos a processamento laboratorial de rotina para análise histológica de cada terço da polpa. Foram atribuídos escores de acordo com a quantidade de células inflamatórias, condição da camada odontoblástica, alterações vasculares e desorganização tecidual. Os dados foram analisados estatisticamente usando os testes de Kruskal-Wallis e Dunn ($p<0.05$). As três concentrações de PH alteraram significativamente as características pulpares. Houve necrose envolvendo os terços oclusal, médio e cervical da polpa coronária e o terço cervical da polpa radicular nos grupos 2B, 3A e 3B. A camada odontoblástica foi alterada em todos os terços dos grupos 2B, 3A, 3B e 3C. Áreas de edema, hemorragia e vasos dilatados e congestos apareceram até o terço médio da polpa radicular dos grupos 1B, 2A, 2B, 3A e 3B. Os grupos C não apresentaram desorganização tecidual significativa. Em conclusão, PH a 35%, 38% e 40% induziram dano imediato a polpas vitais de molares de ratos. PH 40% causou alterações mais severas. Após 72 horas, a polpa demonstrou sinais de reparo e envelhecimento.

Palavras-chave: clareamento dental; inflamação; modelos animais; peróxido de hidrogênio; polpa dentária.

ABSTRACT

The demand for rapid esthetic conservative prodecures in Dentistry has made in office bleaching be ascendingly requested. Besides the bleaching effect, hydrogen peroxide used in the protocol also lead to tooth sensibility and injuries to the pulp tissue, making it necessary to keep the techniques under study. The aim of the study was to evaluate *in vivo* the acute effects in vital dental pulps of rat molars subjected to high concentrations of hydrogen peroxide (HP) bleaching gels in different periods of analysis. Therefore, 120 maxillary and mandibular molars from 30 Wistar rats were assigned to groups according to the bleaching agent: 1–35% HP, 2–38% HP, 3-40% HP, 4-untreated (control) and to the period of analysis: A-a single session (3X15 minutes) with immediate analysis, B- two sessions (3X15 minutes) with immediate analysis and C- two sessions (3X15 minutes) with analysis after 72 hours (n=12/group). The animals were sacrificed immediately after bleaching or 72 hours later and jaws were subjected to routine laboratory processing for histological analysis of each third of the pulp. Scores were attributed to inflammatory cell count, condition of odontoblastic layer, vascular changes and tissue disorganization. Data were statistically analysed using Kruskal-Wallis and Dunn's tests ($p<0.05$). The three HP concentrations significantly changed pulp characteristics. There was necrosis extensive to the occlusal, middle and cervical thirds of coronal pulp and to the cervical third of radicular pulp in groups 2B, 3A and 3B. Odontoblastic layer was altered in all thirds of groups 2B, 3A, 3B and 3C. Areas of edema, bleeding, congested and dilated blood vessels appeared up to the middle third of radicular pulp of groups 1B, 2A, 2B, 3A and 3B. Groups C did not show significant tissue disorganization. In conclusion, 35%, 38% and 40% HP induced immediate damage vital dental pulps of rat molars. 40% HP led to more severe alterations. After 72 hours, pulp demonstrated signs of repair and aging.

Key words: animal models; dental pulp; hydrogen peroxide; inflammation; tooth bleaching.

LISTA DE ILUSTRAÇÕES

INTRODUÇÃO E METODOLOGIA

Figura 1 - A) Posicionamento, abertura bucal e afastamento da mucosa jugal do animal em mesa operacional. (B) Afastador para mucosa jugal confeccionado com fio ortodôntico..... 14

Figura 2 - Agentes clareadores utilizados. (A) Peróxido de Hidrogênio 35%/Whiteness HP Blue. (B) Peróxido de Hidrogênio 38%/Opalescence Boost. (C) Peróxido de Hidrogênio 40%/Opalescence Boost..... 14

Figura 3 - (A) Equipamento para processamento histotécnico. (B) Micrótomo manual. (C) Banho histológico..... 17

Figura 4 - Equipamento utilizado para escaneamento das lâminas..... 18

Figura 5 - Divisão do tecido pulpar em terços..... 18

Quadro 1 - Divisão dos grupos experimentais..... 13

Quadro 2 - Agentes clareadores utilizados..... 15

Quadro 3 - Condição da camada odontoblástica..... 19

Quadro 4 - Alteração vascular..... 19

Quadro 5 - Desorganização tecidual..... 20

Quadro 6 - Intensidade do infiltrado celular inflamatório..... 21

ARTIGO

Figura 1 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the control group. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, and black arrows show the balanced distribution of extracelelular matrix components, cells and blood vessels..... 29

Figura 2 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G1A. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, yellow arrows indicate odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells, white asteristics indicate dilated and congested blood vessels and black circles indicate areas of bleeding..... 31

Figura 3 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G1B. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, N = necrotic areas, yellow arrows indicate odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells, white asterisks indicate dilated and congested blood vessels and black circles indicate areas of bleeding..... 32

Figura 4 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G1C. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, white asteristics indicate dilated and congested blood vessels and black circles indicate areas of bleeding..... 33

Figura 5 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G2A. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, N = necrotic areas, yellow arrows show odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells, white asteristics indicate dilated and congested blood vessels and yellow circles indicate inflammatory cells..... 35

Figura 6 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G2B. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, N = necrotic areas, yellow arrows indicate odontoblastic cells sucked into pre-dentin layer, white asterisks indicate dilated and congested blood vessels, black circles indicate areas of bleeding and yellow circles indicate inflammatory cells..... 36

Figura 7 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the group 2C. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer and black asteristics indicate areas of edema and absence of cells..... 37

Figura 8 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G3A. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, yellow arrows show odontoblastic cells sucked into pre-dentin layer and black asterisks indicate areas of edema and absence of cells..... 39

Figura 9 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G3B. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, yellow arrows present odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells and white asterisks indicate dilated and congested blood vessels..... 40

Figura 10 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G3C. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, N = necrotic areas, black asterisks indicate areas of edema and absence of cells, white asteristics indicate dilated and congested blood vessels and yellow circles indicate inflammatory cells..... 41

LISTA DE TABELAS

ARTIGO

Tabela 1 - Comparison among medians observed for inflammatory cell infiltrate in the pulp thirds..... 42

Tabela 2 - Comparison among medians observed for the condition of odontoblastic layer in the pulp thirds..... 43

Tabela 3 - Comparison among medians observed for vascular alterations in the pulp thirds..... 44

Tabela 4 - Comparison among medians observed for tissue disorganization..... 44

SUMÁRIO

1 PREÂMBULO..... 18

2 INTRODUÇÃO..... 19

2.1 MOTIVOS PELA BUSCA E ASCENSÃO DO USO DO CLAREAMENTO DENTAL..... 19

2.2 ETIOLOGIA DAS PIGMENTAÇÕES, TÉCNICAS DE CLAREAMENTO DENTAL E MECANISMO DE AÇÃO DOS AGENTES CLAREADORES..... 20

2.3 EFEITOS NOCIVOS DO CLAREAMENTO DENTAL SOBRE O TECIDO PULPAR..... 21

3 OBJETIVOS..... 26

3.1 OBJETIVO GERAL..... 26

3.2 OBJETIVOS ESPECÍFICOS..... 26

4 METODOLOGIA..... 27

5 ACUTE EFFECTS OF HYDROGEN PEROXIDE IN HIGH CONCENTRATIONS IN PULPS OF RAT MOLARS: A DETAILED HISTOLOGICAL STUDY..... 37

5.1 INTRODUCTION..... 37

5.2 MATERIAL AND METHODS..... 38

5.3 RESULTS..... 40

5.4 DISCUSSION..... 57

5.5 CONCLUSION..... 61

REFERENCES..... 62

6 CONCLUSÕES..... 67

REFERÊNCIAS..... 69

APÊNDICE A - FICHA PARA ANÁLISE QUALITATIVA DA CONDIÇÃO DA CAMADA ODONTOBLÁSTICA..... 74

APÊNDICE B - FICHA PARA ANÁLISE QUALITATIVA DA CONDIÇÃO DAS ALTERAÇÕES VASCULARES..... 75

APÊNDICE C - FICHA PARA ANÁLISE QUALITATIVA DA CONDIÇÃO DA DESORGANIZAÇÃO TECIDUAL..... 76

APÊNDICE D - FICHA PARA ANÁLISE SEMIQUANTITATIVA DO INFILTRADO INFLAMATÓRIO..... 77
ANEXO A - CONSENTIMENTO ÉTICO DO CEUA PARA REALIZAÇÃO DO ESTUDO..... 78
ANEXO B - NORMAS DA REVISTA (INTERNATIONAL ENDODONTIC JOURNAL)..... 79

1 PREÂMBULO

O clareamento dental se configura como uma modalidade de tratamento odontológico estético de grande popularidade e de intensa e crescente procura pelos pacientes, uma vez que representa um procedimento considerado conservador quando comparado à realização de restaurações diretas, facetas e coroas. Dentre as modalidades de tratamentos clareadores disponíveis, a técnica de consultório, que emprega o peróxido de hidrogênio como principal componente ativo de seus produtos, representa o tratamento de eleição nas situações em que resultados estéticos rápidos e satisfatórios são pretendidos pelo paciente.

A indústria, considerando a intensa demanda por procedimentos clareadores, explora ativamente esse mercado através do contínuo desenvolvimento de materiais destinados a esse fim. Nesse sentido, produtos em cuja composição encontram-se agentes clareadores em concentrações cada vez altas estão disponíveis no mercado sem, no entanto, que haja estudos que demonstrem seus efeitos biológicos.

Há, na literatura, uma lacuna quanto à disponibilidade de estudos que investigaram, sob uma mesma metodologia, os efeitos pulpares do clareamento dentário provocados por agentes clareadores a base de peróxido de hidrogênio de 35, 38 e 40% e em períodos de tempo imediatos. Além disso, o apelo comercial envolvendo o uso de agentes clareadores em concentrações cada vez maiores impulsiona a necessidade de estudos que esclareçam seus efeitos sobre o tecido pulpar. Desse modo, essa pesquisa teve como objetivo fazê-lo por meio de avaliação histológica de polpa dentária de ratos, que representa um modelo válido para a realização de estudos dessa natureza.

Para tanto, no período de 2015 a 2017, mediante obtenção de consentimento ético, foram realizados procedimentos clareadores nos primeiros molares de 30 ratos Wistar com géis de peróxido de hidrogênio a 35, 38 e 40% e processamento desses para análises histológicas. Todos os achados do estudo comporão artigo a ser submetido à revista International Endodontic Journal (Qualis A1) de modo que o conhecimento gerado seja compartilhado com toda a comunidade científica. Os achados provenientes dessa investigação contribuirão com a identificação de efeitos ainda não relatados na literatura, subsidiando o estabelecimento de protocolos eficientes e biologicamente mais seguros para a prática clínica.

2 INTRODUÇÃO

2.1 MOTIVOS PELA BUSCA E ASCENSÃO DO USO DO CLAREAMENTO DENTAL

O sorriso é fundamental na determinação da atratividade facial e supera, em grau de importância, a cor da pele, olhos, cabelos, forma da face e do nariz (Valo, Ohio, 1995), tendo, portanto, um importante aspecto das relações sócio-culturais, uma vez que provoca grandes repercussões psicológicas no indivíduo, sobretudo na auto-estima (Al-Qunaian, 2005). Assim, a busca por procedimentos estéticos odontológicos que se traduzam em sorrisos brancos, estéticos e harmoniosos tem sofrido relevante ascensão nas últimas décadas (Sasaki *et al*., 2009; Poonan, 2011).

Ao divulgar a beleza em sorrisos tidos como perfeitos e relacioná-los com a saúde e o bem-estar físico e mental, os meios de comunicação também fomentam esse processo (Saver, Ackerman, 2003). Ademais, um maior critério na auto-percepção do paciente no que diz respeito ao seu sorriso associado ao consequente impulso fornecido à indústria de produtos odontológicos cosméticos, tem fundamentado o crescimento da demanda por tratamentos estéticos na clínica odontológica (Oliveira, Leme, Giannini, 2005).

Nesse sentido, o clareamento dentário ganhou grande notabilidade e passou a ser o tratamento de eleição em virtude de sua aceitabilidade, popularidade, aspecto conservador (Cervantes *et al*., 2006; Meireles *et al*., 2010; Briso *et al*., 2016) e facilidade de acesso, representando uma solução para os pacientes que apresentam problemas estéticos compatíveis com esta modalidade de tratamento (El-Murr, Ruel, St-Georges, 2011).

Em estudos que investigaram a satisfação dos pacientes quanto à estética dentária e o desejo de realizar algum procedimento para reversão desse quadro, foi possível constatar que, na Malásia, 56,2% da amostra pesquisada apresentou descontentamento com a cor dos dentes, e 48,1% relatou ser o clareamento dentário o tratamento pretendido (Tin-OO *et al*., 2011). Em Israel, esses percentuais foram de 62,7% e 81,8%, respectivamente (Samorodnitzky-Naveh *et al*., 2007). No Brasil, um estudo realizado na cidade de Pelotas – Rio Grande do Sul, revelou que os indivíduos que se declararam insatisfeitos com a cor dos seus dentes apresentaram prevalência de desejo de se submeter ao clareamento dentário 30% maior do que aqueles que se declararam satisfeitos (Silva, 2015).

2.2 ETIOLOGIA DAS PIGMENTAÇÕES, TÉCNICAS DE CLAREAMENTO DENTAL E MECANISMO DE AÇÃO DOS AGENTES CLAREADORES

As alterações de cor podem acometer a estrutura dentária por meios intrínsecos ou extrínsecos. As pigmentações intrínsecas são consequências de fatores congênitos (eritroblastose fetal, amelogênese e dentinogênese imperfeitas, fluorose e uso de tetraciclina) ou adquiridos (hemorragia intrapulpar decorrente de traumas e necrose pulpar). Manchamentos dessa natureza apresentam prognóstico mais desfavorável às técnicas de clareamento dental, ao contrário dos manchamentos extrínsecos, os quais são provenientes da deposição de pigmentos e corantes oriundos da dieta, a exemplo do café, bebidas à base de cola, chá preto, tabaco e vinhos tintos (Sulieman, 2008).

Os produtos para clareamento dental mais empregados na atualidade são à base de peróxido de carbamida em concentração de 10 a 37% e peróxido de hidrogênio de 3 a 38%, cuja seleção deve ser feita de acordo com a técnica de clareamento a ser executada (Joiner, 2006). Na composição de tais produtos, o peróxido representa o componente ativo, enquanto que espessantes, condutores, surfactantes, dispersantes, conservantes e aromatizantes compõem a parte inativa (Samorodnitzkynaveh *et al*., 2007).

O mecanismo de ação do clareamento dental se processa por meio da degradação da estrutura orgânica dos cromógenos pigmentantes presentes no tecido dentinário (Markovic *et al*., 2007). Estruturalmente, os agentes pigmentantes são moléculas longas, complexas e compostas por anéis de carbono com altas taxas de absorção de comprimentos de onda eletromagnéticas, o que as torna altamente pigmentadas. O agente clareador possui baixo peso molecular (Joiner, 2007) e, uma vez aplicado sobre o esmalte dental, inicia processo de difusão através da estrutura dentária até atingir a dentina, onde reage quimicamente com os pigmentos orgânicos por meio de oxidação. A reação produz radicais livres compostos por oxigênio e hidroxilas, havendo abertura dos anéis de carbono, o que torna as moléculas menores, menos complexas, com menor taxa de absorção e, portanto, mais claras (Joiner, 2006; Ubaldini *et al*., 2013).

Diferentes modalidades de clareamento dental estão disponíveis na clínica odontológica, sendo aplicáveis tanto em dentes polpados ou vitais como em dentes despolpados ou não-vitais, como um procedimento seguro e eficaz. O tratamento

clareador pode ser executado através da técnica de consultório, cujo procedimento depende exclusivamente do profissional e apresenta maior custo para o paciente, técnica caseira, na qual o paciente executa o clareamento em casa mas com supervisão do cirurgião-dentista, demandando colaboração e custo inferior, ou ainda, uma associação entre elas (Auschill *et al*., 2005; Joiner, 2006; Meirelles *et al*., 2010). Na técnica de consultório, o agente clareador apresenta maior concentração e é utilizado durante curto intervalo de tempo, já na técnica caseira, a concentração do produto é bem inferior e o período de uso é superior em relação à técnica de consultório (Attin *et al*., 2003).

Apesar da crescente popularidade da técnica caseira, o clareamento de consultório tem sido notadamente procurado pela falta de adaptação dos pacientes aos protocolos caseiros, que requerem uso de moldeira por tempo determinado, demanda por resultados mais rápidos e pela necessidade de monitoramento na ocasião de existência de lesões de abfração profundas não restauradas ou recessões gengivais extensas (Barghi, 1998; Blankenau; Goldstein; Haywood, 1999; Gottardi, Brackett, Haywood, 2006; Zekonis *et al*., 2003). Nesses casos, a técnica de consultório utilizando concentrações de peróxido de hidrogênio de 30 a 40%, cuja eficácia está bem documentada na literatura com obtenção de mudança de cor de cinco a oito unidades de escala vita (considerando essa escala organizada por ordem de valor) após duas sessões de clareamento, representa uma alternativa apropriada à técnica caseira (Marson *et al*., 2008; Bernardon *et al*., 2010; Reis *et al*., 2011).

Quando se trata da obtenção de rápidos resultados estéticos, a técnica de clareamento de consultório é o tratamento de eleição (Zekonis *et al*., 2003; Gottardi, Bracket, Haywood, 2006; Buchalla *et al*., 2007; Reis *et al*., 2011(B). Em estudo realizado com clínicos dos Estados Unidos, foi revelado que 33% dos cirurgiões-dentistas adotam a técnica de consultório (CRA, 2000). Essa técnica é realizada através da aplicação de géis de alta concentração (30 – 40%) durante 30 a 60 minutos por sessão, que podem ou não ser ativados por luz para acelerar o processo de clareamento (Sulieman *et al*., 2005; Sulieman *et al*., 2006; Briso *et al*., 2016). O uso do peróxido de hidrogênio em altas concentrações confere resultados estéticos visíveis pelo paciente após uma única sessão clínica (CRA, 2000).

2.3 EFEITOS NOCIVOS DO CLAREAMENTO DENTAL SOBRE O TECIDO PULPAR

Apesar dos benefícios da técnica de clareamento de consultório, estudos mostram que mais de 70% dos pacientes que se submetem a essa modalidade de tratamento desenvolvem sensibilidade dental durante a realização do procedimento e cerca de 48 horas após, o que ocasionalmente os impede de completar o tratamento clareador (TAY *et al*., 2009; Alomari, El Daraa, 2010; Reis *et al*., 2011). O grau de sensibilidade desenvolvido pode ainda ser potencializado quando a técnica empregada adota o uso de luz (Reis *et al*., 2011 (A); Reis *et al*., 2011 (B); Tay *et al*., 2012).

Graças ao seu baixo peso molecular e alta capacidade de difusão pelos tecidos dentários, cerca de dez minutos após o contato do agente clareador com o esmalte, o peróxido de hidrogênio já atinge a interface dentina-polpa (Ubaldini *et al*., 2013). Em razão do seu baixo potencial de oxidação, grande quantidade de moléculas de peróxido de hidrogênio permanece não reagida na dentina, constituindo o chamado peróxido de hidrogênio residual (Soares *et al*., 2016), o que induz a ocorrência de estresse oxidativo nas células pulpares (Sato *et al*., 2013). O estresse oxidativo pode ser compreendido como um desequilíbrio entre a produção de espécies reativas de oxigênio e a presença de antioxidantes de natureza endógena e exógena (Halliwell, 2007). Os radicais hidroxila atacam as duplas ligações dos ácidos graxos presentes na membrana lipídica das células causando imediata lesão oxidativa (Halliwell, 2006). Os resultados de estudos com cultura de células ainda mostram a ocorrência de ativação de metaloproteinases teciduais e diminuição do metabolismo, proliferação e viabilidade celular (Min *et al*., 2008; Sato *et al*., 2013*;* Soares *et al*., 2014*;* Soares *et al*., 2015). Havendo dano ao tecido pulpar, ocorre produção de mediadores de hiperalgesia e excitação ou sensibilização de nociceptores pulpares provocados pela liberação de mediadores da inflamação, como prostaglandinas (Park *et al*., 2006; Andersson *et al*., 2008; Markowitz *et al*., 2010).

Estudos *in vivo* em humanos e em modelo animal com avaliação histopatológica apontam para a ocorrência de morte de células da camada odontoblástica, reações inflamatórias de leve à intensa, desorganização tecidual, aumento da permeabilidade vascular e necrose (Costa *et al*., 2010; Kina *et al*., 2010; Cintra *et al*., 2013; Ferreira *et al*., 2013; Roderjan *et al*., 2014). Ferreira *et al*. (2013) identificaram processo inflamatório agudo pulpar frente ao tratamento clareador, enquanto que Cintra *et al*. (2013) observaram severos danos pulpares com

características majoritariamente irreversíveis e de necrose, variando de acordo com a área da polpa estudada.

A rápida difusão trans-amelodentinária do peróxido de hidrogênio está intimamente relacionada a alta prevalência de sensibilidade dental (Benetti *et al*., 2004; Camargo *et al*., 2007). Tal difusão depende da concentração original do agente clareador e do tempo durante o qual o agente permanece em contato com o substrato dentário, sendo diretamente proporcionais aos efeitos deletérios consequentes no tecido pulpar (Hanks *et al*., 1993; Benetti *et al*., 2004; Meireles *et al*., 2008).

Estudos *in vitro* usando câmaras pulpares artificiais associadas a discos de esmalte e dentina demonstraram elevado efeito citotóxico em células odontoblastóides submetidas à ação de géis clareadores à base de peróxido de hidrogênio com concentrações variando de 20 a 38% (Coldobella *et al*., 2009; Dias Ribeiro *et al*., 2009; Sacono *et al*., 2010). O mesmo tipo de estudo foi realizado utilizando discos de esmalte e dentina em diferentes espessuras de modo a simular diferentes elementos dentários (2,3 mm para incisivos mandibulares e 4,0 mm para molares mandibulares), tendo sido possível observar maior difusão de subprodutos do agente clareador e efeitos citotóxicos mais pronunciados nas células pulpares cultivadas junto aos discos de menor espessura, denotando a influência da espessura do substrato dentário nesse processo (De Oliveira Duque *et al*., 2017). A espessura do substrato dentário também foi relacionada aos efeitos negativos dos géis clareadores *in vivo*. Em um estudo no qual três aplicações de peróxido de hidrogênio a 38% foram realizadas em incisivos e pré-molares humanos, houve necrose de coagulação da polpa coronária dos incisivos inferiores, mas não nos pré-molares, nos quais não foram vistas alterações significantes. Os incisivos apresentavam 1,8 mm de espessura de substrato dentinário, enquanto os pré-molares apresentavam 3,1 mm, sugerindo uma real influência da espessura dos substratos dentários na difusão dos géis clareadores e, consequentemente, nos efeitos deletérios no tecido pulpar (Costa *et al*., 2010).

Mesmo com os pronunciados efeitos citotóxicos evidenciados pela literatura como consequência da ação de agentes clareadores sobre o tecido pulpar, estudos *in vitro* apontam para a ocorrência de recuperação da viabilidade e morfologia de células odontoblastóides e humanas após 72 horas (Soares *et al*., 2014; Soares *et al*., 2015). Estudos *in vivo* realizados em modelo animal também indicam a ocorrência de recuperação pulpar. Lima *et al*. (2016) observaram que áreas de necrose pulpar foram

substituídas por tecido conjuntivo viável e que células odontoblastóides diferenciadas foram depositadas subjacente a dentina na maioria dos elementos estudados após sete dias de procedimento experimental com peróxido de hidrogênio. Benetti *et al* (2017) e Cintra *et al*. (2016) verificaram a ocorrência de deposição de dentina terciária e ausência de infiltrado celular inflamatório após 30 dias da realização do experimento com peróxido de hidrogênio a 20 e 35%, aspectos contrários aos vistos após dois dias da ação clareadora, denotando sinais de reparo.

Muitos são os estudos que evidenciam a citotoxicidade dos procedimentos clareadores sobre as células pulpares (Dias Ribeiro *et al*., 2009; Sacono *et al*., 2010; Soares *et al*., 2014; SOARES *et al*., 2015; De Oliveira Duque *et al*., 2017), no entanto, por adotarem metodologias *in vitro* nas quais os sistemas vascular e imune do hospedeiro estão ausentes, seus achados se tornam menos transponíveis à realidade (Ferreira *et al*., 2013). Nesse sentido, metodologias *in vivo* com modelo animal utilizando ratos possibilitam resultados análogos aos que seriam vistos em humanos. Os molares dos ratos representam um modelo válido para avaliações pré-clínicas de biocompatibilidade em Odontologia, uma vez que apresentam características anatômicas, histológicas, biológicas e fisiológicas semelhantes ao dente humano (Dammaschke, 2010), o que já foi especialmente verificado para estudos acerca dos efeitos nocivos do clareamento dentário (Cintra *et al*., 2016). Ademais, estudos com ratos possibilitam padronização, fácil reprodutibilidade, controle e obtenção de amostra satisfatória, dentro de princípios éticos estabelecidos, aspectos esses que seriam menos praticáveis com estudos em humanos (Cintra *et al*., 2016; Dammaschke, 2010). No entanto, é necessário critério quando do estabelecimento dos períodos de avaliação aplicados nesses estudos uma vez que, apesar de as reações biológicas essenciais do tecido pulpar do molar do rato serem comparáveis às dos humanos, polpas de molares de ratos apresentam excepcional reatividade, resiliência e capacidade de reparo. Comparativamente, um mês de vida de um rato, equivale a 30 meses de um humano (Dammaschke, 2010).

Os estudos de avaliação de efeitos pulpares do clareamento dentário em ratos publicados na literatura adotam, predominantemente, períodos de análises de dois, sete e 30 dias após finalização do procedimento clareador experimental (Cintra *et al*., 2013; Cintra *et al*., 2016; Lima *et al*., 2016; Benetti *et al*., 2017), o que corresponde, por equivalência, a tempos de até mais de 2 anos em humanos, deixando uma lacuna nos achados em curto prazo.

Considerando a pronunciada ocorrência de efeitos citotóxicos provocados pela realização da técnica de clareamento de consultório com o peróxido de hidrogênio, alguns autores têm desenvolvido estudos aplicando protocolos alternativos com concentrações e tempos de contato reduzidos (Bortolatto *et al*., 2014; Soares *et al*., 2014; Lima *et al*., 2016; De Oliveira Duque *et al*., 2017). No entanto, mesmo diante dos favoráveis aspectos de compatibilidade verificados por tais autores, a eficácia do efeito clareador obtido não é satisfatória, uma vez que inferiores concentrações e tempos de contato do peróxido de hidrogênio com o substrato dentário conduzem a menor eficácia clareadora, restringindo as possibilidades de aplicação clínica desses protocolos (Soares *et al*., 2014). Assim, considerando as situações nas quais haja necessidade de obtenção de resultados clareadores rápidos e eficazes por parte dos pacientes, terapias que utilizem peróxido de hidrogênio na técnica de consultório permanecem em demanda, e, portanto, devem continuar sendo alvo de estudos.

Ainda não há, na literatura, estudos que tenham investigado, com a mesma metodologia, os efeitos pulpares do clareamento dentário provocados por peróxido de hidrogênio com concentrações de 35, 38 e 40%, concentrações essas cercadas, também de o apelo comercial por resultados supostamente mais eficientes num tempo clínico menor. O que há disponível, ora compara os efeitos resultantes de géis a 20 e 35%, de significativa diferença de concentração, ora analisa a aplicação isolada do gel a 38%, o que impede comparações fidedignas. Além disso, estudos *in vivo* com peróxido de hidrogênio a 40% ainda não estão disponíveis na literatura. Assim, o objetivo do presente estudo foi de avaliar os efeitos agudos do peróxido de hidrogênio em altas concentrações sobre a polpa dentária de ratos por meio de avaliação histológica.

3 OBJETIVOS

3.1 OBJETIVO GERAL

- Avaliar os efeitos imediatos do peróxido de hidrogênio em altas concentrações sobre a polpa dentária de ratos por meio de avaliação histológica.

3.2 Objetivos específicos

- Avaliar o grau de inflamação provocado pelo peróxido de hidrogênio a 35%, 38% e 40% sobre a polpa dentária de ratos;
- Verificar a condição da camada odontoblástica da polpa dentária de ratos frente à aplicação de o peróxido de hidrogênio a 35%, 38% e 40%;
- Investigar a ocorrência de alterações vasculares na polpa dentária de ratos frente à aplicação de o peróxido de hidrogênio a 35%, 38% e 40%;
- Analisar a ocorrência de desorganização tecidual, considerando a totalidade do tecido, na polpa dentária de ratos frente à aplicação de o peróxido de hidrogênio a 35%, 38% e 40%.

4 METODOLOGIA

4.1 CONSIDERAÇÕES ÉTICAS

Este estudo foi estruturado em consonância com os preceitos da Lei n° 11.794, de 08 de outubro de 2008 (Brasil, 2008), sob protocolo de número 64/15 e submetido à apreciação da Comissão de Ética no Uso de Animais da Universidade Federal de Pernambuco (CEUA - UFPE), a qual aprovou sua realização (Processo n° 23076.013107/2015-24) (Anexo A).

4.2 PREPARO DOS ANIMAIS

Para o estudo, foram utilizados 30 ratos albinos machos da linhagem Wistar (*Rattus norvegicus albinus*) provenientes do biotério de criação do Departamento de Nutrição da UFPE pesando em média 150 g. Os animais foram recebidos no biotério de experimentação do Núcleo de Cirurgia Experimental (NCE) da UFPE, onde foram distribuídos, em grupos de três, em gaiolas com maravalha, até que atingissem o peso médio de 250 g, o que acontecia quando o animal tinha, em média 70 dias de vida. Durante esse intervalo e ao longo de todo o período experimental, os animais foram mantidos em sala com temperatura controlada (22 ± 1°C), ciclo claro-escuro de 12 h e fornecimento de ração e água filtrada *ad libitum*, sempre sob supervisão de um técnico especializado (Costa *et al*., 2012; Cintra *et al*., 2013; Cintra *et al*., 2016; Lima *et al*., 2016).

4.3 GRUPOS EXPERIMENTAIS

De cada animal, foram utilizados os quatro primeiros molares para os procedimentos experimentais. Assim, 120 molares foram divididos em 10 grupos experimentais (n = 12 por grupo) em função do agente clareador utilizado, sessões de clareamento realizadas e período de eutanásia, conforme descrito no quadro 1 (Costa *et al*., 2012; Ferreira *et al*., 2013).

Quadro 1 - Divisão dos grupos experimentais.

Grupo	Procedimento experimental		
	A	B	C
1 - PH 35%	1 sessão de clareamento com eutanásia imediata	2 sessões de clareamento com intervalo de 72h e eutanásia imediata	2 sessões de clareamento com intervalo de 72h e eutanásia após 72h
2 - PH 38%	1 sessão de clareamento com eutanásia imediata	2 sessões de clareamento com intervalo de 72h e eutanásia imediata	2 sessões de clareamento com intervalo de 72h e eutanásia após 72h
3 - PH 40%	1 sessão de clareamento com eutanásia imediata	2 sessões de clareamento com intervalo de 72h e eutanásia imediata	2 sessões de clareamento com intervalo de 72h e eutanásia após 72h
4 - Controle	Sem intervenção (com eutanásia ao final do período experimental dos grupos C)		

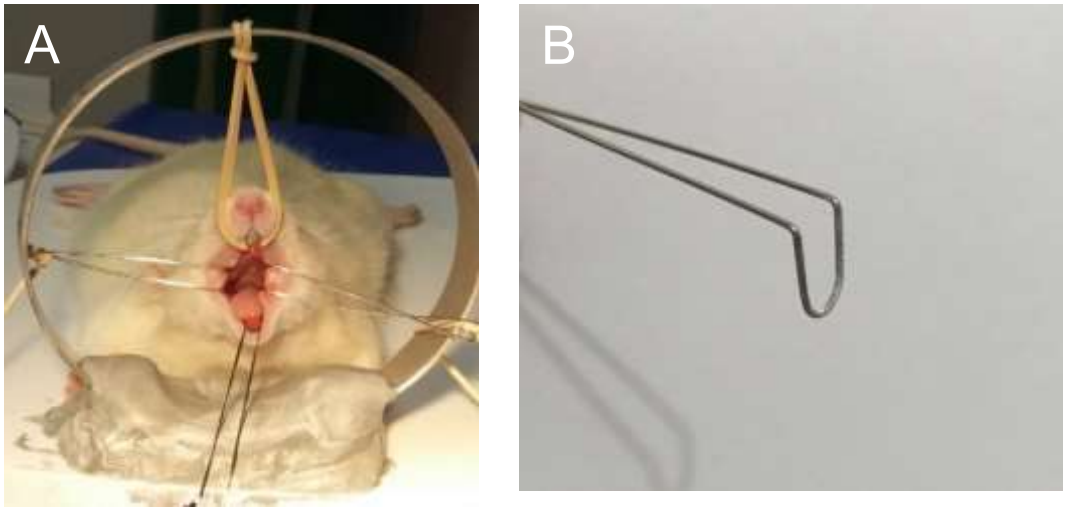
Fonte: A autora, 2018.

4.4 PROCEDIMENTOS EXPERIMENTAIS

Os animais foram submetidos aos procedimentos experimentais sob ação de anestesia. Para tanto, foram anestesiados através de injeção intramuscular de uma mistura de 0,05 mg/Kg de xilazina (Rompum®, Bayer do Brasil, SP, Brasil) e 0,1 mg/Kg de ketamina (Ketalar®, Cristália, SP, Brasil) (Costa *et al*., 2012; Cintra *et al*., 2013; Ferreira *et al*., 2013; Cintra *et al*., 2016; Lima *et al*., 2016).

Uma vez realizada a indução anestésica, os animais foram posicionados em mesas operacionais com o ventre voltado para baixo. A abertura bucal e o afastamento da mucosa jugal foram obtidos, respectivamente, por meio do uso de elásticos de silicone e do uso de fio ortodôntico 08 (Morelli, Sorocaba, SP, Brasil) (Figura 1A) moldado em forma de alça e dobrado no ângulo de 90° (Figura 1B) (Nabeshima, 2015).

Figura 1 - (A) Posicionamento, abertura bucal e afastamento da mucosa jugal do animal em mesa operacional. (B) Afastador para mucosa jugal confeccionado com fio ortodôntico.



Fonte: A autora, 2018.

Na sequência, foram submetidos a profilaxia e aplicação de barreira gengival resinosa fotoativada (Opal Dam, Ultradent, South Jordan, EUA). Os agentes clareadores (Figuras 2A, 2B e 2C, Quadro 2) foram, então, manipulados e aplicados, conforme recomendações do fabricante, na superfície vestibular dos primeiros molares, no regime de três aplicações de 15 minutos cada.

Figura 2 - Agentes clareadores utilizados. (A) Peróxido de Hidrogênio 35% / Whiteness HP Blue. (B) Peróxido de Hidrogênio 38% / Opalescence Boost. (C) Peróxido de Hidrogênio 40% / Opalescence Boost.

Fonte: A autora, 2018.

Quadro 2 - Agentes clareadores utilizados (Conforme informações do fabricante).

Material /Marca comercial	**Composição**	**Fabricante**
Gel clareador - Peróxido de Hidrogênio 35% / Whiteness HP Blue	Peróxido de hidrogênio a 35% (após mistura das fases), espessantes, pigmento violeta, agentes neutralizantes, gluconato de cálcio, glicol e água deionizada.	FGM (Joinvile, Brasil)
Gel clareador - Peróxido de Hidrogênio 38% / Opalescence Boost	Peróxido de hidrogênio a 38% (após mistura das fases), ativador químico, 1,1% de fluoreto (após mistura das fases) e 3% de nitrato de potássio (após mistura das fases).	Ultradent (South Jordan, EUA)
Gel clareador - Peróxido de Hidrogênio 40% / Opalescence Boost	Peróxido de hidrogênio a 40% (após mistura das fases), ativador químico, 1,1% de fluoreto (após mistura das fases) e 3% de nitrato de potássio (após mistura das fases).	Ultradent (South Jordan, EUA)

Fonte: A autora, 2018

Finalizados os tempos de aplicação, os agentes clareadores foram removidos através de sucção, gaze estéril e água e a barreira gengival destacada com o auxílio de uma sonda exploradora n° 5 (SSWhite, Rio de Janeiro, RJ, Brasil).

4.5 SACRIFÍCIO DOS ANIMAIS

Os sacrifícios dos animais foram realizados em dois diferentes períodos: imediatamente após as sessões de clareamento – grupos A e B, e após 72 h da finalização das sessões de clareamento – grupos C. Os animais alocados no grupo controle foram sacrificados ao final do período experimental dos animais dos grupos C.Para tanto, foram submetidos a sobredose anestésica com tiopental sódico (Thiopentax®, Cristália, SP, Brasil).

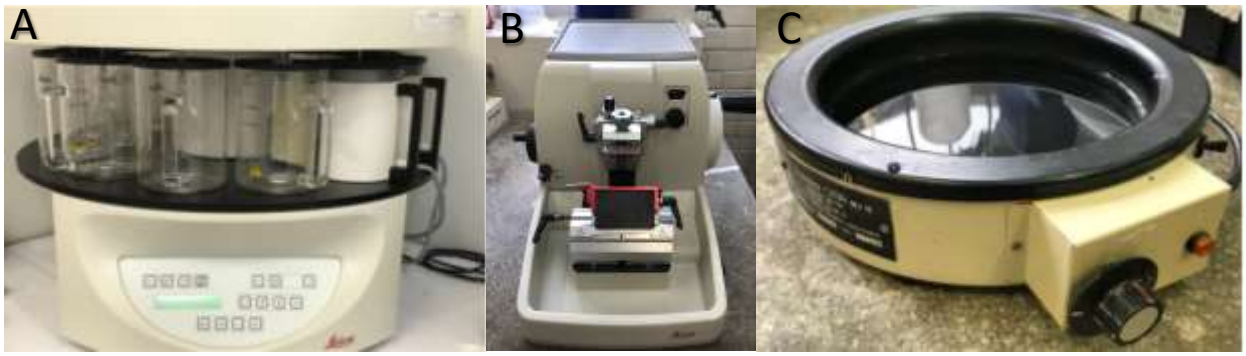
As mandíbulas foram extraídas por dissecação e as maxilas por decapitação. Na sequência, foram imersas em recipientes identificados contendo solução tamponada de formol a 10%, onde foram mantidas por 24 h para fixação (Cintra *et al*., 2013; Cintra *et al*., 2016; Benetti *et al*., 2017).

4.6 PROCESSAMENTO HISTOLÓGICO

Após o período de fixação, as mandíbulas e maxilas foram lavadas durante 1 h em água corrente para eliminação de resíduos da solução de formol. Os espécimes foram, então, descalcificados por meio de imersão em solução de EDTA (ácido etileno diaminotetracético) com concentração de 5% por um período de 30 dias, sendo a solução trocada a cada 24 h. Em seguida, os espécimes foram lavados em água corrente durante 1 h de modo a eliminar resíduos da solução descalcificadora, seccionados em hemi-mandíbula e hemi-maxila, acondicionados em cassetes identificados e processados em equipamento histotécnico Leica Histoembedder® (Leica Instruments GmbH, Nussloch- Alemanha) (Figura 3A).

Para inclusão, as amostras foram inseridas em moldes os quais foram preenchidos por parafina aquecida, obtendo-se, após resfriamento, blocos padronizados que seguiram para microtomia. Para tanto, os blocos foram posicionados em micrótomo manual (Leica RM 2235 - Leica Instruments GmbH, Nussloch- Alemanha) (Figura 3B) para aquisição de secções transversais seriadas de 5 µm de espessura no sentido mésio-distal. Essa direção de cortes foi adotada considerando o fato de que, como a aplicação do produto foi feita na face vestibular dos elementos dentários dos animais e, numa análise mais imediata, o produto poderia ter ação mais concentrada na porção vestibular da polpa, a direção mésio-distal foi preferida para proporcionar uma visão mais ampla de toda a polpa afetada pelo protocolo clareador. Os cortes histológicos foram distendidos em banho histológico (ANCAP, São Paulo, SP, Brasil) (Figura 3C) a 32°C e colocados em lâminas de vidro previamente identificadas. Em cada lâmina, foram colocados três cortes intervalados. As lâminas foram acondicionadas em estufa a 60°C durante 30 minutos para remoção dos excessos de parafina.

Figura 3 - (A) Equipamento para processamento histotécnico. (B) Micrótomo manual. (C) Banho histológico.



Fonte: A autora, 2018.

De posse das lâminas livres de excesso de parafina, partiu-se para a técnica de coloração com hematoxilina e eosina (HE). Desse modo, o processo foi iniciado com dois banhos de xilol de cinco minutos cada, seguido de banho de álcool xilol, álcool absoluto, álcool 90%, álcool 70% e água por dez segundos cada. Na sequência, banho de Hematoxilina de Harris durante três minutos, água por dez segundos, mergulho rápido em solução de álcool e ácido clorídrico, que age como diferenciador, e água corrente durante três minutos. Então, procedeu-se banho em eosina por 3 minutos, sequência de três mergulhos em álcool absoluto e mais dez mergulhos em álcool xilol, finalizando com dois banhos em xilol e montagem com entellan.

4.7 ANÁLISE HISTOLÓGICA

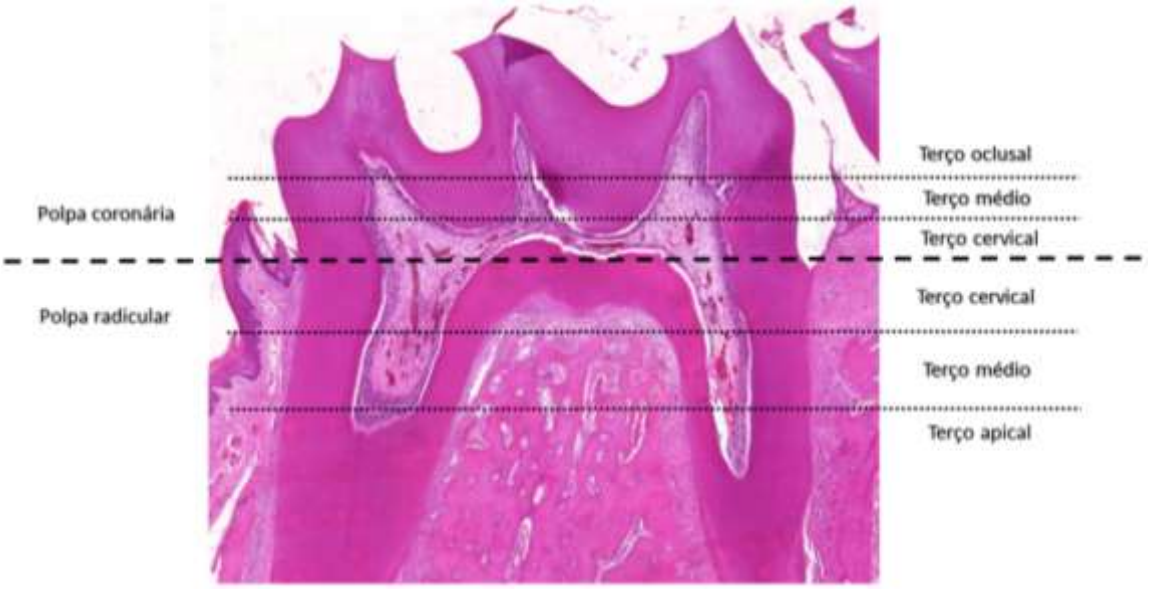
Foram considerados cortes que atingiram, além da coroa, pelo menos uma raiz do primeiro molar em sua extensão longitudinal. A partir disso, para avaliação, foram selecionadas cinco lâminas histológicas distintas contendo três cortes para cada amostra. Essas lâminas foram escaneadas e digitalizadas através do escaner 3DHistech modelo Panoramic MIDI® (Figura 4) e analisadas com aumento total de 400x por meio do programa Panoramic Viewer 1.15.4 (3DHistech®, Konkoly-Thege M. str. Budapest, Hungary).

Figura 4 - Equipamento utilizado para escaneamento das lâminas.

Fonte: A autora, 2018.

Para as análises propostas, a polpa coronária foi dividida em terços oclusal, médio e cervical e a polpa radicular em terços cervical, médio e apical, exceto para a realização da análise de desorganização tecidual, na qual a totalidade do tecido foi avaliada (Figura 5).

Figura 5 - Divisão do tecido pulpar em terços.



Fonte: A autora, 2018.

Os dados foram analisados por um avaliador com experiência, previamente calibrado e de maneira cega. Para calibração, o avaliador foi treinado por um patologista experiente apontando as alterações microscópicas que foram avaliadas. Eventuais dúvidas na avaliação foram dirimidas pela consulta ao patologista que realizou o treinamento.

4.7.1 Condição da camada odontoblástica

Foram atribuídos escores de 1 a 3 para cada terço do elemento em análise (Quadro 3) (Costa *et al*., 2010; Cintra *et al*., 2016).

QUADRO 3. Condição da camada odontoblástica

Escore	Condição da camada odontoblástica
1	Intacta – Células odontoblásticas com aparência íntegra dispostas em camadas ordenadamente subjacentes à pré-dentina
2	Desorganizada - Células odontoblásticas com aparência íntegra ou apresentando aspecto mais achatado dispostas desordenadamente subjacentes à pré-dentina
3	Interrompida - Células odontoblásticas parcial ou totalmente ausentes

Fonte: A autora, 2018.

Os dados foram anotados em uma ficha para posterior análise dos resultados (Apêndice A).

4.7.2 Alterações vasculares

Foram atribuídos escores de 1 a 3 para cada terço do elemento em análise (Quadro 4) (adaptado de Costa *et al*., 2017).

Quadro 4 - Alteração vascular

Escore	Alteração vascular
1	Ausente – Vasos sanguíneos com aspecto de normalidade ou alterações insignificantes

2	Intermediária – Vasos dilatados e congestionados associados ou não à áreas de edema
3	Severa – Vasos dilatados e congestionados associados ou não à áreas de edema com presença de áreas de hemorragia

Fonte: A autora, 2018.

Os dados foram anotados em uma ficha para posterior análise dos resultados (Apêndice B).

4.7.3 Desorganização tecidual

Foram atribuídos escores de 1 a 4 para cada elemento em análise, considerando a totalidade do tecido pulpar avaliado (Quadro 5) (Costa *et al*., 2010; Kina *et al*., 2010; Lima *et al*., 2016).

Quadro 5 - Desorganização tecidual

Escore	**Desorganização do tecido pulpar**
1	Tecido normal
2	Camada odontoblástica desorganizada, mas polpa central normal
3	Necrose parcial da polpa coronária associada a discreta desorganização do tecido pulpar remanescente
4	Necrose da polpa coronária associada (ou não) com calcificação distrófica

Fonte: A autora, 2018.

Os dados foram anotados em uma ficha para posterior análise dos resultados (Apêndice C).

4.7.4 Intensidade do infiltrado inflamatório

Para cada corte avaliado, a partir do *software* Panoramic Viewer, foram geradas imagens em aumento de 40x, de acordo com as especificações do programa, e em formato “.tiff” de campos consecutivos de cada terço do dente analisado. Essas imagens foram abertas no *software* Imaging Processing and Analysis in Java (ImageJ®, National Institute of Mental Health, Bethesda, Maryland, USA), com cuja ferramenta *multi-point* foi realizada a contagem das células inflamatórias, de modo que a mesma célula fosse contada uma única vez. Foram atribuídos escores de 1 a 5 para cada terço do elemento sob análise (Quadro 6) (Cintra *et al*., 2013; Benetti *et al*., 2017).

Quadro 6 - Intensidade do infiltrado celular inflamatório

Escore	**Condição do tecido pulpar**
1	Ausência de células inflamatórias ou número insignificante
2	Infiltrado inflamatório discreto (<25 células por campo)
3	Infiltrado inflamatório moderado (entre 25 e 125 células por campo)
4	Infiltrado inflamatório severo (>125 células por campo)
5	Necrose

Fonte: A autora, 2018.

Os dados foram anotados em uma ficha para posterior análise dos resultados (Apêndice D).

4.8 ANÁLISE ESTATÍSTICA

Os escores obtidos através das análises realizadas e digitados em fichas próprias foram transpostos para planilhas eletrônicas no programa Excel (Microsoft Office 2007®) e exportados para o programa Statistical Package for the Social Sciences (versão 22.0.0.0; SPSS Inc., Chicago, IL, USA), no qual foram realizadas as análises estatísticas. Os dados foram submetidos ao teste não-paramétrico de Kruskall-Wallis, considerando a distribuição não-normal dos dos dados. Quando observadas diferenças estatisticamente significativas entre os grupos, adicionalmente foi realizado o pós-teste de Dunn, para investigar diferenças dois a dois. Para todos os testes, o nível de significância estabelecido foi de 5% ($p < 0,05$).

5 ACUTE EFFECTS OF HYDROGEN PEROXIDE IN HIGH CONCENTRATIONS IN PULPS OF RAT MOLARS: A DETAILED HISTOLOGICAL STUDY

5.1 INTRODUCTION

The growing request for conservative dentistry procedures, which leads to esthetic smiles and increased self-esteem, has contributed to make dental bleaching techniques popular and to boost manufacturers in the continuous development of products for this purpose (Al-Qunaian, 2005, Oliveira, Leme, Giannini, 2005, Cervantes *et al*. 2006, Meireles *et al*. 2010, Briso *et al*. 2016). In-office bleaching techniques are the ones to be chosen when rapid and effective results are expected for the pacients, with high demand in dental routine (Zekonis *et al*., 2003, Gottardi, Bracket, Haywood, 2006, Buchalla *et al*., 2007, Reis *et al*., 2011(B). Hydrogen peroxide (HP) is the main active compound of most bleaching agents, and, for the in-office technique, is available in concentrations ranging from 20 to 40% (Costa *et al.* 2010, Briso *et al.* 2015). For such procedure, bleaching gels are applied in the buccal surface of the teeth (Attin *et al*., 2003, Reis *et al*., 2011). Because of its low molecular weight and ability to denature proteins, it diffuses through enamel and dentin, where reactive oxygen species are released and oxidation of organic pigments responsible for discoloring teeth happens (Joiner, 2006, Joiner, 2007, Ubaldini *et al*., 2013).

In spite of its beneficial effects, bleaching techniques are related to high rates of tooth sensitivity occurence (TAY *et al*., 2009, Alomari, El Daraa, 2010, Reis *et al*., 2011), given HP rapid transenameldentin diffusion (Benetti *et al*., 2004, Camargo *et al*., 2007). In about ten minutes after the contact of the bleaching agent with dental enamel, residual HP and its by-products reach dentin-pulp interface and interact with pulp cells, whose double bounds of membrane fatty acids are broken causing oxidative stress (Sato *et al.* 2013; Halliwell, 2006, Ubaldini *et al*. 2013, Soares *et al*. 2016). Several studies has also correlated HP action with the activation of tissue metalloproteinases and reduction of metabolism, proliferation and cell viability (Min *et al*. 2008, Sato *et al*. 2013*;* Soares *et al*. 2014*;* Soares *et al*. 2015). Application time and concentration of HP present in the bleaching agent are directly proportional to pulp damage (Hanks *et al*. 1993, Benetti *et al*. 2004, Meireles *et al*. 2008). Considering in-office bleaching citotoxicity pulp effects, alternative protocols with reduced concentration and time of application have being proposed (Bortolatto *et al*. 2014,

Soares *et al*. 2014, Lima *et al*. 2016, De Oliveira Duque *et al*. 2017). However, contrasting with the favorable biocompatibility results, bleaching efficacy of such protocols is not satisfactory, making it little clinically applicable. (Soares *et al.* 2014).

Studies performed with human incisors and premolars point to the occurence of odontoblastic layer cells death, inflammatory reactions from mild to severe, tissue disorganization, vascular alterations and necrosis of coronal pulp (Costa *et al*. 2010, Kina *et al*. 2010, Roderjan *et al*. 2014). Costa *et al.* (2010) observed that three applications of 38% HP cause coagulative necrosis in the coronal pulp of incisors but not in premolars, given its thicker substrate, which demonstrated that toxicity effects may vary according to the group of teeth studied.

In face of the greater possibility of standardization, reprodutibility, control and obtention of satisfactory samples (Dammaschke, 2010, Cintra *et al*. 2016), rat models have been widely adopted to study bleaching protocols, mainly molar teeth (Kina *et al*. 2010, Cintra *et al*. 2013, Cintra *et al.* 2016, Lima *et al.* 2016, Benetti *et al.* 2017).

However, literature lacks studies investigating pulp tissue changes resulting from bleaching therapies with 35, 38 and 40% HP, whose increasing and continuous demand require studies on its consequences. Therefore, the aim of this study was to evaluate the acute effects of HP in high concentrations (35, 38 and 40%) in dental pulp of rat molars.

5.2 MATERIAL AND METHODS

5.2.1 Ethical considerations and animals

The procedures performed were approved by the Ethics Committee of Animal Use (CEUA 23076.013107/2015-24) of Universidade Federal de Pernambuco, Brazil. All procedures and treatments on animals were carried out according to the Ethical Principles for Animal Research, recommended by the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures, and the National Council for Control of Animal Experimentation in Brazil. In addition, the entire study was designed in accordance to the ARRIVE guidelines (Kilkenny *et al*. 2010).

One hundred and twenty first maxillary and mandibular molars from 30 male Wistar rats (200 – 250g) were used in this study. The animals were housed in plastic

cages in a temperature controlled environment (22°C±1°C and 12h light-dark cycle) and received food and water *ad libitum.*

5.2.2 Bleaching procedure

The rats were anesthetized with intramuscular injection of an association of 0.05mL of xylazin (Rompum®, Bayer do Brasil, SP, Brasil) and 0.1 mL of ketamin (Ketalar®, Cristália, SP, Brasil) per 100 g of body weight. Four first molars of each rat, totalizing 120 teeth, were assigned to ten experimental groups (n=12 per group). The experimental groups were divided according to HP concentration (1: HP 35% Whiteness HP Blue, FGM Dental Products, Joinville, SC, Brazil; 2: HP 38% Opalescence Boost, Ultradent, South Jordan, USA; 3: HP 40% Opalescence Boost, Ultradent, South Jordan, USA) and to number of bleaching sessions and period of euthanasia (A: one bleaching session with immediate euthanasia; B: two bleaching sessions with 72h of interval and immediate euthanasia; C: two bleaching sessions with 72h of interval and euthanasia after 72h). Group 4 consisted of the animals which did not receive any treatment (controls).

Prior to the bleaching procedure, the animals were fixed on an operational table, where prophylaxis and instalation of gengival barrier (Opal Dam, Ultradent, South Jordan, USA) were performed. The bleaching gels were handled according to the manufacturer's instructions, and were applied to the buccal surface of the teeth in a regime of three applications of 15 minutes each. At the end of the bleaching sessions, the bleaching gel was removed by aspiration and air/water spray, and the teeth were dried using sterile gauze.

5.2.3 Histology

The animals were euthanized with an overdose of ketamine and xylazin following the periods of euthanasia determined in the experimental protocols. The bilateral maxillae and mandible were separated, dissected, fixed in a solution of 10% buffered formalin for 24 h and decalcified in a 5% ethylenediaminetetraacetic (EDTA) solution for 1 month. Then, the specimens were dehydrated through a graded series of ethanol, and embedded in paraffin. Histological sections of 5 µm were obtained from tissues embedded in paraffin and stained with hematoxylin-eosin.

The slides were scanned using the Pannoramic MIDI scanner (3DHISTECH®, Budapest, Hungary). Histomorphometry was performed under magnification of 400x through the software Panoramic Viewer 1.15.4 (3DHistech®, Budapest, Hungary). For carrying out the analysis, the pulp chamber was divided into thirds (occlusal, middle and cervical) as well as the radicular pulp (cervical, middle and apical). The intensity of inflammation was scored in each third according to the inflammatory cell count: 1 – inflammatory cells absent or negligible in number; 2 – mild inflammatory infiltrate (<25 cells per field); 3 – moderate inflammatory infiltrate (between 25 and 125 cells per field); 4 – severe inflammatory infiltrate (>125 cells per field); or 5 – necrosis. The condition of the odontoblastic layer was also assessed in each third: 1 – intact; 2 – disorganized; or 3 – disrupted. Scores for vascular changes were attributed in accordance with the following criteria: 1 – absent (normal blood vessels or insignificant alterations); 2 – intermediate, dilated and congested blood vessels associated (or not) with edema areas; or 3 – severe, dilated and congested blood vessels associated (or not) with edema areas, and presence of bleeding areas. For tissue disorganization, the scores were assigned considering the tissue as a total, as follows: 1 – normal tissue; 2 – odontoblastic layer disorganized but central pulp normal; 3 – partial coronal pulp necrosis associated with discrete disorganization of the reminiscente pulp tissue; or 4 – coronal pulp necrosis associated (or not) with dystrophic calcification.

5.2.4 Statistical analysis

Data were collected and analysed by a single calibrated and blinded researcher. The scores obtained were analyzed by the non-parametric Kruskal-Wallis and Dunn's tests, using the Statistical Package for the Social Sciences (version 22.0.0.0; SPSS Inc. Chicago, IL, USA), considering a significance level of 5%.

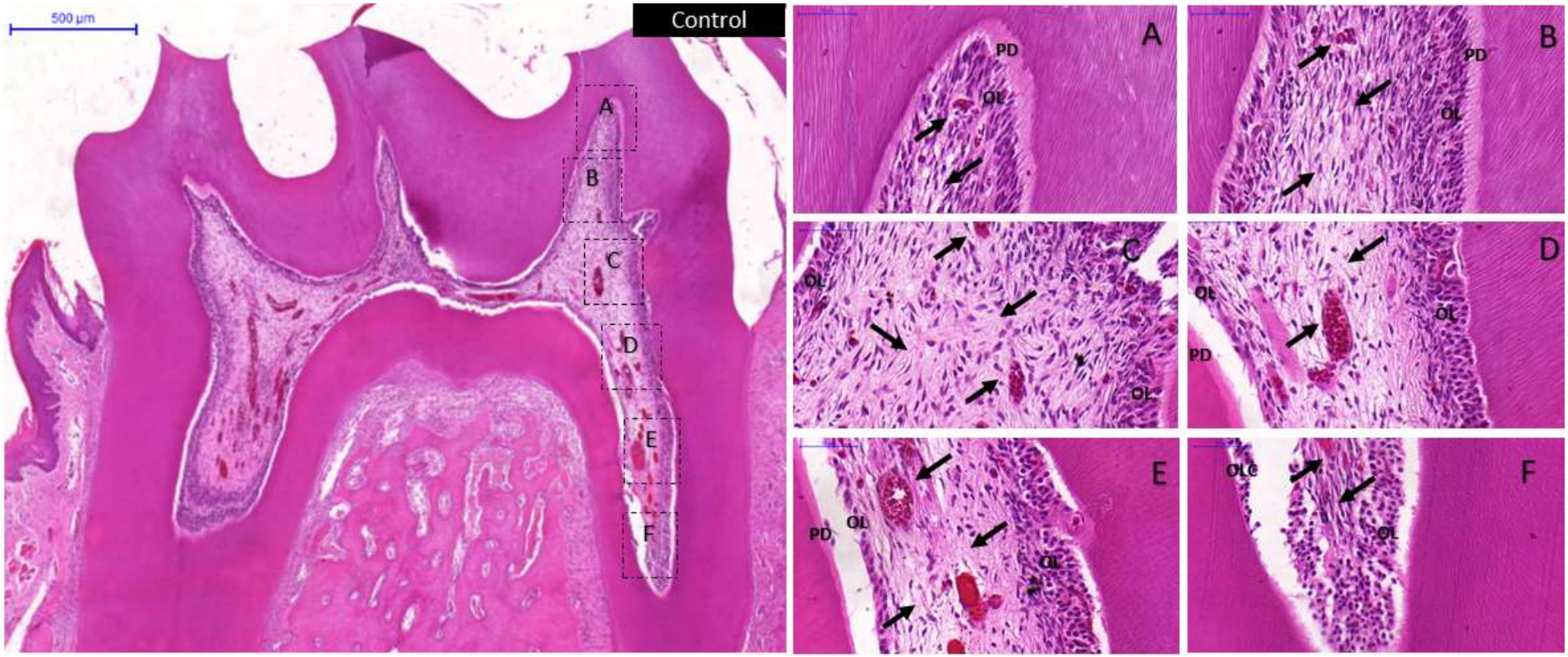
5.3 RESULTS

5.3.1 Control group

The control group presented normal pulp tissue, with continuous odontoblastic layer underlying dentin substrate, normal cell-free, cell-rich and central areas, and

equilibrium in the distribution of extracelular matrix componentes, cells and blood vessels. Thus, enabling comparisons with experimental groups (Figure 1).

Figure 1 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the control group. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, and black arrows show the balanced distribution of extracelelular matrix components, cells and blood vessels.



Source: The author, 2018.

5.3.2 G1

5.3.2.1 G1A

This group exhibited the odontoblastic layer severely disorganized in the occlusal and middle thirds of coronal pulp, with presence of odontoblastic cells sucked into pre-dentin layer. There was necrosis in the coronal pulp, with edema and loss of tissue architecture. Blood vessels showed dilatation and congestion in all thirds, which was less intense in the apical third (Figure 2).

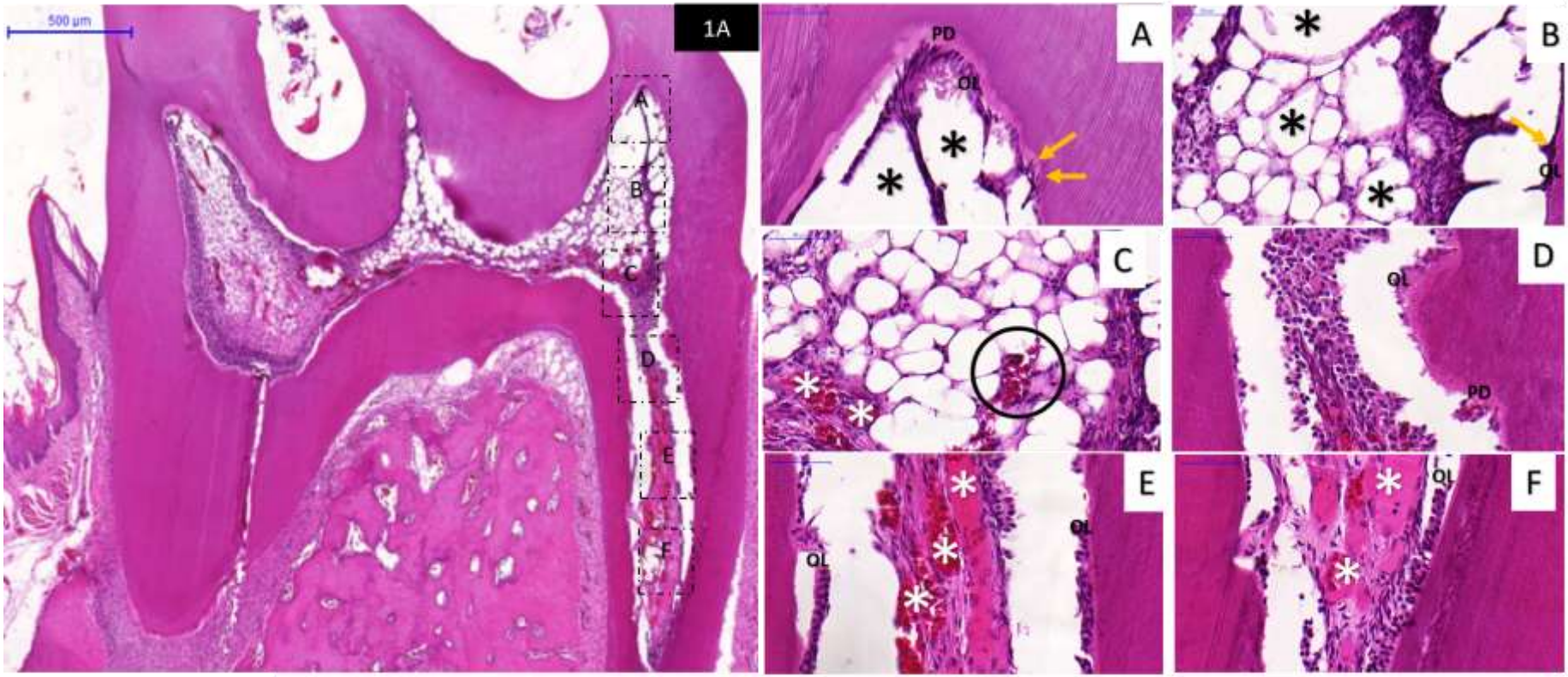
5.3.2.2 G1B

The odontoblastic layer was interrupted in the occlusal and middle thirds of the coronal pulp (with presence of odontoblastic cells sucked into pre-dentin layer) and disorganized in the other thirds of the pulp tissue. There was necrosis in the coronal pulp and cervical third of radicular pulp, with presence of liquefied material in the pulp horns. Bleeding areas were seen throughout the coronal pulp (Figure 3).

5.3.2.3 G1C

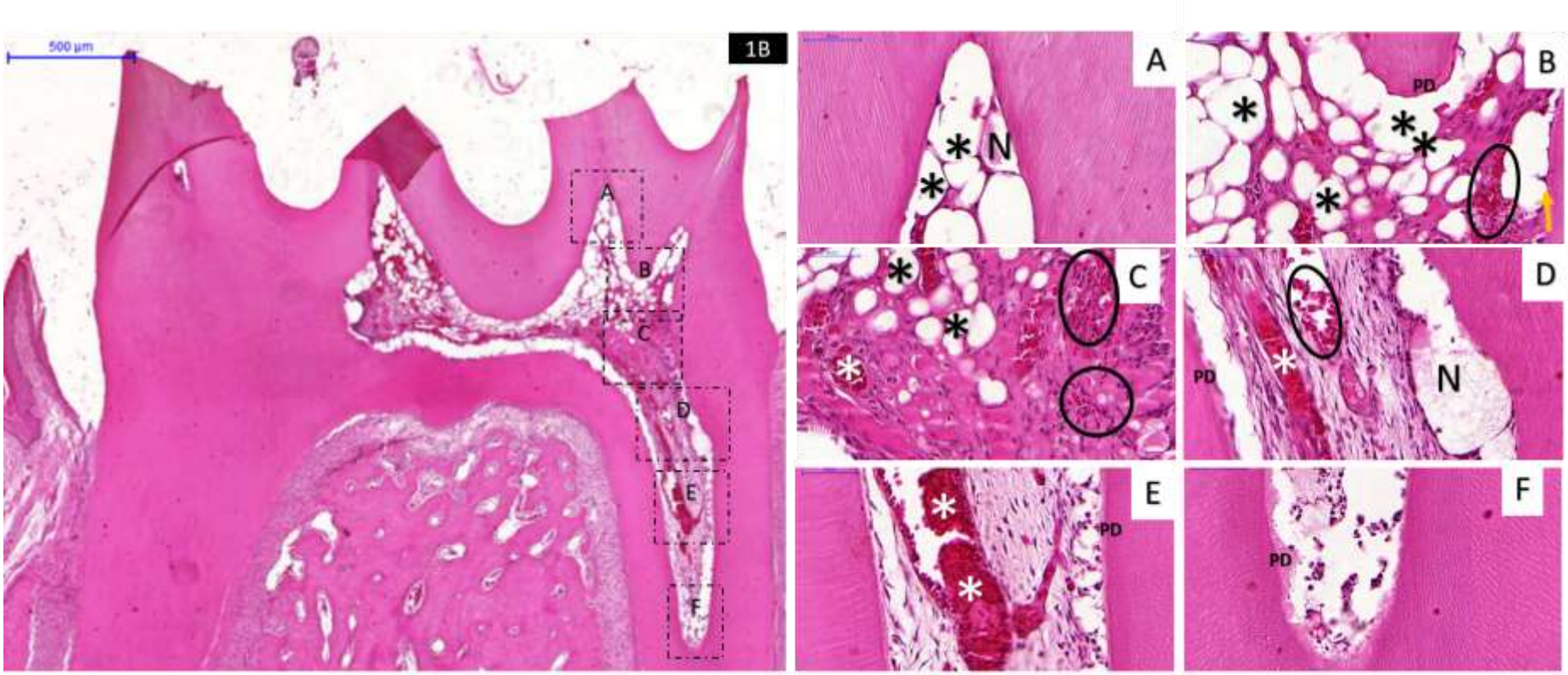
In the specimens of this group, the odontoblastic layer was absent in the occlusal and middle thirds of coronal pulp. Greatly dilated and congested blood vessels were present throughout pulp tissue, except in the apical third, with ocasional areas of bleeding in the pulp horns. Near the pulp floor, points of endotelial neoformation could be noted. The pulp as a whole exhibited an aspect of estructured tissue, despite of predominance of fibrous components (Figure 4).

Figure 2 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G1A. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, yellow arrows indicate odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells, white asteristics indicate dilated and congested blood vessels and black circles indicate areas of bleeding.



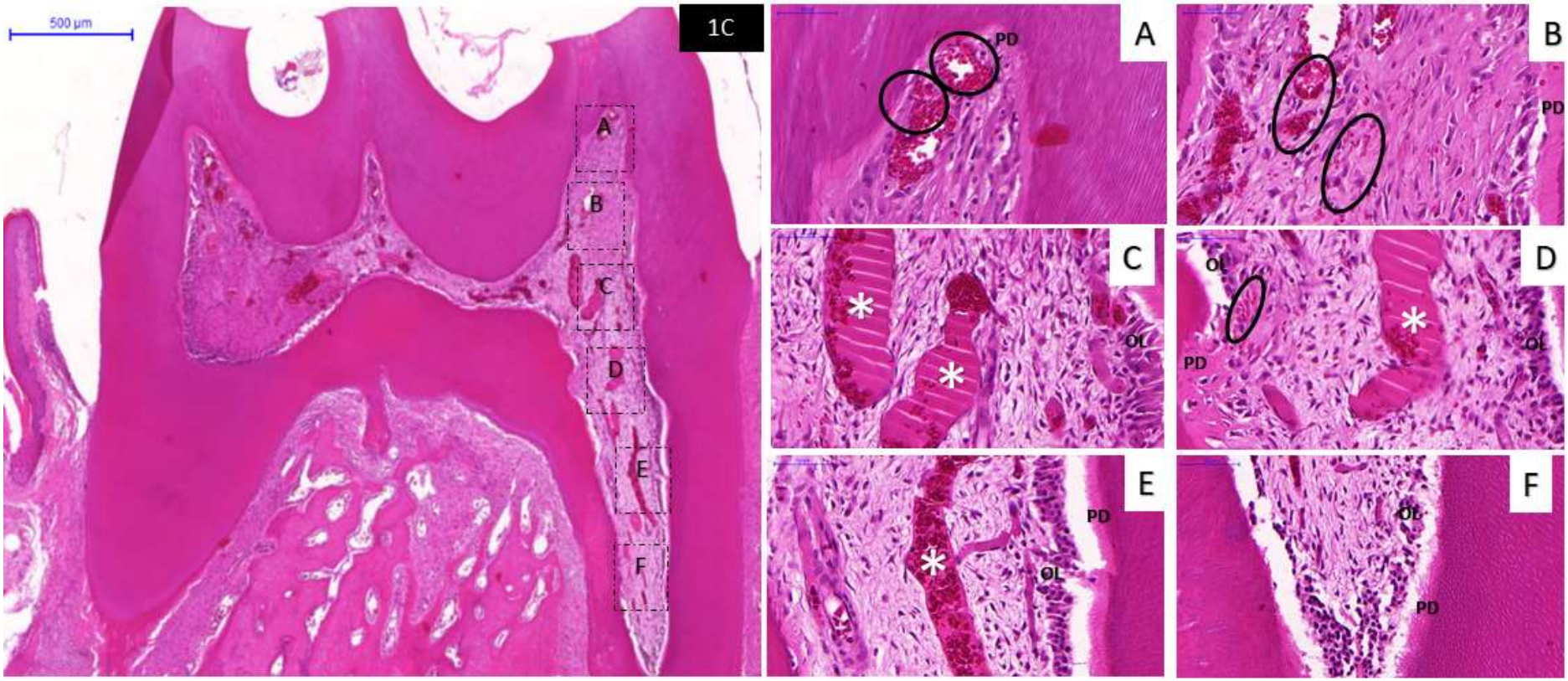
Source: The author, 2018.

Figure 3 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G1B. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, N = necrotic areas, yellow arrows indicate odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells, white asterisks indicate dilated and congested blood vessels and black circles indicate areas of bleeding.



Source: The author, 2018.

Figure 4 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G1C. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, white asteristics indicate dilated and congested blood vessels and black circles indicate areas of bleeding.



Source: The author, 2018.

5.3.3 G2

5.3.3.1 G2A

This group exhibited interrupted odontoblastic layer in the pulp horns and odontoblastic cells sucked into pre-dentin layer of coronal pulp. Necrotic areas are present throughout coronal pulp, in whose cervical third there was liquefied tissue and inflammatory cell infiltrate predominantly composed of polymorphonuclear cells. The cervical third of the radicular pulp also exhibited a mild number of polymorphonuclear cells. Dilatation and congestion of blood vessels were more concentrated in the cervical thirds. Radicular pulp tissue was mildly disorganized (Figure 5).

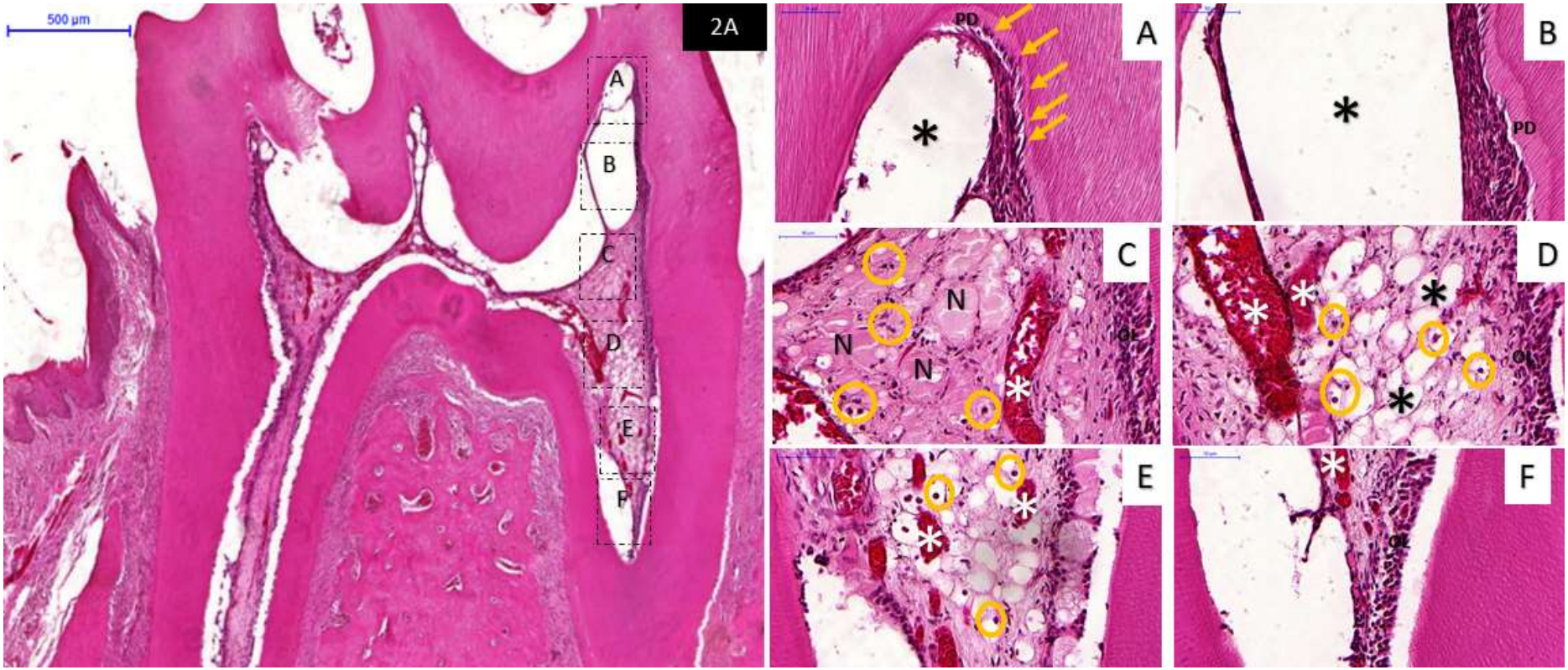
5.3.3.2 G2B

In this group, the coronal pulp and the cervical third of radicular pulp showed complete interruption of the odontoblastic layer, and necrosis. Liquefied tissue was present mainly in the pulp horns, but also in the middle and cervical thirds of coronal pulp. In the central area of coronal pulp, there was presence of edema and polymorphonuclear cells. There were bleeding areas in the cervical thirds (Figure 6).

5.3.3.3 G2C

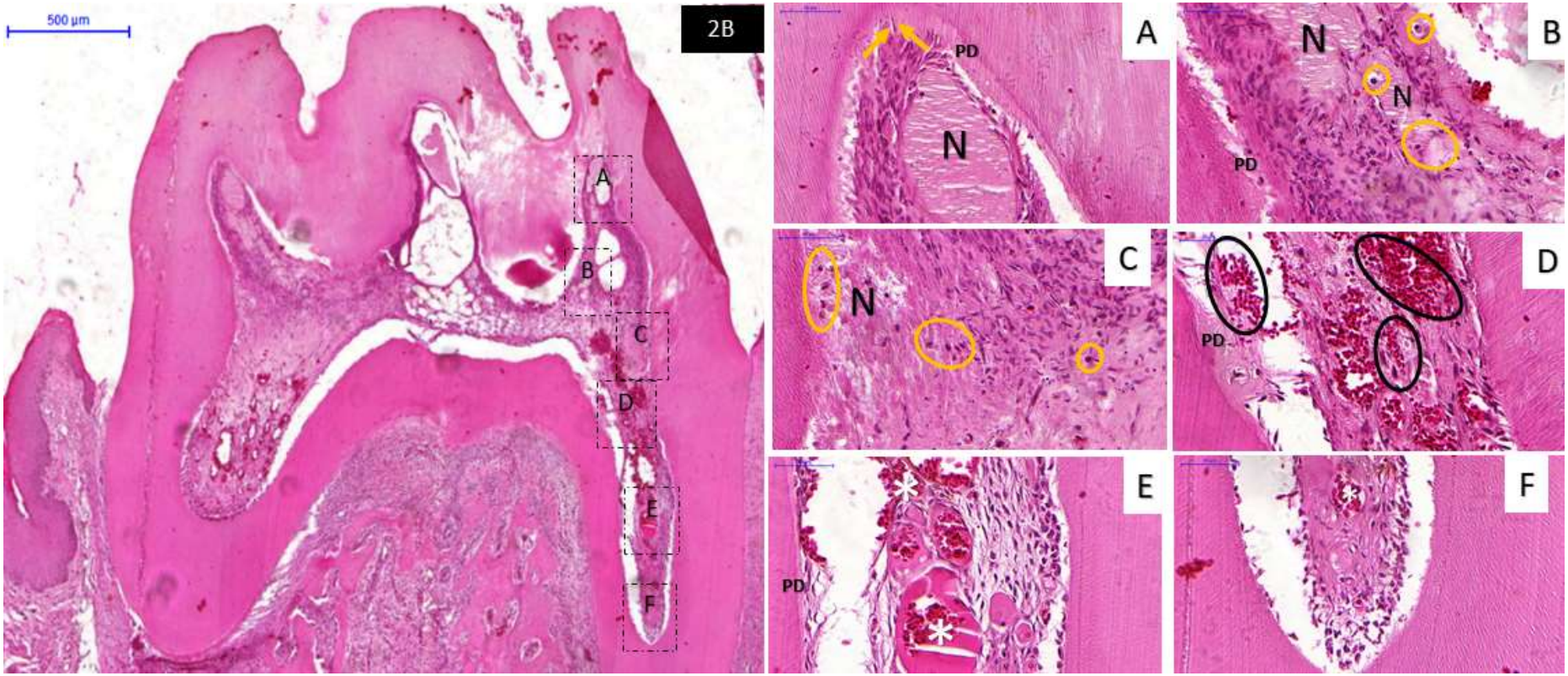
The specimens of this group exhibited interrupted odontoblastic layer in the coronal pulp. In the middle and cervical thirds of coronal pulp, there was mild inflammatory response. Pulp tissue, as a whole, showed a more fibrous aspect (Figure 7).

Figure 5 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G2A. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, N = necrotic areas, yellow arrows show odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells, white asteristics indicate dilated and congested blood vessels and yellow circles indicate inflammatory cells.



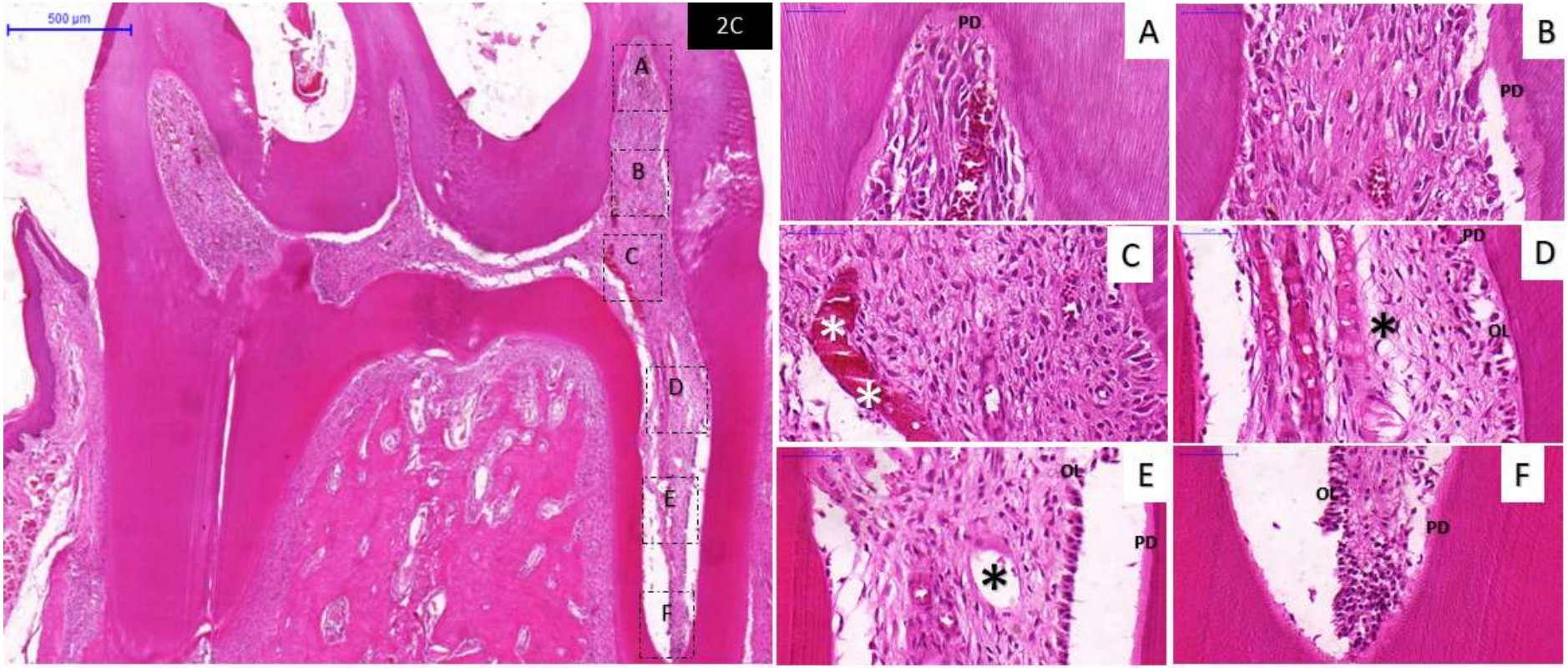
Source: The author, 2018.

Figure 6 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G2B. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, N = necrotic areas, yellow arrows indicate odontoblastic cells sucked into pre-dentin layer, white asterisks indicate dilated and congested blood vessels, black circles indicate areas of bleeding and yellow circles indicate inflammatory cells.



Source: The author, 2018.

Figure 7 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the group 2C. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer and black asteristics indicate areas of edema and absence of cells.



Source: The author, 2018.

5.3.4 G3

5.3.4.1 G3A

In the coronal pulp and cervical third of radicular pulp, the odontoblastic layer was interrupted, with regions where sparse odontoblasts stucked into pre-dentin. These areas also exhibited necrosis and absence of cells. Pulp tissue was shown to be wholly disorganized. These characteristics are not applicable to the apical third of radicular pulp, once that it exhibited the slightest changes (Figure 8).

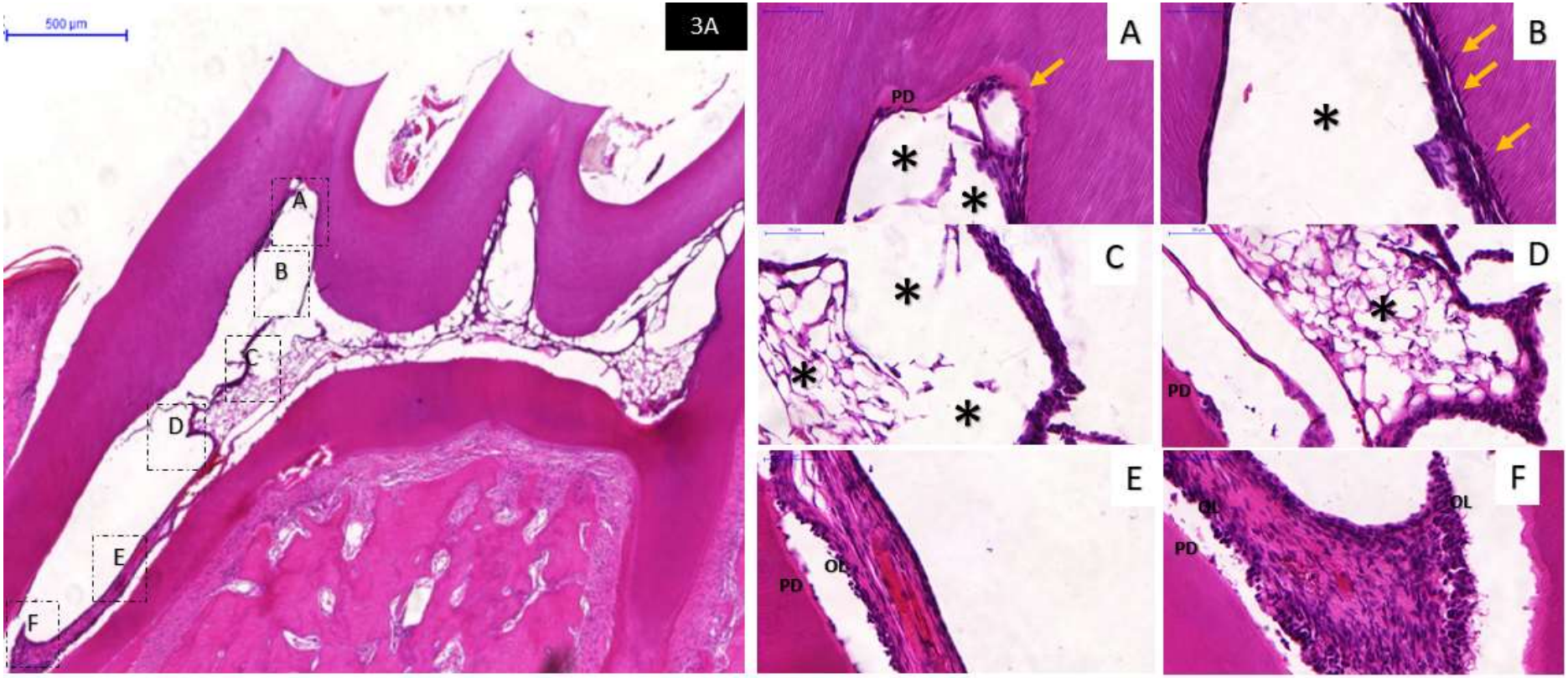
5.3.4.1 G3B

The specimens of this group showed interruption in the odontoblastic layer from the occlusal third of coronal pulp up to the cervical third of radicular pulp. Odontoblasts stucked into the pre-dentin were present throughout coronal pulp. In the same area, necrosis was also observed. Areas of edema were present in the whole pulp tissue, together with an aspect of complete tissue disorganization (Figure 9).

5.3.4.2 G3C

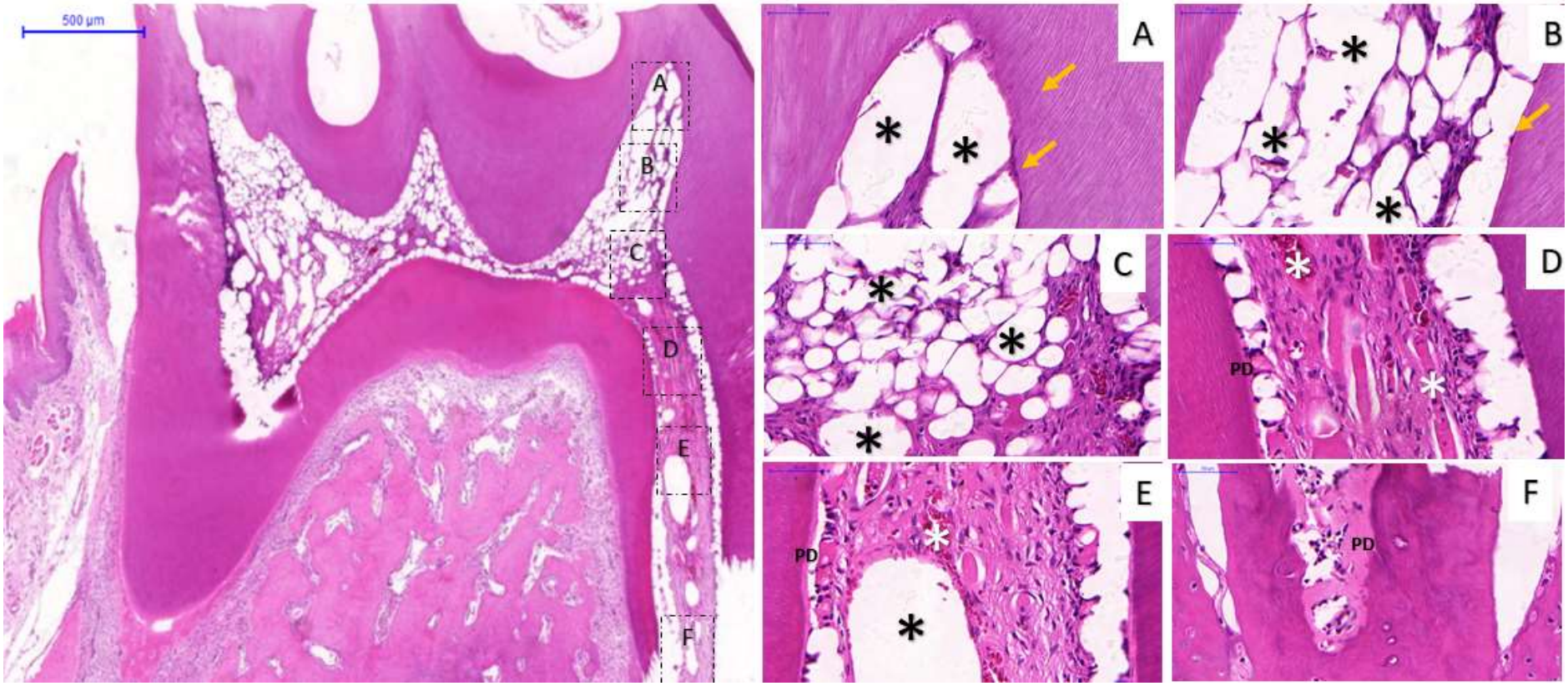
There was no odontoblastic layer in the coronal pulp. Pulp horns exhibited liquefied tissue, associated with polymorphonuclear and mononuclear cells. There was edema up to the middle third of radicular pulp. Despite of pulp tissue appeared organized, it was fibrous (Figure 10).

Figure 8 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G3A. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, yellow arrows show odontoblastic cells sucked into pre-dentin layer and black asterisks indicate areas of edema and absence of cells.



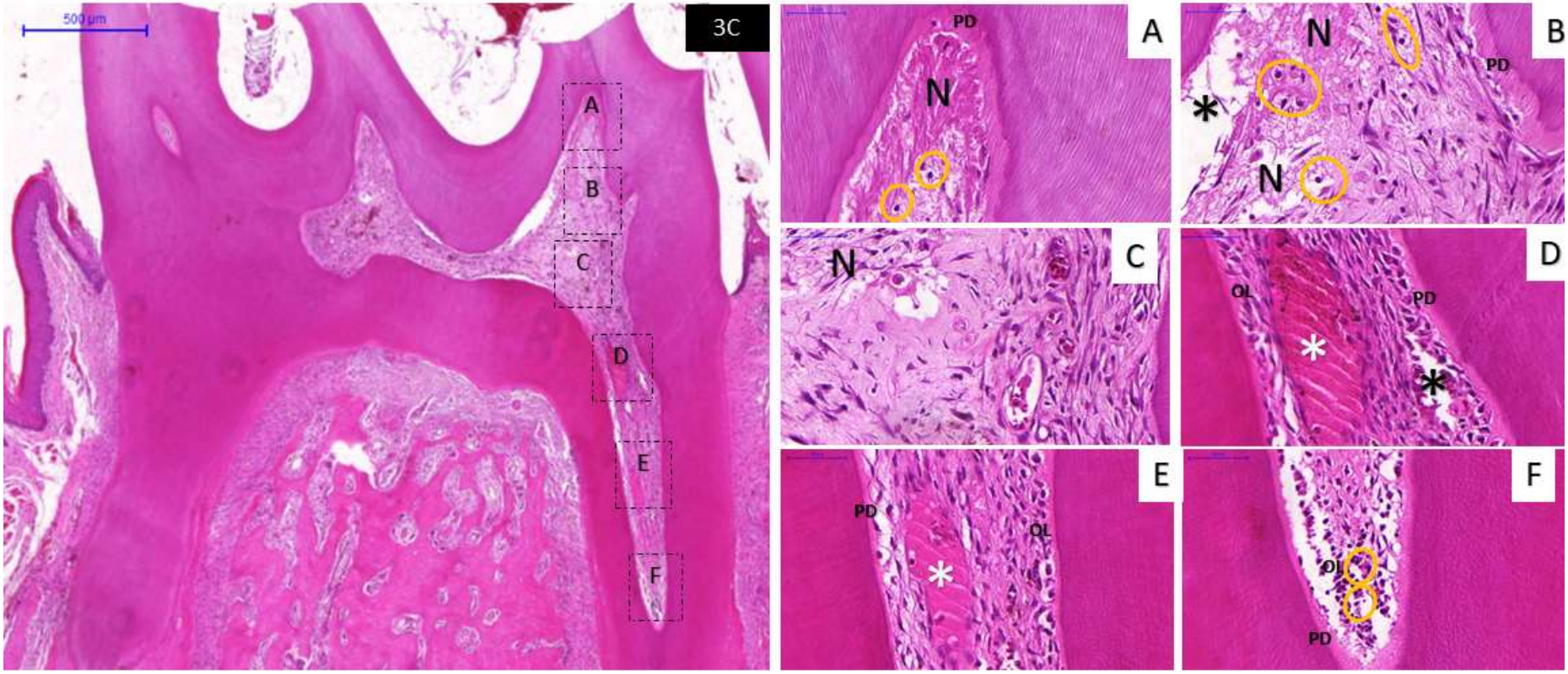
Source: The author, 2018.

Figure 9 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G3B. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, yellow arrows present odontoblastic cells sucked into pre-dentin layer, black asterisks indicate areas of edema and absence of cells and white asterisks indicate dilated and congested blood vessels.



Source: The author, 2018.

Figure 10 - Representative photomicrographs stained with hematoxylin-eosin of the specimens of the G3C. Panels A to F are magnifications (400x) corresponding to each demarcated area of the panoramic image. PD = pre-dentin, OL = odontoblastic layer, N = necrotic areas, black asterisks indicate areas of edema and absence of cells, white asteristics indicate dilated and congested blood vessels and yellow circles indicate inflammatory cells.



Source: The author, 2018.

5.3.5 Statistical analysis

Analysing the scores assigned to inflammatory cell infiltrate, there was significant statistically difference between most of the groups ($p<0.05$) (Table 1). In the occlusal and middle thirds, only 1C and 2C groups did not show statistically significant difference in comparison to the control group ($p>0.05$). In the cervical third of coronal pulp, besides the C groups, the specimens subjected to 1A modality of treatment also did not show statistically significant difference in comparison to the control group ($p>0.05$). These thirds presented wide areas of liquefative necrosis and absence of cells, mainly in the pulp horns. In the cervical third of radicular pulp, there were significant diferences when comparions between 2A, 2B, 3A, 3B and control group were made ($p<0.05$). Middle and apical thirds of radicular pulp did not differ significantly from the control.

Table 1 - Comparison among medians observed for inflammatory cell infiltrate in the pulp thirds.

Group		Coronal Pulp			Radicular Pulp		
		Occlusal	Middle	Cervical	Cervical	Middle	Apical
Control		1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}
1 - 35% H_2O_2	A	5^{b}	5^{c}	5^{abcd}	3^{abcd}	1^{a}	1^{a}
	B	5^{b}	5^{c}	5^{bcd}	5^{abd}	1^{a}	1^{a}
	C	2^{a}	2^{a}	1^{ab}	1^{ab}	1^{a}	1^{a}
2 - 38% H_2O_2	A	5^{b}	5^{c}	5^{cd}	2^{bcd}	1^{a}	1^{a}
	B	5^{b}	5^{c}	5^{cd}	5^{d}	1^{a}	1^{a}
	C	2^{a}	2^{ab}	1^{a}	1^{a}	1^{a}	1^{a}
3 - 40% H_2O_2	A	5^{b}	5^{c}	5^{d}	5^{cd}	1^{a}	1^{a}
	B	5^{b}	5^{c}	5^{d}	5^{d}	1^{a}	1^{a}
	C	5^{b}	5^{bc}	1^{abc}	1^{abc}	1^{a}	1^{a}

Different letters in the columns indicate that there was significant difference among the groups (Kruskal-Wallis and Dunn's test, $p<0.05$).

Source: The author, 2018.

In the occlusal third of 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C groups, there was significant difference from 1A and control groups ($p<0.05$). In the middle third, only 1A and 2A groups did not differed from control ($p>0.05$). The occurrencce of disrupted odontoblastic layers was predominant in these thirds. In the cervical thirds, significant differences were not observed in 1A and 1C groups, in coronal pulp, and in 1A and 1B

groups, in the radicular pulp, compared to control (p>0.05). All thirds of groups 2B, 2C, 3A, 3B and 3C showed significant statistically difference to control group (p<0.05), having the middle and apical thirds of radicular pulp presented predominance of a disorganized odontoblastic layer, differently from the other thirds, which showed odontoblastic layers disrupted.

Table 2. Comparison among medians observed for the condition of odontoblastic layer in the pulp thirds.

Group		Coronal Pulp			Radicular Pulp		
		Occlusal	Middle	Cervical	Cervical	Middle	Apical
Control		1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}
1 - 35% H_2O_2	A	2^{a}	2^{ab}	1^{ab}	1^{ab}	1^{a}	1^{a}
	B	3^{b}	3^{c}	2^{bc}	2^{abc}	2^{bc}	2^{bc}
	C	3^{b}	3^{bc}	2^{abc}	2^{c}	1^{ab}	1^{ab}
2 - 38% H_2O_2	A	3^{b}	2^{ab}	2^{c}	2^{bc}	1^{abc}	1^{a}
	B	3^{b}	3^{c}	3^{c}	3^{c}	2^{c}	2^{c}
	C	3^{b}	3^{c}	3^{c}	3^{bc}	2^{abc}	1^{a}
3 - 40% H_2O_2	A	3^{b}	3^{c}	3^{c}	3^{c}	2^{c}	2^{bc}
	B	3^{b}	3^{c}	3^{c}	3^{c}	2^{bc}	2^{bc}
	C	3^{b}	3^{c}	3^{c}	2^{c}	2^{c}	2^{c}

Different letters in the columns indicate that there was significant difference among the groups (Kruskal-Wallis and Dunn's test, p<0.05).

Source: The author, 2018.

For the vascular alterations, significant diferences were observed in the occlusal and middle thirds of coronal pulp when comparing the control and 1A group, with the 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C groups (p<0.05) (Table 3). In the cervical third of coronal pulp, only 2C group showed the same distribution of control (p>0.05). In the radicular pulp of groups 1C, 2C and 3C, differences were not observed (p>0.05). None of groups differed from each other in the apical third (p>0.05).

Table 3 - Comparison among medians observed for vascular alterations in the pulp thirds.

Group		Coronal Pulp			Radicular Pulp		
		Occlusal	Middle	Cervical	Cervical	Middle	Apical
Control		1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}
1 - 35% H_2O_2	A	2^{a}	2^{a}	2^{b}	2^{bc}	2^{bc}	1^{a}
	B	2^{b}	2^{b}	2^{b}	2^{c}	2^{b}	1^{a}
	C	3^{b}	3^{b}	3^{b}	2^{abc}	2^{abc}	1^{a}
2 - 38% H_2O_2	A	2^{b}	2^{b}	2^{b}	2^{bc}	2^{bc}	1^{a}
	B	2^{b}	2^{b}	2^{b}	2^{c}	2^{c}	1^{a}
	C	2^{b}	2^{b}	2^{ab}	2^{abc}	2^{abc}	1^{a}

3 - 40% H_2O_2	A	2^{b}	2^{b}	2^{b}	2^{bc}	2^{b}	1^{a}
	B	2^{b}	2^{b}	3^{b}	2^{bc}	2^{bc}	1^{a}
	C	2^{c}	2^{b}	2^{b}	2^{ab}	1^{ab}	1^{a}

Different letters in the columns indicate that there was significant difference among the groups (Kruskal-Wallis and Dunn's test, p<0.05).

Source: The author, 2018.

Table 4 shows the comparisons among tissue disorganization, whose analysis was performed considering the totality of the pulp tissue instead of each third. Groups 1A, 1B, 2A, 2B, 3A and 3B significantly differed from the control group (p<0.05). In the C modality of treatment, in which analysis occurred after 72 hours of the bleaching session regardless of the concentration of HP, no statistically significant difference could be noted (p>0.05).

Table 4 -Comparison among medians observed for tissue disorganization.

Group		Medians
Control		1^{a}
1 - 35% H_2O_2	A	3^{bc}
	B	4^{c}
	C	2^{ab}
2 - 38% H_2O_2	A	3^{bc}
	B	4^{c}
	C	2^{ab}
3 - 40% H_2O_2	A	4^{c}
	B	4^{c}
	C	2^{ab}

Different letters in the columns indicate that there was significant difference among the groups (Kruskal-Wallis and Dunn's test, p<0.05).

Source: The author, 2018.

5.4 DISCUSSION

In office bleaching techniques can provide rapid results of teeth darkening reversal, with considerable changes in shade guide units after two sessions (Reis *et al*. 2011) through the application of bleaching gels with high concentration of HP (Attin *et al*. 2003; Reis et al. 2011 B). Considering the ascending request for faster outcomes, such techniques need to be kept under study. The higher the concentration, the greater the bleaching effectiveness when short periods are considered (Almeida *et al.* 2015).

However, high concentrations of HP in bleaching gels lead to tooth sensibility and injuries to the pulp tissue (Costa *et al*. 2010, Cintra *et al.* 2013, Cintra *et al*. 2016 (A), Cintra *et al*. 2016 (B), Roderjan *et al*. 2015, Soares *et al*. 2014, Vaz *et al*. 2016).

The present study evaluated the acute effects of 35, 38 and 40% HP in the pulp tissue, considering the inflammatory response, condition of odontoblastic layer, vascular alterations and tissue disorganization after bleaching procedures with different periods. Most studies investigated bleaching pulp tissue effects, using HP in the concentrations of 20% and/or 35% (Cintra *et al*. 2013, Cintra *et al.* 2016, Cintra *et al.* 2016, Costa et al, 2012, Ferreira *et al.* 2013, Lima *et al.* 2016, Roderjan *et al.* 2015) or 38% in isolation (Costa *et al.* 2010, Kina *et al.* 2010, Vaz *et al.* 2018). *S*tudies employing HP at 40% or comparing the effects of high concentrated bleaching agents under the same metodology still were not performed. The different types of study and bleaching protocols may impair the comparisons among them (Cintra *et al.* 2013, Ferreira *et al.* 2013, Kina *et al.* 2010). In this study, an *in vivo* model using rat molars was preferred due to its degree of physiological similarity with human teeth (Dammaschke 2010), besides, the aforementioned model has already proved to be adequate for studies of bleaching protocols (Cintra *et al.* 2013, Cintra *et al.* 2016).

However, caution must be taken in the establishment of evaluation periods of study with rats molar. Althought its biological reactions are comparable to humans, rats molar pulps present exceptional reactivity, resilience and repair capability. Proportionally, a day of life of a rat correspond to 30 days of a human (Dammaschke 2010). Studies evaluating tooth bleaching pulp damage using rat model have adopted, predominantly, analysis period of two, seven and thirty days after experimental bleaching procedures (Cintra *et al*. 2013, Cintra *et al*. 2016, Lima *et al*. 2016, Benetti *et al*. 2017), which corresponds, by equivalence, to too prolonged times in humans, leaving a gap when it comes to findings in the short term.

In this study, there was inflammatory response resulting from the application of HP in all three concentrations studied, having the pulp effects been more pronounced in the occlusal and middle thirds of coronal pulp and in the groups in which 40% HP was applied. Besides inflammatory cell infiltrate, liquefactive necrosis was observed, similarly to other authors (Cintra *et al.* 2013, Cintra *et al.* 2016 (A), Cintra *et al.* 2016 (B), Lima *et al.* 2016, Benetti *et al*. 2017). Such events result from the diffusion of the bleaching agent through enamel and dentinal tubules reaching pulp tissue (Soares *et*

al. 2014). In rats' molars, dental hard substrates present low mineral density, more porosity and enamel of a thinner thickness, allowing, thus, a higher degree of diffusion (Tanaka 1989). The degree of diffusion of bleaching agents is directly proportional to the HP concentration and the contact time of the gel with enamel (Benetti *et al.* 2004, Camargo *et al.* 2007, Cintra *et al.* 2016). Given that, in this study, the contact time of the gel was standardized, the degree of penetration of HP only varied according to its concentrations. Thus, it is fair to assume that 40% HP presented a higher degree of penetration in the pulp chamber in comparison with 35% and 38% HP, and, therefore, led to more damage. Once HP penetrate tooth estructure, it degrades into free radicals, such as reactive oxygen species (ROS) and hydroxil ions (Walsh 2000, Martindale & Holbrook, 2002), which induce cell membrane damage and protein fragmentation, resulting in cell death and decreased capacity of proliferation (Davies 1999, Martindale & Holbrook 2002, Dias Ribeiro *et al.* 2009).

In groups 1A, 1B, 2A, 2B, 3A and 3B, the presence of odontoblastic cells sucked into pre-dentin layer was a common finding, which was not reported in other similar studies, probably because they did not adopt immediate period of analysis (Cintra *et al.* 2013, Cintra *et al.* 2016, Cintra *et al.* 2016, Lima *et al.* 2016, Benetti *et al.* 2017). Odontoblasts are the first cells to become in contact with aggressor agents that diffuse through dentin tubules (Sloan & Smith 2007). When a high intensity agression reaches the dentin-pulp complex, odontoblasts death or even aspiration of these cells into the dentin tubules are events that may occur initially, triggering a process of autolysis (Sloan & Smith 2007).

Regarding vascular alterations, according to Ferreira *et al.* (2013), the increase of vascular permeability produced by a substance is a mean of assessing the capacity of such substance to induce inflammatory responses. In this study, significant vascular alterations were noted mainly in coronal pulp, having been a finding more related to the period of analysis than to the concentration of the bleaching agent. Edema, dilated and congested blood vessels, and hemorrhagic areas were also observed in the coronal pulp tissue, as observed in other assays (Cintra *et al.* 2013, Cintra *et al*. 2016, Lima *et al.* 2016). However, differently from the most studies (Cintra *et al.* 2013, Cintra *et al.* 2016, Lima *et al.* 2016, Benetti *et al.* 2017), our results revealed the occurrence of vascular alterations extensive to the cervical and middle thirds of radicular pulp from the first bleaching session. This reinforces the aggressive manner of the bleaching

agents employed in the present study and the importance of also analyzing the changes occurring in immediate periods in such a way as to elucidate the extension of all events resulting from these protocols. Vaz *et al.* (2016) did not found changes in the vascular condition of the pulp tissue subject to 38% HP applied for 45 minutes throughout 3 sessions or 15% carbamide peroxide applied for 16 days for 2 hours a day. Kina *et al*. (2010) also did not report pulp vascular alterations following the application of 38% HP for 30 minutes. In the aforementioned cases, the analysis were performed after 7 days and up to 15 days, respectively. Thus, it is not possible to affirm whether the specimens assessed in these studies have experienced vascular alterations immediately after the procedure (Ferreira *et al.* 2013).

The aspect of tissue disorganization was evident in groups 1A, 1B, 2A, 2B, 3A and 3B, being that in the two groups in which 40% HP was applied, were assigned to the highest scores. Lima *et al.* (2016) also reported tissue disorganization ever since the first 6 hours following a single session of application of the bleaching gel, while Cintra *et al.* (2013) could only observe this after the fourth bleaching session. In the study of Vaz *et al.* (2016), there was no differences in pulp tissue organization between groups because of the methodology used of mechanically remove dental pulp. In the present study, groups C, whose analysis were performed 72 h after the last bleaching session, did not differ significantly from the control. According to Soares *et al.* (2014), after 72 hours of exposition to bleaching agents' byproducts, pulp cells start to exhibit cell viability recovery. In these groups, there was a recovery of the tissue architecture and filling of the areas where absence of cells had taken place. Even with this tissue restructuring, odontoblastic layer was not readily restored, which might be explained by the fact that odontoblastic differentiation is impaired up to 7 days after the contact with toxic concentrations of HP (Soares *et al.* 2014). Additionally, although these events are indicative of repair, a prolonged level of apoptosis might be maintained, giving continuity to the sequence of cells death (Benetti *et al.* 2017). Lima *et al.* (2016) describes a process of pulp repair as a continuous replacement of the necrotic areas by new connective tissue of high cellularization and vascularization. In both cases, these histopathological characateriscts can be interpreted as signs of repair. However, comparisons between such processes between rat and human teeth must be made with caution as rat molar pulps demonstrate exceptional resilience and healing capacity (Dammaschke 2010).

Attempting to soften damage to the teeth, manufactures add some ingredients in the formulation of the bleaching agents. According to the manufacterers, among the gels used in this study, one contains 2% of calcium gluconate (Whitness HP Blue© 35%) and the others contain 1,1% of fluoride and 3% of potassium nitrate (Opalescence Boost© 38% and 40%). Calcium decreases dentin permeability reducing the rate of HP penetration in the pulp tissue and providing more time to the pulp cells defend from the damage (Kossatz *et al.* 2012). Fluoride occludes dentinal tubules and potassium nitrate reduces nerve excitability and ability of the nerve to trasmit pain (Mrkowitz, Bilotto, Kim, 1991). The pulp tissue itself presents means of self-defense represented by lymphatic drenage, which eliminates toxic products, and by the release of endogenous antioxidant agents (peroxidases and catalases) able to degrade HP (Esposito *et al.* 2003). Dentinal fluid flow, cytoplasmatic extensions of odontoblasts and other intratubular components also represent means of limitating the diffusion of HP through dentinal tubules and hit the pulp tissue (Kina *et al.* 2010). Even with these defense resources, bleaching gels application resulted in inflammatory response, necrosis, vascular alterations and tissue disorganization. According to previous studies, the higher the concentration of HP, the greater the damage induced in the pulp tissue (Benetti *et al.* 2004, Cintra *et al.* 2016). However, this was just partially true for the present study, as 40% HP led to damage of a higher degree, while 35% and 38% induced similar and less severe alterations.

Clinically, patients undergoing bleaching treatment report high levels of tooth sensitivity initially, which decreases as time goes by, corresponding to the process of pulp recovery after the procedure. Despite this recovery, pulp tissue becomes aged and with less responsiveness to new aggressors (Benetti *et al.* 2017). The literature has not yet reported the occurrence of complete loss of tooth vitality following bleaching procedures as well as have not investigated the clinical effects of 40% HP in human teeth to clarify if pulp behavior will be similar. The findings presented in this study will be useful to drive further *in vivo* studies to elucidate the pulp responses to bleaching agents of high concentrations not yet investigated.

5.5 CONCLUSION

In conclusion, in-office bleaching with 35%, 38% and 40% HP resulted in immediate inflammatory response, necrosis, and alterations in the odontoblastic layer, in the vascular condition, and in the pulp tissue organization. HP in the concentration of 40% induced more severe alterations, while 35% and 38% HP led to milder damage. After 72 hours, pulp damage starts to be repaired along with the appearence of signs of aging. Further clinical studies including short periods of analysis are needed to confirm whether the events found in this study would also be found in human teeth. For the time being, it would be advisable for clinicals to opt for bleaching protocols which lead to satisfactory esthetic results through the adoption of lower concentrations of HP.

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6 CONCLUSÕES

Pode-se concluir que:

O peróxido de hidrogênio a 35%, 38% e 40% provocou alterações significativas quanto à inflamação na polpa dentária de ratos:

- Na concentração de 35%, houve necrose na polpa coronária nos terços oclusal e médio nos espécimes do grupo A e nos terços oclusal, médio de cervical nos espécimes do grupo B.
- Na concentração de 38%, houve necrose da polpa coronária e radicular nos terços oclusal, médio e cervicais nos espécimes do grupo A, e nos terços oclusal, médio e cervical nos espécimes do grupo B. No terço cervical da polpa radicular do grupo B, houve infiltrado celular inflamatório significativo.
- Na concentração de 40%, houve necrose da polpa coronária e radicular nos terços oclusal, médio e cervicais nos espécimes dos grupos A e B, e nos terços oclusal e médio nos espécimes do grupo C.

O peróxido de hidrogênio a 35%, 38% e 40% provocou alterações significativas quanto à condição da camada odontoblástica da polpa dentária de ratos:

- Na concentração de 35%, apresentaram desorganização significativa os terços cervical da polpa coronária e médio e apical da polpa radicular do grupo B e cervical da polpa radicular do grupo C, enquanto que os terços oclusal e médio da polpa coronária dos grupos B e C apresentaram interrupção significativa.
- Na concentração de 38%, apresentaram desorganização significativa os terços cervicais do grupo A e os terços médio e apical do grupo B, enquanto que os terços oclusal do grupo A e terços oclusal à médio da polpa radicular dos grupos B e C apresentaram interrupção significativa.
- Na concentração de 40%, apresentaram desorganização significativa os terços médio e apical da polpa radicular dos grupos A, B e C e cervical da polpa radicular do grupo C, enquanto que os terços de oclusal à cervical da polpa radicular e de oclusal à cervical da polpa coronária apresentaram interrupção significativa.

O peróxido de hidrogênio a 35%, 38% e 40% provocou alterações vasculares significativas na polpa dentária de ratos:

- Na concentração de 35%, apresentaram alterações significativas os terços cervical e médio da polpa radicular do grupo A, os terços de oclusal à médio da polpa radicular do grupo B, e os terços de oclusal à cervical da polpa radicular do grupo C.
- Na concentração de 38%, apresentaram alterações significativas os terços de oclusal à médio da polpa radicular grupos A e B, e terços oclusal e médio da polpa coronária do grupo C.
- Na concentração de 38%, apresentaram alterações significativas os terços de oclusal à médio da polpa radicular dos grupos A e B e terços oclusal, médio e cervical da polpa coronária do grupo C.

O peróxido de hidrogênio a 35%, 38% e 40% provocou alterações significativas quanto à desorganização tecidual da polpa dentária de ratos nos grupos 1A, 1B, 2A, 2B, 3A e 3B.

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APÊNDICE A - FICHA PARA ANÁLISE QUALITATIVA DA CONDIÇÃO DA CAMADA ODONTOBLÁSTICA

Condição da camada odontoblástica						
Lâmina	Polpa coronária			Polpa radicular		
	Terço oclusal	Terço médio	Terço cervical	Terço cervical	Terço médio	Terço apical

APÊNDICE B - FICHA PARA ANÁLISE QUALITATIVA DAS ALTERAÇÕES VASCULARES

Alterações vasculares						
Lâmina	Polpa coronária			Polpa radicular		
	Terço oclusal	Terço médio	Terço cervical	Terço cervical	Terço médio	Terço apical

APÊNDICE C - FICHA PARA ANÁLISE QUALITATIVA DA DESORGANIZAÇÃO TECIDUAL

Desorganização tecidual	
Lâmina	**Escore atribuído**

APÊNDICE D - FICHA PARA ANÁLISE SEMIQUANTITATIVA DO INFILTRADO INFLAMATÓRIO

Número de células por campo																																
Lâmina	Polpa coronária																	Polpa radicular														
	Terço oclusal					Terço médio					Terço cervical							Terço cervical					Terço médio					Terço apical				
	1	2	3	T	E	1	2	3	T	E	1	2	3	4	5	T	E	1	2	3	T	E	1	2	3	T	E	1	2	3	T	E

T = Total; E = Escore

ANEXO A - CONSENTIMENTO ÉTICO DO CEUA PARA REALIZAÇÃO DO ESTUDO

Universidade Federal de Pernambuco
Centro de Ciências Biológicas
Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8340 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Recife, 26 de junho 2015.

Ofício nº 64/15

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: Profº. Danyel Elias da Cruz Perez
Departamento de Clínica Odontologia Preventiva/ Patologia Oral
Universidade Federal de Pernambuco
Processo nº 23076.013107/2015-24

Os membros da Comissão de Ética no Uso de Animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEUA-UFPE) avaliaram seu projeto de pesquisa intitulado "**Avaliação do peróxido de hidrogênio em diferentes concentrações sobre a polpa dentária de ratos: estudo histológico e imunohistoquímico".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEUA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 11.794 de 08 de outubro de 2008, que trata da questão do uso de animais para fins científicos e didáticos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais a serem realizados.

Origem dos animais: Biotério do Departamento de Nutrição/ UFPE; Animais: rato heterogênico; Linhagem: rattus novergicus, albinus Wistar; Idade: 4 semanas; Peso: 180-200g; Sexo: macho; Nº total de Animais: 36

Atenciosamente,

[redacted]

Prof. Dr. Pedro V. Carelli
Presidente da CEUA / CCB - UFPE
SIAPE 1801584

ANEXO B - NORMAS DA REVISTA (INTERNATIONAL ENDODONTIC JOURNAL)

1. Manuscript format and structure

1.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Presentation: Authors should pay special attention to the presentation of their research findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

1.2. Structure

All manuscripts submitted to *International Endodontic Journal* should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:

• **Aim**: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• **Methodology**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.

• **Results**: Give the main results of the study, including the outcome of any statistical

analysis.

• **Conclusions**: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles should be non-structured of no more than 250 words giving details of what was done including the literature search strategy.

Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.

Abstract for Case Reports should be no more than 250 words using the following structure:

• **Aim**: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Summary**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• **Key learning points**: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 250 words using the following structure:

• **Aim:** Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Methodology:** Describe the methods adopted.

• **Results:** Give the main results of the study.

• **Conclusions:** State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the

fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.
All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.
Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.
Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.
Conclusion: should contain a summary of the findings.
Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.
Main Text of Mini Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should briefly introduce the subject matter and justify the need and timeliness of the literature review. The Review section should be divided into logical sub-sections to enhance readability and understanding and may be supported by up to 5 tables and figures. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The Conclusions section should present clear statements/recommendations and suggestions for further work. The manuscript, including references and figure legends should not normally exceed 4000 words.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

Acknowledgements: *International Endodontic Journal* requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

1.3. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by *et al.* is sufficient, e.g. (Tobias *et al.* 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias *et al*. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add *et al.*
(ii)Year of publication in parentheses
(iii) Full title of paper followed by a full stop (.)
(iv) Title of journal in full (in italics)
(v) Volume number (bold) followed by a comma (,)
(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. *International Endodontic Journal* **24**, 8-14.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* **16**, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). *Blood* **54** (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) *Surgical Endodontics*, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. *Endodontics in Clinical Practice*, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.
Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html [accessed on 7 November 2003]

1.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part

figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.
Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.
Permissions: If all or part of previously published illustrations are to be used, permission must be obtained from the copyright holder concerned. This is the responsibilty of the authors before submission.
Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley Blackwell's guidelines for figures: http:/authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp.

1.5. Supporting Information

Publication in electronic formats has created opportunities for adding details or whole sections in the electronic version only. Authors need to work closely with the editors in developing or using such new publication formats.
Supporting information, such as data sets or additional figures or tables, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted. It should be clearly stated at the time of submission that the supporting information is intended to be made available through the online edition. If the size or format of the supporting information is such that it cannot be accommodated on the journal's website, the author agrees to make the supporting information available free of charge on a permanent Web site, to which links will be set up from the journal's website. The author must advise Wiley Blackwell if the URL of the website where the supporting information is located changes. The content of the supporting information must not be altered after the paper has been accepted for publication.

The availability of supporting information should be indicated in the main manuscript by a paragraph, to appear after the References, headed 'Supporting Information' and providing titles of figures, tables, etc. In order to protect reviewer anonymity, material posted on the authors Web site cannot be reviewed. The supporting information is an integral part of the article and will be reviewed accordingly.

Preparation of Supporting Information: Although provision of content through the web in any format is straightforward, supporting information is best provided either in web-ready form or in a form that can be conveniently converted into one of the standard web publishing formats:
• Simple word-processing files (.doc or .rtf) for text.
• PDF for more complex, layout-dependent text or page-based material. Acrobat files can be distilled from Postscript by the Publisher, if necessary.
• GIF or JPEG for still graphics. Graphics supplied as EPS or TIFF are also acceptable.
• MPEG or AVI for moving graphics.

Subsequent requests for changes are generally unacceptable, as for printed papers. A charge may be levied for this service.

Video Imaging: For the on-line version of the Journal the submission of illustrative video is encouraged. Authors proposing the use such media should consult with the Editor during manuscript preparation.

2. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

2.1. Figures

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