UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Julio Cesar Sánchez Puetate

Avaliação da superfície hidrofílica na osseointegração e do protocolo PenTo (Pentoxifilina + α-Tocoferol) na progressão da periodontite induzida: estudo pré-clínico em ratos tratados com bisfosfonatos

Araraquara

2023

UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Julio Cesar Sánchez Puetate

Avaliação da superfície hidrofílica na osseointegração e do protocolo PenTo (Pentoxifilina + α-Tocoferol) na progressão da periodontite induzida: estudo pré-clínico em ratos tratados com bisfosfonatos

Tese apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia de Araraquara para obtenção do título de Doutor em Odontologia, na Área de concentração em Periodontia.

Orientadora: Profa. Dra. Rosemary Adriana Chierici Marcantonio

Araraquara

2023

S211a

Sánchez-Puetate, Julio Cesar

Avaliação da superfície hidrofílica na osseointegração e do protocolo PenTo (Pentoxifilina + -Tocoferol) na progressão da periodontite induzida: estudo pré-clínico em ratos tratados com bisfosfonatos / Julio Cesar Sánchez-Puetate. -- Araraquara, 2023

83 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara

Orientadora: Rosemary Adriana Chierici Marcantonio

1. Difosfonatos. 2. Osseointegração. 3. Periodontite. 4. Pentoxifilina. 5. alfa-Tocoferol. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Odontologia, Araraquara. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

Julio Cesar Sánchez Puetate

Avaliação da superfície hidrofílica na osseointegração e do protocolo PenTo (Pentoxifilina + α-Tocoferol) na progressão da periodontite induzida: estudo pré-clínico em ratos tratados com bisfosfonatos

Comissão Julgadora

Tese para obtenção do grau de Doutor em Odontologia

Presidente e orientadora: Profa. Dra. Rosemary Adriana Chierici Marcantonio

2o Examinador: Prof. Dr. Joni Augusto Cirelli

3o Examinadora: Profa. Dra. Suzane Pigossi

4o Examinador: Prof. Dr. Nicolau Conte Neto

Araraquara, 23 de setembro de 2023.

DADOS CURRICULARES

Julio Cesar Sánchez Puetate

NASCIMENTO	02 de Maio de 1990 – Quito – Equador
FILIAÇÃO	Rosa de las Nieves Puetate Llerena Julio César Sánchez Zambrano
2008-2013	Graduação em Odontologia Universidad Central del Ecuador - Equador
2015-2017	Especialização em Periodontia Universidad San Francisco de Quito - Equador
2017-2019	Especialização em Implantodontia Fundação Araraquarense de Ensino e Pesquisa em Odontologia. Faculdade Herrero
2018-2020	Curso de Pós-Graduação em Odontologia, Área de Concentração em Periodontia – Nível Mestrado Faculdade de Odontologia de Araraquara (FOAr) Universidade Estadual Paulista (UNESP)
2020-Atual	Curso de Pós-Graduação em Odontologia, Área de Concentração em Periodontia – Nível Doutorado Faculdade de Odontologia de Araraquara (FOAr) Universidade Estadual Paulista (UNESP)

Aos meus Pais, Rosita e Julio

que iluminam meu caminho com o coração cheio de amor e paciência, pela compreensão, por aceitarem e apoiarem a minha escolha nesta longa jornada, não há palavras suficientes para descrever todo o amor e admiração que sinto por vocês, muito obrigado por sonharem comigo.

Aos meus irmãos, Cristian e Berenice

pelo suporte incondicional, vocês sempre serão parte das minhas conquistas.

Aos meus sobrinhos, Renata e Joaquin

que me inspiram com a ingenuidade de seus sorrisos, vocês são a minha grande inspiração.

Às minhas avós, Dioselina e Gloria.

Ao meu avô, Cristobal (*in memoriam*).

AGRADECIMENTOS

À minha família, por compreenderem a minha ausência para poder alcançar este sonho.

À minha querida e admirada orientadora, Profa. Dra. Rosemary Adriana Chierici Marcantonio, pela confiança e suporte durante todas as fases dessa pesquisa. Obrigado pelas suas contribuições científicas e humanas para me formar plenamente durante o meu doutorado. Obrigado pela sua paciência.

Ao mestre e grande amigo Guilherme de Oliveira, a quem tenho grande admiração, obrigado por me auxiliar na interpretação das análises da imuno-histoquímica e por me ajudar a sedimentar várias ideias no âmbito científico e pessoal.

Aos meus grandes amigos Bruno, Felipe e Camila, irmãos que o Brasil me deu, obrigado pelos valiosos aportes no desenvolvimento das fases mais sensíveis na condução dos projetos.

À Karoline, Gabriel e Bruna, pela ajuda durante as fases experimentais no biotério e no laboratório.

À Isabella e Fernanda, pela ajuda no desenho e planejamento de várias fases desse projeto.

À Suleima, por todo o treinamento e auxílio em várias fases laboratoriais, principalmente com o treinamento no EXAKT e no micrótomo.

À Faculdade de Odontologia de Araraquara (UNESP), na pessoa do seu diretor Prof. Dr. Edson Alves de Campos. e da Vice-diretora, Profa. Dra. Patrícia Petromilli Nordi Sasso Garcia, pelas condições oferecidas para a realização desta pesquisa.

Ao Coordenador atual do Programa de Pós-Graduação em Odontologia, Prof. Dr. Paulo Sergio Cerri e ao anterior Prof. Dr. Joni Augusto Cirelli.

Aos professores da Disciplina de Periodontia, Profa. Dra. Daniela Leal Zandim-Barcelos, Prof. Dr. Joni Augusto Cirelli, Prof. Dr. Carlos Rossa Júnior pela confiança e suporte

no desenvolvimento do estágio supervisionado nas disciplinas de Periodontia I, II e Tópicos especiais em Periodontia, assim como por todos os ensinamentos recebidos durante as atividades clínicas, sem dúvidas foi um ciclo intenso de muito aprendizado teórico e clínico que levarei para sempre.

Ao Prof. Dr. Élcio Marcantonio Júnior, professor da disciplina de Implantodontia e colaborador nos desenhos dos estudos apresentados nesta tese, também pelos ensinamentos teóricos e clínicos sobre a osseointegração, muito obrigado professor.

Ao Toninho, secretário do departamento de Diagnóstico e Cirurgia, pelo pronto atendimento e gentileza para atender todas as minhas solicitações.

Aos funcionários da Seção de Pós-Graduação, Alessandra, José Alexandre e Cristiano pela gentileza e paciência.

À funcionara da biblioteca, Ana Cristina, pela minuciosidade e cuidado na formatação deste trabalho.

Aos meus amigos da turma de Doutorado: Lorena, Carolina, Natalie, Mariely, Ingra, Renata, Javier, Gabriel, Marco, Jonathan e Victor, por todos os momentos de aprendizados que passamos juntos.

Ao meu grande amigo e irmão José Rodolfo, pela amizade incondicional, e pelo apoio sincero que têm caracterizado a nossa amizade desde que cheguei na Pós-graduação.

Aos meus amigos David e Luis Alberto, com quem mantemos uma amizade de longa data apesar da distância, minha gratidão pelo apoio nos momentos mais difíceis.

À Laís e sua família, pelo suporte e acolhimento durante a realização deste trabalho na fase mais crítica da pandemia, minha eterna gratidão.

À CAPES: o presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001.

À FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo (Processo nº 2020/06287-3) pelo apoio financeiro essencial para realização dessa pesquisa.

Ultimately, clinical medicine wasn't for me, as it seemed more concerned with the answers, whereas I was always more drawn to the questions. For me, answers were simply a way to get to the next question, so I decided to study for a PhD.

Matthew Walker*

* Walker M. Why we sleep: unlocking the power of sleep and dreams. Harlow: Penguin Books; 2017.

Sánchez Puetate JC. Avaliação da superfície hidrofílica na osseointegração e do protocolo PenTo (Pentoxifilina + α-Tocoferol) na progressão da periodontite induzida: estudo pré-clínico em ratos tratados com bisfosfonatos [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2023.

RESUMO

Objetivo: Este estudo teve como objetivo avaliar os efeitos das superfícies de implantes hidrofóbicas (HFB) e hidrofílicas (HFL) na osseointegração, e a influência da pentoxifilina e α-tocoferol na remodelação do osso alveolar durante a progressão da Periodontite Experimental (PD), em ratos tratados com altas doses de bisfosfonatos (BP). **Materiais e Métodos:** Estudo I – Osseointegração: Foram incluídos 64 ratos (n=8) divididos em 2 grupos: Grupo CG (controle): ratos tratados com solução salina; Grupo BP: ratos tratados com BP. Após 60 dias do tratamento, os ratos de cada grupo foram subdivididos em 2 subgrupos de acordo com o tipo de implante instalado na tíbia [superfície hidrofóbica (grupos CG-HFB e BP-HFL) e hidrofílica (grupos CG-HFL e BP-HFL)]. Os animais foram submetidos à eutanásia após 15 e 45 dias para análises biomecânicas, microtomográficas, histomorfométricas e imuno histoquímicas. Estudo II – Periodontite experimental: 120 ratos (n=8) receberam solução salina (SS) ou BP durante 60 dias, seguido pela indução de PD por ligadura, os animais foram alocados de forma aleatória em cinco grupos: GC: recebeu SS durante todo o experimento, BPG-B: recebeu 1mg/kg/dia de BP durante todo o experimento, BPG-Pent: 50 mg/kg/dia de pentoxifilina (Pent) pós-ligadura após tratamento prévio com BP, BPG-To: 40 mg/kg/dia de α-tocoferol (αTo) pós-ligadura após o tratamento prévio com BP, BPG-PenTo: 50 mg/kg/dia de Pentoxfilina mais 40 mg/kg/dia de α-Tocoferol pós-ligadura após tratamento prévio com BP. A eutanásia foi realizada aos 7, 15 e 30 dias, seguida de avaliações por microtomografia computadorizada (μCT) e análises histológicas, histomorfométricas e estereométricas. Nos dois experimentos à indução da alta concentração de BP foi realizada por injeção subcutânea diária (1 mg/kg) iniciada 60 dias antes da colocação do implante ou instalação da ligadura. **Resultados:** No estudo I, tanto as superfícies HFB quanto as HFL permitiram a osseointegração em ratos sob terapia com BP. As superfícies HFL demonstraram melhor desempenho biomecânico, maior contato osso-implante e maior volume ósseo. No estudo II, o grupo BPG-PenTo exibiu consistentemente percentagens mais altas de fração de volume ósseo (BVF) ao longo do estudo, preservando o osso alveolar. A combinação de pentoxifilina e α-tocoferol mitigou a inflamação em todos os grupos tratados. **Conclusões:** As superfícies de implantes HFL demonstraram uma osseointegração aprimorada em ratos em terapia com BP. Além disso, a combinação de pentoxifilina e α-tocoferol mostrou redução na perda óssea alveolar durante o tratamento com BP na PD experimental. Esses achados destacam o potencial para melhorar e preservar o osso alveolar em indivíduos submetidos ao tratamento com BP.

Palavras-chave: Difosfonatos. Osseointegração. Periodontite. Pentoxifilina. alfa-Tocoferol.

Sánchez Puetate JC. Evaluation of hydrophilic surface on osseointegration and the PenTo protocol (Pentoxifylline + Alpha-Tocopherol) on the progression of induced periodontitis: preclinical study in bisphosphonate-treated rats [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2023.

ABSTRACT

Objective: This study aimed to evaluate the effects of hydrophobic (HFB) and hydrophilic (HFL) implant surfaces on osseointegration, and the influence of pentoxifylline and α-tocopherol on alveolar bone remodeling during the progression of Experimental Periodontitis (PD), in rats. treated with high doses of bisphosphonates (BP). **Materials and Methods:** Study I – Osseointegration: 64 rats (n=8) were included, divided into 2 groups: Group CG (control): rats treated with saline solution; BP group: BP-treated rats. After 60 days of treatment, the rats in each group were subdivided into 2 subgroups according to the type of implant installed in the tibia [hydrophobic surface (groups CG-HFB and BP-HFL) and hydrophilic (groups CG-HFL and BP-HFL)]. The animals were euthanized after 15 and 45 days for biomechanical, microtomographic, histomorphometric, and immunohistochemical analyses. Study II - experimental Periodontits study, 120 rats (n=8) received either SS or BP for 60 days, followed by ligature-induced PD. The animals were then randomly assigned to five groups: CG: which received SS throughout the experiment, BPG-B: received 1mg/kg/day BP throughout the experiment, BPG-Pent: 50 mg/kg/day pentoxifylline (Pent) post-ligature following prior BP treatment, BPG-To: 40 mg/kg/day α-tocopherol (αTo) post-ligature following prior BP treatment, BPG-PenTo: 50 mg/kg/day pentoxifylline plus 40 mg/kg/day α-tocopherol post-ligature following prior BP treatment. Euthanasia was performed at 7, 15, and 30 days, followed by evaluations using microcomputed tomography (μCT) and histological, histomorphometric, and stereometric analyses. In both experiments, high-dose BP induction was achieved through daily subcutaneous injections (1 mg/kg) initiated 60 days before implant placement or ligature installation. **Results:** In the study I, both HFB and HFL surfaces enabled osseointegration in rats under BP therapy. HFL surfaces demonstrated better biomechanical performance, greater bone-implant contact, and increased bone volume. In the study II, the BPG-PenTo group consistently exhibited higher Bone Volume Fraction (BVF) percentages throughout the study, preserving alveolar bone. **Conclusions:** HFL implant surfaces demonstrated enhanced osseointegration in rats on BP therapy. Furthermore, the combination of pentoxifylline and α-tocopherol showed a reduction in alveolar bone loss during BP treatment in experimental PD. These findings highlight the potential to improve and preserve alveolar bone in individuals undergoing BP treatment.

Keywords: Diphosphonates. Osseointegration. Periodontitis. Pentoxifylline. alpha-Tocopherol.

SUMÁRIO

1 INTRODUÇÃO

Os bisfosfonatos (BP) foram inicialmente introduzidos para o tratamento de distúrbios ósseos metabólicos[1,2] e são conhecidos por seus potentes efeitos antirreabsortivos. Atuam inibindo a função dos osteoclastos por meio da indução de apoptose[3,4], inibição da diferenciação, adesão e recrutamento dessas células para a superfície óssea[5-7] e diminuição do transporte intracelular. Seu mecanismo de ação envolve o impedimento da prenilação de pequenas proteínas de sinalização da guanosina tri-fosfatase por meio da inibição da farnesil difosfato sintase na via de biossíntese do colesterol[8]. Várias pesquisas demonstraram impactos adicionais dos BP, incluindo seu potencial para modificar os processos angiogênicos[9] e reduzir os níveis do fator de crescimento endotelial vascular (VEGF) com o uso prolongado[10]. Consequentemente, a redução da atividade dos osteoclastos induzida pelos BP influencia indiretamente a vascularização do osso[11]. Atualmente, os BP estão posicionados como agentes bem estabelecidos para o tratamento de condições relacionadas ao câncer, incluindo fraturas patológicas associadas a metástases ósseas no contexto de tumores sólidos (como cânceres de mama, próstata e pulmão) e mieloma múltiplo e outras doenças metabólicas ósseas, como osteoporose e doença de Paget[12,13].

Os BP também têm sido investigados no contexto da terapia periodontal por seu potencial de atenuar a perda óssea alveolar associada à Doença Periodontal (PD), devido à sua capacidade de inibir a atividade osteoclástica[14-22]. No entanto, as evidências demonstraram uma eficácia limitada, sendo um tópico de controvérsia na literatura[16,22]. Essa controvérsia é particularmente evidente à luz dos graves efeitos colaterais sistêmicos e locais associados à terapia de longo prazo com altas doses de alguns tipos de BP administrados por via intravenosa. Um dos efeitos colaterais descritos é a ocorrência da osteonecrose maxilofacial relacionada a medicamentos (MRONJ)[13,23], após procedimentos cirúrgicos odontológicos, como extração dentária, tratamento periodontal e instalação de implantes[24-26]. Essa condição é caracterizada pela presença de osso exposto não cicatrizado e acessível por meio de uma sonda (fístula) na região maxilofacial persistindo por um período superior a 8 semanas em indivíduos submetidos a terapias antirreabsortivas. Apesar de sua prevalência relativamente baixa, conforme delineado no posicionamento da AAOMS (American Association of Oral and Maxillofacial Surgeons)[13], a MRONJ pode ocorrer em vários grupos de pacientes que recebem BP intravenoso, com frequências variando de 0% a 18% no tratamento de malignidades com zoledronato, 0,02% no tratamento da osteoporose com zoledronato e entre 0,043% a 0,05% na administração oral de

BP. Outros estudos[27-29] também relataram essas ocorrências. Por essas razões, opções terapêuticas têm sido propostas com o objetivo de mitigar alguns dos efeitos colaterais do BP.

Nos últimos anos, os procedimentos de implante dentário tornaram-se cada vez mais populares, especialmente entre a população idosa que busca restaurar a dentição perdida por meio de próteses suportadas por implantes. No entanto, esse aumento na busca por tratamentos com implantes em pacientes idosos tem trazido à tona várias condições sistêmicas e doenças que se tornam mais prevalentes com o envelhecimento, incluindo as alterações no metabolismo ósseo e a osteoporose. Dentre essas condições, a osteoporose se destaca como a doença metabólica crônica mais comum, afetando mais de 200 milhões de pessoas em todo o mundo, especialmente mulheres na pós-menopausa[30].

Modificar a superfície por jateamento e ataque ácido e manter em contato com solução de cloreto de sódio até o momento da instalação do implante, mostrou acelerar e aprimorar o processo de osseointegração[31-34]. Essa aceleração ocorre precisamente devido à manutenção das camadas de óxido na superfície do implante promovida pela presença da solução de cloreto de sódio que promove o aumento e a molhabilidade da superfície[32]. Pesquisas demonstraram a superioridade dessa superfície hidrofílica em comparação com outros tipos de modificações de superfície[33-36]. Portanto, pode ser considerada uma solução para melhorar a osseointegração em condições que o metabolismo ósseo é comprometido pelo uso de medicamentos como os BP.

A PD, uma doença inflamatória crônica que afeta as estruturas de suporte dos dentes, continua sendo uma preocupação global de saúde com implicações significativas para a saúde bucal e bem-estar geral[37,38]. A intrincada interação entre a resposta imune do hospedeiro, citocinas, atividade dos osteoclastos e fatores disbióticos do biofilme microbiano orquestra o complexo processo de reabsorção óssea alveolar, uma característica da doença que, em última instância, compromete a estabilidade dentária, função e leva à perda dentária[39,40].

Esforços para elucidar os mecanismos fisiopatológicos subjacentes à PD revelaram algumas abordagens terapêuticas potenciais que poderiam atenuar a progressão da reabsorção óssea alveolar e aprimorar o potencial regenerativo do periodonto[41,42]. Entre essas estratégias, as intervenções farmacológicas têm despertado interesse devido ao seu potencial para modular processos inflamatórios e de remodelação óssea[43-47]. Nas últimas décadas, tem sido relatado o uso de medicamentos farmacológicos e compostos naturais, com o objetivo de suprimir a destruição óssea durante a PD em modelos animais[47]. Um desses agentes é a pentoxifilina, um derivado de metilxantina e inibidor de fosfodiesterase que promove o aumento do fluxo sanguíneo periférico por meio do aumento da vasodilatação[48]. Também existem numerosos relatos de que o tratamento com pentoxifilina reduz a concentração de citocinas pró-

inflamatórias [1] , como o fator de necrose tumoral-α (TNF-α)[49-53] interleucina-1 beta (IL-1β)[54] e interleucina-6 (IL-6)[51,55], o que posiciona a pentoxifilina como um candidato plausível para atenuar o ambiente inflamatório e influenciar as características da PD.

Além disso, os tocoferóis são uma classe de compostos químicos orgânicos que consistem em vários fenóis metilados[56,57]. Nesse grupo, o α-tocoferol (vitamina E) produz efeitos antioxidantes para estabilizar a estrutura da membrana oral, reduzindo a peroxidação lipídica[58] e aumentando os níveis de superóxido dismutase (SOD)[56,57]. Ademais, diminui a expressão de iNOS, um mediador de inflamação e estresse oxidativo em células gengivais[59], interrompendo assim as reações de radicais livres e mostrando potencial para combater danos teciduais induzidos pelo estresse oxidativo[60].

Embora os benefícios individuais da pentoxifilina e do α-tocoferol, coletivamente conhecidos como o protocolo PenTo, tenham encontrado apoio na literatura com uma análise abrangente de suas ações sinérgicas[38,60-69] e revelado um perfil de segurança favorável e resultados geralmente bem tolerados no manejo de efeitos colaterais específicos associados aos BP, seu impacto na progressão da PD experimental, especialmente na presença de BP, permanece relativamente inexplorado e requer uma elucidação mais aprofundada.

À medida que a população que recebe tratamento com BP continua a crescer, compreender como esses fatores interagem torna-se crucial para o desenvolvimento de estratégias clínicas eficazes para procedimentos de implante dentário em pacientes com saúde sistêmica desafiadora. Este trabalho apresenta os resultados de dois estudos independentes que lançam luz sobre essa complexa dinâmica, oferecendo insights valiosos para aprimorar o sucesso dos implantes e preservar o osso alveolar em um cenário clínico cada vez mais relevante.

Compreender a complexa interação dinâmica entre as intervenções de superfícies hidrofílicas e protocolo PenTo e seu impacto na remodelação óssea no contexto da Osseointegração e PD experimental no modelo de animais sob os efeitos das altas concentrações de BP, é crucial para avançar nossas possíveis estratégias terapêuticas no manejo pacientes sob os efeitos dos BP. O conhecimento obtido deste estudo pode fornecer insights para o desenvolvimento de abordagens de tratamento inovadoras.

2 PROPOSIÇÃO

Este estudo teve como objetivo avaliar os efeitos das superfícies de implantes hidrofóbicas (HFB) e hidrofílicas (HFL) na osseointegração, e a influência da pentoxifilina e α-tocoferol na remodelação do osso alveolar durante a progressão da Periodontite Experimental (PD), em ratos tratados com altas doses de bisfosfonatos (BP).

2.1 Estudo 1

Avaliar os efeitos das superfícies de implantes hidrofóbicas (HFB) e hidrofílicas (HFL) na osseointegração em ratos tratados com altas doses de bisfosfonatos (BP).

2.2 Estudo 2

Avaliar a influência do uso isolado e combinado da pentoxifilina e α-tocoferol (protocolo PenTo) na remodelação do osso alveolar durante a progressão da Periodontite Experimental (PD) induzida por ligadura, em ratos tratados com altas concentrações de bisfosfonatos (BP).

3 PUBLICAÇÕES

Os dados obtidos em nossa pesquisa são apresentados a seguir no formato de artigos científicos. O primeiro estudo resultante do estudo 1 já está sendo revisado por pares na revista *Clinical Oral Investigations*, e o segundo artigo, resultante do estudo 2, segue o formato da revista *Journal of Periodontal Research*, na qual pretendemos submetê-lo.

3.1 Publicação 1*

Assessing Bone Formation on Hydrophilic and Hydrophobic Implant Surfaces in a Murine Model Treated with Bisphosphonates.

Authors:

Julio Cesar Sánchez-Puetate[1]; Bruno Luís Graciliano Silva[1]; Felipe Eduardo Pinotti[1]; Camila Chierici Marcantonio[1]; Guilherme José Pimentel Lopes de Oliveira[2]; Elcio Marcantonio Junior[1]; Rosemary Adriana Chierici Marcantonio[1]

Institutional affiliations:

[1] São Paulo State University (UNESP), School of Dentistry of Araraquara, Department of Diagnosis and Surgery, Araraquara, Brazil

[2] Federal University of Uberlândia, School of Dentistry at Uberlândia, Department of Periodontology and Implantology, Uberlândia, Brazil.

Corresponding author:

Rosemary Adriana Chierici Marcantonio.

São Paulo State University (UNESP), School of Dentistry of Araraquara, Department of Diagnosis and Surgery, Araraquara, Brazil.

Rua Humaitá, 1680. CEP 14801-130, Araraquara, Brazil.

Contact number: +55 (16) 3301-6376/ Fax: +55 (16) 3301-6369

Email address: adriana.marcantonio@unesp.br

Artigo em revisão na revista *Clinical Oral Investigations* (Qualis A1. Fator de Impacto 3.4) (Anexo A) Submission ID: ef389ace-afc4-49b6-958e-8e92de49d97d

Acknowledgments

The authors thank to Neodent® Research Support Program for supplying the customized dental implants used in the study.

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by J.C.S.P, B.L.G.S., F.E.P., C. C. M., G.J.P.L.O. The first draft of the manuscript was written by J.C.S.P, and R.A.C.M., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Abstract

Objective: To evaluate the osseointegration of implants with hydrophobic (HFB) and hydrophilic (HFL) surfaces in a murine model of high-dose bisphosphonates (BPs). **Materials and Methods:** Sixty-four rats were randomly allocated into four groups: control group with HFB implants (CG-HFB), control group with HFL implants (CG-HFL), BP group with HFB implants (BP-HFB), and BP group with HFL implants (BP-HFL). Animals were euthanized after 15 and 45 days (n=8). The dependent variables assessed were the removal torque (biomechanical analysis), the bone volume around the implants (%BV/TV) (microtomographic analysis), the bone-implant contact (%BIC), the bone between the threads (%BBT) (histomorphometric analysis), and the expression of bone metabolism markers (immunohistochemistry analysis). **Results:** The CG-HFL and BP-HFL groups presented higher removal torque than the CG-HFB and BP-HFB implants. The %BIC of the CG-HFL surfaces was slightly higher than that of the CG-HFB implants. The BP-HFB and BP-HFL groups presented a higher %BIC than that of the CG-HFB and CG-HFL groups ($p<0.001$). BP therapy also increased the %BBT at both implant surfaces. Higher levels of ALP were observed in the matrix region of bone tissue on the HFL surfaces than on the HFB surfaces. **Conclusion:** Both surfaces enable osseointegration in rats under BP therapy. **Clinical Relevance:** The study demonstrates that hydrophobic (HFB) and hydrophilic (HFL) implant surfaces can promote osseointegration in rats undergoing bisphosphonate therapy. The HFL surfaces exhibited improved biomechanical performance, higher bone-implant contact, and increased bone volume, suggesting their potential clinical relevance for implant success in individuals on bisphosphonate treatment.

Keywords: dental implants; surface properties; osseointegration; bisphosphonates.

1. Introduction

The increase in life expectancy has resulted in a growing number of elderly patients undergoing dental implant placement procedures to restore lost dentition with implant-supported prostheses[1]. Certain diseases and systemic conditions in this population become more evident daily, such as changes in bone metabolism and osteoporosis. Osteoporosis is this group's most common chronic metabolic disease, affecting more than 200 million people worldwide, mainly postmenopausal women [2, 3]. Medications such as bisphosphonates treat some systemic conditions, such as osteoporosis and cancers, which can directly interfere with osseointegration [3-5].

Bisphosphonates (BPs) are molecular drugs and endogenous regulators of bone mineralization that can inhibit bone resorption by osteoclastic cells through four mechanisms: induction of apoptosis[2, 3], inhibition of recruitment to the bone surface [6], inhibition of adhesion of these cells[4], and osteoclastic activity on the bone surface[5, 6]. In addition to osteoclasts, BPs indirectly act on osteoblasts, inhibiting bone turnover or neoformation[7–9]. Inhibition of resorption stabilizes bone loss and increases mineral density[10]. Some studies have shown other effects of bisphosphonates, such as their ability to alter the mechanism of angiogenesis [7, 8] and decrease levels of vascular endothelial growth factor (VEGF) when used chronically[11, 12]. Therefore, the decrease in osteoclasts by bisphosphonates indirectly alters bone vascularization [9, 10]. These drugs have been used in patients with osteoporosis and cancers because they change the metabolism of this tissue by the abovementioned mechanisms [3, 11, 12].

Despite the risk of BPs inducing severe side effects after oral surgeries (i.e., osteonecrosis of the jaws)[13–15], this event is uncommon[16–18]. However, previous studies have shown that BPs can delay bone formation around implants[19–23] and bone grafts[24–26]. Implants in the rat maxilla treated with zoledronic acid presented 2-3 times lower bone-implant contact values than those in control rats[27]. Osteoconductive bone substitutes (i.e., deproteinized bovine bone and biphasic ceramics based on hydroxyapatite and β-tricalcium phosphate) presented less bone formation in critical-sized calvaria defects of rats treated with alendronate than the control rats[26].

Modifying the surface by sandblasting and acid etching and keeping it in a sodium chloride solution has accelerated and improved the osseointegration process[28–31]. This acceleration occurs precisely as described by Sartoretto et. al. [29] "due to the maintenance of oxide layers on the implant surface promoted by the presence of the sodium chloride solution in contact with the surface until the moment of implant installation, which maintains the oxide

layer and increases the surface wettability". Research has demonstrated the superiority of this surface modification compared to other types of surface modifications. Therefore, it can be considered a solution for conditions where bone metabolism is modified using drugs such as bisphosphonates.

This study aimed to evaluate osseointegration around titanium implants with two different surfaces (hydrophobic and hydrophilic) in a murine model of high-dose bisphosphonates.

2. Materials and Methods

At approximately three months of age, sixty-four male rats (Rattus norvegicus, Holtzman Albinus variation) were used. The animals were kept under controlled conditions of humidity (65%-70%), light (12-h light cycle), and temperature (21±1 °C). The animals were fed solid rodent chow and had access to water ad libitum before and throughout the experimental period. This study was submitted and approved by the Ethics Committee on Animal Use of the São Paulo State University (Unesp), School of Dentistry, Araraquara, Brazil (CEUA: 07/2019). This study was conducted according to the ARRIVE 2.0 guidelines.

The sample size calculation was performed according to the data of a previous study that showed that the slightest difference between the means of groups that showed a statistically significant difference was 19.29%, with a standard deviation of 6.59%. Thus, a sample of seven animals per group/period was sufficient for applying statistical tests with an α-type error of 0.05 and a β-power of 0.90. Eight animals per group were employed to account for a minimum loss of 14%, which is considered acceptable[30].

2.1. Group division

A total of sixty-four animals were randomly divided into four groups and evaluated in two different experimental periods: 15 and 45 days (n=8). The groups were divided according to the application of BP therapy and implant surface type: **CG-HFB** – Hydrophobic implant surface modified by oxide blasting and acid etching (NeoPoros®, Neodent®, Curitiba, PR, Brazil) placed in control animals; **CG-HFL –** Hydrophilic implant surface modified by oxide blasting and acid etching in a vacuum environment and kept in sodium chloride solution (Acqua®, Neodent®, Curitiba, PR, Brazil) placed in control animals; **BP-HFB –** Hydrophobic implant surface modified by oxide blasting and acid etching (NeoPoros®, Neodent®, Curitiba, PR, Brazil) placed in animals challenged with a high dose of BPs; **BP-HFL** – Hydrophilic implant surface modified by oxide blasting and acid etching in a vacuum environment and kept

in sodium chloride solution (Acqua®, Neodent®, Curitiba, PR, Brazil) placed in animals challenged with a high dose of BPs.

2.2. Bisphosphonate therapy

The animals were subjected to BP therapy with sodium alendronate (ALCON Laboratory, São Paulo, SP, Brazil) by daily subcutaneous injection (1 mg/kg). BP administration started 60 days before implant placement and was maintained throughout the experimental periods of 15 or 45 days after the surgical procedure [32] (Figure 1a).

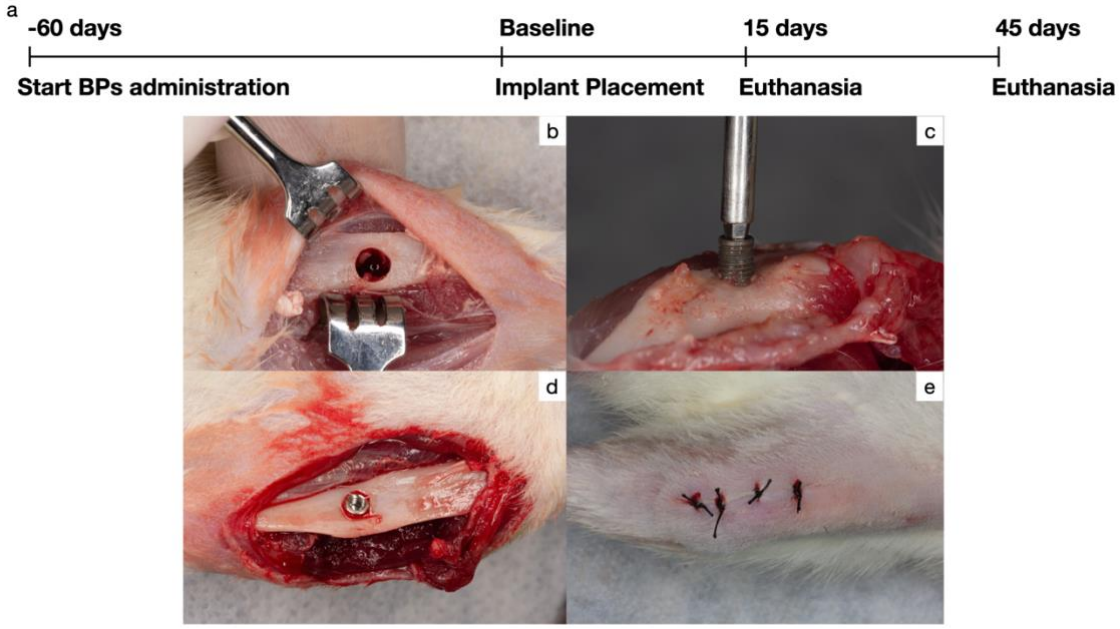


Fig. 1 a) Flowchart of the study containing the experimental model. BP administration was started 60 days before implant placement. The animals were submitted to euthanasia 15 and 45 days after implant installation; (b-e) Surgical procedure of implant installation. b) Aspect of the perforation where the implants were placed; c) Implant placement; d) Implants in the final position; e) Final aspect of the sutures

2.3. Surgical procedure

Animals were anaesthetized with an intramuscular injection of ketamine (Agener União Ltda, São Paulo, SP, Brazil) at a dose of 0.08 ml/100 g body mass and xylazine (Rompum, Bayer S.A., São Paulo, SP, Brazil) at a dose of 0.04 ml/100 g body mass. Subsequently, the animals were submitted to trichotomy of the internal region of the right and left tibiae, where antisepsis had already been performed.

An incision of approximately 10 mm was made over the tibial tuberosity, and delicate tissue dissection was performed. The surgical site was prepared for implant placement by a progressive sequence of drills (spear drill; 2.0 mm spiral drill - Neodent®; Curitiba, PR, Brazil)

to install a 4 × 2.2 mm titanium implant (NeoPoros and Acqua surface implants, Neodent®, Curitiba, PR, Brazil). All perforations were performed with an electric motor adjusted to 1200 rpm (BLM 600, Driller, São Paulo, Brazil) under abundant irrigation with sterile saline solution. The implant was placed with the help of a digital key (1.2-mm hexagonal digital key – Neodent®, Curitiba, PR, Brazil). The tissue was sutured internally with 5.0 resorbable suture (Vicryl Ethicon, Johnson & Johnson, São José dos Campos, Brazil) and externally with 4.0 silk thread (Ethicon, Johnson & Johnson, São José dos Campos, Brazil). The animals received a single dose of intramuscular penicillin with streptomycin at a dosage of 0.1 ml/kg body mass (Pentabiotic® (Wyeth-Whitehall Ltda, São Paulo, Brazil) and subcutaneous ketoprofen at a dosage of 0.03 ml/kg body mass (Ketoflex; Mundo Animal, São Paulo, Brazil) (Figure 1 b-e).

The implants were placed on the left tibiae and on the right in each animal, a total of one hundred and twenty-eight implants were installed (64 on the left tibiae and 64 on the right one). There were no unexpected adverse effects. At 15 and 45 days after implant placement, the animals were euthanized by an overdose of anaesthetic. The tibiae were removed and separated; one was used for microtomographic and histomorphometric analysis, while the other was used for biomechanical and immunohistochemistry analysis.

2.4. Biomechanical analysis

2.5.

A hexagonal wrench (1.2-mm diameter) was connected to both the implant and the torque wrench (Tohnichi, model ATG24CN-S, Toquio, Japan). An anti-clockwise movement was performed to unscrew the implant. The maximum peak torque required to move the implant was the peak of the removal torque value (Ncm) (Figure 2 a-b).

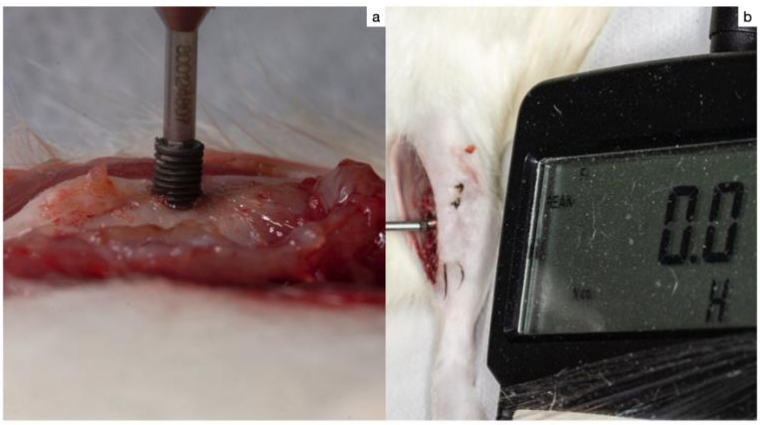


Fig. 2 (a-b) Representative image of the removal torque value (Ncm) registration of the implants

2.6. Microtomographic analysis (µCT)

The tibiae selected for this analysis were fixed in 4% paraformaldehyde for 48 hours and then stored in 70% alcohol. To perform the microtomographic evaluation, the tibiae were scanned by a microtomographic (Skyscan, Aatselaar, Belgium) with the following parameters: camera pixel, 12.45; X-ray tube power, 65 kVP; X-ray intensity, 385 µA; integration time, 300 ms; filter, Al-1 mm; and voxel size, 18 μm^3. After scanning the samples, the images were reconstructed, spatially repositioned, and analysed by specific software (NRecon, Data Viewer, CTAnalyser, Aatselaar, Belgium).

The region of interest (ROI) was defined as a 0.5-mm circular region around the entire implant diameter. This ROI was defined as the total volume (0.5-mm margin around the implants - 4.5 mm × 3.2 mm). As the implants placed did not have a cover screw, in some cases, bone formation occurred within the prosthetic platform. A second ROI was defined to remove volume from the platform to prevent this bone formation from interfering with the analysis around the implant (Figure 3 a-b). With the results obtained from the two ROIs, it was possible to define the volume of bone formation using the formula: total volume – platform volume = volume of bone tissues. The threshold used in the analysis was 25-90 greyscale, and the volume values of the mineralized tissue around the implants were obtained as a percentage (BV/TV%) (Figure 3 c). A trained examiner blinded to the experimental groups performed this analysis (BLGS).

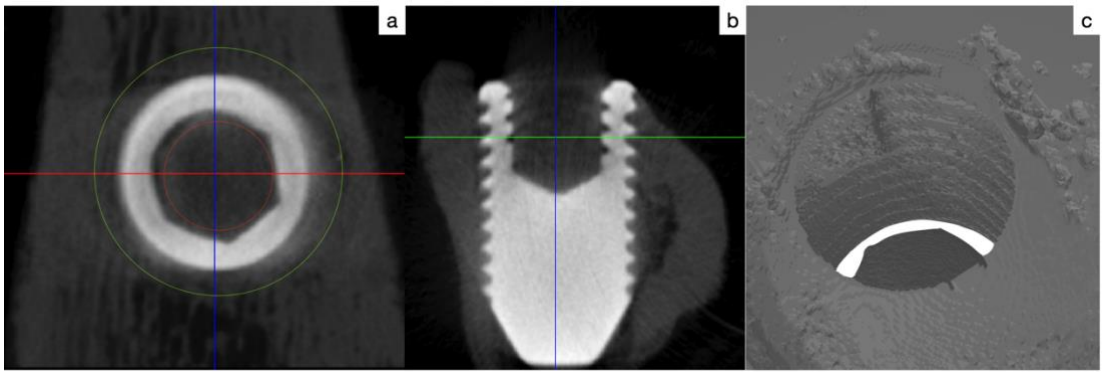


Fig. 3 a) Representative figure of the ROIs of sections for uCT analysis. The more significant ROI is represented by the green circle and the radiolucent area within it, and the smaller ROI is represented by the red circle and the radiolucent area comprising it; b) Axial view of alignment the ROIs; c) 3D reconstruction of the analyzed area of the ROIs.

2.7. Histomorphometric analysis

After microtomographic scanning, the tibiae that were not decalcified were dehydrated in an increasing ethanol series (60-100%) and infiltrated and polymerized in light-curing resin

(Technovit 7200 VLC, Kultzer Heraus GmbH & CO, Wehrheim, Germany). The blocks containing the implant and the surrounding bone tissue were cut at a central point using a cutting and grinding system (Exakt Apparatebeau, Hamburg, Germany). The 45-µm-thick sections were stained with Stevenel's blue associated with acid fuchsin and analysed under a light microscope (DIASTAR - Leica Reichert & Jung products, Wetzlar, Germany) at 100× magnification. Histomorphometric evaluations were performed with image analysis software (FIJI, San Rafael, CA, USA). The percentages of bone-to-implant contact (BIC%) and the bone area between threads (BBT%) were assessed separately in the first three threads of the implants inserted into the cortical bone[30] (Figure 4). A blinded and trained examiner (BLGS) performed these analyses.

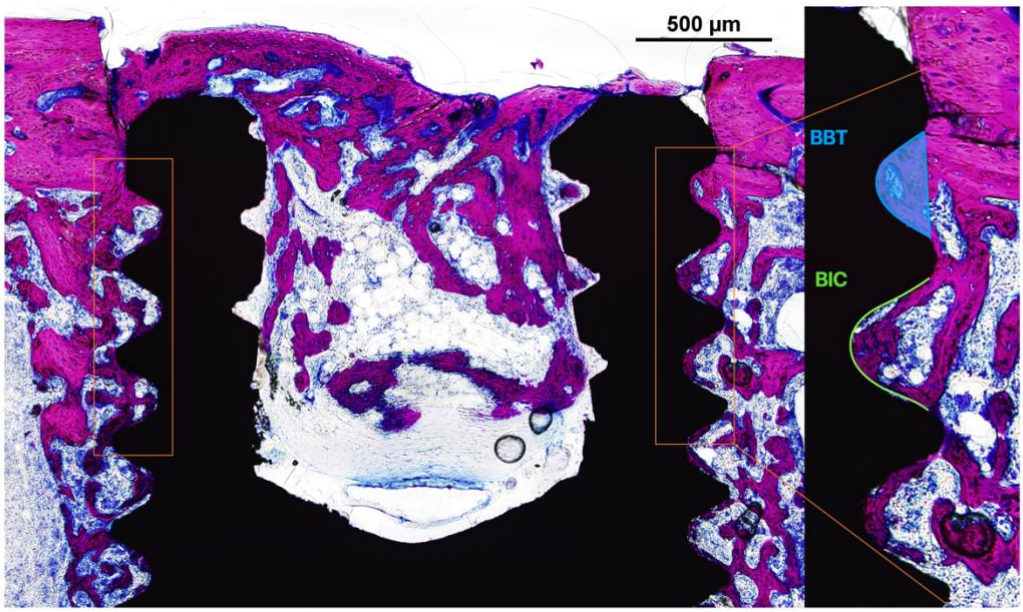


Fig. 4 Representative figure of a non-decalcified section. Selection of the first three implant threads inserted into the cortical bone, bilaterally, for BIC (%) (green line) and BBT (%) (blue area)

2.8. Immunohistochemical analysis

Immunohistochemical analyses were performed on the tibiae where the implants were removed after biomechanical analysis to identify and localize the expression of proteins related to bone remodelling, namely, alkaline phosphatase (ALP) and bone morphogenic protein-2 (BMP-2). Histological sections were mounted on silanized slides, followed by routine laboratory procedures for deparaffinization and rehydration. Subsequently, the sections were subjected to nonspecific epitope blockade by applying 3% hydrogen peroxide for 30 min and

3% bovine albumin protein for 120 min. Then, the sections were incubated for 16 hours in the following primary antibodies: ALP (1:100) and BMP-2 (1:50). As a negative control, histological sections were treated with 1% phosphate buffered saline (PBS). Subsequently, the sections were treated with avidin-biotin-peroxidase complex and stained with diaminobenzidine (Dako, Glostrup, Denmark). The sections were counterstained with Carrazi's haematoxylin solution to visualize the cell nuclei. The images were obtained using a camera coupled to a light microscope (Leica Reichert Diastar Products & Jung, Wetzlar, Germany). The expression levels of the proteins were assessed by applying a protein labelling extension index[33] that was performed in the coronal half of the surgical implant bed at 15 days: (0) no labelling (0% of cells/matrix); (1) weak labelling (<25% of cells/matrix); (2) moderate labelling (25%–50% of cells/matrix); and (3) intense labelling (<50% of cells/matrix). The analyses were performed by a blinded and trained examiner (GJO).

2.9. Statistics

Jamovi 2.4.1 (Sidney, Australia) was used for statistical analysis. The data obtained from the biomechanical, microtomographic, and histomorphometric analyses were evaluated for normality using the Shapiro–Wilk test, demonstrating, in all evaluations, a normal distribution (α=0.05). The relationship between the implant surface type vs. experimental period vs. BP therapy condition and the differences between groups were examined using three-way ANOVA followed by Tukey's post hoc test ($p<0.05$). The comparison of the protein expression was performed by the nonparametric Kruskal–Wallis test followed by the Dunn test. All the statistical tests used in this study were applied with a significance level set at 95%.

3. Results

3.1. Biomechanical analysis

The experimental period, BP therapy, and implant surface influenced the removal force torque. The HFL surface implants had higher removal torque values than the HFB surface implants in the CG (9.63 ± 0.98 Ncm vs. 2.81 ± 1.43 Ncm at 15 days; 24.31 ± 5.46 Ncm vs. 20.39 ± 3.02 Ncm at 45 days) and BP therapy animals (32.48 ± 3.80 Ncm vs. 28.76 ± 6.53 Ncm at 45 days) ($p<0.0001$). When comparing the same surface in the two experimental BP therapy conditions, it was shown that the BP-HFB group presented higher removal torque than the CG-HFB group at 15 days (10.01 ± 2.57 Ncm vs. 2.81 ± 1.43 Ncm; $p = 0.0068$) and 45 days (28.76 ± 6.53 Ncm vs. 20.39 ± 3.02 Ncm; $p = 0.0088$). The same pattern was observed with the HFL

surface since the BP-HFL group exhibited a higher removal torque than the CG-HFL group at 45 days (32.48 ± 3.80 Ncm vs. 24.31 ± 5.46 Ncm; p = 0.0194) (Table 1).

Table 1 Median and standard deviation of the removal torque data assessed by biomechanical analysis. * p<0.05 - Higher removal torque than the HFB groups; # p<0.05 - Higher removal torque than the CG groups – Three-Way ANOVA complemented by Tukey test

Groups / Period	15 days	45 days
CG - HFB	2.81 ± 1.43	20.39 ± 3.02
CG - HFL	9.63 ± 0.98*	24.31 ± 5.46*
BPs - HFB	10.01 ± 2.57#	28.76 ± 6.53#
BPs - HFL	12.68 ± 2.79	32.48 ± 3.80*#

3.2. Microtomographic analysis (µCT)

Although there were no statistically significant differences, the HFL surface led to a slightly increased percentage of bone tissue surrounding the implants (%BV/TV) in all the groups. Additionally, a more significant %BV/TV was observed after 45 days compared to at 15 days in all groups (p<0.05) (Table 2).

Table 2 Median and standard deviation of the %BV/TV data assessed by microtomographic analysis. * p<0.05 - Higher %BV/TV than the 15-day period within the same group – Three-Way ANOVA complemented by Tukey test

Groups / Period	15 days	45 days
CG - HFB	78.02 ± 3.20	79.45 ± 2.34
CG - HFL	79.48 ± 4.29	81.46 ± 3.36
BPs - HFB	81.77 ± 0.63	83.83 ± 0.65
BPs - HFL	82.96 ± 0.90	84.24 ± 1.65

3.3 Histomorphometric analysis

The experimental period, BP therapy, and implant surface influenced osteointegration. The %BIC of the HFL surface was slightly higher than that of the HFB surface in the CG groups at both experimental timepoints (44.35 ± 11.82% vs. 35.20 ± 12.66% at 15 days; 52.80 ±

22.39% vs. 43.98 ± 14.53% at 45 days; p<0.001). BP therapy increased the %BIC associated with the HFB (47.16 ± 6.14% vs. 35.20 ± 12.66% at 15 days; 67.13 ± 15.97% vs. 43.98 ± 14.53% at 45 days) and HFL surface (70.98 ± 9.27% vs. 52.80 ±22.39% at 45 days) compared with that of the CG groups (p<0.001) (Table 3). BP therapy also increased the %BBT at both types of implant surfaces and at both experimental periods of evaluation (~15% difference in %BBT in each experimental period between the CG and BP groups; p<0.001) (Table 4). Representative images are shown in Figure 5.

Table 3 Median and standard deviation of the %BIC data assessed by histomorphometric analysis. *p<0.001 – Higher %BIC than the CG-HFB group at 15-day period; # p<0.001 – Higher %BIC than the CG-HFB group at 45-day period; δ Higher %BIC than the CG-HFL group at 45-day period – Three-way ANOVA complemented by Tukey test

Groups / Period	15 days	45 days
CG - HFB	35.20 ± 12.66	43.98 ± 14.53
CG - HFL	44.35 ± 11.82*	52.80 ±22.39#
BPs - HFB	47.16 ± 6.14*	67.13 ± 15.97#
BPs - HFL	49.20 ± 6.93	70.98 ± 9.27δ

Table 4 Median and standard deviation of the %BBT data assessed by histomorphometric analysis. *p<0.001 – Higher %BBBT than the CG-HFB and CG-HFL groups at 15-day period; # p<0.001 – Higher %BBT than the CG-HFB and CG-HFL groups at 45-day period – Three-way ANOVA complemented by Tukey test

Groups / Period	15 days	45 days
CG - HFB	30.62 ± 7.27	31.76 ± 9.98
CG - HFL	31.14 ± 4.41	34.12 ± 8.96
BPs - HFB	45.78 ± 5.47*	50.62 ± 7.17#
BPs - HFL	47.10 ± 10.24*	55.54 ± 8.09#

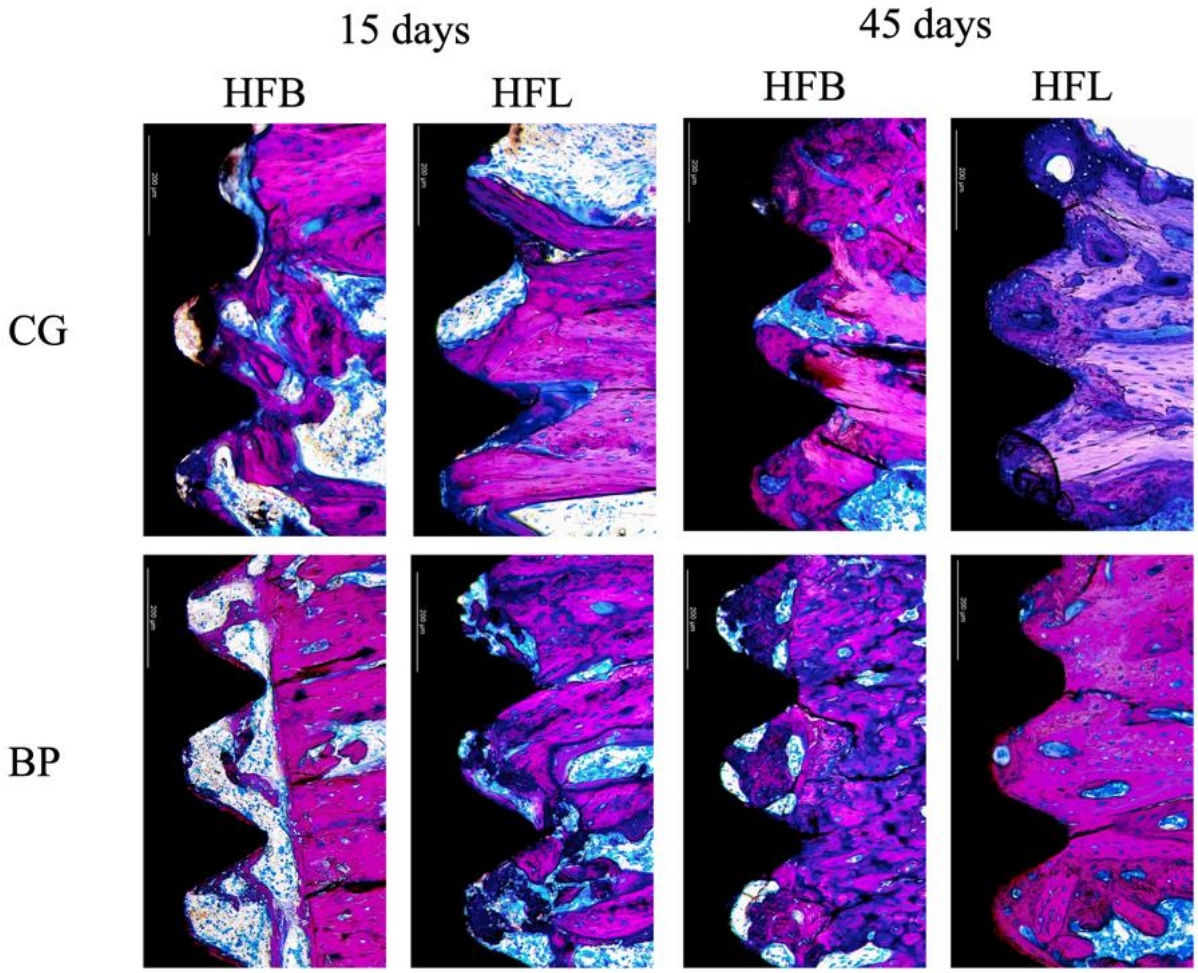


Fig. 5 Representative figures of the non-decalcified sections containing the first three implant threads inserted into the cortical bone.

3.4. Immunohistochemistry analysis

Higher levels of ALP were observed in the matrix region of bone tissue on the HFL surface than on the HFB surface, albeit with the administration of BPs ($p<0.05$) (Figure 6). In the BP group, this labelling was observed only in the matrix, with no evidence of labelling in the cells. BMP-2 appeared as diffuse labelling in the matrix and in cells. Although cell labelling was more evident in the control group, this difference was not statistically significant. * $p<0.05$ Higher protein expression than the HFB surface; Kruskal–Wallis followed by the Dunn test.

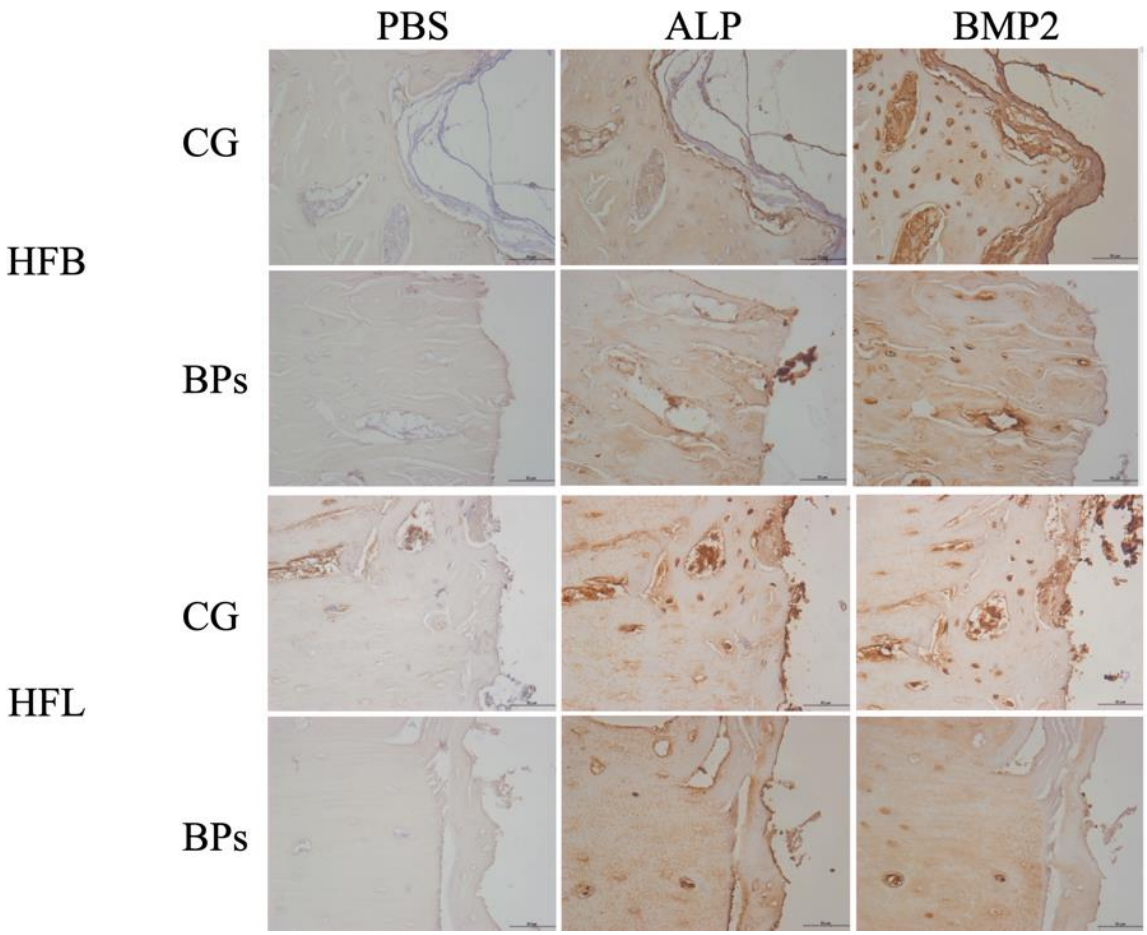


Fig. 6 Representative images and graphs of protein expression analysis at 15 days. It is possible to note a higher expression of ALP associated with the HFL surface. BMP2 expression was not different between the groups

4 Discussion

This study showed that the HFL surface enhances osseointegration in CG and BP animals. Thus, this study confirms the benefits of the HFL surface in improving osseointegration in hosts with systemic conditions/diseases who may be at risk for early failure of dental implants. On the other hand, BP therapy was associated with improved bone formation, which may be related to a lower resorption of the bone after implant placement.

The effect of the HFL on bone formation noted in this study (improving the removal torque and osseointegration) is consistent with previous studies that indicate that HFL implants significantly modulate pathways involved in bone formation, likely due to the surface's wettability[28, 29, 34]. An in vitro study evaluating the response of titanium discs with different surfaces revealed a reduction in proinflammatory cytokine levels around hydrophilic surface discs, suggesting the modulatory effect of the surface's properties on the inflammatory

response[35]. These findings are reinforced by another study that assessed protein expression during the early stages of osseointegration of dome-shaped membranes of titanium placed in the calvaria of rats with HFL (SLActive) and HFB (SLA) surfaces, which identified the downregulation of a group of proteins related to inflammation and the upregulation of proteins related to cell migration, osteoblast differentiation, and osteoblast metabolism associated with the HFL surface[36].

The improvement in the %BIC and %BBT associated with using the HFL was previously demonstrated in clinical and preclinical studies. One clinical study showed that using the HFL surface increased osteointegration compared with HFB at 2 and 4 weeks after implant placement[34]. Other preclinical studies with similar experimental models conducted by our team showed that the HFL increases osseointegration in bone defects grafted with osteoconductive bone substitutes[30], in hyperglycaemic animals[31] and in animals submitted to nicotine application[37]. This study confirms the effect of HFL implants on improving bone formation around dental implants despite the host's systemic condition. Clinical studies to evaluate implant osseointegration and clinical success must be performed in the future to test HFL surface implants in challenging situations.

Another important finding of this study was the improvement in the osseointegration and removal torque of implants placed in animals under BP therapy. These findings suggest that BPs have a favourable impact on both short- and long-term osseointegration. These outcomes may be related to the mechanism of action of the BPs and the experimental model used in this study. Some studies[38, 39] revealed that bisphosphonates effectively reduced bone turnover rates and correspondingly raised bone mineral density in osteoporotic patients within the initial year of treatment, reaching a plateau. Several investigations [13-15] have demonstrated a significant increase in bone mineral density among ovariectomized animals subjected to bisphosphonate treatment, but these effects did not occur in healthy animals. The decrease in bone remodelling rates associated with bisphosphonate treatment corresponds with the outcomes of other investigations on the BP mode of action, demonstrating a reduction in new bone formation[40]. These results provide further evidence supporting the effectiveness of BPs in combating conditions related to increased bone resorption rates.

It is important to note that the experimental model can influence the outcomes of the studies that evaluated the effect of BP therapy on osseointegration. Long bones are more prone to presenting benefits regarding BP therapy than is the jawbone [16, 17]. Preclinical studies that evaluated the effects of BPs on long bones in healthy rats showed that BP therapy improved osseointegration[20, 41]. BP therapy also enhanced osseointegration in implants placed in

ovariectomized animals[42, 43]. However, the effect of BP therapy on the osseointegration of implants placed in the oral environment tends to induce a reduction in osseointegration, especially in control animals. A preclinical study using an ovariectomized rat model with jaw implant placement found a significant decrease in the BIC in the BP group after 14 days compared to the control group[44]. A recent study showed that zoledronic acid therapy reduces the %BIC of rough and turned implants placed on the postextraction sockets of maxillary first molars in rats at 14-28 days of evaluation[27]. The findings of this study may be related to the experimental model used where the implants were placed in long bones in nonovariectomized rats, which tend to show a benefit with BP therapy on osseointegration.

The effects of BP therapy and the HFL surface on implant osseointegration shown in this study may occur due to the close interactions of the surface with the vicinity of the bone. The %BV/TV data showed no differences between the groups regarding the bone around the dental implants, despite the differences in this study on the %BIC and removal torque. Micro-CT analysis presents a limitation in the evaluation of osteointegration due to metallic artefacts[45, 46], so in this study, this method was used to evaluate bone that was not in contact with the implant's surface. Interestingly, ALP expression was higher on the bone associated with HFL implants during the 15-day period, albeit with BP therapy. ALP is a bone biological marker of osteoblastic cell function. It is associated with bone formation[47, 48], and this finding may be the reason for the higher osseointegration associated with the HFL surface. This finding was not observed to be associated with BP therapy, and perhaps the higher osseointegration was associated with BPs due to the lower bone resorption after implant placement rather than the improvement in bone synthesis.

There are some limitations in our study. One such limitation is that the implants were placed in the tibiae of rats rather than in the maxilla or mandible, which is the intraoral environment in humans. Although this animal model is commonly used to evaluate osseointegration, other factors specific to the intraoral environment, such as biofilm, implant loading, and masticatory function, may affect the outcomes analysed. Therefore, future studies should consider intraoral clinical situations to better assess the osseointegration process.

5 Conclusion

Both implant surfaces enable osseointegration in rats under BP therapy. The HFL and BPs improved osseointegration; however, the effect of these factors may occur in different ways. HFL may improve osteointegration by improving bone formation, while BPs may improve osseointegration due to the reduction in bone resorption after implant placement.

Declarations

Funding

This study was supported by the São Paulo Research Foundation (FAPESP-2020/06287-3), Coordination for the Improvement of Higher Education Personnel (CAPES-001), and National Council for Scientific and Technological Development – CNPq.

Conflicts of interest

Co-author E.M.J is a consultant for the company Neodent. The other authors have no relevant financial or non-financial interests to disclose.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by J.C.S.P, B.L.G.S., F.E.P., C. C. M., G.J.P.L.O. The first draft of the manuscript was written by J.C.S.P, and R.A.C.M., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Bosshardt DD, Chappuis V, Buser D (2017) Osseointegration of titanium, titanium alloy and zirconia dental implants: current knowledge and open questions. Periodontol 2000 73:22–40. https://doi.org/10.1111/prd.12179

2. Hughes DE, Wright KR, Uy HL, et al (1995) Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. J Bone Miner Res 10:1478–1487. https://doi.org/10.1002/jbmr.5650101008

3. Reszka AA, Halasy-Nagy JM, Masarachia PJ, Rodan GA (1999) Bisphosphonates Act Directly on the Osteoclast to Induce Caspase Cleavage of Mst1 Kinase during Apoptosis. J Biol Chem 274:34967–34973. https://doi.org/10.1074/jbc.274.49.34967

4. Colucci S, Minielli V, Zambonin G, et al (1998) Alendronate Reduces Adhesion of Human Osteoclast-like Cells to Bone and Bone Protein-Coated Surfaces. Calcified Tissue Int 63:230–235. https://doi.org/10.1007/s002239900519

5. Flanagan AM, Chambers TJ (1991) Inhibition of bone resorption by bisphosphonates: Interactions between bisphosphonates, osteoclasts, and bone. Calcified Tissue Int 49:407–415. https://doi.org/10.1007/bf02555852

6. Piper K, Boyde A, Jones SJ (1994) The effect of 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (ADP) on the resorptive function of osteoclasts of known nuclear number. Calcified Tissue Int 54:56–61. https://doi.org/10.1007/bf00316291

7. Maruotti N, Corrado A, Neve A, Cantatore FP (2012) Bisphosphonates: effects on osteoblast. Eur J Clin Pharmacol 68:1013–8. https://doi.org/10.1007/s00228-012-1216-7

8. Ohe J-Y, Kwon Y-D, Lee H-W (2011) Bisphosphonates modulate the expression of OPG and M-CSF in hMSC-derived osteoblasts. Clin Oral Invest 16:1153–9. https://doi.org/10.1007/s00784-011-0614-z

9. Viereck V, Emons G, Lauck V, et al (2002) Bisphosphonates Pamidronate and Zoledronic Acid Stimulate Osteoprotegerin Production by Primary Human Osteoblasts. Biochem Bioph Res Co 291:680–686. https://doi.org/10.1006/bbrc.2002.6510

10. Roelofs AJ, Thompson K, Gordon S, Rogers MJ (2006) Molecular Mechanisms of Action of Bisphosphonates: Current Status. Clin Cancer Res 12:6222s–6230s. https://doi.org/10.1158/1078-0432.ccr-06-0843

11. Santini D, Vincenzi B, Cuonzo GD, et al (2001) Pamidronate (P) induces modifications of circulating angiogenetic factors in cancer patients. Eur J Cancer 37:S262–S263. https://doi.org/10.1016/s0959-8049(01)81465-9

12. Vincenzi B, Santini D, Rocci L, Tonini G (2003) Bisphosphonates: new antiangiogenic molecules in cancer treatment? Ann Oncol 14:806–807. https://doi.org/10.1093/annonc/mdg194

13. Estilo CL, Poznak CHV, Wiliams T, et al (2008) Osteonecrosis of the Maxilla and Mandible in Patients with Advanced Cancer Treated with Bisphosphonate Therapy. Oncol 13:911–920. https://doi.org/10.1634/theoncologist.2008-0091

14. Kellesarian SV, Abduljabbar T, Vohra F, et al (2017) Role of local alendronate delivery on the osseointegration of implants: a systematic review and meta-analysis. Int J Oral Max Surg 46:912–921. https://doi.org/10.1016/j.ijom.2017.03.009

15. Yajima N, Munakata M, Fuchigami K, et al (2017) Influence of bisphosphonates on implant failure rates and characteristics of postmenopausal woman mandibular jawbone. J Oral Implant 43:345–349. https://doi.org/10.1563/aaid-joi-d-17-00015

16. Malden N, Lopes V (2012) An epidemiological study of alendronate-related osteonecrosis of the jaws. A case series from the south-east of Scotland with attention given to case definition and prevalence. J Bone Miner Metab 30:171–182. https://doi.org/10.1007/s00774-011-0299-z

17. Qi W-X, Tang L-N, He A-N, et al (2014) Risk of osteonecrosis of the jaw in cancer patients receiving denosumab: a meta-analysis of seven randomized controlled trials. Int J Clin Oncol 19:403–410. https://doi.org/10.1007/s10147-013-0561-6

18. Hallmer F, Andersson G, Götrick B, et al (2018) Prevalence, initiating factor, and treatment outcome of medication-related osteonecrosis of the jaw—a 4-year prospective study. Oral Surg, Oral Med, Oral Pathol Oral Radiol 126:477–485. https://doi.org/10.1016/j.oooo.2018.08.015

19. Oh KC, Hwang W, Park YB, et al (2017) Effects of Alendronate on Bone Remodeling Around Osseointegrated Implants in Rats. Implant Dent 26:46–53. https://doi.org/10.1097/id.0000000000000497

20. Verzola MHA, Frizzera F, Oliveira GJPL, et al (2015) Effects of the long-term administration of alendronate on the mechanical properties of the basal bone and on osseointegration. Clin Oral Implant Res 26:1466–1475. https://doi.org/10.1111/clr.12492

21. Frizzera F, Verzola MHA, Molon RS de, et al (2019) Evaluation of bone turnover after bisphosphonate withdrawal and its influence on implant osseointegration: an in vivo study in rats. Clin Oral Investig 23:1733–1744. https://doi.org/10.1007/s00784-018-2612-x

22. Oh K, Moon H, Lee J, et al (2015) Effects of alendronate on the peri-implant bone in rats. Oral Dis 21:248–256. https://doi.org/10.1111/odi.12258

23. Yu RQ, Wang JY, Rao NJ, et al (2021) Effects of Bisphosphonates on Osseointegration of Dental Implants in Rabbit Model. BioMed Res Int 2021:6689564. https://doi.org/10.1155/2021/6689564

24. Cohen DJ, Lohmann CH, Scott KM, et al (2022) Osseointegration and Remodeling of Mineralized Bone Graft Are Negatively Impacted by Prior Treatment with Bisphosphonates. J Bone Jt Surg 104:1750–1759. https://doi.org/10.2106/jbjs.21.01489

25. Silva JR da, Balbas MC de M, Corrêa CÁ, et al (2022) The Role of Bone Grafts in Preventing Medication-Related Osteonecrosis of the Jaw: Histomorphometric, Immunohistochemical, and Clinical Evaluation in Animal Model. Craniomaxillofacial Trauma Reconstr 15:304–311. https://doi.org/10.1177/19433875211048367

26. Prisinoto NR, Molon RS de, Scardueli CR, et al (2020) Alendronate Impairs Healing of Calvaria Critical Defects After Bone Graft With Different Bone Substitute Materials. J Oral Maxillofac Surg 78:2184–2194. https://doi.org/10.1016/j.joms.2020.08.022

27. Hou M, Lee RSB, Du Z, et al (2019) The influence of high-dose systemic zoledronate administration on osseointegration of implants with different surface topography. J Periodontal Res 54:633–643. https://doi.org/10.1111/jre.12664

28. Buser D, Broggini N, Wieland M, et al (2004) Enhanced Bone Apposition to a Chemically Modified SLA Titanium Surface. J Dent Res 83:529–533. https://doi.org/10.1177/154405910408300704

29. Sartoretto SC, ALVES ATNN, RESENDE RFB, et al (2015) Early osseointegration driven by the surface chemistry and wettability of dental implants. J Appl Oral Sci 23:279–287. https://doi.org/10.1590/1678-775720140483

30. Pinotti FE, Oliveira GJPL de, Aroni MAT, et al (2018) Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: A Preclinical study. Clin Oral Implant Res 29:963–972. https://doi.org/10.1111/clr.13361

31. Pinotti FE, Aron MAT, Oliveira GJPL de, et al (2022) Implants with hydrophilic surfaces equalize the osseointegration of implants in normo- and hyperglycaemic rats. Braz Dent J 33:71–77. https://doi.org/10.1590/0103-6440202204793

32. Neto NC, Spolidorio LC, Andrade CR, et al (2013) Experimental development of bisphosphonate-related osteonecrosis of the jaws in rodents. Int J Exp Pathol 94:65–73. https://doi.org/10.1111/iep.12007

33. Oliveira GJPL de, Paula LGF de, Spin-Neto R, et al (2014) Effect of avocado/soybean unsaponifiables on osseointegration: A proof-of-principle preclinical in vivo study. Int J Oral Maxillofac Implant 29:949–957. https://doi.org/10.11607/jomi.3498

34. Lang NP, Salvi GE, Huynh-Ba G, et al (2011) Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. Clin Oral Implan Res 22:349–356. https://doi.org/10.1111/j.1600-0501.2011.02172.x

35. Lee RSB, Hamlet SM, Ivanovski S (2017) The influence of titanium surface characteristics on macrophage phenotype polarization during osseous healing in type I diabetic rats: a pilot study. Clin Oral Implan Res 28:e159–e168. https://doi.org/10.1111/clr.12979

36. Calciolari E, Mardas N, Dereka X, et al (2018) Protein expression during early stages of bone regeneration under hydrophobic and hydrophilic titanium domes. A pilot study. J Periodontal Res 53:174–187. https://doi.org/10.1111/jre.12498

37. Pinotti FE, Aroni MAT, Oliveira GJPL de, et al (2023) Osseointegration of implants with superhydrophilic surfaces in rats with high serum levels of nicotine. Braz Dent J 34:105–112. https://doi.org/10.1590/0103-6440202305096

38. Karlsson J, Martinelli A, Fathali HM, et al (2016) The effect of alendronate on biomineralization at the bone/implant interface. J Biomed Mater Res A 104:620–629. https://doi.org/10.1002/jbm.a.35602

39. Abtahi J, Tengvall P, Aspenberg P (2012) A bisphosphonate-coating improves the fixation of metal implants in human bone. A randomized trial of dental implants. Bone 50:1148–1151. https://doi.org/10.1016/j.bone.2012.02.001

40. Wehner C, Lettner S, Moritz A, et al (2020) Effect of bisphosphonate treatment of titanium surfaces on alkaline phosphatase activity in osteoblasts: a systematic review and meta-analysis. Bmc Oral Health 20:125. https://doi.org/10.1186/s12903-020-01089-4

41. Ayan M, Dolanmaz D, Mihmanlı A, et al (2012) The effect of systemically administrated zoledronic acid on the osseointegration of dental implants. Oral Dis 18:802–808. https://doi.org/10.1111/j.1601-0825.2012.01948.x

42. Giro G, Coelho PG, Pereira RMR, et al (2011) The effect of oestrogen and alendronate therapies on postmenopausal bone loss around osseointegrated titanium implants. Clin Oral Implan Res 22:259–264. https://doi.org/10.1111/j.1600-0501.2010.01989.x

43. Apostu D, Lucaciu O, Mester A, et al (2020) Tibolone, alendronate, and simvastatin enhance implant osseointegration in a preclinical in vivo model. Clin Oral Implan Res 31:655–668. https://doi.org/10.1111/clr.13602

44. Cardemil C, Omar OM, Norlindh B, et al (2013) The effects of a systemic single dose of zoledronic acid on post-implantation bone remodelling and inflammation in an ovariectomised rat model. Biomaterials 34:1546–1561. https://doi.org/10.1016/j.biomaterials.2012.11.003

45. Bohner L, Meier N, Gremse F, et al (2020) Magnetic resonance imaging artifacts produced by dental implants with different geometries. Dentomaxillofacial Radiol 49:20200121. https://doi.org/10.1259/dmfr.20200121

46. He T, Cao C, Xu Z, et al (2017) A comparison of micro-CT and histomorphometry for evaluation of osseointegration of PEO-coated titanium implants in a rat model. Sci Rep 7:16270. https://doi.org/10.1038/s41598-017-16465-4

47. Zancanela DC, Simão AMS, Matsubara EY, et al (2016) Defective Multilayer Carbon Nanotubes Increase Alkaline Phosphatase Activity and Bone-Like Nodules in Osteoblast Cultures. J Nanosci Nanotechnol 16:1437–1444. https://doi.org/10.1166/jnn.2016.10753

48. Hadi HA, Smerdon GR, Fox SW (2015) Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation. J Dent 43:382–388. https://doi.org/10.1016/j.jdent.2014.10.006

3.2 Publicação 2*

Alveolar Bone Remodeling Effects of Pentoxifylline and α-Tocopherol in Rat experimental Periodontitis under Bisphosphonate Treatment

Authors: Julio Cesar Sánchez-Puetate[1]; Bruno Luís Graciliano Silva[1]; Karoline Bernardes do Nascimento[1]; Rosemary Adriana Chierici Marcantonio[1]

Institutional affiliations:
[1] São Paulo State University (UNESP), School of Dentistry of Araraquara, Department of Diagnosis and Surgery, Araraquara, Brazil

Corresponding author:
Rosemary Adriana Chierici Marcantonio.
São Paulo State University (UNESP), School of Dentistry of Araraquara, Department of Diagnosis and Surgery, Araraquara, Brazil.
Rua Humaitá, 1680. CEP 14801-130, Araraquara, Brazil.
Contact number: +55 (16) 3301-6376/ Fax: +55 (16) 3301-6369
Email address: adriana.marcantonio@unesp.br

Acknowledgments
São Paulo Research Foundation (FAPESP-2020/06287-3), Coordination for the Improvement of Higher Education Personnel (CAPES-001), and National Council for Scientific and Technological Development – CNPq.
Funding
This study was supported by the São Paulo Research Foundation (FAPESP-2020/06287-3), Coordination for the Improvement of Higher Education Personnel (CAPES-001), and National Council for Scientific and Technological Development – CNPq.

Conflicts of interest
The other authors have no relevant financial or non-financial interests to disclose.

Ethical approval
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author contributions
All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by J.C.S.P, B.L.G.S, K. B. N, I. F. S, F. C. G. The first draft of the manuscript was written by J.C.S.P, and R.A.C.M., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

* Artigo formatado segundo as normas do *Journal of Periodontal Research* para o qual pretende-se submeter.

Abstract

Objective: Evaluate the effects of pentoxifylline and α-tocopherol on alveolar bone remodeling during the progression of experimental Periodontitis (PD) in rats treated with Bisphosphonates (BP).

Materials and Methods: One hundred twenty rats had PD induced by bilateral ligature placement on their lower second molars. After 60 days of daily administration of either saline solution (SS) or BP, animals were randomly assigned to one of five groups (n = 8/group), CG: received SS throughout the experiment, BPG-B: received 1mg/kg/day BP throughout the experiment. BPG-Pent: 50 mg/kg/day Pentoxifylline (Pent) post-ligature following prior BP treatment. BPG-To: 40 mg/kg/day α-tocopherol (αTo) post-ligature following prior BP treatment, BPG-PenTo: 50 mg/kg/day Pent plus 40 mg/kg/day αTo post-ligature following prior BP treatment. Euthanasia was performed at 7, 15, and 30 days. Outcomes were assessed by evaluating the bone remodeling process, including Bone Volume Fraction (BVF) using microcomputer tomography (μCT) and histological, histomorphometric, and stereometric analyses. **Results:** In μCT analysis, the BPG-PenTo group consistently exhibited higher BVF percentages throughout the study. Histological examination revealed notable changes at seven days, including inflammation and gingival migration. All treatment groups differed significantly from the CG, preserving alveolar crest bone. Over time, similar histological changes occurred with increased inflammation and bone resorption. BPG-PenTo maintained bone crest levels, with a significant increase in bone area ($p < .001$). Interproximal CEJ-to-tissue distance decreased ($p < 0.05$) in treated animals compared to the CG and gradually increased over the study duration. The stereometric analysis supported increased volumetric bone (VBo) values compared to the CG ($p < .05$). **Conclusions:** Combining pentoxifylline and α-tocopherol showed preserving alveolar bone in rats with PD under bisphosphonate treatment. Although all treated groups experienced early inflammation, combination therapy mitigated these effects. These findings suggest a potential utility of combined pentoxifylline and α-tocopherol therapy in preserving alveolar bone health during bisphosphonate treatment, warranting further clinical exploration.

Keywords: Periodontitis, Alveolar bone remodeling, Bisphosphonates, Pentoxifylline, α-tocopherol

Introduction

Periodontitis, a chronic inflammatory disease affecting the supporting structures of the teeth, remains a global health concern with significant implications for oral health and overall well-being[1,2]. The intricate interplay of host immune response, cytokines, osteoclast activity, and dysbiotic microbial biofilm factors orchestrates the complex process of alveolar bone resorption, a hallmark of disease that ultimately compromises tooth stability, function and eventual tooth loss[3,4].

Efforts to elucidate the pathophysiological mechanisms underlying PD have revealed some potential therapeutic approaches that could attenuate the progression of alveolar bone resorption and enhance the regenerative potential of the periodontium[5,6]. Among these strategies, pharmacological interventions have garnered interest due to their potential to modulate inflammation and bone remodeling processes[7-11]. In parallel, the identification of alternative pharmacological agents that can modulate bone metabolism and inflammation to minimize the adverse effects associated with BP remains a pertinent research avenue.

Bisphosphonates (BP), were initially introduced for the treatment of metabolic bone disorders[12,13] and are known for their potent antiresorptive effects by inhibiting the function of the osteoclasts by impairing differentiation, induction of apoptosis[14,15], inhibition of recruitment to the bone surface[16,17], inhibition of adhesion of these cells[18], decreasing the intracellular transport and do so by hindering the prenylation of small guanosine tri-phosphatase signaling proteins via the inhibition of farnesyl diphosphate synthase in the cholesterol biosynthesis pathway[19]. Several investigations have demonstrated additional impacts of bisphosphonates, including their potential to modify angiogenic processes [20] and reduce vascular endothelial growth factor (VEGF) levels with prolonged usage[21]. Consequently, the reduction in osteoclast activity induced by bisphosphonates indirectly influences the vascularization of bone[22]. Actually BP positioned as well-established agents for managing cancer-related conditions, including pathologic fractures associated with bone metastases in the context of solid tumors (such as breast, prostate, and lung cancers) and multiple myeloma and other bone metabolic diseases such as osteoporosis, bone neoplasia, and Paget disease[23,24].

The BP also have been investigated in the context of periodontal therapy for their potential to attenuate alveolar bone loss associated with PD due to their capacity to inhibit osteoclastic activity[25-33]. However, there remain concerns regarding the long-term systemic and local side effects of high doses of some intravenous types of BP, like medication-related osteonecrosis of the jaws (MRONJ)[24,34], a condition characterized by the presence of unhealed exposed bone in the maxillofacial region persisting beyond an 8-week period in individuals

undergoing antiresorptive therapies. As stated in the 2022 Update of the AAOMS Position Paper on Medication-Related Osteonecrosis of the Jaw[24], the risk of developing MRONJ among osteoporotic patients exposed to BP is low. The observed cases can be attributed to an uncommon rare event within a substantial patient population, comprising 5.1 million individuals over 55, who have been subjected to these medications. For these reasons, careful consideration has led to the search for adjunctive therapeutic agents that can mitigate some of the side effects of BP.

In recent decades, the use of pharmacological drugs and natural compounds (like vitamins) aiming to suppress bone destruction during experimental PD in animal models has been reported[11]. In this context, one such agent is pentoxifylline, a methylxanthine derivative and phosphodiesterase inhibitor with established peripheral blood flow by increasing vasodilation properties [35]. There are also numerous reports that pentoxifylline treatment reduces the concentration of proinflammatory cytokines[36] such as including tumor necrosis factor-α (TNF- α)[37-41], interleukin-1 beta (IL-1β)[42] and interleukin-6 (IL-6)[39,43], thereby these attributes position pentoxifylline as a plausible candidate for dampening the inflammatory milieu and influencing characteristic of PD under bone with high-dose of BP. Additionally, tocopherols are a class of organic chemical compounds consisting of various methylated phenols[44,45]. In this group, α-tocopherol (vitamin E) produces antioxidant effects to stabilize the structure of the oral membrane by reducing lipid peroxidation[46], increasing superoxide dismutase (SOD) levels[47,48], and reduced the expression of iNOS, a mediator of inflammation and oxidative stress in gingival cells[49], thereby stopping free radical reactions and holds promise in counteracting oxidative stress-induced tissue damage[50].

While the individual benefits of pentoxifylline and α-tocopherol, collectively known as the PenTo protocol, a comprehensive analysis of their synergistic actions has found support in the literature [51-61] and revealing a favorable safety profile and generally well-tolerated outcomes in managing specific side effects associated with BP. Nevertheless, their impact on the progression of experimental PD, particularly in the presence of BP, remains relatively unexplored and demanding further elucidation.

Understanding the dynamic interplay between these pharmacological interventions and their impact on alveolar bone remodeling within the context of experimental PD is crucial for advancing our therapeutic strategies in the management of this complex disease. The knowledge garnered from this study could offer insights into the development of novel treatment approaches that not only address inflammation and bone resorption but also consider the intricacies of systemic medication administration.

This study aims to comprehensively evaluate the effects of pentoxifylline and α-tocopherol (PenTo protocol) in the context of alveolar bone remodeling during the progression of experimental PD under BP treatment. Through a multifaceted approach utilizing microcomputer tomography (μCT), histological, histomorphometric, and stereometric analyses, this investigation seeks to shed light on the potential therapeutic contributions of these agents to periodontal health.

Material and Methods

Samples

The experimental protocol was approved by the Ethical Committee for Animal Use (CEUA) of the School of Dentistry at Araraquara— UNESP (permit number: 16-2018) and performed in accordance with the guidelines from the National Council for Animal Experiment Control (CONCEA) and Animal Research: Reporting of In Vivo Experiments (ARRIVE 2.0) guidelines. One hundred and twenty male Holtzman rats (Rattus norvegicus, variation Albinus, Holtzman) weighing 180-220 g (18-week-old) were used. Animals were maintained in appropriate cages (4 animals/cage) at the animal facility of the School of Dentistry at Araraquara—UNESP and exposed to standardized conditions of temperature and humidity (22°C ± 1°C, 65%–70%), 12 h light/dark cycle (lights on at 7:00 AM), with access to food and water ad libitum.

The sample size calculation was based on the variability observed in our previous studies[62-65] that assessed the bone volume (as the major outcome) in rats submitted to experimental periodontal disease. Thus, a sample of seven animals per group/period was sufficient for applying statistical tests and detecting meaningful differences between groups with an α-type error of 0.05 and a β-power of 0.90. Eight animals per group were employed to account for a minimum loss of 14%, which is acceptable. This calculation ensures that the study has a sufficient sample to detect meaningful differences between groups, enhancing the reliability and robustness of our results.

Medications and BP induction

The animals received sterile SS and other medications with different types of administration due to the nature of the compounds and the time of administration. When SS was administered, it was in an amount of 0.3 ml via subcutaneous applications. The bisphosphonate used was sodium alendronate at a concentration of 1mg/kg and was administered systemically by daily subcutaneous injections following the methodology

previously reported by our research group[62]. The experimental medications were administered systemically by daily intraperitoneal injections at a concentration of 50 mg/kg of Pentoxifylline[66] and 40 mg/kg of αTocopherol[50]. To induce the high dose of BP, animals in the BP experimental groups received 60 days of systemic administration of 1mg/kg of Bisphosphonates (BP).

Periodontitis and experimental design of the treatment protocols

PD was induced by ligature placement in the subgingival region around the lower second molars bilaterally, under general anesthesia by intraperitoneal administration of ketamine (Ketamine Agener; Agener União Ltda, São Paulo, SP, Brazil; 0.08 mL/100 g body weight) and xylazine 2% (Rompum; Bayer S.A., São Paulo, SP, Brazil; 0.04 mL/100 g body weight). Then animals were randomly assigned to the following five experimental groups (n = 8/group): CG: a control group with animals that received SS during the whole experiment, BPG-B: animals that received 1mg/kg/day of BP during the whole experiment, BPG-Pent: animals that received 50 mg/kg/day of Pentoxifylline after installation of ligature and previously treated with BP. BPG-To: animals that received 40 mg/kg of α-tocopherol after installation of ligature and previously treated with BP, BPG-PenTo: animals that received a combination of 50 mg/kg/day of pentoxifylline plus 40 mg/kg α-tocopherol after installation of ligature and previously treated with BP. In the three groups that received the experimental medications (BPG-Pent, BPG-To, and BPG-PenTo). BP applications were withdrawn after the installation of the ligatures. After 7, 15, and 30 days of placement of the ligatures, the animals were euthanized by lethal overdose of anesthetic, and the mandibles were divided into two hemi-mandibles for different analysis processing (Figure 1).

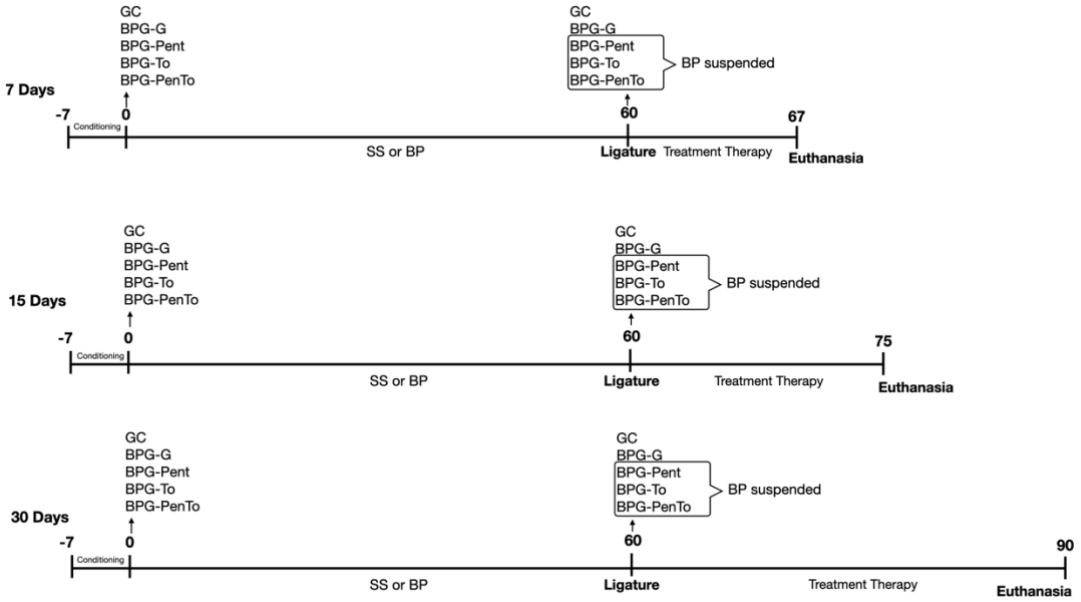


Figure 1- Experimental Design

Specimen processing

Subsequently, right and left hemi-mandibles were fixed with 4% paraformaldehyde for 48 h and washed under running water for 24 h. The right hemi-mandibles were transferred to 70% ethanol for microcomputer tomography (μCT) scanning. The left hemi-mandibles were submitted to decalcification in 10% EDTA (pH 7.2) for 16 weeks.

Alveolar bone remodeling by three-dimensional radiographic analysis (uCT)

The right hemi-mandibles were scanned by a microtomography— μCT scan (Skyscan 1176, Bruker, Aatselaar, Belgium) following the parameters: Camera Pixel Size (um)=7.4; Image Pixel Size (um)=12.000006; voxel size: 18 μm3; Exposure (ms)=500; Source Voltage (kV)=80; X-ray tube potency: 65 kVP; X-ray intensity: 385 μA; Filter=Al 1mm; rotation step 0.4° across 360°.

The images were reconstructed by NRecon Software (NRecon 1.6.1.5; Bruker, Aatselaar, Belgium), the following parameters were used: ring artifact correction, 4; pixel defect mask, 5%; smoothing, 1%; beam hardening correction, 26%; and compressed sensing (CS) to image conversion, 0.0–0.8. Then the images spatially repositioned by DataViewer (Bruker, Aatselaar, Belgium) and analyzed by CTAnalyser (CTAnalyser, Aatselaar, Belgium).

A two-standardized region of interest (ROI) was meticulously defined in a sagittal orientation. ROI 1: was delimited from the distal first molar root to the mesial third molar root and extended from the top of the furcation and alveolar crest to the root apices, and ROI 2:

delimited the roots. The ROIs were delimited every ten planes extending 100 slices. Once the ROI values had been obtained, ROI 1 - ROI 2 were subtracted, resulting in the fraction volume of the ROI occupied by mineralized tissue (bone volume fraction [BVF]) that was quantified with a threshold of 90–225. This rigorous strategy maximizes bone quantification and discarding tissues of the roots and teeth. A single trained blinded examiner performed all the measurements. The results are representative of eight animals per group.

Histological Processing

After the decalcification process, the left hemi-mandibles were embedded in paraffin. For each sample, approximately 30 semi-serial sections (4μm thickness) were obtained from the sagittal plane towards the lingual/vestibular axis along the second mandibular molar and divided into slides containing 3 sections each. The semi-serial sections were stained with Hematoxilin and Eosin (H/E).

Histological analyses

Images from semi-serial section slides stained with H/E were taken using standardized settings for image acquisition with an optical light microscope Leica DM2500 (Leica Microsystems GmbH, Wetzlar, Germany) coupled to a color digital camera Leica DFC500 (Leica Microsystems GmbH, Wetzlar, Germany), under a 40x objective. The analysis of morphological tissue structures was performed by a single trained blinded examiner through the evaluation of the bone tissue remodeling processes and inflammatory reactions in each experimental group, the results are representative of one semi-serial section per sample.

Histometric analyses

Analyses were carried out on images obtained under 20x objective and the same standardized settings for image acquisition previously described in the manuscript and with a similar methodology published by our group[64]. The ROI was delimited in the furcation and interproximal regions. In the furcation region, after measuring 1000μm from the top of the furcation, an area was delimited between the roots. In this region, the area with the bone present was measured. In the interproximal regions (mesial and distal), linear measurements were taken between the cemento-enamel junction and the bone crest using straight lines. Measurements were made using the image processing toolkit FIJI (National Institutes of Health, Maryland, USA) by a trained blinded examiner with 3 semi-serial representative sections per sample.

Stereometric analyses

The stereometric analysis was performed using the same images for histometric analyses with methods employed by our previous research[64,65]. The three ROI was defined as follows: Interproximal tissues that were located between the first and second molars (mesial) and between the third and second molars (distal), delimited coronally by the most apical portion of the epithelial tissue; apically by the top of the bony crest; and laterally by the most prominent region of the root of the second molar. The furcation region, which was located between the roots, was delimited coronally by the top of the furcation, apically by the top of the bony crest, and laterally by the roots of the second molar. Using a image processing tool FIJI (National Institutes of Health, Maryland, USA), a 154-point grid was overlaid digitally on the region of interest (ROI), and the proportion of Vital bone tissue (VBo) was performed in relation to the total number of points counted, and the percentage of each tissue component was calculated in relation to the total number of points by a trained blinded examiner with 3 semi-serial representative sections per sample.

Statistical analysis

Data obtained from each experiment were analyzed using GraphPad Prism version 10.0.2 for Mac (GraphPad Software Inc., Boston, MA, USA). The data were evaluated for normality using the Shapiro–Wilk test, demonstrating, in all evaluations, a normal distribution (α=0.05).

Two-way ANOVA followed by Tukey's test was performed to verify the existence of statistical differences among treatment periods (inter-period analyses), and two-way ANOVA followed by Dunnett's test to verify statistical differences between the groups (intra-period analyses). The significance level was set at 95% ($p < 0.05$) in all analyses.

Results

While conducting μCT analysis, we observed no statistically significant differences in bone volume fraction measurements (Figure 2). However, it is noteworthy that the BG-PenTo group exhibited a higher percentage of BVF compared to the other experimental groups along the whole experiment.

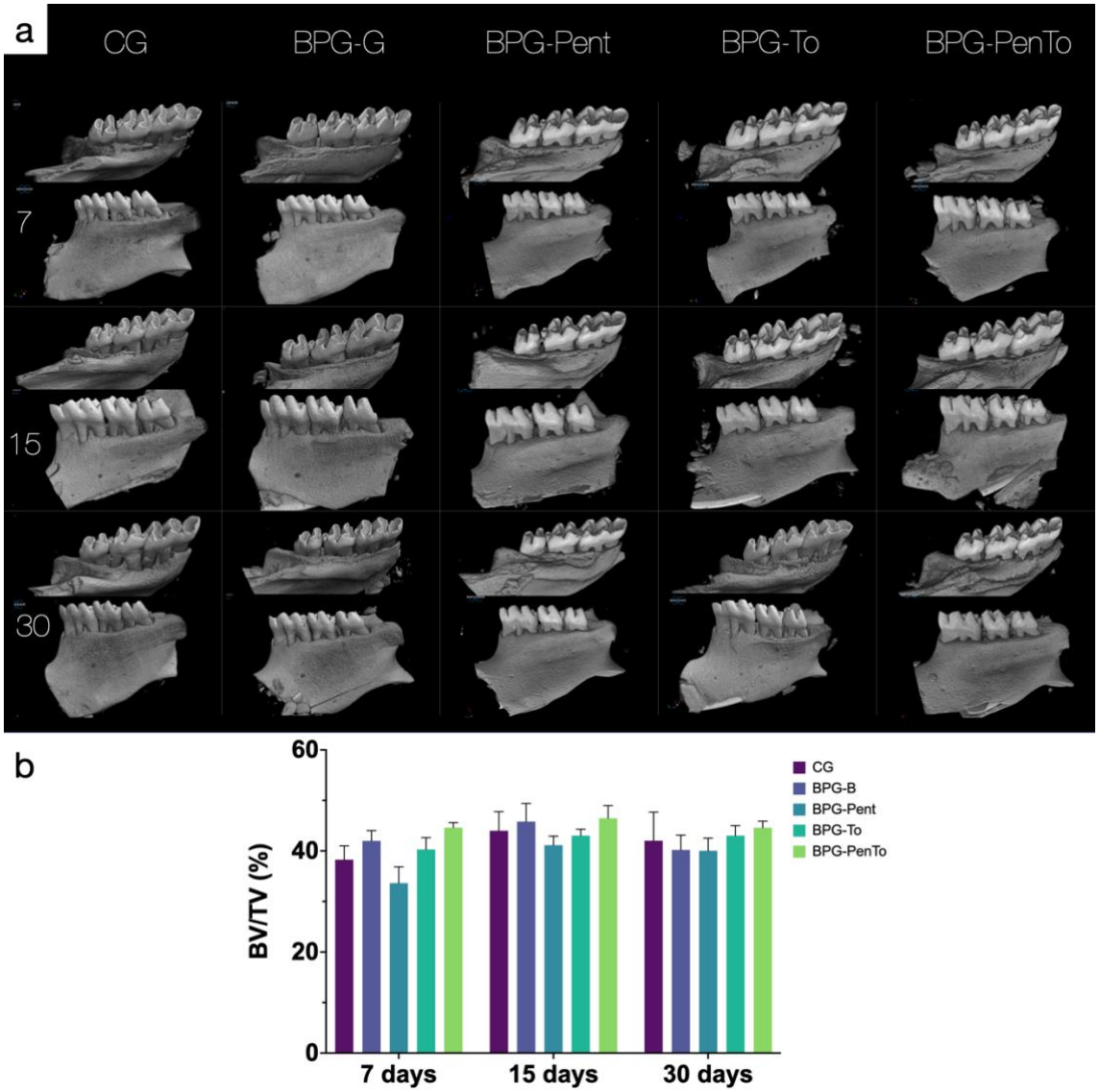


Figure 2 - 3D-μCT analysis of bone volume fraction (BVF) measurements in the ligature-induced model of periodontitis. a) Representative 3D reconstructed images of each experimental group in the lingual and vestibular views. b) The bar graph represents the quantitative results of the ratio of mineralized tissue (BV/TV %) in the defined and standardized region of interest (ROI), according to the experimental condition from 8 animals in each experimental group and period. No statistically significant differences observed among the experimental groups and periods.

Upon histological examination, notable alterations were observed in the periodontal region. At the 7-day mark, a robust inflammatory process characterized by ulceration was evident in the interproximal and furcation areas, concurrent with an apical migration of the gingival epithelium relative to the cement-enamel junction. Notably, the four treatment groups exhibited distinct histological characteristics compared to the CG, with the most prominent distinction being the preservation of alveolar crest bone in the interproximal regions. In subsequent time periods, the histological findings exhibited similarities across all experimental

groups, albeit with a progressively heightened inflammatory response and increased bone resorption (Figure 3a).

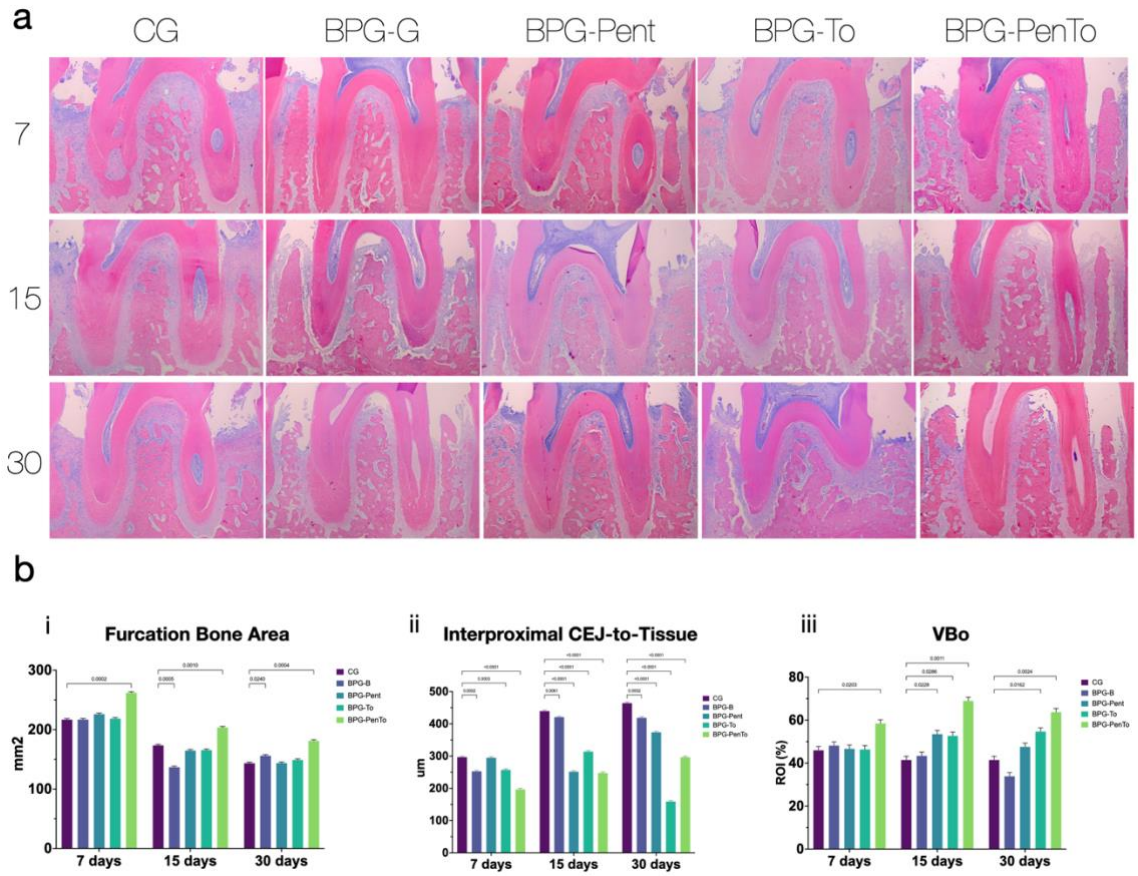


Figure 3 - Comprehensive Histological, Histometric, and Stereometric analysis of the gingival tissues among treatment groups. a) Histological Evaluation (200× magnification): Histological aspects of the experimental groups are depicted, highlighting tissue characteristics. b) Histometric and Stereometric Analyses: various parameters were assessed. i) Furcation Bone Area (mm²): The BPG-PenTo group maintained higher bone crest levels throughout the experimental periods. ii) Interproximal CEJ-to-Tissue Measurements (µm): A significant decrease was observed in animals treated with the experimental medications compared to the CG. iii) Vital Bone Percentage (VBo%): An evident increase in the BPG-PenTo group compared to the CG, indicating enhanced bone vitality. Error bars represent the average values, and vertical lines indicate standard deviations (SD). Significance was determined at $p < 0.05$ compared to the CG group within each period.

Remarkably, BPG-PenTo demonstrated the maintenance of bone crest levels in the furcation region, accompanied by a notable absence of inserted collagen fibers. This observation aligns with the highly significant increase in bone area measurements compared to the CG group ($p < .001$).

Notably, the distance between the cementoenamel junction (CEJ) and the tissue in the interproximal areas exhibited a notable decrease ($p < 0.05$) in animals treated with the experimental medications as compared to the CG. Intriguingly, these values demonstrated a progressive increase throughout the three experimental periods.

Stereometric analysis reveals a statistically significant ($p < .05$) augmentation in the BPG-PenTo group in volumetric bone (VBo) values when compared to the CG, in line with the findings of other analyses.

Discussion

The present study sought to elucidate the effects of pentoxifylline and α-tocopherol (PenTo protocol) on alveolar bone remodeling in a rat model of experimental PD treated with bisphosphonates (BP). The findings provide valuable insights into potential therapeutic approaches for mitigating the possible side effects of BP treatments on alveolar bone health.

One of the notable findings in this study was the preservation of alveolar crest bone in all treatment groups compared to the CG. The histological and μCT data consistently showed that all treatment groups, including those with pentoxifylline and α-tocopherol, exhibited better preservation of alveolar crest bone compared to the CG. This preservation is a vital indicator of the potential efficacy of these compounds in countering the adverse effects of BP on bone health. This finding is consistent with the hypothesis that these agents may help maintain bone integrity[41,46,50,55,61].

The histological analysis revealed an early inflammatory response in all treatment groups, characterized by inflammation and gingival migration aligns with the known pathophysiology of PD [3,4]. Periodontal ligature placement initiates an inflammatory cascade, a hallmark of periodontal diseases[67]. This observation underscores the challenging nature of the progression of PD. While the combination proposed therapy appeared to mitigate the alveolar bone resorption.

Several studies have demonstrated some effects of the experimental medications utilized in this investigation, which align with our findings. Pentoxifylline has been shown to possess notable anti-inflammatory properties, as evidenced by its ability to reduce proinflammatory cytokines[36,37,39,43], enhance vascular perfusion and stimulate angiogenesis[35], and exhibit a protective effect against alveolar bone loss, as reported in the study by Lima et al. (2004)[41]. Additionally, α-tocopherol's well-documented mediator of inflammation and oxidative stress in gingival cells[49], while their antioxidant capabilities are evident as demonstrated by Bas et al. (2021)[50] in its research. Additionally, emerging evidence suggests a potential synergistic effect of these drugs. Several studies indicate that the PenTo protocol plays a pivotal role in promoting tissue repair and modulating the inflammatory response [53-59,61].

Bisphosphonates are recognized for their ability to interfere with the bone remodeling process, principally by inhibiting osteoclast activity, potentially leading to aberrant bone

turnover[19,68]. The utilization of BP as a therapeutic approach to ameliorate PD has shown limited evidence of efficacy[27,33] and has become a topic of controversy. This controversy is particularly pronounced in light of reported severe adverse effects associated with long-term BP therapy, most notably medication-related osteonecrosis of the jaw (MRONJ). Despite its relatively low prevalence, as outlined in the AAOMS Position Paper on Medication-Related Osteonecrosis of the Jaw – 2022 Update[24], MRONJ can occur in various patient groups receiving intravenous BP, with frequencies ranging from 0% to 18% for malignancy treatment with zoledronate, 0.02% for osteoporosis treatment with intravenous zoledronate, and between 0.043% to 0.05% for oral BP administration. Other studies [69-71] have also reported on these occurrences.

The clinical implications of our findings hold significant relevance, especially given the extensive utilization of bisphosphonates in diverse medical scenarios, including , osteopenia, osteoporosis and cancer-associated bone disorders [23]. Our study contributes to this expanding body of knowledge by shedding light on the promising prospects of a PenTo protocol. Nevertheless, a comprehensive understanding of the exact mechanisms through which pentoxifylline and α-tocopherol manifest their anti-inflammatory effects in experimental PD demands further exploration.

While this study primarily focuses on alveolar bone remodelling process, it opens avenues for exploring the broader effects of pentoxifylline and α-tocopherol in bone metabolism. Given the systemic nature of these compounds, their impact on other skeletal sites and overall bone density warrants investigation. Additionally, their potential role in enhancing bone healing or managing bone disorders beyond PD and MRONJ could be explored.

The response to therapeutic interventions may vary among individuals due to genetic factors and comorbidities. Future research could delve into personalized medicine approaches, identifying biomarkers that predict treatment response. This would enable clinicians to tailor treatment strategies for patients receiving bisphosphonate therapy, optimizing outcomes while minimizing potential risks.

Conclusion

This study elucidated the potential benefits of pentoxifylline and α-tocopherol in mitigating alveolar bone remodeling changes induced by experimental periodontitis in rats under bisphosphonate treatment. The BPG-PenTo group consistently demonstrated superior preservation of bone parameters, including higher BVF percentages and reduced interproximal CEJ-to-tissue distance, suggesting a protective effect on alveolar crest bone. Notably, early

histological changes, marked by inflammation and gingival migration, were observed across all treatment groups, emphasizing the complex nature of PD progression. These findings underscore the potential utility of combined pentoxifylline and α-tocopherol therapy in preserving alveolar bone health in the context of bisphosphonate treatment, warranting further investigation in clinical settings.

References

1. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *J Clin Periodontol.* 2018;45 Suppl 20:S1-S8.
2. Lang NP, Bartold PM. Periodontal health. *J Clin Periodontol.* 2018;45(S20):S9-S16.
3. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. *Periodontol 2000.* 2015;69(1):7-17.
4. Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. *Periodontol 2000.* 2020;84(1):14-34.
5. Tonetti MS, Sanz M. Implementation of the new classification of periodontal diseases: Decision-making algorithms for clinical practice and education. *J Clin Periodontol.* 2019;46(4):398-405.
6. Sanz M, Herrera D, Kebschull M, et al. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. *J Clin Periodontol.* 2020;47 Suppl 22(Suppl 22):4-60.
7. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. *Ann Periodontol.* 2003;8(1):12-37.
8. Golub LM, Lee HM. Periodontal therapeutics: Current host-modulation agents and future directions. *Periodontol 2000.* 2020;82(1):186-204.
9. Preshaw PM. Host modulation therapy with anti-inflammatory agents. *Periodontol 2000.* 2018;76(1):131-149.
10. H RR, Dhamecha D, Jagwani S, et al. Local drug delivery systems in the management of periodontitis: A scientific review. *J Control Release.* 2019;307:393-409.
11. Pavanelli ALR, de Menezes BS, Pereira EBB, de Souza Morais FA, Cirelli JA, de Molon RS. Pharmacological Therapies for the Management of Inflammatory Bone Resorption in Periodontal Disease: A Review of Preclinical Studies. *Biomed Res Int.* 2022;2022:5832009.
12. Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J Bone Miner Res.* 1998;13(4):581-589.
13. Kimmel DB. Mechanism of Action, Pharmacokinetic and Pharmacodynamic Profile, and Clinical Applications of Nitrogen-containing Bisphosphonates. *J Dent Res.* 2007;86(11):1022-1033.
14. Hughes DE, Wright KR, Uy HL, et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J Bone Miner Res.* 1995;10(10):1478-1487.
15. Reszka AA, Halasy-Nagy JM, Masarachia PJ, Rodan GA. Bisphosphonates act directly on the osteoclast to induce caspase cleavage of mst1 kinase during apoptosis. A link between inhibition of the mevalonate pathway and regulation of an apoptosis-promoting kinase. *J Biol Chem.* 1999;274(49):34967-34973.

16. Flanagan AM, Chambers TJ. Inhibition of bone resorption by bisphosphonates: Interactions between bisphosphonates, osteoclasts, and bone. *Calcified Tissue Int.* 1991;49(6):407-415.

17. Piper K, Boyde A, Jones SJ. The effect of 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (ADP) on the resorptive function of osteoclasts of known nuclear number. *Calcified Tissue Int.* 1994;54(1):56-61.

18. Colucci S, Minielli V, Zambonin G, et al. Alendronate Reduces Adhesion of Human Osteoclast-like Cells to Bone and Bone Protein-Coated Surfaces. *Calcified Tissue Int.* 1998;63(3):230-235.

19. Russell RGG. Bisphosphonates: The first 40years. *Bone.* 2011;49(1):2-19.

20. Ledoux D, Hamma-Kourbali Y, Benedetto MlD, et al. A new dimethyl ester bisphosphonate inhibits angiogenesis and growth of human epidermoid carcinoma xenograft in nude mice. *Anti-cancer Drug.* 2006;17(4):479-485.

21. Vincenzi B, Santini D, Rocci L, Tonini G. Bisphosphonates: new antiangiogenic molecules in cancer treatment? *Ann Oncol.* 2003;14(5):806-807.

22. Cackowski FC, Anderson JL, Patrene KD, et al. Osteoclasts are important for bone angiogenesis. *Blood.* 2010;115(1):140-149.

23. Drake MT, Cremers S, Russell RG, Bilezikian JP. Drugs for the treatment of metabolic bone diseases. *Br J Clin Pharmacol.* 2019;85(6):1049-1051.

24. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. *J Oral Maxillofac Surg.* 2022;80(5):920-943.

25. Brunsvold MA, Chaves ES, Kornman KS, Aufdemorte TB, Wood R. Effects of a Bisphosphonate on Experimental Periodontitis in Monkeys. *J Periodontol.* 1992;63(10):825-830.

26. Weinreb M, Quartuccio H, Seedor JG, et al. Histomorphometrical analysis of the effects of the bisphosphonate alendronate on bone loss caused by experimental periodontitis in monkeys. *Journal of Periodontal Research.* 1994;29(1):35-40.

27. Almeida JD, Ervolino E, Bonfietti LH, et al. Adjuvant Therapy With Sodium Alendronate for the Treatment of Experimental Periodontitis in Rats. *J Periodontol.* 2015;86(10):1166-1175.

28. Moreira MM, Bradaschia-Correa V, Marques ND, Ferreira LB, Arana-Chavez VE. Ultrastructural and immunohistochemical study of the effect of sodium alendronate in the progression of experimental periodontitis in rats. *Microsc Res Tech.* 2014;77(11):902-909.

29. Storrer CLM, Deliberador TM, Giovanini AF, Crivellaro V, Zielak JC, Romito GA. Effect of alendronate on the progression of periodontitis induced by Porphyromonas gingivalis and Fusobacterium nucleatum: a study in rats. *Clinical Oral Investigations.* 2016;20(9):2565-2573.

30. Menezes AM, Rocha FA, Chaves HV, Carvalho CB, Ribeiro RA, Brito GA. Effect of sodium alendronate on alveolar bone resorption in experimental periodontitis in rats. *J Periodontol.* 2005;76(11):1901-1909.

31. Oktay S, Chukkapalli SS, Rivera-Kweh MF, Velsko IM, Holliday LS, Kesavalu L. Periodontitis in rats induces systemic oxidative stress that is controlled by bone-targeted antiresorptives. *J Periodontol.* 2015;86(1):137-145.

32. Santinoni CS, Silveira FM, Caldeira ML, et al. Topical sodium alendronate combined or not with photodynamic therapy as an adjunct to scaling and root planing: Histochemical and immunohistochemical study in rats. *Journal of Periodontal Research.* 2020;55(6):850-858.

33. Muniz F, Silva BFD, Goulart CR, Silveira TMD, Martins TM. Effect of adjuvant bisphosphonates on treatment of periodontitis: Systematic review with meta-analyses. *J Oral Biol Craniofac Res.* 2021;11(2):158-168.

34. Sacco R, Calasans-Maia MD, Woolley J, et al. 18 Years of Medication-Related Osteonecrosis of the Jaw (MRONJ) Research: Where Are We Now?—An Umbrella Review. *Appl Sci.* 2021;11(19):8818.

35. McCarty MF, O'Keefe JH, DiNicolantonio JJ. Pentoxifylline for vascular health: a brief review of the literature. *Open Hear.* 2016;3(1):e000365.

36. Abdel-Salam OM, Baiuomy AR, El-Shenawy SM, Arbid MS. The anti-inflammatory effects of the phosphodiesterase inhibitor pentoxifylline in the rat. *Pharmacol Res.* 2003;47(4):331-340.

37. Queiroz-Junior CM, Bessoni RL, Costa VV, Souza DG, Teixeira MM, Silva TA. Preventive and therapeutic anti-TNF-alpha therapy with pentoxifylline decreases arthritis and the associated periodontal co-morbidity in mice. *Life Sci.* 2013;93(9-11):423-428.

38. Pollice PF, Rosier RN, Looney RJ, Puzas JE, Schwarz EM, O'Keefe RJ. Oral Pentoxifylline Inhibits Release of Tumor Necrosis Factor-Alpha from Human Peripheral Blood Monocytes: A Potential Treatment for Aseptic Loosening of Total Joint Components. *J Bone Jt Surg-Am Vol.* 2001;83(7):1057-1061.

39. González-Espinoza L, Rojas-Campos E, Medina-Pérez M, Peña-Quintero P, Gómez-Navarro B, Cueto-Manzano AM. Pentoxifylline decreases serum levels of tumor necrosis factor alpha, interleukin 6 and C-reactive protein in hemodialysis patients: results of a randomized double-blind, controlled clinical trial. *Nephrol Dial Transplant.* 2012;27(5):2023-2028.

40. Colson A, Willems B, Thissen JP. Inhibition of TNF-alpha production by pentoxifylline does not prevent endotoxin-induced decrease in serum IGF-I. *J Endocrinol.* 2003;178(1):101-109.

41. Lima V, Vidal FDP, Rocha FAC, Brito GAC, Ribeiro RA. Effects of Tumor Necrosis Factor-α Inhibitors Pentoxifylline and Thalidomide on Alveolar Bone Loss in Short-Term Experimental Periodontal Disease in Rats. *J Periodontol.* 2004;75(1):162-168.

42. Gutierrez-Reyes G, Lopez-Ortal P, Sixtos S, et al. Effect of pentoxifylline on levels of pro-inflammatory cytokines during chronic hepatitis C. *Scand J Immunol.* 2006;63(6):461-467.

43. Ferrari P, Mallon D, Trinder D, Olynyk JK. Pentoxifylline improves haemoglobin and interleukin-6 levels in chronic kidney disease. *Nephrology (Carlton).* 2010;15(3):344-349.

44. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med.* 2014;72:76-90.

45. Azzi A. Many tocopherols, one vitamin E. *Mol Aspects Med.* 2018;61:92-103.

46. Shadisvaaran S, Chin K-Y, Shahida M-S, Ima-Nirwana S, Leong X-F. Effect of vitamin E on periodontitis: Evidence and proposed mechanisms of action. *J Oral Biosci.* 2021;63(2):97-103.

47. Singh N, Chander Narula S, Kumar Sharma R, Tewari S, Kumar Sehgal P. Vitamin E supplementation, superoxide dismutase status, and outcome of scaling and root planing in patients with chronic periodontitis: a randomized clinical trial. *J Periodontol.* 2014;85(2):242-249.

48. Carvalho Rde S, de Souza CM, Neves JC, et al. Vitamin E does not prevent bone loss and induced anxiety in rats with ligature-induced periodontitis. *Arch Oral Biol.* 2013;58(1):50-58.

49. Hatipoglu M, Alptekin N, Avunduk MC. Effects of alpha-tocopherol on gingival expression of inducible nitric oxide synthase in the rats with experimental periodontitis and diabetes. *Niger J Clin Pr.* 2016;19(4):480.

50. Bas N, Kayar NA, Baba ZF, Avunduk MC, Haliloğlu S, Alptekin NÖ. Systemic treatment with alpha-tocopherol and/or sodium selenite decreases the progression of experimental periodontitis. *Clinical Oral Investigations.* 2021;25(5):2677-2688.

51. Boerma M, Roberto KA, Hauer-Jensen M. Prevention and Treatment of Functional and Structural Radiation Injury in the Rat Heart by Pentoxifylline and Alpha-Tocopherol. *Int J Radiat Oncol Biology Phys.* 2008;72(1):170-177.

52. Epstein MS, Wicknick FW, Epstein JB, Berenson JR, Gorsky M. Management of bisphosphonate-associated osteonecrosis: pentoxifylline and tocopherol in addition to antimicrobial therapy. An initial case series. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol, Endodontology.* 2010;110(5):593-596.

53. Magremanne M, Reychler H. Pentoxifylline and tocopherol in the treatment of yearly zoledronic acid-related osteonecrosis of the jaw in a corticosteroid-induced osteoporosis. *J Oral Maxillofac Surg.* 2014;72(2):334-337.

54. Owosho AA, Estilo CL, Huryn JM, Yom SK. Pentoxifylline and tocopherol in the management of cancer patients with medication-related osteonecrosis of the jaw: an observational retrospective study of initial case series. *Oral Surg, Oral Med, Oral Pathol Oral Radiol.* 2016;122(4):455-459.

55. Heifetz-Li JJ, Abdelsamie S, Campbell CB, Roth S, Fielding AF, Mulligan JP. Systematic review of the use of pentoxifylline and tocopherol for the treatment of medication-related osteonecrosis of the jaw. *Oral Surg, Oral Med, Oral Pathol Oral Radiol.* 2019;128(5):491-497.e492.

56. Cavalcante RC, Tomasetti G. Pentoxifylline and tocopherol protocol to treat medication-related osteonecrosis of the jaw: A systematic literature review. *J Cranio-Maxillofac Surg.* 2020;48(11):1080-1086.

57. Colapinto G, Goker F, Nocini R, et al. Outcomes of a Pharmacological Protocol with Pentoxifylline and Tocopherol for the Management of Medication-Related Osteonecrosis of the Jaws (MRONJ): A Randomized Study on 202 Osteoporosis Patients. *J Clin Med.* 2023;12(14):4662.

58. Arqueros-Lemus M, Mariño-Recabarren D, Niklander S, Martínez-Flores R, Moraga V. Pentoxifylline and tocopherol for the treatment of osteoradionecrosis of the jaws. A systematic review. *Med Oral, Patol Oral y Cirugía Bucal.* 2023;28(3):e293-e300.

59. Delfrate G, Mroczek T, Mecca LEA, et al. Effect of pentoxifylline and alpha-tocopherol on medication-related osteonecrosis of the jaw in rats: Before and after dental extraction. *Arch Oral Biol.* 2022;137:105397.

60. Banjar A, Patel V, Abed H. Pentoxifylline and tocopherol (vitamin E) with/without clodronate for the management of osteoradionecrosis: A scoping review. *Oral Diseases.* 2023;29(1):29-39.

61. Paiva GLA, Campos WGd, Rocha AC, Júnior CAL, Migliorati CA, Silva ARdS. Can the prophylactic use of pentoxifylline and tocopherol before dental extractions prevent osteoradionecrosis? A systematic review. *Oral Surg, Oral Med, Oral Pathol Oral Radiol.* 2023;136(1):33-41.

62. Conte Neto N, Spolidorio LC, Andrade CR, A SB, Guimaraes M, Marcantonio E, Jr. Experimental development of bisphosphonate-related osteonecrosis of the jaws in rodents. *Int J Exp Pathol.* 2013;94(1):65-73.

63. Macedo PD, Corbi ST, Kitagawa FA, Perussi JR, Ribeiro AO, Marcantonio RAC. Evaluation of the effects of photodynamic therapy with hypericin-glucamine in the treatment of periodontal disease induced in rats. *Lasers Dent Sci.* 2018;2(4):255-263.

64. Corbi SCT, Macedo PD, Perussi JR, Ribeiro AO, Marcantonio RAC. Evaluation of antimicrobial photodynamic therapy (aPDT) effects using zinc tetracarboxy-phthalocyanine N-methylglucamine salt photosensitizer as an adjunct therapy in the treatment of induced periodontal disease in rats. *Lasers Dent Sci.* 2020;4(1):43-52.

65. Goncalves FC, Mascaro BA, Scardueli CR, de Oliveira G, Spolidorio LC, Marcantonio RAC. Strontium ranelate improves post-extraction socket healing in rats submitted to the administration of bisphosphonates. *Odontology.* 2022;110(3):467-475.

66. Yalcin-Ulker GM, Cumbul A, Duygu-Capar G, Uslu Ü, Sencift K. Preventive Effect of Phosphodiesterase Inhibitor Pentoxifylline Against Medication-Related Osteonecrosis of the Jaw: An Animal Study. *J Oral Maxillofac Surg.* 2017;75(11):2354-2368.

67. Kuhr A, Popa-Wagner A, Schmoll H, Schwahn C, Kocher T. Observations on experimental marginal periodontitis in rats. *J Periodontal Res.* 2004;39(2):101-106.

68. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. *Mayo Clin Proc.* 2008;83(9):1032-1045.

69. Schwech N, Nilsson J, Gabre P. Incidence and risk factors for medication-related osteonecrosis after tooth extraction in cancer patients-A systematic review. *Clin Exp Dent Res.* 2023;9(1):55-65.

70. Srivastava A, Gonzalez GMN, Geng Y, et al. Prevalence of medication related osteonecrosis of the jaw in patients treated with sequential antiresorptive drugs: systematic review and meta-analysis. *Support Care Cancer.* 2021;29(5):2305-2317.

71. Hallmer F, Bjarnadottir O, Götrick B, Malmström P, Andersson G. Incidence of and risk factors for medication-related osteonecrosis of the jaw in women with breast cancer with bone metastasis: a population-based study. *Oral Surg, Oral Med, Oral Pathol Oral Radiol.* 2020;130(3):252-257.

4 CONCLUSÃO

Apesar das limitações de nossos estudos, eles oferecem uma compreensão valiosa sobre a osseointegração de implantes com superfícies hidrofílicas e os efeitos da pentoxifilina e α-tocoferol na remodelação óssea alveolar, em animais com altas concentrações de BP.

No primeiro estudo, demonstrou-se que ambas as superfícies testadas permitem a osseointegração em ratos com altas concentrações de BP. Enquanto a superfície HFL e os BP melhoraram a osseointegração, seus efeitos podem se manifestar de maneiras distintas. A superfície HFL parece favorecer a osseointegração ao promover uma formação óssea mais precoce, enquanto os BP podem melhorar a osseointegração ao reduzir a reabsorção óssea após a colocação do implante.

No segundo estudo, foram esclarecidos os potenciais benefícios da pentoxifilina e α-tocoferol na atenuação das alterações na remodelação óssea alveolar induzidas pela periodontite experimental em ratos tratados com BP. O grupo BPG-PenTo demonstrou consistentemente uma preservação superior dos parâmetros ósseos, indicando um efeito protetor sobre o osso alveolar. Importante notar que os grupos de tratamento exibiram alterações histológicas precoces, caracterizadas por inflamação e migração gengival, enfatizando a natureza complexa da progressão da periodontite.

As implicações clínicas destas descobertas podem orientar o desenvolvimento de estratégias clínicas mais eficazes, com o propósito de otimizar as taxas de sucesso dos implantes e melhorar os resultados para pacientes com periodontite que enfrentam desafios sistêmicos associados ao uso de BP. No entanto, é importante ressaltar que pesquisas adicionais e ensaios clínicos são necessários para validar e ampliar esses resultados.

REFERÊNCIAS*

1. Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. J Bone Miner Res. 1998; 13(4): 581-9.
2. Kimmel DB. Mechanism of action, pharmacokinetic and pharmacodynamic profile, and clinical applications of nitrogen-containing bisphosphonates. J Dent Res. 2007; 86(11): 1022-33.
3. Hughes DE, Wright KR, Uy HL, Sasaki A, Yoneda T, et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. J Bone Miner Res. 1995; 10(10): 1478-87.
4. Reszka AA, Halasy-Nagy JM, Masarachia PJ, Rodan GA. Bisphosphonates act directly on the osteoclast to induce caspase cleavage of mst1 kinase during apoptosis. A link between inhibition of the mevalonate pathway and regulation of an apoptosis-promoting kinase. J Biol Chem. 1999; 274(49): 34967-73.
5. Flanagan AM, Chambers TJ. Inhibition of bone resorption by bisphosphonates: interactions between bisphosphonates, osteoclasts, and bone. Calcif Tissue Int. 1991; 49(6): 407-15.
6. Piper K, Boyde A, Jones SJ. The effect of 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (ADP) on the resorptive function of osteoclasts of known nuclear number. Calcif Tissue Int. 1994; 54(1): 56-61.
7. Colucci S, Minielli V, Zambonin G, Cirulli N, Mori G, et al. Alendronate reduces adhesion of human osteoclast-like cells to bone and bone protein-coated surfaces. Calcif Tissue Int. 1998; 63(3): 230-5.
8. Russell RGG. Bisphosphonates: The first 40years. Bone. 2011; 49(1): 2-19.
9. Ledoux D, Hamma-Kourbali Y, Di Benedetto M, Foucault-Bertaud A, Oudar O, et al. A new dimethyl ester bisphosphonate inhibits angiogenesis and growth of human epidermoid carcinoma xenograft in nude mice. Anticancer Drugs. 2006; 17(4): 479-85.
10. Santini D, Vincenzi B, Avvisati G, Dicuonzo G, Battistoni F, et al. Pamidronate induces modifications of circulating angiogenetic factors in cancer patients. Clin Cancer Res. 2002; 8(5): 1080-4.
11. Cackowski FC, Anderson JL, Patrene KD, Choksi RJ, Shapiro SD, et al. Osteoclasts are important for bone angiogenesis. Blood. 2010; 115(1): 140-9.
12. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin Proc. 2008; 83(9): 1032-45.
13. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022; 80(5): 920-43.

* De acordo com o Guia de Trabalhos Acadêmicos da FOAr, adaptado das Normas Vancouver. Disponível no site da Biblioteca: http://www.foar.unesp.br/Home/Biblioteca/guia-de-normalizacao-atualizado.pdf

14. Brunsvold MA, Chaves ES, Kornman KS, Aufdemorte TB, Wood R. Effects of a bisphosphonate on experimental periodontitis in monkeys. J Periodontol. 1992; 63(10): 825-30.

15. Weinreb M, Quartuccio H, Seedor JG, Aufdemorte TB, Brunsvold M, et al. Histomorphometrical analysis of the effects of the bisphosphonate alendronate on bone loss caused by experimental periodontitis in monkeys. J Periodontal Res. 1994; 29(1): 35-40.

16. De Almeida J, Ervolino E, Bonfietti LH, Novaes VC, Theodoro LH, et al. Adjuvant Therapy With Sodium Alendronate for the Treatment of Experimental Periodontitis in Rats. J Periodontol. 2015; 86(10): 1166-75.

17. Moreira MM, Bradaschia-Correa V, Marques ND, Ferreira LB, Arana-Chavez VE. Ultrastructural and immunohistochemical study of the effect of sodium alendronate in the progression of experimental periodontitis in rats. Microsc Res Tech. 2014; 77(11): 902-9.

18. Storrer CLM, Deliberador TM, Giovanini AF, Crivellaro V, Zielak JC, Romito GA. Effect of alendronate on the progression of periodontitis induced by Porphyromonas gingivalis and Fusobacterium nucleatum: a study in rats. Clin Oral Investig. 2016; 20(9): 2565-73.

19. Menezes AM, Rocha FA, Chaves HV, Carvalho CB, Ribeiro RA, Brito GA. Effect of sodium alendronate on alveolar bone resorption in experimental periodontitis in rats. J Periodontol. 2005; 76(11): 1901-9.

20. Oktay S, Chukkapalli SS, Rivera-Kweh MF, Velsko IM, Holliday LS, Kesavalu L. Periodontitis in rats induces systemic oxidative stress that is controlled by bone-targeted antiresorptives. J Periodontol. 2015; 86(1): 137-45.

21. Santinoni CS, Silveira FM, Caldeira ML, Genaro V, Martins TM, et al. Topical sodium alendronate combined or not with photodynamic therapy as an adjunct to scaling and root planing: Histochemical and immunohistochemical study in rats. Journal of Periodontal Research. 2020; 55(6): 850-8.

22. Muniz F, Silva BFD, Goulart CR, Silveira TMD, Martins TM. Effect of adjuvant bisphosphonates on treatment of periodontitis: Systematic review with meta-analyses. J Oral Biol Craniofac Res. 2021; 11(2): 158-68.

23. Sacco R, Calasans-Maia MD, Woolley J, Akintola O, de Almeida Barros Mourão CF, et al. 18 Years of Medication-Related Osteonecrosis of the Jaw (MRONJ) Research: Where Are We Now?—An Umbrella Review. Appl Sci. 2021; 11(19): 8818.

24. Estilo CL, Van Poznak CH, Wiliams T, Bohle GC, Lwin PT, et al. Osteonecrosis of the maxilla and mandible in patients with advanced cancer treated with bisphosphonate therapy. Oncologist. 2008; 13(8): 911-20.

25. Kellesarian SV, Abduljabbar T, Vohra F, Malignaggi VR, Malmstrom H, et al. Role of local alendronate delivery on the osseointegration of implants: a systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2017; 46(7): 912-21.

26. Yajima N, Munakata M, Fuchigami K, Sanda M, Kasugai S. Influence of Bisphosphonates on Implant Failure Rates and Characteristics of Postmenopausal Woman Mandibular Jawbone. J Oral Implantol. 2017; 43(5): 345-9.

27. Schwech N, Nilsson J, Gabre P. Incidence and risk factors for medication-related osteonecrosis after tooth extraction in cancer patients-A systematic review. Clin Exp Dent Res. 2023; 9(1): 55-65.

28. Srivastava A, Gonzalez GMN, Geng Y, Won AM, Cabanillas ME, et al. Prevalence of medication related osteonecrosis of the jaw in patients treated with sequential antiresorptive drugs: systematic review and meta-analysis. Support Care Cancer. 2021; 29(5): 2305-17.

29. Hallmer F, Andersson G, Gotrick B, Warfvinge G, Anderud J, Bjornland T. Prevalence, initiating factor, and treatment outcome of medication-related osteonecrosis of the jaw-a 4-year prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018; 126(6): 477-85.

30. Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. Am J Obstet Gynecol. 2006; 194(2 Suppl): S3-11.

31. Bosshardt DD, Chappuis V, Buser D. Osseointegration of titanium, titanium alloy and zirconia dental implants: current knowledge and open questions. Periodontol 2000. 2017; 73(1): 22-40.

32. Sartoretto SC, Alves AT, Resende RF, Calasans-Maia J, Granjeiro JM, Calasans-Maia MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. J Appl Oral Sci. 2015; 23(3): 279-87.

33. Pinotti FE, de Oliveira G, Aroni MAT, Marcantonio RAC, Marcantonio E, Jr. Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: A Preclinical study. Clin Oral Implants Res. 2018; 29(10): 963-72.

34. Pinotti FE, Aron MAT, Oliveira G, Marcantonio Junior E, Marcantonio RAC. Implants with hydrophilic surfaces equalize the osseointegration of implants in normo- and hyperglycaemic rats. Braz Dent J. 2022; 33(6): 71-7.

35. Pinotti FE, Aroni MAT, Oliveira G, Silva BLG, Marcantonio Junior E, Marcantonio RAC. Osseointegration of implants with superhydrophilic surfaces in rats with high serum levels of nicotine. Braz Dent J. 2023; 34(2): 105-12.

36. Silva BLG, Sanchez-Puetate JC, Pinotti FE, Marcantonio CC, Pedroso GG, et al. Influence of obesity on osseointegration of implants with different surface treatments: A preclinical study. Clin Implant Dent Relat Res. 2023.

37. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Clin Periodontol. 2018; 45 Suppl 20: S1-S8.

38. Heifetz-Li JJ, Abdelsamie S, Campbell CB, Roth S, Fielding AF, Mulligan JP. Systematic review of the use of pentoxifylline and tocopherol for the treatment of medication-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019; 128(5): 491-7 e2.

39. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. Periodontol 2000. 2015; 69(1): 7-17.

40. Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020; 84(1): 14-34.

41. Tonetti MS, Sanz M. Implementation of the new classification of periodontal diseases: Decision-making algorithms for clinical practice and education. J Clin Periodontol. 2019; 46(4): 398-405.

42. Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, et al. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020; 47 Suppl 22(Suppl 22): 4-60.

43. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. Ann Periodontol. 2003; 8(1): 12-37.

44. Golub LM, Lee HM. Periodontal therapeutics: Current host-modulation agents and future directions. Periodontol 2000. 2020; 82(1): 186-204.

45. Preshaw PM. Host modulation therapy with anti-inflammatory agents. Periodontol 2000. 2018; 76(1): 131-49.

46. H RR, Dhamecha D, Jagwani S, Rao M, Jadhav K, et al. Local drug delivery systems in the management of periodontitis: A scientific review. J Control Release. 2019; 307: 393-409.

47. Pavanelli ALR, de Menezes BS, Pereira EBB, de Souza Morais FA, Cirelli JA, de Molon RS. Pharmacological Therapies for the Management of Inflammatory Bone Resorption in Periodontal Disease: A Review of Preclinical Studies. Biomed Res Int. 2022; 2022: 5832009.

48. McCarty MF, O'Keefe JH, DiNicolantonio JJ. Pentoxifylline for vascular health: a brief review of the literature. Open Hear. 2016; 3(1): e000365.

49. Queiroz-Junior CM, Bessoni RL, Costa VV, Souza DG, Teixeira MM, Silva TA. Preventive and therapeutic anti-TNF-alpha therapy with pentoxifylline decreases arthritis and the associated periodontal co-morbidity in mice. Life Sci. 2013; 93(9-11): 423-8.

50. Pollice PF, Rosier RN, Looney RJ, Puzas JE, Schwarz EM, O'Keefe RJ. Oral pentoxifylline inhibits release of tumor necrosis factor-alpha from human peripheral blood monocytes : a potential treatment for aseptic loosening of total joint components. J Bone Joint Surg Am. 2001; 83(7): 1057-61.

51. Gonzalez-Espinoza L, Rojas-Campos E, Medina-Perez M, Pena-Quintero P, Gomez-Navarro B, Cueto-Manzano AM. Pentoxifylline decreases serum levels of tumor necrosis factor alpha, interleukin 6 and C-reactive protein in hemodialysis patients: results of a randomized double-blind, controlled clinical trial. Nephrol Dial Transplant. 2012; 27(5): 2023-8.

52. Colson A, Willems B, Thissen JP. Inhibition of TNF-alpha production by pentoxifylline does not prevent endotoxin-induced decrease in serum IGF-I. J Endocrinol. 2003; 178(1): 101-9.

53. Lima V, Vidal FDP, Rocha FAC, Brito GAC, Ribeiro RA. Effects of Tumor Necrosis Factor-α Inhibitors Pentoxifylline and Thalidomide on Alveolar Bone Loss in Short-Term Experimental Periodontal Disease in Rats. J Periodontol. 2004; 75(1): 162-8.

54. Gutierrez-Reyes G, Lopez-Ortal P, Sixtos S, Cruz S, Ramirez-Iglesias MT, et al. Effect of pentoxifylline on levels of pro-inflammatory cytokines during chronic hepatitis C. Scand J Immunol. 2006; 63(6): 461-7.

55. Ferrari P, Mallon D, Trinder D, Olynyk JK. Pentoxifylline improves haemoglobin and interleukin-6 levels in chronic kidney disease. Nephrology (Carlton). 2010; 15(3): 344-9.

56. Singh N, Chander Narula S, Kumar Sharma R, Tewari S, Kumar Sehgal P. Vitamin E supplementation, superoxide dismutase status, and outcome of scaling and root planing in patients with chronic periodontitis: a randomized clinical trial. J Periodontol. 2014; 85(2): 242-9.

57. Carvalho Rde S, de Souza CM, Neves JC, Holanda-Pinto SA, Pinto LM, et al. Vitamin E does not prevent bone loss and induced anxiety in rats with ligature-induced periodontitis. Arch Oral Biol. 2013; 58(1): 50-8.

58. Shadisvaaran S, Chin K-Y, Shahida M-S, Ima-Nirwana S, Leong X-F. Effect of vitamin E on periodontitis: Evidence and proposed mechanisms of action. J Oral Biosci. 2021; 63(2): 97-103.

59. Hatipoglu M, Alptekin NO, Avunduk MC. Effects of alpha-tocopherol on gingival expression of inducible nitric oxide synthase in the rats with experimental periodontitis and diabetes. Niger J Clin Pract. 2016; 19(4): 480-5.

60. Arqueros-Lemus M, Marino-Recabarren D, Niklander S, Martinez-Flores R, Moraga V. Pentoxifylline and tocopherol for the treatment of osteoradionecrosis of the jaws. A systematic review. Med Oral Patol Oral Cir Bucal. 2023; 28(3): e293-e300.

61. Boerma M, Roberto KA, Hauer-Jensen M. Prevention and treatment of functional and structural radiation injury in the rat heart by pentoxifylline and alpha-tocopherol. Int J Radiat Oncol Biol Phys. 2008; 72(1): 170-7.

62. Epstein MS, Wicknick FW, Epstein JB, Berenson JR, Gorsky M. Management of bisphosphonate-associated osteonecrosis: pentoxifylline and tocopherol in addition to antimicrobial therapy. An initial case series. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 110(5): 593-6.

63. Magremanne M, Reychler H. Pentoxifylline and tocopherol in the treatment of yearly zoledronic acid-related osteonecrosis of the jaw in a corticosteroid-induced osteoporosis. J Oral Maxillofac Surg. 2014; 72(2): 334-7.

64. Owosho AA, Estilo CL, Huryn JM, Yom SK. Pentoxifylline and tocopherol in the management of cancer patients with medication-related osteonecrosis of the jaw: an observational retrospective study of initial case series. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016; 122(4): 455-9.

65. Cavalcante RC, Tomasetti G. Pentoxifylline and tocopherol protocol to treat medication-related osteonecrosis of the jaw: A systematic literature review. J Craniomaxillofac Surg. 2020; 48(11): 1080-6.

66. Colapinto G, Goker F, Nocini R, Albanese M, Nocini PF, et al. Outcomes of a Pharmacological Protocol with Pentoxifylline and Tocopherol for the Management of Medication-Related Osteonecrosis of the Jaws (MRONJ): A Randomized Study on 202 Osteoporosis Patients. J Clin Med. 2023; 12(14): 4662.

67. Delfrate G, Mroczek T, Mecca LEA, Andreis JD, Fernandes D, et al. Effect of pentoxifylline and alpha-tocopherol on medication-related osteonecrosis of the jaw in rats: Before and after dental extraction. Arch Oral Biol. 2022; 137: 105397.

68. Banjar A, Patel V, Abed H. Pentoxifylline and tocopherol (vitamin E) with/without clodronate for the management of osteoradionecrosis: A scoping review. Oral Dis. 2023; 29(1): 29-39.

69. Paiva GLA, de Campos WG, Rocha AC, Junior CAL, Migliorati CA, Dos Santos Silva AR. Can the prophylactic use of pentoxifylline and tocopherol before dental extractions prevent osteoradionecrosis? A systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2023; 136(1): 33-41.

APÊNDICE A - MATERIAL E MÉTODOS

Comitê de Ética

Os dois projetos foram aprovados pelo Comitê de Ética em Experimentação Animal da Faculdade de Odontologia de Araraquara – UNESP. Projeto 1 (CEUA: 07/2019) (Anexo B), e projeto 2: (CEUA: 16/2018) (Anexo C). Os experimentos foram realizados de acordo com as diretrizes do Conselho Nacional de Controle de Experimentação Animal (CONCEA), e as normas internacionais da Animal Research Reporting of In Vivo Experiments (ARRIVE 2.0).

Amostra

Neste estudo foram utilizados 184 ratos machos adultos (Rattus norvegicus, variação Albinus, Holtzman) com peso variando entre 180-220 gramas (18 semanas de idade) provenientes do Biotério da Faculdade de Odontologia de Araraquara (FOAr) – UNESP. Os animais foram mantidos em caixas apropriadas (4 animais/caixa) no biotério da Faculdade de Odontologia de Araraquara-UNESP e expostos a condições padronizadas de temperatura e umidade (22°C ± 1°C, 65%-70%), ciclo claro/escuro de 12 horas (luzes acesas às 7:00 da manhã), com acesso a alimentos e água ad libitum.

O cálculo do tamanho da amostra foi baseado na variabilidade observada em nossos estudos anteriores[1-4] que avaliaram o volume ósseo (como o principal resultado) em ratos submetidos à doença periodontal experimental. Assim, uma amostra de sete animais por grupo/período foi suficiente para aplicar testes estatísticos e detectar diferenças significativas entre os grupos com um erro do tipo α de 0,05 e um poder β de 0,90. Foram utilizados oito animais por grupo para contabilizar uma perda mínima de 14%, o que é aceitável. Esse cálculo garante que o estudo tenha uma amostra suficiente para detectar diferenças significativas entre os grupos, aumentando a confiabilidade e a robustez dos nossos resultados.

Grupos Experimentais de Acordo com os Estudos

Estudo 1 (Publicação 1): Avaliar os efeitos das superfícies de implantes hidrofóbicas (HFB) e hidrofílicas (HFL) na osseointegração no modelo de ratos tratados com altas doses de bisfosfonatos (BP).

Medicamentos e indução de BP

Os animais receberam 0,3 ml de solução salina estéril (SS) ou alendronato de sódio (BP) na concentração de 1mg/kg/día por meio de aplicações subcutâneas seguindo a metodologia relatada anteriormente pelo nosso grupo de pesquisa[1]. Para induzir a alta dose de BP, os animais dos grupos experimentais receberam 60 dias de administração sistêmica de 1mg/kg de Alendronato de sódio.

Desenho experimental

Um total de 64 animais foram divididos aleatoriamente em quatro grupos e avaliados em dois períodos experimentais diferentes: 15 e 45 dias (n=8). Os grupos foram divididos de acordo com a aplicação da terapia com BP e o tipo de superfície do implante em (Figura A1)

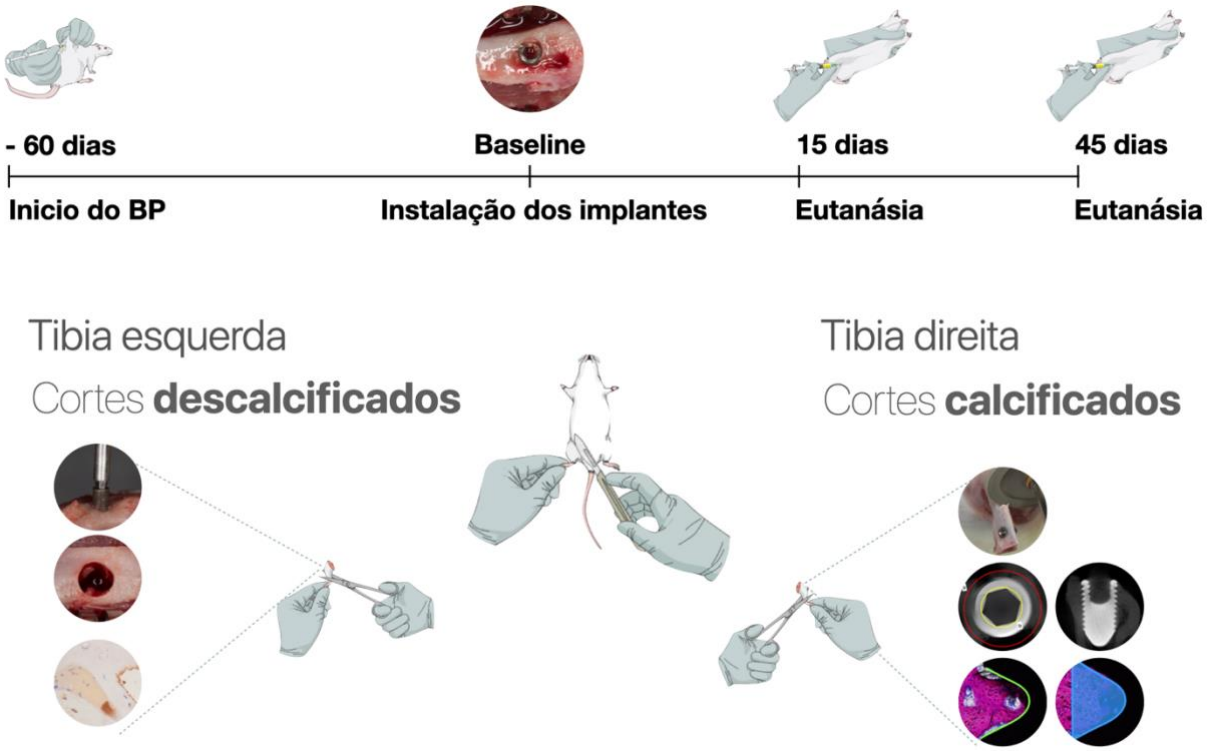
CG-HFB: Superfície de implante hidrofóbica modificada por jateamento de óxido e condicionamento ácido (NeoPoros®, Neodent®, Curitiba, PR, Brasil) colocada em animais sem uso prévio de BP.

CG-HFL: Superfície de implante hidrofílica modificada por jateamento de óxido e condicionamento ácido em ambiente a vácuo e mantida em solução de cloreto de sódio (Acqua®, Neodent®, Curitiba, PR, Brasil) colocada em animais sem uso prévio de BP.

BP-HFB: Superfície de implante hidrofóbico modificada por jateamento de óxido e condicionamento ácido (NeoPoros®, Neodent®, Curitiba, PR, Brasil) colocada em animais com aplicação prévia de BP.

BP-HFL: Superfície de implante hidrofílico modificada por jateamento de óxido e condicionamento ácido em um ambiente a vácuo e mantida em solução de cloreto de sódio (Acqua®, Neodent®, Curitiba, PR, Brasil) colocada em animais com aplicação prévia de BP.

Figura A1- Desenho experimental do projeto 1



Fonte: Elaboração própria.

Procedimento cirúrgico da instalação de implantes customizados

Para esse procedimento seguimos a metodologia desenvolvida anteriormente e bem estabelecida na literatura pelo nosso grupo de pesquisa[5-8]. Os animais foram anestesiados com uma injeção intramuscular de cetamina (Agener União Ltda., São Paulo, SP, Brasil) na dose de 0,08 ml/100 g de massa corporal e xilazina (Rompum, Bayer S.A., São Paulo, SP, Brasil) na dose de 0,04 ml/100 g de massa corporal. Em seguida, os animais foram submetidos à tricotomia da região interna das tíbias direita e esquerda, onde previamente foi realizada a antissepsia.

Uma incisão de aproximadamente 10 mm foi feita sobre a tuberosidade da tíbia e uma dissecção delicada do tecido foi realizada. O local da cirurgia foi preparado para a colocação do implante por meio de uma sequência progressiva de brocas (broca de lança; broca espiral de 2,0 mm - Neodent®; Curitiba, PR, Brasil) para instalar um implante de titânio customizado de 4 × 2,2 mm (implantes de superfície NeoPoros e Acqua, Neodent®, Curitiba, PR, Brasil). Todas as perfurações foram realizadas com um motor elétrico ajustado para 1200 rpm (BLM 600, Driller, São Paulo, Brasil) sob irrigação abundante com solução salina estéril. O implante foi

colocado com o auxílio de uma chave digital (chave digital hexagonal de 1,2 mm - Neodent®, Curitiba, PR, Brasil). O tecido foi suturado com fio de seda 4.0 (Ethicon, Johnson & Johnson, São José dos Campos, Brasil). Os animais receberam uma dose única de penicilina intramuscular com estreptomicina na dosagem de 0,1 ml/kg de massa corporal (Pentabiotic® (Wyeth-Whitehall Ltda., São Paulo, Brasil) e cetoprofeno subcutâneo na dosagem de 0,03 ml/kg de massa corporal (Ketoflex; Mundo Animal, São Paulo, Brasil).

Os implantes foram colocados na tíbia esquerda e direita de cada animal, em um total de cento e vinte e oito implantes instalados (64 na tíbia esquerda e 64 na direita). Não houve efeitos adversos inesperados. Aos 15 e 45 dias após a colocação do implante, os animais foram sacrificados com uma overdose de anestésico. (Figura A2).

Figura A2 - Procedimento cirúrgico da instalação de implantes customizados na tíbia dos animais

Fonte: Arquivo pessoal do autor.

Processamento das amostras

Após as eutanásias as tíbias foram removidas, separadas e armazenadas da seguinte forma: As tíbias do lado direito foram transferidas para etanol a 70% para posterior escaneamento por tomografia computadorizada (μCT) e avaliar Bone Volume/Tissue Volume (BV/TV%) e posterior processamento do corte não descalcificado mediante sistema de corte e retificação (Exakt Apparatebeau, Hamburgo, Alemanha) para realizar as análises histomorfométricas Bone-to-implant Contact (BIC%) e Bone Between Threads (BBT%). E as tíbias do lado esquerdo, foram usadas para análise biomecânica (remoção do implante) e imuno-histoquímica. Após a remoção do implante essas amostras foram fixadas com paraformaldeído a 4% por 48 horas e lavadas em água corrente por 24 horas para posterior descalcificação em EDTA 10% (pH 7,2) por 16 semanas.

Análise biomecânico

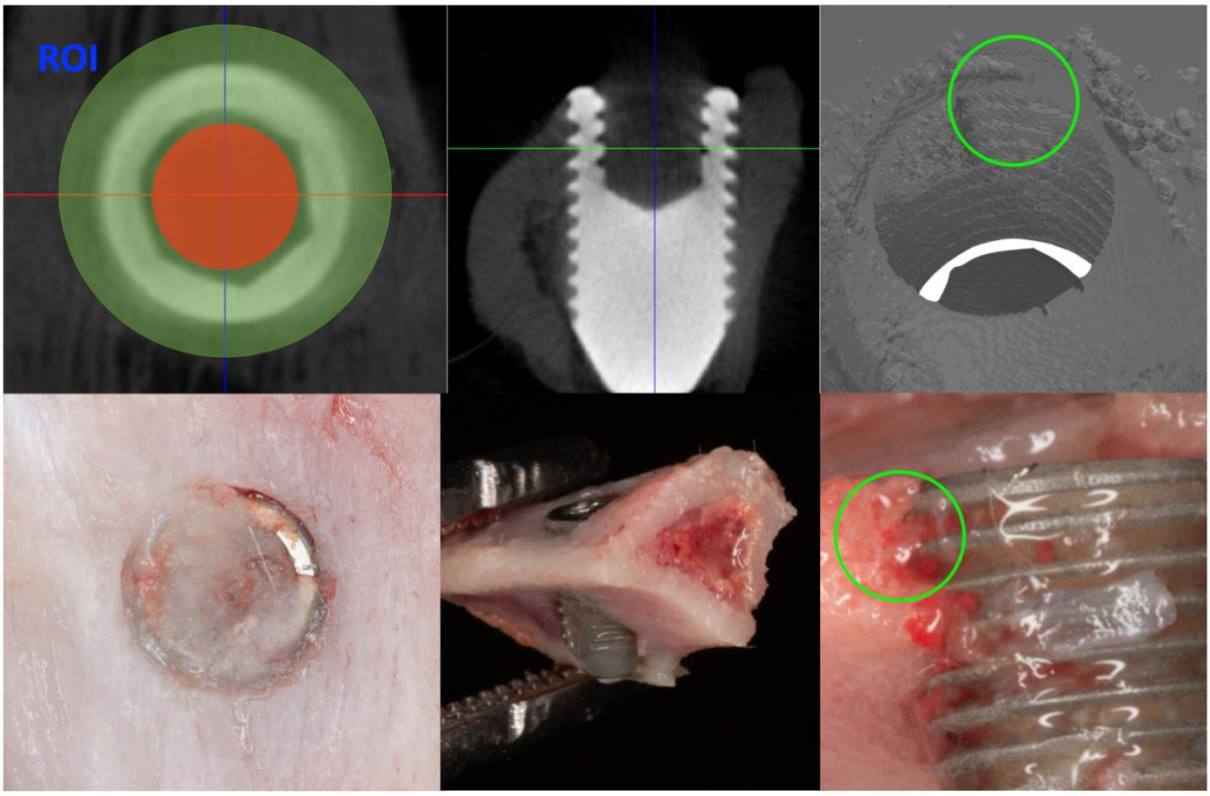
Uma chave hexagonal (1,2 mm de diâmetro) foi conectada ao implante e à chave de torque (Tohnichi, modelo ATG24CN-S, Toquio, Japão). Foi realizado um movimento no sentido anti-horário para remover o implante. O pico máximo de torque necessário para mover o implante foi o valor do torque de remoção (Ncm).

Análise microtomográfica (µCT)

Para realizar a avaliação microtomográfica, as tíbias foram escaneadas por um microtomógrafo (Skyscan, Aatselaar, Bélgica) com os seguintes parâmetros: pixel da câmera, 12,45; potência do tubo de raios X, 65 kVP; intensidade de raios X, 385 µA; tempo de integração, 300 ms; filtro, Al-1 mm; e tamanho do voxel, 18 µm3. Após o escaneamento das amostras, as imagens foram reconstruídas, reposicionadas espacialmente e analisadas por um software específico (NRecon, Data Viewer, CTAnalyser, Aatselaar, Bélgica).

A região de interesse (ROI) foi definida como uma região circular de 0,5 mm em torno de todo o diâmetro do implante. Essa ROI foi definida como o volume total (margem de 0,5 mm ao redor dos implantes - 4,5 mm × 3,2 mm). Como os implantes colocados não tinham um parafuso de cobertura, em alguns casos, ocorreu formação óssea dentro da plataforma protética. Uma segunda ROI foi definida para remover o volume da plataforma a fim de evitar que essa formação óssea interferisse na análise ao redor do implante. Com os resultados obtidos das duas ROIs, foi possível definir o volume da formação óssea usando a fórmula: volume total - volume da plataforma = volume dos tecidos ósseos. O limiar usado na análise foi de 25-90 em escala de cinza, e os valores de volume do tecido mineralizado ao redor dos implantes foram obtidos como uma porcentagem (BV/TV%). (Figura A3).

Figura A3- Análise microtomográfica (µCT)



Delimitação das regiões de interesse (ROI) e comparação com fotografias clínicas.

Fonte: Arquivo pessoal do autor.

Análise histomorfométrica

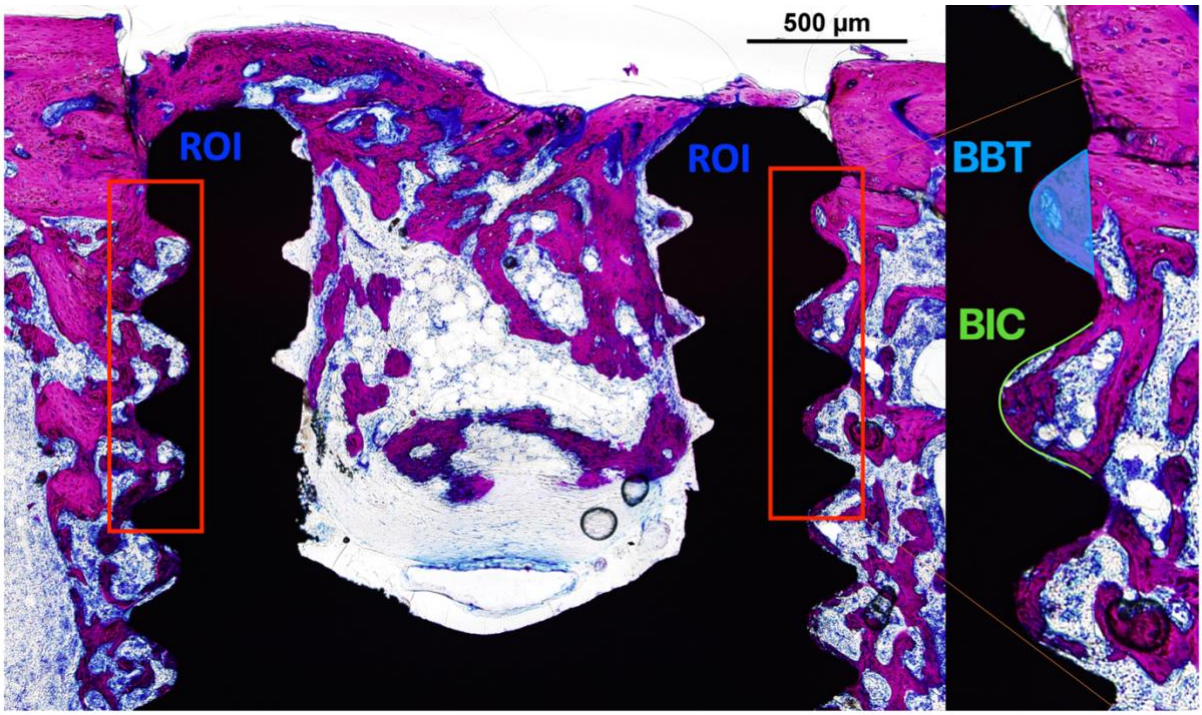
Após o escaneamento microtomográfico, as tíbias que não foram descalcificadas foram desidratadas em uma série crescente de etanol (60-100%) e infiltradas e polimerizadas em resina fotopolimerizável (Technovit 7200 VLC, Kultzer Heraus GmbH & CO, Wehrheim, Alemanha). Os blocos contendo o implante e o tecido ósseo circundante foram cortados em um ponto central usando um sistema de corte e retificação (Exakt Apparatebeau, Hamburgo, Alemanha). As seções de 45 µm de espessura foram coradas com azul de Stevenel associado à fucsina ácida e analisadas em um microscópio óptico de luz Leica DM2500 (Leica Microsystems GmbH, Wetzlar, Alemanha) com magnificación de 200x (Objetiva 20x e amplificador de 10x). As avaliações histomorfométricas foram realizadas com o kit de ferramentas de processamento de imagens FIJI (National Institutes of Health, Maryland, EUA).

As porcentagens de contato osso-implante (BIC%) e a área óssea entre as roscas (BBT%) foram avaliadas separadamente nas três primeiras roscas dos implantes inseridos no osso cortical[8] (Figura A4 e A5).

Figura A4 – Fotografias representativas do processamento dos cortes não descalcificados

Fonte: Arquivo pessoal do autor.

Figura A5 - Análise histomorfométrica



Fotografia representativa de lâmina corada com Stevenel's blue e esquema da delimitação das regiões de interesse (ROI) para análise do BIC% e BBT%

Fonte: Elaboração própria.

Análise imuno-histoquímica

As análises imuno-histoquímicas foram realizadas nas tíbias onde os implantes foram removidos após a análise biomecânica para identificar e localizar a expressão de proteínas relacionadas à remodelação óssea, ou seja, fosfatase alcalina (ALP) e proteína morfogênica óssea-2 (BMP-2). As seções histológicas foram montadas em lâminas silanizadas, seguidas de procedimentos laboratoriais de rotina para desparafinização e reidratação. Posteriormente, as seções foram submetidas a um bloqueio de epítopo inespecífico com a aplicação de peróxido de hidrogênio a 3% por 30 minutos e proteína de albumina bovina a 3% por 120 minutos. Em seguida, as seções foram incubadas por 16 horas com os seguintes anticorpos primários: ALP (1:100) e BMP-2 (1:50). Como controle negativo, as seções histológicas foram tratadas com solução salina tamponada com fosfato (PBS) a 1%. Posteriormente, as seções foram tratadas com o complexo avidina-biotina-peroxidase e coradas com diaminobenzidina (Dako, Glostrup, Dinamarca). As seções foram coradas com a solução de hematoxilina de Carrazi para visualizar os núcleos das células. As imagens foram obtidas com uma câmera acoplada a um microscópio

de luz (Leica Reichert Diastar Products & Jung, Wetzlar, Alemanha). Os níveis de expressão das proteínas foram avaliados pela aplicação de um índice de extensão de marcação de proteínas[9] que foi realizado na metade coronal do leito do implante cirúrgico aos 15 dias: (0) nenhuma marcação (0% de células/matriz); (1) marcação fraca (<25% de células/matriz); (2) marcação moderada (25%-50% de células/matriz); e (3) marcação intensa (<50% de células/matriz).

Estudo 2 (Publicação 2): Avaliar a influência da pentoxifilina e α-tocoferol (protocolo PenTo) na remodelação do osso alveolar durante a progressão da Periodontite Experimental (PD) induzida por ligadura, no modelo de ratos sob os efeitos das altas concentrações de bisfosfonatos (BP).

Medicamentos e indução de BP

Os animais receberam solução salina estéril (SS) e outros medicamentos com diferentes tipos de administração devido à natureza dos compostos e ao tempo de administração. Quando a SS foi administrada, foi em uma quantidade de 0,3 ml por meio de aplicações subcutâneas. O BP usado foi o alendronato de sódio na concentração de 1mg/kg e foi administrado por via sistêmica por meio de injeções subcutâneas diárias, seguindo a metodologia relatada anteriormente pelo nosso grupo de pesquisa[1]. Os medicamentos experimentais foram administrados sistemicamente por injeções intraperitoneais diárias em uma concentração de 50 mg/kg de pentoxifilina[10] e 40 mg/kg de α-tocoferol, até o momento das eutanásias (7,15 e 30 días)[11]. Para induzir a alta dose de BP, os animais dos grupos experimentais de BP receberam 60 dias de administração sistêmica de 1mg/kg de BP.

Indução da Periodontite por ligadura

A Periodontite foi induzida pela colocação de ligaduras na região subgengival ao redor dos segundos molares inferiores bilateralmente, sob anestesia geral por meio da administração intraperitoneal de cetamina (Ketamine Agener; Agener União Ltda., São Paulo, SP, Brasil; 0,08 mL/100 g de peso corporal) e xilazina 2% (Rompum; Bayer S.A., São Paulo, SP, Brasil; 0,04 mL/100 g de peso corporal).

Desenho experimental

Um total de 120 animais foram distribuídos aleatoriamente nos cinco grupos experimentais a seguir (n = 8/grupo) (Figura A6):

GC: grupo controle com animais que receberam SS durante todo o experimento.

BPG-B: animais que receberam 1mg/kg/dia de PB durante todo o experimento.

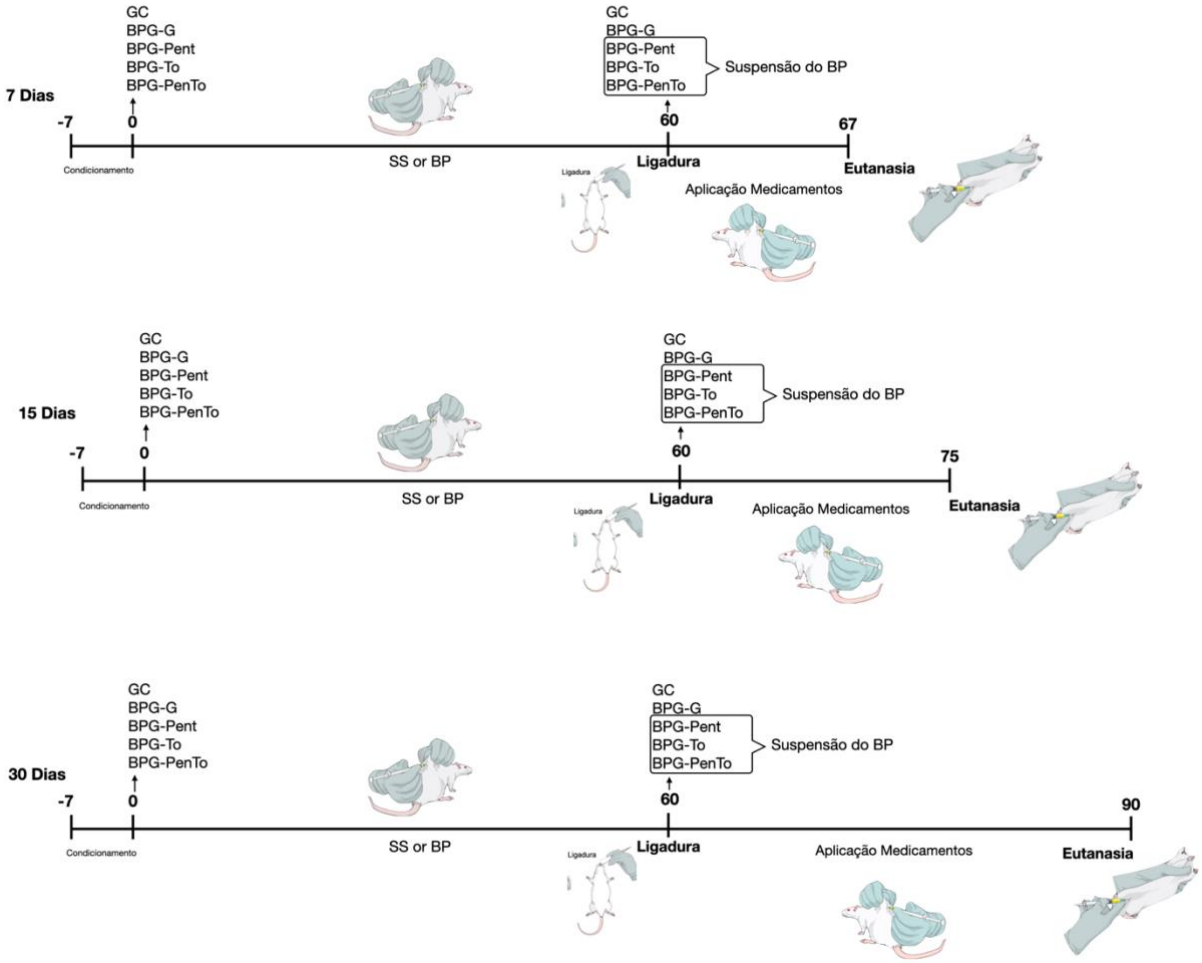
BPG-Pent: animais que receberam 50 mg/kg/dia de Pentoxifilina após a instalação da ligadura e previamente tratados com BP.

BPG-To: animais que receberam 40 mg/kg de α-tocoferol após a instalação da ligadura e previamente tratados com BP.

BPG-PenTo: animais que receberam uma combinação de 50 mg/kg/dia de pentoxifilina mais 40 mg/kg de α-tocoferol após a instalação da ligadura e previamente tratados com BP.

Nos três grupos que receberam os medicamentos experimentais (BPG-Pent, BPG-To e BPG-PenTo). As aplicações de BP foram retiradas após a instalação das ligaduras. Após 7, 15 e 30 dias da colocação das ligaduras, os animais foram eutanasiados por overdose letal de anestésico, e as mandíbulas foram divididas em duas hemi-mandíbulas para processamento de análises diferentes.

Figura A6- Desenho experimental do projeto 2



Fonte: Elaboração própria.

Processamento das amostras

Posteriormente, as hemi-mandíbulas direita e esquerda foram fixadas com paraformaldeído a 4% por 48 horas e lavadas em água corrente por 24 horas. As hemi-mandíbulas direitas foram transferidas para etanol a 70% para escaneamento por tomografia computadorizada (μCT). As hemi-mandíbulas esquerdas foram submetidas à descalcificação em EDTA 10% (pH 7,2) por 16 semanas.

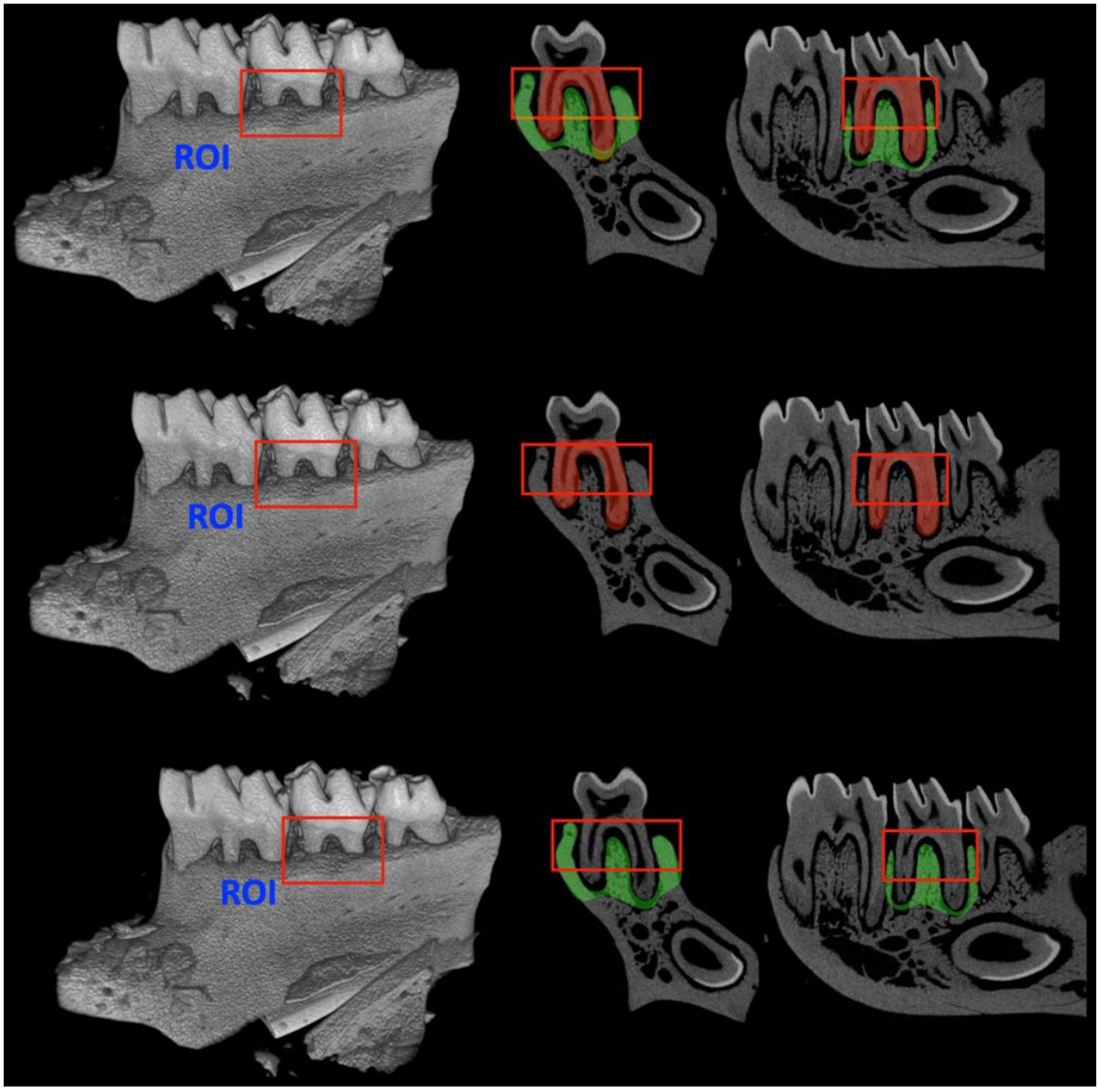
Remodelação do osso alveolar por análise radiográfica tridimensional (uCT)

As hemi-mandíbulas direitas foram escaneadas por uma microtomografia - µCT (Skyscan 1176, Bruker, Aatselaar, Bélgica) seguindo os parâmetros: Tamanho do pixel da câmera (um)=7,4; tamanho do pixel da imagem (um)=12,000006; tamanho do voxel: 18 µm3; exposição (ms)=500; tensão da fonte (kV)=80; potência do tubo de raios X: 65 kVP; intensidade dos raios X: 385 µA; filtro=Al 1mm; etapa de rotação 0,4° em 360°.

As imagens foram reconstruídas pelo software NRecon (NRecon 1.6.1.5; Bruker, Aatselaar, Bélgica), os seguintes parâmetros foram usados: correção de artefato de anel, 4; máscara de defeito de pixel, 5%; suavização, 1%; correção de endurecimento do feixe, 26%; e conversão de sensoriamento comprimido (CS) para imagem, 0,0-0,8. Em seguida, as imagens foram reposicionadas espacialmente pelo DataViewer (Bruker, Aatselaar, Bélgica) e analisadas pelo CTAnalyser (CTAnalyser, Aatselaar, Bélgica).

Uma região de interesse (ROI) de dois padrões foi meticulosamente definida. ROI 1: foi delimitada da raiz distal do primeiro molar até a raiz mesial do terceiro molar e se estendeu do topo da furca e da crista alveolar até 100 fatias em sentido apical, e ROI 2: delimitou as raízes. As ROIs foram delimitadas a cada dez planos. Após a obtenção dos valores de ROI, a ROI 1 - ROI 2 foi subtraída, resultando na fração de volume da ROI ocupada por tecido mineralizado (fração de volume ósseo [BVF]) que foi quantificada com um limite de 90-225. Essa estratégia rigorosa maximiza a quantificação óssea e descarta os tecidos das raízes e dos dentes. Os resultados são representativos de oito animais por grupo (Figura A7).

Figura A7 - Análise radiográfica tridimensional (uCT). Delimitação esquemática do ROI



Delimitação das regiões de interesse (ROI) nas imagens representativas da reconstrução 3D e cortes sagital e coronal.

Fonte: Elaboração própria.

Processamento histológico

Após o processo de descalcificação, as hemi-mandíbulas esquerdas foram incluídas em parafina. Para cada amostra, aproximadamente 30 seções semi-seriadas (4μm de espessura) foram obtidas a partir do plano sagital em direção ao eixo lingual/vestibular ao longo do

segundo molar mandibular e divididas em lâminas contendo 3 seções cada. As seções semi-seriadas foram coradas com Hematoxilina e Eosina (H/E).

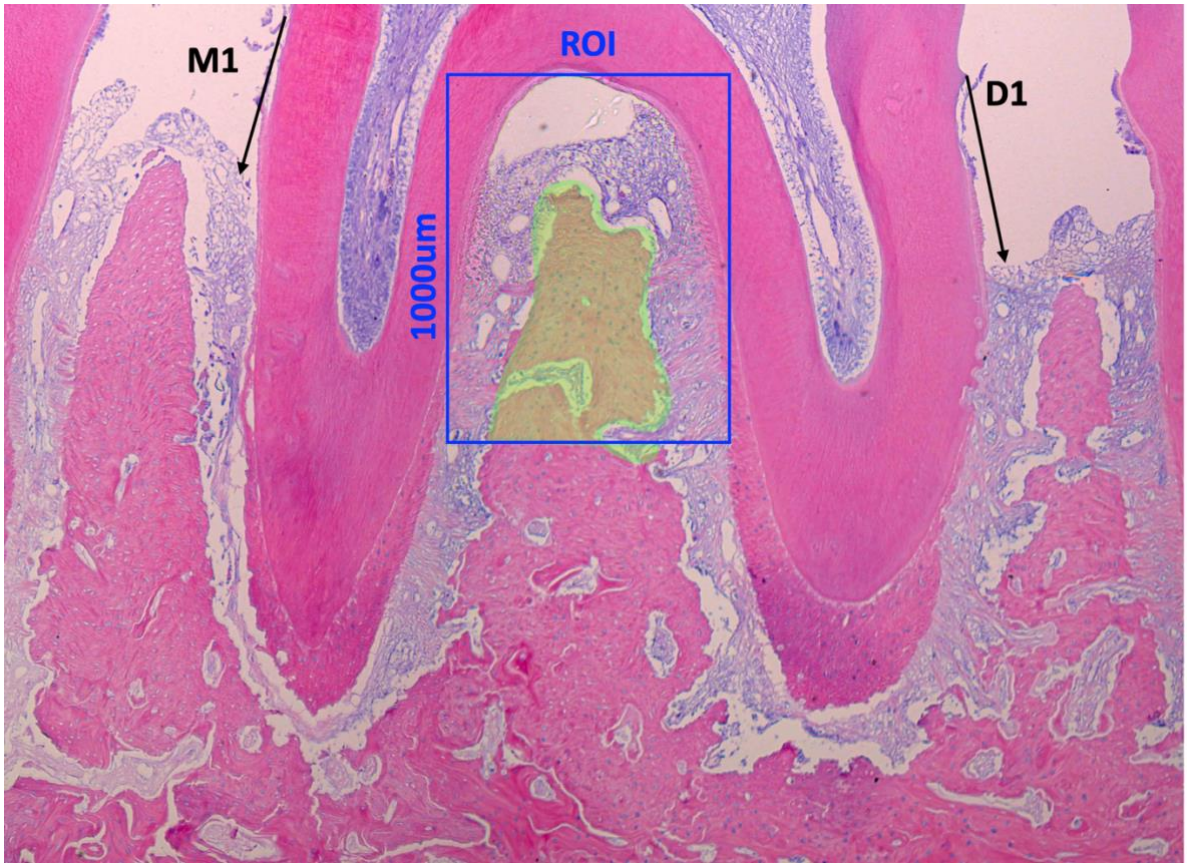
Análises histológicas

As imagens das lâminas das seções semi-seriadas coradas com H/E foram obtidas usando configurações padronizadas para aquisição de imagens com um microscópio óptico de luz Leica DM2500 (Leica Microsystems GmbH, Wetzlar, Alemanha) acoplado a uma câmera digital colorida Leica DFC500 (Leica Microsystems GmbH, Wetzlar, Alemanha), com uma objetiva de 40x. A análise das estruturas morfológicas dos tecidos foi realizada por meio da avaliação dos processos de remodelação do tecido ósseo e das reações inflamatórias em cada grupo experimental; os resultados são representativos de uma seção semi-seriada por amostra.

Análises histométricas

As análises foram realizadas em imagens obtidas com a objetiva de 20x e as mesmas configurações padronizadas para aquisição de imagens previamente descritas. A ROI foi delimitada nas regiões de furca e interproximal. Na região da furca, após medir 1000μm do topo da furca, foi delimitada uma área entre as raízes. Nessa região, foi medida a área com o osso presente. Nas regiões interproximais (mesial e distal), foram feitas medições lineares entre a junção cemento-esmalte e a crista óssea usando linhas retas. As medições foram feitas usando o kit de ferramentas de processamento de imagens FIJI (National Institutes of Health, Maryland, EUA) com 3 seções representativas semi-seriadas por amostra (Figura A8).

Figura A8 - Análises histométricas



Fotografia reconstruída a partir de imagens com aumento de 20x de cortes corados com H/E, na região do segundo molar inferior. Setas M1 e D1, indicando as medidas lineares realizadas nesta região interproximal. Em destaque verde, a área analisada para histometria de região de furca, Em azul, delimitação da furca por ROI previamente definido.

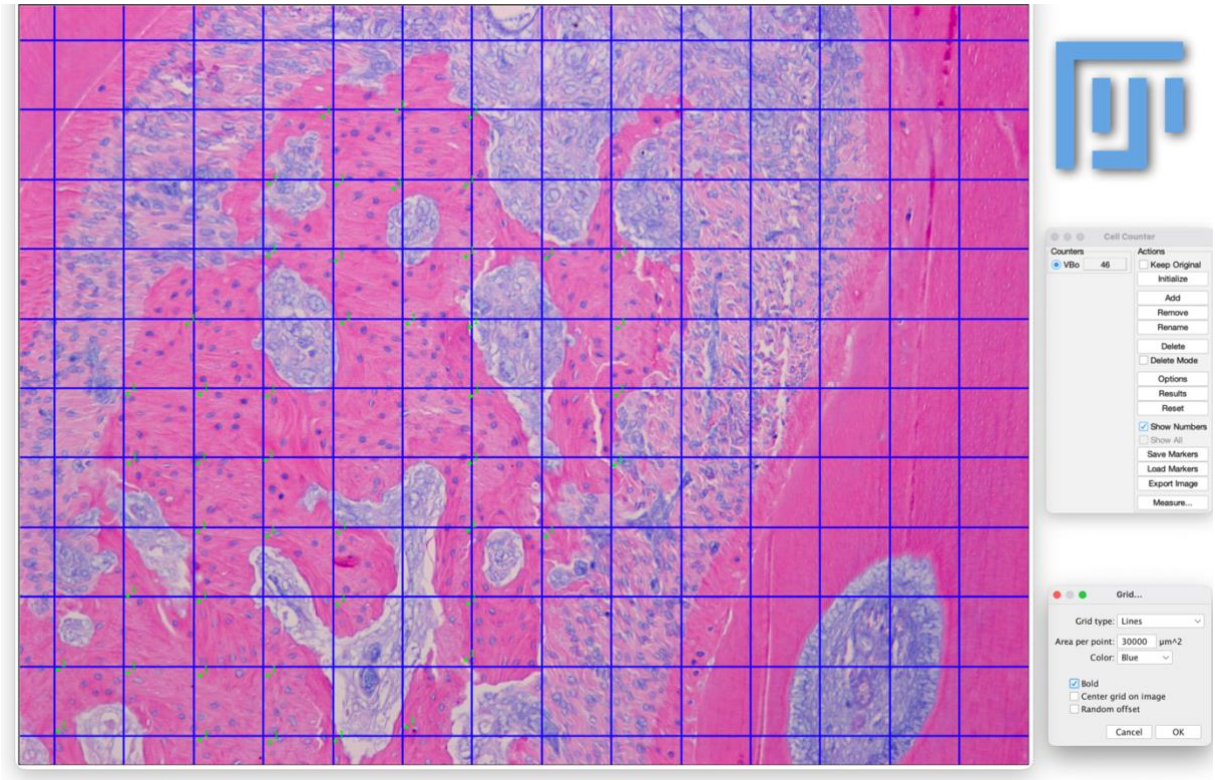
Fonte: Elaboração própria.

Análises estereométricas

A análise estereométrica foi realizada usando as mesmas imagens para análises histométricas com métodos empregados em nossa pesquisa anterior[2,3]. As três ROI foram definidas da seguinte forma: Tecidos interproximais localizados entre o primeiro e segundo molares (mesial) e entre o terceiro e segundo molares (distal), delimitados coronalmente pela porção mais apical do tecido epitelial; apicalmente pelo topo da crista óssea; e lateralmente pela região mais proeminente da raiz do segundo molar. A região da furca, localizada entre as raízes, foi delimitada coronalmente pelo topo da furca, apicalmente pelo topo da crista óssea e lateralmente pelas raízes do segundo molar. Usando uma ferramenta de processamento de imagens FIJI (National Institutes of Health, Maryland, EUA), uma grade de 154 pontos foi sobreposta digitalmente na região de interesse (ROI), e a proporção de tecido ósseo vital (VBo)

foi realizada em relação ao número total de pontos contados, e a porcentagem de cada componente do tecido foi calculada em relação ao número total de pontos com 3 seções representativas semi-seriadas por amostra (Figura A9).

Figura A9 – Análises estereométricas



Imagens histológicas representativas coradas com H/E da metodologia utilizada para a análise estereométrica de contagem de constituintes teciduais. Colocação da grade com 154 pontos na ROI previamente definida. Magnificação 200x.

Fonte: Elaboração própria.

Análise estatística

Projeto 1:

O Jamovi 2.4.1 (Sidney, Austrália) foi usado para análise estatística. Os dados obtidos das análises biomecânica, microtomográfica e histomorfométrica foram avaliados quanto à normalidade por meio do teste de Shapiro-Wilk e homoscedasticidade pela análise das variâncias, demonstrando, em todas as avaliações, uma distribuição normal (α=0,05). A relação entre o tipo de superfície do implante vs. período experimental vs. condição de terapia com PB e as diferenças entre os grupos foram examinadas por meio do three-way ANOVA seguido pelo teste post hoc de Tukey ($p<0,05$). A comparação da expressão proteica foi realizada pelo teste não paramétrico de Kruskal-Wallis seguido pelo teste de Dunn. Todos os testes estatísticos usados neste estudo foram aplicados com um nível de significância definido em 95%.

Projeto 2:

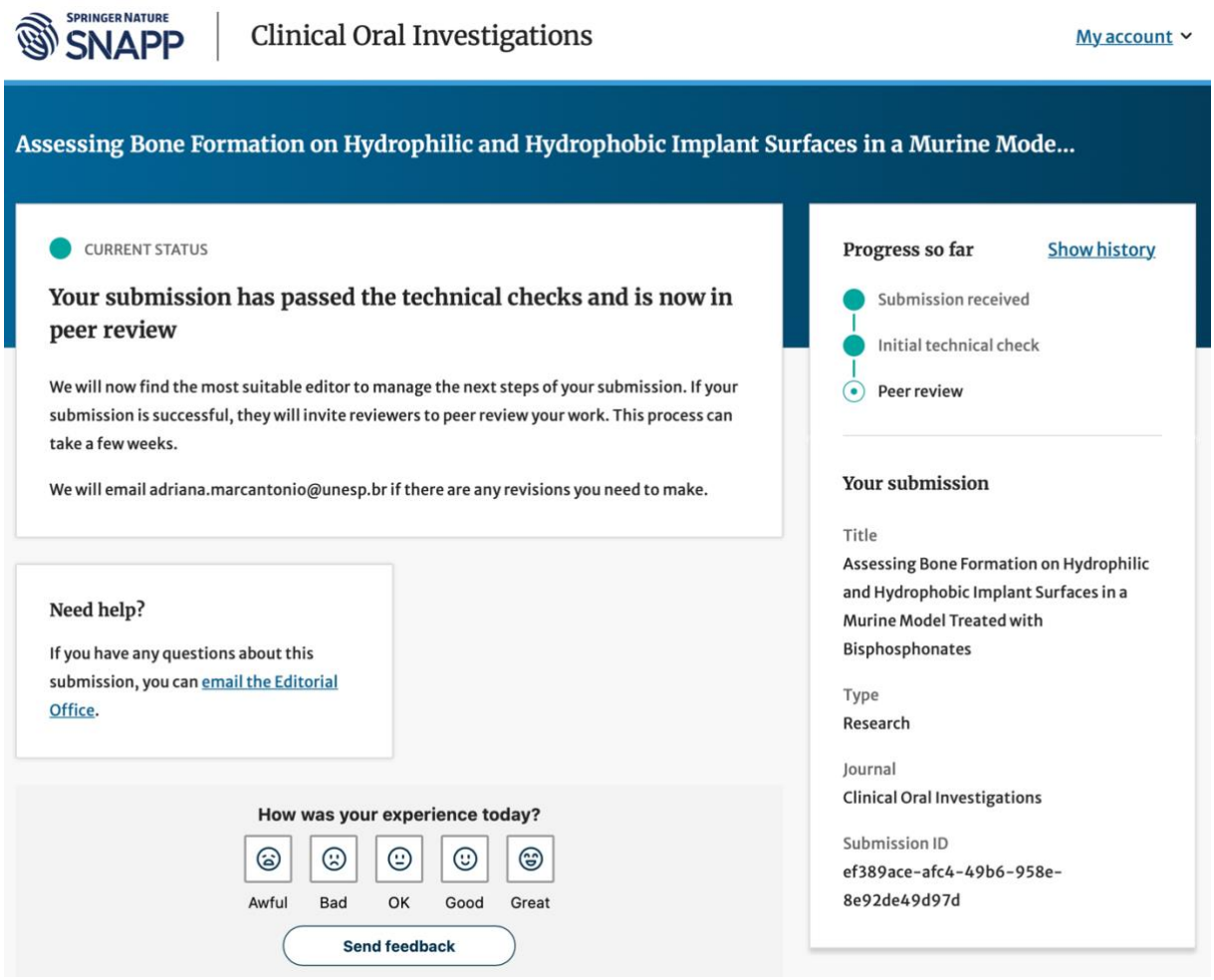
Os dados obtidos de cada experimento foram analisados com o GraphPad Prism versão 10.0.2 para Mac (GraphPad Software Inc., Boston, MA, EUA). Os dados foram avaliados quanto à normalidade homocedasticidade usando o teste de Shapiro-Wilk e análise de variâncias, demonstrando, em todas as avaliações, uma distribuição normal (α=0,05).

O two-way ANOVA seguido do teste de Tukey foi realizado para verificar a existência de diferenças estatísticas entre os períodos de tratamento (análises entre períodos) e o two-way ANOVA seguido do teste de Dunnett para verificar as diferenças estatísticas entre os grupos (análises intraperíodo). O nível de significância foi fixado em 95% ($p < 0,05$) em todas as análises.

REFERÊNCIAS DA METODOLOGIA

1. Conte Neto N, Spolidorio LC, Andrade CR, A SB, Guimaraes M, Marcantonio E, Jr. Experimental development of bisphosphonate-related osteonecrosis of the jaws in rodents. Int J Exp Pathol. 2013; 94(1): 65-73.
2. Corbi SCT, Macedo PD, Perussi JR, Ribeiro AO, Marcantonio RAC. Evaluation of antimicrobial photodynamic therapy (aPDT) effects using zinc tetracarboxy-phthalocyanine N-methylglucamine salt photosensitizer as an adjunct therapy in the treatment of induced periodontal disease in rats. Lasers Dent Sci. 2020; 4(1): 43-52.
3. Goncalves FC, Mascaro BA, Scardueli CR, de Oliveira G, Spolidorio LC, Marcantonio RAC. Strontium ranelate improves post-extraction socket healing in rats submitted to the administration of bisphosphonates. Odontology. 2022; 110(3): 467-75.
4. Macedo PD, Corbi ST, Kitagawa FA, Perussi JR, Ribeiro AO, Marcantonio RAC. Evaluation of the effects of photodynamic therapy with hypericin-glucamine in the treatment of periodontal disease induced in rats. Lasers Dent Sci. 2018; 2(4): 255-63.
5. Pinotti FE, Aroni MAT, Oliveira G, Silva BLG, Marcantonio Junior E, Marcantonio RAC. Osseointegration of implants with superhydrophilic surfaces in rats with high serum levels of nicotine. Braz Dent J. 2023; 34(2): 105-12.
6. Silva BLG, Sanchez-Puetate JC, Pinotti FE, Marcantonio CC, Pedroso GG, et al. Influence of obesity on osseointegration of implants with different surface treatments: A preclinical study. Clin Implant Dent Relat Res. 2023.
7. Pinotti FE, Aron MAT, Oliveira G, Marcantonio Junior E, Marcantonio RAC. Implants with hydrophilic surfaces equalize the osseointegration of implants in normo- and hyperglycaemic rats. Braz Dent J. 2022; 33(6): 71-7.
8. Pinotti FE, de Oliveira G, Aroni MAT, Marcantonio RAC, Marcantonio E, Jr. Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: A Preclinical study. Clin Oral Implants Res. 2018; 29(10): 963-72.
9. de Oliveira GJ, de Paula LG, Spin-Neto R, Stavropoulos A, Spolidorio LC, et al. Effect of avocado/soybean unsaponifiables on osseointegration: a proof-of-principle preclinical in vivo study. Int J Oral Maxillofac Implants. 2014; 29(4): 949-57.
10. Yalcin-Ulker GM, Cumbul A, Duygu-Capar G, Uslu U, Sencift K. Preventive Effect of Phosphodiesterase Inhibitor Pentoxifylline Against Medication-Related Osteonecrosis of the Jaw: An Animal Study. J Oral Maxillofac Surg. 2017; 75(11): 2354-68.
11. Bas N, Kayar NA, Baba ZF, Avunduk MC, Haliloglu S, Alptekin NO. Systemic treatment with alpha-tocopherol and/or sodium selenite decreases the progression of experimental periodontitis. Clin Oral Investig. 2021; 25(5): 2677-88.

ANEXO A - DOCUMENTO COMPROBATÓRIO DO ARTIGO EM PROCESSO DE REVISÃO POR PARES



ANEXO B – CERTIFICADO DO COMITÊ DE ÉTICA DO PROJETO 1

UNIVERSIDADE ESTADUAL PAULISTA
Faculdade de Odontologia
Campus de Araraquara

Comissão de Ética no Uso de Animais - CEUA

C E R T I F I C A D O

Certificamos que o protocolo nº 07/2019 referente à pesquisa **"AVALIAR O EFEITO DE SUPERFÍCIE DE IMPLANTES MODIFICADA POR JATEAMENTO E ATAQUE ÁCIDO SOBRE A OSSEOINTEGRAÇÃO DE IMPLANTES EM RATOS COM DIFERENTES ALTERAÇÕES SISTÊMICAS"** sob a responsabilidade da **Profª Drª Rosemary Adriana Chierici Marcantonio** está de acordo com os Princípios Éticos em Experimentação Animal adotado pela legislação brasileira atualmente em vigor, tendo sido aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Faculdade de Odontologia de Araraquara-UNESP.

C E R T I F I C A T E

We certify that the protocol 07/2019 referring to the research **"EVALUATION OF THE OSSEOINTEGRATION OF IMPLANTS WITH THE HYDROPHILIC SURFACE IN RATS WITH DIFFERENT SYSTEMIC ALTERATIONS"** under responsibility of **Profª Drª Rosemary Adriana Chierici Marcantonio** in agreement with the nowadays specific Brazilian laws and was approved by the Araraquara Dental School-UNESP Ethical Committee for Animal Research (CEUA).

Araraquara, 24 de junho de 2022.

Profª Drª CARINA A. FABRICIO DE ANDRADE
Coordenadora da CEUA/FOAr/UNESP

ANEXO C – CERTIFICADO DO COMITÊ DE ÉTICA DO PROJETO 2

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Araraquara

FACULDADE DE ODONTOLOGIA

Proc. CEUA nº 16/2018

Araraquara, 21 de junho de 2023.

Senhor Pesquisador:

A Comissão de Ética no Uso de Animal - CEUA desta Faculdade procedeu à análise do formulário de alteração do projeto de pesquisa de sua responsabilidade intitulado ***"Avaliação de medicamentos que interferem na modulação óssea associado ou não ao bisfosfonato. Estudo na gengivite experimental em ratos"***, e APROVOU a alteração na metodologia e prorrogação de prazo até Março/2026 .

Lembramos que o Relatório Final deste projeto deverá ser entregue em **Abril/2026.**

Atenciosamente.

Assinado de forma digital por Debora Simoes de Almeida Colombari:12257980867
Dados: 2023.06.21 11:18:09 -03'00'

Profª Drª DÉBORA SIMÕES DE ALMEIDA COLOMBARI
Coordenadora da CEUA/FOAr/UNESP

A
Profª Drª Rosemary Adriana Chiérici Marcantonio
DD. Pesquisador Responsável
Departamento de Diagnóstico e Cirurgia

Araraquara, 23 de setembro de 2023.

Julio Cesar Sánchez Puetate