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LETÍCIA ANTONELO CAMPOS

CARACTERIZAÇÃO DE UM MODELO DE RECESSÃO GENGIVAL EM RATOS PARA O ESTUDO DA HIPERSENSIBILIDADE DENTINÁRIA

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Tese apresentada à Universidade Estadual de Ponta Grossa para obtenção do título de Doutora em Clínica Integrada - Terapêutica Clínica Aplicada à Odontologia.

Orientador: Prof. Dr. Fábio André dos Santos

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Caracterização de um modelo de recessão gengival em ratos para estudo da hipersensibilidade dentinária

Tese apresentada ao Programa de Pós-graduação Stricto sensu em Odontologia da Universidade Estadual de Ponta Grossa, como requisito parcial à obtenção do título de Doutora em Odontologia, área de concentração em Clínica Integrada, linha de Etiologia, diagnóstico e tratamendo das doenças bucais.

Ponta Grossa, 31 de agosto de 2018

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DADOS CURRICULARES

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RESUMO

Campos, L.A. **Caracterização de um modelo de recessão gengival em ratos para o estudo da hipersensibilidade dentinária.** [Tese] Doutorado em Clínica Integrada. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2018.

A hipersensibilidade dentinária pode ser definida como dor derivada da dentina exposta em resposta a substâncias químicas, táteis, térmicas ou estímulos osmóticos que não podem ser explicados como decorrentes de qualquer outro defeito ou doença dentária. Dentre os fatores etiológicos, encontra-se a recessão gengival, que gera exposição dos túbulos dentinários da região cervical para o meio bucal. Portanto, o objetivo deste estudo foi estabelecer um modelo de recessão gengival em ratos para o estudo da hipersensibilidade dentinária e avaliar a aplicação de diferentes formulações de biovidros na dentina exposta. A recessão gengival foi induzida cirurgicamente em 56 ratos, os quais foram divididos em grupos que receberam de E.D.T.A. e E.D.T.A./Ácido fosfórico 10%, controle Naive, controle sem tratamento apenas com a Recessão Gengiva induzida, e os tratamentos: Verniz, Biossilicato, Biossilicato com Estrôncio e Biossilicato com Potássio. Dentre as análises estão de morfologia da dentina exposta por meio de microscopia eletrônica de varredura, análise do peso corporal dos animais, a fluxometria da polpa, análise histológica e comportamental pelos testes de campos aberto e labirinto em cruz elevado. Os resultados mostram que o modelo empregado não interferiu na dieta alimentar dos animais pois não houve diminuição do peso corporal. Na fase prévia aos tratamentos observou-se uma diminuição do fluxo sanguíneo do tecido pulpar ao final da fase experimental para todos os grupos. E os grupos com a aplicação de E.D.T.A. e E.D.T.A./Ácido fosfórico 10% apresentaram uma remoção da *smear layer* e debris, por meio da abertuta dos túbulos dentinários. No grupo com E.D.T.A./ácido houve redução significativa da frequência de locomoção e do aumento do tempo em menores ambulações nos testes comportamentais. Na análise histológica, nenhum grupo apresentou mudanças detectáveis na estrutura dos tecidos moles da polpa, caracterizado como infiltrado infamatório intenso. Sendo este modelo de recessão gengival induzida cirurgicamente válido para a exposição da dentina cervical ao meio

bucal, tornando os túbulos dentinários abertos, onde alterou o comportamento animal quando foi submetido ao ataque ácido, porém sem causar alterações no tecido pulpar. E quando tratados com diferentes formulações de biovidros, todos os grupos reduziram o fluxo sanguíneo pulpar. E o grupo tratado com estrôncio apresentou sinais de menor ansiedade.

Palavras-chave: Retração gengival, Sensibilidade da dentina, Dessensibilizantes dentinários.

ABSTRACT

Campos, L.A. **Characterization of a gingival recession model in rats for the study of dentin hypersensitivity.** [Tese] Doutorado em Clínica Integrada. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2018.

Dentin hypersensitivity may be defined as pain derived from exposed dentin in response to chemical, tactile, thermal, or osmotic stimuli that can not be explained as arising from any other defect or dental disease. Among the etiological factors, there is gingival recession, which generates exposure of the dentinal tubules from the cervical region to the buccal environment. Therefore, the objective of this study was to establish a model of gingival recession in rats for the study of dentin hypersensitivity and to evaluate the application of different bioglass formulations in the exposed dentin. Gingival recession was surgically induced in 56 rats, which were divided into groups receiving E.D.T.A. and E.D.T.A / 10% phosphoric acid, Naive control, control without treatment only with induced Gingival Recession, and the treatments: Varnish, Biosilicate, Biosilicate with Strontium and Biosilicate with Potassium. Among the analyzes were the morphology of exposed dentin by scanning electron microscopy, analysis of the animals' body weight, pulp flowmetry, histological and behavioral analysis by open field and high cross maze tests. The results show that the model employed did not interfere in the animals' diet as there was no decrease in body weight. In the pre-treatment phase a decrease in the blood flow of the pulp tissue was observed at the end of the experimental phase for all groups. And the groups with application of E.D.T.A. and E.D.T.A./ 10% phosphoric acid showed a removal of the smear layer and debris, through the opening of the dentinal tubules. In the E.D.T.A./Acid group there was a significant reduction in the frequency of locomotion and the increase in time in the lower ambulances in the behavioral tests. In the histological analysis, no group showed detectable changes in the structure of the soft tissues of the pulp, characterized as intense inflammatory infiltrate. This model of surgically induced gingival recession is valid for the exposure of the cervical dentin to the buccal environment, making the dentinal tubules open, where it altered the animal behavior when it was submitted to acid attack, but without causing changes in the pulp tissue. And

when treated with different bioglass formulations, all groups reduced pulp blood flow. And the strontium group showed signs of less anxiety.

Key words: Gingival Recession, Dentin Sensitivity, Dentin Desensitizing Agents.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

HD	Hipersensibilidade dentinária
RG	Recessão gengival
E.D.T.A.	Ácido etilenodiamino tetra-acético
tto	Tratamento
LTDA	Limitada
MEV	Microscopia eletrônica de varredura

LISTA DE SÍMBOLOS

%	Porcentagem
±	Mais ou menos
°C	Grau Celsius
H	Hora
G	Grama
mg	Miligrama
Kg	Kilograma
mL	Mililitro
mm	Milímetro
n°	Número
x	vezes
Kv	KiloVolts
mW	MegaWatts
cm	Centímetro
s	Segundo
nm	Nanômetro

SUMÁRIO

INTRODUÇÃO

A hipersensibilidade dentinária (HD) é uma condição multifatorial em que a dentina encontra-se exposta e permeável, em que a resposta do paciente varia de acordo com os diferentes estímulos. Estes compreendem agentes térmicos, evaporativos, osmóticos, táteis, químicos, elétricos e bacterianos[1-3]. A variabilidade com que a HD se apresenta para cada indivíduo dificulta a quantificação da mesma, pois alguns indicam dor, mas esta não afeta sua qualidade de vida, enquanto outros podem solicitar intervenção, a fim de obter alívio da dor[4]. A subjetividade da dor, aliada aos fatores causais e predisponentes podem interferir na resposta do indivíduo[5]. Sua prevalência varia de 3 a 98% e pode ser explicada - em parte - por diferentes métodos de avaliação e diferentes populações de pacientes[6,7]. A etiologia da HD é multifatorial, dentre os fatores predisponentes estão a recessão gengival, além da perda de esmalte na porção cervical da coroa por erosão, abrasão e abfração ou uma combinação destes[5-7], além de tratamento periodontal cirúrgico e não cirúrgico. A teoria mais aceita relacionada à HD é a da hidrodinâmica, descrita por Brännström, em que considera a movimentação dos fluídos presentes nos túbulos dentinários como consequência dos estímulos não nocivos, os quais contribuem para a deformação dos odontoblastos e/ou de seus prolongamentos, ao ativar os mecanorreceptores das terminações nervosas pulpares, que leva ao processo doloroso.[8,9] Portanto, o tratamento da HD visa suprimir o impulso nervoso por meio de interação neurológica ou bloqueio mecânico dos túbulos.

Diferentes metodologias foram descritas para tratar HD relacionada à causa, com vistas a proteger mecânica-quimicamente o complexo dentino-pulpar ou modificar a estimulação do nervo, concomitante ao controle dos fatores predisponentes[10,11]. No entanto, nenhum tratamento foi encontrado até o momento, que pudesse servir como um padrão-ouro terapêutico, ao eliminar de forma previsível e completa a percepção da dor, especialmente a longo prazo. De forma que, ao estabelecer um modelo de tratamento de HD, por meio da exposição cervical com recessão gengival em animais, facilmente reprodutível, capaz de promover informação sobre HD na presença de desafios alimentares, força mastigatória, temperatura, pH e saliva.

PROPOSIÇÃO

OBJETIVO GERAL

- Validar um modelo de recessão gengival para o estudo da hipersensibilidade dentinária em ratos.

OBJETIVOS ESPECÍFICOS

- Avaliar a estabilidade do modelo ao longo da fase experimental.
- Avaliar a exposição dos túbulos dentinários da região em que foi induzida a recessão gengival.
- Verificar se ocorre alteração do fluxo sanguíneo pulpar nos dentes com a dentina exposta.
- Analisar se o modelo ocasionou mudança no comportamento animal.
- Avaliar alterações inflamatórias no tecido pulpar dos dentes com recessão gengival.
- Avaliar a efetividade de diferentes biovidros no tratamento da hipersensibilidade dentinária.

Material e métodos

Animais

Foram utilizados ratos Wistar machos, de 3 meses de idade, com peso de 300-400 g, mantidos em ambiente com temperatura de 22 ± 2 ºC e sob ciclo de claro/escuro (12/12 h) controlado automaticamente. Os animais tinham livre acesso à alimentação e água. O projeto foi aprovado pelo comitê local de ética em pesquisa com animais da Universidade Estadual de Ponta Grossa (Protocolo 10039/2015). O cálculo amostral foi realizado com o programa GPower 3.1 (G*Power, Universität Düsseldorf, Düsseldorf, North Rhine-Westphalia, Alemanha).

Procedimento de recessão gengival (RG)

Os animais foram sedados com hidrato de cloral a 4%, sendo 400 mg/kg (1mL/100 g de peso). Em seguida, sobre a mesa cirúrgica foi realizada uma incisão na região palatina do 2º molar superior esquerdo até mesial do 1º molar superior esquerdo, com lâmina de bisturi (n.15c), de 1 a 1,5 mm abaixo da margem gengival, com remoção das fibras gengivais com cureta periodontal (Gracey 7-8). Sequencialmente, a osteotomia foi realizada com microcinzel ósseo Oschmbein nº 2, removendo-se 1 mm de osso na parte mesial e palatina do primeiro molar superior esquerdo. Posteriormente à exposição da dentina radicular foi instalado um dispositivo constituído por fio de amarrilho de aço 0,025 e tela-malha de aço (Morelli® Ortodontia, Sorocaba, São Paulo, Brasil) com posicionamento da tela na região cervical da recessão provocada cirurgicamente com torção do fio de amarrilho na porção mesial do 1º molar superior esquerdo e corte (com tesoura) do fio de amarrilho, de 1 a 1,5 mm de distância do dente (Figura 1). Este dispositivo permaneceu por 14 dias.

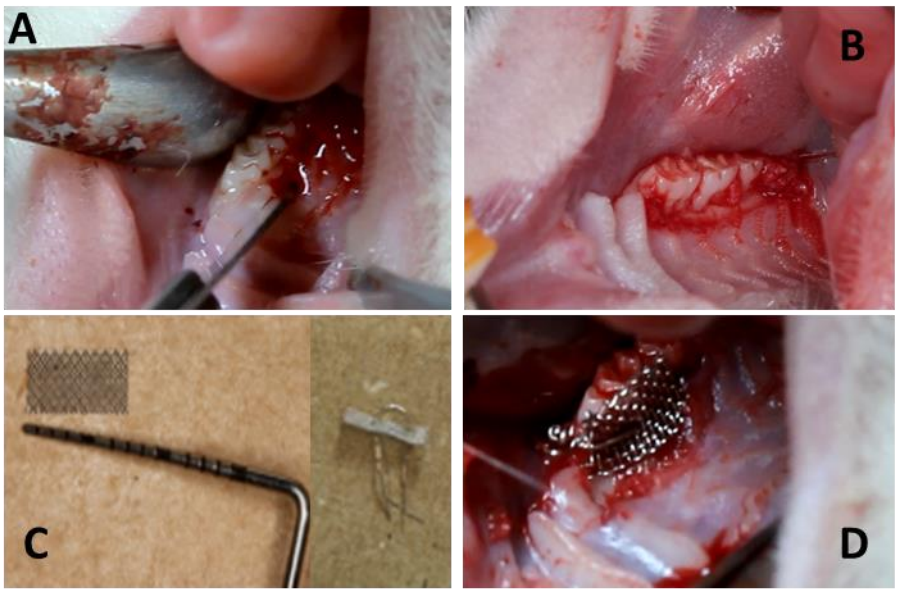

Figura 1. Procedimento de recessão gengival. A: Incisão gengival. **B:** Osteotomia. **C:** Dispositivo. **D:** Instalação do dispositivo.

EXPERIMENTO 1 e 2

Delineamento do estudo

Após o procedimento cirúrgico para exposição radicular por meio da recessão gengival induzida e instalação do dispositivo para a sua manutenção, aguardou-se 14 dias para a cicatrização gengival. No dia 0 (após o período de cicatrização) foi removido o dispositivo, e iniciaram-se os respectivos tratamentos com E.D.T.A. (ácido etilenodiamino tetra-acético), E.D.T.A. e ácido fosfórico 10% para estes grupos a cada quatro dias, totalizando sete tratamentos na fase experimental e as análises de fluxometria do tecido pulpar a cada oito dias, totalizando quatro leituras no tempo experimental de vinte e cinco dias, segundo o fluxograma abaixo (Figura 2).

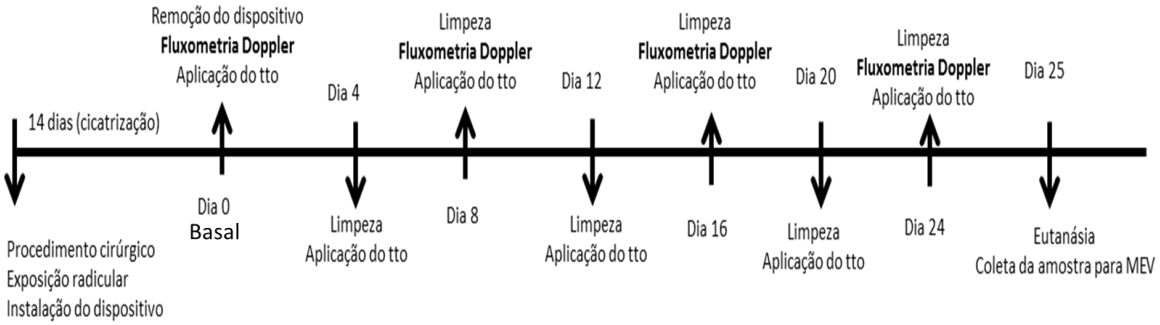

Figura 2. Delineamento do estudo.

Nota. tto = tratamento

Os animais foram divididos em 4 grupos (n= 08 por grupo):

- Naive – Sem recessão gengival e sem tratamento. Não foi realizado o procedimento de recessão gengival e para limpeza e remoção do biofilme da coroa do dente foi utilizado apenas solução salina por 3 minutos.
- Recessão gengival – Com recessão gengival e sem tratamento. Foi realizado o procedimento de recessão gengival e para limpeza e remoção do biofilme da dentina exposta foi utilizado apenas solução salina por 3 minutos.
- E.D.T.A. – Com recessão gengival e tratamento com E.D.T.A. Foi realizado o procedimento de recessão gengival e sobre a dentina exposta foi aplicado E.D.T.A. 24% (Reatec®, Reagen Ltda, Colombo, Paraná, Brasil), em bolinha de algodão com fricção por 5 segundos, manutenção em posição sobre a área exposta por 3 minutos.
- E.D.T.A. e Ácido Fosfórico – Com recessão gengival e tratamento com E.D.T.A. e ácido fosfórico. Foi realizado o procedimento de recessão gengival e sobre a dentina exposta foi aplicado E.D.T.A. 24% em bolinha de algodão com fricção por 5 segundos, manutenção em posição sobre a área exposta por 3 minutos. Em seguida, foi aplicada solução de ácido fosfórico 10% em bolinha de algodão e manutenção em posição por 20 segundos.

Os tratamentos foram realizados a cada quatro dias, sendo nos dias 0, 4°, 8°, 12°, 16°, 20° e 24°. No 25° dia os animais foram anestesiados e eutanasiados com punção cardíaca e obtidas as hemimaxilas para análises da morfologia da região de RG induzida e análise histológica do tecido pulpar.

Para os tratamentos os animais foram sedados com hidrato de cloral.

Peso corporal

O peso dos animais foi registrado no dia da cirurgia, no dia em que foi removido o dispositivo (dia 0), e nos dias 4°, 16° e 24° da fase experimental.

Análise da Fluxometria Pulpar - Dopller

As análises de fluxometria do tecido pulpar foram realizadas no dia da remoção do dispositivo (basal - dia 0 do fluxograma), a cada intervalo de oito dias, sendo nos dias 8°, 16° e 24°. Com o animal sedado a sonda era posicionada manualmente na região mesial do primeiro molar superior esquerdo a 2 mm da

margem gengival pelo tempo de 1 minuto. Previamente foi realizado após a limpeza da dentina exposta com algodão e antes da aplicação de E.D.T.A. e E.D.T.A. + ácido nos referentes grupos. O fluxo sanguíneo do tecido alvo é calculado, ao processar as informações contidas no sinal de fotocorrente[19]. Os registros foram efetuados com um fluxômetro moorVMS-LDF (Vascular Monitoring System - Laser Doppler Perfusion and Temperature Monitor - Moor Instruments, Axminster, UK) equipado com um laser diodo que emite no infravermelho, no comprimento de onda de 785 nm. O fluxômetro laser Doppler é um laser de baixa intensidade (aproximadamente 1 mW), e a sonda usada do mesmo fabricante acondicionada em um tubo de aço de 1,5 mm.

Análise por Microscopia Eletrônica de Varredura (MEV)

Para a análise por MEV foram utilizados 3 dentes (primeiro molar superior esquerdo) para cada grupo com o objetivo de verificar a morfologia da dentina exposta. Para o preparo ao MEV os dentes foram lavados com água destilada (20 mL por 15 segundos) e limpos em uma cuba ultrassônica durante 10 minutos, na temperatura de 47° C. A desidratação dos espécimes foi feita com uma sequência de imersão em álcool 25%, 50%, 70%, 90% e álcool absoluto (99,5%), permanecendo por 10 minutos em cada concentração. Em seguida, as amostras foram mantidas em uma estufa a 37° C por 24 horas, e então, permaneceram em um dessecador a vácuo por 48 horas. A metalização das amostras foi realizada em um aparelho Shimadzu C-50 (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brasil) por 10 minutos. Posteriormente, foram obtidas imagens de cada dente por meio do MEV Shimadzu SSX- 550 Superscan (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brasil), ao empregar voltagens de aceleração de 1,8; 10 e 20 Kv. As fotomicrografias foram analisadas de forma qualitativa de acordo com a característica da superfície dentinária e condição dos túbulos dentinários.

Teste Comportamental - Campo Aberto

O teste de campo aberto, o qual avalia a atividade motora e ansiedade do animal, foi desenvolvido em 1934 por Calvin Hall[14]. O aparelho é feito de madeira coberta com fórmica impermeável branca com o chão branco de 100 X 100 cm (dividida por linhas azuis em 25 quadrados de 20 x 20 cm) e paredes

brancas (40 cm de altura). O animal foi colocado na porção central do aparelho e os seguintes comportamentos foram registrados durante 5 minutos (tanto na sessão basal, antes de realizar o procedimento cirúrgico de recessão gengival e nos tempos experimentais dos dias 9° e 25°), número de ambulações na periferia, número de ambulações no centro, tempo na periferia, tempo no centro, número de elevações nas patas dianteiras, tempo de autolimpeza. O número de ambulações foi definido pelas linhas cruzadas com as quatro patas.

Teste Comportamental - Labirinto em Cruz Elevado

Desenvolvido por Handley e Mithani[15] e posteriormente validado por Pellow et al.[16], constitui de dois braços abertos e opostos, medindo 50x10 cm, e dois fechados em suas três faces externas por paredes de 40 cm de altura. As plataformas possuem as mesmas medidas dos braços abertos, cruzando-os perpendicularmente, o que delimita uma área central de 10 cm. O aparelho dista 50 cm do solo e os braços abertos não possuem bordas. O animal foi posicionado na plataforma central de frente para um dos braços abertos e observado por 5 minutos. Os comportamentos foram registrados no dia anterior ao procedimento cirúrgico de recessão gengival, e nos dias 9° e 25°. Foi considerado o número de entradas nos braços abertos; número de entradas nos braços fechados; tempo de permanência nos braços abertos; tempo de permanência nos braços fechados e tempo de permanência no centro.

Avaliação do tecido pulpar por histologia

Para avaliar as células do infiltrado inflamatório e a vascularização, os cortes histológicos dos fragmentos de maxila, o primeiro, segundo e terceiro molares foram fixados em solução de formol neutro a 10%. Após um período máximo de 48 horas de fixação, as amostras seguiram para a descalcificação, em solução de E.D.T.A. 9% a pH 7,2 aproximadamente 21 dias. As peças foram então preparadas para a inclusão em parafina com a face vestibular paralela ao plano de corte. Foram realizados cortes seriados com cerca de 5 µm de espessura e coradas com Hematoxilina e Eosina. Para cada amostra, foram selecionados 3 cortes histológicos, sendo que de cada corte foram fotografadas 4 regiões diferentes da polpa coronária e cervical, com a lente objetiva de 40x e ocular de 10x, com imagens em aumento de 400x. O critério para captura das imagens

foi a observação, no campo de visão da objetiva de 40x, da maior quantidade de área pulpar, acompanhada de margem em dentina. A análise da resposta inflamatória do tecido pulpar considerou a presença de neutrófilos polimorfonucleares, eosinófilos polimorfonucleares, infiltração de linfócitos, infiltração de células plasmáticas, macrófagos e células gigantes. A pontuação da resposta inflamatória foi definida de acordo com as células e vasos sanguíneos, em que 0. Ausente: ausência de inflamação; 1. Leve: células mononucleares escassas; 2. Moderado: infiltrado mononuclear e/ou dispersão neutrófilos e eosinófilos; 3. Intenso: infiltrado polimorfonuclear de neutrófilos e eosinófilos (Ramos, 2012[17]). A análise histológica foi realizada sob microscópio óptico de luz (Olympus, Tóquio - Japão), sendo observada a reação inflamatória do tecido pulpar qualitativamente.

Análise estatística

Os resultados foram expressos por média e desvio padrão para o peso corporal dos animais, análise de fluxometria sanguínea da polpa e testes comportamentais pela análise de variância (ANOVA) duas-vias, tendo como fatores fixos associados o tempo experimental e o tratamento. Nos casos em que foram encontradas diferenças significativas, as comparações múltiplas foram realizadas com o pós-teste de Bonferroni. Para morfologia dos túbulos dentinários, foi realizada análise descritiva. Para as características histológicas os dados foram avaliados qualitativamente para presença ou ausência de alterações patológicas e vasculares, por meio de estatística descritiva e por meio dos escores para a resposta inflamatória com o teste Kruskal-Wallis.O nível de significância adotado foi de 5% (α=0,05). Todos os cálculos foram realizados com programa de computador (GraphPadPrismversion 5.00 for Windows, GraphPad Software, La Jolla California, USA).

EXPERIMENTO 3

Delineamento do estudo

Após o procedimento cirúrgico para exposição radicular por meio da RG induzida e instalação do dispositivo para a sua manutenção, aguardou-se 14 dias para a cicatrização gengival. No dia 0 (após o período de cicatrização) foi removido o dispositivo, e iniciou-se os respectivos tratamentos, estes a cada

quatro dias, total de sete tratamentos na fase experimental. Foram realizadas análises de fluxometria do tecido pulpar a cada oito dias, no total de quatro leituras no tempo experimental de vinte e cinco dias. As avaliações comportamentais foram realizadas nos dias basal, 9º e 25º dia, total de três registros segundo o fluxograma abaixo (Figura 3).

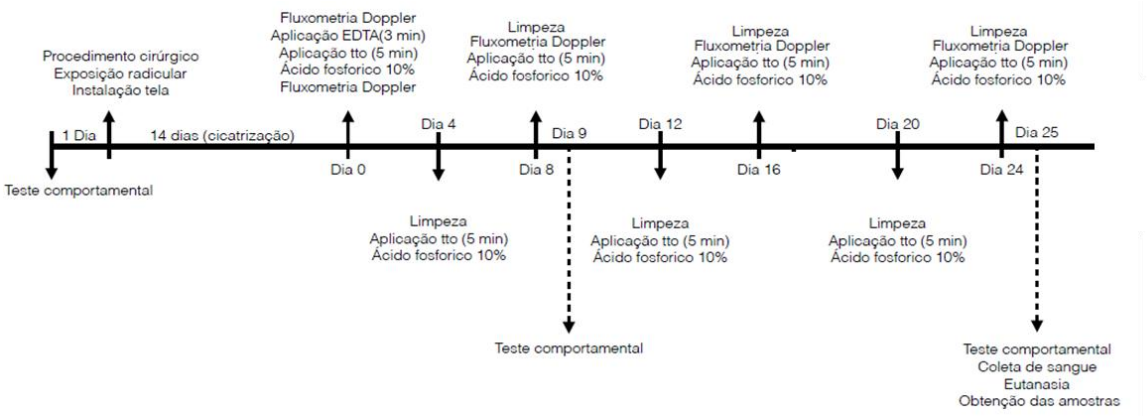

Figura 3. Delineamento do estudo

Os animais foram divididos em 6 grupos com (n= 06 por grupo):

- Naive – Sem recessão gengival e sem tratamento. Não foi realizado o procedimento de recessão gengival e para limpeza e remoção do biofilme da coroa do dente foi utilizado apenas solução salina por 3 minutos.
- Recessão Gengival – Com recessão gengival e sem tratamento. Foi realizado o procedimento de recessão gengival e para limpeza e remoção do biofilme da dentina exposta foi utilizado apenas solução salina por 3 minutos.
- Verniz – Com recessão gengival e tratamento com verniz não fluoretado. Foi realizado o procedimento de recessão gengival e sobre a dentina exposta foi realizada a limpeza com algodão embebido em soro fisiológico para remoção do biofilme e em seguida, aplicado o tratamento com verniz cavitário (0,1 mL) por 5 minutos e em seguida, realizado o ataque ácido com ácido fosfórico a 10% por 20 segundos.
- Biossilicato – Com recessão gengival e tratamento com biossilicato. Foi realizado o procedimento de recessão gengival e sobre a dentina exposta foi realizada a limpeza com algodão embebido em soro fisiológico para remoção do biofilme e em seguida, aplicado o tratamento com 7,5 mg de nanopartículas de biossilicato com veículo verniz cavitário (0,1 mL) por 5 minutos e em seguida, realizado o ataque ácido com ácido fosfórico a 10 % por 20 segundos.

- Biossilicato + Estrôncio (Sr) – Com recessão gengival e tratamento com biossilicato acrescido de estrôncio. Foi realizado o procedimento de recessão gengival e sobre a dentina exposta foi realizada a limpeza com algodão embebido em soro fisiológico para remoção do biofilme e em seguida, aplicado o tratamento com 7,5 mg de nanopartículas de biossilicato acrescido de estrôncio com veículo verniz cavitário (0,1mL) por 5 minutos e em seguida, realizado o ataque ácido com ácido fosfórico a 10 % por 20 segundos.
- Biossilicato + Potássio (K) – Com recessão gengival e tratamento com biossilicato acrescido de potássio. Foi realizado o procedimento de recessão gengival e sobre a dentina exposta foi realizada a limpeza com algodão embebido em soro fisiológico para remoção do biofilme e em seguida, aplicado o tratamento com 7,5 mg de nanopartículas de biossilicato acrescido de potássio com veículo verniz cavitário (0,1 mL) por 5 minutos e em seguida, realizado o ataque ácido com ácido fosfórico a 10% por 20 segundos.

Para a realização dos tratamentos, os animais foram sedados com hidrato de cloral a 4% e os tratamentos foram aplicados com fricção por um aplicador descartável (KG Brush Regular, KG Sorensen®, Cotia, São Paulo, Brasil).

Biovidros experimentais nanoparticulados

As formulações dos biovidros experimentais utilizadas foram elaboradas e produzidas pelo Laboratório de Materiais Vítreos (LAMAV) do Departamento de Engenharia de Materiais (DEMa) da UFSCar. Os biovidros utilizados são variações da formulação: $2Na_2O.1CaO.3SiO_2$-P_2O_5, na qual 6% do peso correspem que a P_2O_5. Foram utilizados os seguintes biovidros experimentais: a. $2Na_2O.1CaO.3SiO_2$-6% P_2O_5-K_2CO_3, sendo 5% do peso correspem quente a K_2CO_3; b. $2Na_2O.1CaO.3SiO_2$-6% P_2O_5-SrO, sendo 5% do peso correspem quente a SrO. Além destes dois biovidros experimentais, também utilizou-se o Biosilicato®, um biovidro de altíssimo grau de cristalinidade (99,5%), cuja fórmula é $SiO_2.P_2O_5.Na_2O.CaO$ A produção destes biovidros deu-se pelo método de fusão, e a obtenção da forma nanoparticulada pelo método de moagem. O biovidro contendo K_2CO_3 possui nanopartículas de diâmetro entre 1500 nm e 20.000 nm, sendo que apresentou maior frequência o tamanho de 6.000 nm. E o biovidro contendo SrO em sua composição possui nanopartículas entre 3.000 nm e 25.000 nm, sendo que a maioria das

partículas possui 9.000 nm de diâmetro. As substâncias precursoras usadas na obtenção dos biovidros foram: a. carbonato de cálcio ($CaCO_3$), marca Synth, teor de pureza de 99,0%; b. carbonato de sódio (Na_2CO_3), marca Synth, teor de pureza de 99,5%; c. sílica (SiO_2), marca Zetasil 3; d. pentóxido de fósforo (P_2O_5); e. carbonato de potássio (K_2CO_3); e f. óxido de estrôncio (SrO). O processo de fusão dos precursores envolveu a secagem dos pós de carbonato em estufa a 100ºC durante 8 horas, seguida da fusão propriamente dita, em cadinho de platina, em forno a 1400ºC por 3 horas. O resfriamento deu-se pela técnica do *splash cooling*, o qual foi seguido de recozimento a 455ºC. Um segundo resfriamento, para alívio das tensões residuais, foi feito à taxa de 2ºC por minuto. A cristalização ocorreu a 560ºC, com intervalos de tempo de 8, 15, 30, 60 e 120 horas. Por fim, para ao preparo das nanopartículas, realizou-se a moagem em moinho de alta energia.

Análise da Fluxometria Pulpar - Dopller

O animal foi sedado com hidrato de cloral e posicionado no aparato cirúrgico, em que a sonda era posicionada manualmente na região mesial do primeiro molar superior esquerdo a 2 mm da margem gengival para avaliação do fluxo sanguíneo da polpa pelo tempo de 1 minuto. No dia 0 (basal) foi realizada a leitura da fluxometria do tecido pulpar logo após a remoção do dispositivo, e a média de duas leituras, senda uma antes e outra logo após o tratamento. Nos dias 8°, 16° e 24° realizou-se a limpeza da dentina exposta com algodão e a leitura da fluxometria pulpar previamente aos tratamentos daquele dia.

Os registros foram efetuados com um fluxômetro moorVMS-LDF (Vascular Monitoring System - Laser Doppler Perfusion and Temperature Monitor - Moor Instruments, Axminster, UK) equipado com um laser diodo que emite no infravermelho, no comprimento de onda de 785 nm. O fluxômetro laser Doppler é um laser de baixa intensidade (aproximadamente 1 mW), e a sonda usada do mesmo fabricante acondicionada em um tubo de aço de 1,5 mm.

Teste Comportamental - Campo Aberto

O aparelho é feito de madeira coberta com fórmica impermeável branca com o chão branco de 100 X 100 cm (dividida por linhas azuis em 25 quadrados de 20 x 20 cm) e paredes brancas (40 cm de altura). O animal foi colocado na porção

central do aparelho e os seguintes comportamentos foram registrados durante 5 minutos (basal, antes de realizar o procedimento cirúrgico de recessão gengival e nos tempos experimentais dos dias 9° e 25°), número de ambulações na periferia (áreas com paredes laterais); número de ambulações no centro (áreas sem contato com paredes laterais); tempo na periferia; tempo no centro. O número de ambulações era definido pelos quadrados percorridos ao passarem pela linha com as quatro patas.

Teste Comportamental - Labirinto em Cruz Elevado

O arranjo dos braços permite que os animais percebam simultaneamente o precipício e o espaço aberto. Constitui de dois braços abertos e opostos, medindo 50x10 cm, e dois fechados em suas três faces externas por paredes de 40 cm de altura, e as plataformas com a mesma medida dos braços abertos, cruzando-os perpendicularmente, o que delimita uma área central de 10 cm. O aparelho dista 50 cm do solo e os braços abertos não possuem bordas. O animal foi posicionado na plataforma central de frente para um dos braços abertos e observado por 5 minutos. Os comportamentos foram registrados no dia anterior ao procedimento cirúrgico de recessão gengival (basal), e nos dias 9° e 25° da fase experimental. Foi considerado o número de entradas nos braços abertos; número de entradas nos braços fechados; tempo de permanência nos braços abertos; tempo de permanência nos braços fechados e tempo de permanência no centro.

Análise estatística

Os resultados foram expressos por média e desvio padrão para a análise de fluxometria sanguínea da polpa. Os testes comportamentais pela variância (ANOVA) dois-fatores, tendo como fatores fixos associados o tempo experimental e o tratamento. Nos casos em que foram encontradas diferenças significativas, as comparações múltiplas foram realizadas com o pós-teste de Bonferroni. O nível de significância adotado foi de 5% (α=0,05). Todos os cálculos foram realizados com programa de computador (GraphPadPrismversion 5.00 for Windows, GraphPad Software, La Jolla California, USA).

ARTIGO 1

Título: Characterization of a gingival recession model in rats for the study of dentin hypersensitivity

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Characterization of a gingival recession model in rats for the study of dentin hypersensitivity

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Abstract: Dentin hypersensitivity may be defined as pain derived from exposed dentin in response to chemical, tactile, thermal or osmotic stimuli that can not be explained as arising from any other defect or dental disease. Among the etiological factors, there is gingival recession, which generates exposure of the dentinal tubules from the cervical region to the buccal environment. Therefore, the purpose of this study was to establish a model of gingival recession in rats for the study of dentin hypersensitivity. Gingival recession was surgically induced in 32 rats, which were divided into some groups that received E.D.T.A. and E.D.T.A. / 10% phosphoric acid, in which the animals' body weight, pulp flowmetry and analysis by scanning electron microscopy were evaluated for exposed dentin morphology. It was obtained as results, that the employed model did not interfere in the diet of the animals because there was no decrease in body weight. There was a decrease in blood flow from pulp tissue at the end of the experimental phase for all groups. And the groups treated with E.D.T.A. and E.D.T.A./ 10% phosphoric acid showed a removal of the smear layer and debris, through the opening of the dentinal tubules. It was concluded that the surgically induced gingival recession model exposed the dentin to the buccal medium, making the dentinal tubules open, so that it is validated as a model for the study of dentin hypersensitivity, with the need for the continuity of the research to evaluate other parameters in the animal model.

Key words: Gingival Recession, Dentin Sensitivity, Dentin Permeability

Introduction

Dentin hypersensitivity (HD) can be defined as pain derived from exposed dentin in response to chemical, tactile, thermal or osmotic stimuli that can not be explained as arising from any other defect or dental disease[1,2]. Its prevalence varies from 3 to 98% and can be explained - in part - by different methods of evaluation and different populations of patients[3,4].

The etiology of HD is multifactorial, among the predisposing factors are gingival recession, as well as the loss of enamel in the cervical portion of the crown by erosion, abrasion and abduction or a combination of these[5-7].

The prevalence of human teeth with gingival recession, exposed cervical areas, with HD ranges from 4% to 74% [8,9]. Another aggravating factor due to HD is radicular sensitivity to external stimuli, after periodontal scaling due to exposure of the dentinal tubules to the buccal environment and, therefore, hydrodynamic forces occur[10].

Non-carious cervical lesions, gingival recession and cervical dentin hypersensitivity all had a positive correlation, in which the depth and morphology of the lesions contributed to high levels of sensitivity and severity of recessions[11]. The HD level was significantly higher in recessionary teeth (1, 2, 3 and 4-8 mm) than in teeth without gingival recession (0 mm). The highest HD level was observed in teeth with gingival recession extension (4-8 mm)[12].

The pain resulting from HD can be variable related to the patient's tolerance to pain, and to emotional and physical factors[13]. Most patients describe the pain as being rapid at the onset, sharp and of short duration[14]. Being acute in the short term, it can be localized or generalized, by affecting the surface of one or more teeth simultaneously, usually ceases immediately after the removal of the stimulus[15]. The sensitivity of the dentin correlates positively with the permeability of the dentinal tubules, the capacity and speed of the restorative processes of the tooth, the occlusion of the tubule[16].

The treatment of HD is aimed at suppressing the nerve impulse by means of neurological interaction or mechanical blockade of the dentinal tubules. Different methodologies have been described to treat HD related to the cause, which aims to mechanically or chemically protect the dentin-pulp complex or modify nerve stimulation while controlling the predisposing factors[17,18]. However, no gold standard treatment has been found so far, which could

predictably and completely eliminate pain perception, especially over the long term. In this way, when establishing a model of HD in animals, which represents the cervical exposure of the dentinal tubules through the gingival recession, easily reproducible and to promote close and detailed information allied to the conditions of the oral cavity, such as food challenges, masticatory force, temperature, pH and saliva. Therefore, the purpose of this study was to establish a model of gingival recession in rats for the study of dentin hypersensitivity.

Material and methods

Animals

Thirty-three male Wistar rats weighing 300-400 g were used, kept in an environment with a temperature of 22 ± 2 ºC and under a light / dark cycle (12/12 h), controlled automatically. The animals had free access to food and water. The project was approved by the local animal research ethics committee (Protocol 10039/2015). The sample calculation was performed with the GPower 3.1 program (G*Power, Universität Düsseldorf, Düsseldorf, North Rhine-Westphalia, Germany).

Gingival recession procedure (RG)

The animals were sedated with 4% chloral hydrate, being 400 mg / kg (1 mL/100 g weight). Then, on the surgical table, an incision was made in the palatine region of the left upper 2nd molar to mesial of the upper left 1st molar, with a scalpel blade (n.15c), 1 to 1.5 mm below the gingival margin, with removal of gingival fibers with periodontal curette (Gracey 7-8). Sequentially, the osteotomy was performed with Oschmbein # 2 microchamber, removing 1 mm of bone in the mesial and palatine part of the first left upper molar. Subsequent to the exposure of the root dentin was installed a device composed of 0.025 steel wire and steel mesh (Morelli® Ortodontia, Sorocaba, SP, Brazil) with tissue placement in the cervical region of the surgically provoked recession with twisting of the wire the mesial portion of the upper left molar and the cut (with scissors) of the alloy wire, 1 to 1.5 mm away from the tooth (Figure 1). This device remained for 14 days.

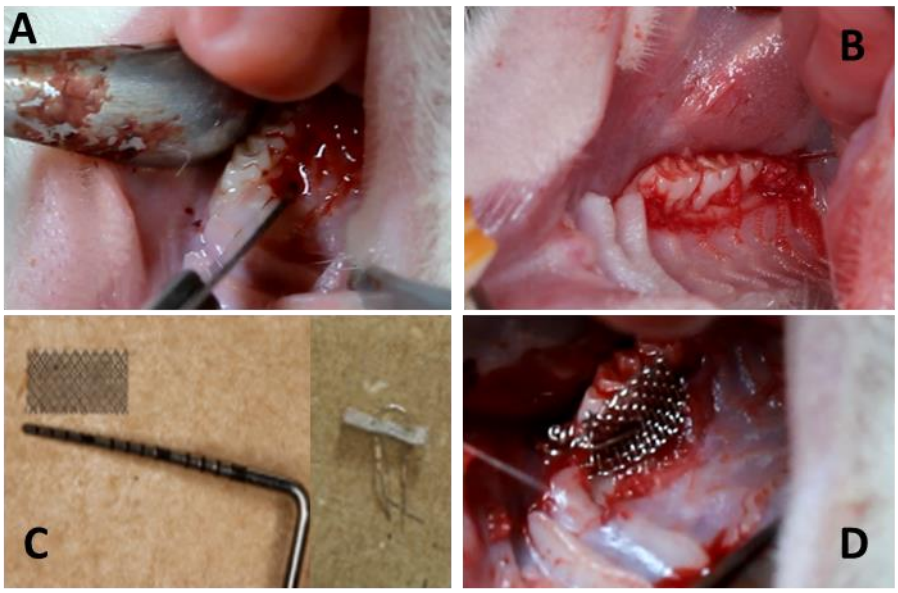

Figure 1. **Gingival recession procedure. A:** Gingival incision. **B:** Osteotomy. **C:** Device. **D:** Device installation.

Design of the study

After the surgical procedure for root exposure through the induced gingival recession and installation of the device for its maintenance, it was waited 14 days for gingival healing. At day 0 (after the healing period) the device was removed, and the respective treatments were started with E.D.T.A. (ethylenediamine tetra acetic acid), E.D.T.A. and acid phosphoric acid 10% for these groups every four days, totaling seven treatments in the experimental phase and the analysis of flowmetry of the pulp tissue every eight days, totalizing four readings in the experimental time of twenty five days, according to the flow chart below (Figure 2).

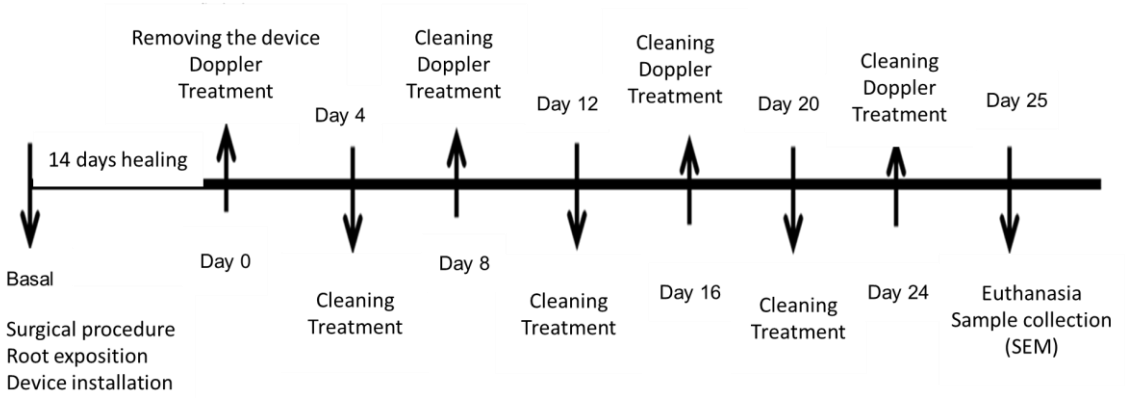

Figure 2. Study design.

The animals were divided into 4 groups (n = 08 per group):

● Naive - No gum recession and no treatment. The gingival recession procedure was not performed and for cleaning and removal of the tooth crown biofilm only saline solution was used for 3 minutes.

● Gingival recession - With gingival recession and no treatment. The gingival recession procedure was performed and for cleaning and removal of the exposed dentin biofilm only saline solution was used for 3 minutes.

● E.D.T.A. - With gingival recession and treatment with E.D.T.A. The gingival recession procedure was performed and on the exposed dentin was applied E.D.T.A. 24% (Reatec®, Reagen Ltda, Colombo, Paraná, Brazil), in cotton ball with friction for 5s, maintained in position on the exposed area for 3 minutes.

● E.D.T.A. and Phosphoric Acid - With gingival recession and treatment with E.D.T.A. and phosphoric acid. The gingival recession procedure was performed and on the exposed dentin was applied E.D.T.A. 24% cotton ball with friction for 5s, hold in position on the exposed area for 3 minutes. Subsequently, 10% phosphoric acid solution was applied to cotton ball and held in place for 20 seconds.

The treatments were performed every four days, on days 0, 4, 8, 12, 16, 20 and 24. On the 25th day the animals were anesthetized and euthanized with cardiac puncture and the hemimaxils were obtained for analysis of the morphology of the induced RG region.

For all treatments the animals were sedated with chloral hydrate.

Body weight

The weight of the animals was recorded on the day of surgery (basal), the day the device was removed (day 0), and days 4, 16 and 24 of the experimental phase.

Pulp Fluxometry Analysis - Doplller

Pulmet tissue flowmetry analyzes were performed on the day of device removal (day 0), at each eight - day interval, on days 8, 16 and 24. With the sedated animal the catheter was positioned manually in the mesial region of the upper left first molar 2 mm from the gingival margin for 1 minute. It was previously performed after the cleaning of the exposed dentin with cotton and before the

application of E.D.T.A. and E.D.T.A. + acid in the referring groups. Blood flow from the target tissue is calculated by processing the information contained in the photocurrent signal[19]. The records were made with a moorVMS-LDF (Vascular Monitoring System - Laser Doppler Perfusion and Temperature Monitor, Axminster, UK) equipped with a diode laser that emits in the infrared, at wavelength of 785 nm. The Doppler laser flowmeter is a low-intensity laser (approximately 1 mW), and the probe used from the same manufacturer is housed in a 1.5 mm steel tube.

Analysis by Scanning Electron Microscopy (SEM)

For the SEM analysis, 3 teeth (first left molar) were used for each group to verify the morphology of the exposed dentin. To prepare the SEM, the teeth were washed with distilled water (20 mL for 15 seconds) and cleaned in an ultrasonic vessel for 10 minutes at a temperature of 47 ° C. Dehydration of the specimens was done with a 25%, 50%, 70%, 90% and absolute alcohol (99.5%), remaining for 10 minutes at each concentration. The samples were then kept in a 37 ° C oven for 24 hours, and then remained in a vacuum desiccator for 48 hours. The metallization of the samples was performed in a Shimadzu C-50 apparatus (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brazil) for 10 minutes. Afterwards, images of each tooth were obtained by MEV Shimadzu SSX-550 Superscan (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brazil), using acceleration voltages of 1.8; 10 and 20 Kv. The photomicrographs were analyzed in a qualitative way considering the characteristic of the dentin surface and condition of the dentinal tubules.

Statistical analysis

The results were expressed as means with standard deviation for the animals body weight and pulp blood fluxometry analysis by two-factor analysis of variance (ANOVA), with fixed factors associated with experimental time and treatment as fixed factors. In cases where significant differences were found, multiple comparisons were performed with the Bonferroni post-test. For the morphology of the dentinal tubules, a descriptive analysis was performed. The level of significance was 5% ($\alpha = 0.05$). All calculations were performed with computer program (GraphPadPrismversion 5.00 for Windows, GraphPad

Software, La Jolla California, USA).

Results

The surgically induced RG was maintained throughout the experimental phase period (Figure 3).

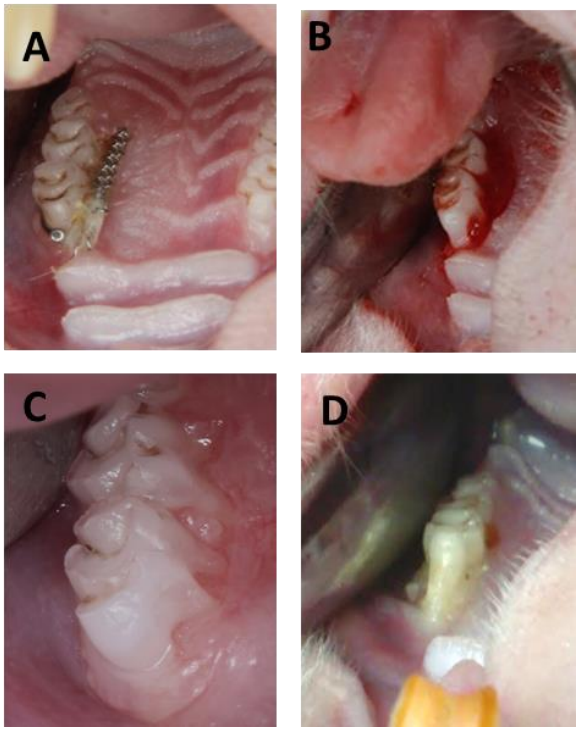

Figure 3. Gingival recession. A: Device on the 14th day on the exposed dentin. **B:** Removal of the device on the 14th day of healing. **C:** Dentin exposed by induced gingival recession. **D:** Dentin exposed by gingival recession in the mesial of the first left upper molar on the 24th day of the experimental phase.

Body weight

There was a statistically significant difference between the weight of the animals of the Gingival Recession group of the day of the surgical procedure of the GG induced at days 16 and 24 ($p < 0.05$) with a weight gain. In the other groups, there was no weight loss in the animals, but maintenance or a slight gain of body mass, however without a statistically significant difference (Figure 4).

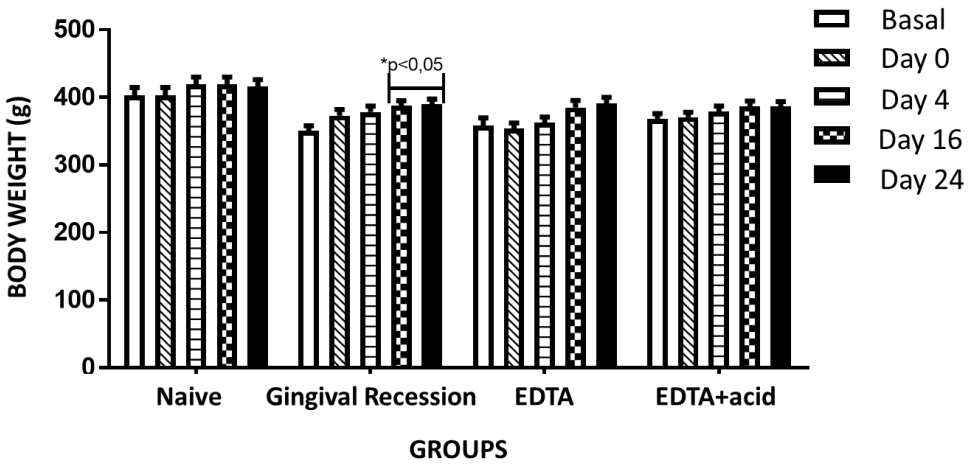

Figure 4. Body Weight Assessment (mean ± standard deviation). In the Gingival Recession group, there was a statistically significant difference for the day of gingival recession (GR) for days 16 ° and 24 ° (* p <0.05) with a gain of body weight. In the other groups, there was no significant difference in gain or loss of weight. Anova two-factors and post-test Bonferroni.

Pulp Fluxometry – Doppler

The dentinal tubules act by guiding the light from the dental surface to the pulp. The reading of the pulp blood flow on the day the device was removed (basal) showed a statistically significant positive difference for the Gingival Recession and E.D.T.A., and E.D.T.A. + acid when compared to Naive. Throughout the experiment, there was a decrease in blood flow on the eighth day for the Gingival Recession and E.D.T.A. groups. + acid, on the sixteenth day for Gingival Recession, E.D.T.A. + acid and Naive, and for the twenty-fourth day for all groups when compared to the initial pulp flow (Figure 5).

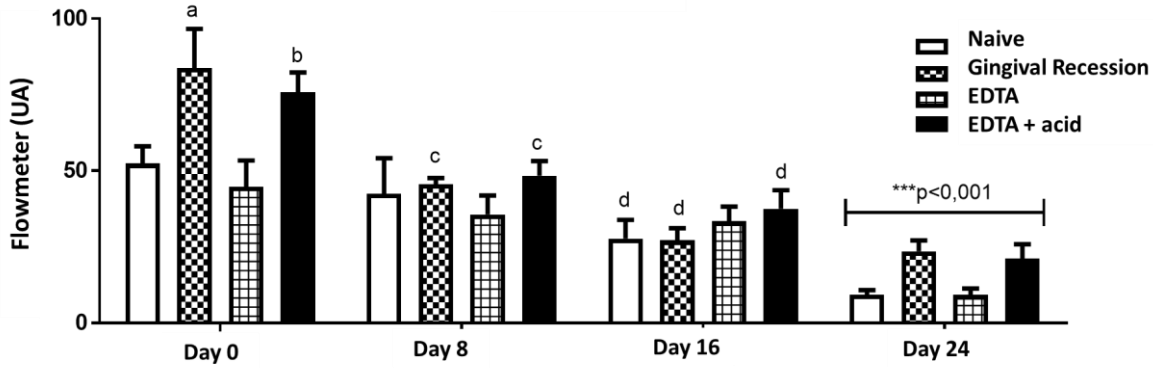

Figure 5. **Fluxometry of the pulp (mean ± standard deviation).** At baseline: a with E.D.T.A. (p <0.01), b with Naive (p <0.05) and E.D.T.A. (p <0.001). There were differences for the time: baseline vs 8th day for groups c (p <0.01), baseline vs 16th day for groups d (p <0.05), baseline

and 24th day for all groups *** (p <0.01). Anova two-factors and post-test Bonferroni.

Scanning Electron Microscopy

In the photomicrographs obtained by scanning electron microscopy (Figure 6), the morphology of the exposed dentin tubules was observed in the groups in which the surgical gingival recession, the odontoblastic prolongations, was observed. In the groups where the application of E.D.T.A. and E.D.T.A. + acid, it was evident the opening of the dentin tubules, due to the removal of the smear layer by the action of the acid that increases the dentin permeability. In the Gingival Recession group the partial exposure of the dentinal tubules occurred, due to being closed by debris, peritubular dentin and smear layer. In the Naive group there was no exposure of the dentinal tubules because the gingival recession procedure was not performed in this group, where the visualized cervical dentin was covered by root cementum.

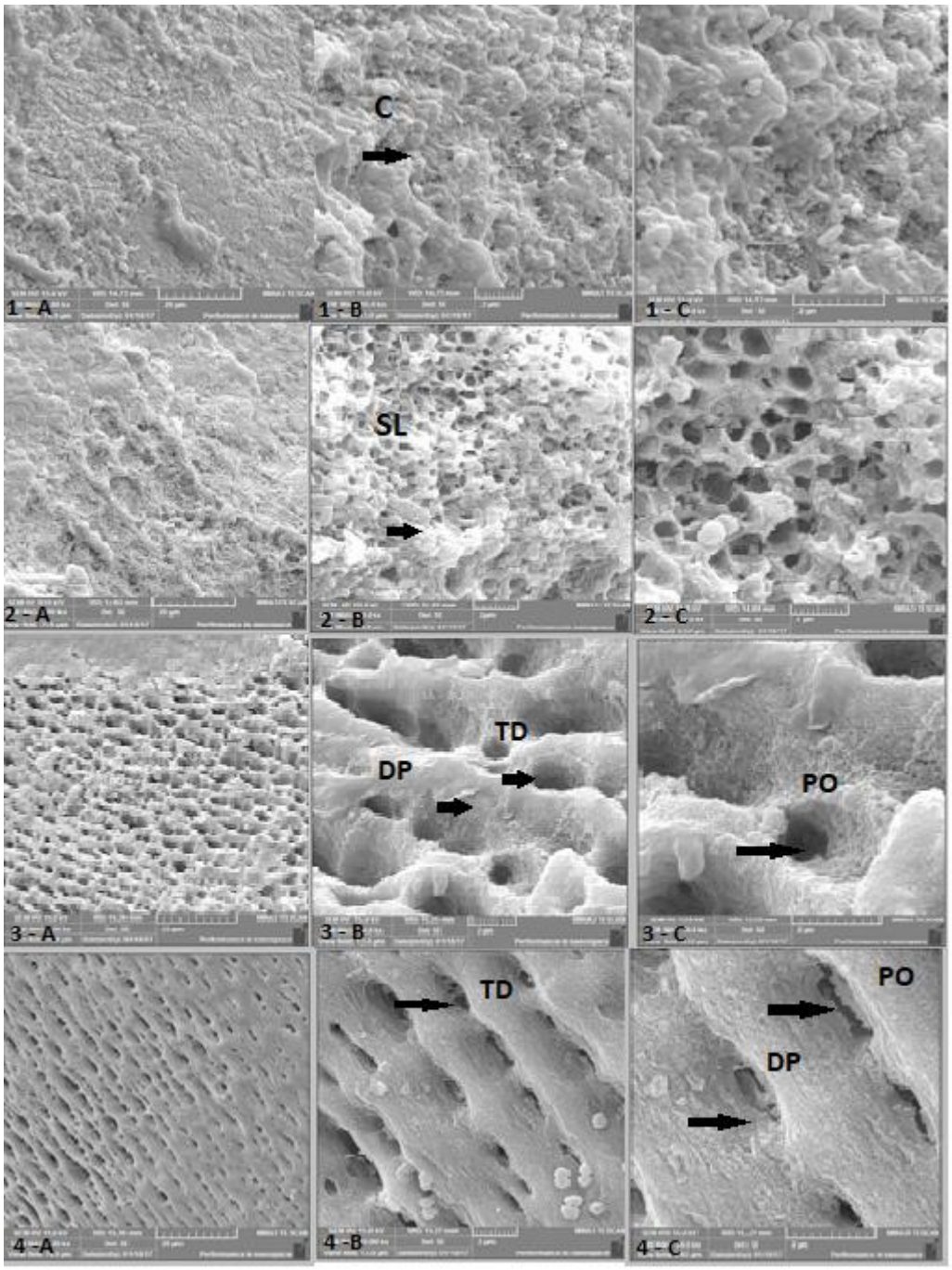

Figure 6. Photomicrograph: 1 - A, B, C: Naive 1,8; 10 and 20 Kv, respectively. **2 - A, B, C: Gingival Recession** 1.8; 10 and 20 Kv, respectively. **3-A, B, C: E.D.T.A.** 1.8; 10 and 20 Kv, respectively. **4-A, B, C: E.D.T.A. + ACID** 1.8; 10 and 20 Kv, respectively. C: Cement SL: Smear layer. DP: Peritubular dentin. TD: Dentinous tubule. PO: odontoblastic prolongations.

Discussion

According to the results obtained, it was observed that the surgical procedure of gingival recession and the installation of the metallic device did not interfere in the diet of the animal, since these had no loss of body weight, so that there was no great impact on the animal welfare. Therefore, the model used to induce the

gingival recession associated with the exposure of dentin tubules and the application of treatments in the groups (E.D.T.A. and E.D.T.A. + acid) was not generally harmful to the systemic health of the animals. Among the physiological signs of chronic stress measured in an animal, weight loss is considered a good indicator[20].

When evaluated if the exposure of the dentin would interfere in the blood flow of the pulp, it was obtained that at the end of the experimental phase all the groups had reduction in the flowmetry. Initially, reading on the day the device was removed showed increased blood flow to the Gingival Recession and E.D.T.A. groups. + acid, these may have increased flow due to exposure of the dentin by the induced gingival recession and the application of phosphoric acid as a way to simulate oral conditions, such as the drop in pH due to acid feed or the accumulation of biofilm[21,22], altering the opening of the tubules and the hydraulic conductivity of the dentin[23,24].

Throughout the experimental phase the decrease in blood flow may have occurred because the root exposure is located in small regions, generating only a slight inflammatory response. Corrosive with the findings, other studies reported a decrease in flow after the seventh day, suggestive of a tissue repair process in which there is a maximal increase in flow, and after the seventh day its decrease[25], indicating that there was only a slight inflammatory response. Among the limitations of this method is the sensitivity of the Doppler laser fluxometer at small displacements of 0.01 mm / s, in which small movements between the probe and the dentine, due to respiration, involuntary muscular contractions of the animals and movements of the optical fiber, result in Doppler interference. Thus, given the great variability of flow, the obtained results were evaluated, considering that each animal was its own control.

Analysis of the surface morphology showed that the gingival recession procedure exposed the dentinal tubules, and when E.D.T.A. was applied, they remained open, due to the removal of the smear layer. The morphological aspect of the exposed area shows the natural variation in the dentinal tubules. The dentinal tubule is composed of peritubular dentin, odontoblastic process, collagen, nerves and dentin fluid, components that help dentin permeability. The odontoblastic extensions fill the entire path of the main dentinal tubules[26]. The

smear layer is a form of protection so that the tubules do not open and cause dentin hypersensitivity[27]. The root dentine is covered by cement, avascular mineralized connective tissue, which in the layer closest to the cementoenamel junction is afribilar, thin and acellular, being easily removed when exposed to the oral environment by gingival recession[28], as found in the Naive group in that the root dentin was not exposed to the buccal environment, is covered by cement. E.D.T.A. has a chelating capacity, making it able to promote tubule obliteration by penetration, by removing the mineral layers of the peritubular dentin and increasing the dentin permeability through exposure of the collagen fibers. In addition to being essential for almost complete removal of the smear layer[29,30].

The dentinal tubules taper to a diameter of about 2 μm at their pulp end at about 0.5 μm or less peripherally, depending on age[31]. The formation of the dentin occurs in a centripetal (from outside to inside) form, so that the density of tubules and the diameter of the same increase towards the pulp[32]. Thus, the dentin closest to the pulp is more permeable (greater tubule density and larger diameter) than the dentin closest to the dentin-bond junction[33]. In this study, no cut of the exposed dentin was observed in the proximity of the pulp tissue, only the morphological surface of the regions in which the recessions were induced surgically, evaluating if they were exposed to the buccal environment, without considering the diameters of the dentinal tubules exposed by only if they were open. When applied E.D.T.A. and E.D.T.A. + acid to exposed dentin, this showed with more open tubules due to the removal of debris and smear layer. In the literature it is reported that hypersensitive exposed dentin presents a greater amount of open dentin tubules and larger diameter when compared to non-sensitive dentin, in which the tubules are obliterated with peritubular dentine or debris[34]. Animal models may contribute to better elucidation of the pain process due to dentin hypersensitivity. Studies validated a model for induction of erosion and dentin hypersensitivity in rats by ingestion of an isotonic solution for 30 days, in which the beverage caused total loss of the supragingival enamel and exposure of the dentin[35,36]. Another model of dentin hypersensitivity in animals was studied with occlusal cavities prepared in the second and third maxillary molars, to the dentin[37]. In another in vivo study,

cavities were prepared on the surfaces of the upper and lower incisors in the cervical region[38]. As there is a positive correlation of teeth with gingival recessions and dentin hypersensitivity, by means of an animal model of root dentin exposure by the gingival recession surgically induced and maintained by device during the period of gingival healing, it becomes valid when evaluated the exposure of the tubules dentin.

However, as well as the caveats concerning the limitations of this work, it is understood that the in vivo model presented has reproducibility over conditions in humans. Therefore, it is fundamental to continue the research in order to elucidate the animal's responses to the exposure of the dentin root, thus being a preliminary model for the study of dentin hypersensitivity in rats, through the surgically induced gingival recession, generating a exposure of the dentinal tubules.

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ARTIGO 2

Título: Evaluation of animal behavior and pulp response in a gingival recession model for the study of dentin hypersensitivity

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Evaluation of animal behavior and pulp response in a gingival recession model for the study of dentin hypersensitivity

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Abstract:

Dentin hypersensitivity is a multifactorial condition in which the dentin is exposed and permeable, in which the patient's response varies according to the different stimuli. These comprise thermal, evaporative, osmotic, tactile, chemical, electrical and bacterial agents. Therefore the objective of this study was to evaluate the animal behavior and pulp response to the gingival recession model for the study of dentin hypersensitivity. In 32 adult male rats, gingival recession was induced surgically and this was maintained by means of a metal device for 14 days, after its removal, treatments were initiated with E.D.T.A. and E.D.T.A./Acid, and behavioral open field and labyrinth cross-over and histological analyzes were performed. In the E.D.T.A./Acid group there was a significant reduction in the frequency of locomotion and the increase in the time of fewer ambulances in the behavioral test. In the histological analysis, no group showed detectable changes in the structure of the soft tissues of the pulp, characterized as intense inflammatory infiltrate. It was concluded that the gingival recession model proposed for the study of dentin hypersensitivity altered the animal behavior when it was submitted to acid attack without causing changes in the pulp tissue.

Key words: Gingival Recession, Dentin Sensitivity, Behavior Animal

Introduction

Dentin hypersensitivity (HD) is a multifactorial condition in which the dentin is exposed and permeable, in which the host response varies according to the different stimuli. These comprise thermal, evaporative, osmotic, tactile, chemical, electrical and bacterial agents[1-3]. The variability with HD presents for each individual makes it difficult to quantify it, since some indicate pain, without affecting its quality of life, while others may request intervention in order to obtain pain relief[4]. In addition to pain being subjective, there are causal and predisposing factors, such as amount of affected teeth, previous dental interventions performed, may interfere with the individual's response[5].

Among the multifactorial conditions, gingival recessions, abrasions, attritions, erosions, surgical and non-surgical periodontal treatment stand out. Dentin hypersensitivity presents a high prevalence, from 8 to 57%[6] of the population, with prevalent involvement in women, individuals in the age range of 20-40 years, and in patients with periodontal disease (72.5 - 98%)[7]. The most prevalent dental faces are the vestibular regions, most frequently canines, premolars followed by incisors and molars[8].

Once the gingival recession occurs, the cement that covers the dentin surface is easily removed by physical and chemical forces with exposure to dentin sensitivity[9] due to the acidic environment produced by the bacteria promoting the opening of the dentinal tubules[10].

Among the treatments proposed for HD are specific dentifrices, fluoride, desensitizers, dentin adhesives, laser use, biomaterials, restorations, mucogingival surgeries and endodontic treatment[11-13]. The treatments presented seek to obliterate the dentinal tubules by reducing or ceasing hypersensitivity. However, there is no short-term effective gold standard that promotes the elimination of the painful sensitivity of HD.

Studies are needed to evaluate the efficacy of new materials by means of animal models in order to mimic some elementary features of a specific disease state and reduce the number of variables whose control is inaccessible. So, the greater degree of experimental control allows for experimental manipulations that could be impossible under other circumstances.

Therefore, the objective of this study was to evaluate the animal behavior and the pulp response to a pre-established gingival recession model for the study of

dentin hypersensitivity.

Material and methods

Animals

Thirty-three male Wistar rats weighing 300-400 g were used, kept in an environment with a temperature of 22 ± 2 ºC and under a light / dark cycle (12/12 h), controlled automatically. The animals had free access to food and water. The project was approved by the animal research ethics committee University (Protocol 10039/2015). The sample calculation was performed with the GPower 3.1 program (G * Power, Universität Düsseldorf, Düsseldorf, North Rhine-Westphalia, Germany).

Gingival recession procedure (RG)

The animals were sedated with 4% chloral hydrate, being 400 mg / kg (1 mL / 100 g of weight), then, on the surgical table, an incision was made in the palatine region of the left upper 2nd molar until mesial of the (n.15c), 1 to 1.5 mm below the gingival margin, and the gingival fibers removed with a periodontal curette (Gracey 7-8). Afterwards, the osteotomy was performed with Oschmbein # 2 microchamber, removing 1 mm of bone in the mesial and palatal part of the first left upper molar. Subsequently the exposure of the root dentin was installed a device consisting of 0.025 steel wire mesh and steel mesh fabric (Morelli® Ortodontia, Sorocaba, SP, Brazil) with tissue placement in the cervical region of the recession surgically provoked with the mesial portion of the upper left molar and the cut (with scissors) of the alloy wire, from 1 to 1.5 mm away from the tooth (Figure 1). This device remained for 14 days.

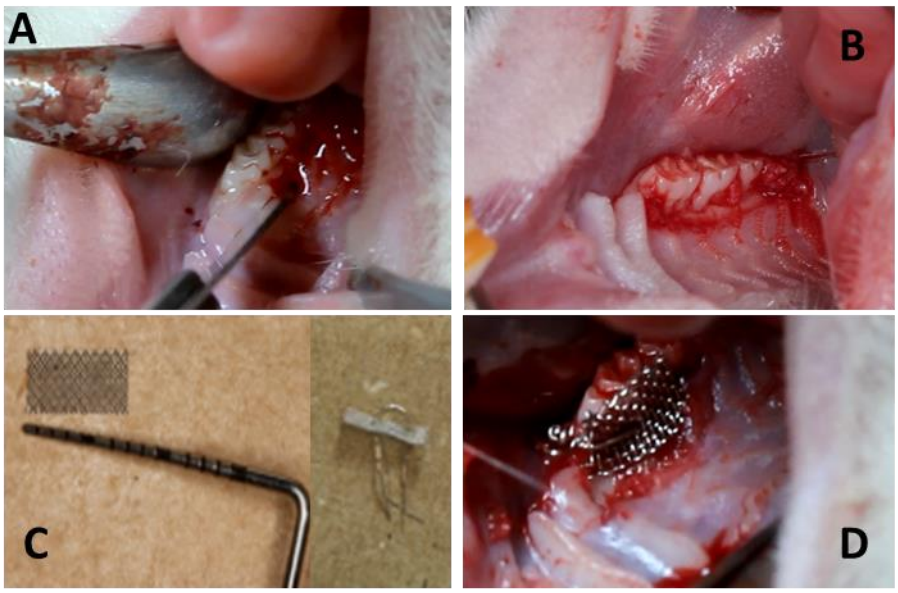


Figure 1. Gingival recession procedure. A: Gingival incision. **B:** Osteotomy. **C:** Device. **D:** Device installation.

Design of the study

After the surgical procedure for radicular exposure by means of the induced RG and installation of the device for its maintenance, it was waited 14 days for the gingival cicatrization. At day 0 (after the healing period) the device was removed, the respective treatments were started with E.D.T.A. (ethylenediamine tetra acetic acid), E.D.T.A. and acid phosphoric acid 10% for these groups every four days, totaling seven applications, together with the analyzes of the behavioral evaluations and histological evaluation of the pulp tissue, according to the flow chart below (Figure 2).

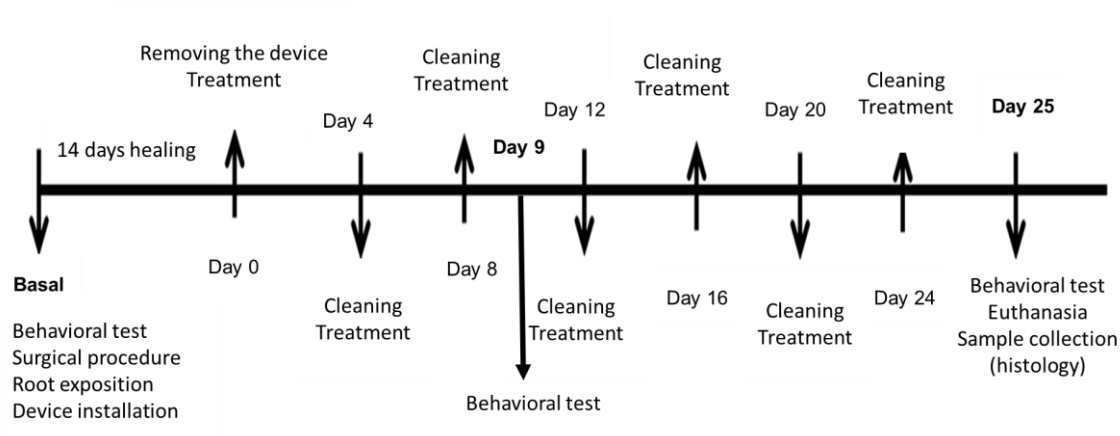


Figure 2. Study design.

The animals were divided into 4 groups (n = 08 per group):

● Naive - No gum recession and no treatment. The gingival recession procedure was not performed and for cleaning and removal of the tooth crown biofilm only saline solution was used for 3 minutes.

● Gingival recession (G.R.) - With gingival recession and no treatment. The gingival recession procedure was performed and for cleaning and removal of the exposed dentin biofilm only saline solution was used for 3 minutes.

● E.D.T.A. - With gingival recession and treatment with E.D.T.A. The gingival recession procedure was performed and on the exposed dentin was applied E.D.T.A. 24% (Reatec®, Reagen Ltda, Colombo, Paraná, Brazil), in cotton ball with friction for 5s, maintained in position on the exposed area for 3 minutes.

● E.D.T.A. and Phosphoric Acid - With gingival recession and treatment with E.D.T.A. and phosphoric acid. The gingival recession procedure was performed and on the exposed dentin was applied E.D.T.A. 24% cotton ball with friction for 5s, hold in position on the exposed area for 3 minutes. Then applied 10% phosphoric acid solution in cotton ball and held in position for 20 seconds.

The treatments were performed every four days, being 0 °, 4 °, 8 °, 12 °, 16 °, 20° and 24 °. On the 25th day the animals were anesthetized and euthanized with cardiac puncture and the samples were obtained for analyzes of the pulp tissue of the tooth in which the GR was induced.

For the treatments the animals were sedated with chloral hydrate.

Behavioral Testing - Open Field

The open field test, which assesses the animal's motor activity and anxiety, was developed in 1934 by Calvin Hall[14]. The apparatus is made of wood covered with white waterproof formica with white floor of 100 X 100 cm (divided by blue lines in 25 squares of 20 x 20 cm) and white walls (40 cm high). The animal was placed in the central portion of the apparatus and the following behaviors were recorded for 5 minutes (both at the baseline session, before performing the surgical procedure of gingival recession and at the experimental times of days 9 and 25), number of ambulances in the periphery, number of ambulances in the center, time in the periphery, time in the center, number of elevations in the front legs, time of self-cleaning. The number of ambulances was defined by the crossed lines with all four legs.

Behavioral Test - High Cross Labyrinth

Developed by Handley and Mithani[15] and later validated by Pellow et al.[16], it consists of two open and opposing arms, measuring 50x10 cm, and two closed on its three external faces by walls 40 cm high. The platforms have the same measures of the open arms, crossing them perpendicularly, which delimits a central area of 10 cm. The device is 50 cm from the ground and the open arms have no edges. The animal was positioned on the central platform facing one of the open arms and observed for 5 minutes. The behaviors were recorded the day before the surgical procedure of gingival recession, and days 9 and 25. The number of entries in the open arms was considered; number of entries in the closed arms; length of stay in the open arms; time of stay in the closed arms and time of permanence in the center.

Evaluation of pulp tissue by histology

To evaluate the cells of the inflammatory infiltrate and vascularization, the histological sections of the maxilla fragments, the first, second and third molars were fixed in 10% neutral formalin solution. After a maximum period of 48 hours of fixation, the samples were followed for decalcification in E.D.T.A. solution. 9% at pH 7.2 about 21 days. The pieces were then prepared for inclusion in paraffin with the vestibular face parallel to the cut plane. Serial cuts of about 5 µm thick were stained with Hematoxylin and Eosin. For each sample, 3 histological sections were selected. Four different regions of the coronary and cervical pulp were photographed from each cut, with the objective lens 40x and 10x eyepiece, with images increasing by 400x. The criterion for capturing the images was the observation, in the field of view of the 40x objective, of the largest amount of pulp area, accompanied by margin in dentin. The analysis of the inflammatory response of the pulp tissue considered the presence of polymorphonuclear neutrophils, polymorphonuclear eosinophils, lymphocyte infiltration, infiltration of plasma cells, macrophages and giant cells. The inflammatory response score was defined according to the cells and blood vessels, where 0 Absent: absence of inflammation; 1. Mild: scarce mononuclear cells; 2. Moderate: mononuclear infiltrate and / or neutrophil and eosinophilic dispersion; 3. Intense: polymorphonuclear infiltrate of neutrophils and eosinophils[17]. The histological analysis was performed under light microscopy

(Olympus, Tokyo - Japan), and the inflammatory reaction of the pulp tissue was observed qualitatively.

Statistical analysis

The results of the behavioral tests were expressed by mean and standard deviation by two-way ANOVA, with fixed factors associated to the variable (time and groups). In cases where significant differences were found, multiple comparisons were performed with the Bonferroni post-test. The level of significance was 5% ($\alpha = 0.05$). For the histological characteristics the data were qualitatively evaluated for the presence or absence of pathological and vascular changes, by means of descriptive statistics and by means of the scores for the inflammatory response with the Kruskal-Wallis test. All calculations were performed using a computer program (GraphPad Prism version 5.00 for Windows, GraphPad Software, La JollaCalifornia USA).

Results

Behavioral Assessment - Open Field

The open field test demonstrated that after the gingival recession procedure, on day 9, in the E.D.T.A. + acid, the animals remained less time in the periphery and more time in the center when compared with the Naive group. When comparing the times before the gingival recession procedure and the treatments (baseline time) with those after (days 9 and 25), there was a decrease in the time spent standing up and in the number of squares traveled in the periphery, in which ambulated less in the group EDTA + acid. Low locomotor activity indicates increased anxiety in the animal.

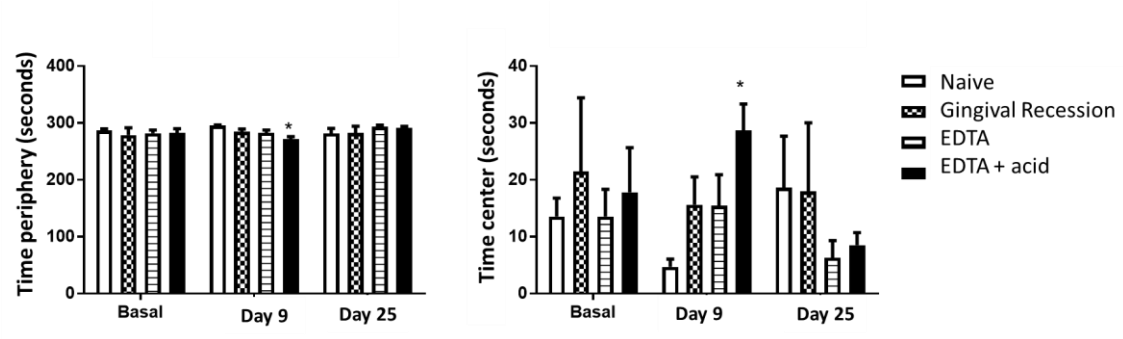


Figure 3. Time at the periphery and at the center (mean ± standard deviation). In the evaluation of the groups with basal times (before the gingival recession procedure), and after removal of the device in the experimental phase on days 9 and 25, there was a statistically significant difference between Naive vs E.D.T.A. + acid on day 9 of the experimental phase ($p<0.05$) in both peripheral and central time. Anova two-way and post-test Bonferroni.

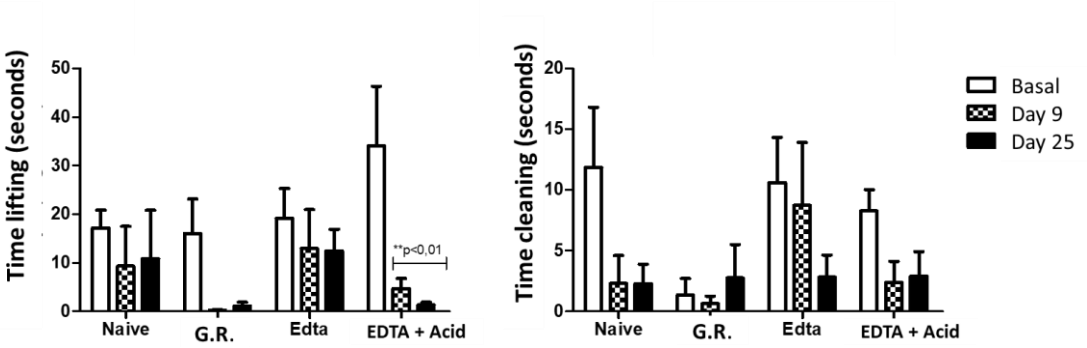


Figure 4. Lifting and cleaning time (mean ± standard deviation). In the evaluation of the time that the animals remained raising there was a statistically significant difference in the group E.D.T.A. + acid for baseline time (before the gingival recession procedure) for days 9 and 25 ($p <0.01$). In the evaluation of the time that the animals remained cleaning, there was no statistically significant difference for groups and times. Anova two-way and post-test Bonferroni.

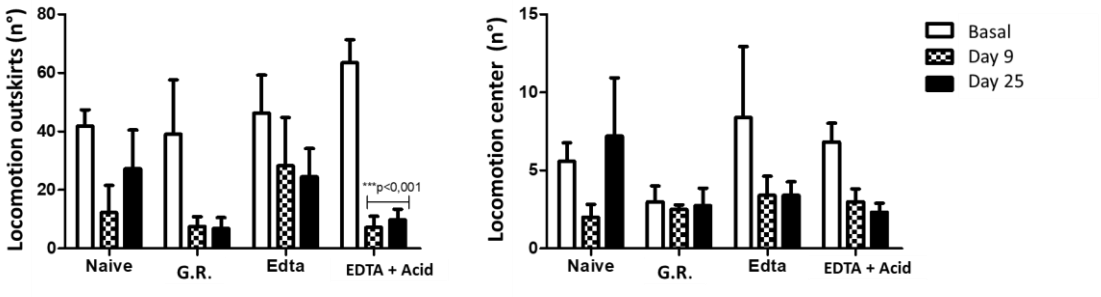


Figure 5. Number of ambulances in the periphery and in the center (mean ± standard deviation). In the number of squares traversed in the periphery, there was a statistically significant difference for the group E.D.T.A. + acid in basal times (before the gingival recession procedure) for days 9 and 25 ($p <0.001$). For the number of squares traversed in the center, there was no statistically significant difference between groups and times. Anova two-way and post-test Bonferroni.

Behavioral Testing - High Cross Labyrinth

In the high cross-maze test, the Gingival Recession group remained longer in the closed arm after the surgical procedure (days 9 and 25) when compared to the baseline period. The frequency of entry into the closed and open arms decreased for the E.D.T.A group. + acid after the surgical procedure and treatment, where the animal remained in smaller ambulances.

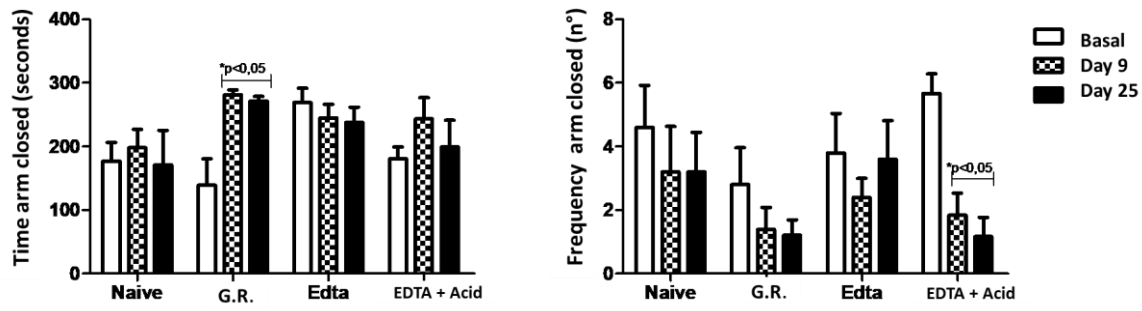


Figure 6. Time and frequency in the closed arm (mean ± standard deviation). In the evaluation of the time spent in the closed arm, there was a statistically significant difference for the Gingival Recession group at baseline versus days 9 and 25 ($p < 0.05$). For the frequency of entry into the closed arm there was a statistically significant difference for the group E.D.T.A. + acid at baseline time vs days 9 and 25 ($p < 0.05$). Anova two-way and post-test Bonferroni.

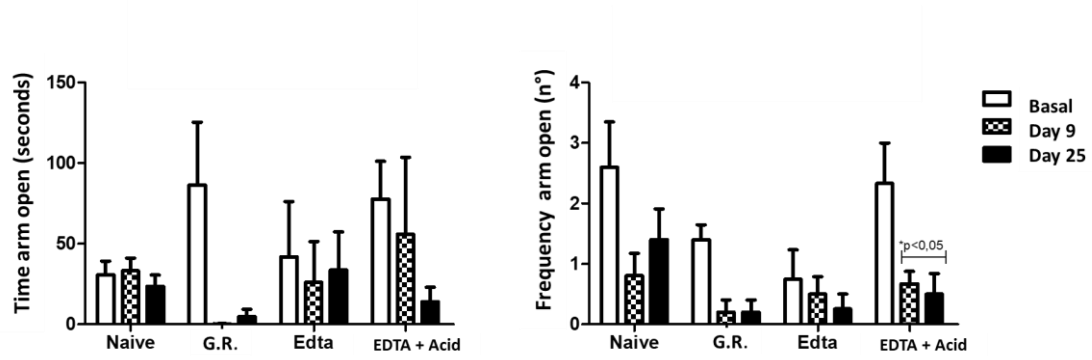


Figure 7. Time and frequency in the open arm (mean ± standard deviation). In the evaluation of the time remaining in the open arm there was no statistically significant difference for groups and times. For the frequency of entry into the open arm there was a statistically significant difference for the group E.D.T.A. + acid at baseline time vs days 9 and 25 ($p < 0.05$). Anova two-way and post-test Bonferroni.

Evaluation of pulp tissue by histology

Intergroup comparisons showed mean values below score 1, and there was no statistically significant difference between groups. The inflammatory reaction

was considered mild due to the presence of sparse mononuclear cells (Figure 9). In the E.D.T.A. and acid, in some cuts, the presence of mononuclear infiltrate and sparse neutrophils was found, which classifies the inflammation as moderate but not predominant in the group with no significant statistical difference.

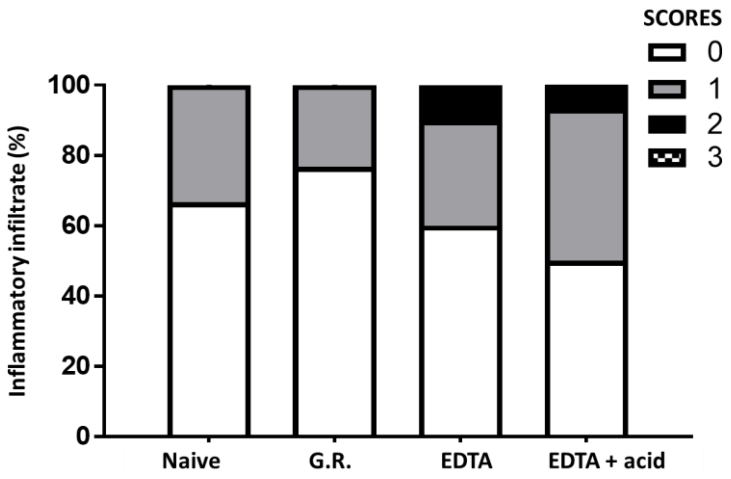
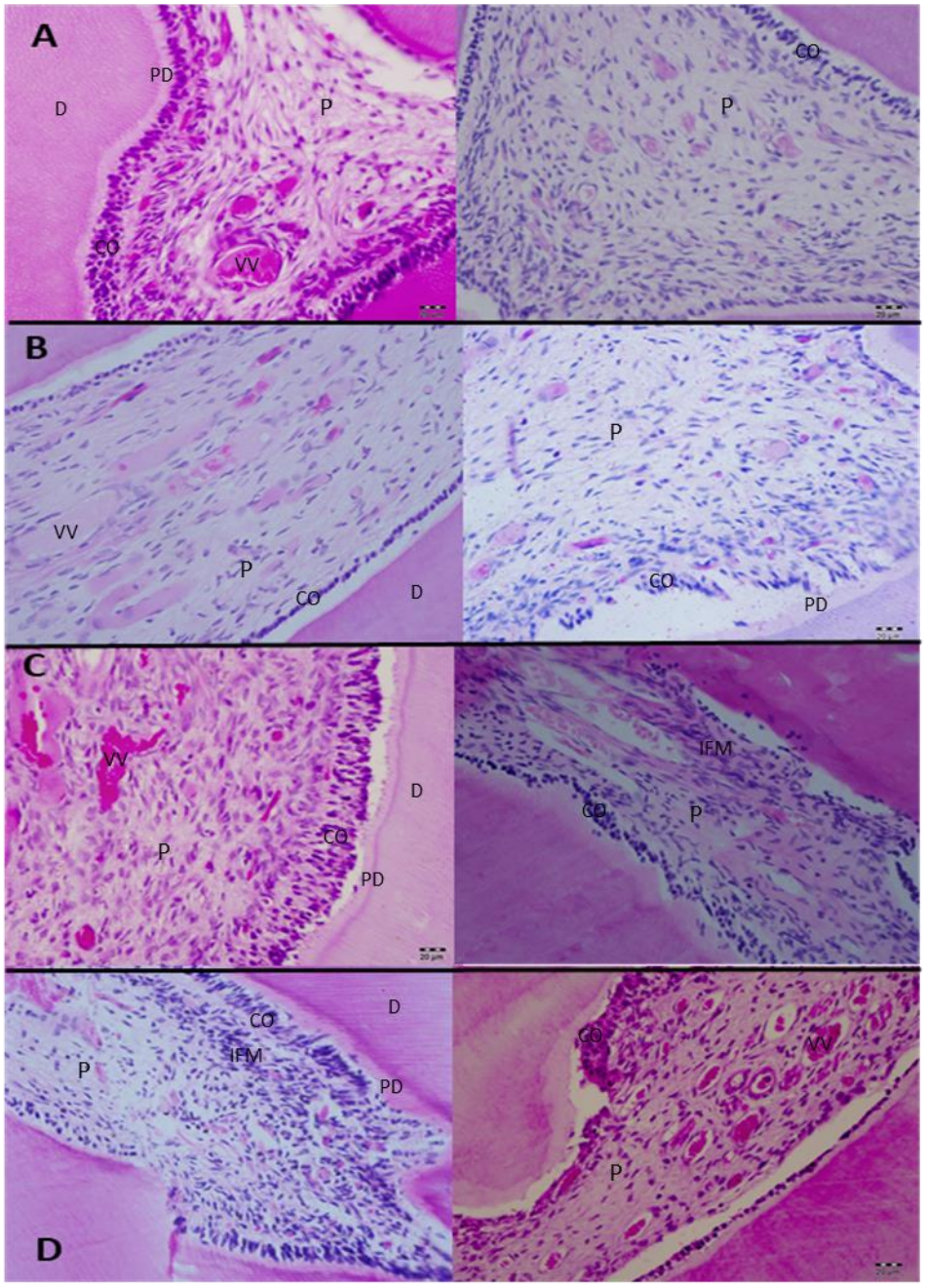


Figure 8. Inflammatory infiltrate evaluation in percentage by scores (percentage distribution). Score 0 - None or few inflammatory cells in the pulp area; Score 1 - Light infiltration of inflammatory cells; Score 2 - Moderate inflammatory cell infiltrate; Score 3 - Severe infiltrate of inflammatory cells. There was no statistically significant difference. Kruskal-Wallis



test.

Figure 9. Histological analysis of pulp tissue. At 400x magnification. A: Naive Group **B:** Gingival Recession Group **C:** E.D.T.A. Group **D:** Group E.D.T.A. + acid. In all groups it was possible to observe markings on the pre-dentin layer (PD), the odontoblasts (CO) layer, blood vessels (VV), pulp tissue (P), dentin (D). In groups C (E.D.T.A.) and D (E.D.T.A. + acid) the presence of moderate inflammatory infiltrate (MFI).

In none of the groups were there detectable changes in the soft tissue structure of the pulp, the odontoblasts layer was intact for the Naive, Gingival Recession, E.D.T.A. + acid and slightly disorganized for the E.D.T.A group. Analysis of the inflammatory response of the pulpal tissue considered the presence of a moderate inflammatory infiltrate for the E.D.T.A. groups. and E.D.T.A. + acid.

Discussion

The results of this work showed that in the open field test the experimental groups with E.D.T.A. and E.D.T.A. + acid had behaviors indicative of greater anxiety. For the high cross maze test it was shown that the surgically and untreated RG group remained longer in the closed arms and in the E.D.T.A group. and acid there was a significant reduction in the frequency of locomotion and the increase in the time of less ambulances.

In the experimental area it is possible to evaluate the anxiety indexes by means of different behavioral tests, such as the open field test, used in the evaluation of the exploratory behavior of rats, and the high cross maze, which is considered a valid instrument to measure anxiety[18].

In the open field, the locomotor activity of the animal can be used in the center of the open field as a selective measure of anxiety, while the locomotor activity in the periphery of the apparatus, in which low locomotor activity would indicate the anxiety of the animal[19]. Vertical movement (lifting) is also an index of locomotor activity[20].

Corroborating with the findings, a study in which the behavior of the animals in the model of dentin hypersensitivity through acid erosion was evaluated, it was shown that stressed animals show freezing behavior and in less ambulances[21].

In the high-labyrinth test, the results show that the groups in which the surgical gingival recession was performed only, and in which E.D.T.A. and acid there was a longer stay in the closed arm and a lower frequency of entry into the closed and open arms. This allows us to evaluate that they were more anxious than in the initial phase (before recession - baseline time). Whereas, the higher the levels of anxiety, the lower the frequency of entries in the open arms and the time spent in them[22].

Animal stress assessment can be performed by complementary means of behavioral assessment, such as analysis of corticosterone in the plasma, and is directly involved in the response to stress[23].

In the histological sections evaluated, a predominance was found for the absence of inflammation or, when present, it was mild for groups without gingival recession and with gingival recession, but without treatment. When the treatments of E.D.T.A., E.D.T.A. A moderate inflammatory infiltrate was found with acid but no statistically significant difference. The state of the pulp in dentin

hypersensitivity is not known, although the symptoms suggest that there is unlikely to be an acute or chronic inflammation due to the time the symptoms persist[24]. This indicates that this model of dentin hypersensitivity through induced gingival recession did not allow the observation of significant cellular changes in the pulp tissue. Perhaps because of the size of the induced gingival recession and the stimulation with acid after E.D.T.A. to remove smear layer and to open the dentinal tubules have been applied punctually and of short duration. Since most patients describe the pain of HD as being fast at the onset, marked and short duration[25].

It is important to note that not every tooth with exposed dentin has HD. Electron microscopy studies have shown that, in general, the exposed dentin that is associated with HD presents larger and larger open tubules with dissolution of the peritubular dentin and consequent exposure to the buccal medium[26].

As a complement to the histological analysis, a polymerase chain reaction (PCR) study could evaluate the distribution of mechanotransducers in pulp neurons during pulp inflammation, when evaluating the possibility that this sensitization reflects on a positive regulation of proteins responsible for the mechanotransduction[27].

The model studied in HD for gingival recession may be a method to study HD, due to the exposure of the dentinal tubules to the oral environment, being the first factor to make a dentin sensitive. In addition, the model simulates the human oral environment, reproduces the conditions of presence of saliva, biofilm, occlusion, feeding and mastication, as well as possible changes in the masticatory function. Removal of the gingival, bone and cementum tissues to create the gingival recession, due to the exposure of the root dentin, was permanent throughout the experimental phase.

The study performed the treatment every 4 days, in the experimental time of 25 days, based on data from the literature of times of in vitro and clinical treatments for HD. These range from 7 to 28/30 days[28-30], where application frequencies varied from daily[31], weekly[32] at intervals of 48 or 72 hours between each application[33].

In another HD model in vivo, however, through dentin erosion, in which mineral loss was induced by the replacement of water by low pH beverages, shows that the animals remained in a shorter time and frequency of entries in the open

arms and did not there were alterations in histological evaluations[24,34,35].

The scarcity of experimental models that have similarity to the recurrent clinical situation of HD associated with gingival recessions and non-carious cervical lesions promotes the relevance of this study. The model can be applied in future tests to assess whether these exposed dentinal tubules can cause hypersensitivity, assessing pulpal neurotransmitters by means of an immunohistochemical assay and evaluation of dentin permeability through dyes such as methylene blue.

It was concluded that the gingival recession model proposed for the study of dentin hypersensitivity altered the animal behavior when it was submitted to acid attack without causing changes in the pulp tissue.

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ARTIGO 3

Título: Evaluation of different bio-glasses in the treatment of dentin hypersensitivity in an animal model of gingival recession

Status: Submetido

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Evaluation of different bio-glasses in the treatment of dentin hypersensitivity in an animal model of gingival recession

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Abstract:

The purpose of the treatments for dentin hypersensitivity is to decrease the movement of the dentin fluid to block the pulp nerve response by inducing smear layer formation, so that it is obliterating or desensitizing. The use of bioglass in the treatment of HD is justified by its obliteration action, which occurs along the remineralization from the formation of apatite. This action can also be complemented by the potential of the bioglass to stimulate odontoblasts to secrete dentin that diminished the hydrodynamic mechanisms responsible for pain. Therefore the objective of this work is to evaluate the application of different bioglass formulations in the exposed dentin by means of a model of gingival recession in rats. Thirty-six animals were used and gingival recessions were induced surgically. Different treatments were carried out with bioglass formulations, added with strontium and potassium, as non-fluoride varnished vehicle. Pulp flowmetry analyzes and behavioral evaluations were performed during the experimental phase of twenty five days. It was possible to conclude in this model of gingival recession for the study of dentin hypersensitivity that, among the treatments with different bio-tests performed, in the evaluated experimental time, all the groups reduced the pulp blood flow. And the strontium group showed signs of less anxiety.

Key words: Gingival Recession, Dentin Sensitivity, Dentin Desensitizing Agents

Introduction

Dentin hypersensitivity (HD) is considered a painful sensation due to the exposure of dentin, in response to mechanical, thermal, evaporative, osmotic, chemical, electrical and / or bacterial stimuli[1]. Exposed dentin associated with HD presents open tubules in greater number and in greater diameter, with dissolution of the peritubular dentine and consequent exposure to the buccal medium to the pulp[2].

Among the theories to explain the neurophysiological mechanisms involved in the HD process, the hydrodynamic theory described by Brännström, is the most accepted currently[3]. This theory considers the movement of the fluids present in the dentinal tubules as a consequence of the non-harmful stimuli, responsible for the deformation of the odontoblasts and / or their prolongations, which activates the mechanoreceptors of the pulp nerve endings resulting in the painful process.

The treatments for HD are aimed at decreasing the movement of the dentin fluid by blocking the pulp nerve response induced by smear layer formation, so that it is obliterating or desensitizing[4].

The obliteration of dentinal tubules can be done by applying substances on the exposed dentin region, in which the effectiveness of the obliterating agents depends on their resistance to the oral acid environment. Among these, the bio-glass, which has the capacity to release ions and promote the formation of hydroxyapatite, from the occlusion of the dentinal tubules, due to mechanical obliteration or superficial mineralization[5] stands out.

However, no treatment for HD is effective for durability, being easily displaced by mechanical actions. It is necessary the study of different formulations of bioglass in the treatment of HD. Therefore, the objective of this work is to evaluate the application of different bioglass formulations in the exposed dentin by means of a gingival recession model induced in rats.

Material and methods

Animals

Thirty-six male Wistar rats, weighing 300-400 g, were kept in an environment with a temperature of 22 ± 2 ºC and under a light / dark cycle (12/12 h). The animals had free access to food and water. The project was approved by the

local animal research ethics committee (Protocol 10039/2015). The sample calculation was performed with the GPower 3.1 program (G * Power, Universität Düsseldorf, Düsseldorf, North Rhine-Westphalia, Germany).

Gingival recession procedure (RG)

The animals were sedated with 4% chloral hydrate, being 400 mg / kg (1 mL/100 g of weight), then, on the surgical table, an incision was made in the palatine region of the left upper 2nd molar until mesial of the (n.15 c), 1 to 1.5 mm below the gingival margin, and the gingival fibers removed with a periodontal curette (Gracey 7-8). Afterwards, the osteotomy was performed with Oschmbein # 2 microchamber, removing 1 mm of bone in the mesial and palatal part of the first left upper molar. Subsequently the exposure of the root dentin was installed a device consisting of 0.025 steel wire mesh and steel mesh fabric (Morelli® Ortodontia, Sorocaba, SP, Brazil) with tissue placement in the cervical region of the recession surgically provoked with the mesial portion of the upper left molar and the cut (with scissors) of the alloy wire, from 1 to 1.5 mm away from the tooth (Figure 1). This device remained for 14 days.

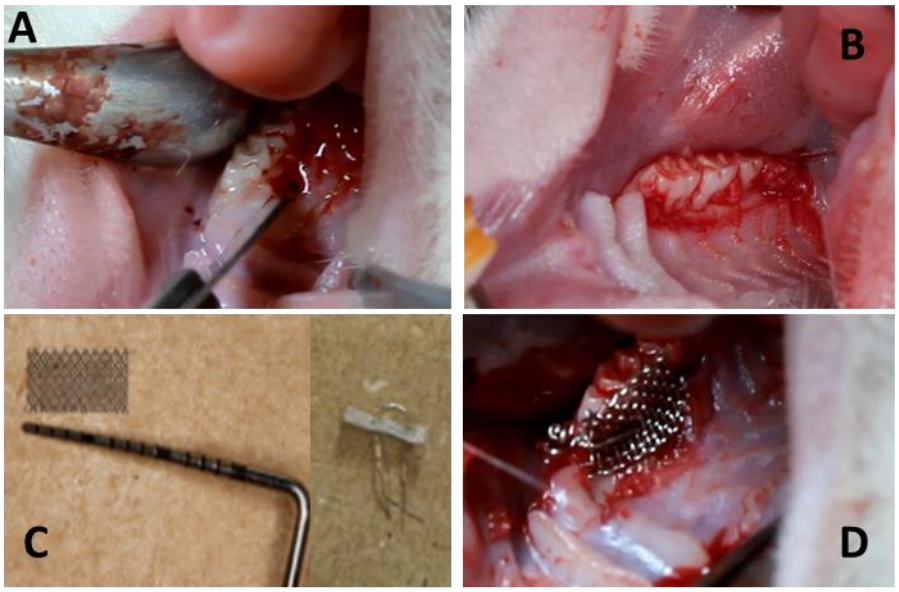

Figure 1. Gingival recession procedure. A: Gingival incision. **B:** Osteotomy. **C:** Device. **D:** Device installation.

Design of the study

After the surgical procedure for radicular exposure by means of the induced RG

and installation of the device for its maintenance, it was waited 14 days for the gingival cicatrization. On day 0 (after the healing period) the device was removed, and the respective treatments were started, every four days, totaling seven treatments in the experimental phase. Pulmet tissue flowmetry analyzes were performed every eight days, in a total of four readings in the experimental time of twenty five days. Behavioral assessments were performed on basal days, 9° and 25° days, totaling three records according to the flow chart below (Figure 2).

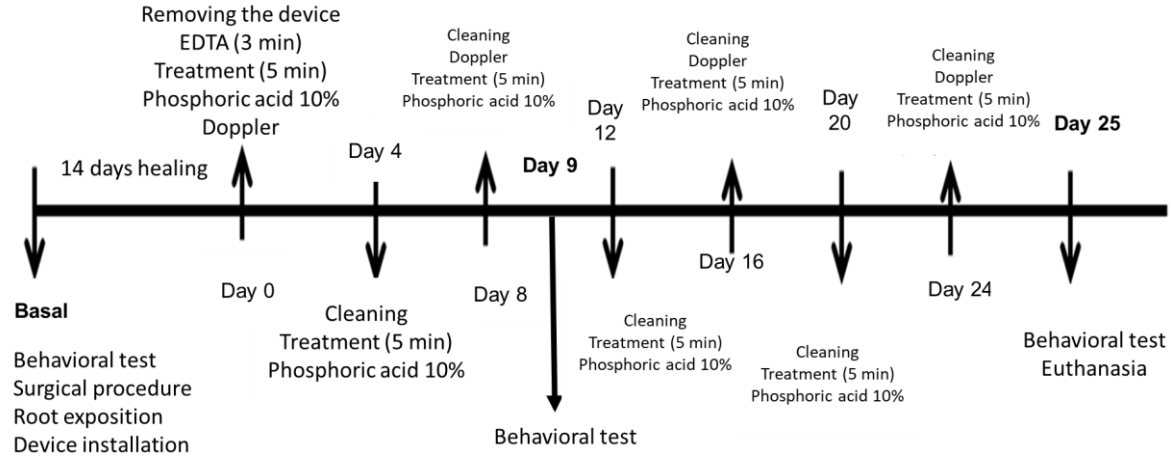

Figure 2. Study design

The animals were divided into 6 groups with (n = 06 per group):

● Naive - No gum recession and no treatment. The gingival recession procedure was not performed and for cleaning and removal of the tooth crown biofilm only saline solution was used for 3 minutes.

● Gingival Recession - With gum recession and no treatment. The gingival recession procedure was performed and for cleaning and removal of the exposed dentin biofilm only saline solution was used for 3 minutes.

● Varnish - With gingival recession and treatment with non-fluoridated varnish. The gingival recession procedure was performed and the exposed dentin was cleaned with cotton wool soaked in saline solution to remove the biofilm and then applied the cavity varnish treatment (0.1 mL) for 5 minutes and then performed the acid attack with 10% phosphoric acid for 20 seconds.

● Biossilicate - With gingival recession and biosilicate treatment. The gingival recession procedure was performed and the exposed dentin was cleaned with

cotton wool soaked in saline to remove the biofilm and then applied with 7.5 mg of biosilicate nanoparticles with cavity varnish vehicle (0.1 mL) for 5 minutes and then the acid attack with 10% phosphoric acid was performed for 20 seconds.

● Biossilicate + Strontium (Sr) - With gingival recession and treatment with biosilicate plus strontium. The gingival recession procedure was performed and the exposed dentin was cleaned with cotton wool soaked in saline to remove the biofilm, followed by treatment with 7.5 mg of biosilicate nanoparticles plus strontium with cavity varnish vehicle (0.1mL) for 5 minutes and then the acid attack with 10% phosphoric acid was performed for 20 seconds.

● Biossilicate + Potassium (K) - With gingival recession and treatment with biosilicate plus potassium. The gingival recession procedure was performed and the exposed dentin was cleaned with cotton wool soaked in saline to remove the biofilm, followed by treatment with 7.5 mg of biosilicate nanoparticles plus potassium with cavity varnish vehicle (0.1 mL) for 5 minutes and then acid etching with 10% phosphoric acid for 20 seconds.

To perform the treatments, the animals were sedated with 4% chloral hydrate and the treatments were applied by rubbing a disposable applicator (KG Brush Regular, KG Sorensen®, Cotia, São Paulo, Brazil).

For the treatments the animals were sedated with chloral hydrate.

Experimental nanoparticulate nanoparticles

The formulations of the experimental bioglass used were elaborated and produced by the Laboratory of Vitreous Materials (LAMAV) of the Department of Materials Engineering (DEMa) of UFSCar. The bioglass used are variations of the formulation: 2Na2O.1CaO.3SiO2-P2O5, in which 6% of the weight corresponds to P2O5. The following experimental bioassays were used: a. 2Na 2 O 1 Ca 3 .SiO 2 - 6% P 2 O 5 -K 2 CO 3, 5% of the weight corresponding to K 2 CO 3; B. 2Na 2 O.CaO.3SiO 2-6% P 2 O 5-SrO, with 5% of the weight corresponding to SrO. Biosilicate®, a very high crystallinity (99.5%) bioglass, whose formula is SiO2 .P2O5.Na2O.CaO, was also used. The production of these bio-glasses was done by the fusion method, and obtaining the nanoparticulate form by the grinding method. The bioglass containing K2CO3 has nanoparticles of diameter between 1500 nm and 20,000 nm, with a higher

frequency of 6,000 nm. And the bioglass containing SrO in its composition has nanoparticles between 3,000 nm and 25,000 nm, with most particles having 9,000 nm in diameter. The precursor substances used to obtain the bioglass were: a. calcium carbonate (CaCO3), brand Synth, purity content 99.0%; B. sodium carbonate (Na 2 CO 3), brand Synth, purity content 99.5%; W. silica (SiO 2), brand Zetasil 3; d. phosphorus pentoxide (P2O5); and. potassium carbonate (K2 CO3); and f. strontium oxide (SrO). The precursor melting process involved the drying of the carbonate powders in an oven at 100°C for 8 hours followed by the melting itself in a platinum crucible in a furnace at 1400°C for 3 hours. Cooling was done by the splash cooling technique, which was followed by annealing at 455°C. A second cooling, to relieve the residual stresses, was done at the rate of 2°C per minute. Crystallization occurred at 560°C, with time intervals of 8, 15, 30, 60 and 120 hours. Finally, for the preparation of the nanoparticles, milling was carried out in a high energy mill.

Pulp Fluxometry Analysis - Dopller

The animal was sedated with chloral hydrate and positioned in the surgical apparatus where the probe was positioned manually in the mesial region of the upper left first molar 2 mm from the gingival margin for evaluation of the blood flow of the pulp for 1 minute. On day 0 (baseline), the flowmetry of the pulp tissue was read immediately after the removal of the device, and the average of two readings, one before and one after the treatment. On days 8, 16 and 24 the exposed dentin was cleaned with cotton and the pulp flowmetry was read before the treatments of that day.

The records were made with a moorVMS-LDF (Vascular Monitoring System - Laser Doppler Perfusion and Temperature Monitor, Axminster, UK) equipped with a diode laser that emits in the infrared, at wavelength of 785 nm. The Doppler laser flowmeter is a low-intensity laser (approximately 1 mW), and the probe used from the same manufacturer is housed in a 1.5 mm steel tube.

Behavioral Testing - Open Field

The apparatus is made of wood covered with white waterproof formica with white floor of 100 X 100 cm (divided by blue lines in 25 squares of 20 x 20 cm) and white walls (40 cm high). The animals were placed in the central portion of

the device and the following behaviors were recorded for 5 minutes (baseline, before performing the surgical procedure of gingival recession and at the experimental times of days 9 and 25), number of ambulances in the periphery with side walls); number of ambulances in the center (areas without contact with lateral walls); time on the periphery; time in the center. The number of ambulances was defined by the squares traversed as they crossed the line with all four legs.

Behavioral Test - High Cross Labyrinth

The arrangement of the arms allows the animals to simultaneously perceive the cliff and the open space. It consists of two open and opposing arms, measuring 50x10 cm, and two closed in its three external faces by walls of 40 cm in height, and the platforms with the same measure of the arms open, crossing them perpendicularly, which delimits a central area of 10 cm. The device is 50 cm from the ground and the open arms have no edges. The animal was positioned on the central platform facing one of the open arms and observed for 5 minutes. The behaviors were recorded the day before the surgical procedure of gingival recession (basal), and days 9 and 25 of the experimental phase. The number of entries in the open arms was considered; number of entries in the closed arms; length of stay in the open arms; time of stay in the closed arms and time of permanence in the center.

Statistical analysis

The results were expressed by mean and standard deviation for the analysis of blood flowmetry of the pulp. The behavioral tests by the two-factor variance (ANOVA), having as fixed factors associated the experimental time and the treatment. In cases where significant differences were found, multiple comparisons were performed with the Bonferroni post-test. The level of significance was 5% ($\alpha = 0.05$). All calculations were performed with computer program (GraphPadPrismversion 5.00 for Windows, GraphPad Software, La Jolla California, USA).

Result

Pulp Fluxometry - Doppler

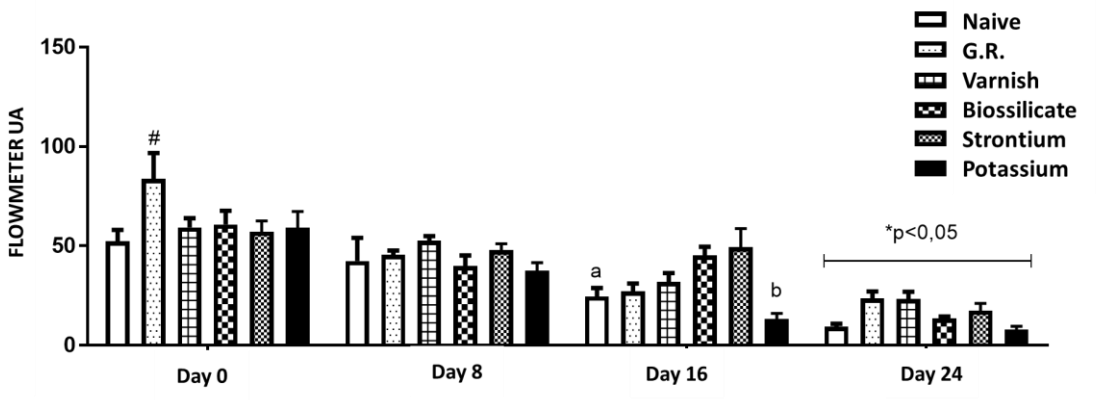

Figure 3. Fluxometry of the pulp (mean ± standard deviation). There was a statistically significant difference for the groups at baseline (#) Gingival Recession vs Naive (p = 0.0063), Varnish (p = 0.0323), Strontium (p = 0.0164) and Potassium (p = 0.0288). (a) On the 16th day Naive vs. Strontium (p = 0.0457), (b) Potassium vs Biossilicate (p = 0.0027), Strontium (p = 0.0004). At 8th and 24th days there was no statistically significant difference when compared to treatments. (*) On the 25th day vs baseline time (p <0.05) for all groups. Anova two-factors and post-test Bonferroni.

The evaluation soon after removal of the devices showed that the group with induced gingival recession presented a higher flowmetry of the pulp. During the experimental phase, on the 16th day, the group without induced gingival recession presented lower blood flow when compared to the strontium group. Among the bioglass treatments, the bioassay group plus Potassium presented lower blood flow when compared to biosilicate and strontium. For all groups, there was a reduction of flowmetry in the final phase.

Behavioral Assessment - Open Field

In the open field test in the analysis of time spent in the periphery, on the 25th day, the biosilicate group remained longer when compared to the varnish and strontium groups. The varnish group moved more when compared to the Gingival Recession and Biossilicate groups. And among the bio-glass, on the 9th day the biosilicate group moved less than the Strontium. In the final stage 25th day, the strontium group traveled more squares in the periphery than the groups Gingival recession, biosilicate and potassium.

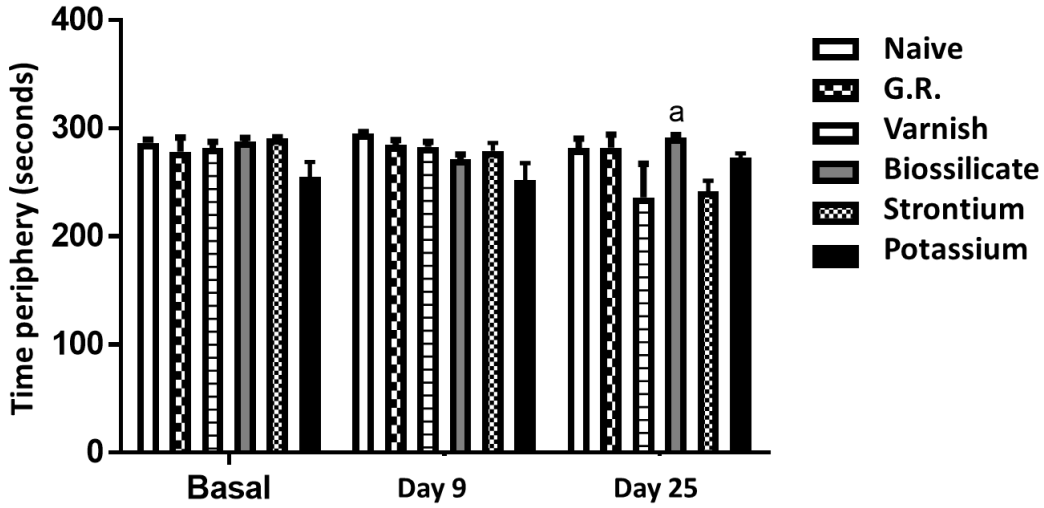

Figure 4. Behavioral evaluation - Open field (mean ± standard deviation). There was a statistically significant difference in the 25th day for the groups (a) Biossilicate vs Varnish (p = 0.0030) and Strontium (p = 0.0026). Anova two-factors and post-test Bonferroni.

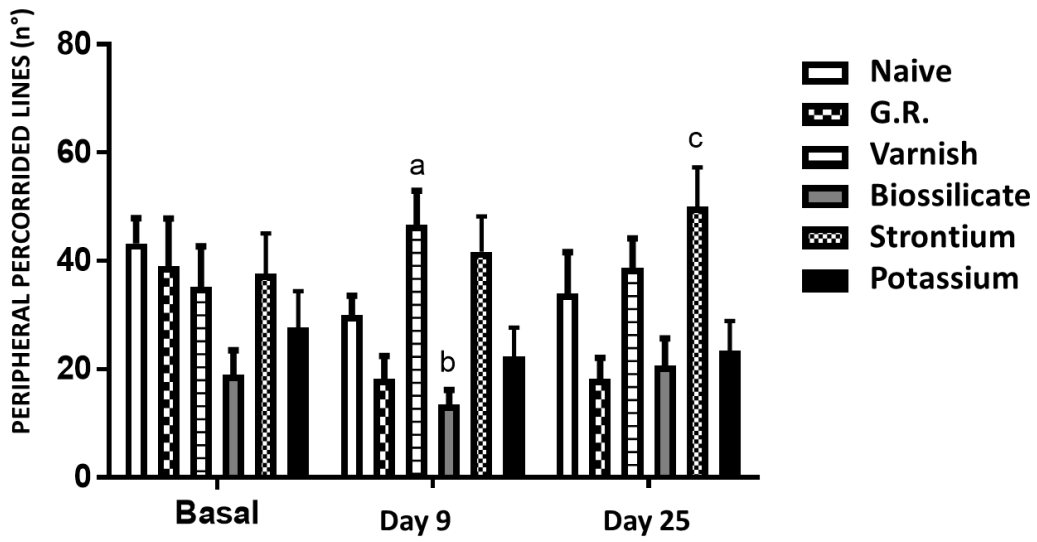

Figure 5. Behavioral evaluation - Open field (mean ± standard deviation). There was a statistically significant difference in the 9th day for the groups (a) Varnish vs Gingival Recession (p = 0.0388) and Biossilicate (p = 0.0033), (b) Biossilicate vs. Strontium (p = 0.0220). On the 25th day (c) Strontium vs. Gingival recession (p = 0.0127), Biossilicate (p = 0.0146), Potassium (p = 0.0245). Anova two-factors and post-test Bonferroni.

Behavioral Assessment - Elevated Cross Labyrinth

In the labyrinth test, the Gingival Recession group remained longer in the closed arm during the experimental phase and when compared to the groups with varnish, biosilicate, strontium and potassium treatments.

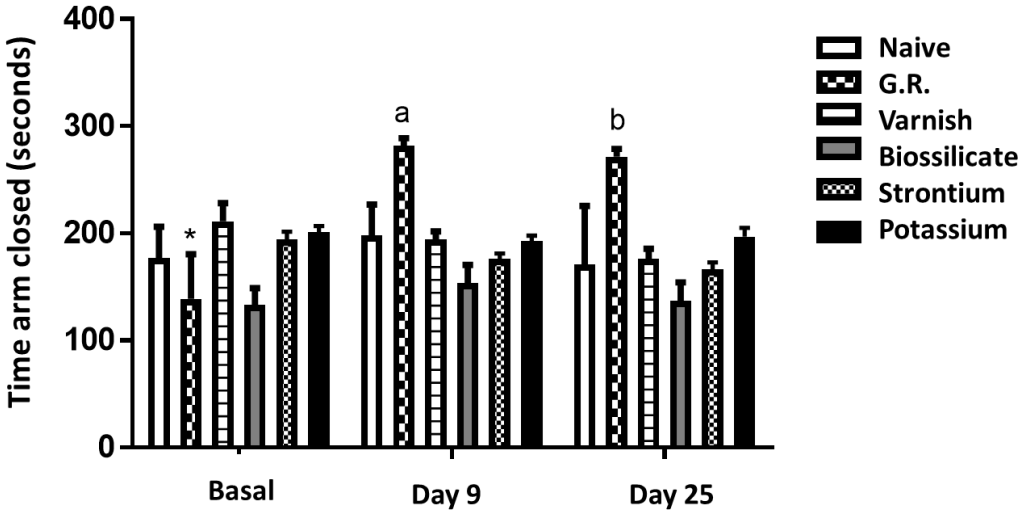

Figure 6. Behavioral evaluation - Labyrinth (mean ± standard deviation). There was a statistically significant difference for the group (*) Gingival recession when compared basal times vs 9º and 25º day (p <0.0001). (a) In the 9th day group Recession Gingival vs. Biossilicate

(p = 0.0003), Strontium (p = 0.0095), Potassium (p = 0.0357). (b) In the 25th day group Gingival Recession vs. Naive (p = 0.0159), Varnish (p = 0.0279), Biossilicate (p = 0.0001), Strontium (p = 0.0094). Anova two-factors and post-test Bonferroni.

Discussion

The pulp flowmetry analysis allows to evaluate if there is alteration in the pulp blood flow, and may be suggestive of the presence or absence of an inflammatory process[6]. Because external stimuli can be driven to the pulp, they generate the activation of the mechanoreceptors in the pulp nerve endings, through chemical mediators and promote a vasodilation. When removing the devices for the induction of gingival recession, the Gingival Recession group with induced gingival recession presented a higher flowmetry of the pulp. And in the middle of the experimental phase the potassium group had lower blood flow than the biosilicate and strontium treatments. However, in the final phase there was no difference between the treatments, because all groups reduced the pulp blood flow. Decreased flow after seventh day, when evaluating pulp blood flow, suggests the process of tissue repair in which there is a maximum increase in flow and after its seventh day its decrease[7], indicating that only a slight inflammatory response occurred. Among the limitation in this method is the sensitivity of the Doppler laser fluxometer at small displacements in the order of 0.01 mm / s, in which small movements between the probe and the dentine, resulting from respiration, involuntary muscular contractions of the animals and movements of the optical fiber result in interference from the Doppler signal. Due to the great variability of flow, the obtained results were evaluated, with each animal being its own control.

In the experimental area, it is possible to evaluate the anxiety indexes by means of different behavioral tests, such as the open field test, which is a behavioral test used to evaluate the exploratory behavior of rats and the high cross maze, which is considered a valid instrument to measure anxiety[8].

In the open field the Biossilicate group remained longer in the periphery, while the strontium group traveled more squares, the low locomotor activity of the animal is indicative of anxiety. In the labyrinth test, the Gingival Recession group remained longer in the closed arm during the experimental phase and when compared to the groups with varnish, biosilicate, strontium and potassium treatments, with no difference between the treatments. Animals with high levels

of stress and anxiety tend to stay longer in the closed arms. The obliteration of the dentinal tubules decreases the intensity of the painful stimulus to the pulp nerve endings and prevents toxins from the biofilm to activate an inflammatory response[9].

The choice of the experimental bio-tests containing strontium or potassium was given because the properties have already been studied in various formulations and applications for the treatment of HD[5,10-12]. In previous studies, both bio-glasses presented satisfactory results regarding the decrease of permeability dentin in vitro[13].

The use of bio-glass in the treatment of HD is justified by its obliterating action, due to remineralization from the formation of apatite. This action can also be complemented by the potential of the bioglass to stimulate odontoblasts to secrete dentin, which promotes the decrease of the hydrodynamic mechanisms responsible for pain[14].

The association of 45S5 Bioglass® with 50% phosphoric acid was tested for penetration into the dentinal tubules, resistance to the simulation of buccal challenges, and its biocompatibility, obtaining results favorable to the treatment of HD[15]. Studies have indicated that the original formulation, although promoting coverage of the dentinal surface and / or occlusion of the tubules, is easily displaced, indicating the need to use higher concentrations of bioglass in vehicles of more appropriate formulations[16].

The mechanism of action of strontium salts is based on the theory that salts have considerable affinity for dentin, through organic precipitation and denaturation of odontoblasts. This process would be responsible for the formation of an obliterating sealing film, which makes it difficult to move liquids in the dentinal tubules[17]. In vitro studies have demonstrated a decrease in the dentin permeability promoted by formulations with strontium, due to the affinity of the strontium ions to the dentin[18,19].

Potassium ions reduce the excitability of the pulp nerve endings along the diffusion through dentinal tubules, since they increase the local extracellular concentration of potassium ions, with consequent blockage of the nervous functions[20]. Bioassay formulations with potassium have a higher release rate than Na + ions, resulting in a higher capacity to initiate the ionic exchanges required to form hydroxycarbonate apatite[21].

Scanning electron microscopy analyzes should be performed to evaluate the incorporation of the bioglass in the dentin tubules. Specific compositional analyzes, such as X-ray spectroscopy, were carried out to verify if the components added in these bio-samples could be present on the surface of the precipitate and for the analysis of the number of open tubules and their diameters, since not all dentin exposed to the buccal medium sensitive.

The application of phosphoric acid present in other studies[22,23], used it as a vehicle for the bioglass, supplying calcium, phosphate and crystals of sodium to the medium. Phosphoric acid would mobilize calcium and phosphate ions from the underlying dentin, which would react by forming salts on the dentin surface, small crystals at the entrance of the dentinal tubules. In another in vitro study, the application of phosphoric acid as a vehicle to bioglass led to the closure of dentinal tubules, even after erosive / abrasive challenge, which was performed with 1% citric acid[24]. The vehicle for bioglass applications in which the non-fluoride varnish was used, which has temporary adhesive properties, so that it increases the time of the bioglass in contact with the dentin. In the literature, other vehicles were found, such as distilled water and gel formulations, which showed efficacy in the clinical use of bioglass[25].

There are other experimental models of HD found in the literature, using low pH beverages in substitution of drinking water in an animal model for mineral loss (erosion)[26], cavities performed in molars or incisors for dentin exposure[27], but no model associated with gingival recession.

Studies are necessary to evaluate the efficacy of new materials, and animal models are fundamental to the research, by mimicking some elementary characteristics of a specific pathological state and reducing the number of variables whose control is inaccessible, thus offering a greater degree of experimental control, besides allowing experimental manipulations that could be impossible under other circumstances.

It was concluded in this model of gingival recession for the study of dentin hypersensitivity that, among the treatments with different bio-tests performed, in the evaluated experimental time, all the groups reduced the pulp blood flow. And the strontium group showed signs of less anxiety.

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CONCLUSÃO

Ao considerar todo o exposto, bem como as ressalvas concernentes às limitações deste trabalho, entende-se que o modelo *in vivo* apresentado possui reprodutibilidade em relação às condições em humanos. Portanto, torna-se interessante dar continuidade às pesquisas, a fim de melhor elucidar as respostas do animal frente a esta exposição da dentina radicular, sendo assim um modelo preliminar para o estudo da hipersensibilidade dentinária em ratos, por meio da recessão gengival induzida cirurgicamente gera uma exposição dos túbulos dentinários. Conclui-se que o modelo de recessão gengival proposto para o estudo da hipersensibilidade dentinária alterou o comportamento animal quando este foi submetido ao ataque ácido, diminuiu o fluxo sanguíneo da polpa, porém sem causar alterações no tecido pulpar. E quando realizados diferentes tratamentos com biovidros, todos os grupos reduziram o fluxo sanguíneo pulpar no tempo experimental avaliado, e o grupo tratado com estrôncio apresentou sinais de menor ansiedade.

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