AMANDA LEAL ROCHA

TERAPIA ANTICOAGULANTE ORAL:
COMPLICAÇÕES HEMORRÁGICAS EM INDIVÍDUOS SUBMETIDOS À CIRURGIA ORAL MENOR E AVALIAÇÃO IN VITRO DO EFEITO SOBRE CÉLULAS ÓSSEAS

Faculdade de Odontologia
Universidade Federal de Minas Gerais
Belo Horizonte
2019

Amanda Leal Rocha

TERAPIA ANTICOAGULANTE ORAL:
COMPLICAÇÕES HEMORRÁGICAS EM INDIVÍDUOS SUBMETIDOS À CIRURGIA ORAL MENOR E AVALIAÇÃO IN VITRO DO EFEITO SOBRE CÉLULAS ÓSSEAS

Tese apresentada ao Colegiado de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Doutor em Odontologia - área de concentração em Estomatologia.

Orientador: Prof.(a) Tarcília Aparecida da Silva
Coorientador: Prof. Lucas Guimarães Abreu
Coorientador: Dr. Daniel Dias Ribeiro

Belo Horizonte
2019

Ficha Catalográfica

R672t 2019 T

Rocha, Amanda Leal.
Terapia anticoagulante oral : complicações hemorrágicas em indivíduos submetidos à cirurgia oral menor e avaliação in vitro do efeito sobre células ósseas / Amanda Leal Rocha. -- 2019.

130 f. : il.

Orientadora: Tarcília Aparecida da Silva.
Coorientador: Lucas Guimarães Abreu.
Coorientador: Daniel Dias Ribeiro.

Tese (Doutorado) - Universidade Federal de Minas Gerais, Faculdade de Odontologia.

1. Anticoagulantes. 2. Varfarina. 3. Dabigatrana. 4. Hemorragia pós-operatória. 5. Extração dentária. I. Silva, Tarcília Aparecida da. II. Abreu, Lucas Guimarães. III. Ribeiro, Daniel Dias. IV. Universidade Federal de Minas Gerais. Faculdade de Odontologia. V. Título.

BLACK - D047

Elaborada pela Biblioteca da Faculdade de Odontologia - UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

COLEGIADO DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

Terapia Anticoagulante Oral: Complicações hemorrágicas em indivíduos submetidos à cirurgia oral menor e avaliação in vitro do efeito sobre células ósseas

AMANDA LEAL ROCHA

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Odontologia, como requisito para obtenção do grau de Doutor, área de concentração Estomatologia.

Aprovada em 22 de julho de 2019, pela banca constituída pelos membros:

Prof(a). Lucas Guimaraes Abreu - Orientador
FO-UFMG

Prof(a). Maria Cassia Ferreira de Aguiar
FO-UFMG

Prof(a). Tulio Pinho Navarro
UFMG

Prof(a). Renata Gonçalves de Resende
Faculdade Padre Arnaldo Janssem

Prof(a). Diele Carine Barreto Arantes
Newton Paiva Instituto de Ensino

Prof(a). Daniel Dias Ribeiro
HC-UFMG

Belo Horizonte, 22 de julho de 2019.

Aos meus pais, José Raimundo e Carlina, minha base, pelo apoio incondicional. Ao Fernando, pelo amor, incentivo e companheirismo diários.

AGRADECIMENTOS

Agradeço à Profa. Tarcília Aparecida Silva, orientadora deste trabalho, pela valiosa orientação e pela confiança depositada em mim.

Aos coorientadores, Prof. Lucas Guimarães Abreu e Dr. Daniel Dias Ribeiro pela acolhida, ensinamentos e paciência.

À Profa. Denise Vieira Travassos, grande apoiadora e facilitadora deste trabalho, pela colaboração.

Ao Prof. Luiz César, Prof. Wagner Castro e aos alunos do projeto de cirurgia bucomaxilofacial da FO/UFMG, pela colaboração.

À Profa. Soraia Macari e Profa. Ivana Diniz pelo aprendizado, horas despendidas no laboratório e paciência.

À Profa. Maria Auxiliadora Parreiras Martins e Profa. Gisele de Assis Goulart (Faculdade de Farmácia-UFMG) pela colaboração, apoio e ensinamentos.

Aos professores da USP-Ribeirão Preto que me acolheram e colaboraram de sobremaneira para a concretização deste trabalho, em especial ao Prof. Márcio Beloti e Profa. Sandra Fukada.

Aos professores, colegas e alunos da FO/UFMG que passaram pelo Ambulatório Borges da Costa (ABC) e que de alguma forma colaboraram para este trabalho.

Aos funcionários do HC e ABC, especialmente à Neli, por toda ajuda e carinho. À equipe de enfermagem e secretaria pelo suporte e paciência durante todos estes anos.

À Sicília e Raíssa, meus dois “braços direitos”, pela ajuda, amizade e dedicação a este trabalho.

À Let, pela acolhida, apoio e carinho em momentos tão importantes.

À Alessandra, pela amizade e parceria de sempre.

Às companheiras queridas de laboratório, Bibi, Janine, Tati e Ana Laura pela amizade, pelas risadas, mas também por todos os ensinamentos.

Aos colegas da pós-graduação da Faculdade de Odontologia e Faculdade de Farmácia que colaboraram para a realização deste trabalho. Em especial à Rayana, Thaise e Bruna, amigas especiais que este trabalho aproximou e que foram essenciais para sua concretização.

À minha querida família e amigos. Em especial meus pais, José Raimundo e Carlina, meu irmão Pedro e Vó Clara.

Ao meu melhor amigo e parceiro em todos os momentos, Fernando.

A todos os pacientes que generosamente aceitaram participar desta pesquisa, tornando possível sua realização, minha homenagem e agradecimentos especiais.

Finalmente, agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio financeiro.

"O correr da vida embrulha tudo, a vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta.O que ela quer da gente é coragem."

Guimarães Rosa

RESUMO

Os objetivos deste trabalho foram 1) avaliar o impacto da terapia anticoagulante oral no sangramento associado à exodontias durante os períodos intraoperatório e pós-operatório; 2) investigar os efeitos do etexilato de dabigatrana, um inibidor direto da trombina, sobre as células ósseas*. Para atender o objetivo 1, f*oram recrutados indivíduos em uso de anticoagulantes orais do tipo antagonista de vitamina K (AVK) e alvo-específico (DOAC, do inglês *direct oral anticoagulant*) e indivíduos sem terapia anticoagulante com indicação de exodontia. As exodontias foram realizadas sem a suspensão da terapia anticoagulante e parâmetros associados a desfechos hemorrágicos foram avaliados. A avaliação quantitativa do sangramento intraoperatório foi realizada por meio da mensuração do volume e análise dos fluidos aspirados durante o procedimento e normalizada por um escore. Obtivemos como resultados que as complicações hemorrágicas pós-operatórias bem como o escore de sangramento intraoperatório foi similar entre os grupos, sendo que nenhum evento hemorrágico foi observado no grupo DOAC. A história prévia de complicações hemorrágicas em procedimentos odontológicos (p=0,001) e uso de medidas hemostáticas locais (p=0,017) foram estatisticamente maiores no grupo AVK. *Para atender o objetivo 2*, experimentos foram conduzidos a partir de modelo *in vitro*, no qual o efeito da terapia anticoagulante foi avaliado diretamente sobre as células ósseas e em modelo animal *ex-vivo*. Neste modelo *ex-vivo*, células de animais previamente tratados com etexilato de dabigatrana foram diferenciadas em osteoclastos. Culturas primárias de células-tronco de camundongos e ratos foram diferenciadas em osteoclastos e osteoblastos e tratadas com o fármaco disponível comercialmente, etexilato de dabigatrana (Pradaxa® 1-6 µg/mL) bem como seu princípio ativo, dabigatrana (0,1, 0,3, 3 e 6 µg/mL). Células não expostas aos medicamentos foram utilizadas como controle. A diferenciação de osteoclastos foi inibida pelo tratamento em ambos os modelos, *in vitro* e *ex-vivo*. Paralelamente, observou-se a redução da expressão gênica e proteica do marcador Catepsina K e da atividade reabsortiva destas células. Nas culturas de osteoblastos, o tratamento inibiu a expressão gênica dos marcadores fosfatase alcalina (ALP) e osteocalcina, reduziu a atividade *in situ* de ALP e a deposição de matriz extracelular, indicando um efeito negativo na diferenciação dos osteoblastos. Concluiu-se que o uso de anticoagulantes orais não aumentou a ocorrência de desfechos hemorrágicos na população estudada, o que reforça a manutenção da terapia para a realização de exodontias. O tratamento sobre culturas celulares utilizando etexilato de dabigatrana impactou negativamente a diferenciação e atividade de osteoclastos e osteoblastos.

Palavras-chave: Anticoagulantes. Varfarina. Dabigatrana. Hemorragia pós-operatória. Extração dentária. Osteoblastos. Osteoclastos.

ABSTRACT

Oral anticoagulant therapy: Bleeding complications in individuals submitted to dental surgery and *in vitro* effects on bone cells

The objectives of this study were *1)* to evaluate the impact of oral anticoagulant therapy on the pattern of intraoperative and postoperative bleeding in dental surgery; *2)* to investigate the effects of dabigatran etexilate, a direct thrombin inhibitor, on bone cells. *To fulfill objective 1*, individuals undergoing oral anticoagulant therapy with vitamin K antagonists (VKA) or direct oral anticoagulants (DOAC) and individuals without anticoagulant therapy, who had indication of dental extraction were included. Dental surgery procedures were performed without interruption of anticoagulant therapy and parameters associated with hemorrhagic outcomes were evaluated. Intraoperative bleeding was evaluated by means of the measurement of the total amount of blood collected during the procedure corrected by absorbance reading and normalized by score. The results showed that the occurrence of bleeding events and the intraoperative blood loss were similar among groups and hemorrhagic episodes were not observed amongst the individuals taking DOACs. The previous history of complications in dental procedures (p=0.001) and the use of additional hemostatic measures (p=0.017) were significantly higher in the VKA group. *To fulfill objective 2*, experiments were conducted by means of an *in vitro* model in which the direct effect of anticoagulant therapy on bone cells was evaluated. An *ex-vivo* animal model in which cells of animals previously treated with dabigatran etexilate were differentiated was also carried out into osteoclasts. Primary cultures of mice and rats cells were differentiated into osteoclasts and osteoblasts and treated with dabigatran etexilate solution (Pradaxa® 1-6 µg/mL) and its active principle dabigatran (0.1, 0.3, 3 and 6 µg/mL). Untreated cells were used as controls and the effects of the treatment on cell viability and differentiation were evaluated. Both dabigatran etexilate and its active principle, dabigatran inhibited osteoclast differentiation and activity *in vitro* and in the *ex-vivo* model, as demonstrated by the reduction of resorption pits and cathepsin K gene and protein expression. In osteoblast cultures, dabigatran etexilate reduced the *in situ* alkaline phosphatase (ALP) activity, matrix mineralization and gene expression of ALP and osteocalcin. These findings indicated osteoblast inhibition. In conclusion, oral anticoagulant therapy did not result in increased bleeding outcomes in this sample, which strengthen the advocacy of the maintenance of the therapy during dental surgery. Dabigatran etexilate treatment impaired the activity and differentiation of osteoclasts and osteoblasts.

Keywords: Anticoagulants. Warfarin. Dabigatran. Postoperative hemorrhage. Tooth extraction. Osteoclasts. Osteoblasts.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

ALP	Do inglês, *alkaline phosphatase* (fosfatase alcalina)
AVK	Antagonista de Vitamina K
α-MEM	Do inglês, *α- Minimal Essential Medium* (Meio Mínimo Essencial α)
^{o}C	Graus Celsius
CA	Estado da Califórnia, EUA
CEUA	Comissão de Ética no Uso de Animais
CETEA	Comitê de Ética em Experimentação Animal
$_{C}DNA$	Do inglês, *Complementary Deoxyribonucleic Acid* (Ácido Desoxirribonucleico Complementar)
CO_2	Gás Carbônico
COEP	Comitê de Ética em Pesquisa com Seres Humanos
Ct	Do inglês, *Cicle Threshold*
CTSK	Catepsina K
DEPE	Diretoria de Ensino, Pesquisa e Extensão
DMSO	Do inglês, *Dimethyl Sulfoxide* (Dimetilsulfóxido)
DNA	Do inglês, *Deoxyribonucleic Acid* (Ácido Desoxirribonucléico)
DOAC	Do inglês, *Direct Oral Anticoagulant* (Anticoagulante Alvo-específico)
EUA	Estados Unidos da América
FA	Fibrilação atrial
FBS	Do inglês, *Fetal Bovine Sérum* (Soro Fetal Bovino)
FXa -	Fator X ativado
FO	Faculdade de Odontologia
GAPDH	Do inglês, *Glycer-Aldehyde-3-Phosphate Dehydrogenase*
HC	Hospital das Clínicas
HCl	Ácido Clorídrico
HPLC	Do inglês, *High Performance Liquid Chromatography*
HRP	Do inglês, *Horseradish Peroxidase*
H_2SO_4	Ácido Sulfúrico
H_2O_2	Peróxido de Hidrogênio
IL	Estado de Illinois, EUA
Kg	Quilogramas
MA	Estado de Massachusetts, EUA

M-CSF	Do inglês, *Macrophage Stimulating-colony Factor*
MD	Estado de Maryland, EUA
mg	Miligramas
MG	Minas Gerais
mL	Mililitros
mm^3	Milímetros cúbicos
mM	Milimolar
mm/Hg	Milímetros de Mercúrio
MN	Estado de Minnesota, EUA
MO	Estado do Missouri, EUA
MTT -	Do inglês, *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylltetrazolium bromide* (3-(brometo de 4,5-dimetiltiazol -2-yl)-2,5-difeniltetrazólio)
µM	Micromolar
µg	Microgramas
Na_2CO_3	Carbonato de Sódio
Nano	Nanotecnologia
ng	Nanograma
nm	Nanômetro
nM	Nanomolar
NY	Estado de Nova York, EUA
NRS	Do inglês, *Numerical Rating Scale*
OC	Osteocalcina
OSX	Osterix
PA	Pressão Arterial
PBS	Do inglês, *Phosphate Buffered Saline* (tampão fosfato-salina)
PVDF	Do inglês, *Polyvinylidene Difluoride*
qPCR	Do inglês, *Reverse Transcriptase-polimerase Chain Reaction in Real Time* (Transcriptase Reversa,Reação em Cadeia da Polimerase em Tempo Real)
RANKL	Do inglês,A*ctivator of Nuclear Factor kappa-B Ligand*
RIPA	Do ingles, *Radioimmunoprecipitation Assay*
RNA	Do inglês, *Ribonucleic Ácid* (Ácido Ribonucléico)
RNI	Razão Normalizada Internacional
rpm	Rotação por minuto

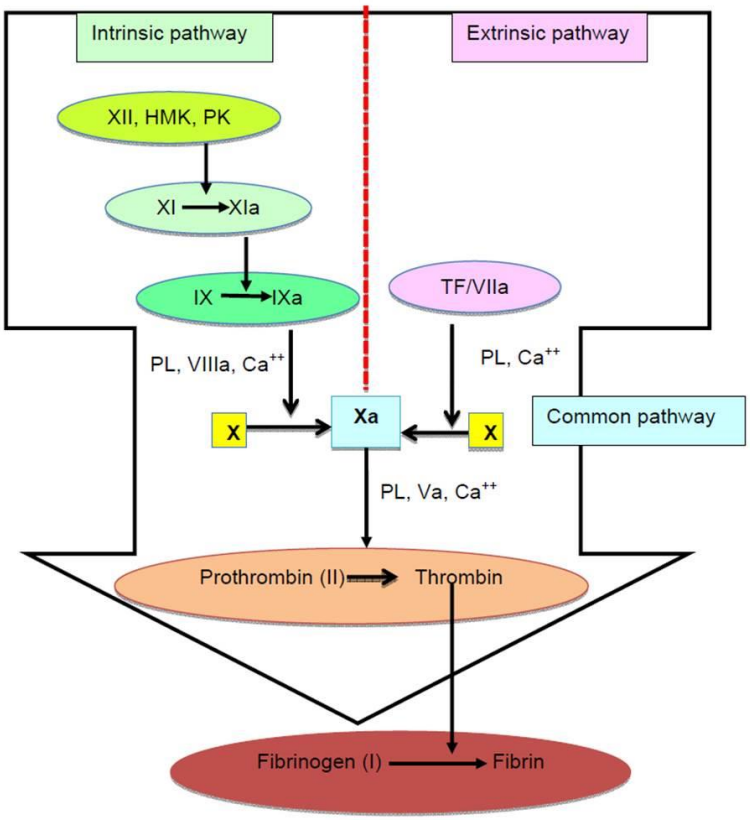
RUNX2	Do inglês, *Runt-related Transcription Factor 2*
SDS/PAGE	Do inglês, *Sodium Dodecyl Sulfate-polyacrylamide Electrophoresis Gel*
SEDTO	Serviço Especial de Diagnóstico e Tratamento em Odontologia
SPSS	Do inglês, *Statistical Package for the Social Sciences*
TCLE	Termo de Consentimento Livre e Esclarecido
Ti	Titânio
TRAP	Do inglês,*Tartrate-resistant Acid Phosphatase* (Fosfatase Ácida Resistente ao Tartarato)
UFMG	Universidade Federal de Minas Gerais
USP	Universidade de São Paulo
VA	Estado da Virgínia, EUA
WI	Estado do Wisconsin, EUA

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

A hemostasia é um processo fisiológico e multifatorial que limita a perda sanguínea associada a um dano tecidual de maneira restrita ao local do estímulo sem prejuízo ao fluxo sanguíneo sistêmico (FAKHRI *et al*., 2013). Em 1964, Macfarlane, Davie e Ratnoff propuseram um modelo com o objetivo de descrever os mecanismos da coagulação que se assemelhavam a uma cachoeira ou cascata. Este modelo, portanto, foi denominado cascata da coagulação (DAVIE e RATNOFF, 1964; MACFARLANE, 1964) (FIGURA 1). O modelo cascata propõe a divisão do processo de coagulação em duas vias; uma via intrínseca cuja ativação dos fatores da coagulação ocorre quando o sangue entra em contato com cargas elétricas negativas, e outra extrínseca, cujo mecanismo é mediado pela ação do fator tecidual (FT) (TANAKA, KEY, LEVY, 2009). No modelo proposto por Macfarlane, Davie e Ratnoff, a ativação dos fatores ocorre sequencialmente em ambas vias que se convergem ao fim da cascata para uma via comum que irá culminar na conversão do fibrinogênio em fibrina (DAVIE e RATNOFF, 1964; MACFARLANE, 1964).

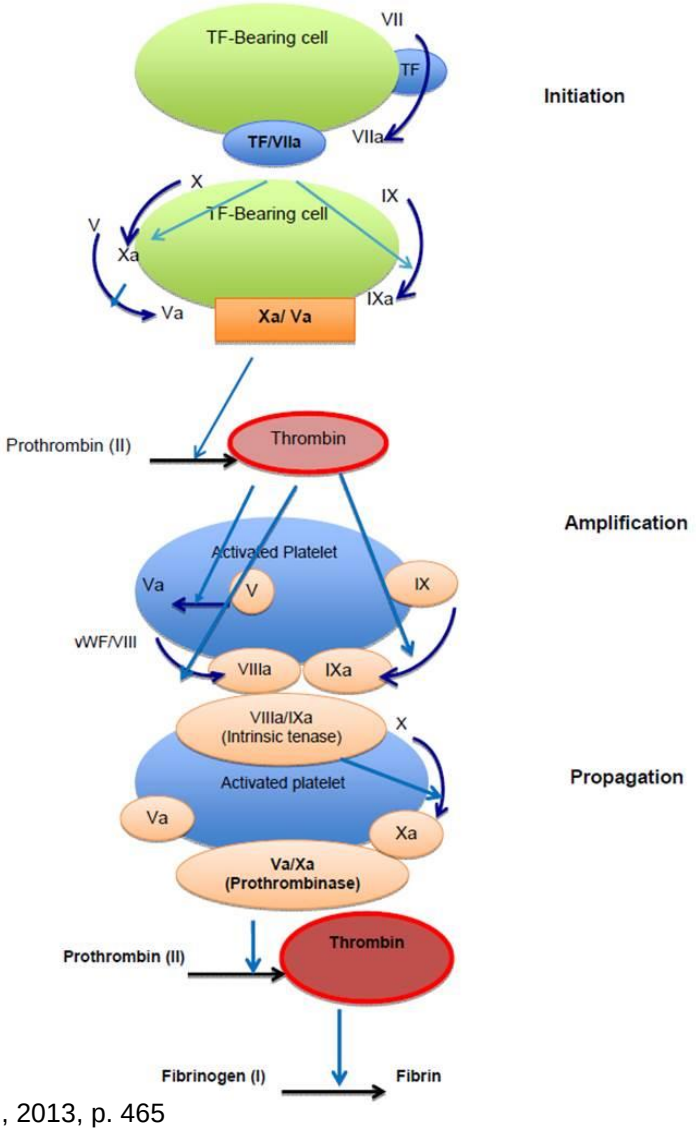
Figura 1 – Modelo cascata da coagulação.



Fonte: FAKHRI *et al*., 2013, p. 463

Embora amplamente utilizado para análises e rastreamento da coagulação sanguínea em testes laboratoriais, o modelo cascata foi considerado inadequado para explicar os mecanismos que levam à coagulação *in vivo*. Diante das limitações demonstradas por este modelo clássico, Hoffman & Monroe (2001) propuseram um novo modelo baseado em descobertas relacionadas à coagulação sanguínea, sobretudo a partir de observações clínicas dos distúrbios da coagulação. O denominado modelo da coagulação baseado em superfícies celulares enfatiza a importância de receptores específicos para as proteínas da coagulação cuja regulação é mediada por propriedades das superfícies celulares. O processo ocorre em três grandes fases; iniciação, amplificação e propagação, e não em duas vias conforme proposto anteriormente. Sendo assim, o novo modelo propiciou um melhor entendimento do processo da coagulação em organismos vivos ao demonstrar que o evento não ocorre como cascata, mas sim em grandes etapas simultâneas (HOFFMAN & MONROE, 2001) (FIGURA 2).

Figura 2 - Modelo da coagulação baseado em superfícies celulares.



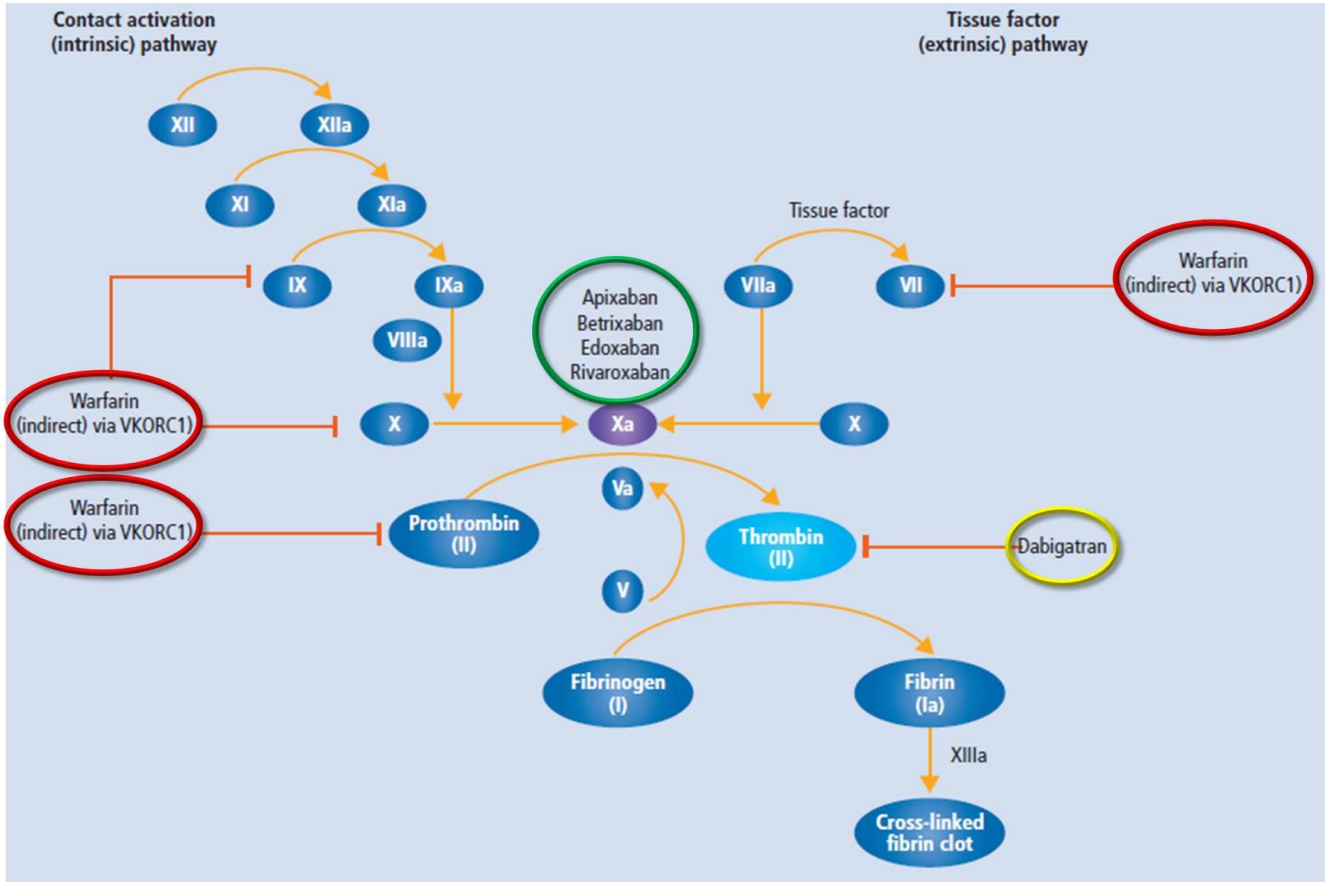
Fonte: FAKHRI *et al*., 2013, p. 465

Desequilíbrios dos mecanismos de hemostasia podem levar a condições de hipocoagulabilidade ou hipercoagulabilidade. Para a segunda, o tratamento por meio da utilização de medicamentos anticoagulantes é o mais indicado, sendo a terapia anticoagulante oral preconizada na prática médica por mais de 50 anos (ANSELL *et al*., 2004). Sua aplicação clínica representa a principal estratégia para a prevenção e tratamento de eventos tromboembólicos e dentre as condições sistêmicas que requerem terapia anticoagulante oral se destacam a fibrilação atrial (FA), os tromboembolismos venosos e a utilização de válvulas cardíacas protéticas (AGENO *et al.,* 2012; DOUKETIS *et al*., 2012; NISHIMURA *et al*., 2014). A FA representa uma das principais causas associadas à complicações tromboembólicas e estima-se que cerca de 1,5 milhões de indivíduos apresentem a doença no Brasil (ZIMERMAN *et al*., 2009).

A varfarina, um antagonista da vitamina K (AVK), é o fármaco mais comumente prescrito para anticoagulação de longo prazo (WAHL, 1998). Embora amplamente utilizados, os AVKs apresentam limitações relacionadas à necessidade de ajustes frequentes da dose, monitoramento constante dos níveis de coagulação sanguínea e múltiplas interações medicamentosas. Estas limitações estimularam o desenvolvimento de novos medicamentos anticoagulantes que não requerem monitoramento e ajustes de dose e que apresentem menos interações medicamentosas (FORTIER, SHROFF, REEBYE, 2018).

Os anticoagulantes orais alvo-específicos ou diretos (DOACs, do inglês, *direct oral anticoagulants*) surgiram para superar as limitações da terapia convencional. Tais medicamentos tem demonstrado vantagens, especialmente no que concerne à sua previsibiliade de resposta e farmacocinética (LITTLE, 2012). De maneira geral, os DOACs inibem seletivamente proteínas específicas da coagulação (FIGURA 3). Atualmente, quatro medicamentos possuem registro no Brasil: o etexilato de dabigatrana; um inibidor direto da trombina ou fator II ativado (Pradaxa® 75 mg, 110 mg, 150 mg, Boehringer, Ingelheim am Rhein, Alemanha), a rivaroxabana (Xarelto® 10 e 20 mg, Bayer, Leverkusen, Alemanha) e apixabana (Eliquis® 2,5 mg, 5 mg, Bristol-Myers, Humacao, Porto Rico); inibidores diretos do fator X ativado (FXa) (CONITEC, 2016), e recentemente a edoxabana (Lixiana® 15 mg, 30 mg, 60 mg, Daiichi Sankyo, Barueri, São Paulo); também inibidor do FXa (ANVISA, 2018).

Figura 3 - Mecanismos de ação dos anticoagulantes do tipo AVK e DOACs.



Fonte: NATHWANI & WANIS, 2017, p. 625 (houve alteração na ilustração para fins didáticos).

Estratégias para o manejo odontológico do paciente em terapia anticoagulante oral tem sido extensivamente exploradas. As primeiras recomendações para os indivíduos em uso de AVK foram interrupção e/ou substituição da terapia anticoagulante previamente a procedimentos cirúrgicos odontológicos (BAJKIN, POPOVIC, SELAKOVIC, 2009; LOCKHART *et al*., 2003; ROSER, ROSENBLOOM, 1975). Atualmente, já se sabe que a exposição do paciente ao risco de novos eventos tromboembólicos com a suspensão da medicação excede o risco de possíveis eventos hemorrágicos em procedimentos odontológicos. Esta premissa é confirmada pela evidência de que a ocorrência de complicações hemorrágicas associadas a cirurgias odontológicas é baixa e os episódios podem ser facilmente solucionados por meio de medidas hemostáticas locais (BAJKIN, BAJKIN, PETROVIK, 2012; DUDEK *et al*., 2016; FEEBO *et al.,* 2016; HONG *et al.,* 2012; MORIMOTO, NIWA, MINEMASTU, 2011; ROCHA *et al.,* 2018). Ademais, a manutenção da terapia anticoagulante durante o tratamento

odontológico está indicada pelas principais diretrizes internacionais (ADA Science Institute, 2018; DOUKETIS *et al.,* 2012; NISHIMURA *et al*., 2014).

Frente à inserção relativamente recente dos DOACs no mercado, ainda não há diretrizes bem definidas acerca do manejo odontológico cirúrgico dos indivíduos submetidos a esta terapia. Um número limitado de estudos clínicos investigou a ocorrência de complicações hemorrágicas associadas a procedimentos odontológicos, e não há consenso sobre o risco de sangramento nesta população (BENSI *et al*., 2018; BERTON *et al*., 2018; MAUPRIVEZ *et al.*, 2016; MICLOTTE *et al.,* 2017; MILLER, MILLER, 2018; MIRANDA *et al.,* 2016). Ensaios clínicos de grande porte como o EINSTEIN–Extension (BAUERSACHS *et al.*, 2010), o *Randomized Evaluation of Long-Term Anticoagulation Therapy* - RE-LY (HEALEY *et al*., 2012) e o ARISTOTLE (GARCIA *et al*., 2014) tem buscado investigar diversos desfechos associados aos DOACs. Todavia, informações sobre os desfechos hemorrágicos associados a procedimentos odontológicos não são descritas de forma detalhada ou estão indisponíveis na literatura (BAUERSACHS *et al.*, 2010; GARCIA *et al*., 2014; HEALEY *et al*., 2012). Assim, a falta de diretrizes clínicas baseadas em evidência científica para a conduta perante indivíduos sob a terapia alvo-específica dificulta a tomada de decisões assertivas durante o manejo odontológico seguro nesta população.

No que tange a anticoagulação, não apenas as complicações hemorrágicas são desfechos explorados na literatura científica. Particularmente na Odontologia, a manutenção adequada do coágulo é imprescindível para que a reparação alveolar ocorra adequadamente após a realização de exodontias. O reparo ósseo do sítio cirúrgico é um processo multifatorial dependente da formação do coágulo sanguíneo, de sua progressão para uma matriz organizada e posterior neoformação óssea. Desequilíbrios na formação e manutenção do coágulo pós-cirúrgico associados a fatores locais, alterações sistêmicas ou ao uso de medicamentos são críticos para a recuperação pós-operatória do indivíduo (SHENOY *et al*., 2015). Na prática médica, o uso de fármacos anticoagulantes após procedimentos cirúrgicos complexos que exigem imobilidade pós-operatória reduz o risco de tromboembolismos. No entanto, a osteopenia e osteoporose são efeitos colaterais reconhecidamente associados ao tratamento a longo prazo com anticoagulantes, especialmente as heparinas (ANSELL *et al.,* 2004; GARCIA *et al*., 2012; GIGI *et al.,* 2012). Sendo assim, embora necessária, a tromboprofilaxia após

cirurgias ortopédicas, por exemplo, pode impactar o processo de remodelação óssea, condição necessária para a recuperação da fratura (KLÜTER *et al.,* 2015). A partir desta constatação, estudos experimentais buscaram demonstrar os potenciais efeitos da heparina e outros anticoagulantes sobre as células ósseas (ARIYOSHI *et al*., 2008; FOLWARCZNA *et al*., 2005; FUSARO *et al.,* 2017; IRIE *et al.*, 2007; MAZZIOTTI, CANALIS, GIUSTINA, 2010). Trabalhos utilizando modelos experimentais com o objetivo de avaliar os efeitos dos DOACs, embora escassos, também revelaram sua ação sobre a diferenciação e atividade de osteoclastos e osteoblatos (GIGI *et al.,* 2012; KLÜTER *et al.,* 2015; MORISHIMA *et al.,* 2013; SOLAYAR, WALSH, MULHALL, 2011; SOMJEN *et al.,* 2013; WINKLER *et al.* 2011).

A necessidade de compreender o real impacto da anticoagulação oral na prática clínica odontológica bem como seus potenciais efeitos adversos ainda pouco explorados foram os fatores que motivaram a realização deste trabalho. Considerando a evidência científica disponível referente à terapia anticoagulante oral e aos desfechos avaliados, as hipóteses deste estudo foram formuladas. Primeiro, indivíduos utilizando terapia anticoagulante oral e sem anticoagulação poderiam apresentar parâmetros de sangramento similiares quando submetidos à exodontias. Segundo, em um modelo experimental, um DOAC, inibidor direto da trombina, poderia interferir no processo de diferenciação de células ósseas.

De forma resumida, a partir deste trabalho, buscou-se apresentar dados, ainda não disponíveis na literatura, acerca da mensuração do sangramento intraoperatório e complicações hemorrágicas em indivíduos em uso de terapia anticoagulante oral, bem como o efeito dos DOACs sobre a diferenciação de células ósseas.

2 OBJETIVO

2.1 Objetivo geral

Avaliar o impacto da terapia anticoagulante oral no sangramento associado à exodontias durante os períodos intraoperatório e pós-operatório e seu efeito sobre as células ósseas.

2.1 Objetivos específicos

a) Comparar parâmetros clínicos, ocorrência de complicações hemorrágicas pós-operatórias e mensuração do sangramento intraoperatório durante exodontias por via alveolar realizadas em três grupos distintos: (1) indivíduos em terapia anticoagulante oral do tipo AVK, (2) indivíduos em terapia anticoagulante do tipo alvo-específico e (3) indivíduos sem uso de anticoagulante oral.

b) Avaliar o efeito de um anticoagulante alvo-específico, inibidor direto da trombina, sobre a viabilidade, diferenciação e atividade de osteoclastos e osteoblastos.

3 MATERIAIS E MÉTODOS

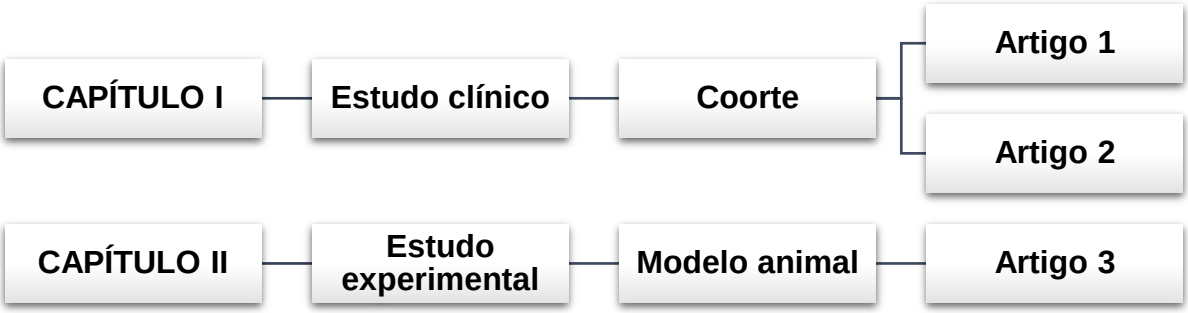
Delineamento

O presente estudo foi dividido em dois capítulos:

Capítulo I. Estudo clínico, do tipo coorte prospectivo, em que desfechos hemorrágicos em indivíduos submetidos à exodontias sem suspensão da terapia anticoagulante oral foram avaliados;

Capítulo II. Estudo experimental, conduzido por meio de modelos animais*, in vitro* e *ex-vivo*, o qual buscou-se avaliar o efeito da terapia anticoagulante sobre as células ósseas (FIGURA 4).

Figura 4- Fluxograma do delineamento do estudo



Fonte: Elaborado pela autora, 2019.

Locais de realização

Este estudo foi idealizado no Departamento de Clínica, Patologia e Cirurgia Odontológicas da Faculdade de Odontologia da Universidade Federal de Minas Gerais (FO/UFMG). A coleta de dados bem como as etapas experimentais do projeto foram executadas nos seguintes locais: Ambulatório Borges da Costa - Serviço Especial de Diagnóstico e Tratamento em Odontologia - vinculado ao Hospital das Clínicas da Universidade Federal de Minas Gerais (HC/UFMG); Laboratórios de Análises Farmacêuticas do Departamento de Produtos Farmacêuticos da Faculdade de Farmácia da UFMG; Laboratórios de Cultura Celular do Departamento de Física e Química da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo (USP-Ribeirão Preto) e do Departamento de Biologia Básica e Oral da Faculdade de Odontologia da USP (Ribeirão Preto).

3.1 Capítulo I

3.1.1 Desenho do estudo

A realização deste estudo longitudinal prospectivo foi aprovada pela Diretoria de Ensino, Pesquisa e Extensão do Hospital das Clínicas UFMG (DEPE/HC) (ANEXO A) e pelo Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Minas Gerais (COEP/UFMG), CAAE: 48122215.4.0000.5149 (ANEXO B, C e D). Todos os indivíduos foram devidamente informados sobre a pesquisa e assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) (APÊNDICE A). A amostra foi composta por pacientes sob terapia anticoagulante oral do tipo AVK ou DOAC e indivíduos sem terapia anticoagulante. Os participantes foram divididos em três grupos cujas variáveis dependentes e independentes foram comparadas.

3.1.2 Local e Seleção dos Pacientes

O estudo foi desenvolvido no Serviço Especial de Diagnóstico e Tratamento em Odontologia (SEDTO) alocado no Ambulatório Borges da Costa do Hospital das Clínicas da Universidade Federal de Minas Gerais (HC/UFMG). Neste serviço, pacientes com alterações sistêmicas em acompanhamento médico no HC/UFMG são encaminhados por meio de um pedido de interconsulta e atendidos em nível ambulatorial.

3.1.3 Cálculo amostral

O cálculo do tamanho da amostra foi baseado na variável contínua (quantidade de sangramento intraoperatório) avaliada em indivíduos de dois grupos (controle e AVK) com um controle para cada indivíduo experimental. Em um estudo piloto, a diferença da média da quantidade de sangramento total aspirado entre os grupos foi de 9,12 mL e o desvio padrão de 18,95. Portanto, levando em consideração um poder de teste de 80%; 69 indivíduos no grupo experimental e 69 no grupo controle foram necessários para rejeitar a hipótese nula de que não haveria

diferença entre esses grupos. A probabilidade de erro do Tipo I associada a este teste foi de 0,05.

3.1.4 Critérios de elegibilidade – Grupo AVK e DOAC

Foram incluídos no estudo todos os indivíduos em uso regular de anticoagulante oral do tipo AVK ou DOAC encaminhados pela equipe médica ao SEDTO entre janeiro de 2016 e janeiro de 2018.

a) Os critérios de inclusão para o grupo AVK e DOAC foram:

- Indivíduos que concordaram em participar do estudo e assinaram o TCLE;
- Indivíduos que necessitaram de procedimento odontológico do tipo exodontia de dente erupcionado;
- Indivíduos que apresentaram valores de Razão Normalizada Internacional (RNI) ≤ 3,5 mensurados no máximo três dias antes do procedimento (apenas para pacientes em uso de AVK).

b) Os critérios de exclusão para os grupos AVK e DOAC foram:

- Indivíduos com RNI fora da faixa terapêutica pré-determinada (para indivíduos em uso de AVK);
- Indivíduos com outras alterações de coagulação não relacionadas ao uso do anticoagulante (doença hepática, doença hematológica, contagem de plaquetas ≤ 50.000 mm^3);
- Indivíduos com idade igual ou superior a 80 anos;
- Procedimentos que envolveram dentes com condições inflamatórias agudas instaladas (abscesso periodontal ou periapical e supuração);
- Exodontias por via não-alveolar.

3.1.5 Critérios de elegibilidade – Grupo sem anticoagulação

a) Foram incluídos todos os pacientes encaminhados ao SEDTO no mesmo período de tempo e que preencheram os seguintes critérios de inclusão:

- Indivíduos sem alterações no processo de coagulação, associadas a doenças de base (alterações hematológicas, hepáticas, condições imunomediadas e/ou inflamatórias) ou ao uso de medicações

(anticoagulantes, antiagregantes plaquetários, antiinflamatórios não esteroidais);

- Indivíduos que necessitaram de procedimento odontológico do tipo exodontia de dente erupcionado;

b) Os critérios de exclusão para o grupo sem anticoagulação foram:

- Indivíduos com idade igual ou superior a 80 anos;

- Procedimentos que envolveram dentes com condições inflamatórias agudas instaladas (abscesso periodontal ou periapical e supuração);

-Exodontias por via não-alveolar.

3.1.6 Coleta de dados

Dados como idade, sexo, história prévia de episódios de sangramento em tratamentos odontológicos e/ou médicos, história de sangramento na família (pais ou irmãos), presença de doença de base, uso de medicamentos e contagem de plaquetas foram coletados a fim de caracterizar a amostra. Para os indivíduos sob terapia anticoagulante, o valor de RNI (para indivíduos em terapia AVK), informações sobre a indicação e tipo de medicamento utilizado (varfarina, dabigatrana, rivaroxabana, apixabana e edoxabana) bem como o uso concomitante de outros medicamentos que afetam a hemostasia também foram registrados.

O número de exodontias realizadas, número de dentes extraídos por procedimento, indicação da exodontia (doença periodontal, lesões cariosas extensas ou terceiros molares), classificação dos dentes (uni ou multirradiculares) e duração do procedimento foram anotados. Na consulta de acompanhamento pós-operatório, os pacientes foram questionados em relação à dor e ao número de gazes utilizadas durante o período.

Em relação às variáveis desfecho, informações sobre a necessidade de utilização de medidas adicionais de hemostasia durante o procedimento, ocorrência de sangramento pós-operatório imediato e tardio, escore de sangramento intraoperatório e condição de reparo do alvéolo (satisfatório, edema/eritema ou exposição óssea) foram registrados. Os eventos hemorrágicos pós-operatórios foram detalhadamente descritos, bem como as medidas necessárias para atendimento de urgência e hemostasia, necessidade de atendimento ambulatorial ou de internação hospitalar.

Os dados foram registrados na ficha de avaliação (APÊNDICE B) e o procedimento cirúrgico será descrito detalhadamente a seguir.

3.1.7 Procedimento odontológico cirúrgico

Todos os procedimentos foram realizados em nível ambulatorial, no início da manhã e executados por cirurgiões-dentistas qualificados, treinados e supervisionados pela pesquisadora principal. As exodontias foram realizadas sem suspensão da terapia anticoagulante.

Um hemograma recente foi solicitado a todos os pacientes incluídos no estudo. Para pacientes em uso de AVK, o tempo de protrombina (RNI) foi mensurado no prazo máximo de três dias antes do procedimento.

A pressão arterial (PA) foi mensurada previamente e imediatamente após todas as intervenções cirúrgicas realizadas, conforme protocolo do serviço (SEDTO). Os parâmetros máximos aceitáveis foram: PA sistólica máxima de 160 mm/Hg e PA diastólica máxima 90 mm/Hg (LITTLE, 2000). Pacientes que apresentaram valores superiores na aferição pré-operatória foram encaminhados ao médico assistente para controle da pressão arterial e o procedimento foi suspenso. Nos casos em que foram detectados valores superiores na aferição pós-operatória, o paciente permaneceu sob observação no Ambulatório Borges da Costa até a normalização dos parâmetros.

A técnica cirúrgica seguiu parâmetros estabelecidos. Tais parâmetros foram observados pela pesquisadora durante todo o procedimento. Intervenções fora do padrão foram excluídas do estudo.

As exodontias foram realizadas sob anestesia local, empregando-se bloqueio regional complementado por infiltrações locais de lidocaína 2% com epinefrina 1:100.000 (Alphacaine 1:100,000; DFL Indústria e Comércio S.A, Rio de Janeiro, Brasil). A quantidade de agente anestésico local utilizado foi como recomenda a literatura; 4,4 mg de lidocaína por Kg de peso, não ultrapassando a dose limite de 300 mg (LARAGNOIT *et al*., 2009; NEVES *et al.,* 2007). Para pacientes com doença cardiovascular, devido a recomendação de limitar a administração de epinefrina, a dose máxima foi de 0,036 mg a 0,054 mg de epinefrina por procedimento, ou seja, 3,6 mL a 5,4 mL de lidocaína a 2% com epinefrina 1:100.000 (BARTOLOTTO, NEVES, MONTANO, 2012).

A técnica cirúrgica padronizada consistiu das seguintes etapas:

a) Antissepsia extrabucal utilizando solução de digluconato de clorexidina 2% (Riohex, Rioquímica, São Paulo, Brasil);
b) Bochecho utilizando solução de digluconato de clorexidina 0,12% (Periogard, Colgate-Palmolive, São Paulo, Brasil) durante 1 minuto;
c) Bloqueio anestésico local utilizando seringa carpule e agulha gengival (curta ou longa);
d) Incisão intrasulcular utilizando lâmina de bisturi nº15;
e) Sindesmotomia utilizando sindesmótomo ou descolador tipo Molt;
f) Luxação do dente com alavancas (reta, seldin);
g) Avulsão com fórceps odontológico;
h) Curetagem do alvéolo utilizando cureta de Lucas;
i) Irrigação com um volume total de 100 mL de solução fisiológica 0,9%;

As medidas padrão de hemostasia local para todos os procedimentos do estudo incluíram sutura com fio 3.0 nylon 14502 T (Mononylon, Ethicon, Somerville, New Jersey) e posterior compressão com gaze do sítio cirúrgico por 20 minutos. O tempo de procedimento foi mensurado com auxilio de um cronômetro, iniciando da primeira incisão para o descolamento gengival até a sutura completa.

Todos os pacientes receberam orientações verbais detalhadas e uma cartilha impressa contendo instruções pós-operatórias (APÊNDICE C). Como medicação pós-operatória, analgésicos (dipirona 500 mg ou paracetamol 500 mg) a cada seis horas em caso de dor foram prescritos. Profilaxia antibiótica foi preconizada para indivíduos com risco de endocardite infecciosa, conforme definido pela *American Heart Association* (NISHIMURA *et al.,* 2014).

3.1.8 Avaliação quantitativa do sangramento intraoperatório

O período intraoperatório, durante o qual a avaliação quantitativa do sangramento foi realizada, se estendeu desde o momento da incisão intrasulcular até a finalização das suturas com ausência de sangramento ativo no sítio cirúrgico.

A quantidade de sangramento total foi mensurada por meio da coleta dos fluidos aspirados durante o procedimento cirúrgico e armazenados em bomba a vácuo portátil (5005 BRS, Nevoni, São Paulo, Brazil). Para todos os pacientes foi utilizado o volume total de 100 mL de solução fisiológica para irrigação do alvéolo. Em função da padronização do limite de solução para irrigação durante os procedimentos, as exodontias de dentes inclusos por via não-alveolar, para as quais volumes maiores de irrigação são utilizados, não foram incluídas na amostra. Dois mL de heparina (Hepamax-S, Blausiegel, São Paulo, Brazil) foram injetados no frasco de aspiração para evitar a formação de coágulos e interferência na análise. Posteriormente, este fluido foi quantificado com auxílio de proveta graduada conforme o método a seguir.

Do volume final do fluido aspirado desconsiderou-se o valor total de 100 mL de solução fisiológica utilizada em todos os procedimentos. Sendo assim, a cada 5 mL de volume de fluido aspirado, a amostra foi categorizada empregando-se o seguinte escore: amostras com até cinco mL foram classificadas com escore 1; amostras com seis a 10 mL foram classificadas com escore 2; amostras com 11 a 15 mL foram classificadas com escore 3; amostras com 16 a 20 mL escore 4; amostras com 21 a 25 mL escore 5; 26 a 30 mL escore 6; 31 a 35 mL escore 7; 36 a 40 mL escore 8; 41 a 45 mL escore 9 e finalmente amostras com 46 a 50 mL foram classificadas com escore 10.

3.1.9 Análise de absorbância do fluido aspirado

A interferência da saliva é um aspecto crítico para a avaliação do sangramento associado a procedimentos odontológicos. O conteúdo aspirado durante a cirurgia odontológica compreende, além do volume de sangramento proveniente do sítio cirúrgico e a solução de irrigação, o fluido salivar produzido constantemente e que também irá compor a amostra final do fluido coletado. Uma vez que o volume de secreção salivar varia entre os indivíduos, pressupõe-se que esta variação possa interferir na quantificação do fluido total aspirado. Amostras com volumes maiores poderiam apresentar quantidades excessivas de secreção salivar e não representar a quantificação real do sangramento. Sendo assim, a medida (mL) do volume final aspirado por si só não é considerada um método confiável para quantificar o sangramento intraoperatório em procedimentos odontológicos.

Com a finalidade de corrigir a influência da secreção salivar no volume total do conteúdo aspirado, os fluidos coletados foram analisados. Após a quantificação do volume, a amostra foi homogeneizada e uma alíquota foi coletada, a partir do fluido total aspirado, para leitura da densidade óptica (medida indireta da concentração de hemácias) utilizando espectrofotômetro em 537 nm de comprimento de onda (RA 50 clinical, Bayer, São Paulo, Brasil). Os valores de absorbância foram categorizados em escores do menor para o maior valor da seguinte forma: absorbância até 1,0 foi classificada como escore 1, o valor de absorbância de 1,1 a 2,0; como escore 2, 2,1 a 3,0; como escore 3 e valores a partir de 3,1 foram considerado como escore máximo de 4.

Os menores escores de absorbância foram observados em amostras de conteúdo translúcido e menor concentração de hemácias, ao passo que amostras de conteúdo vermelho vivo apresentaram os maiores escores de absorbância (FIGURA 5).

Figura 5- Diferença entre concentrações de amostras do fluido aspirado

Fonte: Elaborado pela autora, 2019.

3.1.10 Mensuração do escore de sangramento intraoperatório

Os escores obtidos na *avaliação quantitativa do sangramento* e *absorbância do fluido aspirado* foram somados para alcançar um escore final de sangramento intraoperatório. O valor variou de dois a 14, sendo que os escores mais altos indicavam os procedimentos com maior sangramento intraoperatório, cujo volume elevado de sangramento (mL) foi associado a um alto valor de absorbância

3.1.11 Avaliação do sangramento no período pós-operatório e manejo

Após a realização do procedimento odontológico, o paciente foi monitorado durante 20 minutos. Transcorrido este período, foi realizada nova avaliação para constatar a presença ou ausência de sangramento ativo e formação de coágulo após a remoção da gaze. Em caso de sangramento ativo, caracterizou-se o evento como sangramento pós-operatório imediato e medidas adicionais para hemostasia local foram realizadas.

As medidas padronizadas para hemostasia local foram; utilização de esponja de gelatina absorvível de 10x10x10mm (Hemospon, Technew, Rio de Janeiro, Brasil), ácido tranexâmico (Transamin 250mg, Nikkho, Rio de Janeiro, Brasil) e/ou novas suturas. O ácido tranexâmico foi utilizado em forma de pasta (um comprimido de 250 mg macerado e misturado à solução salina) e aplicado sobre a esponja de gelatina inserida no alvéolo após a exodontia. Uma camada adicional da pasta foi aplicada sobre a ferida cirúrgica após as suturas e coberta com gaze sob compressão. Para estes pacientes, o uso local de enxaguatório bucal a base de ácido tranexâmico foi prescrito da seguinte forma: quatro vezes ao dia durante os sete dias seguintes ao procedimento cirúrgico (um comprimido diluído em 100 mL de solução salina gelada).

O desfecho clínico caracterizado por um evento hemorrágico pós-operatório tardio foi definido como um episódio de sangramento após o paciente ter deixado o serviço e que exigiu pelo menos uma das seguintes medidas: (1) chamada telefônica para o serviço odontológico ou para a pesquisadora principal relatando sangramento pós-operatório; (2) retornar ao serviço de origem ou outro serviço ambulatorial devido a sangramento pós-operatório; ou (3) necessidade de hospitalização. O número do telefone celular da pesquisadora principal foi fornecido aos pacientes para que eles pudessem entrar em contato em caso de complicações no período pós-operatório. Com o objetivo de monitorar os episódios de sangramento desde o dia da cirurgia até uma semana após as exodontias, os pacientes foram instruídos a realizar compressão local com gaze em caso de sangramento e a registrar o número de gazes utilizadas.

No sétimo dia pós-operatório, os pacientes foram reavaliados para remoção de suturas e acompanhamento da cicatrização da ferida operatória. Parâmetros como a presença de eritema/edema local e exposição óssea foram

analisados. Durante a consulta, os pacientes foram questionados quanto às complicações hemorrágicas durante o período pós-operatório e em relação à dor. A dor foi medida por meio da *numerical rating scale* (NRS) (DOWNIE *et al.,* 1978). A escala variou de 0 a 10. O escore 0 indicou ausência de dor e o escore 10 indicou a maior percepção de dor.

Necessidades adicionais de tratamento odontológico foram atendidas de acordo com a capacidade do SEDTO. Pacientes foram encaminhados também para a Faculdade de Odontologia da UFMG.

3.1.12 Análise estatística

a) Variáveis independentes

As seguintes variáveis independentes foram analisadas:

-Idade e sexo;

-História de sangramento em procedimentos médicos ou odontológicos; anteriores e histórico de sangramento entre os membros da família (pais e/ou irmãos);

-Diagnóstico médico;

-Indicação para terapia anticoagulante oral;

-Tipo de terapia anticoagulante oral;

-RNI (para grupo AVK);

-Contagem de plaquetas e hematócrito;

-Número de exodontias (um dente, dois ou mais dentes);

-Tipo de dentes extraídos (dentes unirradiculares ou multirradiculares);

-Indicação da exodontia (doença periodontal, cárie ou terceiro molar);

-Duração do procedimento cirúrgico;

-Escala de dor;

-Número de gazes utilizadas no período pós-operatório.

b) Variáveis dependentes:

Os desfechos avaliados foram:

-Necessidade de medidas hemostáticas adicionais no período intraoperatório (sim ou não);

-Escore do sangramento intraoperatório;

-Sangramento pós-operatório imediato (sim ou não);

-Cicatrização do alvéolo (satisfatória, edema/eritema, exposição óssea);

-Sangramento pós-operatório: (1) chamada telefônica para o serviço odontológico ou para a pesquisadora principal relatando sangramento pós-operatório; (2) retornou ao serviço de origem ou a outro serviço ambulatorial devido a sangramento pós-operatório; ou (3) necessidade de hospitalização.

A análise estatística foi realizada utilizando o Statistical Package for the Social Sciences (SPSS for Windows, versão 23.0, IL, EUA). Primeiramente foi realizada a análise descritiva dos dados. Comparações intergrupos sobre variáveis independentes e variáveis dependentes (desfecho) foram realizadas por meio de análise bivariada. Para as variáveis qualitativas foram utilizados teste qui-quadrado de Pearson e o teste exato de Fisher. O teste de Kolmogorov-Smirnov demonstrou que as variáveis quantitativas apresentaram distribuição não normal. Assim, foi utilizado um teste não paramétrico (teste de Mann-Whitney) para avaliação destas variáveis quantitativas. A unidade de análise era o número de procedimentos e não o número de pacientes. O nível de significância foi estabelecido em $p<0,05$.

3.2 Capítulo II

3.2.1 Desenho do estudo

Trata-se de estudo experimental em modelos animais realizado por meio de culturas de células-tronco e de linhagem celular. O estudo foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Minas Gerais (protocolo: 247/2018) (ANEXO E) e pelo CEUA da Universidade de São Paulo (protocolo: 2018.1.562.58.0) (ANEXO F). Os experimentos foram realizados de acordo com as normas éticas definidas pelo Comitê de Ética em Experimentação Animal (CETEA). Foram utilizados para os experimentos camundongos machos da linhagem C57BL/6J com seis semanas de vida, ratos machos da linhagem Wistar Hannover com três dias de vida e células MC3T3-E1 de linhagem pré-osteoblástica.

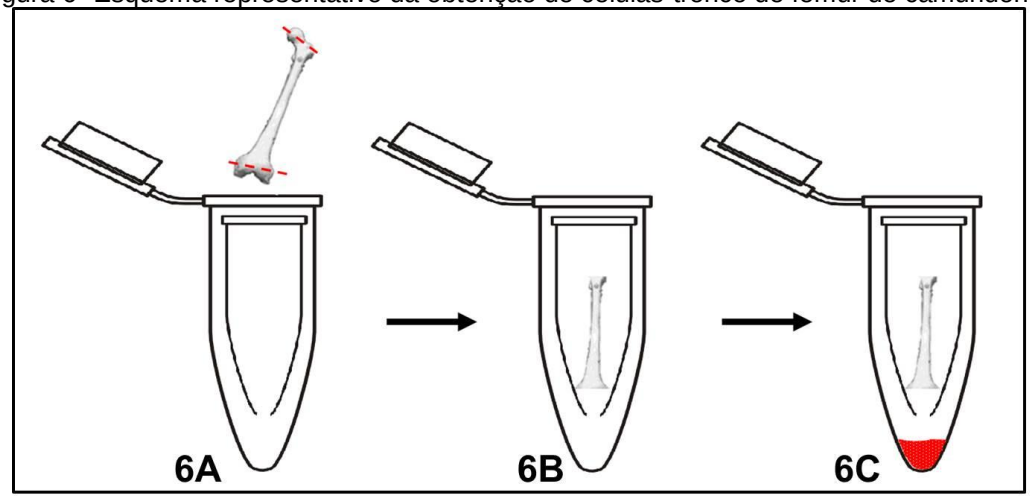
Os experimentos foram conduzidos em modelo *in vitro*, na qual buscou-se avaliar o efeito da terapia anticoagulante diretamente sobre culturas celulares e em modelo animal *ex-vivo*, em que células de animais previamente tratados foram cultivadas. Para os experimentos foram preconizadas duas formulações de um DOAC inibidor direto da trombina: o etexilato de dabigatrana, na formulação disponível comercialmente (mesilato etexilato de dabigatrana) e o seu princípio ativo, a dabigatrana.

3.2.2 Modelo in vitro - Isolamento e cultura de osteoclastos

Para isolamento de osteoclastos foi utilizado um modelo experimental a partir da diferenciação de células-tronco hematopoiéticas derivadas de medula óssea de camundongos. Os animais da linhagem C57BL/6J foram eutanasiados com sobredose de anestésico composto por solução de cloridrato de cetamina (30-50 mg/Kg) e xilasina (5-10 mg/Kg) por via intramuscular, seguido por deslocamento cervical. Posteriormente, procedeu-se com a remoção dos fêmures e tíbias. As epífises dos fêmures e tíbias foram seccionadas na região de maior diâmetro (FIGURA 6A) e os ossos foram colocados em microtubos de centrífuga perfurados na base, inseridos em tubos maiores sem perfuração e centrifugados a 10.000 rpm durante 30 segundos (FIGURA 6B). Os sedimentos concentrados no fundo do tubo*, ou pellets* de células*,* foram desfeitos e 1 mL de solução tampão foi acrescido a eles

(FIGURA 6C). As células obtidas a partir do processo foram contadas utilizando câmara de Neubauer (Laboroptik, London, United Kingdom) e cultivadas em meio alfa completo (α-MEM, Thermo Fisher Scientific, MA, EUA) suplementado com 10% de soro fetal bovino (FBS, Gibco, CA, USA).

Figura 6- Esquema representativo da obtenção de células-tronco de fêmur de camundongo.



Legenda: A- Cortes das extremidades do fêmur e posicionamento do microtubo e tubo de centrífuga. B – Osso seccionado e preparado para centrifugação. C – Após centrifugação observa-se a presença do *pellet* de células no fundo do tubo maior. Fonte: Elaborado pela autora, 2019.

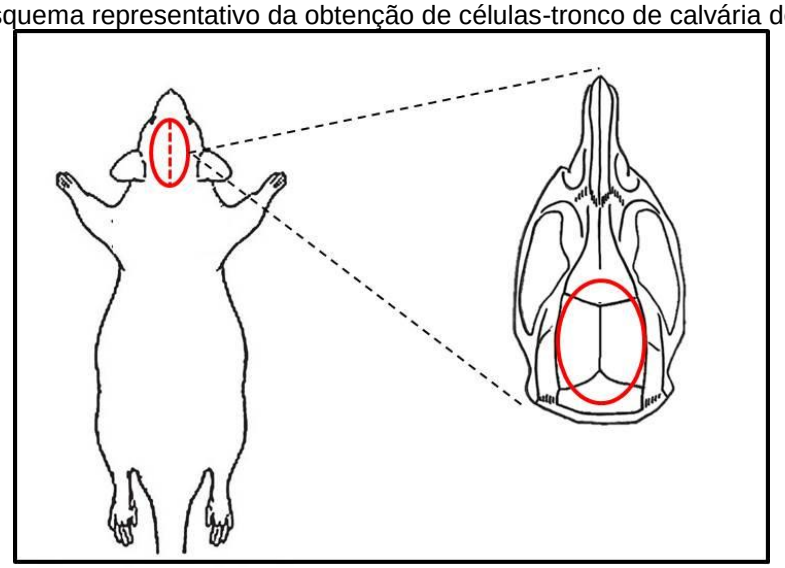
Para diferenciação dos osteoclastos, as células primárias foram cultivadas em meio α-MEM suplementado (Thermo Fisher Scientific) contendo fator estimulador de colônias de macrófagos (M-CSF 30 ng/mL, R&D Systems, MN, EUA) por três dias em estufa úmida a 37^{o}C e 5% CO_2. Após a obtenção da confluência, as células precursoras de osteoclastos foram então plaqueadas em placas de 96 poços (Corning Inc., Corning, NY, EUA) a uma densidade de 2 × 10^4 células por poço e cultivadas em meio contendo M-CSF (30 ng/mL, R&D Systems) e ligante do receptor ativador do fator nuclear kappa B (RANKL, 10 ng/mL, R&D Systems).

3.2.3 Modelo in vitro - Isolamento e cultura de osteoblastos

O modelo experimental adotado para obtenção de osteoblastos foi a partir da diferenciação de células de calvárias de ratos recém-nascidos (BELLOWS & AUBIN, 1989). Para o experimento foram utilizados 8 ratos da linhagem Wistar Hannover, com três dias de vida pós-natal. Os animais foram decapitados com lâmina fria e posteriormente as calvárias foram removidas com a utilização de

tesoura (FIGURA 7). Os osteoblastos foram obtidos por meio da digestão enzimática sequencial com solução de tripsina 0,25% (Gibco) e colagenase do tipo II 0,2% (Gibco) de fragmentos de calvárias. As células enzimaticamente isoladas em suspensão foram contadas, plaqueadas na densidade de 2×10^4 células/poço em placas de 24 poços (Corning Inc.), e cultivadas por períodos de até 14 dias, em meio osteogênico constituído de meio de crescimento α-MEM (Invitrogen Life Technologies, Grand Island, NY, EUA) suplementado com 5 µg/mL de ácido ascórbico (Gibco), 100 µg/mL de gentamicina (Gibco) e beta-glicerofosfato 7 mM (Sigma-Aldrich, Merck KGaA, Darmstadt, Alemanha).

Figura 7-Esquema representativo da obtenção de células-tronco de calvária de ratos recém-nascidos.



Fonte: Elaborado pela autora, 2019.

3.2.4 Modelo in vitro- Diferenciação de células de linhagem pré-osteoblástica MC3T3-E1

Células de linhagem pré-osteoblástica MC3T3-E1 (American Type Culture Collection, VA, EUA) também foram utilizadas para os experimentos em osteoblastos. As células foram cultivadas utilizando-se placas de poliestireno de 24 poços (Corning Inc.) a uma densidade celular de 2×10^4 células/poço por até 14 dias. O ambiente de cultivo para as células foi composto de meio α-MEM essencial mínimo (Invitrogen Life Technologies) suplementado com 10% de FBS (Gibco), 100 U/ml de penicilina (Invitrogen Life Technologies), 100 µg/ml de estreptomicina, 5 µg/ml de ácido ascórbico (Gibco) e 7 µM de β-glicerofosfato (Sigma-Aldrich).

3.2.5 Tratamento com Etexilato de Dabigatrana

Para preparo da solução de estoque de etexilato de dabigatrana a 100 µg/mL, o conteúdo de duas cápsulas de Pradaxa® (mesilato etexilato de dabigatrana, Boehringer, Ingelheim am Rhein, Alemanha) foi triturado em cadinho de porcelana e posteriormente pesado em balança analítica. A quantidade de 142,73 mg do conteúdo das cápsulas, que corresponde a 50 mg de etexilato de dabigatrana foi transferida para um balão de 500 mL e diluído em água ultrapura (Milli-Q Plus system, EMD Millipore, MA, EUA), com auxílio de ultrassonicador por 10 minutos a temperatura ambiente. Posteriormente a solução foi filtrada com filtro de seringa (0.22 µm, 33 mm diametro, PVDF, Millipore, MA, EUA) passivado (Tween™ 20 a 5%, Croda Health Care, East Riding, Inglaterra) e armazenada em temperatura próxima a 2–8°C ao abrigo da luz. Testes para identificação do princípio ativo e estabilidade da solução de estoque, bem como das concentrações de trabalho foram realizados utilizando cromatografia líquida de alta eficiência (do inglês *High Performance Liquid Chromatography* - HPLC- LC system - Shimadzu, Kyoto, Japão).

Após o plaqueamento, as culturas celulares foram expostas à solução aquosa de etexilato de dabigatrana (Pradaxa®) em concentrações de 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL e 6 µg/mL. Tais concentrações foram obtidas a partir da diluição da solução estoque (100 µg/mL) em meio de cultura. Células que não receberam o tratamento com a medicação foram utilizadas como controle. As culturas foram incubadas em estufa úmida a 37^{o}C e 5% CO_2. As trocas de meio foram realizadas a cada 72 horas, quando uma nova estimulação foi realizada.

3.2.6 Tratamento com Dabigatrana

Após o plaqueamento, as culturas celulares foram expostas à solução de Dabigatran-D3 100µg/mL, acetonitrila com 10% 0.01N HCl (Sigma-Aldrich) nas seguintes concentrações: 0,1 µg/mL, 0,3 µg/mL, 3 µg/mL e 6 µg/mL. Células que não receberam o tratamento com a medicação foram utilizadas como controle. As culturas foram incubadas em estufa úmida a 37^{o}C e 5% CO_2. Trocas de meio foram realizadas a cada 72 horas, quando uma nova estimulação foi realizada.

3.2.7 Ensaio de viabilidade celular

O efeito dos tratamentos na viabilidade/proliferação celular foi avaliado nas culturas celulares medindo a redução do brometo de 3-(4,5-dimetiltiazol-2yl)-2,5-difenil brometo de tetrazolina (MTT, Sigma-Aldrich) para cristais de formazan, processo associado à atividade mitocondrial. Quadruplicatas do tratamento com etexilato de dabigatrana foram plaqueadas. Após períodos de três e sete dias de incubação, 20 µL de solução de MTT 5 mg/mL em tampão fosfato-salina (PBS, Sigma-Aldrich) foram adicionados a cada poço, e as placas foram mantidas durante três horas em estufa úmida a 37^{o}C e 5% CO_2. O sobrenadante foi removido e os cristais de MTT foram solubilizados com 200 µl de dimetilsulfóxido (DMSO, Sigma-Aldrich) ou solução de isopropanol ácido (100 mL de isopropanol e 134 µL HCl) à temperatura ambiente. A absorbância foi mensurada em espectrofotômetro com leitura a 540 nm (DMSO) e 570 nm (isopropanol) (µQuant, BioTek Instruments Inc., Vermont, EUA). As médias dos valores de absorbância obtidos foram calculadas em relação ao grupo controle e os valores foram expressos como percentual de células viáveis.

3.2.8 Avaliação da expressão gênica - qPCR (Reverse transcriptase-polimerase chain reaction)

A expressão gênica foi avaliada no terceiro e no sétimo dia de cultura no grupo tratado, o qual foi comparado ao grupo controle sem tratamento. Para este ensaio, o meio de cultura foi removido dos poços e adicionado o reagente Trizol LS (Invitrogen Life Technologies) à temperatura ambiente, por cinco minutos, sob agitação por pipetagem. A extração do ácido ribonucleico (RNA, Do inglês, *ribonucleic acid)* total foi realizada utilizando o kit SV Total RNA Isolation System (Promega, WI, EUA), de acordo com especificações do fabricante. Em seguida, o RNA total foi quantificado em espectrofotômetro (GE Healthcare, Buckinghamshire, Inglaterra) e sua integridade avaliada por meio do aparelho Agilent 2100 BioAnalyzer (Agilent Technologies Stockport, Inglaterra). Em seguida, foi confeccionada a fita de DNA complementar (cDNA, do inglês, *complementar deoxyribonucleic acid*) a partir do RNA total. Este procedimento foi feito no termociclador Mastercycle Gradient (Eppendorf, Alemanha) por meio de reação com a enzima transcriptase reversa,

utilizando o kit High-capacity cDNA Reverse Transcription (Applied Biosystems, CA. EUA). A análise de PCR foi realizada no sistema StepOnePlus Real-Time PCR (Thermo Fisher Scientific, MA, EUA) em triplicata, utilizando SYBR® Green PCR Master Mix kit (Thermo Fisher Scientific). Avaliou-se a expressão gênica dos marcadores ósseos catepsina K (CTSK) para osteoclastos e RUNX2 (do inglês, *runt-related transcription factor 2)*, fosfatase alcalina (ALP, do inglês *alkaline phosphatase*), osteocalcina (OC) e osterix (OSX) para osteoblastos. Como controle endógeno, foi avaliada a expressão do gene para a enzima gliceraldeído-3-fosfato-desidrogenase (GAPDH), que foi utilizada para a normalização dos níveis de expressão dos genes avaliados. Os resultados foram analisados com base no valor de Ct (cicle threshold, ou ciclo limiar), sendo este o ponto correspondente ao número de ciclos em que a amplificação das amostras atinge um limiar (determinado entre o nível de fluorescência dos controles negativos e a fase de amplificação exponencial das amostras), permitindo a análise quantitativa da expressão dos genes avaliados. O método comparativo de $2^{-\Delta\Delta}$Ct foi utilizado para comparar a expressão gênica das células dos diferentes grupos experimentais.

3.2.9 Avaliação da diferenciação de osteoclastos

a) Expressão de proteínas

A análise dos níveis de proteína foi realizada por meio do método Western Blot após três dias de cultura dos osteoclastos provenientes de culturas primárias. As células foram lisadas utilizando tampão RIPA (do inglês, *radioimmunoprecipitation*) (Sigma-Aldrich) e inibidores de protease e fosfatase. A concentração de proteína foi determinada utilizando o kit Bicinchoninic Acid Protein assay (Sigma-Aldrich). Quantidades iguais de proteína (10 µg) foram submetidas a eletroforese SDS/PAGE (do inglês, *sodium dodecyl sulfate-polyacrylamide electrophoresis gel*) e posteriormente transferidas para a membrana PVDF (do inglês, *polyvinylidene difluoride*). A membrana foi incubada com anticorpos específicos contra catepsina K (CTSK) *overnight* a temperatura de 4°C. Após três lavagens, as membranas foram incubadas com solução de anticorpo secundário conjugado HRP (do inglês, *horseradish peroxidase*) (Luminata Forte-Millipore, MA, EUA) durante duas horas à temperatura ambiente. Os experimentos foram realizados em triplicata. A quantificação das bandas foi realizada por meio do

programa ImageJ (National Institutes of Health, MD, EUA) e a β-actina foi utilizada como controle.

b) Fosfatase ácida resistente ao tartarato (TRAP)

Após cinco dias de cultura em condições ideais para diferenciação osteoclástica, o sobrenadante foi removido e as células fixadas. A fixação foi realizada utilizando solução de acetona, citrato e formaldeído a 37% por cinco minutos e lavadas com tampão fosfato-salina (PBS, do inglês *phosphate buffered saline)*. Procedeu-se com a coloração das células utilizando kit comercial TRAP (Sigma-Aldrich) e incubação em estufa úmida a 37^{o}C e 5% CO_2 por 60 minutos, conforme as recomendações do fabricante. Após este período as placas foram lavadas com água destilada e permaneceram em processo de secagem *overnight*. As placas foram digitalizadas utilizando o equipamento Cytation (Cytation Cell Imaging Multi-Mode Reader -BioTek, Vermont, EUA) e obtidas imagens para posterior contagem celular. A contagem celular foi realizada por meio das imagens capturadas utilizando o software Image J (National Institutes of Health, MD, EUA). Os experimentos foram realizados em triplicata. Células com coloração positiva e contendo mais de três núcleos foram consideradas positivas. Os resultados foram expressos como a média de células TRAP +/poço.

c) Ensaio de reabsorção

Os osteoclastos foram cultivados em placas para ensaio de reabsorção (*Corning*™ Osteo Assay Surface, Corning, NY, EUA), sob condições ideais para diferenciação e tratados conforme descrito anteriormente. A aferição da área de reabsorção ou *pits* foi realizada após dez dias da diferenciação dos osteoclastos. Os poços foram fotografados em microscópio invertido com ampliação de 4x. Os experimentos foram realizados em triplicata. A quantificação das áreas de reabsorção foi realizada utilizando ferramentas de contorno no software Leica Application Suite (Leica Microsystems, Wetzlar, Alemanha).

3.2.10 Avaliação da diferenciação de osteoblastos

*a) Atividade da fosfatase alcalina (*ALP, do inglês *alkaline phosphatase*)

Após sete dias de cultura, a atividade da ALP in situ foi avaliada pela coloração Fast red. Primeiramente, o meio de cultura foi removido e as células foram incubadas com 1mL/poço de uma solução contendo 0,9 mM naphthol AS-MX phosphate (Sigma-Aldrich) e 1.8 mMFast red TR (Sigma-Aldrich). Previamente o naphthol foi solubilizado com 4mg/mL de dimetilformamida (Merck KGaA). As placas foram mantidas a 37 °C durante 30 minutos e então a solução foi removida e as placas permaneceram em processo de sacagem por 12 horas. Os dados foram obtidos em quadruplicata e a atividade da ALP in situ foi quantificada pela contagem de pixels utilizando ferramentas de contorno no software Leica Application Suite (Leica Microsystems).

b) Coloração utilizando vermelho de alizarina

Após 14 dias de incubação com os tratamentos, a atividade dos osteoblastos foi avaliada por meio da coloração da matriz mineralizada produzida. O meio de cultura foi removido, os poços fixados com formalina a 10% durante duas horas a temperatura ambiente e posteriormente os depósitos mineralizados formados foram corados utilizando o pigmento vermelho de alizarina a 2% (Sigma-Aldrich), pH 4,2 durante dez minutos. Os poços foram então lavados com PBS e permaneceram em processo de secagem a temperatura ambiente durante 12 horas. Os poços foram fotografados. Para análise quantitativa, os depósitos de cálcio foram dissolvidos em solução a 10% de ácido acético e metanol durante 30 minutos e mantidos em agitador. As amostras foram aquecidas a 85°C durante 10 minutos e depois resfriadas no gelo por cinco minutos. Finalmente as amostras foram centrifugadas e a absorbância foi avaliada utilizando espectrofotômetro (SpectraMax® M Series Multi-Mode Microplate Readers, Molecular Devices, CA, EUA) com leitura a 405nm. Os experimentos foram realizados em quadruplicata.

c) Superfícies de Titânio com Nanotopografia

Discos de titânio (Ti) usinados comercialmente puros, grau 2, de 13 mm de diâmetro e 2 mm de altura (Realum, SP, Brasil) foram lixados com lixas de carbeto de silício de gramatura 180, 320 e 600. Posteriormente os discos foram

lavados em ultrassom e tolueno e submetidos a condicionamento em solução de 10 N H_2SO_4 e 30% de H_2O_2 aquoso (1:1v/v) por quatro horas à temperatura ambiente e sob agitação constante para se obter a superfície com nanotopografia. Os discos foram, em seguida, lavados em água destilada e secos. Discos usinados e não condicionados foram utilizados como controle. Previamente à utilização nos experimentos, os discos de ambos os grupos foram autoclavados.

As células precursoras derivadas de calvária foram isoladas e diferenciadas conforme descrito no item *3.2.3*. Os osteoblastos obtidos foram cultivados nas mesmas condições descritas previamente sobre discos de Ti com nanotopografia e Ti usinado na presença ou não do tratamento com etexilato de dabigatrana. Ao final de sete dias, foi avaliada a expressão gênica de RUNX2, ALP, OC e Osterix, conforme descrito no item *3.2.8* e a atividade de ALP, conforme descrito no item *3.2.10*. Após 14 dias, foi avaliada a deposição de matriz extracelular mineralizada conforme item *3.2.10*.

3.2.11 Modelo ex-vivo

Camundongos machos da linhagem C57BL/6J com seis semanas de vida foram tratados com etexilato de dabrigatrana (Pradaxa®) em formulação aquosa durante o período de 28 dias. Os animais, previamente submetidos à sedação inalatória utilizando Isoflurano a 2%, receberam o medicamento por via oral, pelo método de gavagem, em intervalos de 12 em 12 horas.

A dose de tratamento estabelecida foi calculada proporcionalmente àquela preconizada em humanos, na qual a dose terapêutica recomendada é de uma cápsula de Pradaxa® (150 mg) de 12 em 12 horas (SCHULMAN *et al*., 2009). Considerando-se a dose diária recomendada de 300 mg/dia para indivíduos de 70 Kg e o peso médio dos animais selecionados de 20 gr, preconizou-se a dosagem de 85,7 µg/dia. A dose calculada corresponde a 428,5 µL de uma solução aquosa de etexilato de dabigatrana a 100 µg/mL, preparada conforme descrito no item *3.2.5* administrada duas vezes ao dia.

Após 28 dias de tratamento, os animais foram eutanasiados, conforme método descrito no item *3.2.2*. As células-tronco hematopoiéticas derivadas da medula óssea de fêmures e tíbias foram coletadas e cultivadas em meio α-MEM suplementado (Thermo Fisher Scientific) contendo M-CSF 30 ng/mL (R&D Systems)

por três dias em estufa úmida a 37^{o}C e 5% CO_2. Posteriormente, com a obtenção da confluência, as células precursoras de osteoclastos foram então plaqueadas em placas de 96 poços a uma densidade de 2 × 10^4 células por poço e cultivadas em meio contendo M-CSF 30 ng/mL (R&D Systems) e RANKL 10 ng/mL (R&D Systems). Após três dias de cultura celular, foram avaliados expressão gênica do marcador de atividade osteclástica CTSK e níveis de proteína deste marcador, utilizando-se os métodos de qPCR e Western Blot, respectivamente, conforme descrito nos itens *3.2.8* e *3.2.9.* No sétimo dia de cultura, foi analisada diferenciação celular por meio do método TRAP. A atividade osteoclástica foi mensurada após 10 dias de cultura utilizando o ensaio de reabsorção (ver item *3.2.9*). O experimento foi conduzido com cinco animais selecionados de maneira aleatória. Animais que não receberam tratamento com a solução de etexilato de dabigatrana foram utilizados como grupo comparativo controle.

3.2.12 Análise estatítica

Os dados foram expressos pelas médias e desvio padrão dos resultados obtidos para cada grupo de tratamento e controle. O teste Shapiro-Wilk demonstrou que as variáveis quantitativas apresentavam distribuição normal. As análises foram realizadas utilizando-se o teste *t* de Student. O programa GraphPad Prism versão 8.0 (GraphPad Software, CA, EUA) foi usado. O nível de significância para todos os testes estatísticos foi determinado em $p<0,05$.

4 RESULTADOS E DISCUSSÃO

Os resultados e discussão serão apresentados a seguir no formato de três artigos científicos.

4.1 Artigo I

Artigo científico I publicado no periódico *Journal of Cranio-Maxillofacial Surgery* (Qualis Odontologia A2; Fator de Impacto: 1,252).

ARTICLE IN PRESS 43

Journal of Cranio-Maxillo-Facial Surgery xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group

Amanda Leal Rocha [a], Sicilia Rezende Oliveira [a], Alessandra Figueiredo Souza [a], Denise Vieira Travassos [b], Lucas Guimarães Abreu [c], Daniel Dias Ribeiro [d], Tarcília Aparecida Silva [a, *]

[a] Department of Oral Surgery and Pathology, Faculty of Dentistry, Universidade Federal de Minas Gerais, Minas Gerais, Brazil
[b] Department of Community and Preventive Dentistry, Faculty of Dentistry, Universidade Federal de Minas Gerais, Minas Gerais, Brazil
[c] Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Universidade Federal de Minas Gerais, Minas Gerais, Brazil
[d] Department of Hematology, Faculty of Medicine, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Paper received 15 November 2018
Accepted 31 January 2019
Available online xxx

Keywords:
Blood loss
Surgical
Anticoagulant
Hemorrhage
Tooth extraction

ABSTRACT

Some prospective studies have been designed specifically to investigate perioperative bleeding in dental surgery. The quantitative assessment of intraoperative blood loss can be useful for indicating the real risk of bleeding complications, especially in medically compromised individuals. The aim of this study was to evaluate the pattern of bleeding in individuals under vitamin K antagonist (VKA) therapy and non-anticoagulated individuals submitted to dental extractions. Perioperative bleeding was evaluated by using a total collected bleeding corrected by absorbance reading (dental bleeding score). 138 procedures were performed. When the perioperative dental bleeding score was correlated with the number of extracted teeth, the quantity of bleeding was found to be directly proportional to the procedure. Extractions of two or more teeth presented higher scores than single extractions ($p = 0.003$). In a comparative analysis between the VKA and non-anticoagulated groups, no significant difference in the scores was found. The previous history of complications in dental procedures ($p = 0.001$) and the use of additional hemostatic measures were higher in the VKA group ($p = 0.017$). VKA therapy did not impact significantly the volume of blood lost during dental extractions. Perioperative bleeding assessment might be a useful parameter for evaluating patients under antithrombotic treatment.

1. Introduction

A number of studies evaluating the influence of anticoagulant therapy on dental treatment outcomes have been published in the literature in recent years. In a search in PubMed using the MeSH terms "*anticoagulants and tooth extraction*", more than 300 studies were retrieved. Although thoroughly studied, dental management of individuals undergoing oral anticoagulant therapy (OAT) is still unclear for many clinicians, particularly as regards the need for interruption of medication before dental treatment, international normalized ratio (INR) reference values of for warfarin users, and the risk of bleeding events (Van Diermen et al., 2013; Ringel and Maas, 2016; Chinnaswami et al., 2017).

Warfarin, a vitamin K antagonist (VKA), is the most commonly prescribed anticoagulant drug for prevention and treatment of thromboembolic disorders (Wahl, 1998). Earlier recommendation for individuals using warfarin was the interruption and/or replacement of anticoagulant therapy before dental surgery (Roser and Rosenbloom, 1975; Lockhart et al., 2003; Bajkin et al., 2009). However, a large body of evidence has demonstrated that the incidence of bleeding associated with dental procedures in individuals undergoing anticoagulant therapy is low, and even if any complications occur, management using localized measures is easily applied (Morimoto et al., 2011; Bajkin et al., 2012; Hong et al., 2012; Wahl et al., 2015; Febbo et al., 2016; Dudek et al., 2016, Rocha et al., 2018). Therefore, anticoagulant therapy maintenance during dental treatment has currently been indicated in the most

* Corresponding author. Department of Oral Surgery and Pathology, Faculty of Dentistry, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 — Pampulha, Belo Horizonte, MG, 31270-901, Brazil. Fax: +55 31 34092430.
E-mail address: silva.tarcilia@gmail.com (T.A. Silva).

https://doi.org/10.1016/j.jcms.2019.01.049
1010-5182/

44

important guidelines (Douketis et al., 2012; Nishimura et al., 2014; ADA, 2018).

Although some limitations of VKA therapy have encouraged the development of new drugs, such as direct oral anticoagulants (DOACs), many individuals in need of anticoagulant therapy might be ineligible for treatment with DOACs (Little, 2012; Djulbegovic and Lee, 2018). Moreover, there are clinical situations in which DOACs are not suitable because of insufficient data on their efficacy and safety (Schulman et al., 2009; Einstein Investigators et al., 2010; Liu et al., 2015; Nakamura et al., 2015; Lee, 2016; Moustafa et al., 2018). In addition, DOACs are costly, and affordability and accessibility are an issue for health services in many countries with different healthcare systems, in particular, emerging countries from Asia, Africa, and South America (Lee, 2016; Fortier et al., 2018). Therefore, VKA therapy remains a highly effective strategy for thromboembolism prevention, and the most widely prescribed drug for OAT (Van Gorp and Schurgers, 2015).

Studies using quantitative methods to investigate perioperative bleeding patterns in anticoagulated individuals submitted to dental extractions are scarce in the literature (Karsli et al., 2011; Erden et al., 2015). Furthermore, with robust evidence for the low risk of postoperative bleeding, other questions have arisen: Does OAT significantly impact the bleeding pattern during oral surgery? Do individuals on OAT bleed more than non-OAT individuals when submitted to dental extractions? In addition, increased perioperative bleeding has been shown to be associated with a significant risk of postoperative bleeding in anticoagulated patients (Rocha et al., 2018). Therefore, the assessment of perioperative bleeding might provide additional evidence for predicting and minimizing postoperative outcomes in patients under antithrombotic medication.

In this study, we evaluated the impact of OAT on bleeding, during and after dental extractions, by means of a quantitative method, as well as hemorrhagic outcomes.

2. Methods

2.1. Ethical issues

This was a prospective study, following the STROBE statement guidelines (von Elm et al., 2007). The study was approved by the Department of Education and Research of the Hospital das Clínicas of the Universidade Federal de Minas Gerais (HC/UFMG). Approval from the Institutional Ethics Committee of UFMG (protocol 48122215.4.0000.5149) was also obtained, and the guidelines established in the Declaration of Helsinki (revised version/2002), for research involving humans, were followed. Each participant signed a statement of informed consent to take part in the study. Anonymity was guaranteed to all participants.

2.2. Participants, eligibility criteria, setting, and recruitment period

The sample consisted of all individuals undergoing VKA therapy who met the eligibility criteria, were referred by hematologists and cardiologists, and were admitted for treatment by the HC/UFMG Dental Service between January 2016 and January 2018. Inclusion criteria were as follows: individuals who needed dental extraction of at least one erupted tooth, and had INR values ≤ 3.5. The study also had a control group of non-anticoagulated individuals (non-OAT group), consisting of all those seeking dental treatment at the service, without coagulation disorders or not using antithrombotic drugs, and who needed dental extraction of at least one erupted tooth.

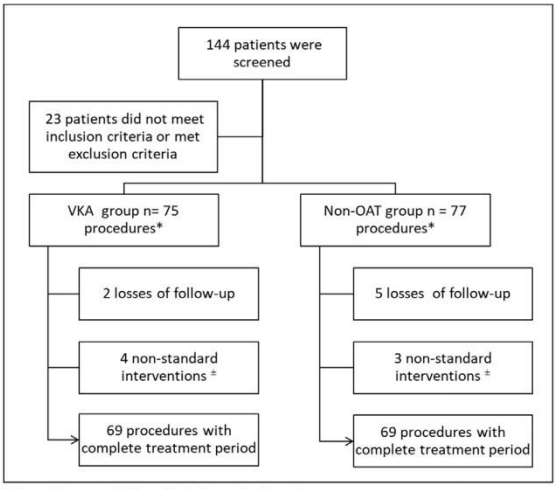
Individuals with INR >3.5, or with any coagulation disorders not related to anticoagulant use (i.e. hepatic diseases, thrombocytopenia); elderly individuals aged >80 years old, and individuals presenting teeth with acute inflammatory conditions (i.e. periodontal or periapical abscess with suppurative process) were excluded from the study. Individuals who underwent non-standard interventions and cases with incomplete follow-up were also excluded (Fig. 1).

A blood count test was ordered for all participants. An INR test was run for all individuals taking VKA. The tests needed to be accomplished 3 days before dental surgery.

2.3. Dental extraction

All procedures were carried out under local anesthesia in the hospital outpatient clinic, early in the morning, by qualified oral surgeons who were trained and supervised by the principal investigator. Dental extractions were performed without interrupting or modifying the OAT regimen. Blood pressure was measured prior to and shortly after the surgical interventions. Local anesthesia was standardized for all patients and consisted of a regional block complemented with local infiltration, using lidocaine hydrochloride 2% with epinephrine (Alphacaine 1:100,000; DFL Indústria e Comércio SA, Rio de Janeiro, Brasil).

The surgical technique followed strictly pre-established parameters, observed by the principal investigator, including the use of forceps and elevator, and was carried out as atraumatically as possible. Hemostatic measures included wound closure with 3.0 nylon 14502 T sutures (Mononylon, Ethicon, Somerville, New Jersey) and a piece of sterile gauze bitten by the participant for 20 min to compress the operated site. Some 20 min later, the participants were examined to ensure that hemostasis was achieved, and the immediate postoperative bleeding outcome was evaluated. When increased levels of immediate postoperative or perioperative bleeding were observed, additional measures of hemostasis were performed, applying a $10 \times 10 \times 10$-mm absorbable gelatin sponge (Hemospon, Technew, Rio de Janeiro Brazil), tranexamic acid (Transamin, Nikkho, Rio de Janeiro, Brazil), and/or new sutures. Tranexamic acid paste (one 250 mg pill macerated and mixed with saline) was used to soak the gelatin sponge filling the alveolar socket. An additional layer of the paste was applied on the wound



* The analysis was conducted considering the number of procedures
± Exceeded the standardized volume of 100 mL of saline solution

Fig. 1. Flowchart for patients recruited to the study groups: screening, inclusion criteria, and follow-up.

Please cite this article as: Rocha AL et al., Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group, Journal of Cranio-Maxillo-Facial Surgery, https://doi.org/10.1016/j.jcms.2019.01.049

after sutures and covered with gauze under compression. For these individuals, local use of tranexamic acid mouthwash (one pill mixed in 100 ml of cold saline solution), four times a day, was recommended during the following 7 postoperative days. The individuals received written postoperative instructions. Procedure time was measured, with a stopwatch, from the first incision for the detachment of the gingiva until the complete suture.

Postoperative pain was managed with 500 mg of metamizole or 500 mg of acetaminophen every 6 h for 3 days. Antibiotic prophylaxis was only used in individuals at risk of infective endocarditis, as defined by the American Heart Association (Nishimura et al., 2014).

2.4. Data collection

2.4.1. Independent variables

The following variables were collected: participants' age and sex; history of bleeding in previous medical or dental procedures, and history of bleeding in family members (parents and/or siblings). Information on medical diagnosis, indication for OAT, concomitant medications affecting hemostasis (e.g. antiplatelet agents, nonsteroidal anti-inflammatory drugs, antibiotics), INR (for the VKA group), hematocrit level, and platelet count were also collected.

The number and indication of surgical procedures (periodontal disease, dental caries, or third molar), as well as a number of teeth extracted, were recorded. The number of tooth extractions (one tooth, two teeth, or three teeth) and type of teeth (single-rooted or multi-rooted) were also recorded. Additional parameters collected were surgical procedure time, pain, and number of gauzes used for hemostasis.

2.4.2. Outcome variables

These variables included: the need for additional hemostatic measures; immediate postoperative bleeding; postoperative bleeding; dental bleeding score; and wound healing (satisfactory, swelling/erythema, or bone exposure). Postoperative bleeding events were recorded, as well as the management of the bleeding (local hemostatic measures in outpatient care or hospital admission).

2.5. Quantitative assessment of perioperative bleeding — dental bleeding score

2.5.1. Bleeding amount

Perioperative bleeding was quantified through the storage of the fluids aspirated during the surgical procedure using a portable vacuum pump (5005 BRS, Nevoni, São Paulo, Brazil). A standardized volume of 100 ml of saline solution was used for wound irrigation in all procedures. To avoid clot formation during aspiration, 2 ml of heparin sodium 5000 IU/ml (Hepamax-S, Blausiegel, São Paulo, Brazil) were added to the final aspirated solution. Subsequently, this fluid was measured with a graduated cylinder. For each five ml of fluid, the sample was categorized from 1 to 10 as follows: samples up to 5 ml were scored 1; samples with 6–10 ml were scored 2; samples with 11–15 mlL were scored 3, and so on.

2.5.2. Absorbance of aspirated fluid

A sample was collected from total aspirated fluids and used to assess optical density (an indirect measurement of red blood concentration) using a spectrophotometer at 537 nm (RA 50 clinical, Bayer, São Paulo, Brazil). With this analysis, control of the bias caused by salivary fluid, which might have had an influence on the total volume of aspirated fluid, was feasible. The values for absorbance were also scored, as follows: absorbance up to 1.0 was scored 1; absorbance of 1.1–2.0 was scored 2; 2.1–3.0 was scored 3, and 3.1 or more was given the maximum score of 4.

2.5.3. Bleeding score assessment

The scores for total aspirated fluid and absorbance were summed to achieve a final score for bleeding. The values varied from 2 to 14, with lower scores indicating less perioperative bleeding.

2.5.4. Postoperative bleeding

A clinical outcome characterized by a postoperative hemorrhagic event was defined as oozing or marked hemorrhage, and required one or more of the following outcomes: (1) telephone call to the dental service or to the principal investigator reporting concern about postoperative bleeding; (2) return to our or other outpatient facility because of postoperative bleeding; (3) need for hospitalization. With the aim of monitoring bleeding episodes from the day of surgery until 1 week after the dental extractions, the participants were instructed to use gauzes for local compression in case of bleeding, and to register the number of gauzes used.

On the seventh postoperative day, participants returned for an appointment to remove the sutures and to evaluate wound healing. Parameters such as the presence of local erythema/edema, bone exposure, and suppuration were also analyzed. During the appointment, the patients were asked to report bleeding complications during the postoperative period and pain, which was measured using a numeric rating scale (NRS) (Downie et al., 1978), ranging from 0 to 10. A score of 0 indicated no pain and a score of 10 indicated the highest perception of pain. Individuals who did not return for a follow-up visit were excluded from the analysis.

2.6. Statistical analysis

The analysis was carried out considering the number of procedures. Comparative analysis was performed between the VKA group ($n = 69$) and the non-anticoagulated group ($n = 69$).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 23.0, Chicago, IL, USA). Intergroup comparisons regarding independent variables and dependent variables (outcome) were carried out by means of bivariate analysis. For qualitative variables, Pearson's chi-squared and Fisher's exact test were used. The Kolmogorov–Smirnov test demonstrated that the continuous variables presented a non-normal distribution. Thus, a non-parametric test (Mann–Whitney test) was used. The level of significance was set at $p < 0.05$.

3. Results

Some 152 dental extractions were performed. Following the application of the remaining inclusion and exclusion criteria, 69 procedures in the VKA group and 69 procedures in the non-anticoagulated group were included for analysis (Fig. 1). As shown in Table 1, the individuals in the VKA group were between 30 and 77 years of age, with a mean age of 51 (±10) years. Most participants in the VKA group were female individuals (63.8%), and all participants were taking warfarin (5 mg). Fifty-six procedures were carried out in individuals under monotherapy and 13 in individuals undergoing dual therapy — anticoagulant plus antiplatelet therapy with acetylsalicylic acid (ASA). A summary of indications for anticoagulation therapy is also provided in Table 1.

In the non-anticoagulated group, the mean age of individuals was 48 (±13) years (range 19–77 years). Most of the participants in this group were female individuals (59.4%). Thirty-seven procedures were performed in individuals without any systemic disease, 20 procedures in individuals with controlled hypertension, seven in

Please cite this article as: Rocha AL et al., Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group, Journal of Cranio-Maxillo-Facial Surgery, https://doi.org/10.1016/j.jcms.2019.01.049

Table 1
Clinical and demographic characteristics for the VKA and non-OAT groups ($n = 138$).

	VKA group (n = 69)	
	n	%
Age (yrs)		
Mean (±SEM)	51.1 (±10.1)	–
Range	30–77	–
Sex		
Male	25	36.2%
Female	44	63.8%
Indication for OAT[a]		
DVT	23	26.1%
AF	20	22.7%
MHV	15	17.1%
PE	15	17.1%
BHV	8	9.1%
CIA	3	3.3%
VD	2	2.3%
PH	2	2.3%
OAT prescribed		
Warfarin	56	81.2%
Warfarin + ASA	13	18.8%
	Non-OAT group (n = 69)	
	n	%
Age (yrs)		
Mean (±SEM)	48.4 (±13.7)	–
Range	19–77	–
Sex		
Male	28	40.6%
Female	41	59.4%

Abbreviations: VKA, vitamin K antagonist; non-OAT, non-anticoagulated; yrs, years; DVT, deep vein thrombosis; AF, atrial fibrillation; MHV, mechanical heart valve; PE, pulmonary embolus; BHV, biological heart valve; CIA, cerebrovascular ischemic accident; VD, valvular disorders; PH, pulmonary hypertension; ASA, acetylsalicylic acid.
[a] The same patient may have more than one diagnosis.

individuals with mental disorders, and five in individuals with thyroid dysfunction.

A comparative analysis of clinical and demographic characteristics (independent variables), taking into account the number of procedures carried out during the study, is presented in Table 2. The continuous variables age and INR value were categorized by median value, and platelet count was grouped according to the minimum reference value of the blood count test. There was no significant difference between groups in relation to participants' age, sex, platelet count, hematocrit level, previous history of bleeding in medical procedures, previous history of bleeding in family members, indication for dental extraction, number and type of teeth extracted, surgical procedure time, or pain ($p > 0.05$). In the VKA group, the previous history of bleeding in dental procedures occurred more often ($p = 0.001$) and more gauzes were used for hemostasis ($p < 0.001$).

Regarding dependent variables (presented in Table 3), in the VKA group, the need for additional hemostatic measures was more frequent than in the non-anticoagulated group ($p = 0.017$). In addition, the amount of wound healing was lower in the non-anticoagulated group ($p = 0.048$). For the outcome of immediate postoperative bleeding and postoperative hemorrhage, no difference between groups was observed ($p > 0.05$). Three episodes of immediate postoperative bleeding were observed in procedures carried out in individuals in the VKA group, and none in individuals in the non-anticoagulated group. Postoperative hemorrhage occurred after three procedures carried out in individuals in the VKA group (4.6%) and after two procedures in individuals in the non-anticoagulated group (3.0%). No bleeding episode in this study required hospitalization or medical/systemic intervention. All individuals returned to the dental service, but those who returned after the 7th day of follow-up were considered follow-up losses (Fig. 1).

Table 2
Comparative analysis of the clinical characteristics of surgical procedures for the VKA versus non-anticoagulated study groups ($n = 138$).

Variables	Non-OAT	VKA	*p*
Age (years)			
≤50	36 (52.2)	37 (53.6)	0.999[a]
>50	33 (47.8)	32 (46.4)	
Sex			
Male	28 (40.6)	25 (36.2)	0.726[a]
Female	41 (59.4)	44 (63.8)	
Previous history of bleeding in a medical procedure			
No	64 (92.8)	63 (91.3)	0.999[a]
Yes	5 (7.2)	6 (8.7)	
Previous history of bleeding in a dental procedure			
No	66 (95.7)	52 (75.4)	**0.001**[a]
Yes	3 (4.3)	17 (24.6)	
Previous history of bleeding in family			
No	68 (98.6)	66 (95.7)	0.619[a]
Yes	1 (1.4)	3 (4.3)	
Platelet count ($10^3/\mu l$)			
≤150	4 (5.8)	7 (10.1)	0.532[a]
>150	65 (94.2)	62 (89.9)	
Hematocrit (%)			
Mean (median)	40.6 (40.8)	40.1 (40.3)	.879[c]
Min–max	30.0–51.4	25.5–51.4	
INR			
≤2.30	–	32 (50.0)	–
>2.30		32 (50.0)	
Tooth extraction			
One tooth	24 (34.8)	34 (49.3)	0.145[b]
Two teeth	24 (33.8)	19 (27.5)	
Three teeth	21 (30.4)	16 (23.2)	
Teeth			
One root	13 (18.8)	14 (20.3)	0.999[a]
Two or more roots	56 (81.2)	55 (79.7)	
Indication			
Periodontal disease	19 (27.5)	22 (31.9)	0.455[b]
Decay	45 (65.2)	44 (63.8)	
Third molar	5 (7.2)	3 (4.3)	
Procedure time			
Mean (median)	38.3 (35.0)	41.1 (40.0)	0.560[c]
Min–max	15–90	10–100	
Pain			
Mean (median)	2.5 (1.0)	2.2 (0.0)	0.276[c]
Min–max	0–10	0–10	
Gauzes			
Mean (median)	0.7 (0.0)	1.9 (1.0)	**0.000**[c]
Min–max	0–10	0–5	

Bold values are those with a *p*-value < 0.05.
Abbreviations: VKA, vitamin K antagonist; non-OAT, non-anticoagulated; INR, international normalized ratio; min, minimum; max, maximum.
[a] Chi-squared test and Fisher exact test.
[b] Linear by linear.
[c] Mann-Whitney U-test.

For the bleeding score outcome, while no difference between the groups was observed ($p > 0.05$). As expected, higher scores (8–14) were more commonly observed in procedures in which more than one tooth had been extracted ($p = 0.003$) (Fig. 2). On the other hand lower scores (2–7) were more observed in procedures with reduced bleeding, as in single extractions. Thus, it was suggested that the method presents sensitivity distinguishing procedures, with increased and reduced bleeding.

4. Discussion

This prospective cohort study was designed to analyze bleeding outcomes after tooth extractions in individuals under OAT. A total of 138 dental procedures were performed in individuals under VKA therapy and in individuals without anticoagulant treatment.

Please cite this article as: Rocha AL et al., Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group, Journal of Cranio-Maxillo-Facial Surgery, https://doi.org/10.1016/j.jcms.2019.01.049

Table 3
Clinical and quantitative assessment of bleeding and outcomes in the VKA versus non-anticoagulated study groups ($n = 138$).

Variables	Non-OAT	VKA	p
Additional hemostatic measures			
No	68 (98.6)	61 (88.4)	**0.033**[a]
Yes	1 (1.4)	8 (11.6)	
Immediate postoperative bleeding			
No	69 (100)	66 (95.7)	0.245[a]
Yes	0 (0.0)	3 (4.3)	
Postoperative bleeding			
No	67 (97.1)	66 (95.7)	0.999[a]
Yes	2 (2.9)	3 (4.3)	
Bleeding score			
Mean (median)	7.7 (7.0)	6.9 (7.0)	0.195[b]
Min–max	3–14	2–14	
Score 2–7	38	46	0.222[a]
Score 8–14	31	23	
Wound healing			
Satisfactory	55 (79.7)	43 (62.3)	**0.045**[c]
Swelling/erythema	13 (18.8)	25 (36.2)	
Bone exposure	1 (1.3)	1 (1.5)	

Bold values are those with a p-value < 0.05.
Abbreviations: VKA, vitamin K antagonist; DOAC, direct oral anticoagulant; non-OAT, non-anticoagulated; min, minimum; max, maximum.
[a] Fisher exact test.
[b] Mann-Whitney U-test.
[c] Linear by linear.

Despite the similar levels of bleeding and postoperative outcomes observed in the controls and anticoagulated patients, the need for hemostatic measures was higher in the VKA group.

VKA therapy — represented by warfarin in this study — is used worldwide, and has been shown to be highly effective for primary and secondary prevention of venous and arterial thromboembolic events (Ageno et al., 2012). Despite the disadvantages associated with the VKAs, such as the need for frequent coagulation blood tests, dose adjustments, and perceived dietary restrictions, warfarin still remains the first choice due to its widespread availability, convenience of administration, and cost-effectiveness (Douketis et al., 2012; Febbo et al., 2016). DOACs have been introduced as a potental replacement for VKAs, however, pivotal trials applying strict exclusion criteria have excluded individuals with a presumed high risk of bleeding (Schulman et al., 2009; Einstein Investigators et al., 2010; Liu et al., 2015; Nakamura et al., 2015).

The occurrence of postoperative bleeding complications in the VKA group compared with the non-OAT group was not statistically different. Only two (2.8%) episodes in procedures performed in individuals in the non-anticoagulated group and three (4.3%) in procedures in the VKA group were observed. Febbo et al. (2016) found nine complications (2.1%) in individuals under anticoagulant therapy and no cases of bleeding in the control group. Moreover, the authors found that all postoperative complications occurred in individuals with an INR ≥2.2 (Febbo et al., 2016). In our study, for analysis purposes, the sample was categorized using an INR median threshold of 2.3, with episodes of postoperative bleeding occurring in procedures carried out in individuals with an INR higher than 2.3 (data not shown). The choice of the INR limit value was based on the fact that the therapeutic ratio for preventing the development of serious thromboembolic events ranges from 2.0 to 3.5, with individuals with an INR value higher than 4.0 having an increased bleeding risk and no medical benefit (Thean and Alberghini, 2016).

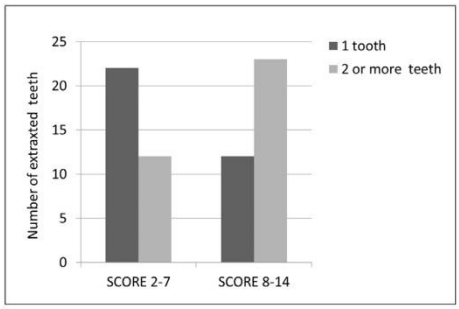


Fig. 2. Distribution of categorized dental bleeding score (set at median = 7). The score bars for 2 to 7 indicate the lower values and those for 8 to 14 the higher values for bleeding score in relation to the number of teeth extracted in the VKA group ($n = 69$).

Previous studies have demonstrated different values for bleeding events following dental surgery, with percentages of incidence from 2.8% to 6.8% (Morimoto et al., 2011; Bajkin et al., 2012; Hong et al., 2012; Wahl et al., 2015; Febbo et al., 2016; Dudek et al., 2016, Rocha et al., 2018). In our study, the percentage of postoperative bleeding complications seems to be within the range of those previously described in the literature, with the occurrence of this outcome in individuals of the VKA group almost equal to that observed in the non-anticoagulated group. It is important to note that, due to the matching strategy, and for a reliable comparative analysis, it was expected that confounding variables would not present significant differences between groups. This assumption was confirmed because groups were equal with regard to the type and indication of dental extraction, and the demographic characteristics of participants. Other confounding variables that may have influenced the outcome, such as non-diagnosed coagulation disorders, measured by platelet count, and duration of dental procedure, were also evaluated, and no differences were observed.

Additional hemostatic measures were used in eight procedures carried out in anticoagulated individuals and one procedure performed in a non-anticoagulated individual. This difference was statistically significant. These data suggest that localized hemostatic measures used after dental extractions could explain the low incidence of postoperative bleeding outcomes. In contrast to our study, in which additional measures were performed only in persistent bleeding situations, other authors have reported the use of hemostatic measures as a reliable strategy for preventing postoperative bleeding after all dental extractions in anticoagulated individuals (Miclotte et al., 2017). The localized hemostatic protocol used in our study was gauze soaked in tranexamic acid, gelatin sponge, successive sutures, and compression of the postextraction site with dry gauze. These were effective in preventing postoperative bleeding (Soares et al., 2015), and may have influenced our results on reducing bleeding outcomes in the VKA group. The clinician should be attentive regarding increased bleeding during surgical procedures, and localized measures should be employed if necessary (Rocha et al., 2018) In addition to localized hemostatic measures, the need for local gauze compression in the postoperative period and previous history of bleeding episodes after dental procedures were also more frequent among individuals in the VKA group. These findings may be related to the concerns of the individuals with respect to bleeding complications, however rare.

The observation of bleeding during the dental procedure is a relevant factor. It has been previously demonstrated that the risk of postoperative complications in procedures with increased perioperative bleeding is 8.8 times higher than those without perioperative bleeding (Rocha et al., 2018). However, the evaluation of this variable is mostly achieved by subjective analysis, with few previous studies providing quantitative data on bleeding amount (Karsli et al., 2011; Erden et al., 2015). Currently, there is no standardized methodology for the quantitative measurement of bleeding in

Please cite this article as: Rocha AL et al., Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group, Journal of Cranio-Maxillo-Facial Surgery, https://doi.org/10.1016/j.jcms.2019.01.049

dental practice, so for this study, a dental bleeding score protocol was proposed to measure the amount of bleeding during tooth extractions. This method was developed to allow for salivary interference in measuring oral perioperative bleeding. The combination of the optical density of the sample with the total amount of aspirated fluid made an estimation attainable.

Results for bleeding score analysis performed in VKA and non-anticoagulated individuals confirmed the clinical outcome of postoperative bleeding, for which no significant difference between the groups was found. Although there was no difference between perioperative bleeding scores for individuals in the VKA group and non-anticoagulated group, the use of additional hemostatic measures was higher in the VKA group. This finding may indicate that, in most individuals undergoing OAT, the alteration in the coagulation process may not significantly influence the amount of bleeding during tooth extraction. However, OAT can prolong the final hemostatic process and, for this reason, additional measures were deemed necessary. Despite this increased time for coagulation, the volume of blood loss in this period did not appear to be significant. In addition, impaired wound healing was more frequent in individuals in the VKA group, which might be associated with the alteration in hemostasis and, consequently, with the formation and stabilization of the clot in the dental socket.

In contrast with our study, Karsli et al. (2011) found that values for bleeding amount in individuals under warfarin therapy were significantly higher than in individuals undergoing bridging therapy and in non-medicated individuals. The authors measured the amount of immediate postoperative bleeding using the weights of gauze swabs placed over the extraction socket, over the course of 20 min, on completion of the extraction (Karsli et al., 2011). The same protocol was applied in the study by Erden et al. (2015), comparing dental extractions under warfarin and bridging therapy. In contrast to Karsli et al. (2011), these authors found that bleeding amount was higher in individuals under bridging therapy than in their peers under warfarin therapy; no control group was enrolled (Erden et al., 2015).

The methods suggested in previous studies differ from that used in our study in two ways. First, our method allows the measurement of bleeding throughout the procedure, not only after removal of the tooth. Second, it takes into account salivary flow during oral surgery, which can increase bias in the measurement of bleeding amount. In addition, the score was correlated with the number of extracted teeth, implying that the method presents sensitivity distinguishing procedures with increased and reduced bleeding. Thus, our study proposes a new method of quantitative measurement of bleeding, by using total collected bleeding corrected using absorbance reading, that could be useful for further studies aiming to analyze amounts of perioperative bleeding.

The study has strengths and limitations. Blinding of the study's personnel and assessors was deemed unfeasible, which might have influenced how the surgeon conducted perioperative procedures. Several analyses were not possible due to the non-occurrence or rarity of the event. The methodology adopted was new, so further studies, with different and larger samples, should be developed in this field to validate it. Nevertheless, this study presents novel information on bleeding outcomes with chronic OAT, and our findings provide an additional and objective parameter for evaluating blood loss in dental surgery.

5. Conclusion

The results of the bleeding score method demonstrated that the amount of blood lost in dental extraction was similar in anticoagulated and non-anticoagulated individuals, in spite of the need for localized hemostatic measures being higher in patients under VKA therapy. Additional measures for hemostasis were effective, and could be used in cases of persistent bleeding. Bleeding tendencies, identified by previous history of hemorrhage and by perioperative bleeding pattern, suggest the need for careful monitoring, and these parameters can be useful for predicting and minimizing postoperative complications.

Funding

This study was supported by FAPEMIG, CNPq, and CAPES.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brazil (CAPES) — finance code 001.

References

ADA Science Institute: Anticoagulant and antiplatelet medications and dental procedures. Available at: https://www.ada.org/en/member-center/oral-health-topics/anticoagulant-antiplatelet-medications-and-dental-, 2018. Acesso em: 06 Nov 2018; 2018

Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G: Oral anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 141: e44S–e88S, 2012

Bajkin BV, Popovic SL, Selakovic SD: Randomized, prospective trial comparing bridging therapy using low-molecular-weight heparin with maintenance of oral anticoagulation during extraction of teeth. J Oral Maxillofac Surg 67: 990–995, 2009

Bajkin BV, Bajkin IA, Petrovic BB: The effects of combined oral anticoagulant–aspirin therapy in patients undergoing tooth extractions: a prospective study. JADA 143: 770–776, 2012

Chinnaswami R, Bagadia RK, Mohan A, Kandaswamy E, Chandrasekaran D: Dentists' knowledge, attitude and practice in treating patients taking oral antithrombotic medications — a survey. J Clin Diagn Res 11: 88–91, 2017

Djulbegovic M, Lee A: An update on the 'novel' and direct oral anticoagulants, and long-term anticoagulant therapy. Clin Chest Med 39: 583–593, 2018

Douketis JD, Spyropoulos AC, Spencer FA, Mayr M, Jaffer AK, Eckman MH, et al: Perioperative management of antithrombotic therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 141: 326–350, 2012

Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA: Studies with pain rating scales. Ann Rheum Dis 37: 378–381, 1978

Dudek D, Marchionni S, Gabriele M, Iurlaro A, Helewski K, Toti P, et al: Bleeding rate after tooth extraction in patients under oral anticoagulant therapy. J Craniofac Surg 27: 1228–1233, 2016

Einstein InvestigatorsBauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, Gallus AS, et al: Oral rivaroxaban for symptomatic venous thromboembolism. N Engl J Med 363: 2499–2510, 2010

Erden İ, Çakcak Erden E, Aksu T, Gölcük ŞE, Turan B, Erkol A, et al: Comparison of uninterrupted warfarin and bridging therapy using low-molecular-weight heparin with respect to the severity of bleeding after dental extractions in patients with prosthetic valves. Anatol J Cardiol 16: 467–473, 2015

Febbo A, Cheng A, Stein B, Goss A, Sambrook P: Postoperative bleeding following dental extractions in patients anticoagulated with warfarin. J Oral Maxillofac Surg 74: 1518–1523, 2016

Fortier K, Shroff D, Reebye UN: Review: an overview and analysis of novel oral anticoagulants and their dental implications. Gerodontology 35(1–9), 2018

Hong C, Napenas JJ, Brennan M, Furney S, Lochart P: Risk of postoperative bleeding after dental procedures in patients on warfarin: a retrospective study. Oral Surg Oral Med Oral Pathol Oral Radiol 114: 464–468, 2012

Karsli ED, Erdogan O, Esen E, Acartürk E: Warfarin and heparin in dental extraction. J Oral Maxillofac Surg 69: 2500–2507, 2011

Lee LH: DOACs — advances and limitations in real world. Thromb J 14: 133–163, 2016

Little JW: New oral anticoagulants: will they replace warfarin? Oral Surg Oral Med Oral Pathol Oral Radiol 113: 575–580, 2012

Liu X, Johnson M, Mardekian J, Phatak H, Thompson J, Cohen AT: Apixaban reduces hospitalizations in patients with venous thromboembolism: an analysis of the apixaban for the initial management of pulmonary embolism and deep-vein thrombosis as first-line therapy (AMPLIFY) trial. J Am Heart Assoc 4: 1–8, 2015

Lockhart PB, Gibson J, Pond SH, Leitch J: Dental management considerations for the patient with an acquired coagulopathy. Part 2: coagulopathies from drugs. Br Dent J 195: 495–501, 2003

Miclotte I, Vanhaverbeke M, Agbaje JO, Legrand P, Vanassche T, Verhamme P, et al: Pragmatic approach to manage new oral anticoagulants in patients undergoing dental extractions: a prospective case-control study. Clin Oral Investig 21: 2183–2188, 2017

Please cite this article as: Rocha AL et al., Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group, Journal of Cranio-Maxillo-Facial Surgery, https://doi.org/10.1016/j.jcms.2019.01.049

Moustafa F, Pesavento R, di Micco P, González-Martínez J, Quintavalla R, Peris ML, et al: RIETE Investigators. Real-life use of anticoagulants in venous thromboembolism with a focus on patients with exclusion criteria for direct oral anticoagulants. Clin Pharmacol Ther 103: 684–691, 2018

Morimoto Y, Niwa H, Minemastu K: Risk factors affecting postoperative hemorrhage after tooth extraction in patients receiving oral antithrombotic therapy. J Oral Maxillofac Surg 69: 1550–1556, 2011

Nakamura M, Wang YQ, Wang C, Oh D, Yin WH, Kimura T, et al: Efficacy and safety of edoxaban for treatment of venous thromboembolism: a subanalysis of East Asian patients in the Hokusai-VTE trial. J Thromb Haemost 13: 1606–1614, 2015

Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, Guyton RA, et al: 2014 AHA/ACC guideline for the management of patients with valvular heart disease: a report of the American college of cardiology/American heart association task force on practice guidelines. Circulation 129: 1–96, 2014

Ringel R, Maas R: Dental procedures in patients treated with antiplatelet or oral anticoagulation therapy — an anonymous survey. Gerodontology 33: 447–452, 2016

Rocha AL, Souza AF, Martins MAP, Fraga MG, Travassos DV, Oliveira ACB, et al: Oral surgery in patients under antithrombotic therapy: perioperative bleeding as a significant risk factor for postoperative hemorrhage. Blood Coagul Fibrinolysis 29: 97–103, 2018

Roser SM, Rosenbloom B: Continued anticoagulation in oral surgery procedures. Oral Surg Oral Med Oral Pathol 40: 448–457, 1975

Schulman S, Kearon C, Kakkar AK, Mismetti P, Schellong S, Eriksson H, et al, RE-COVER Study Group: Dabigatran versus warfarin in the treatment of acute venous thromboembolism. N Engl J Med 361: 2342–2352, 2009

Soares EC, Costa FW, Bezerra TP, Nogueira CB, de Barros Silva PG, Batista SH, et al: Postoperative hemostatic efficacy of gauze soaked in tranexamic acid, fibrin sponge, and dry gauze compression following dental extractions in anticoagulated patients with cardiovascular disease: a prospective, randomized study. Oral Maxillofac Surg 19: 209–216, 2015

Thean D, Alberghini M: Anticoagulant therapy and its impact on dental patients: a review. Aust Dent J 61: 149–156, 2016

Van Gorp RH, Schurgers LJ: New insights into the pros and cons of the clinical use of vitamin K antagonists (VKAs) versus direct oral anticoagulants (DOACs). Nutrients 17: 9538–9557, 2015

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al: Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ 335: 806–808, 2007

Van Diermen DE, van der Waal I, Hoogvliets MW, Ong FN, Hoogstraten J: Survey response of oral and maxillofacial surgeons on invasive procedures in patients using antithrombotic medication. Int J Oral Maxillofac Surg 42: 502–507, 2013

Wahl MJ: Dental surgery in anticoagulated patients. Arch Intern Med 158: 1610–1616, 1998

Wahl MJ, Pinto A, Kilham J, Lalla RV: Dental surgery in anticoagulated patients: stop the interruption. Oral Surg Oral Med Oral Pathol Oral Radiol 119: 136–157, 2015

Please cite this article as: Rocha AL et al., Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group, Journal of Cranio-Maxillo-Facial Surgery, https://doi.org/10.1016/j.jcms.2019.01.049

4.2 Artigo II

Artigo científico II submetido ao periódico *Brazilian Oral Research* (Qualis Odontologia A2; Fator de Impacto: 1,223).

DIRECT ORAL ANTICOAGULANTS IN ORAL SURGERY: A PROSPECTIVE COHORT

Amanda Leal Rocha[a], Sicilia Rezende Oliveira[a], Alessandra Figueiredo Souza[a], Denise Vieira Travassos[b], Lucas Guimarães Abreu[c], Daniel Dias Ribeiro[d], Tarcília Aparecida Silva[a*]

[a] Department of Oral Surgery and Pathology, Faculty of Dentistry, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

[b] Department of Community and Preventive Dentistry, Faculty of Dentistry, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

[c] Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

[d] Department of Hematology, Faculty of Medicine, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

Running Head: DIRECT ORAL ANTICOAGULANTS IN ORAL SURGERY

Key words: antithrombotic agents, anticoagulants, hemorrhage, tooth extraction.

Funding: This study was supported by FAPEMIG, CNPq, and CAPES.

***Conflict of Interest Statement*:** The authors declare that they have no conflict of interest.

Ethics Statement: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

[*]**CORRESPONDING AUTHOR:** Tarcília Aparecida da Silva - Department of Oral Surgery and Pathology, Faculty of Dentistry, Universidade Federal de Minas Gerais – UFMG - Av. Presidente Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270- 901, Brazil. Phone: 55-31-34092478 - Fax: 55-31-34092430 Email: silva.tarcilia@gmail.com.

ABSTRACT

The aim of this study was to evaluate the pattern of bleeding in individuals taking direct oral anticoagulants (DOACs) submitted to dental extractions. Intraoperative bleeding was evaluated by using total collected bleeding corrected by absorbance reading (dental bleeding score), and the postoperative bleeding was characterized by delayed hemorrhagic complications. To monitoring bleeding episodes from the day of surgery, the patients were followed up until the seventh postoperative day. Forty-five procedures were performed in three comparative groups, patients under DOACs, individuals taking vitamin K antagonists (VKAs) and without anticoagulant therapy. No bleeding events were observed in procedures carried out in individuals of the DOAC group. Additional hemostatic measures were required in two procedures in the VKA group and one in the non-anticoagulated group. The dental bleeding scores obtained for the DOAC and VKA groups were similar. Our data suggest that the DOAC therapy did not result in increased bleeding outcomes in this sample.

INTRODUCTION

Anticoagulant therapy has been widely used in the primary and secondary prevention of venous and arterial thromboembolic events.[1] Heparin was first introduced as an anticoagulant agent; however, one of the major drawbacks is the need for parenteral administration.[2] Warfarin has been the most popular oral anticoagulant drug used over the last 60 years, but it has limitations such as dietary and drug interactions, narrow therapeutic range, and the need for monitoring.[3] Therefore, in the last years, new anticoagulant agents have been introduced as alternatives to overcome the limitations of the conventional anticoagulants,[4] improving safety and providing higher therapeutic value.[5]

The direct oral anticoagulants (DOACs) are novel targeted agents that present the advantages of more predictable pharmacokinetics and fewer drug interactions, which improves the quality of care and avoids a requirement for dosage adjustments and monitoring.[6] The DOACs comprise direct thrombin inhibitors that are represented by dabigatran (Pradaxa®) and inhibitors of Xa factor, such as rivaroxaban (Xarelto®), apixaban (Eliquis®), edoxaban (Lixiana®) and betrixaban (Bevyxxa®).[7] The DOACs emerged as an alternative approach for the acute treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE), for the prevention of stroke and systemic embolization in non-valvular atrial fibrillation (NVAF) and for venous thromboembolism (VTE) prophylaxis after orthopedic surgery. [8-12]

Limited evidence on the periprocedural management of DOAC therapy during dental extractions is available, and an optimal surgical protocol remains undefined.[13] Few clinical studies have evaluated bleeding outcomes in individuals under DOAC treatment during oral surgery [7,13-17] and, there is no consensus and no defined clinical guidelines for this population thus far. The lack of evidence-based guidelines for patients on DOACs precludes assertive decisions of health providers about the safest way to perform tooth extractions in this group.[18] Therefore, the aim of this study was to evaluate the impact of DOAC therapy on bleeding outcomes, in individuals undergoing dental extractions, by means of a quantitative method to assess bleeding and hemorrhagic outcomes.

METHODS

Ethical guidelines

The study protocol was approved by the Institutional Ethics Committee of Universidade Federal de Minas Gerais (UFMG) (Protocol 48122215.4.0000.5149). The guidelines established in the Declaration of Helsinki (revised version/2002) for research involving humans were followed. The study also has the approval of the Department of Education and Research of the Hospital das Clínicas of the UFMG (HC/UFMG). Each participant signed a statement of informed consent to take part in the study. Anonymity was guaranteed to all participants.

Participants

The sample consisted of individuals, who had been admitted to treatment at the Dental Service of the HC/UFMG, between January 2017 and January 2018. Patients enrolled in the study consisted of a group of individuals undergoing DOAC therapy (n = 11); a group of individuals under Vitamin K Antagonist (VKA) therapy with International Normalized Ratio (INR) values ≤ 3.5 (n = 15); and a group of non-anticoagulated individuals without coagulation disorders and who were not using an antithrombotic drug (n = 15). The criterion for recruitment of participants was a need for extraction of at least one erupted tooth. The following were excluded from the study: individuals with INR > 3.5; individuals with any coagulation disorders not related to anticoagulant use (i.e., hepatic diseases, thrombocytopenia); elderly individuals over the age of 80 years; and individuals presenting any tooth with acute inflammatory conditions (i.e., periodontal or periapical abscess). Individuals who had undergone non-standard interventions and cases with incomplete follow up were excluded. A blood count test was requested for all participants. Individuals taking VKA were also subjected to INR tests. The tests were performed within three days prior to dental surgery.

Dental extraction

All procedures were carried out in the hospital outpatient clinic, early in the morning, under local anesthesia by qualified oral surgeons, trained and supervised by the principal investigator. For individuals under DOAC therapy, the dental extractions were performed without withdrawal of the daily dose, as long as possible after the last dose. When the medication had been taken in the morning, this dose was delayed until after the procedure, following the protocol proposed by Nathwani & Wanis (2017).[7] In the VKA group, the drug regimen was maintained. Blood pressure was measured previously and shortly after the surgical interventions. Local anesthesia was standardized for all patients and consisted of a regional block complemented with local infiltration of 2% lidocaine hydrochloride with epinephrine (Alphacaine 1:100,000; DFL Indústria e Comércio S.A, Rio de Janeiro, Brazil).

The surgical technique followed strictly pre-established parameters observed by the principal investigator. The surgical technique, including the use of forceps and elevator, was carried out as atraumatic as possible. Hemostatic measures included wound closure with 3.0 nylon 14502 T sutures (Mononylon, Ethicon, Somerville, New Jersey) and a piece of sterile gauze bitten by the participant for 20 minutes to compress the operated site. Twenty minutes later, the participants were examined to ensure that hemostasis was obtained, and the immediate postoperative bleeding outcome was evaluated. When increased levels of immediate postoperative or intraoperative bleeding were observed, additional hemostatic measures were performed, applying a 10 x 10 x 10-mm absorbable gelatin sponge (Hemospon, Technew, Rio de Janeiro Brazil), tranexamic acid (Transamin, Nikkho, Rio de Janeiro, Brazil) and/or new sutures. Tranexamic acid paste (one 250-mg pill macerated and mixed with saline) was used to soak the gelatin sponge. The gelatin sponge was placed in the alveolar socket. An additional layer of the paste was applied on the wound after the sutures and covered with gauze under compression. For these individuals, local use of tranexamic acid mouthwash (one pill mixed in 100 mL of cold saline solution), four times a day, was recommended during the following seven postoperative days. The individuals received written postoperative instructions. Procedure time was measured with a stopwatch from the time of the first incision for the detachment of the gingiva until the complete suture was made.

Postoperative pain was managed with 500 mg of metamizole every six hours or 500 mg of acetaminophen every six hours for three days. Antibiotic prophylaxis was only used in individuals at risk of infective endocarditis as defined by the American Heart Association.[19]

Data collection

Information on participants' age and sex, history of bleeding in previous medical or dental procedures, and history of bleeding among family members (parents and/or siblings) were collected. Information on medical diagnosis, indication for oral anticoagulant therapy (OAT), type of OAT, INR (for VKA group) and platelet count was also obtained. The number of tooth extractions (one tooth, two or more teeth), type of teeth (single-rooted or multi**-**rooted teeth), the indication of surgical procedures (periodontal disease, dental caries or third molar) were recorded**.** Additional collected parameters were surgical procedure time, pain and number of gauzes used for hemostasis.

The outcome variables were as follows: the need for additional hemostatic measures (yes or no), immediate postoperative bleeding (yes or no), postoperative bleeding (yes or no), dental bleeding score, and wound healing (satisfactory, swelling/erythema or bone exposure). The postoperative bleeding events were recorded as well as the management of the bleeding (local hemostatic measures in outpatient care or hospital admission).

Quantitative assessment of intraoperative bleeding – *Dental Bleeding Score*

Bleeding amount

The amount of intraoperative bleeding was quantified by means of the storage of the aspirated fluids during the surgical procedure in a portable vacuum pump (5005 BRS, Nevoni, São Paulo, Brazil). A standardized volume of 100 mL of saline solution was used for wound irrigation in all procedures. To avoid clot formation during aspiration, two mL of heparin sodium (5000 IU/mL) (Hepamax-S, Blausiegel, São Paulo, Brazil) was added to the final aspirated solution. Afterwards, the volume of the fluid was measured with a graduated cylinder. For each five mL of fluid, the sample was categorized from one to 10, from the lowest to the highest volume using

the following score: samples with up to five mL were classified with score 1; samples with six to 10 mL were classified with score 2; samples with 11 to 15 mL were classified with score 3, and then successively until score 10; samples with more than 45 mL.

The absorbance of the aspirated fluid

A sample of the total aspirated fluids was collected and used to assess optical density (an indirect measurement of red blood concentration) with a spectrophotometer at 537 nm (RA 50 clinical, Bayer, São Paulo, Brazil). With this analysis, control of the bias that salivary fluid might have had to influence the total volume of aspirated fluid was feasible. The values of absorbance were also categorized in scores, from the lowest to the highest value as follows: absorbance up to 1.0 was classified as score 1; absorbance value of 1.1 to 2.0 was score 2; 2.1 to 3.0 was score 3 and 3.1 or more was considered as the maximum score of 4.

Bleeding score assessment

The scores of total aspirated fluid and absorbance were summed to achieve a final score of bleeding. The values varied from two to 14, for which lower scores indicated less intraoperative bleeding.

Postoperative bleeding

A clinical outcome characterized by a postoperative hemorrhagic event was defined as a marked hemorrhage that required one or more of the following outcomes: (1) telephone call to the dental service or to the principal investigator reporting concern of postoperative bleeding; (2) return to our or another outpatient facility due to postoperative bleeding; (3) need of hospitalization. With the aim of monitoring bleeding episodes from the day of surgery until one week after the dental extractions, the participants were instructed to use gauzes for local compression in case of bleeding and to record the number of gauzes used.

On the seventh postoperative day, participants returned for an appointment to remove the sutures and to evaluate the wound healing. Parameters such as the presence of local erythema/edema, bone exposure, and suppuration were also analyzed. During the appointment, the patients were surveyed regarding bleeding complications during the postoperative period and whether pain had occurred. The

pain was measured by means of a numerical scale (NRS).[20] The scale ranged from 0 to 10. A score of 0 indicated no pain and a score of 10 indicated the highest perception of pain. Individuals who did not return for a follow-up visit were excluded from the analysis.

Data analysis

The analyses were carried out considering the number of procedures, rather than the number of individuals. Descriptive analyses regarding demographic, clinical and bleeding outcomes were performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 23.0, Armonk, NY, USA). Results were reported with means, standard deviations (SD) and percentages.

RESULTS

In this study, 45 surgical procedures were carried out in three groups. Following the application of the inclusion and exclusion criteria, fifteen dental extractions were performed in 11 individuals under DOAC therapy, fifteen procedures in 15 individuals under VKA therapy and fifteen in 15 non-anticoagulated individuals. One non-standard intervention was excluded from the analysis and 13 individuals using DOAC therapy were not included in the sample because they had not met the criterion for dental extraction.

The main clinical and demographic characteristics of the individuals enrolled in this study are reported in Table 1. In the DOAC sample, six procedures were carried out in individuals taking apixaban (5 mg twice daily), five procedures were performed in individuals taking dabigatran (150 mg twice daily), and four procedures were carried out in individuals taking rivaroxaban (10 mg once daily). In the VKA group, all procedures were performed in individuals under warfarin (5 mg once daily) therapy, and the continuous variable INR was collected for this group in particular. The VKA and the non-anticoagulated individuals were matched to the DOAC group in relation to the individuals' age and sex as well as characteristics of the dental extraction.

Data on the number, type, and indication of extractions are summarized in Table 2. Due to the standardized volume of 100 mL of saline solution stated in the method, extraction of impacted teeth were not included in the sample. The mean time

of surgery was 43 minutes (interquartile range/IQR = 30–55). Individuals of the non-anticoagulated group complained more with respect to pain during the postoperative days, while the use of gauzes was higher among individuals undergoing DOAC therapy during the same period.

No occurrences of additional hemostatic measure, immediate postoperative bleeding or postoperative bleeding were observed in procedures carried out in individuals of the DOAC group (Table 3). In the VKA group, two procedures required additional hemostatic measures. One episode of postoperative bleeding was observed and the participant returned to the outpatient facility. In the non-anticoagulated group, additional hemostatic measures were necessary for one procedure. All bleeding events were easily controlled with local measures and no individual included in the study had severe bleeding requiring hospitalization. Increased bleeding during the dental procedure was managed by applying tranexamic acid paste (one 250 mg pill macerated and mixed with saline) soaked in the gelatin sponge placed in the alveolar socket and digital compression with gauze soaked with tranexamic acid paste. For postoperative bleeding, surgical management of the wound was performed. The above-mentioned additional hemostatic measures, including topical hemostatic agents and new sutures were used to control bleeding.

As regards to the dental bleeding score, values observed among the non-anticoagulated group (8.6 ± 3.4), DOAC (7.5 ± 2.8) and VKA (7.9 ± 2.8) individuals were quite similar. A tendency of unsatisfactory wound healing was observed among individuals undergoing DOAC therapy (Table 3). Data of procedures performed in individuals under DOAC therapy are summarized in Table 4.

DISCUSSION

Few studies have investigated the surgical dental management of individuals under DOAC therapy[7,13-17] Individuals enrolled in this study showed a low incidence of bleeding complications following dental extractions of erupted teeth. Bleeding episodes were not observed amongst the individuals taking DOACs, following a non-cessation protocol. The data regarding bleeding outcomes observed in individuals under DOAC therapy were similar to those obtained by analyses of the VKA and non-anticoagulated groups.

The present study evaluated bleeding during dental extractions by means of a quantitative method, using the total collected bleeding corrected by absorbance reading – *the dental bleeding score*. The assessment of the dental bleeding score was, in general, similar among the three groups, with a slight increase among the individuals in the non-anticoagulated group. Currently, there has been no standardized methodology for the measurement of quantitative bleeding in dental practice. In the present study, the protocol proposed by Rocha *et al*. (2019) was adopted for the measurement of oral intraoperative bleeding that otherwise might be considered unfeasible due to salivary interference. The combination of the optical density of the sample with the total amount of aspirated fluid made the estimation attainable[21].

The evaluation of intraoperative bleeding was performed by Miclotti *et al.* (2016)[13], in their case-control study. The assessment was estimated by a scale, in which 1 was no bleeding and 5 was continued bleeding despite standard measures. The method suggested by Miclotti et al. differs from the current one since our method allowed us to objectively assess bleeding during dental extraction. The authors did not observe a significant difference in intraoperative bleeding between individuals taking DOAC (n = 26) and matched controls (n = 26). In the same study, seven individuals of the DOAC group presented delayed postoperative bleeding episodes. This outcome was not observed in the control group. It is important to emphasize that individuals with bleeding were significantly older than individuals without bleeding.[13] In the present study, elderly individuals (> 80 years old)[1] were excluded from the sample with the intent to mitigate likely bias.

Findings in the literature are heterogeneous regarding the postoperative outcomes of dental procedures. Mauvipirez *et al.* (2016) [15], found no statistical difference in the number of bleeding complications between individuals under DOAC therapy and individuals under VKA therapy. Similarly, in another comparative clinical study, only one individual undergoing DOAC therapy and two individuals undergoing VKA therapy presented postoperative bleeding, and no statistically significant difference was observed between the groups.[17] In a study comparing individuals under DOAC therapy and under bridging anticoagulation (warfarin replaced by low-molecular-weight heparin), the authors reported no complications among individuals in the DOAC group and 15% of bleeding incidence in the bridging group.[22]

Similar to our findings, no bleeding episode was described among DOAC individuals in a retrospective cohort study of Miller & Miller (2018). Interestingly, the authors reported a variability in DOAC discontinuance and in the duration of discontinuance advocated by the practitioners.[14] Bensi *et al.* (2018) pointed out in their systematic review that the included articles followed different protocols in the management of individuals taking DOACs: 46.1% of the providers did not discontinue the therapy, while 53.9% changed the anticoagulant administration.[16] In the present study, the surgery was performed as long as possible after the last dose. When the medication had been taken in the morning, the intake was postponed until after the procedure, following the protocol proposed by Nathwani & Wanis (2017).[7]

DOAC-related postoperative bleeding episodes have also been described in the literature. In a case series, one of the five individuals developed significant postoperative bleeding after multiple extractions, which ceased after dabigatran withdraw.[23] In a cohort of 111 procedures performed in individuals under DOAC therapy, a 13.5% incidence of postoperative complications was observed. In this study, the authors discontinued DOAC doses before and/or after the procedure and they concluded that the drug discontinuation or continuation had not been a factor affecting bleeding outcomes.[24] Similarly, other authors demonstrated that the time between the last DOAC dose and the extraction in patients with or without bleeding was not significantly different.[13]

Large medical trials, such as the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY)[10], EINSTEIN–Extension[9] and ARISTOTLE[11] have investigated several outcomes among individuals under DOAC therapy, including bleeding complications. However, detailed information on dental procedures has been unavailable. The RE-LY study consisted of an evaluation of 7,637 procedures, comparing outcomes regarding the intraoperative use of dabigatran with warfarin. This trial has demonstrated that both drugs are associated with similar rates of bleeding.[10] The Continued Treatment Study EINSTEIN–Extension was a double-blind study, in which the authors included 602 patients in the rivaroxaban group and 594 in the placebo group. Four individuals in the rivaroxaban group had major bleeding (0.7%), versus none in the placebo group.[9] Data from the ARISTOTLE trial, have shown that individuals taking apixaban, may be subjected to low-risk procedures without OAT discontinuance.[11]

For the safe management of individuals under DOAC therapy, caution must be exercised in some critical situations. Impaired renal function, comorbidities, multiple tooth extractions, and poor oral hygiene may negatively influence bleeding outcomes.[17,18] In addition, despite the favorable bleeding profiles of DOACs, the occurrence of major bleeding may, sometimes, be of difficult management, due to the lack of prompt access to techniques for controlling and reversing drug activity.[25] Idarucizumab is the only reversal agent approved by the United States Food and Drug Administration (FDA). Other hemostatic factors that have been studied as potential nonspecific DOAC reversal agents are prothrombin complex concentrates (PCCs), activated PCCs (aPCCs), recombinant activated factor VII, and fresh-frozen plasma (FFP).[26] Laboratory measurement of the anticoagulant effect of DOACs can be necessary in cases of hemorrhage. The monitoring may be accomplished in its best with specialized assays that are expensive and routinely unavailable.[27]

In the present study, the DOAC sample was composed of individuals who had undergone 15 procedures for dental extraction performed under rivaroxaban prophylactic dosage; dabigatran and apixaban in therapeutic form. The small sample depicts issues regarding DOAC implementation in Brazil. DOACs are costly[28] precluding free supply by the national health system. Individuals who have access to expensive treatments with DOAC present higher socioeconomic levels, access to private health services and improved oral health. Twenty-five individuals under DOAC therapy had been evaluated in this study; however, 13 did not meet the dental extraction criterion and were excluded. The strict exclusion criteria justify the small sample in this group, which is the major limitation of this study. It is clear that more research is warranted to develop evidence-based guidelines for the dental management of individuals on DOAC therapy. Furthermore, trials with larger sample sizes should be performed to confirm our results.

CONCLUSION

Our data suggest that bleeding risk during and after dental extractions in individuals under DOAC therapy is low. Bleeding complications could be easily managed in the outpatient setting by using local hemostatic measures.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001). A.L.R. is the recipient of scholarships.

REFERENCES

1- Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Pratice Guidelines. *Chest.* 2012; 141: 44-88.

2- Quader M, Stump L, Sumpio B. Low molecular weight heparins: current use and indications. *J Am Coll Surg.* 1998; 187: 641–658.

3- Lin PJ. Reviewing the reality: why we need to change. *Eur Heart J.* 2005;7:15–20.

4- Little JW.New oral anticoagulants: will they replace warfarin? *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2012; 113:575-80.

5- Johnston S. An evidence summary of the management of patients taking direct oral anticoagulants (DOACs) undergoing dental surgery. *Int J Oral Maxillofac Surg.* 2016; 45:618-630.

6- Elad S, Marshall J, Meyerowitz C, Connolly G. Novel anticoagulants: general overview and practical considerations for dental practitioners. *Oral Dis.* 2016; 22 :23-32.

7- Nathwani S, Wanis C. Novel oral anticoagulants and exodontia: the evidence. *Br Dent J.* 2017; 222 :623-628.

8- Schulman S, Kearon C, Kakkar AK, Mismetti P, Schellong S, Eriksson H, Baanstra D, Schnee J, Goldhaber SZ; RE-COVER Study Group. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. *N Engl J Med.* 2009; 361: 2342-2352.

9- Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, Gallus AS, Lensing AW, Misselwitz F, Prins MH, Raskob GE, Segers A, Verhamme P, Wells P, Agnelli G, Bounameaux H, Cohen A, Davidson BL, Piovella F, Schellong S; EINSTEIN Investigators. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med.* 2010; 363: 2499-2510.

10 Healey JS, Eikelboom J, Douketis J, Wallentin L, Oldgren J, Yang S, Themeles E, Heidbuchel H, Avezum A, Reilly P, Connolly SJ, Yusuf S, Ezekowitz M; RE-LY Investigators.Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) randomized trial. *Circulation.* 2012; 126 :343-348.

11-Garcia D, Alexander JH, Wallentin L, Wojdyla DM, Thomas L, Hanna M, Al-Khatib SM, Dorian P, Ansell J, Commerford P, Flaker G, Lanas F, Vinereanu D, Xavier D, Hylek EM, Held C, Verheugt FW, Granger CB, Lopes RD. Management and clinical outcomes in patients treated with apixaban vs warfarin undergoing procedures. *Blood.* 2014; 124:3692-3698.

12- Liu X, Johnson M, Mardekian J, Phatak H, Thompson J, Cohen AT. Apixaban Reduces Hospitalizations in Patients With Venous Thromboembolism: An Analysis of the Apixaban for the Initial Management of Pulmonary Embolism and Deep-Vein Thrombosis as First-Line Therapy (AMPLIFY) Trial.*J Am Heart Assoc.* 2015; 4: 1-8.

13- Miclotte I, Vanhaverbeke M, Agbaje JO, Legrand P, Vanassche T, Verhamme P, Politis C. Pragmatic approach to manage new oral anticoagulants in patients undergoing dental extractions: a prospective case-control study. *Clin Oral Investig.* 2017; 21: 2183-2188.

14- Miller SG, Miller CS.Direct oral anticoagulants: A retrospective study of bleeding, behavior, and documentation. *Oral Dis.* 2018; 24:243-248.

15- Mauprivez C, Khonsari RH, Razouk O, Goudot P, Lesclous P, Descroix V. Management of dental extraction in patients undergoing anticoagulant oral direct treatment: a pilot study. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2016; 122: 146-155.

16- Bensi C, Belli S, Paradiso D, Lomurno G.Postoperative bleeding risk of direct oral anticoagulants after oral surgery procedures: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg.* 2018; 47:923-932.

17- Berton F, Costantinides F, Rizzo R, Franco A, Contarin J, Stacchi C, Maglione M, Visintini E, Di Lenarda A, Di Lenarda R. Should we fear direct oral anticoagulants

more than vitamin K antagonists in simple single tooth extraction? A prospective comparative study. C*lin Oral Investig.* 2018; 8:1-10.

18- Cocero N, Basso M, Grosso S, Carossa S. Direct Oral Anticoagulants (DOACs) and Medical Comorbidities in Patients Needing Dental Extractions: Management of the Risk of Bleeding. *J Oral Maxillofac Surg.* 2018; 76: 1-16.

19- Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, Guyton RA, O´gara PT, Ruiz CE, Skubas NJ, Soraija P,Thomas JD. 2014 AHA/ACC Guideline for the Management of Patients With Valvular Heart Disease: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation.* 2014; 129: 1-96.

20- Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. *Ann Rheum Dis.* 1978; 37 :378-381.

21- Rocha AL, Oliveira SR, Souza AF, Travassos DV, Abreu LG, Ribeiro DD, Silva TA. Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group. *J Craniomaxillofac Surg.* 2019; 47: 798-804.

22- Miranda M, Martinez LS, Franco R, Forte V, Barlattani A Jr, Bollero P.Differences between warfarin and new oral anticoagulants in dental clinical practice. *Oral Implanto.* 2016; 9:151-156.

23- Breik O, Cheng A, Sambrook P, Goss A.Protocol in managing oral surgical patients taking dabigatran. *Aust Dent J.* 2014; 59 :296-30.

24- Patel JP, Woolcombe SA, Patel RK, Obisesan O, Roberts LN, Bryant C, Arya R.Managing direct oral anticoagulants in patients undergoing dentoalveolar surgery. *Br Dent J.* 2017; 222: 245-249.

25- Djulbegovic M, Lee AI. An Update on the "Novel" and Direct Oral Anticoagulants, and Long-Term Anticoagulant Therapy. *Clin Chest Med*. 2018; 39: 583-593.

26- Ruff CT, Giugliano RP, Antman EM. Management of Bleeding With Non-Vitamin K Antagonist Oral Anticoagulants in the Era of Specific Reversal Agents. *Circulation.* 2016; 134: 248-6.

27- Conway SE, Hwang AY, Ponte CD, Gums JG. Laboratory and Clinical Monitoring of Direct Acting Oral Anticoagulants: What Clinicians Need to Know. *Pharmacotherapy*. 2017; 37: 236-248.

28- Fortier K, Shroff D, Reebye UN. Review: An overview and analysis of novel oral anticoagulants and their dental implications. *Gerodontology*. 2018; 35 :1-9.

Table 1: Clinical characteristics of individuals undergoing surgical procedures in study groups: Non-OAT (n= 15), DOAC (n=15) and VKA (n=15).

Variables	*Non-OAT*	*DOAC*	*VKA*
Age (yrs)			
Mean (SD)	54.0 (11.0)	58.9 (11.1)	53.8 (10.1)
Min – Max	30-66	35-70	34-72
Gender (%)			
Male	12 (80.0)	12 (80.0)	12 (80.0)
Female	3 (20.0)	3 (20.0)	3 (20.0)
Previous history of bleeding in a medical procedure			
No	11 (73.3)	15 (100.0)	14 (93.3)
Yes	04 (26.7)	00 (0.0)	01 (6.7)
Previous history of bleeding in a dental procedure			
No	15 (100.0)	13 (86.7)	13 (86.7)
Yes	0 (0.0)	2 (13.3)	2 (13.3)
Previous history of bleeding in Family			
No	15 (100.0)	15 (100.0)	15 (100.0)
Yes	0 (0.0)	0 (0.0)	0 (0.0)
Platelet count			
Mean (SD)	246.5 (74.2)	273.2 (69.2)	230.7 (105.1)
Min – Max	123-331	182-367	107-535
INR			
Mean (SD)	-	-	2.4 (0.4)
Min – Max	-	-	1.8 - 3.41
Indication for OAT			
DVT	-	4	3
AF	-	7	5
MHV	-	-	4
PE	-	2	3

Variables	*Non-OAT*	*DOAC*	*VKA*
CIA	-	2	-
OAT prescribed			
Warfarin	-	-	15 (100)
Rivaroxaban	-	4 (26.7)	-
Dabigatran	-	5 (33.3)	-
Apixaban	-	6 (40.0)	-

Abbreviations: DOAC, Direct Oral Anticoagulant; VKA, Vitamin K Antagonist; Non-OAT, non-anticoagulated; yrs; years; SD, standard deviation; DVT, Deep Vein Thrombosis; AF, Atrial Fibrillation; MHV, Mechanical Heart Valve; PE, Pulmonary Embolus; CIA, Cerebrovascular Ischemic Accident; INR, International Normalized Ratio; Min, minimum; Max, maximum.

Table 2: Clinical characteristics of procedures for tooth extraction performed in groups: non-anticoagulated (n= 15), DOAC (n=15) and VKA (n=15).

Variables	*Non-OAT*	*DOAC*	*VKA*
Tooth extraction			
1 tooth	6 (40.0)	5 (33.3)	5 (33.3)
2 or more teeth	9 (60.0)	10 (66.6)	10 (66.6)
Teeth			
1 root	3 (20.0)	4 (26.7)	1 (6.7)
More than 1 root	12 (80.0)	11 (73.3)	14 (93.3)
Indication			
Periodontal disease	4 (26.7)	3 (20)	4 (26.7)
Decay	11 (73.3)	11 (73.3)	11 (73.3)
Third molar	-	1 (6.7)	-
Procedure time (minutes)			
Mean (SD)	43.3 (13.5)	46.6 (10.8)	40.3 (15.4)
Min – Max	20-65	30-60	20-60
Pain			
Mean (SD)	4.0 (3.4)	2.53 (2.9)	0.9 (1.3)
Min – Max	0 – 10	0 – 9	0 -4
Gauzes			
Mean (SD)	0.6 (1.4)	3.4 (4.8)	2.6 (2.4)
Min - Max	0-5	0 – 10	0 - 5

Abbreviations: VKA, Vitamin K Antagonist; DOAC, Direct Oral Anticoagulant; Non-OAT, non-anticoagulated; SD, standard deviation; Min, minimum; Max, maximum.

Table 3 - Clinic and quantitative assessment of bleeding and outcomes in study groups: non-anticoagulated (n= 15), DOAC (n=15) and VKA (n=15).

Variables	*Non-OAT*	*DOAC*	*VKA*
Additional hemostatic measures			
No	14 (93.3)	15 (100.0)	13 (86.7)
Yes	1 (6.7)	0 (0.0)	2 (13.3)
Immediate postoperative bleeding			
No	15 (100.0)	15 (100.0)	15 (100.0)
Yes	0 (0.0)	0 (0.0)	0 (0.0)
Postoperative bleeding			
No	15 (100.0)	15 (100.0)	14 (93.3)
Yes	0 (0.0)	0 (0.0)	1 (6.7)
Bleeding score			
Mean (SD)	8.6 (3.4)	7.5 (2.8)	7.9 (2.8)
Min - Max	4 – 14	3 – 13	3 – 13
Wound healing			
Satisfactory	11 (73.3)	7 (46.7)	10 (66.7)
Swelling/erythema	4 (26.7)	8 (53.3)	5 (33.3)

Abbreviations: VKA, Vitamin K Antagonist; DOAC, Direct Oral Anticoagulant; Non-OAT, non-anticoagulated; SD, standard deviation; Min, minimum; Max, maximum.

Table 4 - Baseline characteristics of DOAC individuals on whom the procedures were performed (n=15).

Case	***Age***	***Gender***	***DOAC***	***Indication for OAT***	***Bleeding history***	***Platelet count***	***Number of teeth extracted***	***Dental bleeding score***	***Bleeding complication***
1	M	59	D	AF	No	182	2	13	No
2	M	68	D	CIA	Yes	244	3	12	No
3	M	68	D	CIA	Yes	244	2	7	No
4	M	60	D	PE	No	183	2	6	No
5	M	70	R	DVT	No	261	1	3	No
6	F	49	R	DVT	No	205	1	6	No
7	M	60	D	PE	No	183	2	4	No
8	M	66	R	DVT	No	280	1	9	No
9	M	66	A	AF	No	339	3	5	No
10	M	66	A	AF	No	339	3	8	No
11	M	66	A	AF	No	339	3	7	No
12	M	66	A	AF	No	339	2	6	No
13	F	35	A	AF	No	367	1	10	No
14	M	42	R	AF	No	363	1	7	No
15	F	43	A	DVT	No	231	3	10	No

Abbreviations: M, Male; F, Female; D, Dabigatran; R, Rivaroxaban; A, Apixaban; DVT, Deep Vein Thrombosis; AF, Atrial Fibrillation; PE, Pulmonary Embolus; CIA, Cerebrovascular Ischemic Accident;

4.3 Artigo III

Artigo científico III submetido ao periódico Clinical Orthopaedics and Related Research (Qualis Medicina A2, Fator de Impacto: 4,091)

INHIBITORY EFFECTS OF DABIGATRAN ETEXILATE, A DIRECT THROMBIN INHIBITOR, ON BONE CELLS

Amanda Leal Rocha[1], DDS, PhD Student; Rayana Longo Bighetti Trevisan[2], DDS, PhD Student; Letícia Fernanda Duffles[3], DDS, PhD Student; Bruna Rodrigues Dias Assis[4], MSc Student; Soraia Macari[5], DDS, PhD; Ivana Márcia Alves Diniz[6], DDS, PhD; José Alcides Almeida de Arruda[1], DDS, MSc Student; Marcio Mateus Beloti[2], DDS, PhD; Adalberto Luiz Rosa[2], DDS, PhD; Sandra Yasuyo Fukada[3], DDS, PhD; Thaise Mayumi Taira[3], DDS, PhD Student; Gisele Assis Castro Goulart[4], PhD; Daniel Dias Ribeiro[7], MD, PhD; Lucas Guimarães Abreu[5], DDS, PhD; Tarcília Aparecida Silva[1*] , DDS, PhD.

[1]Department of Oral Surgery and Pathology, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[2]Cell Culture Laboratory, School of Dentistry, University of São Paulo, Ribeirão Preto, SP, Brazil.

[3]Department of Physics and Chemistry, Faculty of Pharmacological Science, University of São Paulo, Ribeirão Preto, SP, Brazil.

[4]Department of Pharmaceutics, Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[5]Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[6]Department of Restorative Dentistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

[7]Department of Hematology, Faculty of Medicine, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[*]**CORRESPONDING AUTHOR:** Tarcília Aparecida da Silva - Department of Oral Surgery and Pathology, Faculty of Dentistry, Universidade Federal de Minas Gerais – UFMG - Av. Presidente Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270- 901, Brazil. Phone: 55-31-34092478 - Fax: 55-31-34092430 Email: silva.tarcilia@gmail.com.

Running head: Dabigatran etexilate effects on bone cells

Keywords**:** Anticoagulants; Orthopedics; In Vitro Techniques; Bone Marrow Cells; Osteoblasts; Osteoclasts.

Funding**:** This study was supported by the Brazilian National Council for Scientific and Technological Development (CNPq, Grant: 302157/2017-4). T.A.S. is a research fellow at CNPq.

Conflict of Interest Statement: Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

ABSTRACT

Background: Anticoagulants are widely used in orthopedic surgery to decrease the risk of deep vein thrombosis. While significant bone impairment is induced by long-term heparin therapy, little is known about the effects of direct oral anticoagulants (DOACs).

Questions/purposes: To investigate the effects of dabigatran etexilate (Pradaxa®), a DOAC inhibitor of thrombin, on bone cells using *in vitro* and *ex vivo* cell culture models.

Methods: Osteoblasts and osteoclasts exposed to different concentrations of dabigatran etexilate and untreated cells were assayed for cell differentiation and activity. Favorable osteogenic conditions for osteoblasts were tested using titanium with nanotopography (Ti-Nano). In addition, mice treated with a dabigatran etexilate solution had bone marrow cells analysed for the ability to generate osteoclasts.

Results: Dabigatran etexilate at concentrations of 1 µg/mL and 2 µg/mL did not impact osteoclast or osteoblast viability. The drug inhibited osteoclast differentiation and activity as observed by the reduction of TRAP+ cells, resorption pits and gene and protein expression of cathepsin K. Consistently, osteoclasts from mice treated with dabigatran showed decreased area, resorptive activity, as well as gene and protein expression of cathepsin K. In osteoblast cultures, grown both on polystyrene and Ti-Nano, dabigatran etexilate reduced alkaline phosphatase (ALP) activity, matrix mineralization, gene expression of ALP and osteocalcin.

Conclusions: Dabigatran etexilate inhibits osteoclast differentiation in *ex vivo* and *in vitro* models in a dose-dependent manner. Moreover, the drug reduced osteoblast activity even under optimal osteogenic conditions. This study provides new evidence regarding the negative overall impact of DOACs on bone cells.

Clinical Relevance: The present findings demonstrated for the first time the negative impact of dabigatran etexilate on osteoblasts and osteoclasts. These effects may further compromise the bone healing after surgery and clinical outcome of orthopedic prostheses .

INTRODUCTION

Long-term oral anticoagulation is indicated for the prevention and treatment of thromboembolic diseases. Coumarins or vitamin K antagonists have been the first choice therapy for over 50 years [1]. Nevertheless, over the last few years, direct oral anticoagulants (DOACs) have been approved, with the advantages of having more predictable pharmacokinetics and fewer drug interactions [2]. These drugs avoid the drawbacks associated with warfarin and the need of dosage adjustments and monitoring [3].

Dabigatran is a direct thrombin inhibitor (DTI) of the DOAC class that binds directly to thrombin and blocks its interaction with its substrates [4]. Commercially, the drug is available in the mesylate salt form, Pradaxa® (dabigatran etexilate mesylate). After oral administration, the prodrug, dabigatran etexilate, is converted by esterases to its active form, dabigatran – a potent, competitive and reversible direct inhibitor of the active site of thrombin [5]. Dabigatran was approved as being effective in the prevention and treatment of venous thromboembolism, stroke and systemic embolism in individuals with nonvalvular atrial fibrillation [6].

DOACs are a novelty on the market and the knowledge of possible risks of their use for treatment is essential in order to improve clinical practice by making them more effective and safer. In this regard, studies investigating the potential side effects of anticoagulants on bone remodeling have been reported elsewhere [7-9]. Bone remodeling is a complex and continuous process maintained as a tighly coupled balance between bone deposition by osteoblasts and bone resorption by osteoclasts [7]. Abnormalities in this mechanism may lead to imbalance in bone homeostasis, triggering skeletal disorders [9]. Osteopenia and osteoporosis are acknowledged side effects of heparins after long-term treatment [1, 10, 11]. Warfarin could have direct negative effects on bone by the inhibition of γ-carboxylation of osteocalcin as well as indirect effects, because individuals treated with warfarin may limit their dietary intake of foods rich in vitamin K [12]. In contrast, the effects of DOACs on bone cells are poorly studied. DTIs and factor Xa inhibitors have been evaluated *in vitro* and *in vivo* and, although no consensus exists, some results have suggested their negative impact on bone cells and structure [11, 13-17].

Since the publication of the RE-MODEL clinical trial [18], the use of dabigatran has increased for the prevention of venous thromboembolism in individuals submitted

to elective hip or knee arthroplasty. These are clinical situations in which a well-preserved bone cell function is required for successful interaction between the cells with the prosthesis interface and hard tissue formation. Therefore, the purpose of the present study was to investigate the effects of dabigatran etexilate (Pradaxa®) on osteoblasts and osteoclasts and the interaction of osteoblasts with titanium (Ti) surfaces.

MATERIAL AND METHODS

Preparation of the dabigatran etexilate (Pradaxa®) solution

A stock solution of dabigatran etexilate (100 µg/mL) was prepared by the accurate weighing of 142.73 mg of the contents of the Pradaxa® capsule (Boehringer, Ingelheim am Rhein, Germany) corresponding to 50 mg of dabigatran etexilate. This amount was diluted in 500 mL of ultrapure water and sonicated for 10 minutes. Prior to the experiments, the dabigatran etexilate stock solution (100 µg/mL) was filtered through a 0.22 µm membrane filter [33 millimeters (mm) in diameter, PVDF, Millipore, Burlington, MA, USA]. Filters were pretreated in order to eliminate the binding of dabigatran etexilate to the membrane filter. The devices were soaked in a passivating solution (Tween™ 20, 5% w/v, Croda Health Care, East Riding, UK) maintained overnight at room temperature and washed with distilled water prior to use. The filtered solutions were stored at 2–8°C protected from light until analysis. The stability of the solutions was evaluated for 30 days by high-performance liquid chromatography (HPLC). The working solutions (1-6 µg/mL) were prepared by the proper dilution of the stock solution (100 µg/mL) in alpha minimum essential medium (α-MEM, Invitrogen Life Technologies, Grand Island, NY, USA). The detection of dabigatran etexilate after dilution in the culture medium was also evaluated by HPLC analysis.

HPLC analysis was performed by adapting the method described by Bernardi et al. [19]. A Shimadzu HPLC system (Kyoto, Japan) consisting of a quaternary pump, an autosampler, and a diode array detector (DAD) was used. An Agilent C18 column (250 mm, 4.6 mm, 5 µm particle size) was also used. Ultraviolet (UV) detection was performed at 225 nm. The mobile phase was an acetonitrile: triethylamine solution, pH 6.0, adjusted with phosphoric acid (65:35 v/v). The

optimized flow rate was 1.0 mL/min, and a 50 µL aliquot of the sample was injected during each run.

To assess the effects of the active principle, cells were treated with dabigatran (Dabigatran-D_3 100 µg/mL solution, acetonitrile with 10% 0.01 N HCl, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) at concentrations of 0.1 µg/mL, 0.3 µg/mL, 3 µg/mL, and 6 µg/mL. Untreated cells under the same conditions were used as controls for the *in vitro* experiments.

Animals

Male C57BL/6 mice (six weeks of age) and Wistar Hannover rats (three days of age) were acquired from the center of animal care of the Federal University of Minas Gerais (UFMG) and University of São Paulo (USP). Animals were treated in conformity with the regulations of the Institutional Ethics Committee of the universities (No. 247/2018 and No. 2018.1.562.58.0).

Bone marrow cell-derived osteoclasts

Bone marrow cells (BMCs) were isolated from the femurs and tibiae of C57BL/6 mice and cultured with α-MEM (Thermo Fisher Scientific, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco®, CA, USA). Cells were grown in a humidified atmosphere containing 5% CO_2 at 37^{o}C and a soluble macrophage colony-stimulating factor (M-CSF, 30 ng/mL, R&D Systems, MN, USA) for three days. The adherent cells (osteoclast precursors) were then plated in 96-well microplates (Corning Inc., Corning, NY, USA) at a density of 2×10^4 cells/well, and cultured in α-MEM (Thermo Fisher Scientific) containing M-CSF (30 ng/mL, R&D Systems) and receptor activator of nuclear factor kappa-B ligand (RANKL 10 ng/mL, R&D Systems).

Tartrate-resistant acid phosphatase staining

The BMC-derived osteoclasts were fixed after five days of culture with acetone, citrate, and 37% formaldehyde and stained with a tartrate-resistant acid phosphatase (TRAP) commercial kit (Sigma-Aldrich) according to the manufacturer's instructions. The experiments were carried out in triplicate and performed at least twice. Multinucleated (three or more nuclei) TRAP+ cells were considered to be osteoclasts. The cells were counted and measured in mm. The images were

captured using ImageJ software (National Institutes of Health, Betesda, MD, USA) and the Cytation Cell Imaging Multi-Mode Reader (BioTek, Winooski, Vermont, USA).

Resorption pit assay

Osteoclasts were generated and cultured in 96-well osteoassay microplates (Corning Inc.). Resorption and pit formation area were measured after 10 days of osteoclast differentiation. The experiments were performed in triplicate. The pits were observed and captured under a microscope at 4× magnification. The results are reported as percent pit areas/well using the contouring tools of Leica Application Suite software (Leica Microsystems, Hessen, Wetzlar, Germany).

Calvaria-derived osteoblasts

Osteoblasts were harvested from calvaria fragments of eight newborn Wistar Hannover rats by sequential enzymatic digestion. Briefly, a 0.25% trypsin solution and 0.2% type II collagenase (all from Gibco®) were added to the fragments to isolate the cells [20]. Cells were counted and plated in 24-well culture microplates (Corning Inc.) at a cell density of 2×10^4 cells/well. The osteoblasts were cultured in α-MEM osteogenic growth medium (Invitrogen Life Technologies) supplemented with 10% FBS (Gibco®), 100 µg/mL gentamicin (Gibco®), 5 µg/mL ascorbic acid (Gibco®), and 7 mM β-glycerophosphate (Sigma-Aldrich) for up to 14 days. During the culture period, the cells were kept at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The medium was changed every three days.

Osteoblast cell line

The MC3T3-E1 pre-osteoblastic cell line (American Type Culture Collection, Manassas, VA, USA) was also used for the experiments. The cells were plated in 24-well polystyrene microplates (Corning Inc.) at a density of 2×10^4 cells/well and cultured for up to 14 days. The medium for MC3T3-E1 cells was α-MEM (Invitrogen Life Technologies) supplemented with 10% FBS (Gibco®), 100 U/mL penicillin (Invitrogen Life Technologies), 100 µg/mL streptomycin (Gibco®), 5 µg/mL ascorbic acid (Gibco®), and 7 mM β-glycerophosphate (Sigma-Aldrich). During the culture period, the cells were kept at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, and the medium was replaced every three days.

Alkaline phosphatase (ALP) activity

After seven days of osteoblast culture *in situ*, ALP activity was qualitatively evaluated by Fast red staining. This staining results in an insoluble Naphthol-Fast Red complex that precipitates where cells show ALP activity. The medium was removed and the cultures were incubated with 1 mL/well of a mixture of 0.9 mM naphthol AS-MX phosphate (Sigma-Aldrich) and 1.8 mM Fast red TR Salt (Sigma-Aldrich). Before mixing, naphthol AS-MX phosphate was solubilized with 4 mg/mL dimethylformamide (Merck KGaA). The plates were kept at 37°C for 30 minutes and the solution was removed and dried overnight. The experiments were undertaken in quadruplicate. *In situ* ALP was quantified by counting pixels with Leica Application Suite software (Leica Microsystems). The results are reported as percent stained areas in relation to the control group.

Alizarin red staining

After 14 days of osteoblast culture, extracellular matrix mineralization was quantified using alizarin red staining. The culture medium was removed and the wells were fixed in 10% formalin for two hours at room temperature, dehydrated and stained with 2% alizarin red (Sigma-Aldrich), pH 4.2, for 10 minutes. For quantitative analysis, calcium content was determined by a colorimetric method. Briefly, 280 mL of 10% acetic acid were added to each well and the plate was incubated for 30 minutes under shaking at room temperature. This solution was vortexed for one minute, heated to 85°C for 10 minutes, and transferred to ice for five minutes. The samples were then centrifuged and 100 mL of the supernatant was mixed with 40 mL of 10% ammonium hydroxide. The optical density values were evaluated spectrophotometrically with the mQuant plate reader (BioTek) at 405 nm. The experiments were carried out in quadruplicate.

Ti surfaces

Machined discs of commercially pure grade two Ti 13 mm in diameter and 2 mm thick (Realum, São Paulo, SP, Brazil) were polished with 180, 320 and 600 grit silicon carbides, cleaned by sonication, and washed with toluene. The discs were conditioned with a solution of 10 N H_2SO_4 and 30% aqueous H_2O_2 (1:1 v/v) for four hours at room temperature under continuous agitation in order to obtain the

nanotopography surface. Non-conditioned discs were used as control (machined). All discs were autoclaved before the cell culture experiments.

Mice treated with dabigatran etexilate solution and ex vivo model

Ten male C57BL/6 mice (six weeks) were randomly divided into two groups: dabigatran etexilate treatment (n=5) and untreated controls (n=5). The standardized dose of treatment was calculated proportionally to that recommended for humans, i.e., one Pradaxa® capsule (150 mg) every 12 hours [19]. Considering the recommended daily dose of 300 mg/day for individuals weighing 70 kg and the mean animal weight of 20 g, a dose of 85.7 µg/day was used in this study. The dose corresponds to 428.5 µL of the aqueous solution of dabigatran etexilate at 100 µg/mL concentration, administered twice daily. The treatment was administered by gavage under inhalatory sedation with 2% isoflurane for 28 days. The animals were housed five to a cage with free access to food and water. At the end of the study, the animals were anesthetized and euthanized. BMCs were isolated from femurs and tibiae and cultured as described above.

Cell viability assay

Cell viability/proliferation of dabigatran etexilate-treated cultures of osteoclasts and osteoblasts were evaluated by measuring the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT, Sigma-Aldrich) to purple formazan crystals, according to manufacturer instructions. Briefly, 20 µL of MTT solution in 96-well and 1 mL in 24-well culture microplates (Corning Inc.), 5 mg/mL in PBS was added to each well, and the plates were incubated for three hours at 37°C. The medium was removed and the MTT crystals were solubilized with 200 µL dimethyl sulfoxide (DMSO, Sigma-Aldrich) or isopropanol acid solution (100 mL isopropanol and 134 µL HCl) at room temperature. The spectrophotometric absorbance of each sample was then measured at 540 nm (DMSO dilution) and 570 nm (isopropanol dilution). The experiments were carried out in quadruplicate and the results are reported as percent optical density of viable cells in relation to the control group.

Real-time polymerase chain reaction (RT-qPCR)

Gene expression was evaluated in osteoclasts and osteoblasts and compared to that of untreated cells. After three and seven days of culture, total ribonucleic acid

(RNA) was extracted with Trizol reagent (Invitrogen Life Technologies) followed by the SV total RNA isolation system (Promega, Madison, WI, USA), according to the manufacturer's instructions. Concentration and purity were determined using a GeneQuant® spectrophotometer (GE Healthcare, Buckinghamshire, UK) and integrity was investigated using a 2100 Bioanalyzer (Agilent Technologies, Stockport, UK). Complementary deoxyribonucleic acid (cDNA) was synthesized using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. RT-qPCR was carried out in triplicate and performed using SYBR® green PCR master mix (Thermo Fisher Scientific). The gene expression of cathepsin K (CTSK) was evaluated in osteoclast cultures, and the expression of bone markers runt-related transcription factor 2 (RUNX2), osterix (OSX), alkaline phosphatase (ALP) and osteocalcin (OC) were evaluated in osteoblast cultures. The results are reported as target genes normalized by the constitutive gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta}$Ct method [21].

Protein levels

Protein analysis was performed by the western blot assay for BMC-derived osteoclasts after three days of treatment. The total cell lysates were obtained using radioimmunoprecipitation assay buffer (RIPA, Sigma-Aldrich) with a cocktail of protease and phosphatase inhibitors. Protein concentration was determined using a bicinchoninic acid protein assay kit (Sigma-Aldrich). Equal amounts of protein (10 µg) were loaded to sodium dodecyl sulfate-polyacrylamide electrophoresis gel (SDS/PAGE) and further transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% nonfat milk for one hour at room temperature and incubated with specific antibodies against CTSK overnight at 4°C. After three washes with tris-buffered saline 0.1% tween 20 (TBS-T, Sigma-Aldrich), the membranes were incubated with appropriate horseradish peroxidase (HRP) and conjugated with secondary antibody solution (Luminata Forte, Millipore, Burlington, MA, USA) for two hours at room temperature. The experiments were carried out in triplicate. Bands were quantified using the ImageJ software. Results were normalized by β-actin as a loading control. Gels were run under the same experimental conditions.

Statistical analysis

Data are reported as mean ± standard deviation (SD). The Shapiro-Wilk test was used to evaluate normality and the statistical analysis was performed using the Student *t*-test. Data obtained from all evaluations were processed with GraphPad Prism, version 8.0 (GraphPad Software, San Diego, CA, USA). The level of significance was set at 5% in all statistical analyses.

RESULTS

Dabigatran etexilate solution: pharmacological and analytical aspects

Since experiments were conducted over 3-30 days, the stability of the dabigatran etexilate stock solution was an important analytical aspect. HPLC analysis showed that the stock solution of dabigatran etexilate (100 µg/mL) remained stable for 30 days at 2-8°C, with less than 2% of drug reduction being observed (Supplementary Figure 1A-C). Moreover, the time course assay (one, two and three days) evaluated by HPLC analysis showed a reduction of dabigatran etexilate peak area in cell culture growth medium, suggesting the conversion of dabigatran etexilate to its active moiety, dabigatran (Supplementary Figure 2A). The capacity of HPLC analysis to detect the dabigatran etexilate peak in cell culture growth medium was determined before the tests (Supplementary Figure 2B, C). Accordingly, preliminary tests using dabigatran etexilate added to the cell cultures demonstrated effects on the differentiation and activity of osteoblasts and osteoclasts (data not shown).

Effects of dabigatran etexilate on osteoclasts in an ex vivo model

We first investigated whether BMCs from mice treated with dabigatran etexilate [at a dose calculated in proportion to that used for human anticoagulant therapy (85.7 µg/day)] preserved the ability to generate osteoclasts, as determined by the analysis of cell differentiation and activity (Figure 1). No difference in the total number of TRAP-atined osteoclasts was observed (Figure 1A); however, the treatment significantly reduced the total area of osteoclasts compared to the untreated control group (p=0.0001) (Figure 1B-D). Consequently, resorptive activity was also reduced in osteoclasts from treated animals (p=0.04) (Figure 1E-G). The gene expression of CTSK (p=0.03) (Figure 1H) and its protein expression (p=0.01) (Figure 1I, J) were significantly reduced in osteoclasts derived from treated animals.

Effects of dabigaran etexilate on osteoclasts treated in vitro

To better characterize the effects of dabigatran etexilate, osteoclasts were treated with different concentrations of the drug (1-6 µg/mL) and the effects on cell differentiation were assessed. Among the tested concentrations, dabigatran etexilate at 2-6 µg/mL exhibited a similar pattern of cell differentiation impairment (Supplementary Figure 3A). Thus, 1 µg/mL and 2 µg/mL were selected as the working standardized concentrations since these concentrations did not interphere with cell viability (Figure 2A).

Dabigatran etexilate treatment (2 µg/mL) resulted in reduced osteoclast differentiation and function, as confirmed by TRAP staining (p<0.0001) (Figure 2B-E) and resorptive activity (p=0.01) (Figure 2F-H). Consistently, the treatment also reduced the gene (p<0.05) (Figure 2I) and protein expression (p=0.01) (Figure 2J, K) of the osteoclast marker CTSK.

To confirm whether the effects of dabigatran etexilate were linked to an active principle, dabigatran was used to treat BMC-derived osteoclasts. At the concentrations of 0.1 µg/mL, 0.3 µg/mL and 3 µg/mL, dabigatran did not influence cell viability (0.1 µg/mL: 112.75±13.67; 0.3 µg/mL: 121.25±9.21; 3 µg/mL: 117.50±10.66; control: 100.00±0.00; p>0.05). The results of TRAP staining demonstrated that dabigatran (3 µg/mL) significantly reduced the number of TRAP+ cells (3 µg/mL: 34.67±6.02; control: 106.74±16.33; p=0.04). The doses of 0.1 µg/mL and 0.3 µg/mL had no effect on osteoclast numbers (0.1 µg/mL: 110.30±11.29; 0.3 µg/mL: 146.00±7.37; control: 106.74±66.33; p>0.05).

Effects of dabigatran etexilate on osteoblasts

Considering the biological mechanisms of interaction between osteoclasts and osteoblasts in bone remodeling/repair and the impairment observed in osteoclasts, our next step was to assess the effects of dabigatran etexilate on osteoblast cell cultures. The tests were performed on cell cultures of calvaria-derived osteoblasts grown on polystyrene, Ti with nanotopography (Ti-Nano) or Ti with machining (Ti-Machined). Ti-Nano has been previously described as a surface with higher osteogenic potential [22].

In a first set of experiments, the MC3T3-E1 pre-osteoblastic cell line was used to define the working dose of dabigatran etexilate. The analysis of matrix mineralization revealed that all the tested concentrations (1-6 µg/mL) inhibited

MC3T3-E1 differentiation (Supplementary Figure 3B). Concentrations of 3 µg/mL showed a pattern of osteoblast impairment that could make tests unfeasible. Doses of 1 µg/mL and 2 µg/mL resulted in a similar pattern. Thus, 2 µg/mL was selected as the working standardized concentration.

Viability was also tested for calvaria-derived osteoblasts. The MTT assay showed that dabigatran etexilate (2 µg/mL) had no effect on cell viability compared to control. Optical density values were determined as percent control absorbance at three (2 µg/mL: 92.29±5.53; control: 100.00±0.00; $p>0.05$) and seven days (2 µg/mL: 91.53±7.68; control: 100.00±0.00; $p>0.05$) of treatment.

Dabigatran etexilate (2 µg/mL) treatment inhibited osteoblast differentiation and function in all tested surfaces; in cells grown on polystyrene, on Ti-Machined and on Ti-Nano (Figure 3). This effect was verified by the reduction of ALP activity of cells grown on polystyrene after seven days of treatment ($p<0.0001$) (Figure 3A-C) and the reduction of mineralized matrix formation ($p<0.0001$) (Figure 3D-F) after 14 days of treatment. In calvaria-derived osteoblasts grown on Ti-Machined, the presence of dabigatran etexilate (2 µg/mL) also reduced ALP activity ($p<0.0001$) (Figure 3G-I) and mineralized matrix formation ($p=0.001$) (Figure 3J-L). Similarly, ALP activity ($p<0.0001$) (Figure 3M-O) and the calcium deposits ($p<0.0001$) (Figure 3P-R) were significantly lower in treated cells grown on Ti-Nano.

Corroborating the phenotypic findings, osteoblasts grown on polystyrene and on both Ti-Machined and Ti-Nano surfaces exhibited reduced gene expression of ALP and OC in the presence of dabigatran etexilate (2 µg/mL) ($p<0.0001$) (Figure 4A-C). No statistically significant difference in the expression of early osteoblast markers, RUNX2 and OSX, was observed between the dabigatran etexilate and control groups.

The active principle, dabigatran, was also used to treat osteoblast cell cultures. Accordingly, inhibition of cell differentiation was also observed. A significant reduction in mineralized matrix formation detected by optical density values of calcium was observed at the highest concentrations of dabigatran, i.e., 3 µg/mL and 6 µg/mL, compared to control (3 µg/mL: 0.42±0.09; 6 µg/mL: 0.38±0.07; control: 0.51±0.06; $p<0.05$). The doses of 0.1 µg/mL and 0.3 µg/mL had no effect on mineralization (0.1 µg/mL: 0.44±0.08; 0.3 µg/mL: 0.55±0.04; control: 0.51±0.06; $p<0.05$).

DISCUSSION

Bone healing consists of complex biological mechanisms involving local and systemic factors. The recruitment of progenitor cells and their proliferation and differentiation into osteoblasts and osteoclasts are essential during bone repair [23]. Knowledge about the effects of medications on bone metabolism, in particular postoperatively used drugs, is of clinical relevance. In surgical orthopedic practice, this is particularly applicable since the prescription of drugs for thromboprophylaxis is usually necessary after major procedures. These medications have effects on bone cells, impairing the surgical outcome, which is strictly dependent on bone remodeling [16]. Herein, we evaluated the effects on osteoclast and osteoblast cells of a DTI in two presentations: the mesylate salt form dabigatran etexilate (Pradaxa®) and its active principle dabigatran. Our main findings showed that both dabigatran and Pradaxa® inhibited osteoclast differentiation and resorptive activity in a dose and time-dependent manner, were also negatively affected, as demonstrated by the reduced expression of ALP and OC, as well as the shortened ALP activity and mineralized matrix formation. Particularly, osteoblasts growing under favorable osteogenic conditions such as on a Ti-Nano surface, still showed impairment of their differentiation ability and function.

Osteopenia, a recognized side effect of long-term therapy with heparin and low-molecular-weight heparin, may impair the healing of bone fractures and the osseointegration of prostheses [1, 10, 11]. Therefore, several studies have investigated the effects of anticoagulants on cell differentiation and function, as well as on bone structure [7-9, 11, 13-16]; however, the specific effects of these drugs on the complex mechanism of bone healing remain unclear. Previous *in vitro* experiments have tested the impact of several anticoagulants on bone cells. These experiments have focused on heparin-related drugs [7, 8, 24-26] and, more recently, on DOACs [11, 13, 15, 17]. Nevertheless, no data on the effects of dabigatran on cell cultures have been described in the literature thus far.

Regarding the impact of anticoagulant therapy on cultured osteoclasts, while data about DOACs are not available, the heparin-related impairment has been well described. In a co-culture system, heparin enhanced osteoclastic activity but did not change osteoclastogenesis [7]. Muir et al. [26] have observed that heparin increased bone resorption by inflating both osteoclast number and activity. Conversely, Folwarczna et al. [24] have shown that heparins affected osteoclast formation in rat

BMCs in two directions depending on the drug concentration. At the highest concentrations, heparins reduced the number of osteoclasts and at lower concentrations they increased osteoclast formation [24]. In the present study, dabigatran and the prodrug dabigatran etexilate reduced osteoclast differentiation at their highest concentrations, i.e., 2 μg/mL and 3 μg/mL. The resorption pit assay also showed reduction of function in the treated group. Furthermore, dabigatran etexilate downregulated the expression of CTSK, a key marker of osteoclast differentiation and activity. Consistent with our *in vitro* data, we also observed that the differentiation and function of osteoclasts was impaired in mice treated with dabigatran etexilate. Nevertheless, when dabigatran-treated rats were compared to warfarin-treated rats it was observed that dabigatran did not interfere with bone structural parameters [12].

In vitro effects of DOACs have been mostly reported in experiments with osteoblasts [11, 13, 15, 17]. Somjen et al*.* [15] have shown that rivaroxaban at different concentrations (0.01-50 μg/mL) inhibited the proliferation of osteoblasts from female individuals. This effect was maintained even in the presence of molecules that stimulate DNA synthesis and ALP specific activity [15]. Similarly, in the present investigation, the upregulation of osteoblast differentiation markers induced by Ti-Nano [27-30] was inhibited in the presence of dabigatran etexilate.

The effect of melagatran, a DTI similar to dabigatran, was investigated in human osteoblasts [13]. In line with our findings, melagatran at the highest concentration (50 nmol/mL) caused a significant reduction of collagen type I deposition and ALP activity [13]. The inhibition of ALP activity by DOACs was also confirmed in two previous studies [11, 17] in which rivaroxaban induced a reduction of ALP activity in osteoblast cultures. However, in one study [11], the osteoblast function remained unaffected. In the other study [17], rivaroxaban and enoxaparin treatment led to a reduction in bone morphogenetic protein-2 (BMP-2), OC and RUNX2 mRNA expression. In contrast, in our experiment, there was no change in RUNX2 or OSX expression after dabigatran etexilate treatment.

Several mechanisms have been assessed to demonstrate how anticoagulants impair bone physiology. Experimental *in vivo* models have been proposed in order to explain how these agents may impair healing of fractures or may cause bone loss, [12, 14, 16, 26]. In a histomorphometric analysis comparing warfarin-treated rats and dabigatran-treated animals, an increase in osteoclast activity was observed when warfarin was used [12]. Klüter et al*.* [16] have demonstrated that there was no

significant differences between the control and rivaroxaban groups in femur bone healing. Despite inconclusive results from the limited number of *in vivo* models with DOACs, there is a consensus from *in vivo* studies on bone metabolism that heparins reduce bone mineral mass [31] and increase bone resorption, as a consequence of the increase of both osteoclast number and activity [26]. The effects on osteoblasts, such as the inhibition of ALP activity and the impairment of OC carboxylation have been demonstrated in rats treated with heparin [26] and warfarin [14], thus strengthening the *in vitro* findings [17].

In summary, the findings of our *in vitro* and *ex vivo* models clearly demonstrated for the first time the negative impact of the prodrug dabigatran etexilate and its active principle, dabigatran, on osteoblasts and osteoclasts. Further *in vivo* drug-comparative studies are encouraged to disclose the response of the set of cells in tissues or organs. The present study provides new evidence about the potential negative impact of dabigatran etexilate on bone turnover and repair.

ACKNOWLEDGMENTS

We wish to thank Mr. Roger R. Fernandes and Ms. Fabiola S. de Oliveira for technical assistance during the experiments. The Centro de Aquisição e Processamento de Imagens - CAPI (https://www2.icb.ufmg.br/capi/) at the Universidade Federal de Minas Gerais, Brazil is also acknowledged for providing the equipment and technical support for experiments involving data collection and image acquisition. The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001), Brazil. A.L.R. and J.A.A.A. are the recipients of scholarships. Mrs. E. Greene provided English editing of the manuscript.

REFERENCES

1. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest. 2008;*133:160-198.

2. Elad S, Marshall J, Meyerowitz C, Connolly G. Novel anticoagulants: general overview and practical considerations for dental practitioners. *Oral Dis. 2016;* 22:23-32.

3. Green B, Mendes RA, Van der Valk R, Brennan PA. Novel anticoagulants -an update on the latest developments and management for clinicians treating patients on these drugs. *J Oral Pathol Med. 2016;* 45:551-556.

4. Di Nisio M, Middeldorp S, Büller HR. Direct thrombin inhibitors. *N Engl J Med. 2005;* 353:1028-1040.

5. Stangier J, Clemens A. Pharmacology, pharmacokinetics, and pharmacodynamics of dabigatran etexilate, an oral direct thrombin inhibitor. *Clin Appl Thromb Hemost. 2009;* 15:9-16.

6. Hankey GJ, Eikelboom JW. Dabigatran etexilate: a new oral thrombin inhibitor. *Circulation. 2011;* 123:1436-1450.

7. Irie A, Takami M, Kubo H, Sekino-Suzuki N, Kasahara K, Sanai Y. Heparin enhances osteoclastic bone resorption by inhibiting osteoprotegerin activity. *Bone. 2007;* 41:165-174.

8. Ariyoshi W, Takahashi T, Kanno T, Ichimiya H, Shinmyouzu K, Takano H, Koseki T, Nishihara T. Heparin inhibits osteoclastic differentiation and function. *J Cell Biochem. 2008;* 103:1707-1717.

9. Mazziotti G, Canalis E, Giustina A. Drug-induced osteoporosis: mechanisms and clinical implications. *Am J Med. 2010;* 123:877-884.

10. Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest. 2012;* 141:24-43.

11. Gigi R, Salai M, Dolkart O, Chechik O, Katzburg S, Stern N, Somjen D. The effects of direct factor Xa inhibitor (Rivaroxaban) on the human osteoblastic cell line SaOS2. *Connect Tissue Res. 2012;* 53:446-450.

12. Fusaro M, Mereu MC, Aghi A, Iervasi G, Gallieni M. Differential Effects of Dabigatran and Warfarin on Bone Volume and Structure in Rats with Normal Renal Function. *PLoS ONE. 2015;* 10:1-15.

13. Winkler T, Perka C, Matziolis D, Matziolis G. Effect of a direct thrombin inhibitor compared with dalteparin and unfractionated heparin on human osteoblasts. *Open Orthop J. 2011;* 16:52-58.

14. Morishima Y, Kamisato C, Honda Y, Furugohri T, Shibano T. The effects of warfarin and edoxaban, an oral direct factor Xa inhibitor, on gammacarboxylated (Gla-osteocalcin) and undercarboxylated osteocalcin (uc-osteocalcin) in rats. *Thromb Res. 2013;* 131:59-63.

15. Somjen D, Katzburg S, Gigi R, Dolkart O, Sharon O, Salai M, Stern N. Rivaroxaban, a direct inhibitor of the coagulation factor Xa interferes with hormonal-induced physiological modulations in human female osteoblastic cell line SaSO2. *J Steroid Biochem Mol Biol. 2013;* 135:67-70.

16. Klüter T, Weuster M, Brüggemann S, Menzdorf L, Fitschen-Oestern S, Steubes and N, Acil Y, Pufe T, Varoga D, Seekamp A, Lippross S. Rivaroxaban does not impair fracture healing in a rat femur fracture model: an experimental study. *BMC Musculoskelet Disord. 2015*; 16:1-8.

17. Solayar GN, Walsh PM, Mulhall KJ. The effect of a new direct Factor Xa inhibitor on human osteoblasts: an in-vitro study comparing the effect of rivaroxaban with enoxaparin. *BMC Musculoskelet Disord. 2011*; 12:1-8.

18. Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk, CN, Frostick SP, Ka¨ lebo P, Christiansen AV, Hantel S, Hettiarachchi R, Schnee J, Bu¨ ller HR, for the RE-MODEL Study Group. Oral dabigatran etexilate vs. subcutaneous enoxaparin for the prevention of venous thromboembolism after total knee replacement: the RE-MODEL randomized trial. *J Thromb Haemost. 2007*; 5: 2178-85.

19. Bernardi RM, Fröehlich PE, Bergold AM. Development and validation of a stability-indicating liquid chromatography method for the determination of dabigatran etexilate in capsules. *Journal of AOAC International. 2013;* 96: 37-41.

20. Bellows CG, Aubin JE. Determination of numbers of osteoprogenitors present in isolated fetal rat calvaria cells in vitro. *Dev Biol. 1989*; 133:8-13.

21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods. 2001;* 25:402-408.

22. Rosa AL, Kato RB, Castro Raucci LM, Teixeira LN, de Oliveira FS, Bellesini LS, de Oliveira PT, Hassan MQ, Beloti MM. Nanotopography drives stem cell fate toward osteoblast differentiation through a1b1 integrin signaling pathway*. J Cell Biochem. 2014*; 115:540-548.

23. Pilge H, Fröbel J, Mrotzek SJ, Fischer JC, Prodinger PM, Zilkens C, Bittersohl B, Krauspe R. Effects of thromboprophylaxis on mesenchymal stromal cells during osteogenic differentiation: an in-vitro study comparing enoxaparin with rivaroxaban. *BMC Musculoskelet Disord. 2016;* 108:1-7.

24. Folwarczna J, Sliwiński L, Janiec W, Pikul M. Effects of standard heparin and low-molecular-weight heparins on the formation of murine osteoclasts in vitro. *Pharmacol Rep. 2005;* 57:635-645.

25. Li B, Lu D, Chen Y, Zhao M, Zuo L. Unfractionated Heparin Promotes Osteoclast Formation in Vitro by Inhibiting Osteoprotegerin Activity. *Int J Mol Sci. 2016;* 17:3-15.

26. Muir JM, Hirsh J, Weitz JI, Andrew M, Young E, Shaughnessy SG. A histomorphometric comparison of the effects of heparin and low-molecular-weight heparin on cancellous bone in rats. *Blood*. *1997;* 89: 3236-3242.

27. de Oliveira PT, Zalzal SF, Beloti MM, Rosa AL, Nanci A. Enhancement of in vitro osteogenesis on titanium by chemically produced nanotopography. *J Biomed Mater Res A*. *2007*; 80:554-564.

28. Kato RB, Roy B, De Oliveira FS, Ferraz EP, De Oliveira PT, Kemper AG, Hassan MQ, Rosa AL, Beloti MM. Nanotopography directs mesenchymal stem cells to osteoblast lineage through regulation of microRNA-SMAD-BMP-2 circuit. *J Cell Physiol*. *2014*; 229:1690-1696.

29. Castro-Raucci LMS, Francischini MS, Teixeira LN, Ferraz EP, Lopes HB, de Oliveira PT, Hassan MQ, Losa AL, Beloti MM. Titanium with nanotopography induces osteoblast differentiation by regulating endogenous bone morphogenetic protein expression and signaling pathway. *J Cell Biochem. 2016*; 117:1718-1726.

30. Souza ATP, Bezerra BLS, Oliveira FS, Freitas GP, Bighetti Trevisan RL, Oliveira PT, Rosa AL, Beloti MM. Effect of bone morphogenetic protein 9 on osteoblast differentiation of cells grown on titanium with nanotopography. *J Cell Biochem. 2018*; 119:8441-8449.

31. Mätzsch T, Bergqvist D, Hedner U, Nilsson B, Ostergaard P. Effects of low molecular weight heparin and unfragmented heparin on induction of osteoporosis in rats. *Thromb Haemost. 1990;* 28;63:505-509.

Figure 1

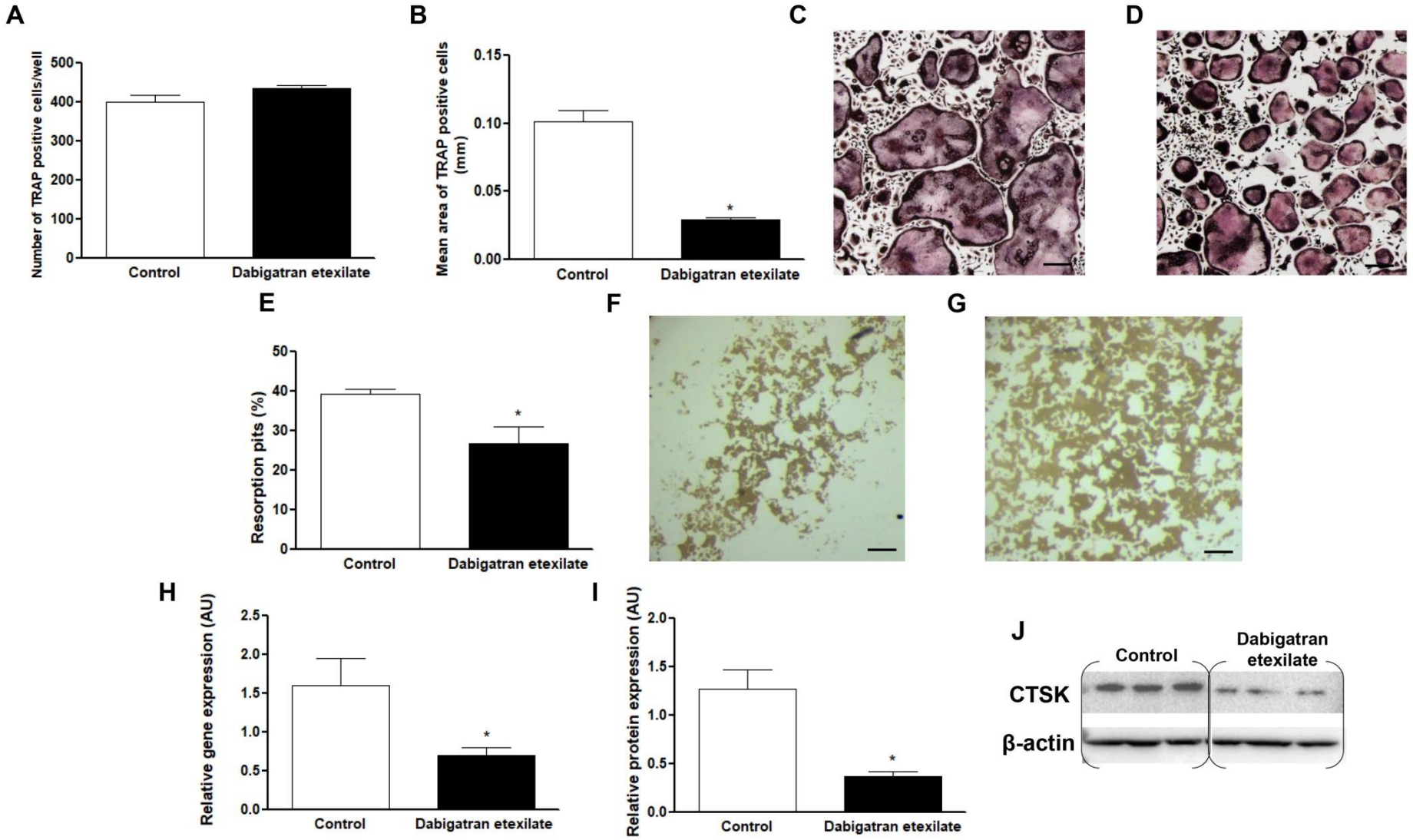

A
Number of TRAP positive cells/well
500
400
300
200
100
0
Control
Dabigatran etexilate
B
Mean area of TRAP positive cells (mm)
0.15
0.10
0.05
0.00
*
Control
Dabigatran etexilate
C
D
E
Resorption pits (%)
50
40
30
20
10
0
*
Control
Dabigatran etexilate
F
G
H
Relative gene expression (AU)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Dabigatran etexilate
I
Relative protein expression (AU)
2.0
1.5
1.0
0.5
0.0
*
Control
Dabigatran etexilate
J
Control
Dabigatran etexilate
CTSK
β-actin

Figure 1. Assessment of the differentiation of bone marrow cell (BMC)-derived osteoclasts from mice treated with dabigatran etexilate administered by gavage twice daily for 28 days. Number **(A)** and mean area **(B)** of tartrate-resistant acid phosphatase (TRAP)-positive cells identified as osteoclasts after five days of culture. TRAP+ cells of non-treated animals **(C)**, and of animals treated with dabigatran etexilate **(D)**. Resorptive activity was measured by the pit formation area in the osteoassay microplate after 10 days of culture **(E)**. Resorption pit of non-treated animals **(F)**, and of animals treated with dabigatran etexilate **(G)**. For the analysis of gene expression and protein levels of cathepsin K, an osteoclast marker, the total cell lysates were subjected to real-time polymerase chain reaction **(H)** and western blot analysis **(I, J)** after three days of treatment. Data are reported as mean and expressed in relation to the control β-Actin and the constitutive gene glycer-aldehyde-3phosphate dehydrogenase (GAPDH), respectively. Statistical analysis was performed by the Student *t*-test. **p*<0.05 compared to control. Acquisition of images with the Cytation Cell Imaging Multi-Mode Reader (BioTek, Winooski, Vermont, USA). Bands were quantified using The ImageJ Software and the Leica Application Suite for pit area determination using contouring tools. AU, arbitrary unit; scale bars = 40 μM.

Figure 2

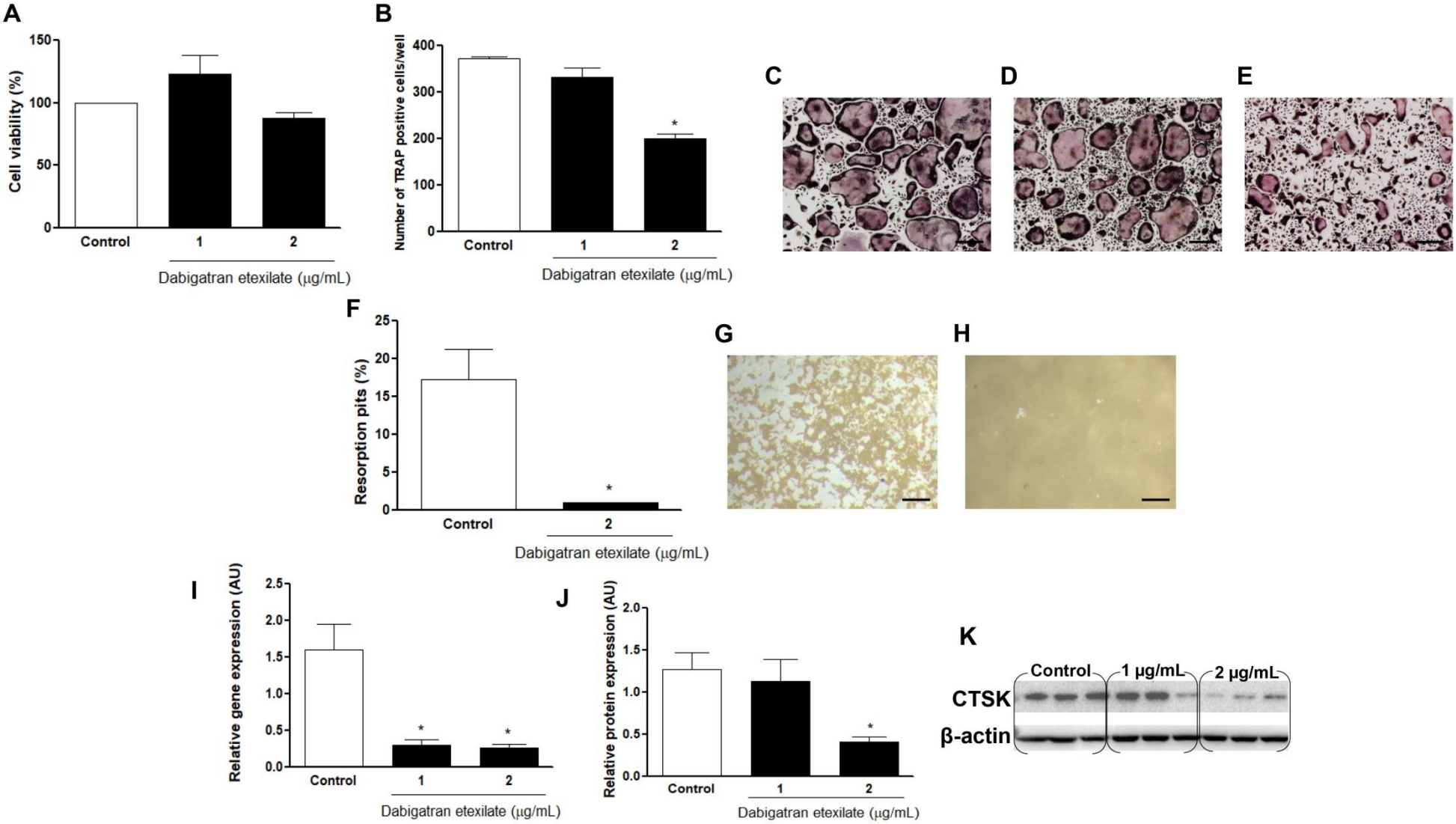

A
Cell viability (%)
150
100
50
0
Control
1
2
Dabigatran etexilate (μg/mL)
B
Number of TRAP positive cells/well
400
300
200
100
0
*
Control
1
2
Dabigatran etexilate (μg/mL)
C
D
E
F
Resorption pits (%)
25
20
15
10
5
0
*
Control
2
Dabigatran etexilate (μg/mL)
G
H
I
Relative gene expression (AU)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Control
1
2
Dabigatran etexilate (μg/mL)
J
Relative protein expression (AU)
2.0
1.5
1.0
0.5
0.0
*
Control
1
2
Dabigatran etexilate (μg/mL)
K
Control
1 μg/mL
2 μg/mL
CTSK
β-actin

Figure 2. Effect of dabigatran etexilate (1 μg/mL and 2 μg/mL) on the viability and differentiation of BMC-derived osteoclasts. Results of the optical density values of the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay at three days of treatment expressed as percentage relative to the absorbance of the control **(A)**. Number of tartrate-resistant acid phosphatase (TRAP)-positive cells after five days of culture **(B)**. TRAP+ cells of the control group **(C)** and of the group treated with dabigatran etexilate at 1 μg/mL **(D)** and 2 μg/mL **(E)**. Resorptive activity was measured by the pit formation area after 10 days of treatment **(F)**. Resorption pit of the control group **(G)** and of the group treated with dabigatran etexilate at 2 μg/mL **(H)**. Gene **(I)** and protein expression **(J, K)** of cathepsin K (CTSK) after three days of treatment. Results were reported in relation to the control β-Actin and to the constitutive gene glycer-aldehyde-3-phosphate dehydrogenase (GAPDH), respectively. Data are reported as mean. Statistical analysis was performed by the Student *t*-test. **p*<0.05 compared to control. AU, arbitrary unit; scale bars = 40 μM.

Figure 3

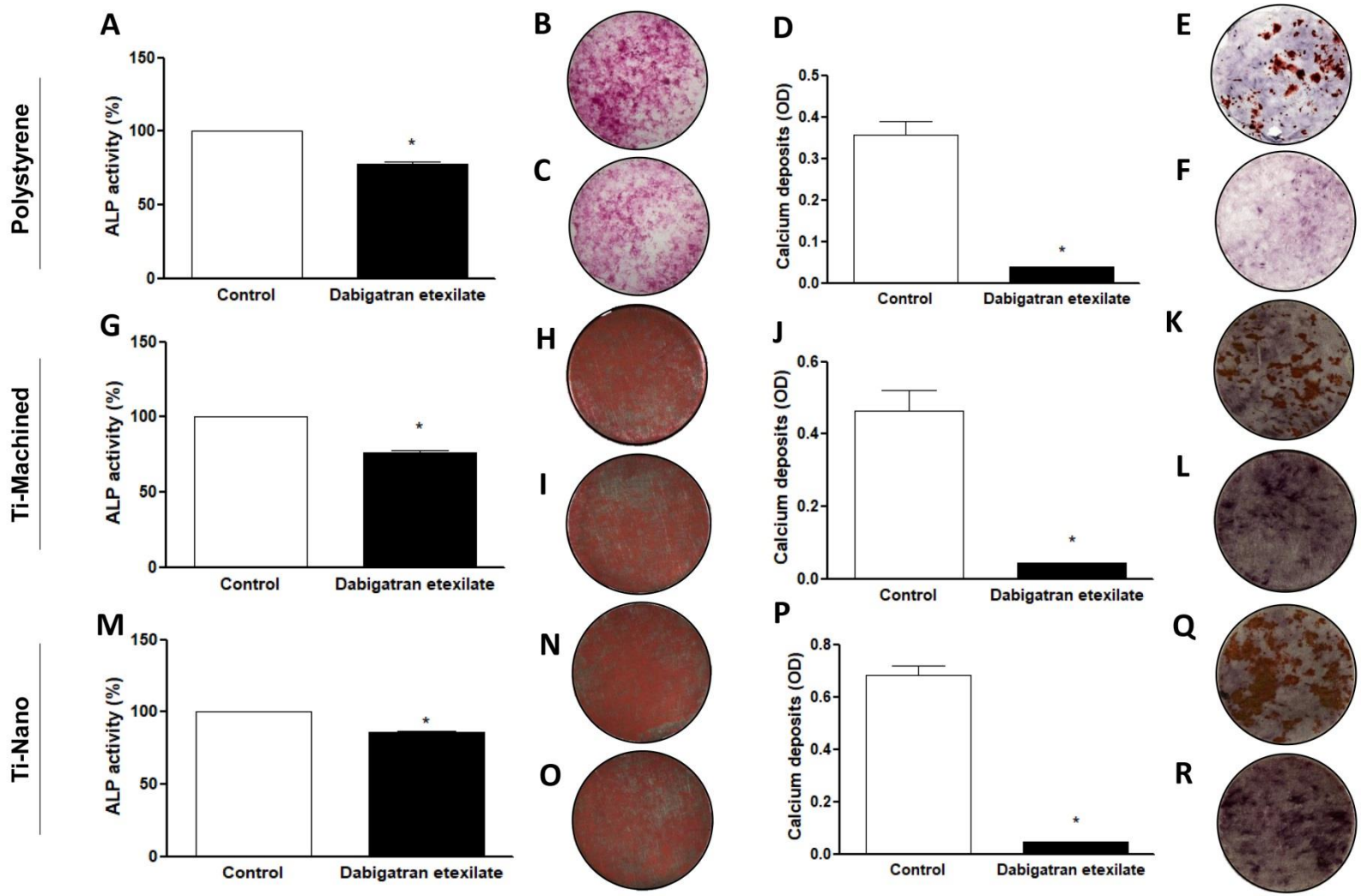

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
Polystyrene
Ti-Machined
Ti-Nano
ALP activity (%)
Calcium deposits (OD)
Control
Dabigatran etexilate
*

Figure 3. Effect of dabigatran etexilate (2 μg/mL) on the differentiation of calvaria-derived osteoblasts grown on polystyrene, titanium (Ti) with nanotopography (Ti-Nano) or machined Ti (Ti-Machined). *In situ* alkaline phosphatase (ALP) activity measured by percent stained area after seven days of treatment of cells grown on polystyrene **(A).** Stained area of *in situ* ALP activity of the control group **(B)** and of th dabigatran etexilate group **(C).** Extracellular matrix mineralization measured by optical density (OD) values of calcium deposit mineralization after 14 days of treatment of cells grown on polystyrene **(D).** Alizarin red-stained calcium deposits in the control group **(E)** and in the dabigatran etexilate group **(F)**. *In situ* ALP activity of cells grown on Ti-Machined **(G),** stained ALP area in the control group **(H)** and in the dabigatran etexilate group **(I).** Extracellular matrix mineralization of cells grown on Ti-Machined **(J)**, alizarin red**-**stained calcium deposits in the control group **(K)** and the dabigatran etexilate group **(L).** *In situ* ALP activity of cells grown on Ti-Nano **(M),** stained ALP area in the control group **(N)** and in the dabigatran etexilate group **(O).** Extracellular matrix mineralization of cells grown on Ti-Nano **(P)**, alizarin red**-**stained calcium deposits in the control group **(Q)** and in the dabigatran etexilate group **(R).** Statistical analysis was performed by the Student *t*-test. **p*<0.05 compared to control.

Figure 4

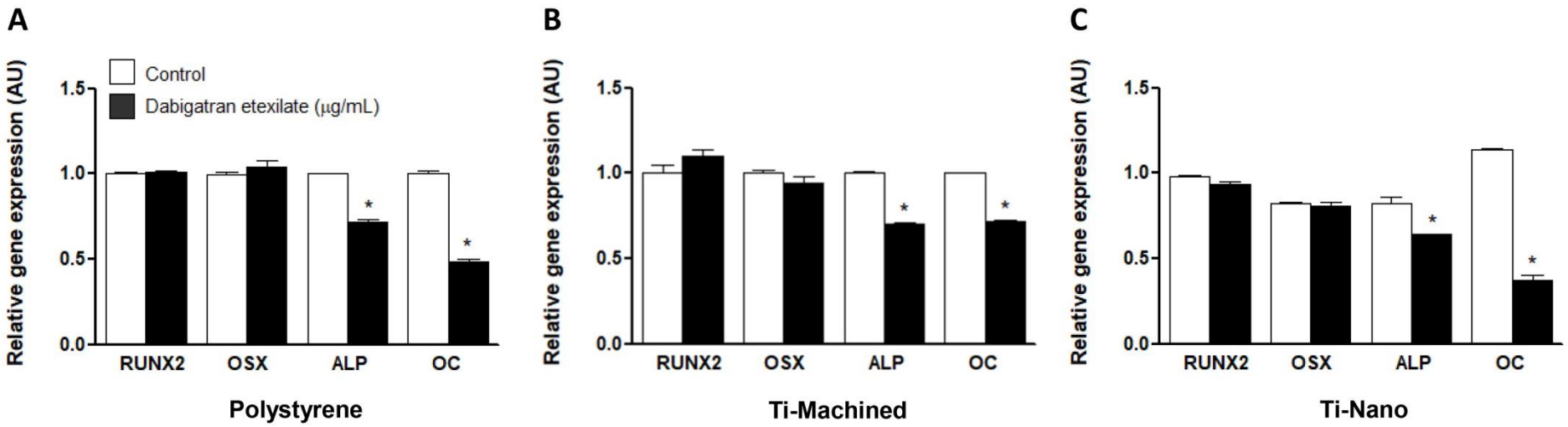
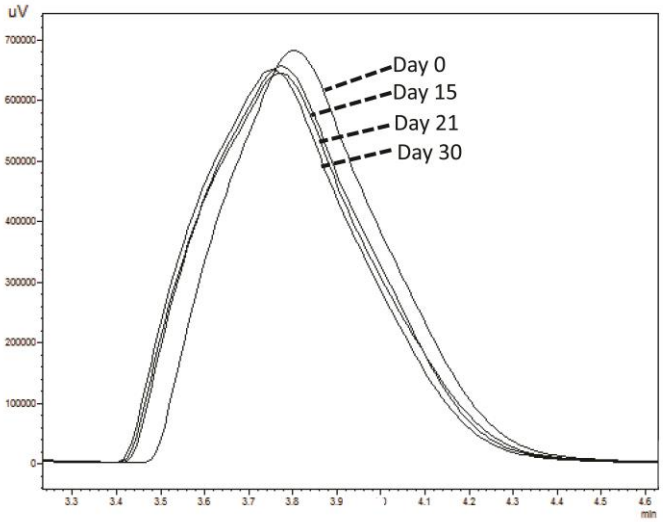


Figure 4. Gene expression of the bone markers runt-related transcription factor 2 (RUNX2), osterix (OSX), alkaline phosphatase (ALP), and osteocalcin (OC) in calvaria-derived osteoblasts treated with dabigatran etexilate (2 μg/mL) and grown on polystyrene **(A)**, machined titanium (Ti-Machined) **(B)** or Ti with nanotopography (Ti-Nano) **(C)**. Data are reported as mean and expressed as fold change in relation to the constitutive gene glycer-aldehyde-3-phosphate dehydrogenase (GAPDH). Statistical analysis was performed by the Student *t*-test. **p*<0.05 indicating a statistically significant difference between control and dabigatran etexilate (2 μg/mL) for each gene evaluated gene. AU, arbitrary unit.

Supplementary Figure 1

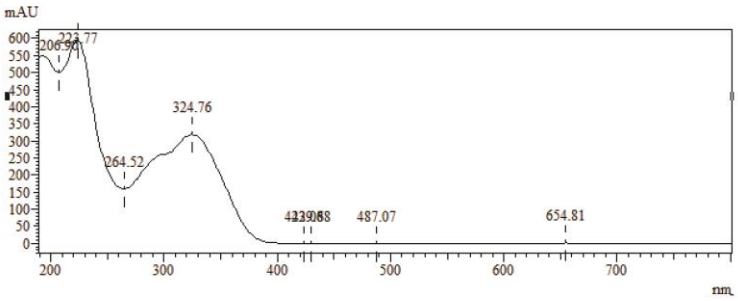
A



B

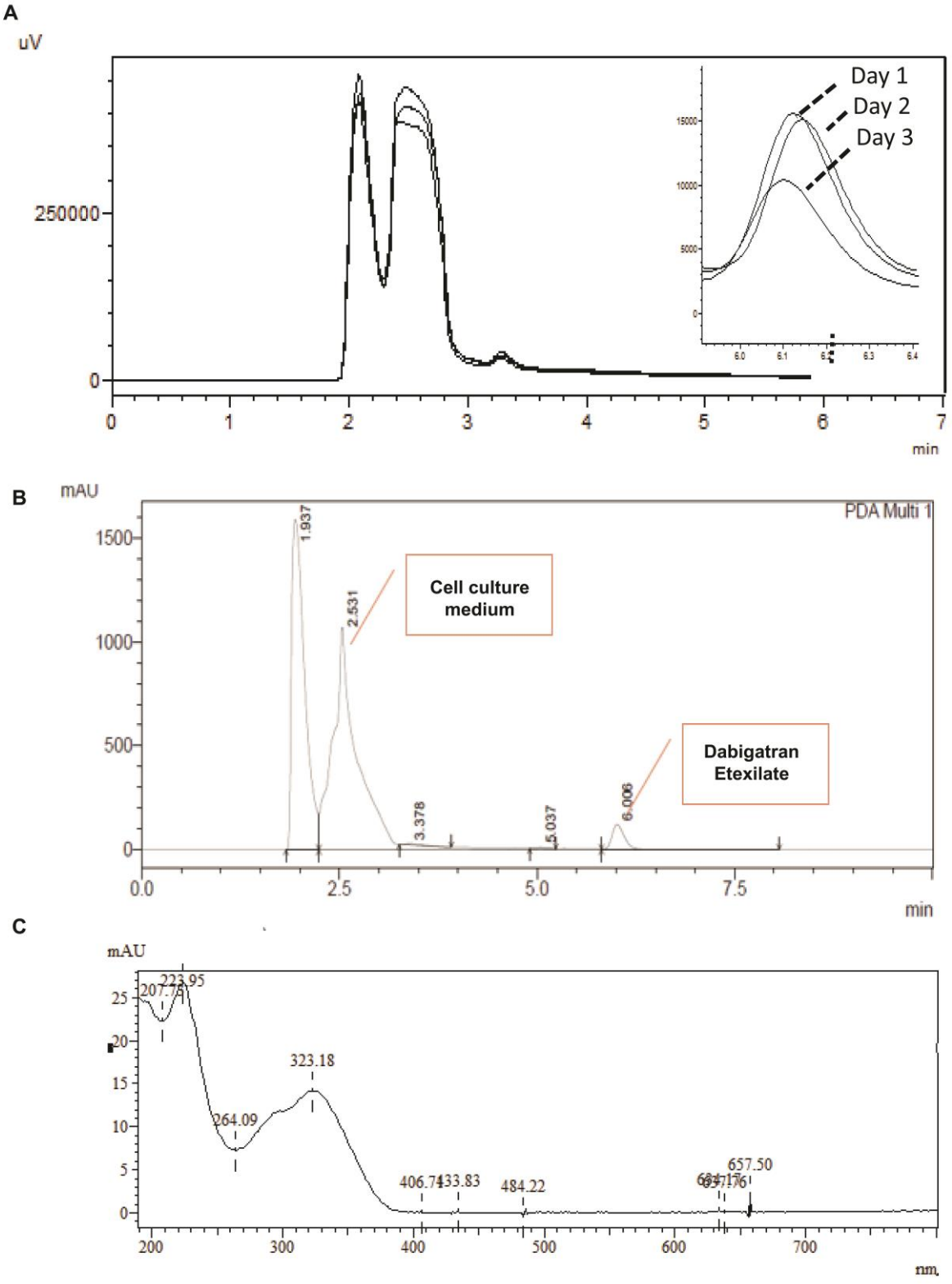
Sample (day)	Peak AUC (mean ± SD)	%
0	17940062 ± 1.30	100.00
15	17790954 ± 1.15	99.16
21	17665246 ± 0.35	98.46
30	17654096 ± 1.57	98.40

C



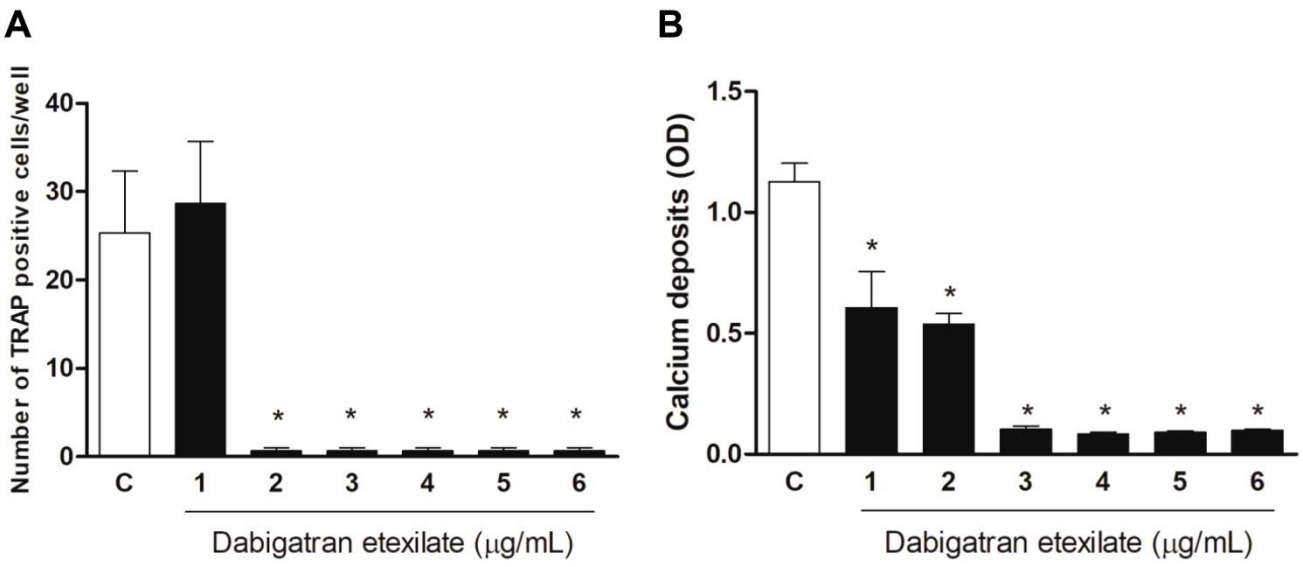
Supplementary Figure 1. Stability analysis of dabigatran etexilate stock solution (100 µg/mL) at day 0, 15, 21 and 30, stored at 2-8°C **(A)**. The parameter was evaluated by high-performance liquid chromatography (HPLC) and presented as the mean area under the curve (AUC) of three samples and percentage relative to day 0 **(B)**. Ultraviolet (UV) scanning spectrum of the dabigatran etexilate interest peak and characteristic bands of dabigatran etexilate at 223 nm and 324 nm **(C).** HPLC parameters: the detector was set at 225 nm and peak areas were integrated automatically by a computer using Shimadzu Class VP V6.12 software. The HPLC system was operated isocratically at room temperature using a mobile phase consisting of acetonitrile and a solution of 0.1% triethylamine, pH 6.0, adjusted with phosphoric acid (65 + 35, v/v). The solution was then filtered through a 0.45 µm membrane filter and run at a flow rate of 1.0 mL/min. The injection volume was 50 µL.

Supplementary Figure 2



Supplementary Figure 2. Analytical aspects of the dabigatran etexilate (Pradaxa®) solution. Time course analysis of dabigatran etexilate at 6 µg/mL in cell culture growth medium. The decrease of the peak area suggests the conversion of dabigatran etexilate in the orally available form to its active moiety, dabigatran **(A)**. Chromatogram of a dabigatran etexilate solution (Pradaxa®, at 100 µg/mL) sample in cell culture growth medium (alpha minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum). The interest peak of dabigatran etexilate was detected next to the retention time of 6.0 minutes **(B)**. Ultraviolet (UV) scanning spectrum of the dabigatran etexilate interest peak and characteristic bands of dabigatran etexilate at 223 nm and 323 nm **(C)**. The analysis was performed by high-performance liquid chromatography (HPLC). HPLC parameters: the detector was set at 225 nm, and peak areas were integrated automatically by a computer using Shimadzu Class VP V6.12 software. The LC system was operated isocratically at room temperature using a mobile phase consisting of acetonitrile and a solution of 0.1% triethylamine, pH 6.0, adjusted with phosphoric acid (65 + 35, v/v). The solution was then filtered through a 0.45 µm membrane filter and run at a flow rate of 1.0 mL/min, and the injection volume was 50 µL.

Supplementary Figure 3.



Supplementary Figure 3. Dose-response effect of dabigatran etexilate (1-6 μg/mL) on the differentiation of BMCs-derived osteoclasts and MC3T3-E1 osteoblasts. Number of tartrate-resistant acid phosphatase (TRAP)-positive cells identified as osteoclasts counted after five days of culture **(A)**. Optical density (OD) values of solubilized calcium deposits after 14 days of treatment **(B)**. Data are reported as mean. Statistical analysis was performed by the Student *t*-test. **p*<0.05 compared to control.

5 CONCLUSÕES

Pelos resultados deste trabalho, podemos concluir que:

Os desfechos relacionados ao sangramento intraoperatório, bem como as complicações hemorrágicas pós-operatórias foram semelhantes entre os grupos AVK, DOACs e sem anticoagulação o que reforça a não suspensão da terapia anticoagulante oral para a realização de exodontias. Ainda que eventos raros, as complicações hemorrágicas podem ser minimizadas por meio da utilização de medidas hemostáticas locais bem como a monitorização cuidadosa dos parâmetros reconhecidamente associados a tendências para sangramento.

O tratamento sobre culturas celulares utilizando etexilato de dabigatrana impactou negativamente as células ósseas, reduzindo a diferenciação e atividade de osteoclastos e osteoblastos.

REFERÊNCIAS

ADA Science Institute. Anticoagulant and Antiplatelet Medications and Dental Procedures. Disponível em: <https://www.ada.org/en/member-center/oral-health-topics/anticoagulant antiplatelet-medications-and-dental->. Acesso em: 17 Jan. 2019.

AGENO, W. *et al*. Oral anticoagulant therapy - Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. **Chest**, v. 141, n.2, p. 44S-88S, Feb. 2012.

ANSELL, J. *et al.* The Pharmacology and Management of the Vitamin K Antagonists. **Chest**, v. 126, n. 3, p. 204S–233S, Set. 2004.

ANVISA. Ministério da Saúde. Novo anticoagulante oral é aprovado no Brasil. Disponível em:<http:/portal.anvisa.gov.br/rss//asset_publisher/Zk4q6UQCj9Pn/content/id/413620>. Acesso em: 17 Jan. 2019.

ARIYOSHI, W. *et al.* Heparin inhibits osteoclastic differentiation and function. **J Cell Biochem.** v, 103, n. 6, p. 1707-1017, Apr. 2008.

Comissão Nacional de Incorporação de Tecnologias no SUS (CONITEC). Apixabana, rivoraxabana e dabigratana em pacientes com fibrilação atrial não valvar: Relatório de recomendação. n.195. Distrito Federal. 2016.

BARTOLOTTO, L. A.; NEVES, I. L. I.; MONTANO, T. C. P. Cardiopatias: Complexidades envolvidas com procedimentos odontológicos. In: SANTOS, P. S.; SOARES, L. A. V. **Medicina Bucal:** A prática na odontologia hospitalar. São Paulo: Santos, 2012. p.157-182.

BAJKIN, B. V; POPOVIC, S. L.; SELAKOVIC, S. D. Randomized, prospective trial comparing bridging therapy using low-molecular-weight heparin with maintenance of oral anticoagulation during extraction of teeth. **J Oral Maxillofac Surg**. v. 67, n. 5, p.990-995, May. 2009.

BAJKIN, B. V.; BAJKIN I. A.; PETROVIC B. B. The effects of combined oral anticoagulant–aspirin therapy in patients undergoing tooth extractions: A prospective study. **JADA**. v. 143, n. 7,p.770-776, Jul. 2012.

BAUERSACHS, R. *et al*. Oral rivaroxaban for symptomatic venous thromboembolism. **N Engl J Med.** v. 363, n. 26, p.2499-2510, Dec. 2010.

BELLOWS, C.G.; AUBIN J. E. Determination of numbers of osteoprogenitors present in isolated fetal rat calvaria cells in vitro. **Dev Biol**; v. 133, p.8-13, Nov. 1989.

BENSI, C. *et al.*Postoperative bleeding risk of direct oral anticoagulants after oral surgery procedures: a systematic review and meta-analysis. **Int J Oral Maxillofac Surg**. v. 47, n. 7, p.923-932, Apr. 2018.

BERTON, F. *et al*. Should we fear direct oral anticoagulants more than vitamin K antagonists in simple single tooth extraction? A prospective comparative study. **Clin Oral Investig**. v.22, n. 8, p.1-10, Nov. 2018.

DAVIE, E. W.; RATNOFF, O. D. Waterfall sequence for intrinsic blood clotting. **Science**. v. 145, p.1310-2, Jan. 1964.

DOUKETIS, J. D. *et al*. Perioperative management of antithrombotic therapy. Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. **Chest**, v. 141, n. 2, p. 326S-350S, Feb. 2012.

DOWNIE, W. W. *et al.* Studies with pain rating scales. **Ann Rheum Dis**. v. 37, n. 4, p. 378-381, Aug. 1978.

DUDEK, D. *et al*. Bleeding Rate After Tooth Extraction in Patients Under Oral Anticoagulant Therapy. **J Craniofac Surg**. v. 27, n.5, p. 1228-1233, Jul. 2016.

FAKHRI, H. R. *et al.*Tutorial in oral antithrombotic therapy: Biology and dental implications.**Med Oral Patol Oral Cir Bucal**. v. 18, n. 3, p.461-72, Jan. 2013.

FEBBO, A. *et al*. Postoperative Bleeding Following Dental Extractions in Patients Anticoagulated With Warfarin. **J Oral Maxillofac Surg**. V. 14, n. 8, p. 1518-1523, Aug. 2016.

FOLWARCZNA, J. *et al*. Effects of standard heparin and low-molecular-weight heparins on the formation of murine osteoclasts in vitro. **Pharmacol Rep.** v. 57, n. 5, p. 635-645, Sep. 2005.

FORTIER, K.; SHROFF, D.; REEBYE, U. N. Review: An overview and analysis of novel oral anticoagulants and their dental implications. **Gerodontology**, v. 35, n. 2 p. 1–9, Jun. 2018.

FUSARO, M. *et al.* Vitamin K and bone. **Clin Cases Miner Bone Metab**. v. 14, n. 2, p. 200-206, May-Aug. 2017.

GARCIA, D. *et al.* Management and clinical outcomes in patients treated with apixaban vs warfarin undergoing procedures. **Blood**. v. 124, n. 25, p. 3692-3698, Dec. 2014.

GARCIA, D. A. *et al.* Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. **Chest**. v. 141, n. 2, p. 24S-43S, Feb. 2012.

GIGI, R. *et al.* The effects of direct factor Xa inhibitor (Rivaroxaban) on the human osteoblastic cell line SaOS2. **Connect Tissue Res.** v. 53, n. 6, p. 446-450, Aug. 2012.

HEALEY, J. S. *et al*. Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) randomized trial. **Circulation**. v. 126, n. 3, p. 343-348, Jul. 2012.

HOFFMAN, M.; MONROE, M. D. A cell-based model pf hemostasis. **Thromb. Haemost.** v. 85, p.958-965, Feb. 2001.

HONG, C. *et al.* Risk of postoperative bleeding after dental procedures in patients on warfarin: a restrospective study. **Oral Surg Oral Med Oral Pathol Oral Radiol.** v. 114, n. 4, p.464-468, Oct. 2012.

IRIE, A. *et al.* Heparin enhances osteoclastic bone resorption by inhibiting osteoprotegerin activity. **Bone**. v. 41, n. 2, p. 165-174, Aug. 2007.

KLÜTER, T. *et al.* Rivaroxaban does not impair fracture healing in a rat femur fracture model: an experimental study. **BMC Musculoskelet Disord**. v. 16, n. 79, p. 1-8, Apr. 2015.

LARAGNOIT, A. B. *et al.* Locoregional anesthesia for dental treatment in cardiac patients: a comparative study of 2% plain lidocaine and 2% lidocaine with epinephrine (1:100,000). **Clinics**. v. 64, n. 3, p. 177-182, 2009.

LITTLE, J. W. The impact on dentistry of recent advances in the management of hypertension. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod.** v. 90, n. 5, p. 591-9, Jan. 2000.

LITTLE, J. W. New oral anticoagulants: Will they replace warfarin? **Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology**. v. 113, n. 5, p. 575–580, May. 2012.

LOCKHART, P. B. *et al*. Dental management considerationd for the patient with an acquired coagulopathy. Part 2: Coagulopathies from drugs. **Br Dent J**. v. 195, n. 9, p. 495-501, Nov. 2003.

MACFARLANE, R. G. An enzyme cascade in the blood clotting mechanism, and its function as a biological amplifier. **Nature**. v. 202, p.498-9, Apr. 1964.

MAUPRIVEZ, C. *et al*. Management of dental extraction in patients undergoing anticoagulant oral direct treatment: a pilot study. **Oral Surg Oral Med Oral Pathol Oral Radiol.** v. 122, n. 5, p. 146-455, Nov. 2016.

MAZZIOTTI, G., CANALIS, E., GIUSTINA, A. Drug-induced osteoporosis: mechanisms and clinical implications. **Am J Med**. v. 123, n. 10, p. 877-884, Oct. 2010.

MICLOTTE, I. *et al*. Pragmatic approach to manage new oral anticoagulants in patients undergoing dental extractions: a prospective case-control study. **Clin Oral Investig**. v. 21, n. 7, p. 2183-2188, Sep. 2017.

MILLER, S.G.; MILLER, C.S. Direct oral anticoagulants: A retrospective study of bleeding, behavior, and documentation. **Oral Dis**. v. 24, n. 1-2, p. 243-248, Mar. 2018.

MIRANDA, M. *et al.* Differences between warfarin and new oral anticoagulants in dental clinical practice. **Oral Implantol**. v. 9, n. 3, p. 151-156, Nov. 2016.

MORIMOTO, Y.; NIWA, H.; MINEMASTU, K. Risk Factors Affecting Postoperative Hemorrhage After Tooth Extraction in Patients Receiving Oral Antithrombotic Therapy. **J Oral Maxillofac Sur**. v. 69, n. 6, p.1550-1556, Jun. 2011.

MORISHIMA, Y. *et al.* The effects of warfarin and edoxaban, an oral direct factor Xa inhibitor, on gammacarboxylated (Gla-osteocalcin) and undercarboxylated osteocalcin (uc-osteocalcin) in rats. **Thromb Res.** v. 131, n. 1, p. 59-63, Jan. 2013.

NATHWANI, S.; WANIS, C. Novel oral anticoagulants and exodontia: the evidence. **Br Dent J***.* v. 222, n. 8, p. 623-628, Apr. 2017.
NEVES, R. S. *et al*. Effects of Epinephrine in Local Dental Anesthesia in Patients with Coronary Artery Disease. **Arq Bras Cardiol**. v. 88, n. 5, p. 482-487, 2007.

NISHIMURA, R. A. *et al*. 2014 AHA/ACC guideline for the management of patients with valvular heart disease. a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. **Circulation**, v. 129, n. 23, p. 1–96, Jul. 2014.

ROCHA, A.L. *et al.* Oral surgery in patients under antithrombotic therapy: perioperative bleeding as a significant risk factor for postoperative hemorrhage. **Blood Coagul Fibrinolysis**. v. 29, n. 1, p. 97-103, Jan. 2018.

ROSER, S. M.; ROSENBLOOM, B. Continued anticoagulation in oral surgery procedures. **Oral Surg Oral Med Oral Pathol**. v. 40, n. 4, p. 448-457, Oct. 1975.

SCHULMAN, S. *et al*. RE-COVER Study Group. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. **N Engl J Med**. v. 361, p. 2342-2352, Dec. 2009.

SHENOY, K. V. *et al*. The Effects of Topical Hemocoagulase Solution on the Healing Process of Post-extraction Wounds: A Split Mouth Design. ***J. Maxillofac*** **Oral Surg.** v. 14, n. 3, p. 586-93, Sep. 2015.

SOLAYAR, G. N.; WALSH, P. M.; MULHALL, K. J. The effect of a new direct Factor Xa inhibitor on human osteoblasts: an in-vitro study comparing the effect of rivaroxaban with enoxaparin. **BMC Musculoskelet Disord**. v. 247, n. 12, p. 1-8, Oct. 2011.

SOMJEN, D. *et al.* Rivaroxaban, a direct inhibitor of the coagulation factor Xa interferes with hormonal-induced physiological modulations in human female osteoblastic cell line SaSO2. **J Steroid Biochem Mol Biol.** v. 135, p. 67-70, May. 2013.

TANAKA, A. K.; KEY, N. S.; LEVY, J. H. Blood coagulation: Hemostasias and trombin regulation. **Anesthesia & Analgesia**. v. 108. n.5, p.1433-1446, Mar. 2009.

ZIMERMAN, L. I. *et al*. Sociedade Brasileira de Cardiologia. Diretrizes Brasileiras de Fibrilação Atrial**. Arq Bras Cardiol,** v. 92, n. 6, p. 1-39, 2009.

WAHL, M. J. Dental surgery in anticoagulated patients. **Arch Intern Med**, v. 158, n. 15, p.1610-1616, Aug. 1998.

WINKLER, T. *et al.* Effect of a direct thrombin inhibitor compared with dalteparin and unfractionated heparin on human osteoblasts. **Open Orthop J.** v. 16, n. 5, p. 52-58, Mar. 2011.

APÊNDICE A – Termo de Consentimento Livre e Esclarecido

Você está sendo convidado (a) a participar, como voluntário (a), de uma pesquisa. Após ser esclarecido (a) sobre as informações, se aceitar fazer parte da pesquisa, assine este documento, que está em duas vias. Uma delas é sua e a outra é da pesquisadora responsável. Caso não aceitar participar, você não será penalizado (a) de forma alguma. Em caso de dúvida, você poderá procurar o Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6677, Unidade Administrativa II, 2º andar, sala 2005; 3409-4592).

INFORMAÇÕES SOBRE A PESQUISA

Título da Pesquisa: AVALIAÇÃO DO RISCO DE SANGRAMENTO E COMPLICAÇÕES PÓS-OPERATÓRIAS EM PACIENTES EM USO DE ANTICOAGULANTE ORAL SUBMETIDOS À EXODONTIAS

Pacientes em uso de anticoagulantes orais (Varfarina, Marevan®, Rivaroxabana, Xarelto®, Dabigatrana, Pradaxa®, Apixabana, Eliquis® Edoxabana, Lixiana®) frequentemente enfrentam dificuldades em conseguir tratamento odontológico, principalmente para realização de cirurgia (extrações de dentes). O tratamento dentário é cercado de mitos sobre do risco de complicações durante e após a cirurgia, principalmente o risco de hemorragias. Atualmente, já se sabe que parar o uso da medicação pode causar problemas sérios à saúde do paciente. Por isso, o recomendado é que seja feito o tratamento odontológico sem a necessidade de parar a medicação, desde que seu RNI esteja adequado.

Para melhorar e facilitar o atendimento aos pacientes que usam anticoagulantes, você está sendo convidado a participar de uma pesquisa que tem como objetivo:

- Avaliar o risco de sangramento aumentado em pacientes que utilizam anticoagulantes orais e necessitam de extração dentária.
- O seu tratamento odontológico será realizado normalmente no consultório odontológico do Ambulatório Borges da Costa. Todas as medidas para evitar sangramentos e hemorragias serão utilizadas. Alguns dados serão coletados durante o tratamento e você deverá retornar após 7 dias para retirada dos pontos e reavaliação.
- Os riscos à sua saúde serão os mesmos caso você não estivesse participando da pesquisa.

Pesquisadores responsáveis: Profª Tarcília Aparecida da Silva, Profª Denise Vieira Travassos e a Cirurgiã Dentista Amanda Leal Rocha, vinculadas à Faculdade de Odontologia da Universidade Federal de Minas Gerais (FO-UFMG).

Telefones para contato: (31) 3409-9191/ (31) 8839-1959 - Amanda Leal

(31) 3409-2478 - Profª Tarcília Silva

Você não pagará nada para participar da pesquisa. Sua participação é voluntária. Os pesquisadores garantem a você que todas as informações que você der serão sigilosas. Além disso, você pode cancelar sua participação a qualquer momento e isto não vai prejudicar o seu tratamento. Os resultados do estudo serão divulgados por meio de apresentação em congressos ou publicação em revistas médicas ou odontológicas e sua identidade não será divulgada de forma alguma.

CONSENTIMENTO DA PARTICIPAÇÃO

Eu, _____, RG/ CPF nº _____, abaixo assinado, concordo em participar do estudo acima especificado como sujeito. Fui devidamente informado e esclarecido pela pesquisadora responsável sobre a pesquisa, os procedimentos nela envolvidos, assim como os possíveis riscos e benefícios decorrentes de minha participação. Foi-me garantido que posso retirar meu consentimento a qualquer momento, sem que isto leve a qualquer penalidade ou interrupção de meu acompanhamento/ assistência/ tratamento.

Belo Horizonte, _____de _____ de 20_____.

Paciente participante: _____

Pesquisadores: _____.

APÊNDICE B - Ficha de avaliação clínica

FICHA DE AVALIAÇÃO CLÍNICA ☐ GRUPO 1 ☐GRUPO 2 ☐GRUPO 3

NOME: NÚMERO:

DATA DE NASCIMENTO: ___/___/___ SEXO: ☐FEMININO ☐MASCULINO

TELEFONE DE CONTATO: Data do procedimento: __/__/___

INDICAÇÃO PARA ANTICOAGULAÇÃO/COMORBIDADES:

☐ FIBRILAÇÃO ATRIAL

☐PRÓTESE METÁLICA ☐PRÓTESE BIOLÓGICA: ☐aórtica ☐mitral

☐TROMBOEMBOLISMO PULMONAR

☐TROMBOSE VENOSA PROFUNDA

☐ACIDENTE VASCULAR ENCEFÁLICO ISQUÊMICO

☐VALVOPATIA

☐OUTROS

HISTÓRIA PRÉVIA DE EPISÓDIOS SANGRAMENTO:

JÁ APRESENTOU SANGRAMENTO AUMENTADO APÓS TRATAMENTOS ODONTOLÓGICOS:

☐SIM ☐NÃO

Se sim:

☐Sem complicações;

☐Foi necessário contato telefônico com o cirurgião dentista, por iniciativa do paciente;

☐Foi necessário retornar ao consultório odontológico;

☐Foi necessário procurar serviço de emergência hospitalar

JÁ APRESENTOU SANGRAMENTO AUMENTADO APÓS CORTES OU PROCEDIMENTOS CIRÚRGICOS:

☐SIM ☐NÃO

Se sim:

☐Sem complicações;

☐Foi necessário procurar serviço de emergência hospitalar

TEM CASOS NA FAMÍLIA (PAIS OU IRMÃOS) DE SANGRAMENTO AUMENTADO APÓS CIRURGIAS:

☐SIM ☐NÃO

CONTAGEM DE PLAQUETAS: _____mm^3 DATA DO EXAME:

HEMATÓCRITO:_____ DATA DO EXAME:

RNI: _____DATA DO EXAME:

ABSORBÂNCIA: _____

MEDICAMENTOS EM USO

☐ANTIGOAGULANTE ORAL:

☐VARFARINA – REGIME TERAPÊUTICO:_____

☐RIVAROXABANA- REGIME TERAPÊUTICO:_____

☐DABIGATRANA- REGIME TERAPÊUTICO:_____

☐APIXABANA- REGIME TERAPÊUTICO:_____

☐EDOXABANA REGIME TERAPÊUTICO:_____

☐ANTIAGREGANTE PLAQUETÁRIO:☐AAS ☐CLOPIDROGREL ☐TICLOPIDINA ☐DIPIRIDAMOL

☐ANTIINFLAMATÓRIOS:☐NÃO ESTEROIDAL☐NÃO ESTEROIDAL INIBIDOR SELETIVO DE COX-2

☐INIBIDORES SELETIVOS DA RECAPTAÇÃO DE SEROTONINA

☐OUTROS:_____

_____

_____

PROCEDIMENTO ODONTOLÓGICO (**EXODONTIA)**:

☐ 1 DENTE ____ INDICAÇÃO:

☐2 DENTES___ , ____ INDICAÇÃO:

☐3 OU + DENTES___ , ___ , ___ , ___ , ___ INDICAÇÃO:

SANGRAMENTO INTRAOPERATÓRIO: _____ ml

MEDIDAS DE HEMOSTASIA ADICIONAIS: ☐ NÃO ☐ SIM:

☐ESPONJA DE FIBRINA ☐ÁCIDO TRANEXÂMICO

TEMPO CIRÚRGICO: _____ min

SANGRAMENTO NO PÓS-OPERATÓRIO IMEDIATO (APÓS 20 MINUTOS DE COMPRESSÃO COM GAZE):

SANGRAMENTO ATIVO: ☐PRESENTE ☐AUSENTE

FORMAÇÃO DE COÁGULO: ☐PRESENTE ☐AUSENTE

SE SANGRAMENTO ATIVO: (*medidas de hemostasia adicionais)*

☐COMPRESSÃO COM GAZE

☐MAIS SUTURAS

☐ESPONJA DE FIBRINA

☐AC. TRANEXÂMICO

AVALIAÇÃO DO PÓS-OPERATÓRIO TARDIO (7 DIAS APÓS PROCEDIMENTO)

DATA: ___/____/_____

1 - COMPLICAÇÕES HEMORRÁGICAS NO PÓS-OPERATÓRIO:

☐Sem complicações;

☐Foi necessário contato telefônico com o cirurgião dentista, por iniciativa do paciente;

☐Foi necessário retornar ao consultório odontológico;

☐Foi necessário procurar serviço de emergência hospitalar:

Necessária transfusão ☐sim ☐não

Repercussão em parâmetros do hemograma ☐sim ☐não

Número de gazes utilizadas para conter o sangramento: _____

ESCALA DE DOR: (0 A 10) _____

2- REPARAÇÃO ALVEOLAR DO SÍTIO CIRÚRGICO

☐edema/eritema ☐exposição óssea ☐sem alterações

CICATRIZAÇÃO: ☐SATISFATÓRIA ☐INSATISFATÓRIA

APÊNDICE C – Orientações pós-operatórias

INTRUÇÕES PÓS-OPERATÓRIAS:

1) Morder firmemente a gaze por 30 minutos após a cirurgia.
2) Alimentação líquida/pastosa e fria nas primeiras 72 horas. Mastigue com os dentes do lado contrário ao operado.
3) Aplique bolsa de gelo na face sobre o local operado (aplicar 10 minutos e descansar 15 minutos nas primeiras 48 horas). Passe um creme na face, no local onde o gelo for aplicado.
4) Mantenha repouso relativo com a cabeça mais alta que o resto do corpo, nas primeiras 48 horas. Evitando esforços físicos e exposição ao sol.
5) Não cuspir e não fazer nenhum tipo de bochecho nas primeiras 48horas.
6) Não fume ou tome líquidos com canudinho nas primeiras 48 horas
7) Higienização: escovar os dentes normalmente nas regiões distantes do local da cirurgia; remover as possíveis crostas que se formem sobre pontos com cotonete embebido em solução fisiológica.
8) É comum haver um leve sangramento durante os primeiros dias. Em caso de sangramento intenso: lavar a boca com água gelada e morder firmemente um rolo de gaze, por uma hora. Persistindo o sangramento, entrar em contato, ou procurar o Ambulatório Borges da Costa ou um hospital.
9) Poderá haver febre leve nos primeiro dias. Caso persista ou for acima de 38, entrar em contato.
10) A face poderá ter aumento de volume(edema) e ocasionalmente manchas vermelhas ou roxas podem aparecer na face ou no pescoço. Trata-se de problemas sem gravidade e irão desaparecer naturalmente

Retorno em ___/___/___ às___:___horas, para controle do tratamento.

CONTATO: DRA. AMANDA LEAL (31) 98839-1959

ANEXO A – Parecer Diretoria de Ensino, Pesquisa e Extensão do Hospital das Clínicas (DEPE/HC)

Universidade Federal de Minas Gerais
Hospital das Clínicas
Gerente de Ensino e Pesquisa

EBSERH
HOSPITAIS UNIVERSITÁRIOS FEDERAIS

Belo Horizonte, 02 de outubro de 2015.

PROJETO DE PESQUISA nº 088/15: "Avaliação do risco de sangramento e complicações pós-operatórias em pacientes em uso de anticoagulante oral submetidos à cirurgia oral menor em nível hospitalar".

Reportando-nos ao projeto de pesquisa acima referenciado, considerando sua concordância com o parecer da Comissão de Avaliação Econômico-financeira de Projetos de Pesquisa do Hospital das Clínicas e a aprovação pelo COEP/UFMG em 10/09/2015, esta Gerência aprova seu desenvolvimento no âmbito institucional. Solicitamos enviar à GEP ***relatório*** parcial ou final, após um ano.

Atenciosamente,

Prof. Alexandre Rodrigues Ferreira
Gerente de Ensino e Pesquisa do
HC-UFMG - Filial EBSERH
Insc. 1243058 - CRM 27630
PT- 937 de 10/11/14

Prof. Alexandre Rodrigues Ferreira

Gerente de Ensino e Pesquisa do HC-UFMG

À Srª
Profª. Tarcília Aparecida da Silva
Odontologia
Ambulatório Borges da Costa- UFMG

HC/UFMG/Filial EBSERH – Superintendência
Av. Prof. Alfredo Belena, 110 – 1º andar – ala leste – Bairro Santa Efigênia - CEP 30130.100 – BELO HORIZONTE – MG
Telefone: 31)3409-9612 – 3409-9613 FAX: 31)3409-9380 e-mail: dirgeral@hc.ufmg.br

ANEXO B - Parecer do comitê de ética em pesquisa

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 48122215.4.0000.5149

Interessado(a): Profa. Tarcília Aparecida da Silva
Departamento de Odontologia Social e Preventiva
Faculdade de Odontologia

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 02 de setembro de 2015, o projeto de pesquisa intitulado **"Risco de sangramento e complicações pós-operatórias em pacientes em uso de anticoagulante oral submetidos à cirurgia oral menor"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto através da Plataforma Brasil.

Telma Campos Medeiros Lorentz

Profa. Dra. Telma Campos Medeiros Lorentz
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

ANEXO C – Parecer emenda

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 48122215.4.0000.5149

Interessado(a): Profa. Tarcília Aparecida da Silva
Departamento de Odontologia Social e Preventiva
Faculdade de Odontologia

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 16 de setembro de 2016, a emenda com alterações no projeto de pesquisa intitulado **"Risco de sangramento e complicações pós-operatórias em pacientes em uso de anticoagulante oral submetidos à cirurgia oral menor".**

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto através da Plataforma Brasil.

Profa. Dra. Vivian Resende
Coordenadora do COEP-UFMG

ANEXO D – Cadastro Plataforma Brasil

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Risco de sangramento e complicações pós-operatórias em pacientes em uso de anticoagulante oral submetidos á cirurgia oral menor

Pesquisador: Tarcília Aparecida da Silva

Área Temática:

Versão: 1

CAAE: 48122215.4.0000.5149

Instituição Proponente: UNIVERSIDADE FEDERAL DE MINAS GERAIS

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.222.370

ANEXO E – Parecer Comissão de Ética no Uso de Animais (UFMG)

UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

CEUA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Prezado(a):

Esta é uma mensagem automática do sistema Solicite CEUA que indica mudança na situação de uma solicitação.

Protocolo CEUA: 247/2018
Título do projeto: TERAPIA ANTICOAGULANTE ORAL: AVALIAÇÃO DO EFEITO IN VITRO DO ETEXILATO DE DABIGATRANA SOBRE AS CÉLULAS ÓSSEAS
Finalidade: Pesquisa
Pesquisador responsável: Soraia Macari
Unidade: Faculdade de Odontologia
Departamento: Departamento de Odontopediatria e Ortodontia

Situação atual: Decisão Final - Aprovado

Aprovado na reunião do dia 03/09/2018. Validade: 03/09/2018 à 02/09/2023

Belo Horizonte, 04/09/2018.

Atenciosamente,

Sistema Solicite CEUA UFMG
https://aplicativos.ufmg.br/solicite_ceua/

ANEXO F – Parecer Comissão de Ética no Uso de Animais (USP-Ribeirão Preto)

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
Comissão de Ética no Uso de Animais

Of. CEUA 088/2018

Ribeirão Preto, 15 de agosto de 2018.

Ref. processo nº 2018.1.562.58.0

Senhor(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais, em sua 59ª Sessão, realizada em 15/08/2018, **APROVOU** os procedimentos éticos apresentados no Protocolo sobre a Pesquisa intitulada: "Efeito do etexilato de dabigatrana em osteoblastos crescidos sobre titânio com nanotopografia" emitindo o certificado anexo.

Informamos, também, que deverá ser entregue na Secretaria da CEUA, **até 01/10/2019**, o **Relatório Final** contendo os resultados e/ou resumo do trabalho publicado.

Atenciosamente,

Prof. Dr. Michel Reis Messora
Coordenador da Comissão de Ética no Uso de Animais

ATIVIDADES DESENVOLVIDAS DURANTE O DOUTORADO

1- Histórico e disciplinas cursadas

1.1 Progressão direta para o programa de Doutorado.

Sistema Acadêmico da UFMG
Pós-Graduação

17/01/2019
10:11

Consultar Ocorrências Acadêmicas

[Informação para simples consulta, sem validade oficial]

Dados Pessoais

Número de Registro: 2015710307 Nome: AMANDA LEAL ROCHA

Curso: 2781 - ODONTOLOGIA/D Situação Atual: NORMAL

Ocorrências Acadêmicas

Período	Data de Decisão	Ocorrência	Discriminação	Data Lançamento
2016/2	16/09/2016	Mudança de Nível	Curso anterior: 2751 - ODONTOLOGIA/M	23/09/2016

1.2 Relação de Atividades no Histórico.

Período	Nome Atividade	Freq	Nota	Conc.	Sit. Final	Créd.	Integr.
2015/2	ESTUDOS AVANCADOS EM ESTOMATOLOGIA I	S	98	A	A	04	Sim
2015/2	ESTUDOS AVANCADOS EM ESTOMATOLOGIA III	S	90	A	A	04	Sim
2015/2	ESTAGIO DOCENTE I	S	100	A	A	03	Sim
2016/1	ESTUDOS AVANCADOS EM ESTOMATOLOGIA II	S	95	A	A	04	Sim
2016/1	ESTAGIO DOCENTE II	S	95	A	A	03	Sim
2016/1	SEMINARIOS DE PESQ. EM ODONTOLOGIA I	S	100	A	A	03	Sim
2016/2	PESQUISA EM ESTOMATOLOGIA I	S	95	A	A	06	Sim
2016/2	SEMINARIOS DE PESQ. EM ODONTOLOGIA II	S	100	A	A	03	Sim
2016/2	BIOESTAT. APL. À PESQ. ODONTOLÓGICA I	S	92	A	A	04	Sim
2016/2	EPIDEMIOLOGIA I	S	92	A	A	02	Sim
2017/1	SEMINARIOS DE PESQ. EM ODONTOLOGIA III	S	100	A	A	03	Sim
2017/1	BIOESTAT. APL. À PESQ. ODONTOLÓGICA II	S	96	A	A	04	Sim
2017/1	EPIDEMIOLOGIA II	S	96	A	A	03	Sim

2017/2	EST.CLI.E LAB. EM ESTOMAT.E PAT. BUCAL I	S	95	A	A	04	Sim
2017/2	METODOLOGIA DA PESQ. EM ODONTOLOGIA II	S	97	A	A	02	Sim
2017/2	NORMALIZACAO BIBLIOGRAFICA	S	98	A	A	02	Não
2018/1	PESQUISA EM ESTOMATOLOGIA II	S	100	A	A	06	Sim
2018/1	EST. CLÍ. E LAB. EM EST. E PAT. BUCAL II	S	100	A	A	04	Sim
2018/1	EXAME DE QUALIFICAÇÃO				A	0	Sim
2018/2	ELABORACAO DE TRABALHO FINAL					0	Sim

Tipo Mat: Tipo de Matrícula: Normal ou Eletiva
Freq: Frequência
Conc: Conceito
Sit Final: Situação Final na Atividade
A: Aprovado
S: Suficiente
4: Dispensa
Créd: Número de créditos atribuídos
Integr :Indica se a atividade será computada ou não na integralização dos créditos exigidos.

1.3 Integralização de créditos

Créditos em Atividades Acadêmicas	
Exigidos	31
Cursados/Dispensados	64
Aproveitamento de Créditos	00
Utilizados para Integralização	31
Em Curso	00
Situação Curricular	INTEGRALIZADO

2- Artigos completos publicados em periódicos

Durante o doutorado, foram produzidos **cinco** artigos científicos **relacionados ao tema da tese,** sendo **três** deles já publicados:

1) **ROCHA, AMANDA L**.; OLIVEIRA, SICÍLIA R.; SOUZA, ALESSANDRA F.; TRAVASSOS, DENISE V.; RIBEIRO, DANIEL D.; ABREU, LUCAS G.; SILVA, TARCÍLIA A. Bleeding assessment in oral surgery: A cohort study comparing individuals on anticoagulant therapy and a non-anticoagulated group. *J. Craniomaxillofac. Surg*. v.47, p.798-804. 2019.

2) **ROCHA, AMANDA L**.; SOUZA, ALESSANDRA F.; MARTINS, MARIA A.P.; FRAGA, MARINA G.; TRAVASSOS, DENISE V.; OLIVEIRA, ANA C.B.; RIBEIRO, DANIEL D.; SILVA, TARCÍLIA A Oral surgery in patients under antithrombotic therapy. *Blood Coagulation & Fibrinolysis* v.29, p.97 - 103, 2018.

3) SOUZA, ALESSANDRA F.; **ROCHA, AMANDA L.**; CASTRO, WAGNER H.; GELAPE, CLAUDIO L.; NUNES, MARIA CARMO P.; OLIVEIRA, SICILIA R.; TRAVASSOS, DENISE V.; SILVA, TARCÍLIA A Dental management for patients undergoing heart valve surgery. *Journal Of Cardiac Surgery* v.2017, p.1 - 6, 2017.

Artigos submetidos:

4) **ROCHA, AMANDA L.**; OLIVEIRA, SICÍLIA R.; SOUZA, ALESSANDRA F.; TRAVASSOS, DENISE V.; RIBEIRO, DANIEL D.; ABREU, LUCAS G.; SILVA, TARCÍLIA A. Direct oral anticoagulants in oral surgery: a prospective cohort.

5) **ROCHA, AMANDA L**; TREVISAN, RAYANA L; DUFFLES, LETÍCIA F; ASSIS, BRUNA D; MACARI, SORAIA; DINIZ, IVANA M; ARRUDA, JOSÉ A; BELOTI, MARCIO M; ROSA, ADALBERTO L; FUKADA, SANDRA Y; TAIRA, THAISE M; GOULART, GISELE C; RIBEIRO, DANIEL D; ABREU, LUCAS G; SILVA, TARCÍLIA A. The effects of a direct thrombin inhibitor, dabigatran etexilate, on bone cells.

Foram ainda publicados **cinco** artigos científicos de temas relacionados à área de concentração em Estomatologia:

1) OLIVEIRA, SICÍLIA R.; BRANCO, LUCIANA G.; **ROCHA, AMANDA L.**; TRAVASSOS, DENISE V.; MAGALHÃES, GUSTAVO; FONSECA, FELIPE P.; MESQUITA, RICARDO A.; ABREU, LUCAS G.; SILVA, TARCILIA A. Association of oral mucosa hyperpigmentation with imatinib mesylate use: a cross-sectional study and a systematic literature review. *Clin Oral Investig*. p. 1-12, 2019.

2) **ROCHA, AMANDA L.**; NUNES, LAIZ F.; TRAVASSOS, DENISE V.; SILVA, GLEYSON K; FONSECA, FELIPE P.; MESQUITA, RICARDO A.; SILVA, TARCÍLIA A. A sessile nodule in the dorsum of the tongue. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology* v.125, p.1 -5, 2018.

3) **ROCHA, AMANDA L.**; SOUZA, ALESSANDRA F.; NUNES, LAIZ F.; CUNHA, NAYARA D; LANZA, CÉLIA R.; TRAVASSOS, DENISE V.; SILVA, TARCÍLIA A. Treatment of oral manifestations of toxic epidermal necrolysis with low-level laser therapy in a pediatric patient. *Pediatric Dermatology* v.2018, p.1-4, 2018.

4) NUNES, LAIZ F.; **ROCHA, AMANDA L.**; MAGALHÃES, GUSTAVO R.; MELO, FREDERICO H.; TRAVASSOS, DENISE V.; MESQUITA, RICARDO A.; SILVA, TARCÍLIA A. Intraoral granulocytic sarcoma as a manifestation of myelofibrosis: A

case report and review of the literature. *Special Care In Dentistry* v.2018, p.1 - 12, 2018.

5) SOUZA, ALESSANDRA F.; **ROCHA, AMANDA L.**; CASTRO, WAGNER H.; FERREIRA, FERNANDA M.; GELAPE, CLAUDIO L.; TRAVASSOS, DENISE V.; DA SILVA, TARCÍLIA A. Dental care before cardiac valve surgery: Is it important to prevent infective endocarditis?. *IJC Heart & Vasculature* v.12, p.57 - 62, 2016.

3-Resumos publicados em Anais de eventos

Durante o período do doutorado foram publicados **cinco** resumos em anais de eventos científicos, sendo um trabalho relacionado ao tema da tese publicado em anais de evento internacional.

1) **ROCHA, A. L.**; MARTINS, M. A. P.; TRAVASSOS, D. V.; RIBEIRO, D. D.; SOUZA, A. F.; FRAGA, M. G.; OLIVEIRA, A. C. B.; SILVA, T. A. Factors associated with postoperative hemorrhage in patients undergoing dental surgery. In: Abstracts Of The XXVI Congress Of International Society On Thrombosis And Haemostasis (ISTH 2017). ***Res Pract Thromb Haemost***., 2017. v.1.

2) SOUZA, A. F.; **ROCHA, A. L.**; TRAVASSOS, D. V.; SILVA, T. A. Análise dos pedidos médicos de avaliação odontológica em um hospital universitário In: 32ª Reunião SBPQO. *Anais Brasilian Oral Research*., 2016. v.30.

3) **ROCHA, A. L.**; TRAVASSOS, D. V.; SOUZA, A. F.; OLIVEIRA, A. C. B.; SILVA, T. A.Complicações hemorrágicas em pacientes sob terapia antitrombótica In: XIII Encontro Científico da Faculdade de Odontologia da UFMG. *Arquivos em Odontologia*., 2016. v.52. p.1 - 39

4) MARQUES, N.; **ROCHA, A. L.**; TRAVASSOS, D. V.; FARIA, D. M.; Mesquita, RA; MORENO, A. Obturador palatino cirúrgico imediato no pós-operatório de ressecções maxilares em pediatria. Relato de dois casos In: Anais da 23a Jornada Mineira de Estomatologia e 29a semana odontológica, 2016. *Arquivo Brasileiro de Odontologia*., 2016. v.12.

5) **ROCHA, A. L.**; Mesquita, RA; PAULA, R. O.; GOMEZ, R. S.; SILVA, T. A. Relapsed multiple myeloma with primary manifestation in the mandible: a case report In: 42TH Brazilian Congress of Oral Medicine and Oral Patology**.** *Oral surgery oral medicine oral pathology and radiology*., 2016. v.128.

4- Apresentação de trabalhos em eventos científicos

1) **ROCHA, A. L.**; NUNES, L. F. M.; SILVA, G. K. A.; OLIVEIRA, S. R.; FONSECA, F. P.; Mesquita, RA; SILVA, T. A. **Metastatic calcinosis of the tongue: a case report**, 2018. *Evento: 44º Congresso Brasileiro De Estomatologia E Patologia Oral -Xv Reunion De La Academia Iberoamericana De Patologia Y Medicina Bucal - XII Congresso Brasileiro De Câncer Bucal.; Inst.promotora/financiadora: Sociedade Brasileira de Estomatologia e Patologia Oral.*

2) OLIVEIRA, S. R.; **ROCHA, A. L.**; SILVA, R. K. M.; SOUZA, A. F.; ABREU, L. G.; TRAVASSOS, D. V.; SILVA, T. A. **Risk factors for bleeding in liver disease individuals undergoing dental surgery**, 2018. *Evento: 44º Congresso Brasileiro De Estomatologia E Patologia Oral -Xv Reunion De La Academia*

Iberoamericana De Patologia Y Medicina Bucal - XII Congresso Brasileiro De Câncer Bucal.; Inst.promotora/financiadora: Sociedade Brasileira de Estomatologia e Patologia Oral.

3) OLIVEIRA, S. R.; GRAVITO, L.; **ROCHA, A. L.**; FONSECA, F. P.; Mesquita, RA; ABREU, L. G.; SILVA, T. A. **Association of oral pigmentation with hydroxyurea and imatinib mesylate use:analysis of 74 cases and systematic review**, 2018. *Evento: 44º Congresso Brasileiro De Estomatologia E Patologia Oral -Xv Reunion De La Academia Iberoamericana De Patologia Y Medicina Bucal - XII Congresso Brasileiro De Câncer Bucal.; Inst.promotora/financiadora: Sociedade Brasileira de Estomatologia e Patologia Oral.*

4) FARIA, L. S.; ARRUDA, J. A. A.; VIEIRA, P. S.; FARIA, D. M.; **ROCHA, A. L.**; Mesquita, RA; MORENO, A. **Immediate surgical obturador after maxillectomy: review of 112 cases from literature and two illustrative cases**, 2018. *Evento: 44º Congresso Brasileiro De Estomatologia E Patologia Oral -Xv Reunion De La Academia Iberoamericana De Patologia Y Medicina Bucal - XII Congresso Brasileiro De Câncer Bucal.; Inst.promotora/financiadora: Sociedade Brasileira de Estomatologia e Patologia Oral.*

5) KATO, C. O.; **ROCHA, AMANDA L.**; CALDEIRA, P. **Metodologia ativa e ferramentas digitais no ensino de patologia bucal e estomatologia**, 2018. *Evento: XXII Semana do Conhecimento; Inst.promotora/financiadora: Universidade Federal de Minas Gerais.*

6) **ROCHA, AMANDA L. Introdução à odontologia hospitalar**, 2018. *Evento: II Fórum de Odontologia Hospitalar do CROMG; Inst.promotora/financiadora: CRO-MG.*

7) **ROCHA, A. L. Manejo do paciente em terapia anticoagulante oral**, 2018. *Evento: Reunião científica da Liga de Estomatologia e Cirurgia Buco-Maxilo-Facial; Inst.promotora/financiadora: Hospital Odilon Behrens.*

8) **ROCHA, AMANDA LEAL Odontologia Intensiva-o papel do cirurgião dentista na UTI**, 2018. *Inst.promotora/financiadora: AMIB-Associação Mineira Intensiva Brasieira.*

9) **ROCHA, A. L. A saúde começa pela boca**, 2017.*Evento: III Semana da Enfermagem Santa Casa BH; Inst.promotora/financiadora: Santa Casa.*

10) **ROCHA, A. L. Abordagem odontológica em pacientes anticoagulados**, 2017. *Evento: XV Congresso Mineiro De Medicina Intensiva; SOMITI.*

11) **ROCHA, A. L.**. **Atuação da Odontologia no Transplante de medula óssea**, 2017. *Evento: I Congresso de Transplantes da Santa Casa; Inst.promotora/financiadora: Santa Casa*

12) SILVA, R. K. M.; SILVA, T. A.; FRAGA, M. G.; LANZA, C.; **ROCHA, AMANDA L.**; SOUZA, A. F.; TRAVASSOS, D. V. **Avaliação de complicações hemorrágicas em pacientes hepatopatas submetidos a cirurgia oral menor em um serviço de odontologia hospitalar**, 2017. *Evento: 34ª Reunião anual da sociedade brasileira de pesquisa odontológica; Inst.promotora/financiadora: SBPqO.*

13) **ROCHA, AMANDA LEAL**; OLIVEIRA, A. C. B.; TRAVASSOS, D. V.; SOUZA, A. F.; SILVA, T. A. **Complicações hemorrágicas em pacientes sob terapia antitrombótica submetidos a cirurgia oral menor: estudo retrospectivo**, 2016. *Evento: Xiiii Encontro Científico Da Foufmg: Inst.Promotora/financiadora: Faculdade de Odontologia UFMG.*

14) MARQUES, N.; FARIA, D. M.; **ROCHA, AMANDA L.**; TRAVASSOS, D. V.; Mesquita, RA; MORENO, A.**Obturador palatino cirúrgico imeadito no pós-operatório de ressecções maxilares em pediatria. Relato de dois casos**, 2016. *Evento: 23ª Jornada Mineira de Estomatologia; Inst.promotora/financiadora: UFVJM.*

15) **ROCHA, A. L. Odontologia Hospitalar**, 2016. *Evento: II FÓRUM CLÍNICO-ODONTOLOGIA HOSPITALAR; Inst.promotora/financiadora: Programa De Educação Permanente. CROMG*

16) **ROCHA, A. L. PERSPECTIVAS E DESAFIOSA NA ODONTOLOGIA HOSPITALAR**, 2016. *Evento: XXXIX JORNADA ODONTOLÓGICA; Inst.promotora/financiadora: Universidade de Itauna*

17) **ROCHA, A. L.**; GOMEZ, R. S.; SILVA, T. A.; PAULA, R. O.; Mesquita, RA. **RELAPSED MULTIPLE MYELOMA WITH PRIMARY MANIFESTATION IN THE MANDIBLE: A CASE REPORT**, 2016. *Evento: 42º Congresso Brasileiro de Estomatologia e Patologia Oral; Inst.promotora/financiadora: Sociedade Brasileira de Estomatologia e Patologia Bucal*

18) SOUZA, A. F.; TRAVASSOS, D. V.; SILVA, T. A.; **ROCHA, AMANDA L.**. **Análise dos pedidos médicos de avaliação odontológica em um hospital universitário**, 2015.*; Evento: 32ª Reunião Anual da Sociedade Brasileira de Pesquisa em Odontologia; Inst.promotora/financiadora: SBPqO*

19) **ROCHA, A. L**.; SILVA, T. A.; CORREA, J. D.; TRAVASSOS, D. V.; RODRIGUES, L. F. D. **Doença Periodontal Grave Associada ao Tratamento a Longo Prazo com Imunoglobulina Intravenosa**, 2015. *Evento: CIOSP*

20) **ROCHA, A. L. INTRODUÇÃO A ODONTOLOGIA HOSPITALAR**, 2015. *Evento: III SEMANA DA SAÚDE; Inst.promotora/financiadora: CENTRO UNIVERSITÁRIO NEWTON PAIVA*