UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Amanda de Carvalho Silva Leocádio

Osseointegração de implantes com diferentes macro e microestruturas instalados em áreas sem enxertia ou com osso bovino desproteinizado associado ou não à medula óssea fresca: estudo pré-clínico em coelhos

Araraquara

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Orientador: Elcio Marcantonio Júnior
Co-orientador: Guilherme José Pimental de Oliveira

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(Salmos, cap. 119, v. 105)

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"Ensinar não é transferir conhecimentos, mas criar as possibilidades para a sua produção ou a sua construção. Quem ensina aprende ao ensinar e quem aprende ensina ao aprender."

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“O primeiro dever da inteligência é desconfiar dela mesma.”
Albert Einstein

“Poderia ser pior!”
Per-Ingvar Brånemark

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RESUMO

Implantes com diferentes macro (CI-Implante Cilíndrico e HCI-Implante Cônico Híbrido) e microestruturas (NP-Jateamento+ataque ácido e AQ-Jateamento+ataque ácido+imersão em solução isotônica de cloreto de sódio 0,9 %) foram testados em áreas de osso nativo ou enxertadas prévia ou imediatamente com osso bovino desproteinizado associado ou não à medula óssea fresca (DBB e DBB/BM). Na primeira hipótese foi testado a estabilidade primária e o processo de osseointegração em implantes com diferentes macroestruturas (CI vs. HCI) na metáfise tibial de coelhos. 24 coelhos foram divididos em 3 períodos (2, 4 e 8 semanas). Cada animal recebeu bilateralmente 2 implantes de cada grupo. Todos os implantes foram avaliados quanto ao torque de inserção. Um dos implantes foi submetido ao torque de remoção e análise histológica e o outro foi utilizado para análise microtomográfica e histométrica (%BIC-Contato Osso-Implante). Os HCI apresentaram maior torque de inserção (32.93±10.61 Ncm vs. 27.99± 7.80Ncm) e maior %BIC no período de 8 semanas (79.08±11.31% vs. 59.72±11.29%) que CI. CI apresentaram maiores valores de torque de remoção que HCI no período de 8 semanas (91.05 ± 9.32 Ncm vs. 68.62 ± 13.70 Ncm). Não houve diferenças em relação aos dados microtomográficos. Na segunda e na terceira hipóteses, foi avaliado a influência de diferentes macros (CI vs. HCI) e microestruturas de implantes (NP vs. AQ) no processo de osseointegração em áreas previamente (metáfise tibial e seio maxilar) ou imediatamente (metáfise tibial) enxertadas (DBB vs. DBB/BM).16 coelhos foram avaliados em um período de 180 dias. Cada animal foi submetido à criação de um defeito ósseo bilateral na metáfise tibial e preenchidos com DBB ou DBB/BM. Foi executado acesso bilateral à membrana do seio maxilar e enxerto. Após 90 dias, os defeitos da metáfise tibial foram trefinados e submetidos a instalação de implantes (CI vs. HCI). No seio maxilar implantes foram instalados (NP vs. AQ). Um segundo defeito foi confeccionado na metáfise tibial, preenchido e seguido da instalação imediata de implantes (CI vs. HCI). 90 dias após os animais foram eutanasiados. Foi verificado nas biópsias ósseas (DBB vs. DBB /BM) que não houve diferenças estatísticas quanto à porcentagem de tecidos mineralizados (75.42±10.11 vs. 77.42±9.57) e em relação a porcentagem de tecido ósseo (23.08±9.95 vs. 27.37±5.83), biomaterial (20.81±11.34 vs. 26.40±7.16) e tecido conjuntivo (51.81±10.53 vs. 50.52±14.31). BV/TV% dos implantes com diferentes macroestruturas, diferença estatística foi verificada no DBB independente do momento de instalação dos implantes (Imediato vs. Tardio) nos HCI (83.34±5.87 e 85.69±7.91, respectivamente) comparado aos CI (DBB-CI: 70.39±15.18 e 77.60±8.31, respectivamente). BIC%, diferença estatística foi verificada nos CI imediatos comparado ao tardio em áreas enxertadas com DBB somente. Já nas diferentes microestruturas foi verificado que não houve diferenças na quantidade no BV/TV% (DBB-NP:33.25±19.67 ; DBB-AQ:35.15±22.17; DBB/BM-NP:39.71±24.21; DBB/BM-AQ: 36.40±23.07) e na BIC% (DBB-NP:58.94±24.37; DBB-AQ:52.52±24.36; DBB/BM-NP: 61.66±14.60; DBB/BM-AQ:64.06±23.30). HCI apresentaram maior estabilidade primária e melhor padrão de osseointegração que os CI ao final de 8 semanas de

avaliação em osso nativo tipo I e a adição de BM e o tipo de macro ou microestrutura não influenciaram na osseointegração dos implantes instalados em áreas com DBB.

Palavras chave: Osseointegração. Implantes dentários. Transplante ósseo. Medula óssea. Torque. Materiais biocompatíveis.

Leocádio ACS. Osseointegration of implants with different macro and microstructures installed in areas without grafting or with deproteinized bovine bone associated or not with fresh bone marrow - Pre-clinical study in rabbits. [Tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2020.

ABSTRACT

Implants with different macro (CI-Cylindrical Implant and HCI-Hybrid Conical Implant) and microstructures (NP-Sandblasting + acid attack and AQ-Sandblasting + acid attack + immersion in 0.9% sodium chloride isotonic solution) were tested in areas of native bone or grafted previously or immediately with deproteinized bovine bone associated or not with fresh bone marrow (DBB and DBB / BM). In the first hypothesis, primary stability and the osseointegration process in implants with different macrostructures (CI vs. HCI) in the tibial metaphysis of rabbits were tested. 24 rabbits were divided into 3 periods (2, 4 and 8 weeks). Each animal received two implants bilaterally from each group. All implants were evaluated for insertion torque. One of the implants was submitted to removal torque and histological analysis and the other was used for microtomographic and histometric analysis (% BIC-Bone Contact-Implant). The HCI showed a higher insertion torque (32.93 ± 10.61 Ncm vs. 27.99 ± 7.80Ncm) and a higher BIC% in the 8-week period (79.08 ± 11.31% vs. 59.72 ± 11.29%) than CI. CI showed higher removal torque values than HCI in the 8 week period (91.05 ± 9.32 Ncm vs. 68.62 ± 13.70 Ncm). There were no differences in relation to microtomographic data. In the second and third hypotheses, the influence of different macros (CI vs. HCI) and implant microstructures (NP vs. AQ) in the process of osseointegration in areas previously (tibial metaphysis and maxillary sinus) or immediately (tibial metaphysis) was evaluated grafted (DBB vs. DBB / BM) .16 rabbits were evaluated over a period of 180 days. Each animal was submitted to the creation of a bilateral bone defect in the tibial metaphysis and filled with DBB or DBB / BM. Bilateral access to the maxillary sinus membrane and graft was performed. After 90 days, defects in the tibial metaphysis were redefined and implanted (CI vs. HCI). Implants were installed in the maxillary sinus (PN vs. AQ). A second defect was made in the tibial metaphysis, filled in and followed by the immediate installation of implants (CI vs. HCI). 90 days after the animals were euthanized. It was verified in bone biopsies (DBB vs. DBB / BM) that there were no statistical differences regarding the percentage of mineralized tissues (75.42 ± 10.11 vs. 77.42 ± 9.57) and in relation to the percentage of bone tissue (23.08 ± 9.95 vs. 27.37 ± 5.83), biomaterial (20.81 ± 11.34 vs. 26.40 ± 7.16) and connective tissue (51.81 ± 10.53 vs. 50.52 ± 14.31). BV / TV% of implants with different macrostructures, statistical difference was verified in DBB regardless of the moment of implant implantation (Immediate vs. Late) in HCI (83.34 ± 5.87 and 85.69 ± 7.91, respectively) compared to CI (DBB-CI: 70.39 ± 15.18 and 77.60 ± 8.31, respectively). BIC%, statistical difference was found in the immediate IC compared to the late IC in areas grafted with DBB only. In the different microstructures, it was verified that there were no differences in the quantity in BV / TV% (DBB-NP: 33.25 ± 19.67; DBB-AQ: 35.15 ± 22.17; DBB / BM-NP: 39.71 ± 24.21; DBB / BM-AQ: 36.40 ± 23.07) and in BIC% (DBB-NP: 58.94 ± 24.37; DBB-AQ: 52.52 ± 24.36; DBB / BM-NP: 61.66 ± 14.60; DBB / BM-AQ: 64.06 ± 23.30). HCI showed greater primary stability and better pattern of osseointegration than CIs after 8 weeks of evaluation in native bone

type I and the addition of BM and the type of macro or microstructure did not influence the osseointegration of implants installed in areas with DBB.

Keywords: Osseointegration. Dental implants. Bone transplantation. Bone marrow.

Torque. Biocompatible materials.

Lista de Abreviaturas

Abreviaturas usadas no texto:

AQ: Superfície Acqua®

%BIC: Contato osso implante expressa em porcentagem

BM: Medula Óssea

%BV/TV: Relação entre volume ósseo e volume total da amostra expressa em porcentagem

CEUA: Comitê de Ética no uso de animais

CI: Implante Cilíndrico

COBEA: Colégio Brasileiro de Experimentação Animal

DBB: Osso bovino desproteinizado

HA: Hidroxiapatita

HCI: Implante Cônico Híbrido

HE: Hematoxilina Eosina

NP: Superfície Neoporos®

ROI: Região de interess

SUMÁRIO

1 INTRODUÇÃO

A Odontologia, na era da Implantodontia, tem possibilitado a utilização de implantes dentais osseointegráveis no tratamento do edentulismo [1,2]. As reabilitações orais tradicionais, com próteses fixas ou removíveis, apoiadas sobre dentes e/ou mucosa, deixaram de ser o tratamento de escolha dos pacientes totais ou parcialmente edêntulos, uma vez que, esses pacientes tomaram conhecimento e vivenciam a possibilidade de reabilitação do sistema estomatognático com próteses implanto-suportadas, as quais, hoje, representam o tratamento que mais se aproxima da dentição natural [3].

Apesar das altas taxas de sobrevivência clínica dos sistemas de implantes dentais contemporâneos, falhas ainda ocorrem, podendo estar associadas a diversos fatores mecânicos ou biológicos [4]. A falta de formação óssea adequada ou volume ósseo de apoio para facilitar a osseointegração têm sido relatados como principais influências sobre a previsibilidade e falha do implante [5,6]. As taxas de sobrevivência também variam com o local do implante na cavidade bucal e de outros fatores, tais como design do implante, biocompatibilidade, carregamento, densidade óssea, técnica cirúrgica e outros [6, 7].

Portanto, uma série de modificações no projeto de implante e técnicas cirúrgicas tem sido introduzida para melhorar o contato osso-implante, ancoragem e distribuição do estresse [5, 8, 9, 10]. Muitas dessas mudanças levaram a uma bem-sucedida terapia com implantes, mesmo nas mais difíceis situações clínicas. Devido a isso, a busca por protocolos que acelerem o processo de osseointegração, e consequentemente o carregamento protético, tem sido o foco de várias pesquisas na área da Implantodontia [1, 2].

Quanto às modificações estruturais dos implantes, elas podem ser executadas ao nível da macro ou da microestrutura [11, 12]. Essas modificações alteram estágios distintos na estabilidade dos implantes, sendo que alterações na macroestrutura têm sido relacionadas com a obtenção da estabilidade primária dos implantes enquanto que as alterações de microestrutura afetariam o processo de osseointegração, e, consequentemente, a estabilidade secundária dos implantes [13, 14, 15,16].

Neste contexto, a macroestrutura exerce fundamental importância na obtenção da estabilidade primária dos implantes em áreas com osso de pobre qualidade, como na região posterior da maxila, ou em condições de pós extrações com instalação imediata do implante e possível carregamento em áreas estéticas[17]. Estudos com implantes cônicos e com implantes com roscas com ausência de bordas cortantes e com câmeras de cicatrização tem demonstrado não apenas melhorar a estabilidade primária, como também acelerar o processo de osseointegração[18, 19].

Com relação às modificações de microestrutura, têm sido verificado que diversas alterações nas superfícies dos implantes que visam modificar suas propriedades físico-químicas tais como a rugosidade de superfícies, a molhabilidade e a sua composição química demonstram serem capazes de acelerar e melhorar a qualidade da osseointegração, resultando em maior deposição óssea e redução do período de reparo, principalmente em regiões de má qualidade óssea [20, 21, 22].

Mas, apesar de um dos mecanismos mais fascinantes no organismo ser a alta capacidade do tecido ósseo se regenerar, a doença periodontal, tumores, traumas, exodontias, anomalias de desenvolvimento, patologias, ressecções oncológicas e perda fisiológica de massa óssea, podem levar a defeitos ósseos perenes, os quais não possuem a capacidade de se regenerar espontaneamente.

Especialmente na região bucomaxilofacial, a reabsorção severa de mandíbula e maxila, associada à perda dos dentes, pode levar a defeitos anatômicos significativos comprometendo a função e a estética e, em muitos casos inviabilizando a reabilitação oral com implantes [23].

E, uma vez que as cirurgias ósseas reconstrutivas, antes da reabilitação oral com próteses implanto-suportadas, são muitas vezes necessárias para evitar ou até mesmo recuperar a perda óssea vertical e horizontal e, principalmente, obter uma quantidade e qualidade ósseas adequadas, garantindo a estabilidade primária de inserção do implante, tornou-se constante a busca por novos biomateriais que possam modular, alterar ou estimular a atividade osteogênica nos defeitos teciduais[24, 25].

Atualmente, como possíveis soluções utilizam-se enxertos autógenos, alógenos, aloplásticos, xenogênicos e fatores de crescimento. Como o recrutamento de células, a modulação do processo inflamatório e a promoção do reparo, por cicatrização ou regeneração, são influenciados pelas suas características físicas, químicas e biológicas [26], cada material apresenta vantagens e limitações, justificando o grande número de estudos realizados para verificar o efeito de novos biomateriais, associados ou não, à outros biomateriais utilizados rotineiramente.

Dentre os biomateriais alternativos a utilização dos enxertos autógenos, podemos citar os enxertos homólogos, xenógenos, ou aloplásticos sendo que cada material apresenta vantagens e limitações[27, 28, 29, 30, 31]. Os xenoenxertos têm ganhado destaque e o mais utilizado e pesquisado até o momento tem sido o osso bovino desproteinizado. Esse tipo de enxerto tem sido utilizado com sucesso em situações clínicas diversas tais como em elevação do assoalho do seio maxilar [32, 33] e manutenção de alvéolo pós-extração [34]. Mas, assim como os demais, esses substitutos ósseos apresentam como único mecanismo de ação a osteocontutividade

[35, 36, 37] o que causa um retardo na cicatrização dos defeitos ósseos, que pode ter como consequência o retardo na instalação dos implantes [38].

Considerando que a presença de um pequeno subconjunto de células-tronco, células progenitoras e osteoblastos são transferidos juntamente com o enxerto autógeno, em grandes defeitos ósseos sua presença torna a reconstrução mais previsível, mesmo sendo o nível de celularidade baixo [39]. A adição de células ou fatores de crescimento a um biomaterial essencialmente osteocondutor poderia resultar em um biomaterial potencialmente comparável ao enxerto autógeno[40, 41, 42]. Neste contexto, a associação do osso bovino desproteinizado com o osso autógeno e fatores de crescimento tais como o plasma rico em plaquetas[41] e a rhBMP2[42] foram testados com intuito de melhorar a formação óssea.

Outra alternativa que tem sido investigada é a utilização da medula óssea fresca associado a biomateriais osteocondutores, pois teoricamente, as células tronco e os fatores de crescimento presentes nesse adjuvante biológico, quando associadas a um arcabouço, podem adicionar ao mesmo um potencial de osteogênese e osteoindução para formação óssea [39, 43, 44, 45]. Não há um consenso sobre a melhor metodologia para o uso de células estromais da medula óssea. Três alternativas principais têm sido propostas: (1) Uso do enxerto de medula óssea autóloga fresca ("in natura"); (2) Uso de concentrado de células da medula óssea autóloga (por centrifugação); e, (3) Uso de cultivo de células estromais da medula óssea autóloga [39, 40, 41, 42].

Algumas desvantagens foram associadas com a utilização da metodologia de cultura celular: custo, tempo (requer um período de algumas semanas entre a coleta de células, obtenção da cultura e transplante), maior risco de contaminação, e a aplicabilidade clínica rotineira da cultura celular pode esbarrar na necessidade de

dois procedimentos cirúrgicos separados. Outro questionamento sobre a necessidade ou não de se cultivar as células é a falta de informação sobre o número mínimo de células requeridas para promoção do reparo ósseo [39, 46, 47, 49]. Dessa forma, a utilização do enxerto de medula de forma fresca seria uma abordagem mais simples do que outros que já foram propostos[47]. O efeito de áreas enxertadas com biomateriais osteocondutores associados ao aspirado de medula óssea sobre a osseointegração dos implantes foi pouco avaliado até o momento.

Portanto, o objetivo desse estudo foi verificar a influência de um implante experimental com macroestrutura compactante perfurante (HCI - Implante Cônico Híbrido) comparada a um implante controle (CI - Implante Cilíndrico) no processo de osseointegração em osso nativo (Osso tipo I) (**Publicação 1**) ou em defeitos cirúrgicos prévio e imediatamente enxertados com osso bovino desproteinizado isolado (DBB) ou associado com medula óssea fresca (DBB/BM) na metáfise tibial de coelhos; e avaliar a neoformação óssea das biópsias colhidas da região previamente enxertada (**Publicação 2**). Além disso, avaliar o efeito da uma superfície de implante altamente hidrofílica (AQ - Implante Acqua: jateamento de óxido, a subtração ácida e manutenção do implante em solução isotônica de cloreto de sódio 0,9 %) comparada a uma hidrofóbica (NP - Implante NeoPoros: jateamento de óxido e subtração ácida) sobre a osseointegração em seios maxilares de coelhos enxertados com DBB vs. DBB/BM (**Publicação 3**).

2 PROPOSIÇÃO

Hipóteses

A) A macroestrutura compactante perfurante dos implantes Cônicos Híbridos (HCI) favorece o processo de osseointegração comparado a um implante cilíndrico (CI) em osso nativo, prévio ou imediatamente enxertado com osso bovino desproteinizado associado ou não à medula óssea fresca (DBB vs. DBB/BM) na metáfise tibial de coelhos.

B) A superfície hidrofílica (AQ) favorece o processo de osseointegração comparado a uma superfície hidrofóbica (NP) de implantes instalados na região do seio maxilar previamente enxertado (DBB vs. DBB/BM).

C) O enxerto xenogênico de origem bovina, constituído de hidroxiapatita pura (HA) e sem componentes orgânicos, sinterizado à altas temperaturas (DBB), apresenta neoformação óssea e osseointegração melhorados quando associado à medula óssea fresca (BM).

Objetivos específicos

Para avaliar a hipótese esse projeto foi dividido nos seguintes objetivos específicos:

1) Avaliar o efeito da modificação da macroestrutura de implantes (HCI vs. CI) com superfície hidrofílica modificada por jateamento e ataque ácido sobre a osseointegração em osso tipo I (metáfise tibial) de coelhos (**Publicação 1**).

2) Avaliar o efeito da modificação da macroestrutura de implantes (HCI vs. CI) com superfície hidrofílica modificada por jateamento e ataque ácido sobre a osseointegração em defeitos ósseos criados na metáfise tibial prévio ou imediatamente enxertados com osso bovino desproteinizado associado ou não à medula óssea fresca (DBB vs. DBB/BM). Além de comparar a neoformação óssea e osseointegração do enxerto xenogênico, constituído de hidroxiapatita pura (HA) e sem componentes orgânicos nos diferentes grupos (DBB vs. DBB/BM) (**Publicação 2**).

3) Avaliar o efeito de uma superfície hidrofílica e modificada por ataque ácido e por jateamento (AQ) sobre a osseointegração em comparação a uma superfície hidrofóbica com o mesmo tipo de tratamento para modificação de superfície (NP) instalados na região do seio maxilar previamente submetido ao levantamento do assoalho e enxertia (DBB vs. DBB/BM) (**Publicação 3**).

3 PUBLICAÇÕES

3.1 Publicação 1

Original Reseach: Evaluation of implants with different macrostructures in type I bone - Pre-clinical study in rabbits

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Conflicts of Interest:

Professor Doctor Élcio Marcantonio Junior is a consultant for the company Neodent. The other authors have no conflict of interest in this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

*** Artigo publicado no periódico Materials**

materials

MDPI

Article

Evaluation of Implants with Different Macrostructures in Type I Bone—Pre-Clinical Study in Rabbits

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Abstract: The objective of this study was to assess the primary stability and the osseointegration process in implants with different macrostructures (Cylindrical vs. Hybrid Conical) in rabbit tibiae. Twenty-four (24) rabbits were used, divided into 3 experimental periods (2, 4 and 8 weeks) with 8 animals each. Each animal bilaterally received 2 implants from each group in the tibial metaphysis: Cylindrical Implant (CI) and Hybrid Conical Implant (HCI). All implants were assessed for insertion torque. After the experimental periods, one of the implants in each group was submitted to the removal counter-torque test and descriptive histological analysis while the other implant was used for microtomographic and histometric analysis (%Bone-Implant Contact). HCI implants showed higher insertion torque (32.93 ± 10.61 Ncm vs. 27.99 ± 7.80 Ncm) and higher % of bone-implant contact in the 8-week period (79.08 ± 11.31% vs. 59.72 ± 11.29%) than CI implants. However, CI implants showed higher values of removal counter-torque than HCI implants in the 8-week period (91.05 ± 9.32 Ncm vs. 68.62 ± 13.70 Ncm). There were no differences between groups regarding microtomographic data. It can be concluded that HCI implants showed greater insertion torque and bone-implant contact in relation to CI implants in the period of 8 weeks when installed in cortical bone of rabbits.

Keywords: dental implants; macrostructure; osseointegration

1. Introduction

The use of osseointegrated dental implants in the treatment of partial or total edentulism has been a widely used procedure in recent years [1,2]. However, despite the high rates of the clinical survival of contemporary dental implant systems, failures still occur, which may be associated with several mechanical or biological factors [3]. The lack of adequate bone formation or bone support volume to facilitate osseointegration has been reported as the main influence on the predictability and failure of the implant [4,5]. Survival rates also vary with the location of the implant in the oral cavity and other factors, such as implant design, biocompatibility, loading, bone density, surgical technique, and others [5,6].

In this context, a series of changes in the implant design and surgical techniques have been introduced to improve bone-implant contact, anchorage and stress distribution [7–9]. Many of these changes have led to successful implant therapy, even in the most difficult clinical situations. Because of this, the search for protocols that accelerate the osseointegration process, and consequently the prosthetic loading, has been the focus of several research papers in the area of Implantology [1,2].

Therefore, these structural modifications of the implants can be performed at the nano- or microstructure level [10–12], and they would alter different stages in the stability of the implants, as changes in the macrostructure have been related to the achievement of the primary stability of the implants, while the changes in microstructure would affect the osseointegration process and, consequently, the secondary stability [11–14].

Considering that changes in the implant macrostructure have been reported as important for obtaining primary stability of implants in areas with poor quality bone, such as in the posterior region of the maxilla [15], studies with conical implants and threaded implants with no sharp edges and with healing chambers have been shown not only to improve primary stability but also to accelerate the osseointegration process [11]. In this context, the emergence of implants with a conical structure and with changes in the conformation of the threads, in order to make them more compressive, allows the installation of implants with good primary stability [16–18], as well as the use of implants with smaller sizes, since they can be used in areas with limited bone availability [19].

Consequently, that the macrostructure is an extremely important characteristic for obtaining primary stability, a parameter related to the success of the implant osseointegration, changes in the implant macrostructure have been proposed in order to improve the initial locking of the implants. Thus, the objective of this study was to verify the influence of an experimental implant with perforating compacting macrostructure (HCI—Hybrid Conical Implant: Helix Acqua, Neodent®, Grand Morse, Curitiba, Brazil) compared to a control implant (CI—Cylindrical Implant: Titamax Acqua, Neodent®, Cone Morse, Curitiba, Brazil) in the process of osseointegration in cortical bone (tibial metaphysis) of rabbits.

2. Materials and Methods

This project was carried out in accordance with the Ethical Principles for Animal Experimentation after approval by the Ethics Committee on Animal Use (ECAU) of the Faculty of Dentistry of Araraquara (FOAr-UNESP) (11/2016). For the present research, 24 male New Zealand albino rabbits (~5 months old and 4–5 kg) were used. The animals were kept in an environment with a temperature of 22–24 °C, with a controlled light cycle (12 h light and 12 h dark) and consumption of solid food and water ad libitum throughout the experimental period. The study was conducted according to the ARRIVE protocol.

2.1. Experimental Outline

The 24 rabbits were randomly divided into 3 experimental periods (2, 4 and 8 weeks). Each animal bilaterally received two implants from each group in the cortical bone of the animals' tibia, with the side selected at random. Two different macrostructures were assessed: Hybrid Conical Implant (HCI) and Cylindrical Implant (CI).
The CI implant used in this study is characterized by a cervical diameter equal to the diameter of the implant body (3.75 mm in diameter × 11 mm in height). Presence of triangular and double threads that facilitate the quick implant insertion, with minimal trauma, and apex morphology with the presence of self-cutting chambers (Figure 1A,C,E). The HCI implant is characterized by an increased cervical diameter in relation to the implant body (3.75 mm in diameter × 11.5 mm in height). In addition, these implants have compacting and double trapezoidal threads, with a conical apex containing helical chambers designed to optimize secondary stability (Figure 1B,D,F).

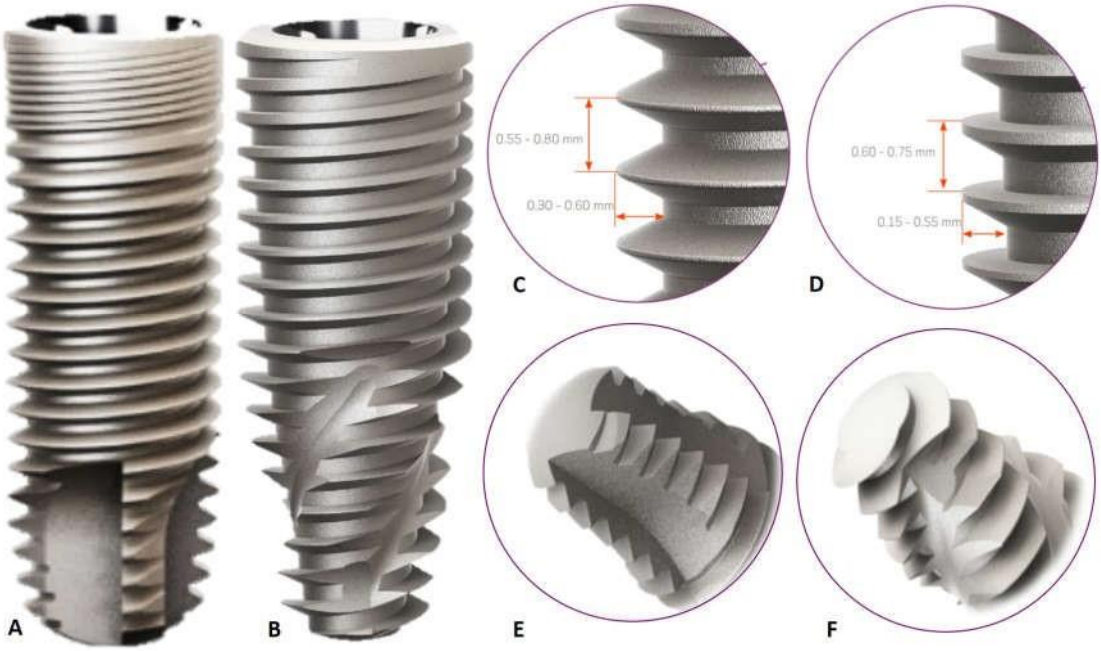

Figure 1. Macrostructure of implants installed in the cortical bone of rabbits**.** Body (**A**), threads (**C**) and apex (**E**) of the Cylindrical Implant; Body (**B**), threads (**D**) and apex (**F**) of the Hybrid Conical Implant.

2.2. Surgical Procedure

The animals were initially weighed and anesthetized intramuscularly, with a combination of ketamine (Quetamina Agener®, Agener União Ltd.a, São Paulo, SP, Brazil—0.35 mg/kg) and xylazine (Rompum, Bayer AS, São Paulo, SP, Brazil—0.5 mg/kg). Subsequently, trichotomy and antisepsis were performed with 10% iodinated polyvinylpyrrolidone (IPVP) with 1% active iodine in the right and left portions of the tibial metaphysis of the animal (Figure 2A). Local anesthesia (Mepivacaine Hydrochloride 2% + Adrenaline 1:100,000) was also applied in the region, to allow peripheral vasoconstriction, reducing local bleeding and optimizing the surgical procedure (Figure 2B). Then, using a No. 15 scalpel blade, a dermo-periosteal incision of approximately 5 cm in length was performed (Figure 2C,D). This allowed for a

delicate dissection so that the bone surface of the tibial metaphysis was exposed, and the surgical beds were prepared according to the manufacturers' recommendations (Neodent®—Curitiba, Brazil), using metal drills under abundant refrigeration with sterile saline solution (Figure 2E,F). Two implants of each type were installed in the tibiae, and the side was selected at random. The upper implants had a distance of 3 cm in relation to the lower ones (Figure 2G–I). Both implants had the same type of surface (Sandblasting + acid attack + immersion in
0.9% sodium chloride isotonic solution) and were manufacture with the same titanium alloy (Ti-6Al4V). The cover screws were installed (Figure 2J) and then the soft tissues were repositioned and sutured plane by plane with resorbable thread (Vicryl®, ETHICON, Sao Paulo, Brazil) and nonresorbable thread (Shalon®-Nylon 3-0, Shalon surgical wires Ltda., Goias, Brazil) (Figure 2K,L). After surgery, all animals received a single intramuscular dose of antibiotic (Pentabiótico®, WyethWhitehall Ltd.a, São Paulo, Brazil—0.1 mL/kg) and analgesic (Tramadol Hydrochloride 50 mg/mL, Tramadol®, Medley, São Paulo, Brazil—5 mg/kg IM). After the periods of 2, 4 and 8 weeks, the animals were euthanized through anesthetic overdose.

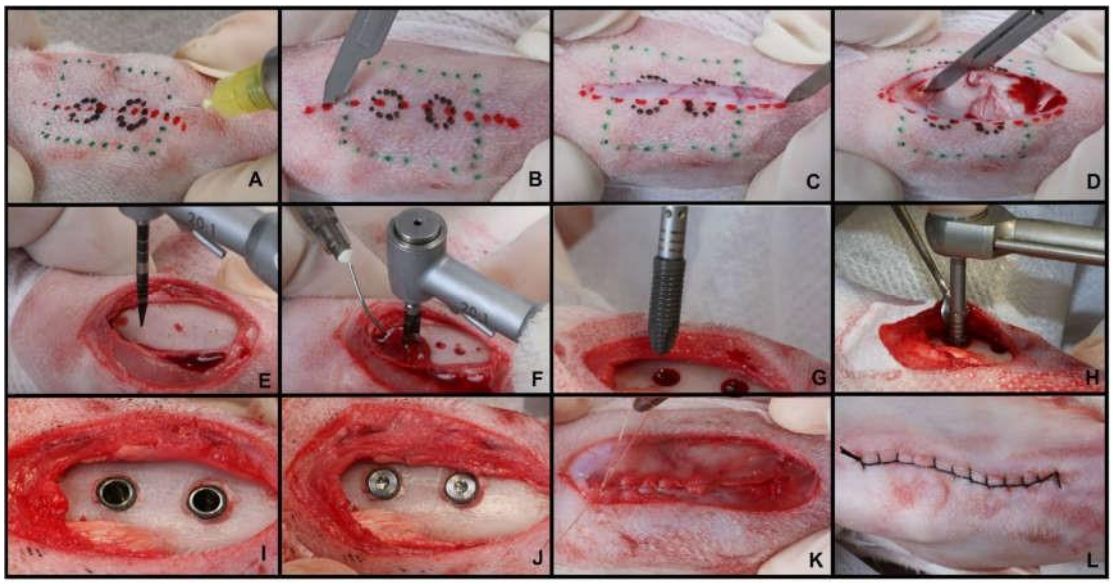

Figure 2. Surgical procedure for installing implants in the tibial metaphysis of rabbits**.** Schematic drawing to define the tibial metaphysis and local anesthesia (**A**); Incision in layers (**B**–**D**); Detachment and exposure of the tibia, and perforation according to the manufacturer's recommendations under abundant sterile saline irrigation (**E**,**F**); Installation of the implants (**G**,**H**); Implants and cover screws installed (**I**,**J**); Internal suture of the muscular fascia with resorbable thread and external suture with non-resorbable thread (**K**,**L**).

2.3. Biomechanical Assessment (Insertion Torque and Removal Torque)

All installed implants were submitted to the assessment of the insertion torque, and for that, the torque was noted after the installation of the implants (at the bone level). After euthanasia, in each analysis period (2, 4 and 8 weeks), the medial portions of the samples obtained from the tibia were reopened to expose the implants and perform the reverse torque. The samples were stabilized in a small vise, and a hexagonal wrench was connected to both the implant and the torque wrench

(Tohnichi, model ATG24CN-S, Tokyo, Japan-with a graduated scale of 0.05 Ncm, measuring the strength from 3 to 24 Ncm), and an anti-clockwise movement was performed to remove the implants, increasing the torque until the rotation of the implant occurred inside the bone tissue, completely disrupting the bone-implant interface, when the torque wrench registered the maximum torque peak necessary for this disruption. This maximum peak required to move the implant was noted as the removal torque value. The remnants of the tibia related to the upper implants that were previously removed for the removal torque analysis were then reduced and immersed in 10% formaldehyde for 48 h, washed in running water for 4 h and then decalcified in Ethylenediaminetetraacetic Acid (E.D.T.A. 7%) for two months.

2.4. Descriptive Histology of Decalcified Sections

After the decalcification period, the parts from the sites where the implants were removed for analysis of the removal torque were embedded in paraffin, cut into a microtome (6 μm-thick) and stained using the hematoxylin-eosin (HE) technique. Five slides were obtained with 3 sections each in the central region of the site where the implant was inserted. Three sections were assessed that were 36 μm apart, and the first section for assessment was selected randomly. The sections were assessed by means of a DIASTAR optical microscope (Leica Reichert & Jung products, Wetzlar, Germany) with 5× and 10× magnifications, and the quality of bone tissue in the vicinity of the implant bed was assessed. This analysis was performed by an experienced trained examiner, blind for the type of implant used.

2.5. Microtomographic Analysis (μCT)

The parts from the sites where the implants were kept (lower tibial implants), after the fixation process with 4% formaldehyde for 48 h and washing with running water for 4 h, were dehydrated in an alcohol solution. Subsequently, they were subjected to the scanning process through the μCT and the following parameters were used: the size of the pixel image was 2000 × 1336 (18 μm), the thickness of the sections was 12 μm, the magnification of the image was 10×, the voltage of the X-ray tube was 50 kV, the beam was 496 μA, and the electrical current was adjusted to 0.1 mA. The three-dimensional images were reconstructed using a reconstruction software (NRecon 1.6.1.5, SkyScan N. V., Belgium). The parameters for reconstruction were: Beam Hardening Correction = 4%, Ring Artifact Correction = 3, Smoothing = 1, Postalignment = 1.00. Subsequently, the scanned images were reoriented in three planes (coronal, axial and sagittal) to standardize the position before performing the volumetric analysis. The measurements for the Volumetric analysis (3D) were carried out using specific software (CT Analyzer 1.10.1.0, SkyScan N. V., Belgium), following the selection of an area of interest (ROI— region of interest) in a cylindrical shape that circumscribed in 0.5 cm the diameter of the implants. Although the implants received a cover screw, there was bone formation inside the cover screw in some cases. In order to prevent this bone formation from interfering with the analysis of the volume of

mineralized tissue around the implant, a second ROI was established to remove the volume of mineralized tissues that could have been formed in this region. With the results obtained in the two ROI's, it was possible to define the volume of mineralized tissues using the following formula:
Total bone volume of mineralized tissues in the main ROI—Volume of mineralized tissues within the cover screw = Volume of mineralized tissues (BV/TV).
The grayscale threshold used was 25–90, and the values of the volume of mineralized tissue around the implant were obtained as a percentage. The entire analysis was performed by a single trained examiner, who was blind for the type of training performed [20].

2.6. Histometric Analysis (BIC)

The biopsies with the lower implants that were submitted to the fixation process and microtomographic analysis were subsequently dehydrated in an alcohol solution in a series of increasing concentrations. The plastic infiltration was performed with mixtures of glycolmethacrylate (Technovit 7200 VLC) and ethyl alcohol, following gradual variations, ending with two infiltrations of pure glycol methacrylate. After plastic infiltration, the specimens were embedded in resin and polymerized. Therefore, the specimens were sectioned longitudinally, along the main axis of the implant, using a high precision diamond disk. The blocks were mounted on an acrylic slide with the help of Tecnovit 4000 resin (Kulzer, Wehrheim, Germany). Using a cutting and micro-wear system (Exact-Cutting, System, Apparatebau Gmbh, Hamburg, Germany), the blades were processed with a section of approximately 50–70 μm thick. The pieces were stained with Stevenel's Blue for histomorphometric analysis. Histometry assessed the percentage of mineralized bone in direct contact with the implant surface (BIC—bone to implant contact) in the extension of the cervical third of the implant. The measurements were made using a DIASTAR optical microscope (Leica Reichert & Jung products, Wetzlar, Germany), with a 10-fold magnification objective lens, through which the images were captured and sent to a PC, with the aid of a video camera (Leica Reichert & Jung products, Wetzlar, Germany). The values were determined using image analysis software (Image J, Jandel Scientific, San Rafael, CA, USA), by a blind examiner, calibrated and trained for this analysis [21].
%BIC = Direct contact of the bone with the implant surface × 100/Extension of the cervical third of the implant

2.7. Statistical Analysis

The data from the biomechanical (insertion and removal torque), microtomographic (BV/TV) and histometric (%BIC) analyses were submitted to the Shapiro-Wilk normality test which confirmed that the data were distributed according to the central distribution theorem. The parametric paired ttest was used for the inferential analysis of the data comparing the different groups of macrostructures (CI vs. HCI). The One-way ANOVA test was applied to compare the different assessment periods within

each group. The GraphPad Prism 6 software (San Diego, CA, USA) was employed in the statistical tests that were applied at the significance level of 5%. Sample calculation was performed using the paired t-test based on the histometric data of bone-implant contact from the study by Faeda et al. 2012 [21], which assessed the effect of different implant surfaces on osseointegration in rabbits. It was found that the difference between the BIC averages among different implant surfaces in order to promote a statistically significant difference was 25.95% (SD = 8.34). Therefore, the use of 8 rabbits per group in each period would be sufficient to obtain a power β and α of the study greater than 0.9 and equal to 0.05, respectively

3. Results

The animals tolerated well the surgical procedure and remained healthy throughout the experimental period.

3.1. Biomechanical Analysis

It was found that the HCI implants had a higher insertion torque than the CI implants. There was a progressive increase in implant removal torques in all groups, regardless of the type of macrostructure assessed. It was found that the CIs presented higher removal torque than HCIs in the period of 8 weeks. Figures 3 and 4 and Table 1 show the data on the mean and standard deviation of the biomechanical analysis.

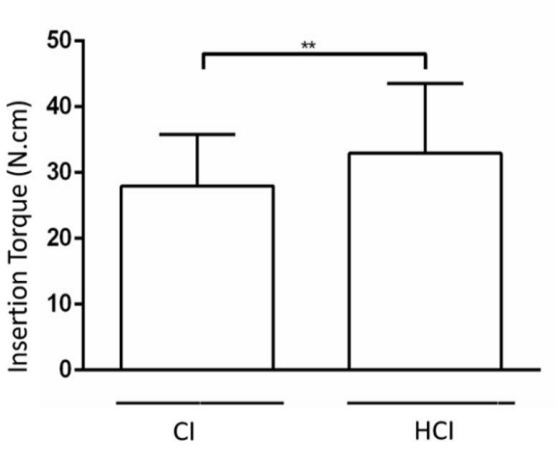

Figure 3. Mean and standard deviation of implant insertion torque with different macrostructures.

**p < 0.01——Differences between groups of implants with different macrostructures——Paired t-test.

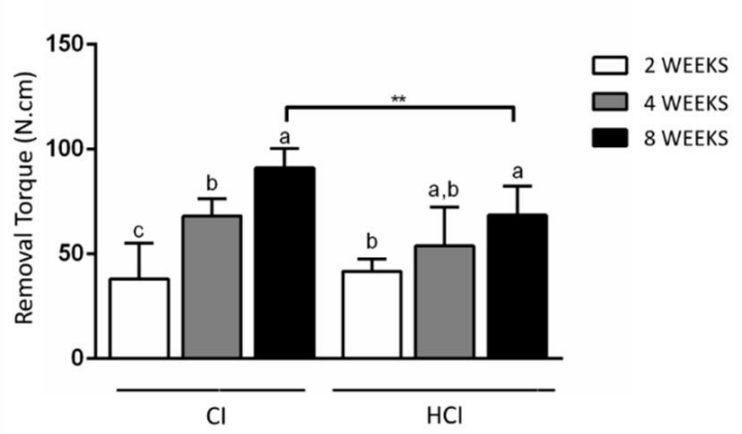

Figure 4. Mean and standard deviation of implant removal torque with different macrostructures. **p < 0.01 – Differences between groups of implants with different macrostructures—Paired t-test. Different letters represent different levels of statistically significant differences among the periods within each group (p < 0.05). One-way ANOVA complemented by the Tukey test.

Table 1. Data on the mean and standard deviation for the insertion and removal torque of all groups.

	Insertion Torque	**Removal Torque**		
Implant type/Period		2 weeks	4 weeks	8 weeks
CI	27.99 ± 7.80 *	38.01 ± 17.09 ^{c}	68.01 ± 8.46 ^{b}	91.05 ± 9.32 **^{,a}
HCI	32.93 ± 10.61 *	41.69 ± 5.98 ^{b}	53.88 ± 18.50 ^{a,b}	68.62 ± 13.70 **^{,a}

*p < 0.01—Differences between groups of implants with different macrostructures—Paired t-test. **p < 0.01—Differences between groups of implants with different macrostructures—Paired t-test. Different letters (a, b and c) represent different levels of statistically significant differences among the periods within each group (p < 0.05). One-way ANOVA complemented by the Tukey test.

3.2. Microtomographic Analysis

There were no differences between groups regarding the BV/TV data. However, there was a progressive increase in this parameter with the increase in the time of the experimental periods in both groups. Figure 5 and Table 2 show the data on the mean and standard deviation from the BV/TV data obtained through microtomographic analysis of all groups. Figure 6 shows the microtomographic images of the implants with the different macrostructures.

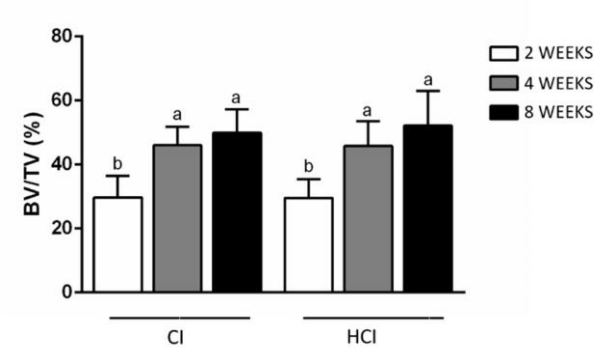

Figure 5. Mean and standard deviation for BV/TV data obtained through microtomographic analysis of all groups. Different letters represent different levels of statistically significant differences among the periods within each group ($p < 0.05$). One-way ANOVA complemented by the Tukey test.

Table 2. Data on the mean and standard deviation for BV/TV data obtained through microtomographic analysis of all groups.

Implant Type/Period	2 weeks	4 weeks	8 weeks
CI	29.68 ± 6.77 [b]	46.06 ± 5.68 [a]	49.95 ± 7.36 [a]
HCI	29.58 ± 5.78 [b]	45.80 ± 7.78 [a]	52.19 ± 10.77 [a]

Different letters (a and b) represent different levels of statistically significant differences among the periods within each group ($p < 0.05$). One-way ANOVA complemented by the Tukey test.

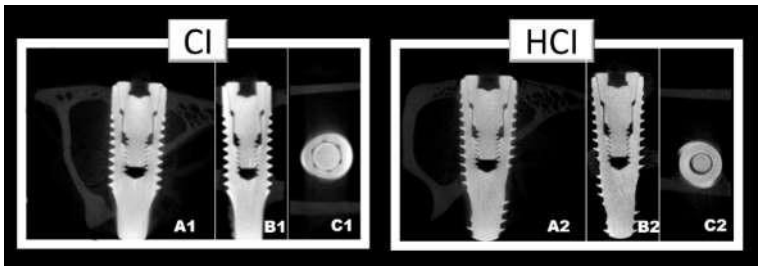

Figure 6. Microtomographic images representative of implants with different macrostructures CI: Cylindrical Implant (1) and HCI: hybrid conical implant (2). Axial slice (**A**); Sagittal slice (**B**); and Coronal slice (**C**).

3.3. Descriptive and Histometric Histological Analysis

The histological description was performed in decalcified sections in the region associated with the first thread of the implants. There were no differences in the histological aspects of bone tissue associated with the different macrostructures of the implants, thus, this description was performed together, varying only the description of the different experimental periods.

At two weeks, it was possible to observe the formation of new bone in the region of the threads that presented a trabecular aspect with rounded osteocytes, immature mineralized matrix without organization in the form of concentric lamellae, active osteoblasts with intense organization of the formation of Haversian channels and presence of medullary tissue. At 4 weeks, it was observed an increase in the formation of the mineralized matrix associated with a change in the appearance of the matrix to a more organized condition with the formation of concentric lamellae and a reduction in the number and diameter of the Haversian channels. There was also a reduction in medullary tissue, presence of rounded osteocytes in large numbers and a lower

number of osteoblasts. The appearance of peri-implant bone tissue has changed little over the 8-week period compared to the 4-week period. The presence of a mineralized matrix was observed in an advanced stage of mineralization, with the presence of well-defined Haversian channels, presence of rounded osteocytes, organization of the mineralized matrix in concentric lamellae and reduced amount of medullary tissue (Figure 7).

It was found that HCI implants (79.08 ± 11.31%) had a higher %BIC in the 8-week period compared to CI implants (59.72 ± 11.29%). Figure 8 and Table 3 show the data of the mean and standard deviation from the %BIC data obtained through the histometric analysis of all groups. Figure 9 shows images representative of the non-decalcified sections of the implants with different macrostructures and in the different experimental periods.

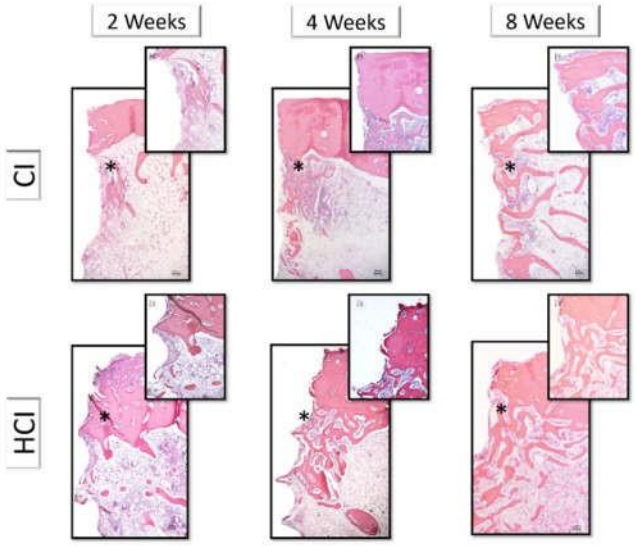

Figure 7. Histological images representative of decalcified sections of implants with different macrostructures and in different experimental periods. CI: Cylindrical Implant and HCI: Hybrid Conical Implant. Both in the periods of 2, 4 and 8 weeks. 5× and 10× magnification.

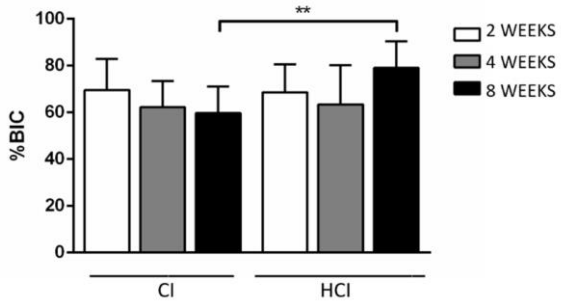

Figure 8. Mean and standard deviation for %BIC data obtained through histometric analysis of all groups. **p < 0.01—Differences between groups of implants with different macrostructures—Paired t-test.

Table 3. Data on the mean and standard deviation for %BIC data obtained through histometric analysis of all groups.

Implant Type/Period	2 weeks	4 weeks	8 weeks
CI	69.64 ± 13.22	62.21 ± 11.19	59.72 ± 11.29 *
HCI	68.62 ± 11.97	63.49 ± 16.77	79.08 ± 11.31 *

*p < 0.01——Differences between groups of implants with different macrostructures——Paired t-test.

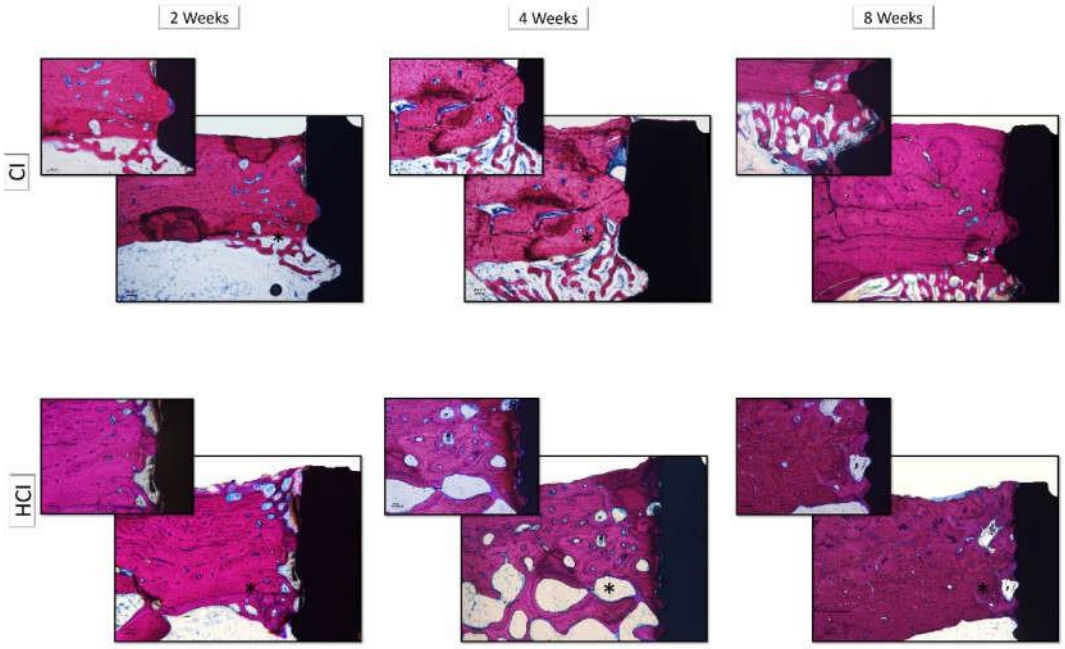

Figure 9. Histological images representative of the non-decalcified sections of the implants with different macrostructures and in different experimental periods. CI: Cylindrical Implant and HCI: Hybrid Conical Implant. Both in the periods of 2, 4 and 8 weeks. 5× and 10× magnification.

4. Discussion

In general, in this study, it was found that HCI implants showed better primary stability, which consequently resulted in greater bone-implant contact at the end of the 8 weeks of assessment, which demonstrates that this type of macrostructure can clinically receive occlusal loads earlier, be used in bones of lower density and accelerate the osseointegration process when compared to CI implants.

It has been determined that the implant macrostructure promotes changes in terms of obtaining primary stability [22,23]. The results of this study support studies that demonstrated that conical implants have superior primary stability when compared to cylindrical implants. However, despite the statistically significant difference obtained in this study, cylindrical implants also showed good results for primary stability [18,24]. This finding may be related to the fact that the experiment was carried out on the tibial bone, which has the characteristics of a type I bone, due to the presence of a thick cortical region that allowed the implants to lock properly [25,26]. It is likely that, in a bone of poorer quality, the differences in primary stability for HCI implants are even greater when compared to CI implants, a hypothesis that needs to be tested in the future.

Conflicting data in this study was that the removal torque of CI implants was higher than that of HCI implants in the period of 8 weeks. A factor that could have interfered with this aspect would be that the higher insertion torque of the HCI implants would

have induced greater necrosis of the cortical bone tissue and delayed the osseointegration process [27,28]. However, the insertion torque values of the HCI implants did not exceed 45 Ncm, which is not related to these adverse events [28]. In addition, the histological assessment of this study proved the similarity between implants with different macrostructures in the same experimental period assessed. The implants in this study had bicortical locking, where their apexes were locked to the posterior cortical bone of the tibia of the rabbits. Despite the fact that the surface area of the implants was not measured, it is possible to observe that the apexes of HCI implants are smaller than those of CI implants, it is possible that this increase in the contact area of the surface of the CI implants may have benefited the increased removal torque of these implants [29]. HCI implants are indicated for all types of bone densities. In the case of type I and II bones, an overrun drill should be used. However, during the installation of these implants, manual torque and counter-torque are often necessary to avoid damage to adjacent tissues. For this reason, the apex of HCI implants (Figure 1F) has already been developed with low intensity helical cameras to facilitate counter torque during manual installation. This fact can also explain the removal torque result of this study.

It has also been reported in the literature that primary stability is essential for the osseointegration process to occur in a predictable way and that the degree of osseointegration is correlated with the quality of this stability [30–32], a fact reinforced by the findings of this study that demonstrated a higher degree of %BIC associated with HCI implants when compared to what was observed in the CI implants in the 8-week period, however, without altering the quality of the newly formed bone. This finding demonstrates that the HCI macrostructure benefits the acceleration of the osseointegration process and that perhaps this difference is even more relevant in more challenging clinical conditions (e.g., low-density native bone, grafted areas), however, the animal model used does not allow to infer on the real impact of the HCI macrostructure on osseointegration in these clinical conditions.

The results of this study should be interpreted with caution because, in addition to the limitations mentioned above, this model cannot extrapolate clinical conditions such as the application of immediate or early loading, which could alter the osseointegration process in these implants. In addition, the bicortical locking offered by the tibia of rabbits, which in fact helps in the primary stability of implants, is not a common event to be expected in daily clinical practice, where implants usually lock in their most coronal portion in the cortical bone of the maxilla or the mandible. Thus, the effect of HCI on osseointegration still requires further investigation.

5. Conclusions

According to the results from and within the limits of this study, it is observed that HCI Implants showed greater insertion torque and bone-implant contact in relation to CI implants in the period of 8 weeks when installed in cortical bone of rabbits. However, the removal torque of CI implants was higher than that of HCI implants in the period of 8 weeks.

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Conflicts of Interest: Professor Doctor Élcio Marcantonio Junior is a consultant for the company Neodent. The other authors have no conflict of interest in this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Papaspyridakos, P.; Mokti, M.; Chen, C.-J.; Benic, G.I.; Gallucci, G.O.; Chronopoulos, V. Implant and Prosthodontic Survival Rates with Implant Fixed Complete Dental Prostheses in the Edentulous Mandible after at Least 5 Years: A Systematic Review. *Clin. Implant. Dent. Relat. Res.* **2013**, *16*, 705–717.
2. Åstrand, P.; Ahlqvist, J.; Gunne, J.; Nilson, H. Implant Treatment of Patients with Edentulous Jaws: A 20Year Follow-Up. *Clin. Implant. Dent. Relat. Res.* **2008**, *10*, 207–217.
3. Lee, J.-T.; Cho, S.A. Biomechanical evaluation of laser-etched Ti implant surfaces vs. chemically modified SLA Ti implant surfaces: Removal torque and resonance frequency analysis in rabbit tibias. *J. Mech. Behav. Biomed. Mater.* **2016**, *61*, 299–307.
4. El-Askary, A.S.; Meffert, R.M.; Griffin, T. Why do dental implants fail? Part I. *Implant. Dent.* **1999**, *8*, 173– 185.
5. Battula, S.; Lee, J.; Wen, H.; Papanicolaou, S.; Collins, M.; Romanos, G. Evaluation of Different Implant Designs in a Ligature-Induced Peri-implantitis Model: A Canine Study. *Int. J. Oral Maxillofac. Implant.* **2015**, *30*, 534–545.
6. Cameron, H.U.; Pilliar, R.M.; Macnab, I. The effect of movement on the bonding of porous metal to bone. *J. Biomed. Mater. Res.* **1973**, *7*, 301–311.
7. Steigenga, J.T.; Al-Shammari, K.F.; Nociti, F.H.; Misch, C.E.; Wang, H.-L. Dental implant design and its relationship to long-term implant success. *Implant. Dent.* **2003**, *12*, 306–317.
8. Junker, R.; Dimakis, A.; Thoneick, M.; Jansen, J.A. Effects of implant surfasse coatings and composition on bone integration: A systematic review. *Clin. Oral Implant. Res.* **2009**, *20,* 185–206.
9. Pilliar, R.M. Overview of surface variability of metallic endosseous dental implants: Textured and porous surface-structured designs. *Implant. Dent.* **1998**, *7*, 305–314.
10. Castilho, G.A.A.; Martins, M.; Macedo, W.A.A. Surface characterization of titanium Based dental implants. *Braz. J. Phys.* **2006**, *36*, 1004–1008.
11. Javed, F.; Romanos, G.E. The role of primary stability for successful immediate loading of dental implants. A literature review. *J. Dent.* **2010**, *38*, 612–620.
12. Salerno, M.; Itri, A.; Frezzato, M.; Rebaudi, A. Surface Microstructure of Dental Implants Before and After Insertion. *Implant. Dent.* **2015**, *24*, 1–255.
13. Chiapasco, M.; Gatti, C.; Rossi, E.; Haefliger, W.; Markwalder, T.H. Implant-retained mandibular overdentures with immediate loading. A retrospective multicenter study on 226 consecutive cases. *Clin. Oral Implant. Res.* **1997**, *8*, 48–57.
14. Chong, L.; Khocht, A.; Suzuki, J.B.; Gaughan, J. Effect of Implant Design on Initial Stability of Tapered Implants. *J. Oral Implant.* **2009**, *35*, 130–135.
15. Wennerberg, A.; Albrektsson, T.; Andersson, B.; Krol, J.J. A histomorghometric study of screw-shaped and removal torque titanium implants with three different surface topographies. *Clin. Oral Implant. Res.* **1995**, *6*, 24–30.
16. Negri, B.; Calvo-Guirado, J.L.; Maté Sánchez de Val, J.E.; Delgado Ruiz, R.A.; Ramirez Fernandez, M.P.; Gomez Moreno, G.; Aguilar Salvatierra, A.; Guardia, J.; Munoz Guzon, F. Biomechanical and Bone Histomorphological Evaluation of Two Surfaces on Tapered and Cylindrical Root Form Implants: An Experimental Study in Dogs. *Clin. Implant. Dent. Relat. Res.* **2012**, *15*, 799–808.
17. Calvo-Guirado, J.L.; Gomez Moreno, G.; Aguilar-Salvatierra, A.; Mate Sanchez de Val, J.E.; Abboud, M.; Nemcovsky, C.E. Bone remodeling at implants with different configurations and placed immediately at different depth into extraction sockets. Experimental study in dogs. *Clin. Oral Implant. Res.* **2014**, *26*, 507– 515.
18. Gehrke, S.A.; Martínez, C.P.-A.; Piattelli, A.; Shibli, J.A.; Markovic, A.; Guirado, J.L.C. The influence of three different apical implant designs at stability and osseointegration process: Experimental study in rabbits. *Clin. Oral Implant. Res.* **2016**, *28*, 355–361.

19. Calvo-Guirado, J.L.; Torres, J.A.L.; Dard, M.; Javed, F.; Martínez, C.P.-A.; De Val, J.E.M.S. Evaluation of extrashort 4-mm implants in mandibular edentulous patients with reduced bone height in comparison with standard implants: A 12-month results. *Clin. Oral Implant. Res.* **2015**, *27*, 867–874.
20. Pinotti, F.E.; De Oliveira, G.J.P.L.; Aroni, M.A.T.; Marcantonio, R.A.C.; Marcantonio, E. Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: A Preclinical study. *Clin. Oral Implant. Res.* **2018**, *29*, 963–972.
21. Faeda, R.S.; Spin-Neto, R.; Marcantonio, E.; Guastaldi, A.C. Laser ablation in titanium implants followed by biomimetic hydroxyapatite coating: Histomorphometric study in rabbits. *Microsc. Res. Tech.* **2012**, *75*, 940–948.
22. Akkocaoglu, M.; Uysal, S.; Tekdemir, I.; Akca, K.; Cehreli, M.C. Implant design and intraosseous stability of immediately placed implants: A human cadaver study. *Clin. Oral Implant. Res.* **2005**, *16*, 202–209.
23. De Oliveira, G.J.P.L.; Barros-Filho, L.A.B.; Queiroz, T.; Marcantonio, Élcio; Barros, L.A.B. In Vitro Evaluation of the Primary Stability of Short and Conventional Implants. *J. Oral Implant.* **2016**, *42*, 458–463.
24. Sakoh, J.; Wahlmann, U.; Stender, E.; Nat, R.; Al-Nawas, B.; Wagner, W. Primary stability of a conical implant and a hybrid, cylindric screw-type implant in vitro. *Int. J. Oral Maxillofac. Implant.* **2006**, *21*, 560– 566.
25. Turkyilmaz, I.; Sennerby, L.; McGlumphy, E.A.; Tozum, T. Biomechanical Aspects of Primary Implant Stability: A Human Cadaver Study. *Clin. Implant. Dent. Relat. Res.* **2009**, *11*, 113–119.
26. Marquezan, M.; Osório, A.; Sant'Anna, E.; Souza, M.M.; Maia, L.C. Does bone mineral density influence the primary stability of dental implants? A systematic review. *Clin. Oral Implant. Res.* **2011**, *23*, 767–774.
27. Markovic, A.; Mišić, T.; Milicic, B.; Calvo-Guirado, J.L.; Aleksić, Z.; Đinić, A. Heat generation during implant placement in low-density bone:effect of surgical technique, insertion torque and implant macro design. *Clin. Oral Implant. Res.* **2012**, *24*, 798–805.
28. Duyck, J.; Roesems, R.; Cardoso, M.V.; Ogawa, T.; Camargos, G.D.V.; Vandamme, K. Effect of insertion torque on titanium implant osseointegration: An animal experimental study. *Clin. Oral Implant. Res.* **2013**, *26*, 191–196.
29. Lee, J.; Frias, V.; Lee, K.; Wright, R.F. Effect of implant size and shape on implant success rates: A literature review. *J. Prosthet. Dent.* **2005**, *94*, 377–381.
30. Montes, C.C.; Pereira, F.A.; Thomé, G.; Alves, E.D.M.; Acedo, R.V.; De Souza, J.R.; Melo, A.C.M.; Trevilatto, P. Failing Factors Associated with Osseointegrated Dental Implant Loss. *Implant. Dent.* **2007**, *16*, 404–412.
31. Gill, A.; Rao, P. Primary stability: The password of implant integration. *J. Dent. Implant.* **2012**, *2*, 103.
32. Bataineh, A.; Al-Dakes, A.M. The influence of length of implant on primary stability: An in vitro study using resonance frequency analysis. *J. Clin. Exp. Dent.* **2017**, *9*, e1–e6.

3.2 Publicação 2

Original Reseach: Analysis osseointegration of implant of diffrent macrostructures in areas previously or immediately grafted with deproteinized bovine bone associated or not with fresh bone marrow

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Conflicts of Interest:

Professor Doctor Élcio Marcantonio Junior is a consultant for the company Neodent. The other authors have no conflict of interest in this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

*** Artigo submetido no periódico Journal of Periodontology**

Abstract

Background: To assess the osseointegration of implants of different macrostructures (CI - Cylindrical and HCI - Hybrid Conical) in areas grafted with deproteinized bovine bone (DBB) alone or associated with fresh bone marrow (DBB/BM), with variation in the time of implant placement (Immediate or Delayed).

Material and methods: Sixteen (16) rabbits were randomly divided into two groups according to the association of biomaterials used to fill the bone defect created in the animals' tibia: DBB vs. DBB/BM. After 90 days, bone biopsies from this region were collected and implants with different macrostructures were installed: CI and HCI. At the same time, a second bilateral defect was created in the tibial metaphysis and filled with the same materials, followed by the immediate implant placement. Euthanasia was performed 90 days after the second surgical procedure. The microtomographic analysis was performed to assess the number of mineralized tissues over the entire length of the bone biopsy and the number of implants over their entire length (%BV/TV). In addition, the histomorphometric analysis was performed to assess the composition of biopsies (%Bone; %Biomaterial and %Connective Tissue), and bone-implant contact (%BIC).

Results: There were no differences in the number of mineralized tissues and the composition of biopsies between the DBB and DBB/BM groups. HCI and CI showed no differences for %BIC, however, HCI showed higher values of %BV/TV in areas grafted with DBB when compared to CI.

Conclusions: Osseointegration in areas grafted with DBB was not affected by the addition of BM or the macrostructure of the implants.

Keywords: Dental implants; Bone marrow; Osseointegration; Bone substitute; Macrostructure.

Introduction

The treatment of edentulism with implant-supported prostheses has been applied in a predictable way in a wide variety of clinical conditions[1]. However, in some cases, it is not possible to install implants due to the limited availability of bone tissue, and guided bone regeneration procedures are necessary before or at the same time as the implant placement[2].

Osteoconductive bone substitutes of xenogeneic origin have been used in bone grafting procedures as an alternative to the use of autogenous bone graft as they promote good clinical results that allow rehabilitation on implants with high success and survival rates in these areas. However, due to the absence of osteogenic and osteoinductive potential for bone tissue formation, these biomaterials have a delayed repair process compared to the autogenous graft[3]. Due to such limitation, it has been proposed the association of biomaterials of xenogeneic origin with other bone substitutes or growth factors that improve their biological potential for bone tissue formation[4-6]. The use of fresh bone marrow (BM) infiltrate can be useful to accelerate bone repair in areas grafted with osteoconductive bone substitutes because it is a store of osteochondral progenitor stem cells that has the potential to differentiate into osteoblasts[7,8].

Obtaining good primary stability of the implants (that is necessary for the osseointegration process to occur) is another problem related to areas grafted with osteoconductive biomaterials[9]. Bone tissue of poor density, such as grafted

bone, makes it difficult to obtain the minimum insertion torque necessary for predictable osseointegration. In order to solve this clinical problem, structural changes in the macrostructure of the implants (e.g. Implant shape, shape and size of threads) have been assessed in order to improve the stability of implants in low-density bone[10, 11]. The emergence of implants with a conical structure and compressive threads allows the installation of implants with good primary stability, as well as the use of implants with smaller sizes since they can be used in areas with limited bone availability [12,13]. However, conical implants can also excessively increase the insertion torque, which can exacerbate the bone remodeling process and consequently impair the osseointegration process [14,15].

Therefore, this preclinical study aimed to verify the influence of an experimental implant of hybrid macrostructure with compacting and perforating threads (HCI - Hybrid Conical Implant), compared to a control implant (CI - Cylindrical Implant), on the osseointegration in areas previously or immediately grafted with deproteinized bovine bone (DBB) alone or associated with fresh bone marrow (BM) in surgical defects created in rabbits' tibiae.

Material and methods

This research was carried out in accordance with the ARRIVE protocol for conducting preclinical studies. The protocol was submitted and approved by the Ethics Committee on Animal Use (ECAU) of the State University of São Paulo (Unesp), Faculty of Dentistry of Araraquara, Brazil (n°15/2017). For this research, 16 male rabbits (Albinos, New Zealand) were used (4-5 kg and 9-12 months of age). These animals were kept in an environment with a temperature of 22-24ºC,

with a controlled light cycle (12 hours light and 12 hours dark) and consumption of solid food and water *ad libitum* throughout the experimental period.

Experimental Outline

To assess the influence of different macrostructures of titanium implants in areas previously or immediately grafted with xenogeneic grafts of bovine origin (Straumann® Cerabone®, Switzerland, Germany/0.5-1.0mm granules) associated or not with fresh bone marrow, 16 rabbits were used in a period of 180 days. The animals were randomly assigned to two experimental groups: Control Group (DBB: Deproteinized bovine bone graft) and Experimental Group (DBB/BM: Deproteinized bovine bone graft associated with fresh autologous bone marrow).

Initially, a bilateral bone defect (5 mm in diameter) was created in the tibial metaphysis of each animal. The defects were filled with DBB or DBB/BM. After 90 days, a bone biopsy was collected and implants of different macrostructures were placed in these previously grafted areas: Hybrid Conical Implant - HCI (Cylindrical body and helical conical apex) and Cylindrical Implant - CI. Each grafted region (right and left side) received an implant with different macrostructures (HCI or CI). In addition, in this surgical procedure, a second bilateral defect (5 mm in diameter) in the tibial metaphysis, located 4 mm from the first defect, was filled with the same materials described for the first defects (DBB or DBB/BM), but with immediate installation of implants of different macrostructures (HCI or CI). Ninety days after the second surgical procedure, all animals were euthanized by anesthetic overdose.

Implants

The CI implant used in this study is characterized by a cervical diameter equal to the diameter of the implant body (3.75mm in diameter x 11mm in height). Presence of triangular and double threads that facilitate the quick implant insertion, with minimal trauma, and apex morphology with the presence of self-cutting chambers (Titamax Acqua, Neodent®, Cone Morse, Curitiba, Brazil) (Figure 1A, 1C, and 1E). The HCI implant is characterized by an increased cervical diameter in relation to the implant body (3.75mm in diameter x 11.5mm in height). In addition, these implants have compacting and double trapezoidal threads, with a conical apex containing helical chambers designed to optimize secondary stability (Helix Acqua, Neodent®, Grand Morse, Curitiba, Brazil) (Figure 1B, 1D, and 1F).

Surgical procedure

The animals were weighed and anesthetized intramuscularly, with a combination of ketamine (Quetamina Agener®; Agener União Ltda, São Paulo, SP, Brazil) - 0.35 mg/kg) and xylazine (Rompum, Bayer AS, São Paulo, SP, Brazil - 0.5 mg/kg). Subsequently, bilateral trichotomy in the region of the tibial metaphysis and antisepsis with 10% polyvinylpyrrolidone (PVPI) with 1% active iodine were performed. Local anesthesia with Mepivacaine Hydrochloride 2% and Adrenaline 1:100,000 was also applied in the region, to allow peripheral vasoconstriction, reducing local bleeding and optimizing the surgical procedure. Then, using a No. 15 scalpel blade, a dermo-periosteal incision of approximately 5 cm in length was performed on the inside of the rear leg, just below the knee (Figure 2A). This allowed for delicate dissection so that the bone surface was exposed. The surgical defect was made using a 5mm diameter trephine drill (3i, Neodent, Brazil), coupled in a counter-angle and micromotor, under abundant

refrigeration with 0.9% sterile saline (Figure 2B, 2C and 2D). Circular markings were also made 2 mm anterior and 2 mm posterior to the margins of the surgical defect and filled with gutta-percha. These markings were located on an imaginary longitudinal line dividing the surgical defect in half. The markings were made with a carbide truncated conical drill (Carbide Truncated Conical Drill for High Rotation Speed no. 16 702, Dentsply, Brazil) coupled in high rotation speed also under continuous irrigation with 0.9% sterile saline. These markings were useful to identify the center of the original surgical defect during implant installation and laboratory processing, allowing to locate the original bone margins of the defect during histological analysis. After filling the defects with the respective materials (DBB or DBB/BM according to the experimental group) (Figure 2E, 2F, 2G, 2H and 2I), a collagen membrane was placed on the surface of the defect (Jason® membrane, Straumann®, Curitiba, Brazil) (Figure 2J). The fresh autologous bone marrow used was obtained from the tibial metaphysis with the aid of a Lucas 86 curette (Figure 2E). Finally, the soft tissues were repositioned and sutured per plane by using simple interrupted stitches with resorbable thread (Vicryl® Ethicon, 4-0, Johnson & Johnson, Brazil) and non-resorbable thread (Nylon 3-0, Shalon®, Brazil) (Figure 2K and 2L). After surgery, all animals received a single dose of antibiotic (Pentabiótico®, Wyeth-Whitehall Ltda, São Paulo, Brazil - 0.1 ml/kgl) and analgesic (Tramadol Hydrochloride 50mg/ml, Tramadol®, Medley, São Paulo, Brazil - 5 mg/Kg IM).

Ninety days after the first surgical procedure, the animals were submitted to the second surgical procedure in which the entire protocol of anesthesia, surgical access, and postoperative care was repeated. When accessing the previously grafted defects, a bone biopsy of the defect was

collected using a 3.3mm trephine drill (Figure 3A, 3B and 3C). The preparation for installing the implants was carried out according to the recommendations of the manufacturers of the implant system (Neodent®, Curitiba, Paraná, Brazil) so that the bone was milled with metal drills under abundant refrigeration with saline. The implants were inserted to the bone level and the cover screws were threaded. One implant was installed on the right side and one on the left side according to the different groups (CI or HCI) (Figure 3D).

After the installation of the implants in the previously grafted areas, the second defect (5mm diameter) in the tibial metaphysis was made and grafted (DBB or DBB/BM according to the experimental group) (Figure 3E, 3F, 3G, 3H, 3I and 3J). The defect was made and filled in the same way as described in the first surgical procedure, however, this defect was performed 2 mm from the distal marking (with gutta-percha) of the previously grafted area and received an implant immediately according to the different groups (CI or HCI) (Figure 3K, 3L and 3M). The soft tissues were repositioned again and sutured per plane as previously described (Figure 3N, 3O, 3P and 3Q).

Microtomographic Analysis (µCT)

The parts from the sites where the bone biopsies were collected and the implants were installed, fixed in 4% formaldehyde for 48 hours and kept in alcohol at 70°. The samples underwent a µCT scan (Skyscan, Aatselaar, Belgium), with the following scanning parameters: 18 µm3 voxel, 10x image magnification, X-ray tube voltage of 50 kV, 496 uA beam and the electrical current adjusted to 0.1 mA. The three-dimensional images were reconstructed, reoriented and analyzed by specific software (NRecon, Data Viewer, Ctan, SkyScan, Belgium).

The biopsies without implants were assessed for the volume of mineralized tissues over their entire length, while the samples with implants were assessed for the volume of mineralized tissues around the implants, and a region of interest was determined covering their entire length and exceeding its diameter by 0.5 mm. The threshold used for both analyses was 25 – 90 [16].

Histomorphometric Analysis

After the decalcification period, the bone biopsies collected from previously grafted areas were embedded in paraffin, cut into a microtome (6µm-thick) and stained using the hematoxylin-eosin (HE) technique. Five slides with 3 sections each were obtained in the central region of the biopsy. Three sections were assessed that were 36 µm apart, and the first section for assessment was selected randomly. The sections were assessed using a DIASTAR optical microscope (Leica Reichert & Jung Products, Germany) with 5x and 10x magnifications and the amount of bone tissue, biomaterial, and connective tissue was assessed, expressed as the average of the percentages of the 3 sections assessed in each sample.

The biopsies with implants were subjected to dehydration in alcohol with increasing concentration and infiltrated later in solutions of glycolmethacrylate (Technovit 7200 VLC) and ethyl alcohol, following gradual increases in the concentration of glycolmethacrylate until infiltration in this pure solution. The specimens were embedded in resin, polymerized and sectioned longitudinally along the main axis of the implant with a high precision diamond disk. The blocks were assembled on an acrylic slide with the help of the Tecnovit 4000 resin (Kulzer, Wehrheim, Germany), and using a cutting and micro-wear system (Exact-Cutting, System, Apparatebau Gmbh, Hamburg, Germany). The slides

were processed so they had a 50-70 µm thick section (approximately). The parts were stained with Stevenel's Blue and fuchsin and were analyzed by histometric analysis that assessed the amount of direct contact between the bone and the surface throughout the implant (%BIC). Measurements were made using photomicrographs obtained by an optical microscope (DIASTAR, Leica Reichert & Jung products, Germany), with 10x magnification, with the aid of a video camera (Leica Reichert & Jung products, Germany). The values were determined using image analysis software (Image J, Jandel Scientific, San Rafael, CA, USA). All analyses in this study were performed by trained examiners, blind for the experimental groups.

Statistical Analysis

The Shapiro-Wilk normality test was applied, which demonstrated that the data were distributed according to the theorem of the central data distribution. The parametric paired t-test was used for the inferential analysis of the data comparing the different groups of macrostructures (CI vs. HCI), to compare the results of the grafted areas (DBB vs. DBB/BM) and the shape of the installed implants (Immediate vs. Delayed). The GraphPad Prism 6 software (San Diego, CA, USA) was employed in the statistical tests that were applied at the significance level of 5%.

Results

The animals tolerated well the surgical procedure and remained healthy throughout the experimental period.

µCT Analysis

There were no statistically significant differences between groups for %BV/TV of biopsies without implants, although the group of DBB associated with BM had higher valuesthan biopsies where DBB was used alone (75.42 ± 10.11 vs. 77.42 ± 9.57). Table 1 show the data from the microtomographic analysis of the groups in this study. Figure 4 shows images representative of the microtomographic analysis of all groups.

It was observed that, regardless of the time of implant placement (Immediate vs. Delayed), HCI implants showed higher %BV/TV values in the areas grafted with deproteinized bovine bone alone (DBB-HCI: 83.34 ± 5.87 and 85.69 ± 7.91, respectively) compared to CI implants (DBB-CI: 70.39 ± 15.18 and 77.60 ± 8.31, respectively). The other groups assessed did not show statistically significant differences. Table 3 show the data on mean and standard deviation from the microtomographic analysis of the areas grafted with deproteinized bovine bone alone or associated with bone marrow. Figure 6 shows images representative of the microtomographic analysis of all groups.

Histomorphometric Analysis

Although the DBB/BM group had higher values regarding the percentage of bone and connective tissue (27.37 ± 5.83 and 51.81 ± 10.53, respectively) compared to the DBB group (23.08 ± 9.95 and 50.52 ± 14.31, respectively) and although the DBB group (26.40 ± 7.16) had higher values regarding the percentage of biomaterial compared to the DBB/BM group (20.81 ± 11.34), no statistical difference was detected between the grafted areas concerning the percentage of bone, biomaterial, and connective tissue. Table 2 show the data on mean and standard deviation for the percentage of bone (2A),

biomaterial (2B) and connective tissue (2C) of the different groups (DBB vs. DBB/BM).

In biopsies of non-decalcified sections, it was found that CIs installed immediately in areas grafted with deproteinized bovine bone alone showed higher %BIC than the same implants installed later in areas grafted with deproteinized bovine bone alone (36.22 ± 6.07 vs. 23.32 ± 5.29). The other groups assessed did not show statistically significant differences. Table 4 show the data on mean and standard deviation from the histometric analysis of %BIC of the areas grafted with deproteinized bovine bone alone or associated with bone marrow. Figure 7 shows the histological images representative of the non-decalcified sections of all groups assessed.

Discussion

In this study, it was proposed to assess the osseointegration of two different implant macrostructures (HCI vs. CI) installed immediately or later in rabbits' tibiae grafted with DBB associated or not with BM. As a general finding, the areas grafted with BM showed a better pattern of osseointegration and new bone formation between the biomaterial particles, however, no statistically significant differences were detected in the analyses employed. As for the different macrostructures assessed, in the grafted areas with DBB, regardless of the time of implant placement (Immediate vs. Delayed), HCI had higher valuesfor the percentage of mineralized tissues around the implant compared to CI. On the other hand, regarding the percentage of bone-implant contact, only in the areas grafted with DBB, CIs showed statistical differences when comparing the

Immediate vs. Delayed implant installation (36.22 ± 6.07 and 23.32 ± 5.29, respectively).

Deproteinized bovine bone (DBB) is widely used and researched as a bone substitute due to its physicochemical properties similar to those of human bone, its osteoconductive potential, and availability. The sintering temperature of bone substitutes is an important parameter that can affect their properties[17]. When sintered at low temperatures (up to 300°C), the mineral crystals of the bone matrix are preserved (Bio Oss®, Geistlich Pharma, Wolhusen, Switzerland, for example)[18]. In contrast, when sintered at high temperatures (Straumann® Cerabone®, Switzerland, Germany) (initial oxidative combustion at temperatures around 800°C followed by a second heat treatment at 1,250°C), this process results in an increase in the crystal size (500-1000%) and crystal density, which makes it comparable to a ceramic-based material[17-19]. The present study used DBB sintered at high temperatures, and bone biopsies collected from previously grafted areas (after 90 days of healing) showed particles of the biomaterial incorporated into the newly formed bone. This indicates that this DBB, in fact, worked as a scaffold for bone apposition.

The use of bone substitutes capable of offering an adequate framework, with emphasis on bovine xenogeneic grafts, has been a good option. But the osteoconduction of these materials, when combined with other mechanisms of action, such as osteoinduction and osteogenesis, seems to have even better results [20]. Therefore, a favorable alternative would be the association of this (essentially osteoconductive) filling material with the fresh bone marrow that has osteogenic and osteoinductive properties because it contains osteochondral progenitor stem cells and growth factors that would induce greater differentiation

and osteoblastic activity. In addition, the use of this biological adjuvant could work as a biological connection between the graft particles, further favoring tissue regeneration [21-25].

There is no consensus on the best methodology for the use of bone marrow stromal cells. Three main alternatives were proposed: (1) Use of fresh autologous bone marrow graft ("in natura"); (2) Use of autologous bone marrow cell concentrate (by centrifugation); and, (3) Use of autologous bone marrow stromal cell culture [21-26]. The use of bone marrow enriched with mononuclear fraction obtained by centrifugation showed better results of bone formation associated with DBB (28.17 ± 3.19%) than the use of fresh bone marrow associated with the same bone substitute (21.14 ± 7.38%) according to a study by Pelegrine et al. (2014)[27]. However, some disadvantages have been associated with the use of the cell culture methodology: cost, time (requires a period of a few weeks between cell collection, obtaining the culture and transplantation), increased risk of contamination, and the routine clinical applicability of the cell culture may require two separate surgical procedures. Another question about the need (or not) to cultivate the cells is the lack of information on the minimum number of cells required to promote bone repair[26-30]. Therefore, the use of fresh bone marrow made in the present study would be a simpler approach than other uses that have already been proposed.

In a study of critical defects in rabbit calvaria, the use of fresh bone marrow associated with DBB showed a greater formation of bone tissue (21.14 ± 7.38%) compared to the use of DBB alone (13.06 ± 5.24 %) 8 weeks after the grafting procedure[26]. As in a study of vertical bone reconstruction in rabbit calvaria, the stem cells derived from the marrow associated with a block of DBB resulted in

increased bone formation when compared to the association of the same block with stem cells from adipose tissue (11.9 ± 7.5% vs. 7.6 ± 5.6%) 8 weeks after the surgical procedure [8]. In the present study, no statistical differences were identified as observed in the studies mentioned in which BM was added to DBB. This may have occurred due to the long experimental period in our study (90 days for the healing of the previous graft and for the post-installation of the implants) compared to the aforementioned studies (60 days). In addition, other forms of application of BM should be tested with regard to the possibility of positively influencing the osseointegration process in areas grafted with DBB, because when BM was used (DBB vs. DBB/BM) the quality of the connection between the particles was evidently higher.

The implant macrostructure directly influences the primary stability of the implants and, consequently, the success of their osseointegration. As for the macrostructure of the implants used (CI vs. HCI), several studies in the literature have shown that implants with conical structure and with changes in the conformation of threads, in order to make them more compressive, allow the installation of implants with good primary stability. Therefore, they are indicated in areas with limited bone availability or in low-density regions [31-33]. The results of this study showed that the percentage of mineralized tissue around the implants was statistically higher in the HCI implant compared to the CI implant when using DBB alone, regardless of the time of implant placement (Immediate or Delayed). In the bone-implant contact, although both tested implants showed good results, the statistical difference in osseointegration was only observed in the CI implant when installed in areas previously or immediately grafted with DBB alone, with better results when immediately installed (36.22 ± 6.07 and 23.32 ±

5.29, immediate and delayed respectively). These results support studies in the literature since the decision to perform previous or immediate grafting will be defined by the type of bone defect found at the beginning of the treatment plan and by the possibility of primary implant locking.

After tooth extraction, the alveolus has an intrinsic cone shape. When it receives immediate implants, the primary locking occurs only in the apical or apical-middle portion of the implant. Therefore, the macrostructure of the selected implant is extremely important. If such locking does not occur, bone regeneration or repair of the alveolus should first be achieved with or without prior grafting depending on the type of defect obtained after tooth loss. And, the alveolus has often dimensions larger than the implant diameter, forming a space between the cervical region of the implant and the bone tissue. Implant locking in these clinical situations occurs basically in the apical portion of the alveolus, which requires the filling of the space between the implant and the alveolus wall with biomaterials[34]. In this study, the second defect produced in the tibial metaphysis of rabbits with a cervical diameter (in the upper cortical area of the tibia) 1.25mm larger than the implant diameter (5mm vs. 3.75mm, respectively), and implant locking only in the apical portion of the (lower cortical) defect simulated a clinical situation of immediate implant after tooth extraction[35]. As the bone width in the cervical portion directly influences the %BIC, it is important to use biomaterials to fill these defects, so we use a bovine xenogeneic graft.

The surfaces modified by sandblasting with abrasive particles followed by acid subtraction have demonstrated good clinical results, success, and survival of the implants. However, they have a low degree of wettability. For this reason, this surface treatment has been associated with the procedure of

maintaining the implant in a 0.9% sodium chloride isotonic solution, resulting in a surface that stands out for being exceptionally hydrophilic, with a contact angle of about 0° against 139.0° (hydrophobic), and 162% increase in the rate of fibronectin adhesion. In addition, the hydrophilic surface promotes an increase in the initial stability of the implants, consequently accelerating the osseointegration, allowing the use of early implant loading protocols[36-39]. Therefore, the use of implants with a hydrophilic surface (Acqua®) in this study, can also explain the lack of statistical differences between the groups assessed.

The deproteinized bovine bone sintered at high temperatures showed good results of incorporation of particles and bone-implant contact regardless of the association of BM and the macrostructures tested in the (delayed) period assessed. However, due to the limitations of this study, further research is encouraged to verify the real influence of BM associated with different biomaterials in the bone regeneration process, especially in short assessment periods and challenging clinical conditions (diabetes, osteoporosis, rheumatoid arthritis, etc.; smokers or users of alendronates; regions of low bone density; and loading of implants).

Conclusion

Osseointegration in areas grafted with DBB was not affected by the addition of BM or the macrostructure of the implants. However, implants with hybrid conical macrostructure increased the number of mineralized tissues around the implants in areas grafted with DBB alone.

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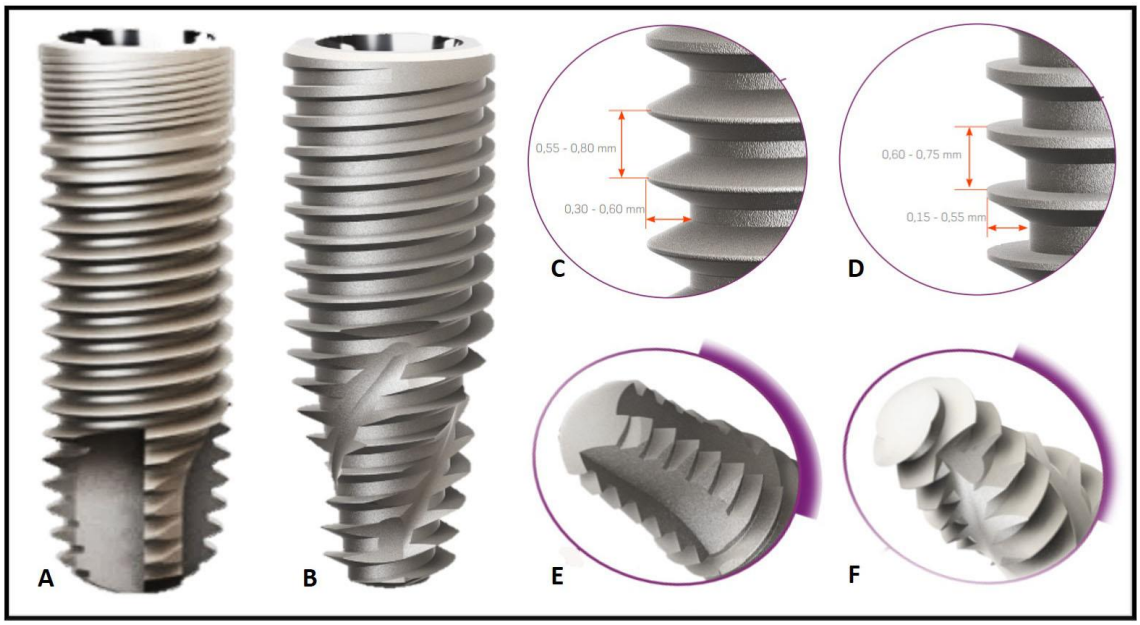
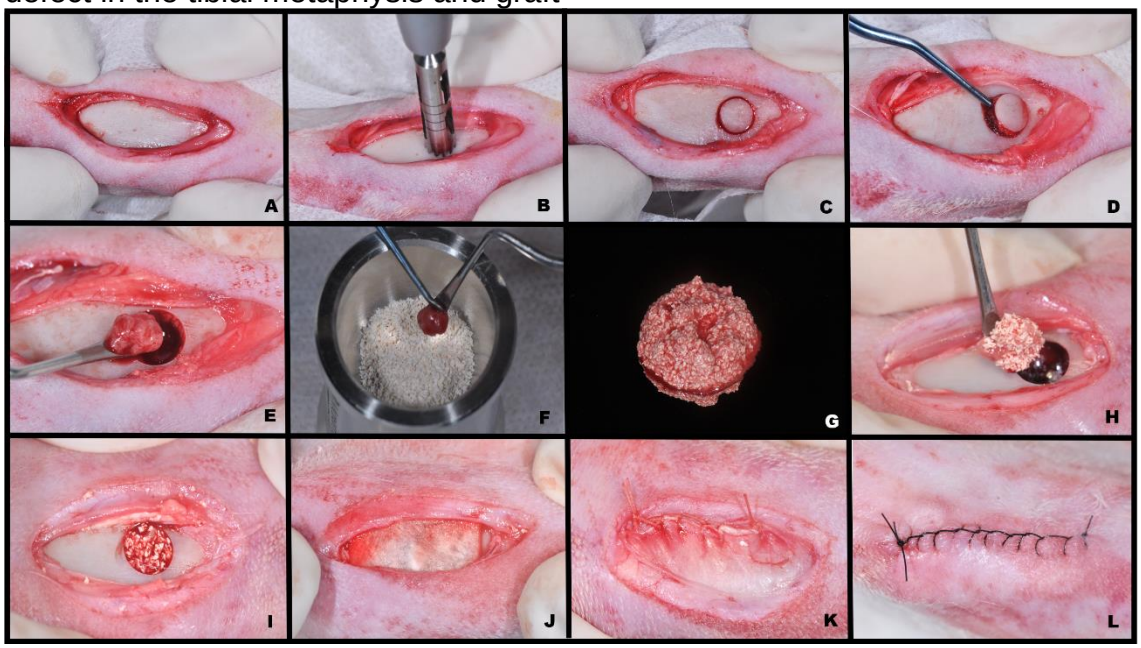
Figure legends

Figure 1: Macrostructure of implants installed in the previously or immediately grafted tibia



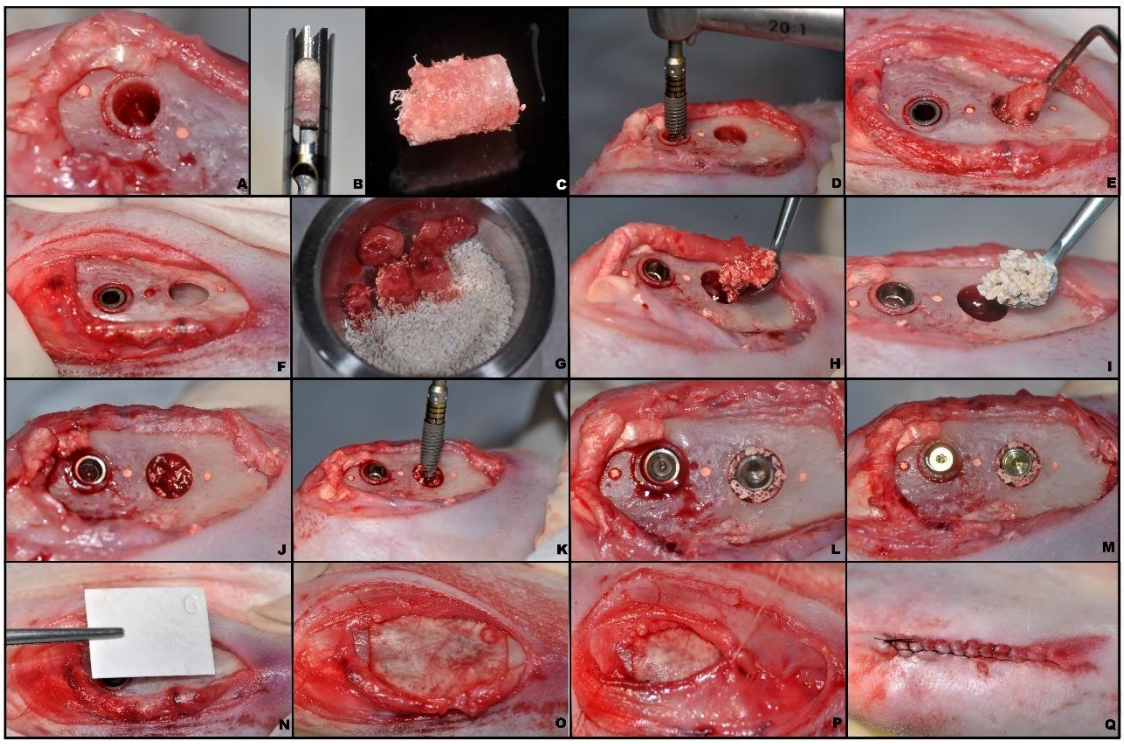
*Body, threads, and apex of Cylindrical Implant (A, C, and E) and Hybrid Conical Implant (B, D, and F)

Figure 2: Day 0 - Surgical procedure for bilateral creation of a 5mm diameter defect in the tibial metaphysis and graft



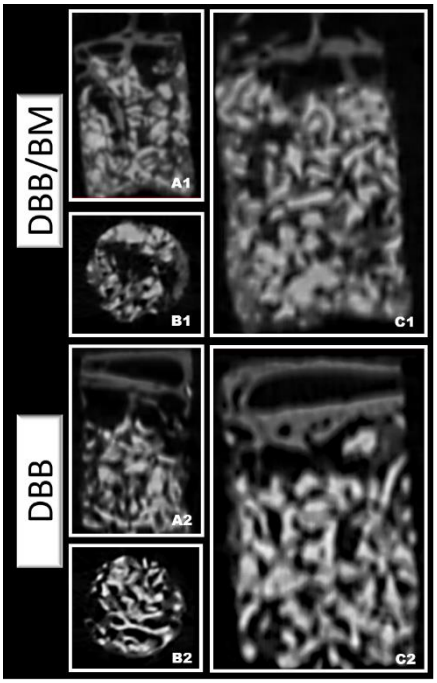
*Stages of the surgical procedure: Incision, detachment, and exposure of the tibial metaphysis (A); Surgical defect made with a 5mm diameter trephine drill (B and C); Excision of ostectomized bone tissue (D); Collection of fresh bone marrow with the aid of a Lucas curette (E); Filling of the bone store with the different biomaterials according to the different groups (Control Group [DBB] and Experimental Group [DBB/BM]) (F, G, H and I); A collagen membrane (Jason® membrane, Straumann®, Curitiba, Brazil) was placed over the bone window (J); Internal suture of the muscular fascia with resorbable thread and external suture with non-resorbable thread (K and L).

Figure 3: Day 90 - Surgical procedure for the installation of the implants in the tibial metaphysis previously grafted and creation of the second 5mm diameter defect, graft, and immediate implant installation



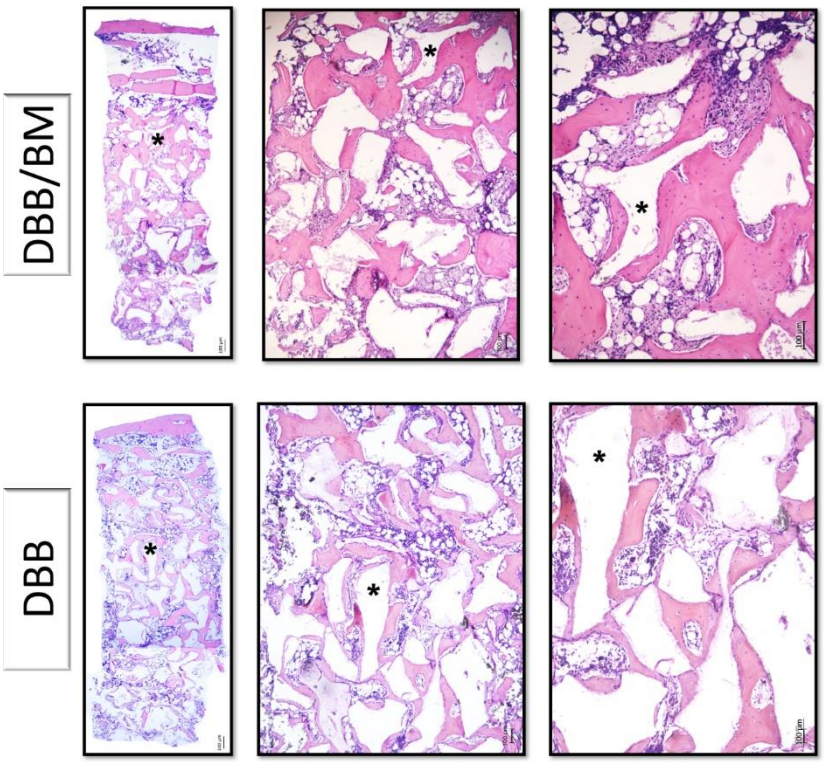
*Collection of bone biopsy from the region previously grafted with a 3.3mm diameter trephine drill (A, B and C); Drilling and installation of the implant in the previously grafted store (D); Collection of fresh bone marrow with the aid of a Lucas curette from the second bone store and emptying of the bone store (E and F); Drilling according to the manufacturer's recommendations for each implant system and filling of the second bone store with the materials according to the different groups (DBB vs. DBB/BM) (G, H, I and J); Immediate installation of the implant in the second bone store (K); Implants and cover screws installed (L and M); A collagen membrane (Jason® membrane, Straumann®, Curitiba, Brazil) was placed over the bone window (N and O); Internal suture of the muscular fascia with resorbable thread and external suture with non-resorbable thread (P and Q).

Figure 4: Microtomographic slices of bone biopsies collected from the previously grafted tibia store



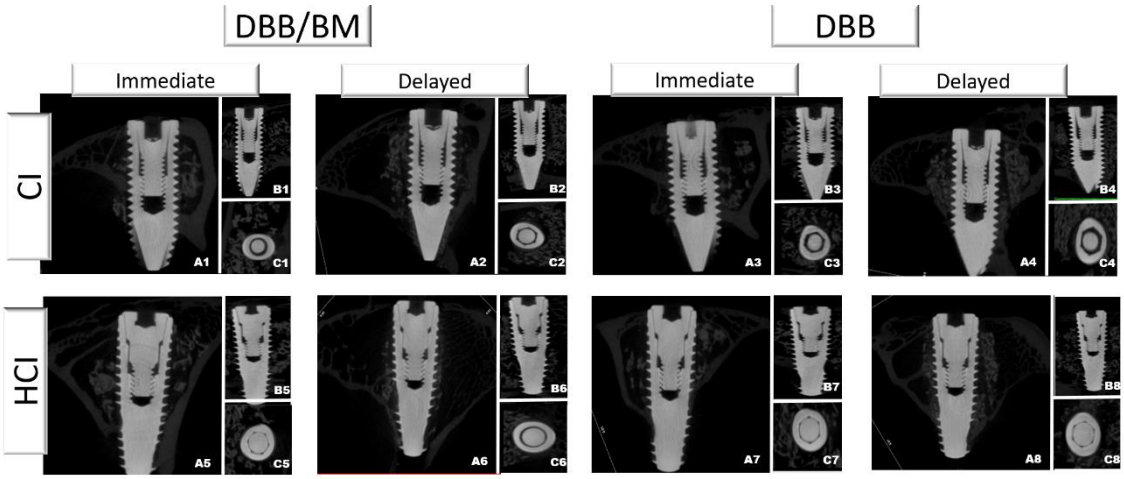
*Sagittal slices (A1 and A2); axial slices (B1 and B2); and coronal slices (C1 and C2) representative of the different experimental groups (1: DBB/BM; and 2: DBB).

Figure 5: Histological images representative of bone biopsies collected from the previously grafted tibia store



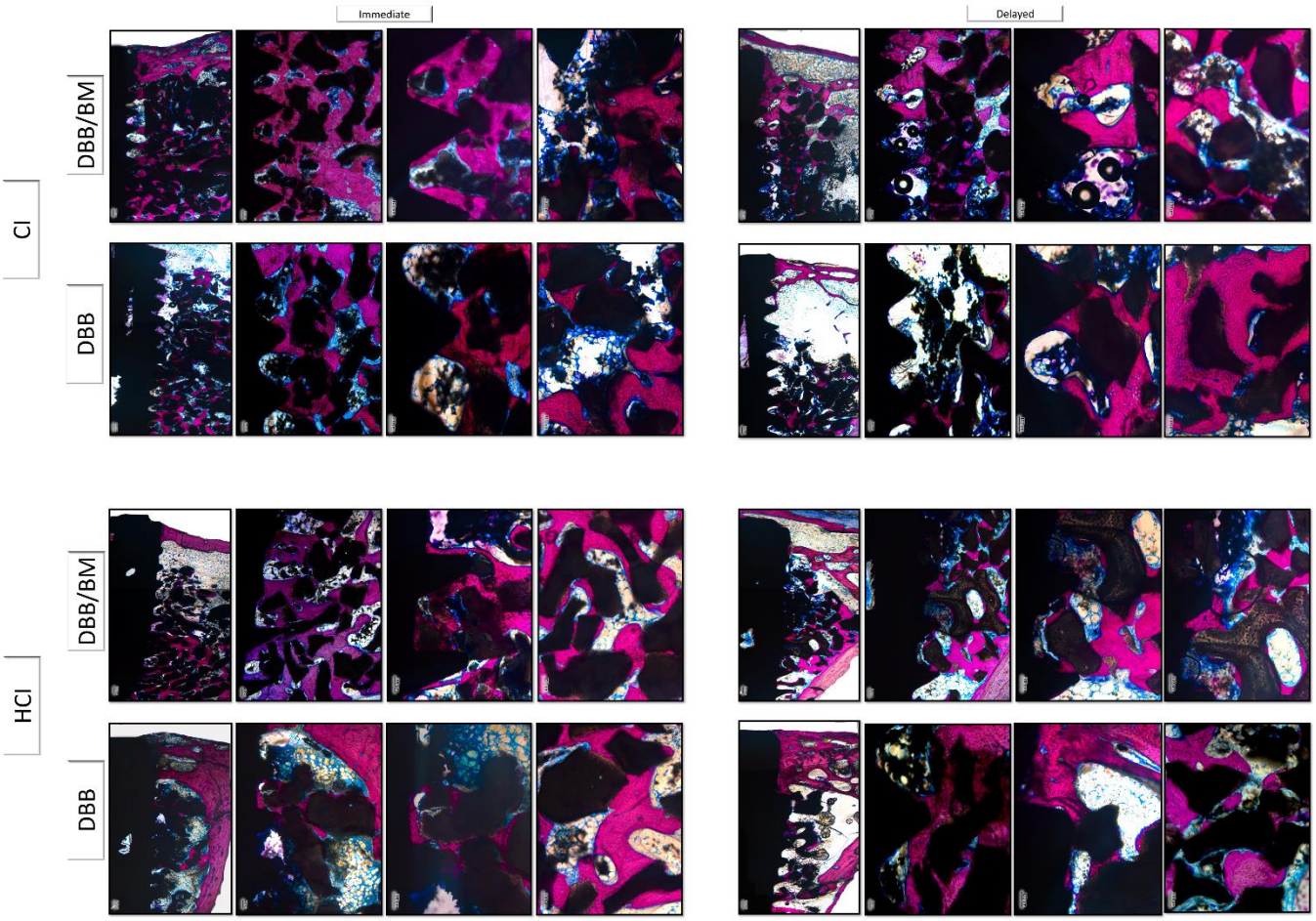
*DBB (Deproteinized bovine bone graft) and DBB/BM (Deproteinized bovine bone graft associated with fresh autologous bone marrow). 2.5x, 5x and 10x magnification.

Figure 6: Microtomographic slices of the tibiae after grafting and installation of the implants according to the different groups in the period of 180 days



*Sagittal slices (A1 and A2); axial slices (B1 and B2); and coronal slices (C1 and C2) representative of the different experimental groups (1: DBB/BM; and 2: DBB).

Figure 7: Histological sections of the tibiae after grafting (DBB/BM vs. DBB) and implant placement according to the different groups (HCI vs. CI and Immediate vs. Delayed) in the period of 180 days



* CI (Cylindrical Implant; HCI (Hybrid Conical Implant); DBB (Deproteinized bovine bone graft) and DBB/BM (Deproteinized bovine bone graft associated with fresh autologous bone marrow). 2.5x, 5x and 10x magnification.

Chart 1 - Data on mean and standard deviation from the microtomographic analysis of the areas grafted with deproteinized bovine bone alone (DBB) or associated with bone marrow (DBB/BM).

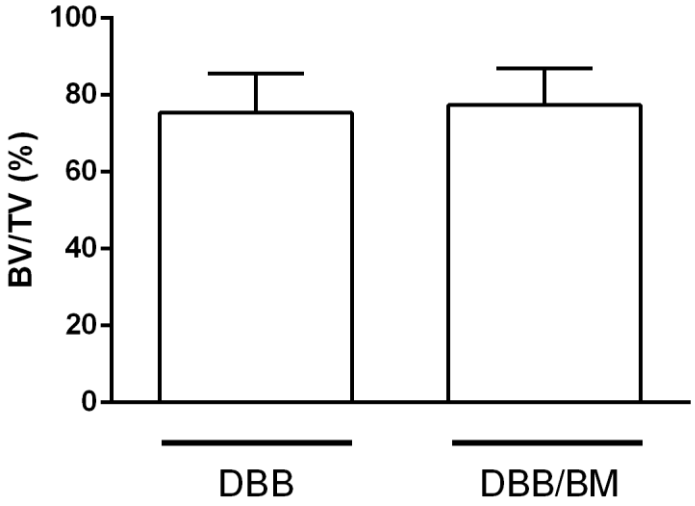


Chart 2 - Data on mean and standard deviation from the analysis of the percentages of bone (A), biomaterial (B) and connective tissue (C) of the areas grafted with the deproteinized bovine bone (DBB) alone or associated with bone marrow (DBB/BM)

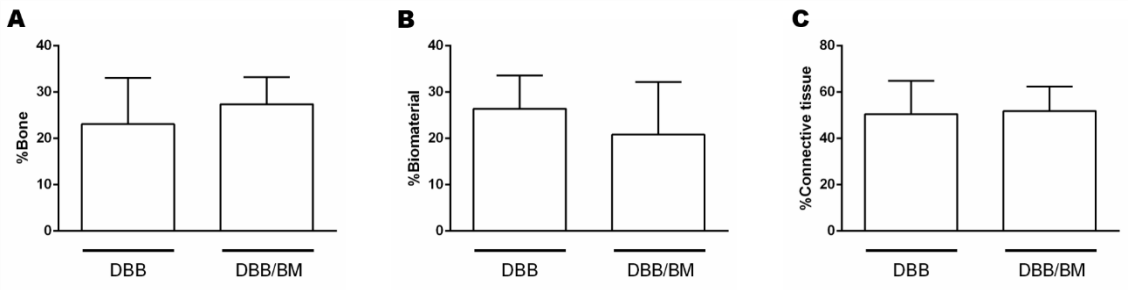
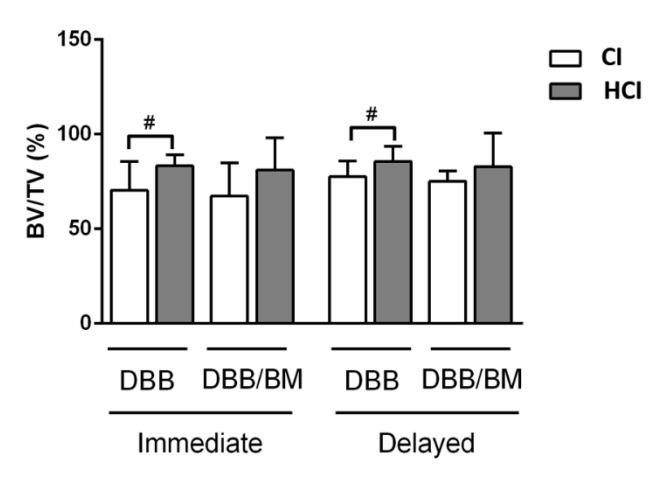
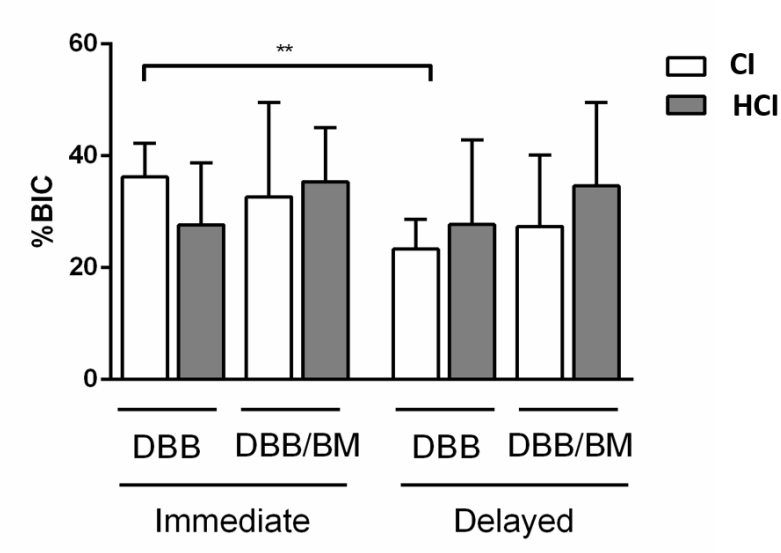


Chart 3 - Data on mean and standard deviation from the microtomographic analysis of the areas grafted with deproteinized bovine bone alone or associated with bone marrow



# p<0.05 - Differences between CI vs. HCI implants (paired t-test).

Chart 4: Data on mean and standard deviation from the histometric analysis of %BIC of the areas grafted with deproteinized bovine bone alone or associated with bone marrow.



** p<0.01 - Differences between implants installed immediately and later - Unpaired t-test.

Table 1 - Data on mean and standard deviation from the microtomographic analysis of the areas grafted with deproteinized bovine bone alone or associated with bone marrow.

Group	BV/TV(%)
DBB	75.42 ± 10.11
DBB/BM	77.42 ± 9.57

Table 2: Data on mean and standard deviation from the histometric analysis of the areas grafted with deproteinized bovine bone alone or associated with bone marrow.

Group	%Bone	% Biomaterial	%Connective Tissue
DBB	23.08 ± 9.95	26.40 ± 7.16	50.52 ± 14.31
DBB/BM	27.37 ± 5.83	20.81 ± 11.34	51.81 ± 10.53

Table 3: Data on mean and standard deviation from the histometric analysis of the areas grafted with deproteinized bovine bone alone or associated with bone marrow.

Installation time for implants	Group	**BV/TV (%)**
Immediate	DBB-CI	70.39 ± 15.18
	DBB-HCI	83.34 ± 5.87
	DBB/BM-CI	67.34 ± 17.44
	DBB/BM-HCI	81.08 ± 17.01
Delayed	DBB-CI	77.60 ± 8.31
	DBB-HCI	85.69 ± 7.91
	DBB/BM-CI	75.22 ± 5.44
	DBB/BM-HCI	82.97 ± 17.74

Table 4: Data on mean and standard deviation from the histometric analysis of %BIC of the areas grafted with deproteinized bovine bone alone or associated with bone marrow.

Installation time for implants	Group	**BIC (%)**
Immediate	DBB-CI	36.22 ± 6.07
	DBB-HCI	27.68 ± 11.11
	DBB/BM-CI	32.69 ± 16.82
	DBB/BM-HCI	35.37 ± 9.68
Delayed	DBB-CI	23.32 ± 5.29
	DBB-HCI	27.71 ± 15.15
	DBB/BM-CI	27.39 ± 12.72
	DBB/BM-HCI	34.60 ± 14.92

REFERENCES

1 Astrand P, Ahlqvist J, Gunne J, Nilson H. Implant treatment of patients with edentulous jaws: a 20-year follow-up. Clin Implant Dent Relat Res. 2008; 10(4): 207-17.

2 Narain RU, Garg S, Narain P. A Prospective study of the quality of removable prostheses and patients' satisfaction in post-prosthetic phase. The Inter J Dent Science. 2010; 9 (1): 1-15.

3 Schmitt CM, Moest T, Lutz R. Anorganic bovine bone (ABB) vs. autologous bone (AB) plus ABB in maxillary sinus grafting. Clin Oral Impl Res. 2014; 1-8.

4 Oliveira TA, Aloise AC, Orosz JE, Mello E, Oliveira R, Carvalho P, Pelegrine AA. Double Centrifugation Versus Single Centrifugation of Bone Marrow Aspirate Concentrate in Sinus Floor Elevation: A Pilot Study. The Int J Oral & Maxillof Impl. 2016; 31(1): 216-22.

5 Wildburger A, Payer M, Jakse N, Strunk D, Etchard-Liechtenstein N, Sauerbier S. First published Impact of autogenous concentrated bone marrow aspirate on bone regeneration after sinus floor augmentation with a bovine bone substitute – a split-mouth pilot study. Clin Oral Impl Res. 2014; 25: 1175-81.

6 Lee S-B, Retzepi M, Petrie A, Hakimi A-R, Schwarz F, Donos N. The effect of diabetes on bone formation following application of the GBR principle with the use of titanium domes. Clin Oral Impl Res. 2012; 24(1): 28–35.

7 Lavareda Corrêa SC, Sousa JE, Pasquali PJ et al. Use of Bone Allograft With or Without Bone Marrow Aspirate Concentrate in Appositional Reconstructions -

A Tomographic and Histomorphometric Study. Implant Dent. 2017; 26(6): 481-485.

8 Carrera-Arrabal T, Calvo-Guirado JL, Santos FP et al. Vertical Bone Construction with Bone Marrow-Derived and Adipose Tissue-Derived Stem Cells. Symmetry. 2019; 59: 1-11.

9 Bertl K, Bertl MH, Heimel P, Burt M, Gahleitner A, Stavropoulos A, Ulm C. Alveolar bone resorption after primary tooth loss has a negative impact on straightforward implant installation in patients with agenesis of the lower second premolar. Clin Oral Implants Res. 2018 Feb;29(2):155-63.

10 Gehrke SA, Marin GW. Biomechanical evaluation of dental implants with three different designs: Removal torque and resonance frequency analysis in rabbits. Ann Anat. 2015 May; 199: 30-5.

11 Negri B, Calvo-Guirado JL, Maté Sánchez de Val JE, et al. Biomechanical and bone histomorphological evaluation of two surfaces on tapered and cylindrical root form implants: an experimental study in dogs. Clin Implant Dent Relat Res. 2013; 15 (6): 799-808.

12 Spin-Neto R, Stavropoulos A, Coletti FL, Pereira LA, Marcantonio E Jr, Wenzel A. Remodeling of cortical and corticocancellous fresh-frozen allogeneic block bone grafts- -a radiographic and histomorphometric comparison to autologous bone grafts. Clin Oral Implants Res. 2015 Jul; 26(7): 747-52.

13 Bertl K, Bertl MH, Heimel P et al. Alveolar bone resorption after primary tooth loss has a negative impact on straightforward implant installation in patients

with agenesis of the lower second premolar. Clin Oral Implants Res. 2018 Feb;29(2):155-163.

14 Alghamdi H, Anand PS, Anil S. Undersized implant site preparation to enhance primary implant stability in poor bone density: a prospective clinical study. J Oral Maxillofac Surg. 2011 Dec;69(12):e506-12.

15 He J, Zhao B, Deng C, Shang D, Zhang C. Assessment of implant cumulative survival rates in sites with different bone density and related prognostic factors: an 8-year retrospective study of 2,684 implants. Int J Oral Maxillofac Implants. 2015 MarApr;30(2):360-71.

16 Pinotti FE, de Oliveira GJPL, Aroni MAT, Marcantonio RAC, Marcantonio E Jr. Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: a preclinical study. Clin Oral Impl Res. 2018; 29(10): 963– 72.

17 Barbeck Ž, Kačarević P, Kavehei F, Rider P, Najman S, Stojanović S, Rimashevskiy D, et al. The effect of temperature treatment of xenogeneic bone substitute on the tissue response – a mini review Acta Medica Medianae. 2019; 58(1): 131-37.

18 Barbeck M, Udeabor S, Lorenz J, et al. High-temperature sintering of xenogeneic bone ubstitutes leads to increased multinucleated giant cell formation: in vivo and preliminary clinical results. J Oral Implantol 2015; 41(5):e212-22

19 Barbeck M, Udeabor SE, Lorenz J, Kubesch A, Choukroun J, Sader RA. Induction of multinucleated giant cells in response to small sized bovine bone

substitute (Bio-Oss) results in an enhanced early implantation bed vascularization. Ann Maxillofac Surg 2014; 4(2):150-7.

20 Lucarelli E, Donati D, Cenacchi A, Fornasari PM. Bone reconstruction of large defects using bone marrow derived autologous stem cells. Transfusion and apheresis science. 2004; 30: 169-74.

21 Oliveira TA, Aloise AC, Orosz JE et al. Double Centrifugation Versus Single Centrifugation of Bone Marrow Aspirate Concentrate in Sinus Floor Elevation: A Pilot Study. The Int J Oral & Maxillof Impl. 2016; 31(1): 216-22.

22 Wildburger A, Payer M, Jakse N, Strunk D, Etchard-Liechtenstein N, Sauerbier S. First published Impact of autogenous concentrated bone marrow aspirate on bone regeneration after sinus floor augmentation with a bovine bone substitute – a split-mouth pilot study. Clin Oral Impl Res. 2014; 25: 1175-81.

23 Lee S-B, Retzepi M, Petrie A, Hakimi A-R, Schwarz F, Donos N. The effect of diabetes on bone formation following application of the GBR principle with the use of titanium domes. Clin Oral Impl Res. 2012; 24(1): 28–35.

24 Pasquali PJ, Teixeira ML, Oliveira TA, Macedo LGS, Aloise AC, Pelegrine AA. Maxillary Sinus Augmentation Combining Bio-Oss with the Bone Marrow Aspirate Concentrate: A Histomorphometric Study in Humans. Int J of Biomat. 2015; 15: 1-7.

25 Aloise AC, Pasquali P, Sperandio M, Macedo LGS, Teixeira ML, Pelegrine AA, Calvo-Guirado JL. Use of Bone Marrow Aspirate Concentrate (BMAC) Associated with Hyperbaric Oxygenation Therapy in Maxillary Appositional

Bone Reconstruction. A Randomized Clinical Trial. Symmetry. 2018; 10(10): 533.

26 Pelegrine AA, Aloise AC, Zimmermann A, Oliveira RM, Ferreira LM. Repair of critical-size bone defects using bone marrow stromal cells: a histomorphometric study in rabbit calvaria. Part I: Use of fresh bone marrow or bone marrow mononuclear fraction. Clin Oral Impl Res. 2014; 25: 567-72.

27 Silva MO, Pelegrine AA, Silva AAP et al. Xenograft Enriched with Autologous Bone Marrow in Inlay Reconstructions: A Tomographic and Histomorphometric Study in Rabbit Calvaria. Int J of Biomat. 2012; 1-8.

28 Pelegrine AA, Costa CES, Correa MEP, Marques Jr JFC. First published: Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft. Clin Oral Impl Res. 2010; 21: 535-42.

29 Nagata MJH, Santinonia CS, Pola NM, Michel NC, Messorab S. Bone marrow aspirate combined with low-level laser therapy: A new therapeutic approach to enhance bone healing. J of Phot and Photobiology B: Biol. 2013; (121): 6-14.

30 Pasquali PJ, Teixeira ML, Oliveira TA, Macedo LGS, Aloise AC, Pelegrine AA. Maxillary Sinus Augmentation Combining Bio-Oss with the Bone Marrow Aspirate Concentrate: A Histomorphometric Study in Humans. Int J of Biomat, 2015.

31 Akkocaoglu M, Uysal S, Tekdemir I, Akca K, Cehreli MC. Implant design and intraosseous stability of immediately placed implants: a human cadaver study. Clin Oral Implants Res. 2005 Apr;16(2):202-09.

32 de Oliveira GJPL, Barros-Filho LAB, Barros LAB, Queiroz TP, Marcantonio E Jr. In vitro evaluation of the primary stability of short and conventional implants. J Oral Impl. 2016 Dec;42(6):458-63.

33 Sakoh J, Wahlmann U, Stender E, Al-Nawas B, Wagner W. Primary stability of a conical implant and a hybrid, cylindric screw-type implant in vitro. Int J Oral Maxillofac Impl. 2006 Jul;21(4)560-66

34 Betoni Jr W, Queiroz, TP, Luvizuto, ER, Valentini-Neto R, Garcia-Júnior IR, Bernabé PFE. Evaluation of Centrifuged Bone Marrow on Bone Regeneration Around Implants in Rabbit Tibia. Implant Dentistry, 2012; 21 (6): 481-485.

35 Akimoto K, Becker W, Persson R, Baker DA, Rohrer MD, O'Neal MB. Evaluation of titanium implants placed into simulated extraction sockets: A study in dogs. Int J Oral Maxillofac Implants. 1999; 14: 351–60.

36 Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. J Biomed Mater Res. 1991; 25(7): 889-902.

37 Ferguson SJ, Broggini N, Wieland M, de Wild M, Rupp F, Geis-Gerstorfer J. Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface. J Biomed Mater Res. 2006; 78A(2): 291–7.

38 Faeda RS, Tavares HS, Sartori R, Guastaldi AC, Marcantonio E. Biological performance of chemical hydroxyapatite coating associated with implant surface

modification by laser beam: biomechanical study in rabbit tibias. J Oral Maxillofac Surg. 2009; 67(8): 1706–15.

39 Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Impl Res. 2009; 20: 172– 84.

3.3 Publicação 3

Original Reseach: Osseointegration of two implant surfaces in areas grafted with deproteinized bovine bone associated or not with fresh bone marrow - Preclinical study in rabbits

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Conflicts of Interest:

Professor Doctor Élcio Marcantonio Junior is a consultant for the company Neodent. The other authors have no conflict of interest in this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Abstract

Objectives: To assess the influence of two implant surfaces on osseointegration in maxillary sinuses of rabbits previously grafted with deproteinized bovine bone (DBB) associated or not with fresh bone marrow (BM).

Material and methods: 16 New Zealand albino rabbits (males, 3.5/4.5 kg and 9-12 months old) were randomly divided into two groups with 8 rabbits each, according to the type of association of biomaterials used to fill the animals' maxillary sinuses: DBB (Deproteinized Bovine Bone) and DBB/BM (Deproteinized bovine bone associated with fresh autologous bone marrow). Ninety (90) days following the grafting procedure, the animals received implants in the area with two different microstructures (NP - Sandblasting + acid attack and AQ - Sandblasting + acid attack + immersion in 0.9% sodium chloride isotonic solution). All rabbits were euthanized 90 days after implant placement. The microtomographic analysis was performed to verify the number of mineralized tissues around the implants throughout their length (%BV/TV), while the histomorphometric analysis was performed to verify the percentage of bone-implant contact around the implants throughout their length (%BIC).

Results: We observed no differences in the quantity for %BV/TV (DBB NP:33.25 ± 19.67; DBB-AQ:35.15 ± 22.17; DBB/BM-NP:39.71 ± 24.21; DBB/BM-AQ:36.40 ± 23.07) and %BIC (DBB-NP:58.94 ± 24.37; DBB-AQ:52.52 ± 24.36; DBB/BM-NP: 61.66 ± 14.60; DBB/BM-AQ: 64.06 ± 23.30) between the groups assessed.

Conclusions: The addition of BM and the type of surface did not influence the osseointegration of implants installed in areas grafted with sintered deproteinized bovine bone at high temperatures in the late period assessed.

Keywords: Animal Experiments, Biomaterials, Bone substitutes, Growth factors, Sinus floor elevation

Introduction

The installation of dental implants is dependent on the bone availability of the host, and it is not always possible to install the implants in a native bone area. This type of situation is especially frequent in the posterior region of the maxilla due to the presence of the maxillary sinus, which, due to a positive pressure after tooth loss, can promote the internal resorption of the alveolar bone and expansion of the sinus in the lower direction, thus reducing bone availability in that region (Smiler, et al., 1992; Chanavaz, 1990; Hürzeler, Kirsch, Ackermann, Quinones, 1996; Xu, Shimizu, Asai, Ooya, 2004; Trindade-Suedam, et al., 2010; Caneva, et al. 2016).

Surgical techniques for elevating the maxillary sinus floor associated with the use of bone substitute biomaterials have been applied with great success rates for increasing bone availability in this region (Klijn, Meijer, Bronkhorst, Jansen, 2010; Sohn, et al. 2010). In order to reduce the morbidity related to the use of autogenous grafts, the deproteinized bovine bone (DBB) has been one of the most suitable bone substitutes as a graft material to resolve this clinical condition, showing high success rates. However, in cases with high degrees of atrophy, the isolated use of this biomaterial produces a delay in bone healing which increases the waiting time for installation or application of an occlusal load of implants compared to implants installed in maxillary sinuses filled with autogenous bone (Klijn, et al.; Schmitt, Doering, Schmitt, 2013; Jensen, et al., 2013; Barbeck, et al., 2019).

As a way to accelerate the bone healing process in areas grafted with osteoconductive bone substitutes, the association of biomaterials with greater potential for bone tissue formation, such as growth factors, has been indicated. Among these alternatives, the use of fresh bone marrow (BM) stands out, which theoretically has osteogenic and osteoinductive properties due to its composition of osteochondroprogenitor stem cells and growth factors that would induce greater differentiation and osteoblastic activity (Lucarelli, Donati, Cenacchi, Fornasari, 2004; Pelegrine, Costa, Correa, Marques Jr, 2010; Pelegrine, Costa, Sendyk, Gromatzky, 2011; Pelegrine, Aloise, Zimmermann, Oliveira, Ferreira, 2014; Pasquali, et al., 2015; Aloise, et al., 2018; Carrera-Arrabal, et al., 2019).

Another approach in the quest to accelerate osseointegration in grafted areas is the evolution of changes in the surfaces of implants seeking to increase the wettability and its chemical composition, which would be able to accelerate and improve the quality of osseointegration, resulting in greater bone deposition and reduction of the repair period, mainly in regions of poor bone quality (Faeda, Tavares, Sartori, Guastaldi, Marcantonio, 2009; Wennerberg, Albrektsson, 2009; Pinotti, Oliveira, Aroni, Marcantonio, Marcantonio, 2018).

Therefore, this preclinical study aimed to assess two approaches to accelerate the osseointegration of implants in maxillary sinuses of rabbits grafted with deproteinized bovine bone: 1) To assess the effect of the association of deproteinized bovine bone (DBB) with fresh bone marrow (BM) infiltrate; 2) To assess the effect of a highly hydrophilic implant surface on the osseointegration of implants in these grafted areas.

Material and methods

The experimental protocol was approved by the Ethics Committee on Animal Use of the State University of São Paulo (Unesp), Faculty of Dentistry of Araraquara, Brazil (CEUA nº -15/2017) and carried out in accordance with the ARRIVE protocol for conducting preclinical studies. Sixteen (16) male rabbits (Albinos, New Zealand) were used (4-5 kg and 9-12 months of age). The animals were kept in an environment with a temperature of 22-24ºC, with a controlled light cycle (12 hours light and 12 hours dark) and consumption of solid food and water ad libitum throughout the experimental period.

Experimental outline

To assess the influence of different microstructures of titanium implants in areas previously grafted with xenogeneic grafts associated or not with autologous bone marrow, 16 rabbits were used in a total period of 180 days. Each animal was initially subjected to bilateral extraoral access to the maxillary sinus floor membrane to raise this membrane and fill it (according to the experimental group) with a xenogeneic graft of bovine origin sintered at high temperatures (Straumann® Cerabone®, Switzerland, Germany/Granules of 0.5-1.0mm) (DBB) associated or not with fresh autologous bone marrow (DBB/BM). After 90 days, these grafted areas were subjected to the installation of implants. The implants with different microstructures installed in the region of the previously grafted maxillary sinus were: NP (Sandblasting + acid attack) and AQ (Sandblasting + acid attack + immersion in 0.9% sodium chloride isotonic solution); the implants in this category were short and had 4mm in diameter x 5mm in height (TitamaxWS NeoPoros® implant vs. TitamaxWS Acqua® implant, Neodent®, Curitiba, Brazil/

Figure 2A). All animals were euthanized 180 days after the first surgical procedure.

Surgical procedure

DAY 0 - Bilateral elevation of the maxillary sinus floor and graft: The animals were weighed and anesthetized intramuscularly, with a combination of ketamine (Quetamina Agener®; Agener União Ltda, São Paulo, SP, Brazil) - 0.35 mg/kg) and xylazine (Rompum, Bayer AS, São Paulo, SP, Brazil - 0.5 mg/kg). Subsequently, they underwent elevation of the maxillary sinus floor and grafting with extra-oral access. For that, the corresponding experimental area was submitted to trichotomy and antisepsis with 10% polyvinylpyrrolidone (PVPI) with 1% active iodine. A 5cm incision was made following the midline of the nasal dorsum, and after detachment on both sides of the nasoincisal suture, the nasal bone was exposed until the nasofrontal suture (Figure 1A). With the aid of a 5 mm diameter trephine drill, with the center located at a distance of about 5 mm lateral to the midline of the nasal dorsum and about 10 mm in front of the apex of the nasofrontal suture, a bone window in both the sides was obtained (Figure 1B). When the sinus mucosa was exposed, the sinus floor elevation instruments were used (Neodent®, Curitiba, Paraná, Brazil). The sinus mucosa was detached 3-4 mm in the medial, lower, upper and distal portions of the bone window obtained. The space created was covered with a collagen membrane (Jason® membrane, Straumann®, Curitiba, Brazil), and it was filled with biomaterials according to the different groups and covered with another collagen membrane (Figure 1C, 1D, 1E and 1F). The fresh bone marrow was obtained from the animals' tibia after cortical perforation with a 5mm diameter trephine drill, and the

collection was performed with the aid of a Lucas curette No. 86. All animals were submitted to the marrow collection regardless of the experimental group to which they belonged. The soft tissues were repositioned and sutured per plane by using a continuous suture scalloped with resorbable thread (Vicryl® ETHICON, 4-0, Johnson & Johnson, Brazil) and non-resorbable thread (Nylon 3-0, Shalon®, Brazil). After surgery, all animals received a single dose of antibiotic (Pentabiótico®, Wyeth-Whitehall Ltda, São Paulo, Brazil - 0.1 ml/kgl) and analgesic (Tramadol Hydrochloride 50mg/ml, Tramadol®, Medley, São Paulo, Brazil - 5 mg/Kg IM).

DAY 90 - Installation of implants in the area of elevation of the maxillary sinus floor with previous grafting: The animals were submitted to the implant installation procedures. The pre-surgical procedures were the same as the previous procedure. After exposure of the grafted area, the installation of short implants (Neodent®, Curitiba, Brazil - Kit WS) was prepared, so that the bone was milled with metal drills under abundant refrigeration with sterile saline. The drilling for installation of the implants was carried out with the standardized sequence of drill bits for implants of 4mm x 5mm (Milling sequence: Spear drill, 2.0 helical drill, 2/3 pilot drill, 3.0 helical drill, 3.3 helical drill, 3.3/4 pilot drill) (Figure 2A). The implants were inserted to the bone level and the cover screws were threaded (Figure 2B, 2C and 2D). Each animal randomly received an implant type in each maxillary sinus (NP - Sandblasting + acid attack and AQ - Sandblasting + acid attack + immersion in 0.9% sodium chloride isotonic solution).

DAY 180 - Euthanasia: The animals were euthanized through anesthetic overdose 90 days after implant placement.

Microtomographic Analysis (µCT)

After euthanasia, the biopsies were removed and fixed in 10% formaldehyde for 48 hours and kept in 70º alcohol until the time of the µCT scan (Skyscan, Aatselaar, Belgium). The following parameters were used to scan the samples: 18 μm^3 voxel, 10x image magnification, X-ray tube voltage of 50 kV, 496 uA beam and the electrical current adjusted to 0.1 mA. The three-dimensional images were reconstructed (NRecon 1.6.1.5 - SkyScan N. V. Belgium) with the following parameters: Beam Hardening Correction 40%, Ring Artifact Correction = 9, Smoothing = 5, Postalignment = 1.00. Subsequently, the scanned images were reoriented in order to perform the volumetric analysis (Data Viewer SkyScan N. V. Belgium). The measurements for the Volumetric analysis (3D) were performed by a single examiner, blind for the type of treatment performed using specific software (CT Analyzer 1.10.1.0 - SkyScan, Belgium), following the selection of a circular area of interest around the implants that exceeded its diameter by 5mm (ROI - region of interest). Although the implants received a cover screw, there was bone formation inside the cover screw in some cases. In order to prevent this bone formation from interfering with the analysis of the volume of mineralized tissue around the implant, a second ROI was established to remove the volume of mineralized tissues that could have been formed in this region. With the results obtained in the two ROI's, it was possible to define the volume of mineralized tissues using the following formula: Total volume of mineralized tissues in the main ROI – Volume of mineralized tissues within the cover screw = Volume of mineralized tissues.

The grayscale threshold used was 25-90, and the values of the volume of mineralized tissue around the implant were obtained as a percentage. The entire

analysis was performed by a single trained examiner, blind for the type of training performed (Pinotti, et al., 2018).

Histometric Analysis (%BIC)

After microtomographic analysis, the parts with the implants were dehydrated in an alcohol solution in a series of increasing concentrations. The plastic infiltration was carried out with mixtures of glycol methacrylate (Technovit 7200 VLC) and ethyl alcohol, following gradual increases in the concentration of glycol methacrylate until the infiltration in this pure solution. The specimens were embedded in resin, polymerized and sectioned longitudinally along the main axis of the implant with a high precision diamond disk. The blocks were assembled on an acrylic slide with the help of the Tecnovit 4000 resin (Kulzer, Wehrheim, Germany), and using a cutting and micro-wear system (Exact-Cutting, System, Apparatebau Gmbh, Hamburg, Germany). The slides were processed so they had a 50-70 μm thick section (approximately). The parts were stained with Stevenel's Blue and fuchsin and were analyzed by histometric analysis that assessed the amount of direct contact between the bone and the surface throughout the implant (%BIC). Measurements were made using photomicrographs obtained by an optical microscope (DIASTAR, Leica Reichert & Jung products, Germany), with 10x magnification, with the aid of a video camera (Leica Reichert & Jung products, Germany). The values were determined using image analysis software (Image J, Jandel Scientific, San Rafael, CA, USA), by a blind examiner, calibrated and trained for this analysis.

a) $\%BIC = \frac{\text{Direct contact of the bone with the implant surface} \times 100}{\text{Total Implant Length}}$

Statistical analysis

The data obtained were submitted to the Shapiro-Wilk normality test followed by parametric paired t-tests for inferential analysis of the data comparing the different groups of microstructures (Neoporous vs. Acqua) and the unpaired t-test to compare the results of the grafted areas with deproteinized bovine bone associated or not with fresh autologous bone marrow. The GraphPad Prism 6 software (San Diego, CA, USA) was employed in the statistical tests that were applied at the significance level of 5%.

Sample calculation was performed using the paired t-test based on the histometric data of bone-implant contact from the study by Faeda et al. 2012, which assessed the effect of different implant surfaces on osseointegration in rabbits. It was found that the difference between the BIC averages among different implant surfaces in order to promote a statistically significant difference was 25.95% (SD=8.34). Therefore, the use of 8 rabbits per group in each period would be sufficient to obtain a power β and α of the study greater than 0.9 and equal to 0.05, respectively.

Results

All animals tolerated well the surgical procedure and remained healthy throughout the experimental period.

µCT

There were no statistically significant differences between groups in relation to %BV/TV, although the groups where BM was associated with DBB presented numerically higher values for %BV/TV. Table 1 and Charts 1 show the data from

the microtomographic analysis of all groups in this study. Figure 3 shows images representative of the microtomographic analysis of all groups.

Hystometry

Although the grafted areas with DBB and BM showed numerically greater results for %BIC, no statistically significant differences were detected between the groups assessed. Table 2 and Charts 2shows data on the mean and standard deviation of %BIC for all groups in this study. Figure 4 shows images representative of the histometry of all groups.

Discussion

Implantology has made implant-supported rehabilitation possible with high success rates. Nowadays, it is the treatment that comes closest to natural dentition. Traditional oral rehabilitation, represented by fixed or removable prostheses, supported on teeth and/or mucous membranes, is no longer the treatment of choice for patients who are totally or partially edentulous (Papaspyridakos, et al., 2013; Astrand, Ahlqvist, Gunne, Nilson, 2008).

However, especially in the posterior region of the maxilla, the low bone density associated with pneumatization of the maxillary sinus often requires bone reconstruction procedures prior to implant placement. The presence of pneumatized sinuses is an anatomical condition that often precludes the immediate installation of the implant. The technique proposed by Taum and Boyne for raising the floor of the maxillary sinus proved to be effective in overcoming this difficulty. Sub-sinus bone regeneration has been successful with several biomaterials (Boyne, James, 1980; Taum, 1986; Caneva, et al., 2016).

In the era of Implantology, Dentistry has intensified research aimed at bone regeneration associated with the use of bone substitutes, a fact that is frequent and fully justified. Among the available materials, none has the characteristics of the ideal material. Recently developed biomaterials must be thoroughly researched and in accordance with the requirements of biocompatibility, stability, safety, and predictability (Klijn, et al., 2010; Schmitt, et al., 2013).

As previously described, this study in rabbits investigated the osseointegration of two different implant microstructures (NP vs. AQ) installed in the maxillary sinus region previously grafted with a xenogeneic graft of bovine

origin (Straumann® Cerabone®, Switzerland, Germany) associated or not with fresh bone marrow.

The experimental model used for this purpose seemed appropriate since rabbits have a well-defined ostium and maxillary sinus ventilation system that opens up to their nasal cavities in a manner very similar to that of humans. The model for assessing sub-sinus regeneration in rabbits is therefore adequate and well-documented to assess the physiology and the effect of space fillers on bone regeneration and osseointegration (Scharf, Lawson, Shapiro, Gannon, 1995; Asai, Shimizu, Ooya, 2002; Ohya, Yamada, Ozawa, Ito, Takahashi, Ueda, 2005; Sun, et al., 2008; Lambert, et al., 2011). In addition, although some studies use intraoral access (Watanabe, Niimi, Ueda, 1999; Trindade-Suedam, et al., 2010) to the maxillary sinus of rabbits, extra-oral access (Xu, et al., 2004; Caneva, et al., 2016) facilitates the procedure, especially with regard to the visibility and handling of the sinus membrane and, therefore, it was the technique used in the present study.

Xenografts from bovine donor tissue or bovine hydroxyapatite are widely used and researched as bone substitutes due to their physicochemical properties similar to those of human bone, their osteoconductive potential, and availability (Jensen, et al., 2013; Pasquali, et al., 2015). During the manufacture of the xenogeneic bone replacement materials available, different purification methods are applied. Barbeck, et al. (2019), in a mini-review, showed that the sintering temperature of bone substitutes, including materials based on bovine hydroxyapatite (BHA), is an important parameter that can affect their properties. In this context, the sintering temperature influences the phase stability,

densification behavior, crystallinity and porosity of BHA. Heat treatment at different temperatures influences the tissue response to the bone matrix.

Deproteinized bovine bone sintered at low temperatures (Bio Oss®, Geistlich Pharma, Wolhusen, Switzerland), for example, initially undergoes a heat treatment (with temperatures up to 300 °C) and cleaning with sodium hydroxide (NaOH - strong alkali) (Barbeck, Unger, Schnettler, Wenisch, Witte, 2017). Milder temperatures (300 °C) lead to the preservation of the mineral crystals of the bone matrix of this biomaterial (Bufler, 2004). For the synthesis of deproteinized bovine bone sintered at high temperatures (Straumann® Cerabone®, Switzerland, Germany), initial oxidative combustion is applied at temperatures around 800 °C followed by a second heat treatment with a temperature around 1,250 °C (sintering). This process results in an increased size of the crystal by 500-1000%, and of the crystal density, a fact that makes it comparable to a ceramic-based material (Barbeck, et al., 2015). In the present study, deproteinized bovine bone sintered at high temperatures was used.

"Natural" (non-synthetic) bone replacement materials, such as bovine and synthetic xenografts, can induce an immune response in the implantation bed of the receptor, called "foreign body reaction to biomaterials". In this cascade of tissue reaction, macrophages and multinucleated giant cells (MNGC) express pro- and anti-inflammatory molecules that guide the cascade and, therefore, the bone healing process (Anderson, Rodriguez, Chang, 2008). In this context, it was demonstrated that the severity and inflammatory alignment of the tissue reaction cascade associated with the material are mainly influenced by different physical and chemical properties of bone substitute materials, such as chemical composition, granule size, granule porosity, etc (Lorenz, et al., 2016). These

physicochemical properties of a bone substitute are extremely important to the clinic since these factors influence the bone regeneration process (Bone regeneration vs. Foreign body reaction) (Anderson, et al., 2008; Cheyn, et al., 2016). Histological images from this study, obtained from non-descaled sections, show the interconnection of DBB particles and bone neoformation.

The quest to accelerate the osseointegration process in areas grafted with osteoconductive bone substitutes aims to reduce the use of autogenous bone grafts, which, despite its limitations, is still considered the gold standard in bone grafting techniques (Klijn, et al., 2010). Because of this, in this study, it was proposed to use BM and highly hydrophilic modified implant surfaces and to assess its effect on osseointegration in maxillary sinuses of rabbits grafted with deproteinized bovine bone. As a general finding, the areas grafted with BM showed a better osseointegration pattern, while the different implant surfaces seemed to behave in a similar way; however, they were not considered statistically significant in any of the analyses employed.

The use of fresh bone marrow promoted a subtle increase in the number of mineralized tissues and the bone-implant contact on both implant surfaces assessed. This fact may be associated with the content of this tissue, which has stem cells that can differentiate into osteoblasts (Lucarelli, et al., 2004; Pelegrine, et al., 2010; Pelegrine, et al., 2011; Pelegrine, et al., 2014; Pasquali, et al., 2015; Aloise, et al., 2018; Carrera-Arrabal, et al., 2019), and the association of osteoprogenitor stem cells with osteoconductive biomaterials has been shown to improve the pattern of bone healing (Pelegrine, et al., 2011; Pasquali, et al., 2015; Carrera-Arrabal, et al., 2019). Carrera-Arrabal, et al. (2019), verified through a histomorphometric analysis that the stem cells derived from the marrow

associated with a block of DBB, in vertical bone reconstruction in rabbit calvaria, increased bone formation (11.9 ± 7.5% vs. 7.6 ± 5.6%) when compared to the association of the same block with adipose tissue-derived stem cells 8 weeks following the surgical procedure. Pelegrine, et al. (2012), demonstrated that the use of fresh bone marrow associated with DBB in critical defects in rabbit calvaria showed a greater formation of bone tissue than the use of DBB alone after 8 weeks of the grafting procedure (21.14 ± 7.38% vs. 13.06 ± 5.24%).

In our study, the use of BM did not produce statistically significant differences as in the aforementioned studies, and this may have occurred due to the long experimental period in our study (180 days) compared to the previous studies (60 days). In addition, there is no consensus on the best methodology for the use of bone marrow stromal cells. Three main alternatives were proposed: (1) Use of fresh autologous bone marrow graft ("in natura"); (2) Use of autologous bone marrow cell concentrate (by centrifugation); and, (3) Use of autologous bone marrow stromal cell culture (Pelegrine, et al., 2014; Wildburger, et al., 2014; Oliveira, et al., 2016). In fact, in the study by Pelegrine et al. (2012), the use of bone marrow enriched with mononuclear fraction obtained by centrifugation showed better results of bone formation associated with DBB (28.17 ± 3.19%) than the use of fresh bone marrow associated with the same bone substitute (21.14 ± 7.38%). Thus, other forms of application of BM should be tested with regard to the possibility of positively influencing the osseointegration process in areas grafted with DBB.

Regarding the type of surface, no statistically significant differences were detected. Both tested surfaces have shown good results in clinical studies (Chiapasco, Gatti, Rossi, Haeflige, Markwaldel, 1997; Crespi, Capparé,

Gherlone, 2009; Hinkle, Rimer, Morgan, Zeman, 2014), however, preclinical studies demonstrate that the highly hydrophilic surface presents an acceleration in the osseointegration process (Ferguson et al., 2006; Wildburger, et al., 2014; Pinotti, et al., 2018). Buser et al. (2004), found that implants with a surface modified by sandblasting oxide particles and acid attack associated with the storage in isotonic NaCl solution showed a higher percentage of bone-implant contact when compared to implants with a surface modified by sandblasting oxide particles and acid attack in the periods of 2 and 4 weeks (49.30 vs 29.42%; p=0.017 and 81.91 vs. 66.57%; p=0.011, respectively), without any statistical difference in the period of 8 weeks. These data can justify the data obtained in the present study which show that no statistical differences were observed for bone-implant contact in the late assessment period (12 weeks after the implant installation).

The deproteinized bovine bone tested in the present study seems to have a good influence on the osseointegration process. However, the real effects of bone marrow on bone regeneration, both in its isolated form and in association with other bone substitutes, should be further investigated. Studies with shorter observation periods, both to assess the different microstructures (hydrophobic and hydrophilic) and the association (or not) of the bovine xenogeneic graft with the bone marrow should also be performed.

Conclusion

The addition of BM and the surface type did not influence the osseointegration of implants installed in areas grafted with deproteinized bovine bone sintered at high temperatures when assessed in the late period.

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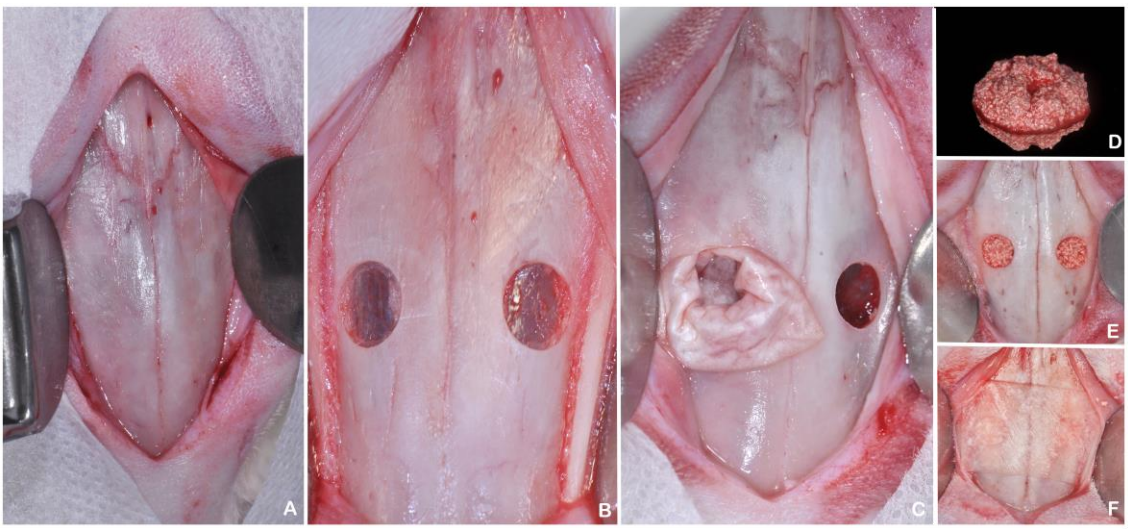
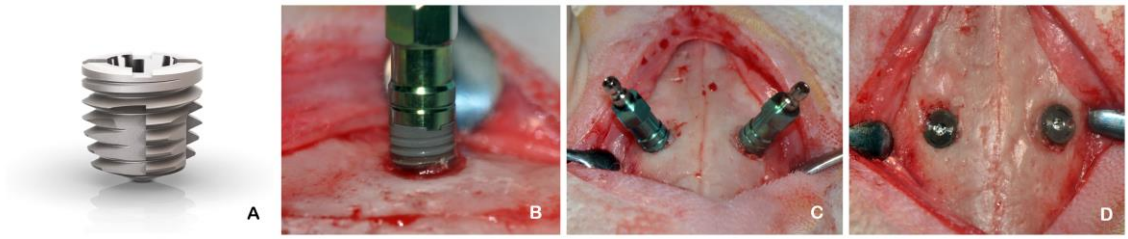
Figure Legends

Figure 1 - Day 0: Surgical procedure for the bilateral elevation of the maxillary sinus floor and graft



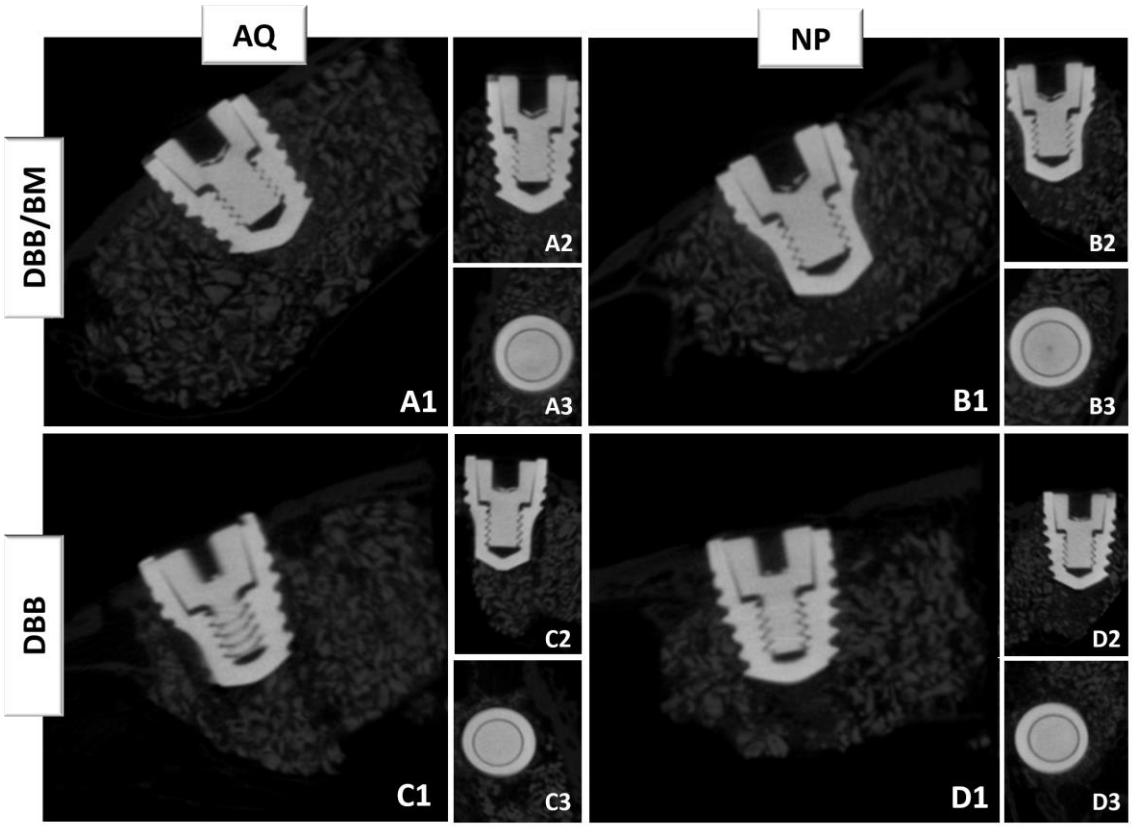
*Stages of the surgical procedure for extra-oral access and elevation of the maxillary sinus floor: Detachment on both sides of the nasoincisal suture and exposure of the nasal bone to the nasofrontal suture (A); Obtaining a bilateral bone window with the aid of a 5mm diameter trephine drill (B); Detachment of the sinus membrane and filling of the bone cavity with a collagen membrane (Jason® membrane, Straumann®, Curitiba, Brazil) (C) and the graft materials according to the different groups (D and E); A collagen membrane (Jason® membrane, Straumann®, Curitiba, Brazil) was also placed over the bone window (F).
Xu et al. (2004) modified.

Figure 2 - Day 90: Installation of implants in the elevation area of the maxillary sinus floor with prior grafting



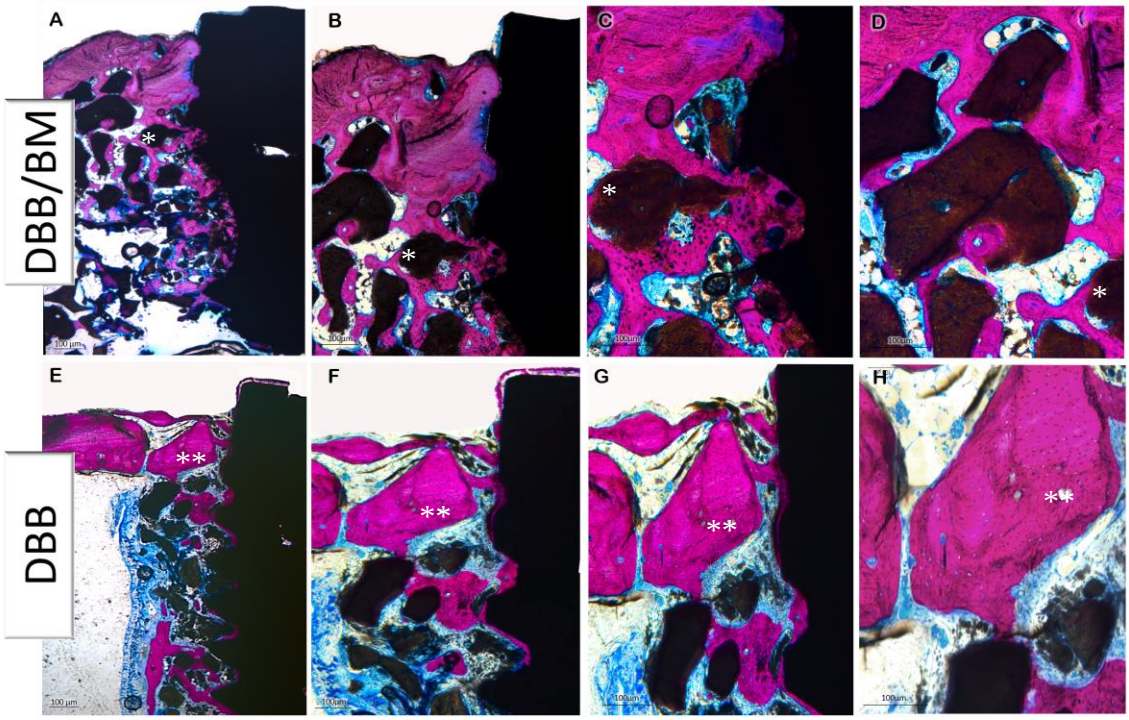
*Stages of the surgical procedure for installing the implants: Titamax WS® implant was used in this study (A); Bilateral installation of the implant and cover screws in the previously grafted maxillary sinuses (B, C, and D)

Figure 3 - Microtomographic slices of the maxillary sinuses after grafting and installation of the implants according to the different groups in the period of 180 days



*****Sagittal slices (A1, B1, C1, and D1); coronal slices (A2, B2, C2, and D2); and axial slices (A3, B3, C3, and D3) representative of the different experimental groups (A: DBB/BM – AQ; B: DBB/BM – NP; C: DBB – AQ; D: DBB – NP).

Figure 4 – Histological sections of the maxillary sinuses after grafting and installation of the implants according to the different groups in the period of 180 days



*Histological images of the non-decalcified sections stained with Stevenel blue plus acid fuchsin, original 2.5x (A and E), 5x (B and F), 10x (C and G), and 20x magnification (D and H). The images (A, B, C, and D) and (E, F, G, and H) represent the DBB/BM group and the DBB group, respectively

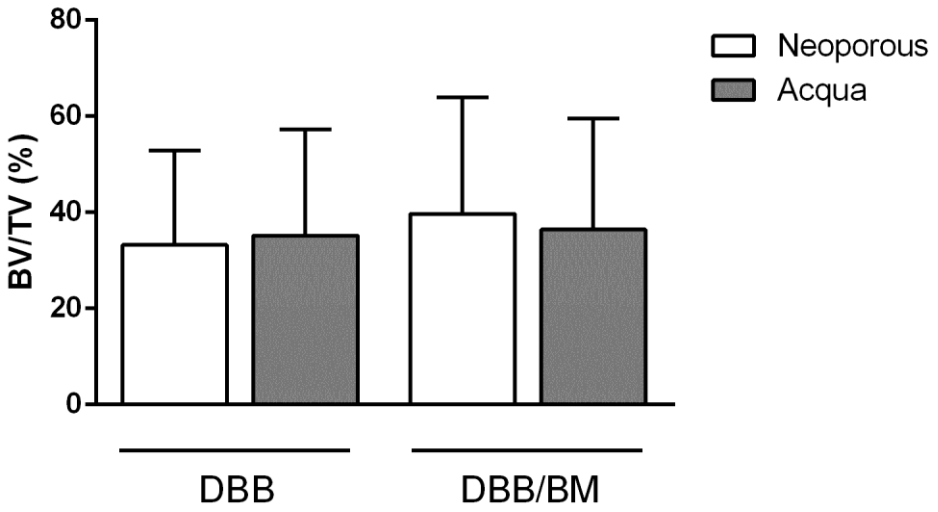


Chart 1: Mean and standard deviation for BV/TV data obtained through microtomographic analysis of all groups.

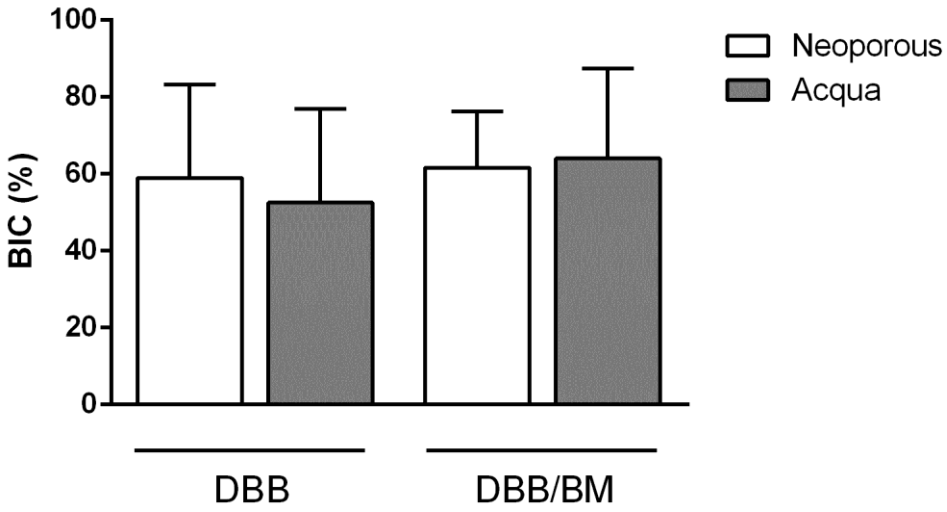


Chart 2: Mean and standard deviation for %BIC data obtained through histometric analysis of all groups

Table 1: Data on the mean and standard deviation for BV/TV data obtained through microtomographic analysis of all groups.

Grupo	BV/TV
DBB – NP	33.25 ± 19.67
DBB -AQ	35.15 ± 22.17
DBB/BM - NP	39.71 ± 24.21
DBB/BM - AQ	36.40 ± 23.07

Table 2: Data on the mean and standard deviation for %BIC data obtained through histometric analysis of all groups.

Grupo	BIC (%)
DBB – NP	58.94 ± 24.37
DBB -AQ	52.52 ± 24.36
DBB/BM - NP	61.66 ± 14.60
DBB/BM - AQ	64.06 ± 23.30

REFERENCES

Aloise, A. C. , Pasquali, P. , Sperandio, M. , Macedo, L. G. S. , Teixeira, M. L. , Pelegrine, A. A. , & Calvo-Guirado, J. L. (2018). Use of Bone Marrow Aspirate Concentrate (BMAC) Associated with Hyperbaric Oxygenation Therapy in Maxillary Appositional Bone Reconstruction. A Randomized Clinical Trial. Symmetry, 10, 533.

Anderson, J. M. , Rodriguez, A. , & Chang, D. T. (2008). Foreign body reaction to biomaterials. Semin Immunol, 20, 86-100.

Asai, S. , Shimizu, Y. & Ooya, K. (2002). Maxillary sinus augmentation model in rabbits: effect of occluded nasal ostium on new bone formation. Clinical Oral Implants Research, 13, 405-09.

Astrand, P. , Ahlqvist, J. , Gunne, J. , & Nilson, H. (2008). Implant treatment of patients with edentulous jaws: a 20-year follow-up. Clin Implant Dent Relat Res., 10, 207-17.

Barbeck, M. , Dard, M. , Kokkinopoulou, M. , Markl, J. , Booms, P. , & Sader, R. A. (2015). Small-sized granules of biphasic bone substitutes support fast implant bed vascularization. Biomatter, 5, e1056943.

Barbeck, M. , Unger, R. , Schnettler, R. , Wenisch, S. , & Witte, F. (2017). Xenogeneic bone grafting materials. Implants. International Magazine of Oral implantology,17, 34-6.

Barbeck, Ž. , Kačarević, P. , Kavehei, F. , Rider, P. , Najman, S. , Stojanović, S. , & Rimashevskiy, D. (2019). The effect of temperature treatment of xenogeneic bone substitute on the tissue response – a mini review Acta Medica Medianae, 58, 131-37.

Boyne, P. J. , & James, R. A. (1980). Grafting of the maxillary sinus floor with autogenous marrow and bone. J. Oral Maxillofac. Surg., 38, 613-16.

Bufler, M. A. (2004). Calciumphosphate Synthese, Reaktionen in Wässrigen Median und Charakterisierung von Oberflachen und Grenzflachen [dissertation]. Siegen: der Universitat Siegen; 2004.

Buser, D. , Broggini, N. , Wieland, M. , Schenk, R. K. , Denzer, A. J. , Cochran D. L. , Hoffmann, B. , Lussi, A. , & Steinemann, S. G. (2004). Enhanced Bone Apposition to a Chemically Modified SLA Titanium Surface. J Dent Res., 83, 529-33.

Caneva, M. , Lang, N. P. , Garcia Rangel, I. J. , Ferreira, S. , Caneva M. , De Santis, E. , & Botticelli, D. (2016). Sinus mucosa elevation using BioOss or Gingistat collagen sponge: an experimental study in rabbits. Clin. Oral Impl. Res., 00, 1–10.

Carrera-Arrabal, T. , Calvo-Guirado, J. L. , Santos, F. P. , Costa, C. E. S. , Costa, F. T. , & Aloise, A. C. (2019). Vertical Bone Construction with Bone Marrow-Derived and Adipose Tissue-Derived Stem Cells. Symmetry, 59, 1-11.

Chanavaz, M. (1990). Maxillary sinus: anatomy, physiology, surgery, and bone grafting related to implantology – eleven years of surgical experience (1979–1990). Journal of Oral Implantology, 16, 199-209.

Cheyn, Z. , Klein, T. , Murray R. Z. , Crawford, R. , Chang, J. , & Wu, C. (2016). Osteoimmunomodulation for the development of advanced bone biomaterials. Mater Today, 19, 304-21.

Chiapasco, M. C, Gatti, E. , Rossi, W. , Haeflige, T. , & Markwaldel, H. (1997). Implant-retained mandibular overdentures with immediate loading: a retrospective multicenter study on 226 consecutive cases. Clin Oral Impl Res., 8, 48-57.

Crespi, R. , Capparé, P. , & Gherlone, E. (2009). Dental implants placed in extraction sites grafted with different bone substitutes: radiographic evaluation at 24 months. J of Periodont., 80, 1616-21.

Faeda, R. S. , Spin-Neto, R. , Marcantonio, E. , Guastaldi, A. C. , & Marcantonio JR, E. (2012). Laser Ablation in Titanium Implants Followed by Biomimeti Hydroxyapatite Coating: Histomorphometric Study in Rabbits. Microscopy Research And Technique, 75, 940-48.

Faeda, R. S. , Tavares, H. S. , Sartori, R. , Guastaldi, A. C. , & Marcantonio, E. (2009). Biological performance of chemical hydroxyapatite coating associated with implant surface modification by laser beam: biomechanical study in rabbit tibias. J Oral Maxillofac Surg, 67, 1706-15.

Ferguson, S. J. , Broggini, N. , Wieland, M. , de Wild, M. , Rupp, F. , & Geis-Gerstorfer, J. (2006) Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface. J Biomed Mater Res., 78, 291-97.

Hinkle, R. M. , Rimer, S. R. , Morgan, M. H. , & Zeman, P. (2014). Loading of Titanium Implants With Hydrophilic Endosteal Surface 3 Weeks After Insertion: Clinical and Radiological Outcome of a 12-Month Prospective. J of Oral and Maxillof Surg., 72, 1495-502.

Hürzeler, M. B. , Kirsch, A. , Ackermann, K. L. , & Quinones, C. R. (1996). Reconstruction of the severely resorbed maxilla with dental implants in the augmented

maxillary sinus: a 5-year clinical investigation. International Journal of Oral & Maxillofacial Implants, 11, 466-75.

Jensen, T. , Schou, S., Gundersen, H. J. G. , Forman, J. L., Terheyden, H. , & Holmstrup, P. (2013) Bone-to-implant contact after maxillary sinus floor augmentation with Bio-Oss and autogenous bone in different ratios in mini pigs. Clin Oral Impl Res., 24, 635-44.

Klijn, R. J., Meijer, G. J., Bronkhorst, E. M. & Jansen, J.A. (2010). Sinus floor augmentation surgery using autologous bone grafts from various donor sites: a meta-analysis of the total bone volume. Tissue Engineering Part B: Reviews, 16, 295-303.

Lambert, F. , Leonard, A. , Drion, P. , Sourice, S. , Layrolle, P. , & Rompen, E. (2011). Influence of space-filling materials in subantral bone augmentation: blood clot vs. autogenous bone chips vs. bovine hydroxyapatite. Clin. Oral Impl. Res., 22, 538-45.

Lorenz, J. , Barbeck, M. , Sader, R. A. , Kirkpatrick, C. J. , Russe, P. , & Choukroun J. (2016). Foreign body giant cell-related encapsulation of a synthetic material three years after augmentation. J Oral Implantol, 42, 273-77.

Lucarelli, E. , Donati, D. , Cenacchi, A. , & Fornasari, P. M. (2004). Bone reconstruction of large defects using bone marrow derived autologous stem cells. Transfusion and apheresis Science, 30, 169-74.

Ohya, M. , Yamada, Y. , Ozawa, R. , Ito, K. , Takahashi, M. & Ueda, M. (2005). Sinus floor elevation applied tissue-engineered bone. Comparative study between mesenchymal stem cells/platelet-rich plasma (prp) and autogenous bone with prp complexes in rabbits. Clinical Oral Implants Research, 16, 622-29.

Oliveira, T. A. , Aloise, A. C. , Orosz, J. E. , Mello, E. , Oliveira, R., Carvalho, P. , & Pelegrine, A. A. (2016) Double Centrifugation Versus Single Centrifugation of Bone Marrow Aspirate Concentrate in Sinus Floor Elevation: A Pilot Study. The Int J Oral & Maxillof Impl., 31, 216-22.

Papaspyridakos, P. , Mokti, M. , Chen, C. J. , Benic, G. I. , Gallucci, G. O. , & Chronopoulos, V. (2013) Implant and prosthodontic survival rates with implant fixed complete dental prostheses in the edentulous mandible after at least 5 Years: a systematic review. Clin Implant Dent Relat Res., 16, 705–17.

Pasquali, P. J. , Teixeira, M. L. , Oliveira, T. A. , Macedo, L. G. S. , Aloise, A. C. , & Pelegrine, A. A. (2015). Maxillary Sinus Augmentation Combining Bio-Oss with the Bone Marrow Aspirate Concentrate: A Histomorphometric Study in Humans. Int J of Biomat, 15, 1-7.

Pelegrine, A. A. , Aloise, A. C. , Zimmermann, A. , Oliveira, R. M. , & Ferreira, L. M. (2014). Repair of critical-size bone defects using bone marrow stromal cells: a histomorphometric study in rabbit calvaria. Part I: Use of fresh bone marrow or bone marrow mononuclear fraction. Clin. Oral Impl. Res., 25, 567-72.

Pelegrine, A. A. , Costa, C. E. S. , Correa, M. E. P. , & Marques Jr, J. F. C. (2010) Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft. Clin. Oral Impl. Res., 21, 535-42.

Pelegrine, A. A. , Costa, C. E. S. , Sendyk, W. R. , & Gromatzky, A. (2011). The comparative analysis of homologous fresh frozen bone and autogenous bone graft, associated or not with autogenous bone marrow, in rabbit calvaria: a clinical and histomorphometric study. Cell Tissue Bank, 12, 171-84.

Pinotti, F. E. , Oliveira, G. J. P. L. , Aroni, M. A. T. , Marcantonio, R. A. C. , & Marcantonio Jr, E. (2018). Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: a preclinical study. Clin Oral Impl Res., 29, 963-72.

Scharf, K. E. , Lawson, W. , Shapiro, J. M. & Gannon, P. J. (1995) Pressure measurements in the normal and occluded rabbit maxillary sinus. Laryngoscope, 105, 570-74.

Schmitt C. M. , Doering, H. , & Schmitt T. (2013) Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone. A randomized controlled clinical trial. Clinical Oral Implants Research, 24, 576-85.

Smiler, D. G. , Johnson, P. W. , Lozada, J. L. , Misch, C. , Rosenlicht, J. L. , Tatum Jr, O. H. & Wagner, J. R. (1992). Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla. Dental Clinics of North America, 36, 151-88.

Sohn, D. S., Kim, W. S., An, K. M., Song, K. J., Lee, J. M. & Mun, Y. S. (2010). Comparative histomorphometric analysis of maxillary sinus augmentation with and without bone grafting in rabbit. Implant Dentistry, 19, 259-70.

Sun, X. J. , Zhang, Z. Y. , Wang, S. Y. , Gittens, S. A. , Jiang, X. Q. & Chou, L. L. (2008). Maxillary sinus floor elevation using a tissue-engineered bone complex with osteobone and bmscs in rabbits. Clinical Oral Implants Research, 19, 804-13.

Tatum Jr. , H. , (1986). Maxillary and sinus implant reconstructions. Dent Clin N Am, 30, 207-29.

Trindade-Suedam, I. K. , de Morais, J. A. N. D. , Faeda, R. S. , Leite, F. R. M. , Tosoni, G. M. , Benatti Neto, C. , Marcantonio Jr, E. , & Scaf, G. (2010). Bioglass Associated With Leukocyte-Poor Platelet-Rich Plasma in the Rabbit Maxillary Sinus: Histomorphometric, Densitometric, and Fractal Analysis. J Oral Implantol, 36, 333-43.

Watanabe, K. , Niimi, A. & Ueda, M. (1999). Autogenous bone grafts in the rabbit maxillary sinus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 88, 26-32.

Wennerberg, A. , & Albrektsson T. (2009) Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Impl Res., 20, 172-184.

Wildburger, A. , Payer, M. , Jakse, N. , Strunk, D. , Etchard-Liechtenstein, N. , & Sauerbier, S. (2014). First published Impact of autogenous concentrated bone marrow aspirate on bone regeneration after sinus floor augmentation with a bovine bone substitute – a split-mouth pilot study. Clin Oral Impl Res., 25, 1175-81.

Xu, H. , Shimizu, Y. , Asai, S. , & Ooya, K. (2004). Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation. Clinical Oral Implants Research, 15, 126-33.

4 DISCUSSÃO

Esse projeto teve como objetivo avaliar sob diversas condições pré-clínicas, em coelhos, a osseointegração de implantes com diferentes macro (Grupo Controle: CI - Implante Cilíndrico e Grupo Experimental: HCI - Implante Cônico Híbrido) e microestruturas (Grupo Controle: NP - Jateamento + ataque ácido e Grupo Experimental: AQ - Jateamento + ataque ácido + imersão em solução isotônica de cloreto de sódio 0,9 %) em áreas de osso nativo (Metáfise tibial) ou enxertadas prévia (Metáfise tibial e seio maxilar) ou imediatamente (Metáfise tibial) com osso bovino desproteinizado associado ou não à medula óssea fresca (Grupo Controle:DBB e Grupo Experimental: DBB/BM).

Como achado geral, foi verificado que os implantes HCI apresentaram melhor estabilidade primária que consequentemente resultou no maior contato osso-implante ao final dos 8 semanas de avaliação, o que demonstra que esse tipo de macroestrutura clinicamente pode receber cargas oclusais de forma mais precoce, ser utilizado em osso de menor densidade e acelera o processo de osseointegração em relação aos implantes CI (**Publicação 1**). As áreas enxertadas com BM apresentaram melhor padrão de osseointegração e neoformação óssea entre as partículas de biomaterial, entretanto, não foram detectadas diferenças estatisticamente significativas nas análises empregadas independentemente da macro (**Publicação 2**) e microestrutura (**Publicação 3**) testadas. Quanto às diferentes macroestruturas avaliadas, nas áreas enxertadas com DBB, independente do momento de instalação dos implantes (Imediata vs. Tardia), o HCI apresentou maiores valores de porcentagem de tecidos mineralizados ao redor do implante comparado ao CI. Por outro lado, quanto a porcentagem de contato osso implante, somente nas áreas enxertadas com DBB, os CI apresentaram diferenças estatísticas quando

comparamos o momento de instalação dos implantes Imediato vs. Tardio (**Publicação 2**). E por fim, as diferentes superfícies de implantes pareceram se comportar de forma semelhante independentemente do material de enxertia prévia e período (tardio) avaliado (**Publicação 2**).

A instalação de implantes na tíbia dos coelhos (**Publicações 1 e 2**) foi o modelo animal adotado, pois, pelo seu porte, pôde receber implantes de comprimento e diâmetro compatíveis com os de uso clínico. Buscando reduzir ao máximo as variáveis relacionadas às características do implante, utilizamos implantes da mesma marca, tamanho e tratamento de superfície, e variamos apenas a sua macroestrutura [50].

O alvéolo após a extração dentária com uma forma intrínseca de cone, quando recebem implantes imediatos, o travamento primário ocorre somente na porção apical ou apical-média do implante, por isso, a macroestrutura também é extremamente importante. Se esse travamento não ocorrer, a regeneração ou reparação óssea do alvéolo deverá ser primeiramente obtida com ou sem enxertia prévia dependendo do tipo de defeito obtido após a perda dentária. E, frequentemente o alvéolo apresenta dimensões maiores que o diâmetro do implante, formando um espaço entre a região cervical do implante e o tecido ósseo e sendo necessário o preenchimento do espaço entre implante e parede do alvéolo com biomateriais[51]. Neste estudo (**Publicação 2**), o segundo defeito produzido na metáfise tibial dos coelhos com diâmetro cervical (na cortical superior da tíbia) 1,25 mm maior que o diâmetro do implante (5mm vs. 3.75mm, respectivamente), e travamento do implante somente na porção apical do defeito (cortical inferior) simulou uma situação clínica de implante imediato após exodontia[52]. Como a largura do osso na porção cervical influencia diretamente o

percentual da BIC%, é importante a utilização de biomateriais para preencher esses defeitos, por isso utilizamos um enxerto xenogênico de origem bovina.

O modelo experimental utilizado para elevação do assoalho do seio maxilar e enxertia também pareceu apropriado (**Publicação 3**), uma vez que os coelhos possuem um sistema de ventilação sinusal maxilar e um óstio bem definido que se abre para suas cavidades nasais de maneira bastante semelhante à dos humanos. O modelo de avaliação da regeneração sub-sinusal no coelho é, portanto, adequado e bem documentado para avaliar a fisiologia e o efeito dos preenchedores de espaço no processo da regeneração óssea e da osseointegração[53-57]. Além disso, apesar de alguns estudos utilizarem o acesso intra oral ao seio maxilar de coelhos[58,59], o acesso extra-oral[60,61] facilita o procedimento, principalmente no que se diz respeito a visibilidade e manuseio da membrana sinusal e por isso, foi a técnica utilizada no presente estudo. Neste caso, utilizamos implantes da mesma marca, tamanho e macroestrutura, e variamos apenas o tratamento de superfície.

Tem sido determinado que a macroestrutura dos implantes promove modificações a nível de obtenção da estabilidade primária[62,63]. Os resultados desse estudo corroboram com estudos que demonstraram que implantes cônicos apresentam estabilidade primária superior aos implantes cilíndricos[64,65]. Entretanto, apesar da diferença estatisticamente significativa obtida nesse estudo, os implantes cilíndricos também apresentaram bons resultados de estabilidade primária. Esse achado pode estar relacionado ao fato de que o experimento foi realizado em osso de tíbia que tem características de osso do tipo I, devido a presença de uma espessa cortical que permitiu o bom travamento dos implantes[66,67]. É provável que em osso de qualidade mais pobre, as diferenças na estabilidade primária entre os implantes HCI

sejam ainda maiores em relação aos implantes CI, hipótese essa que necessita ser testada futuramente

Um dado conflitante nesse estudo (**Publicação 1**) foi que o torque de remoção dos implantes CI foi superior ao dos implantes HCI no período de 8 semanas. Um fator que poderia ter interferido nesse aspecto seria o maior torque de inserção dos implantes HCI teria induzido maior necrose do tecido ósseo cortical e retardado o processo de osseointegração[68,69]. Entretanto, os valores de torque de inserção dos implantes HCI não superaram 45 Ncm, que não é um valor que tem sido relacionado com esses eventos adversos[69]. Além disso, a avaliação histológica deste estudo comprovou similaridade entre os implantes com diferentes macroestruturas no mesmo período experimental avaliado. Os implantes desse estudo tiveram travamento bicortical, aonde o ápice dos mesmos ficaram travados ao osso cortical posterior da tíbia dos coelhos, e levando-se em consideração que o ápice dos implantes HCI são menores do que os implantes CI é possível que esse aumento da área superfície de contato desses últimos implantes possa ter beneficiado o aumento do torque de remoção desses implantes[70]. Os HCI são indicados para todos os tipos de densidades ósseas. No caso de ossos tipo I e II, uma broca de sobrecontorno deve ser usada. No entanto, durante a instalação desses implantes, o torque manual e o contra-torque são frequentemente necessários para evitar danos aos tecidos adjacentes. Por esse motivo, o ápice dos implantes HCI já foi desenvolvido com câmeras helicoidais de baixa intensidade para facilitar o contra-torque durante a instalação manual. Esse fato também pode explicar o resultado do torque de remoção deste estudo.

Também tem sido relatado na literatura que a estabilidade primária é essencial para que o processo de osseointegração ocorra de forma previsível, e que o grau de osseointegração se correlaciona com a qualidade desse estabilidade[71-73],

fato esse reforçado pelos achados desse estudo que demonstraram uma maior grau de %BIC associado aos implantes HCI em relação ao que foi verificado nos implantes CI no período de 8 semanas, porém, sem alteração da qualidade do osso neoformado. Esse achado demonstra que a macroestrutura HCI beneficia a aceleração do processo de osseointegração, e que talvez essa diferença seja ainda mais relevante em condições clínicas mais desafiadoras (eg. osso nativo de baixa densidade, áreas enxertadas), porém o modelo animal utilizado (**Publicação 1**) não permite inferir sobre o real impacto da macroestrutura HCI sobre a osseointegração nessas condições clínicas.

O osso bovino desproteinizado (DBB) é amplamente utilizado e pesquisado como substituto ósseo devido às suas propriedades físico-químicas semelhantes às do osso humano, seu potencial osteocondutivo e disponibilidade. A temperatura de sinterização dos substitutos ósseos é um parâmetro importante que pode afetar suas propriedades[74]. Quando sinterizado a baixas temperaturas (até 300 ° C) [75] os cristais minerais da matriz óssea são preservados (Bio Oss®, Geistlich Pharma, Wolhusen, Switzerland)[76]. Em contrapartida, quando sinterizado a altas temperaturas (Straumann® Cerabone®, Suíça, Alemanha) (combustão oxidativa inicial a temperaturas em torno de 800 ° C seguida de um segundo tratamento térmico com 1.250 ° C), este processo, resulta em um aumento do tamanho do cristal em 500-1000% e da densidade de cristal, fato que o torna comparável a um material à base de cerâmica[76]. No presente estudo foi utilizado o DBB sinterizado a altas temperaturas (**Publicações 2 e 3**) e as biópsias ósseas coletadas das áreas previamente enxertadas (**Publicação 2**), após 90 dias de cura, apresentaram partículas do biomaterial incorporadas ao osso recém-formado. Isso indica que este DBB de fato, agiu como andaime para a aposição óssea.

A utilização de substitutos ósseos capazes de oferecerem um adequado arcabouço, destacando-se aqui os enxertos xenogênicos de origem bovina, tem sido uma boa opção. Mas a osteocondução destes materiais quando agregadas com outros mecanismos de ação, tal como a osteoindução e osteogênese, parecem ter resultados ainda melhores[40]. Portanto, uma favorável alternativa seria a associação deste material de preenchimento, essencialmente osteocondutor, com a medula óssea fresca que possui propriedades osteogênicas e osteoindutoras por possuir em sua composição células-tronco osteocondroprogenitoras e fatores de crescimento que induziriam maior diferenciação e atividade osteoblástica [46-47]. Soma-se a isto, o fato de que a utilização deste adjuvante biológico poderia funcionar como uma conexão biológica entre as partículas do enxerto, favorecendo ainda mais a regeneração tecidual.

Não existe consenso em relação a melhor metodologia para o uso de células estromais da medula óssea. Três alternativas principais têm sido propostas: (1) Uso do enxerto de medula óssea autóloga fresca ("in natura"); (2) Uso de concentrado de células da medula óssea autóloga (por centrifugação); e, (3) Uso de cultivo de células estromais da medula óssea autóloga [39-42]. A utilização da medula óssea enriquecida com fração mononuclear obtida por centrifugação apresentou melhores resultados de formação óssea associado ao DBB (28,17 ± 3,19%) do que o uso da medula óssea fresca associada ao mesmo substituto ósseo (21,14 ± 7,38%) em um estudo de Pelegrine et al. (2014)[39]. Mas, algumas desvantagens foram associadas com a utilização da metodologia de cultura celular: custo, tempo (requer um período de algumas semanas entre a coleta de células, obtenção da cultura e transplante), maior risco de contaminação, e a aplicabilidade clínica rotineira da cultura celular pode esbarrar na necessidade de dois procedimentos cirúrgicos separados. Outro

questionamento sobre a necessidade ou não de se cultivar as células é a falta de informação sobre o número mínimo de células requeridas para promoção do reparo ósseo[46-49]. Portanto, a utilização da medula óssea fresca conforme utilizada no presente estudo, seria uma abordagem mais simples do que outros que já foram propostos (**Publicações 2 e 3**).

Em um estudo de defeitos críticos em calvárias de coelhos a utilização de medula óssea fresca associada a DBB apresentou maior formação de tecido ósseo (21,14 ± 7,38%) do que o uso do DBB isolado (13,06 ± 5,24%) após 8 semanas do procedimento de enxertia[28]. Assim como em um estudo de reconstrução ósseas verticais na calvária de coelhos, as células-tronco derivadas da medula associadas a um bloco de DBB, resultou em aumento da formação óssea em relação a associação do mesmo bloco com células-tronco de tecido adiposo (11,9 ± 7,5% vs. 7,6 ± 5,6%) após 8 semanas do procedimento cirúrgico[78].

No presente estudo (**Publicações 2 e 3**), diferenças estatísticas não foram identificadas como nesses estudos citados quando a BM foi adicionada ao DBB. Isso pode ter ocorrido devido ao longo período experimental em nosso estudo (90 dias de cicatrização do enxerto prévio e para a pós instalação dos implantes) em comparação aos estudos supracitados (60 dias). Além disso, outras formas de aplicação da BM devem ser testadas com relação a possibilidade de influenciar positivamente o processo de osseointegração em áreas enxertadas com DBB, pois quando a BM foi utilizada (DBB vs. DBB/BM) a qualidade da conexão entre as partículas foi evidentemente maior.

Quanto a porcentagem de tecido mineralizado ao redor dos implantes foi estatisticamente maior no implante HCI que no CI quando utilizado o DBB de forma isolada, independentemente do período de instalação do implante (Imediato ou tardio).

Já no contato osso-implante, apesar de ambos implantes testados apresentarem bons resultados, diferença estatística de osseointegração só foi verificada no implante CI quando instalado em áreas previamente ou imediatamente enxertadas com DBB isolado, tendo melhores resultados quando imediatamente instalado (36.22 ± 6.07 e 23.32 ± 5.29, imediato e tardio respectivamente) (**Publicação 2**). Esses resultados corroboram estudos da literatura, uma vez que, a decisão de realizar enxertia prévia ou imediata será definida pelo tipo de defeito ósseo verificado no início do plano de tratamento e da possibilidade de travamento primário do implante.

Os resultados desse estudo devem ser interpretados com cautela pois além das limitações citadas anteriormente, o modelo utilizado não pode extrapolar condições clínicas tais como aplicação de carregamento imediato ou precoce, que poderiam alterar o processo de osseointegração nesses implantes. Além disso, o travamento bicortical oferecido pela tíbia dos coelhos (**Publicação 1**), que de fato ajuda na estabilidade primária dos implantes, não é um evento corriqueiro de se esperar na prática clínica diária, onde normalmente os implantes travam na sua porção mais coronal no osso cortical da maxila ou da mandíbula. Dessa forma, o efeito do HCI sobre a osseointegração ainda requer maiores investigações. Quanto ao osso bovino desproteinizado sinterizado a altas temperaturas, apresentou bons resultados de incorporação das partículas e contato osso implante independente da associação da BM e das macro (**Publicação 2**) ou microestruturas (**Publicação 3**) testadas no período (tardio) avaliado. Contudo, devido às limitações deste estudo, novas pesquisas são encorajadas para verificar a real influência da BM associada a diferentes biomateriais no processo de regeneração óssea, principalmente em períodos curtos de avaliação e em condições clínicas desafiadoras (diabetes,

osteoporose, artrite reumatoide e etc; fumantes ou usuários de alendronatos; regiões de baixa densidade óssea; e, carregamento dos implantes).

4 CONCLUSÕES

De acordo com os resultados obtidos e levando-se em consideração a metodologia aplicada pode-se concluir que:

1) Os HCI apresentaram maior estabilidade primária e contato osso-implante em relação aos CI quando instalados em osso nativo tipo I.
2) Os CI apresentaram maior torque de remoção do que os HCI em osso nativo tipo I.
3) A osseointegração em áreas enxertadas com DBB não foi afetada pela adição de BM nem pela macroestrutura dos implantes no período tardio avaliado. Entretanto, os HCI aumentaram a quantidade de tecidos mineralizados ao redor dos implantes em áreas enxertadas com DBB de forma isolada. O momento de enxertia na tíbia (Prévia vs. Imediata) só afetou a quantidade de contato osso-implante no CI, sendo que melhores resultados foram identificados na enxertia imediata.
4) A adição de BM e o tipo de superfície não influenciaram na osseointegração dos implantes instalados no seio maxilar previamente enxertado com DBB no período tardio avaliado.
5) Estudos clínicos em osso tipo IV são necessários para um melhor entendimento no que se refere à estabilidade a longo prazo bem como do comportamento das macro e microestruturas testadas quando expostas ao biofilme do meio bucal, e submetidas ao carregamento. Além de condições clínicas desafiadoras: alterações sistêmicas

REFERÊNCIAS*

1. Papaspyridakos P, Mokti M, Chen C-J, Benic GI, Gallucci GO, Chronopoulos V. Implant and prosthodontic survival rates with implant fixed complete dental prostheses in the edentulous mandible after at least 5 Years: a systematic review. Clin Implant Dent Relat Res. 2013; 16(5): 705-17.

2. Astrand P, Ahlqvist J, Gunne J, Nilson H. Implant treatment of patients with edentulous jaws: a 20-year follow-up. Clin Implant Dent Relat Res. 2008; 10(4): 207-17.

3. Narain RU, Garg S, Narain P. A prospective study of the quality of removable prostheses and patients' satisfaction in post-prosthetic phase. The Internet Journal of Dental Science. 2010; 9 (1): 1-14.

4. Lee JT, Cho SA. Biomechanical evaluation of laser-etched Ti implant surfaces vs. Chemically modified SLA Ti implant surfaces: removal torque and resonance frequency analysis in rabbit tíbias. J Mech Behav Biomed Mater. 2016; 61: 299-307.

5. El Askary AS, Meffert RM, Griffin T. Why do dental implants fail? Part I. Implant Dent. 1999; 8: 173-85.

6. Battula S, Lee JW, Wen HB, Savvas P, Romanos CM, Georgios E. Evaluation of different implant designs in a ligature-induced peri implantitis model: a canine study. Int J Oral Maxillofac Implants. 2015; 30(3): 534-45.

7. Cameron HU, Pilliar RM, Macnab I. The effect of movement on the bonding of porous metal to bone. J Biomed Mater Res. 1973; 7: 301-11.

8. Steigenga JT, Steigenga JT, Al-Shammari KF, Nociti FH, Misch CE, Wang HL. Dental implant design and its relationship to long-term implant success. Implant Dent. 2003; 12 :306-17.

9. Junker R, Dimakis A, Thoneick M, Jansen JA. Effects of implant surfasse coatings and composition on bone integration: A systematic review. Clin Oral Implants Res. 2009; 20: 185-206.

10. Pilliar, R. Overview of surface variability of metallic endosseous dental implants: Textured and porous surface-structured designs. Implant Dent. 1998; 7: 305-14.

* 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

11. Castilho GAA, Martins MD, Macedo AA. Surface characterization of titanium based dental implant. Brazilian Journal of Physics. 2006; 36 (3): 1004-8.

12. Javed F, Romanos GE. The role of primary stability for successful immediate loading of dental implants: a literature review. J Dent. 2010; 38(8): 612-20.

13. Casar-Espinosa JC, Castillo-Oyagüe R, Serrera-Figallo MÁ, Garrido-Serrano R, Lynch CD, Menéndez-Collar M, Torres-Lagares D, Gutiérrez-Pérez JL. Combination of straight and tilted implants for supporting screw-retained dental prostheses in atrophic posterior maxillae: a 2-year prospective study. J Dent. 2017; 63: 85-93.

14. Esposito M, Zucchelli G, Cannizzaro G, Checchi L, Barausse C, Trullenque-Eriksson A, Felice P. Immediate, immediate-delayed (6 weeks) and delayed (4 months) postextractive single implants: 1-year post-loading data from a randomised controlled trial. Eur J Oral Implantol. 2017; 10(1): 11-26.

15. Alghamdi H, Anand PS, Anil S. Undersized implant site preparation to enhance primary implant stability in poor bone density: a prospective clinical study. J Oral Maxillofac Surg. 2011 Dec; 69(12): e506-12.

16. He J, Zhao B, Deng C, Shang D, Zhang C. Assessment of implant cumulative survival rates in sites with different bone density and related prognostic factors: an 8-year retrospective study of 2,684 implants. Int J Oral Maxillofac Implants. 2015; 30(2): 360-71.

17. Bertl K, Bertl MH, Heimel P, Burt M, Gahleitner A, Stavropoulos A, Ulm C. Alveolar bone resorption after primary tooth loss has a negative impact on straightforward implant installation in patients with agenesis of the lower second premolar. Clin Oral Implants Res. 2018; 29(2): 155-63.

18. Gehrke SA, Marin GW. Biomechanical evaluation of dental implants with three different designs: removal torque and resonance frequency analysis in rabbits. Ann Anat. 2015; 199: 30-5.

19. Negri B, Calvo-Guirado JL, Maté Sánchez de Val JE, Delgado Ruiz RA, Ramírez Fernández MP, Gómez Moreno G, Aguilar Salvatierra A, Guardia J, Muñoz Guzón F. Biomechanical and bone histomorphological evaluation of two surfaces on tapered and cylindrical root form implants: an experimental study in dogs. Clin Implant Dent Relat Res. 2013;15(6): 799-808.

20. Chiapasco MC, Gatti E, Rossi W, Haeflige T, Markwaldel H. Implant-retained mandibular overdentures with immediate loading: a retrospective multicenter study on 226 consecutive cases. Clin Oral Impl Res. 1997; 8: 48-57.

21. Crespi R, Capparé P, Gherlone E. Dental implants placed in extraction sites grafted with different bone substitutes: radiographic evaluation at 24 months. J Periodontol. 2009; 80: 1616-21.

22. Hinkle RM, Rimer SR, Morgan MH, Zeman P. Loading of titanium implants with hydrophilic endosteal surface 3 weeks after insertion: clinical and radiological outcome of a 12-month prospective. J Oral Maxillofac Surg. 2014; 72: 1495-502.

23. Lindhe J, Karring T, Lang NP. Tratado de periodontia clínica e implantodontia oral. 5ª ed. Rio de Janeiro: Guanabara Koogan; 2010.

24. Kim DM, Nevins ML, Lin Z, Fateh A, Kim SW, Schupbach P, Nevins M. The clinical and histologic outcome of dental implant in large ridge defect regenerated with alloplast: a randomized controlled preclinical trial. J Oral Implantol. 2013; 39(2): 148-153.

25. Tetè S, Vinci R, Zizzari VL, Zara S, Scala V, Cataldi A, et al. Maxillary sinus augmentation procedures through equine-derived biomaterial or calvaria autologous bone: immunohistochemical evaluation of OPG/RANKL in humans. Eur J Histochem. 2013; 57 (10): 60-5.

26. Kolk A. Current trends and future perspectives of bone substitute materials e From space holders to innovative biomaterials. J Craniomaxillofac Surg. 2012; 40: 706-18.

27. Schmitt CM, Doering H Schmidt T. Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone: a randomized controlled clinical trial. Clin Oral Implants Res. 2013; 24: 576-85.

28. Pelegrine AA, Costa CES, Sendyk WR, Gromatzky A. The comparative analysis of homologous fresh frozen bone and autogenous bone graft, associated or not with autogenous bone marrow, in rabbit calvaria: a clinical and histomorphometric study. Cell Tissue Bank. 2011; 12: 171-84.

29. Schmitt CM, Moest T, Lutz R. Anorganic bovine bone (ABB) vs. autologous bone (AB) plus ABB in maxillary sinus grafting. Clin Oral Implants Res. 2015; 26(9):1043-50.

30. Santis E, Lang NP, Ferreira S. Healing at implants installed concurrently to maxillary sinus floor elevation with Bio-Oss or autologous bone grafts: a histo-morphometric study in rabbits. Clin Oral Implants Res. 2016; 28(5): 503-11.

31. Sbordone C, Toti P, Guidetti F, Califano L. Volume changes of iliac crest autogenous bone grafts after vertical and horizontal alveolar ridge augmentation of atrophic maxillas and mandibles: a 6-year computerized tomographic follow-up. J Craniomaxillofac Surg. 2012; 70: 2559-65.

32. Meloni SM, Jovanovic SA, Lolli FM, Cassisa C. Grafting after sinus lift with anorganic bovine bone alone compared with 50:50 anorganic bovine bone and autologous bone: results of a pilot randomised trial at one year. Br J Oral Maxillofac Surg. 2015; 53(5): 436-41.

33. Oliveira REL, Hage M, Carrel JP, Lombardi T, Bernard JP. Rehabilitation of the edentulous posterior maxilla after sinus floor elevation using deproteinized bovine bone: a 9-year clinical study. Implant Dent. 2012; 21(5): 422-26.

34. Roccuzzo M, Gaudioso L, Bunino M, Dalmasso P. Long-term stability of soft tissues following alveolar ridge preservation: 10-year results of a prospective study around nonsubmerged implants. Int J Periodontics Restorative Dent. 2014; 34(6): 795-804.

35. Nikoghosyan AS, Ting H, Shen J. Optical properties of human jawbone and human bone substitute cerabone® in the terahertz range. Journal of Contemporary Physics. 2016; 51(3): 256-64.

36. Jensen T. et al. Bone-to-implant contact after maxillary sinus floor augmentation with Bio-Oss and autogenous bone in different ratios in mini pigs. Clin Oral Implants Res. 2013; (24): 635-44.

37. Jensen, T. et al. Maxillary sinus floor augmentation with Bio-Oss or Bio-Oss mixed with autogenous bone as graft in animals: a systematic review. Int J Clin Oral Maxillofac Surg. 2012; 41:114-20.

38. Felice P, Soardi E, Piattelli M, Pistilli R, Jacotti M, Esposito M. Immediate non-occlusal loading of immediate post-extractive versus delayed placement of single implants in preserved sockets of the anterior maxilla: 4-month postloading results from a pragmatic multicentre randomised controlled trial. Eur J Oral Implantol. 2011; 4(4): 329-44.

39. Pelegrine AA, Aloise AC, Zimmermann A, Oliveira RM, Ferreira LM. Repair of critical-size bone defects using bone marrow stromal cells: a histomorphometric study in rabbit calvaria. Part I: Use of fresh bone marrow or bone marrow mononuclear fraction. Clin Oral Implants Res. 2014; 25: 567-72.

40. Oliveira TA, Aloise AC, Orosz JE, Mello E, Oliveira R, Carvalho P, Pelegrine AA. Double Centrifugation Versus Single Centrifugation of Bone Marrow Aspirate Concentrate in Sinus Floor Elevation: A Pilot Study. Int J Clin Oral Maxillofac Surg. 2016; 31(1): 216-22.

41. Wildburger A, Payer M, Jakse N, Strunk D, Etchard-Liechtenstein N, Sauerbier S. First published Impact of autogenous concentrated bone marrow aspirate on bone regeneration after sinus floor augmentation with a bovine bone substitute – a split-mouth pilot study. Clin Oral Implants Res. 2014; 25: 1175-81.

42. Lee S-B, Retzepi M, Petrie A, Hakimi A-R, Schwarz F, Donos N. The effect of diabetes on bone formation following application of the GBR principle with the use of titanium domes. Clin Oral Implants Res. 2012; 24(1): 28–35.

43. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res. 2009; 20: 172-84.

44. Froum SJ, Wallace S, Sang-Choon C, Rosenberg E, Scott F, Schoor R et al. A histomorphometric comparison of Bio-Oss alone versus BioOss and platelet-derived growth factor for sinus augmentation: a postsurgical assessment. Int J Periodontics Restorative Dent. 2013; 33(3): 269-79.

45. Jung RE, Glauser R, Schärer P, Hämmerle CH, Sailer HF, Weber F. E. Effect of rhBMP-2 on guided bone regeneration in humans. Clin Oral Implants Res. 2003; 14(5): 556-68.

46. Silva MO, Pelegrine AA, Silva AAP, Manhães Júnior LR, Oliveira RM, França SG, Aloise AC, Ferreira LM. Xenograft Enriched with Autologous Bone Marrow in Inlay Reconstructions: A Tomographic and Histomorphometric Study in Rabbit Calvaria. Int J Biomater. 2012; 1-7.

47. Pelegrine AA, Costa CES, Correa MEP, Marques Jr JFC. First published: Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft. Clin Oral Implants Res. 2010; 21: 535-42.

48. Nagata MJH, Santinonia CS, Pola NM, Michel NC, Messorab S. Bone marrow aspirate combined with low-level laser therapy: A new therapeutic approach to enhance bone healing. J Photochem Photobiol B. 2013; 121: 6-14.

49. Pasquali PJ, Teixeira ML, Oliveira TA, Macedo LGS, Aloise AC, Pelegrine AA. Maxillary sinus augmentation combining bio-oss with the bone marrow aspirate concentrate: a histomorphometric study in humans. Int J Biomater, 2015; 1-7.

50. Faeda RF, Spin-Neto R, Marcantonio E, Guastaldi AC, Marcantonio Jr E. Laser ablation in titanium implants followed by biomimetic hydroxyapatite coating: histomorphometric study in rabbits. Microsc Res Techniq. 2012; 75: 940-48.

51. Betoni Jr W, Queiroz, TP, Luvizuto, ER, Valentini-Neto R, Garcia-Júnior IR, Bernabé PFE. Evaluation of centrifuged bone marrow on bone regeneration around implants in rabbit tibia. Implant Dent. 2012; 21(6): 481-5.

52. Akimoto K, Becker W, Persson R, Baker DA, Rohrer MD, O'Neal MB. Evaluation of titanium implants placed into simulated extraction sockets: A study in dogs. Int J Oral Maxillofac Implants. 1999; 14: 351-60.

53. Scharf KE, Lawson W, Shapiro JM, Gannon PJ. Pressure measurements in the normal and occluded rabbit maxillary sinus. Laryngoscope, 1995; 105: 570-74.

54. Asai S, Shimizu Y, Ooya K. Maxillary sinus augmentation model in rabbits: effect of occluded nasal ostium on new bone formation. Clin Oral Implants Res. 2002; 13: 405-9.

55. Ohya M, Yamada Y, Ozawa R, Ito K, Takahashi M, Ueda M. Sinus floor elevation applied tissue-engineered bone. Comparative study between mesenchymal stem cells/platelet-rich plasma (prp) and autogenous bone with prp complexes in rabbits. Clin Oral Implants Res. 2005; 16: 622-9.

56. Sun XJ, Zhang ZY, Wang SY, Gittens SA, Jiang XQ, Chou LL. Maxillary sinus floor elevation using a tissue-engineered bone complex with osteobone and bmscs in rabbits. Clin Oral Implants Res. 2008; 19: 804-13.

57. Lambert F, Leonard A, Drion P, Sourice S, Layrolle P, Rompen E. Influence of space-filling materials in subantral bone augmentation: blood clot vs. autogenous bone chips vs. bovine hydroxyapatite. Clin Oral Implants Res. 2011; 22: 538-45.

58. Watanabe K, Niimi A, Ueda M. Autogenous bone grafts in the rabbit maxillary sinus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999; 88: 26-32.

59. Trindade-Suedam IK, de Morais JAND, Faeda RS, Leite FRM, Tosoni GM, Benatti Neto C, Marcantonio-Jr E, Scaf G. Bioglass associated with leukocyte-poor platelet-rich plasma in the rabbit maxillary sinus: histomorphometric, densitometric, and fractal analysis. J Oral Implantol. 2010; 36: 333-43.

60. Xu H, Shimizu Y, Asai S, Ooya K. Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation. Clin Oral Implants Res. 2004; 15: 126-33.

61. Caneva M, Lang NP, Garcia Rangel IJ, Ferreira S, Caneva M, De Santis E, Botticelli D. Sinus mucosa elevation using BioOss or Gingistat collagen sponge: an experimental study in rabbits. Clin Oral Implants Res. 2016; 1-10.

62. Akkocaoglu M, Uysal S, Tekdemir I, Akca K, Cehreli MC. Implant design and intraosseous stability of immediately placed implants: a human cadaver study. Clin Oral Implants Res. 2005;16(2): 202-9.

63. de Oliveira GJPL, Barros-Filho LAB, Barros LAB, Queiroz TP, Marcantonio E Jr. In vitro evaluation of the primary stability of short and conventional implants. J Oral Implantol. 2016; 42(6): 458-63.

64. Calvo-Guirado JL, Gomez Moreno G, Aguilar-Salvatierra A, Mate Sanchez de Val JE, Abboud M, Nemcovsky CE. Bone remodeling at implants with different configurations and placed immediately at different depth into extraction sockets: experimental study in dogs. Clin Oral Implants Res. 2015; 26(5): 507-15.

65. Sakoh J, Wahlmann U, Stender E, Al-Nawas B, Wilfried Wagner W. Primary stability of a conical implant and a hybrid, cylindric screw-type implant in vitro. Int J Oral Maxillofac Implants. 2006; 21: 560-6.

66. Turkyilmaz I, Sennerby L, McGlumphy EA, Tözüm TF. Biomechanical aspects of primary implant stability: A human cadaver study. Clin Implant Dent Relat Res. 2009; 11(2): 113-9.

67. Marquezan M, Osório A, Sant'Anna E, Souza MM, Maia L. Does bone mineral density influence the primary stability of dental implants? A systematic review. Clin Oral Implants Res. 2012; 23(7): 767-74.

68. Marković A, Mišić T, Miličić B, Calvo-Guirado JL, Aleksić Z, Đinić A. Heat generation during implant placement in low-density bone: effect of surgical technique, insertion torque and implant macro design. Clin Oral Implants Res. 2013; 24(7): 798-805.

69. Duyck J, Roesems R, Cardoso MV, Ogawa T, De Villa Camargos G, Vandamme K. Effect of insertion torque on titanium implant osseointegration: an animal experimental study. Clin Oral Implants Res. 2015; 26(2): 191-6.

70. Lee JH, Frias V, Lee KW, Wright RF. Effect of implant size and shape on implant success rates: a literature review. J Prosthet Dent. 2005; 94(4): 377-81.

71. Montes CC, Pereira FA, Thomé G, Alves EDM, Acedo RV, Souza JR, Melo ACM, Trevilatto PC. Failing factors associated with osseointegrated dental implant loss. Implant Dent. 2007; 16: 404-12.

72. Rao PL, Gill A. Primary stability: The password of implant integration. J Oral Implantol. 2012; 2: 103-9.

73. Bataineh AB, Al-Dakes AM. The influence of length of implant on primary stability: An in vitro study using resonance frequency analysis. J Clin Exp Dent. 2017; 9: e1–6.

74. Barbeck Ž, Kačarević P, Kavehei F, Rider P, Najman S, Stojanović S, Rimashevskiy D. The effect of temperature treatment of xenogeneic bone substitute on the tissue response – a mini review. Acta Medica Medianae, 2019; 58: 131-7.

75. Barbeck M, Dard M, Kokkinopoulou M, Markl J, Booms P, Sader, RA. Small-sized granules of biphasic bone substitutes support fast implant bed vascularization. Biomatter, 2015; 5: 1-13.

76. Barbeck M, Unger R, Schnettler R, Wenisch S, Witte F. Xenogeneic bone grafting materials. International Magazine of Oral implantology, 2017; 17: 34-6.

77. Lucarelli E, Donati D, Cenacchi A, Fornasari PM. Bone reconstruction of large defects using bone marrow derived autologous stem cells. Transfus Apher Sci. 2004; 30: 169-74.

78. Carrera-Arrabal T, Calvo-Guirado JL, Santos FP et al. Vertical bone construction with bone marrow-derived and adipose tissue-derived stem cells. Symmetry. 2019; 59: 1-11.

Apêndice

Materiais e métodos

Cada objetivo específico dessa tese foi avaliado nos seguintes estudos:

Publicação 1: Avaliar o efeito da modificação da macroestrutura de implantes (HCI vs. IC) com superfície hidrofílica modificada por jateamento e ataque ácido sobre a osseointegração em osso tipo I (metáfise tibial) de coelhos.

Publicação 2: Avaliar o efeito da modificação da macroestrutura de implantes (HCI vs. IC) com superfície hidrofílica modificada por jateamento e ataque ácido sobre a osseointegração em defeitos ósseos criados na metáfise tibial prévio ou imediatamente enxertados com osso bovino desproteinizado associado ou não à medula óssea fresca (DBB vs. DBB/BM). Além de comparar a neoformação óssea e osseointegração do enxerto xenogênico, constituído de hidroxiapatita pura (HA) e sem componentes orgânicos nos diferentes grupos (DBB vs. DBB/BM)

Publicação 3: Avaliar o efeito de uma superfície hidrofílica e modificada por ataque ácido e por jateamento (AQ) sobre a osseointegração em comparação a uma superfície hidrofóbica com o mesmo tipo de tratamento para modificação de superfície (NP) instalados na região do seio maxilar previamente submetido ao levantamento do assoalho e enxertia (DBB vs. DBB/BM)

As metodologias de cada estudo foram descritas de maneira simultânea enfatizando as particularidades de cada um.

Modelo experimental

Os projetos de pesquisa foram realizados de acordo com os Princípios Éticos para a Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA), após aprovação do Comitê de Ética no uso de animais (CEUA) da Faculdade de Odontologia de Araraquara (FOAr-UNESP) (ANEXO A e B: Protocolo de aprovação 11/2016 – **Publicação 1** e 15/2017 – **Publicações 2 e 3**).

Para a **Publicação 1** foram utilizados 24 coelhos e para as **Publicações 2 e 3** foram utilizados 16 coelhos (Albinos da raça Nova Zelândia, machos, de aproximadamente 9-12 meses de idade e peso entre 4 e 5 quilogramas).

Os animais foram fornecidos pelo Biotério Central da Universidade Estadual Paulista “Júlio de Mesquita Filho”/UNESP - Campus de Botucatu e mantidos no Biotério de Coelhos da Universidade Estadual Paulista "Júlio de Mesquita Filho"/ UNESP - Campus de Araraquara, Faculdade de Ciências Farmacêuticas, situado na Rodovia Araraquara - Jaú – Machados, em um ambiente com temperatura entre 22 e 24°C, com ciclo de luz controlada (12 horas claro e 12 horas escuro) e consumo de ração sólida e água *ad libitum* durante todo período experimental. Foi respeitado um período de 30 dias para aclimatação dos animais nas instalações do biotério.

Delineamento Experimental

Portanto, para avaliação da influência das diferentes macroestruturas dos implantes de titânio no processo de osseointegração na **Publicação 1**, 24 coelhos foram aleatoriamente divididos em 3 períodos experimentais (2, 4 e 8 semanas). Cada animal recebeu dois implantes de cada grupo no osso cortical (tíbia) do lado direito e esquerdo.

Foram avaliadas duas diferentes macroestruturas: Implante Cilíndrico (CI) e Implante Cônico Híbrido (HCI).

Implante Cilíndrico (CI): Caracterizado por apresentar diâmetro cervical igual ao diâmetro do corpo do implante com 3,75mm de diâmetro x 11mm de altura. Presença de roscas piramidais ou triangulares, que também são duplas, ou seja, facilitam a inserção do implante de forma rápida e com o mínimo de trauma. O ápice é cortante ativo com câmaras auto cortantes. É indicado para ossos de densidade tipo I ou II e áreas previamente enxertadas com bloco. Não necessitam do macho de rosca uma vez que o próprio implante corta o osso durante a sua instalação (Titamax Acqua, Neodent®, Cone Morse, Curitiba, Brasil) (Figuras A1A, A1C e A1E). Implante Híbrido Cônico (HCI): é caracterizado por apresentar diâmetro cervical aumentado em relação ao corpo do implante e por apresentar 3,75mm de diâmetro x 11,5mm de altura. Além da presença de roscas trapezoidais compactantes e roscas duplas, as quais permitem uma rápida inserção e com o mínimo de trauma, o ápice cônico deste implante é caracterizado por câmaras de baixa intensidade e câmaras helicoidais desenhadas para otimizar a estabilidade secundária. São indicados para todos os tipos de densidade óssea e pós extração. Lembrando que nos ossos tipo I e II, as brocas de sobrecontorno e piloto final devem ser utilizadas (Helix Acqua, Neodent®, Grand Morse, Curitiba, Brasil) (Figuras A1B, A1D e A1F) (Figura A3).

Para avaliação da influência das diferentes macro (**Publicação 2**) e microestruturas (**Publicação 3**) dos implantes de titânio em áreas (Metáfise tibial – **Publicação 2** e Seio maxilar – **Publicação 3**) prévia (**Publicações 2 e 3**) ou imediatamente (**Publicação 2**) enxertadas com enxerto xenogênico de origem bovina (Straumann® Cerabone®, Suíça, Alemanha / Grânulos de 0,5-1,0mm) associado ou

não à medula óssea fresca (BM), 16 coelhos foram utilizados em um período total de 180 dias.

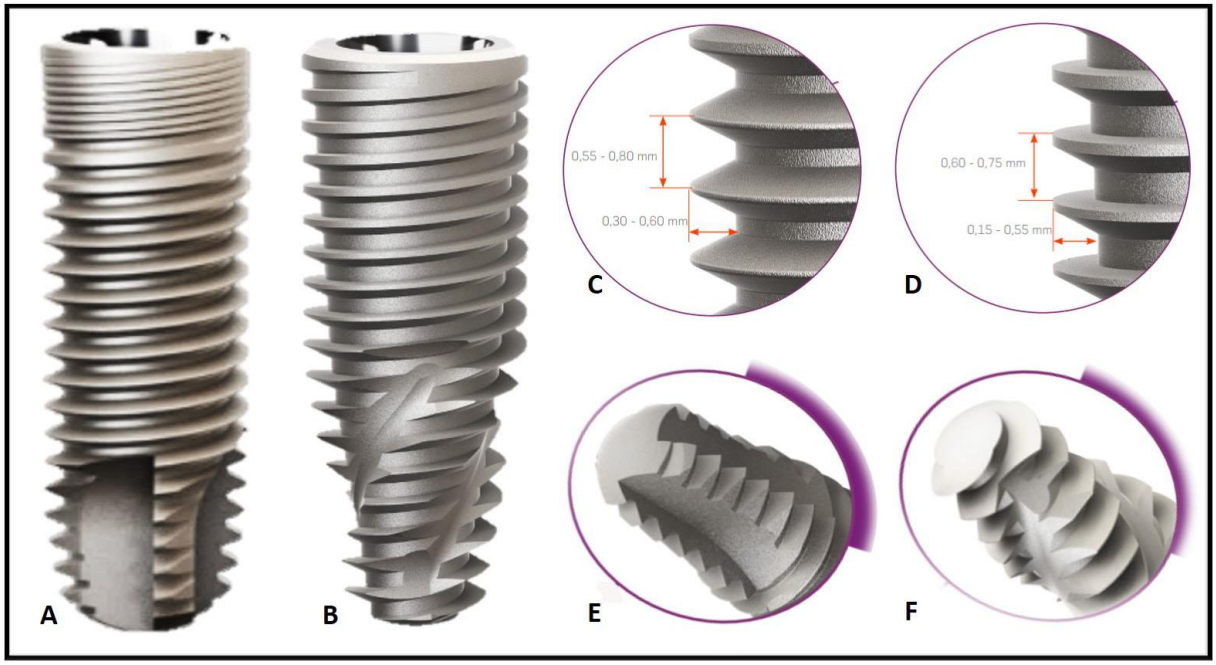
Os animais foram distribuídos aleatoriamente em dois grupos experimentais: Grupo Controle - DBB: Enxerto ósseo bovino desproteinizado e Grupo Experimental - DBB/BM: Enxerto ósseo bovino desproteinizado associado à medula óssea autóloga fresca (**Publicações 2 e 3**).

Na **Publicação 2**, cada animal foi inicialmente submetido à criação de um defeito ósseo bilateral de 5mm de diâmetro na metáfise tibial. Os defeitos foram preenchidos com DBB ou DBB/BM. Após 90 dias, estas áreas previamente enxertadas foram submetidas à coleta de biópsia óssea e instalação de implantes. Cada região enxertada recebeu um implante com diferentes macroestruturas CI vs. HCI (mesmos implantes utilizados na **Publicação 1** e descritos anteriormente). Além disso, neste procedimento cirúrgico, um segundo defeito bilateral de 5mm de diâmetro na metáfise tibial, situado à 4mm do primeiro defeito, foram preenchidos com os mesmos materiais descritos para os primeiros defeitos (DBB ou DBB/BM), porém com instalação imediata de implantes (CI vs. HCI).

Na **Publicação 3**, cada animal foi inicialmente submetido ao acesso extraoral bilateral à membrana do assoalho do seio maxilar para elevação desta membrana e preenchimento de acordo com o grupo experimental com os mesmos biomateriais descritos para a **Publicação 3** (DBB vs. DBB/BM). Após 90 dias, estas áreas enxertadas foram submetidas à instalação de implantes. Os implantes com diferentes microestruturas instalados na região do seio maxilar previamente enxertado foram: NP (Jateamento + ataque ácido) e AQ (Jateamento + ataque ácido + imersão em solução isotônica de cloreto de sódio 0,9 %) (Figura A2); os implantes desta categoria eram curtos e apresentavam com 4mm de diâmetro x 5mm de altura (Figura

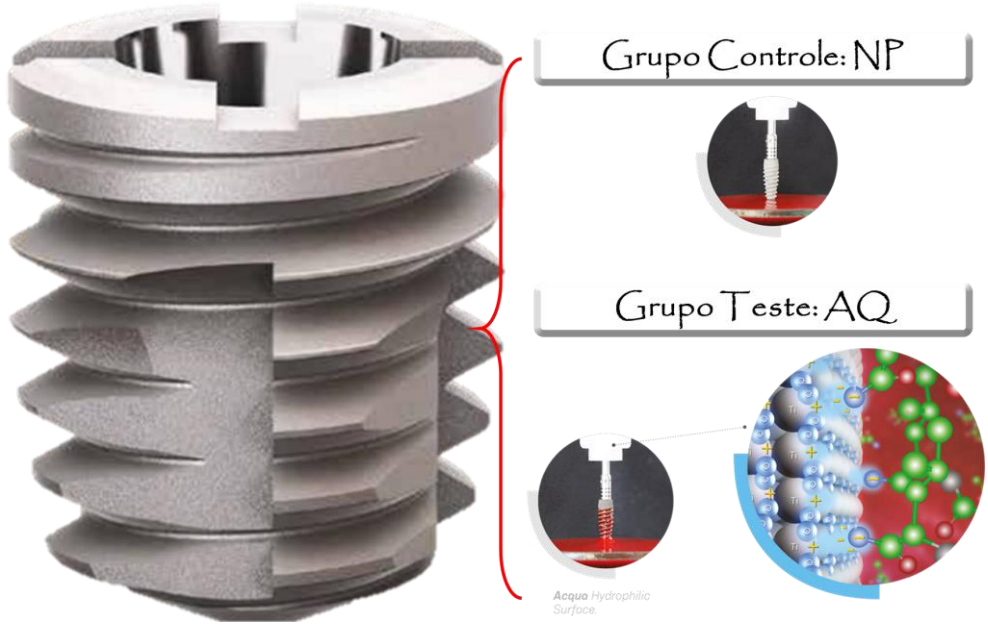
A4 e A5) (Implante TitamaxWS NeoPoros® vs. Implante TitamaxWS Acqua®, Neodent®, Curitiba, Brasil).

Figura A1 - Macroestrutura dos implantes instalados no osso cortical nativo ou enxertado na tíbia dos coelhos nas **Publicações 1 e 2**



*Corpo, roscas e ápice do Implante Cilíndico (A, C e E) e do Implante Cônico Híbrido (B, D e F).
Fonte: Elaboração própria.

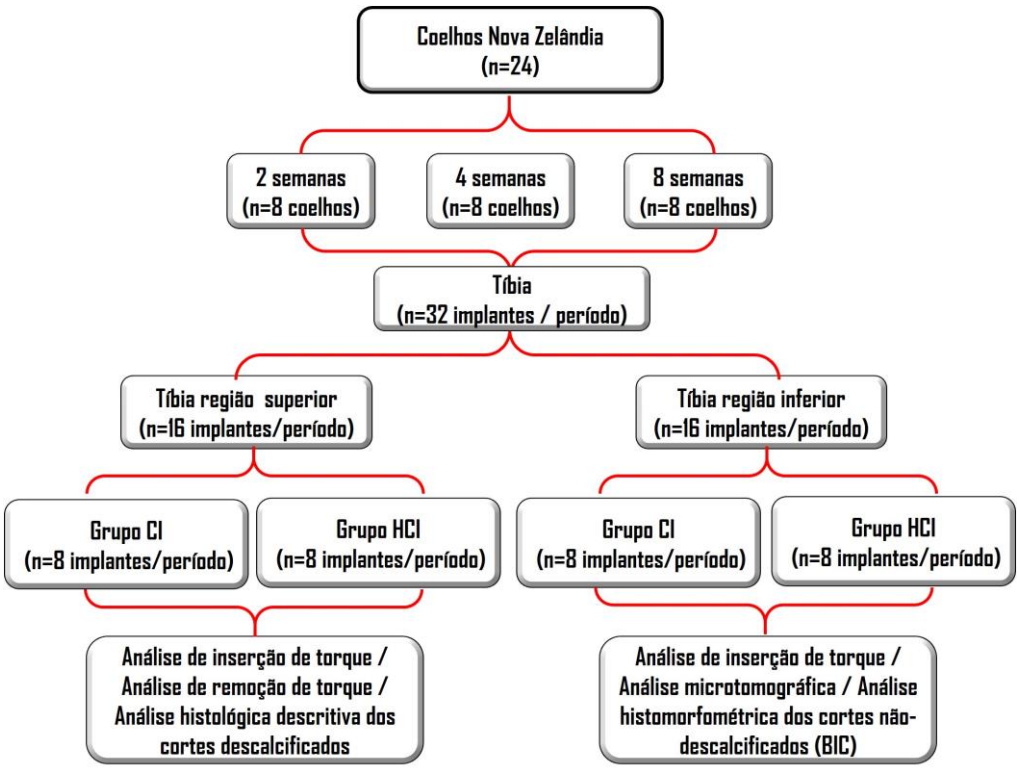
Figura A2: Macro e microestrutura do implante instalado no seio maxilar previamente enxertado dos coelhos no **Publicação 3**



*Implante curto com tratamento de superfície hidrofóbico e hidrofílico.

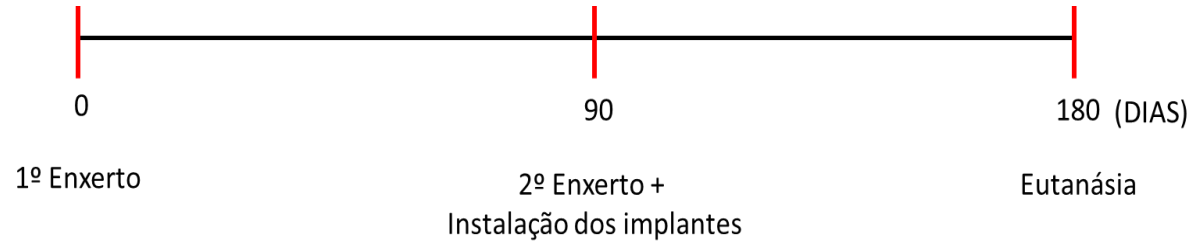
Fonte: Elaboração própria.

Figura A3: Delineamento experimental da **Publicação 1**



Fonte: Elaboração própria.

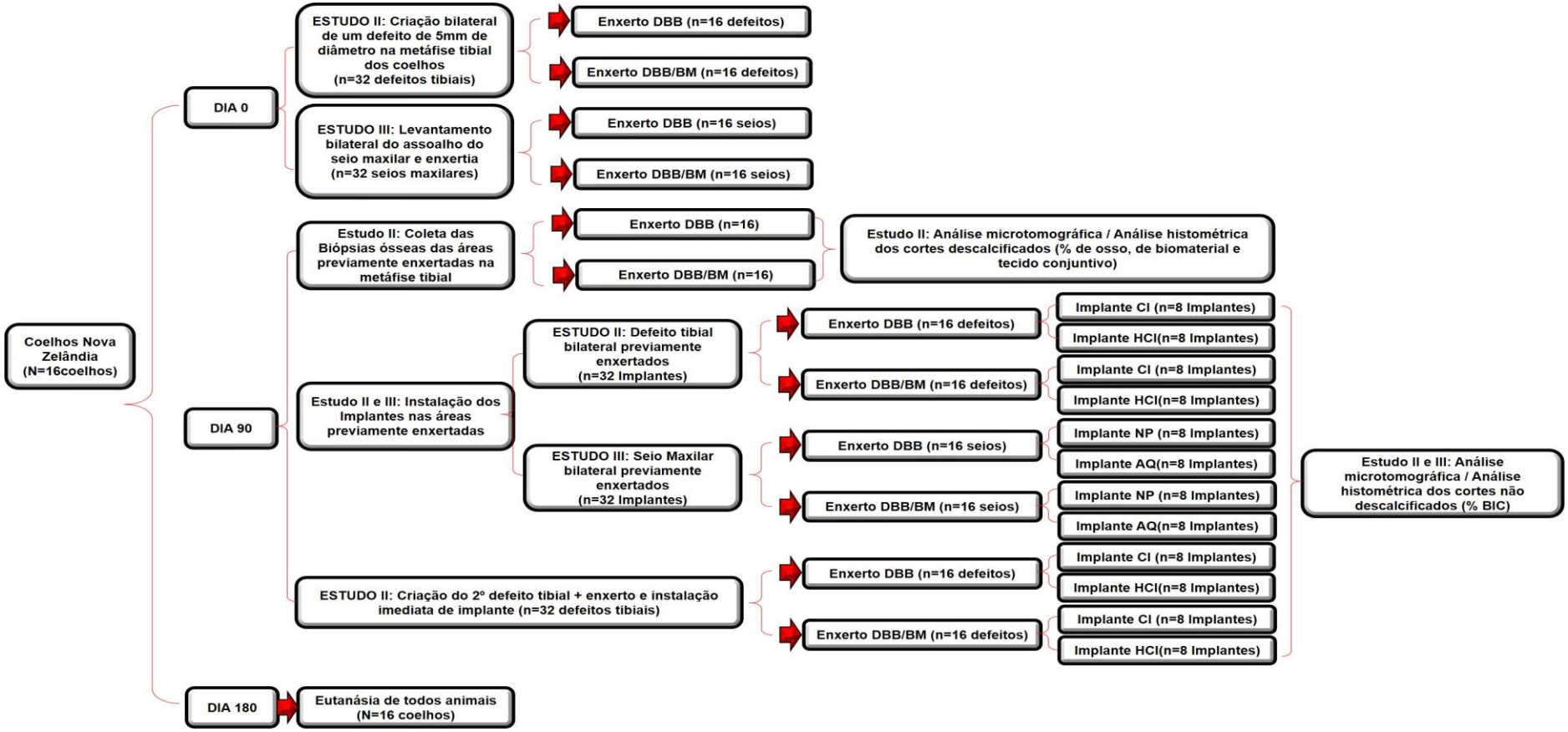
Figura A4 - Fluxograma dos procedimentos realizados nas **Publicações 2 e 3**



*1º Enxerto - Enxerto bilateral realizado no 1º defeito da metáfise tibial e no seio maxilar;
2º Enxerto - Enxerto bilateral realizado no 2º defeito da metáfise tibial situado à 4mm do 1º defeito;
Instalação dos Implantes - Implantes com diferentes macroestruturas instalados bilateralmente no 1º defeito da metáfise tibial previamente enxertado; Implantes com diferentes macroestruturas instalados bilateralmente de forma imediata no 2º defeito da metáfise tibial; Implantes com diferentes microestruturas instalados bilateralmente no seio maxilar previamente enxertado.

Fonte: Elaboração própria.

Figura A5: Delineamento experimental das **Publicações 2 e 3**



Fonte: Elaboração própria.

Procedimento Cirúrgico

Publicação 1:

Inicialmente os animais foram pesados e anestesiados por via intramuscular, com uma combinação de quetamina (Quetamina Agener®; Agener União Ltda, São Paulo, SP, Brasil - 0,35 mg/kg) e xilazina (Rompum, Bayer AS, São Paulo, SP, Brasil - 0,5 mg/kg). Posteriormente, foi realizada tricotomia e antissepsia com polivinilpirrolidona-iodado (PVPI) a 10% com 1% de iodo ativo na porção da metáfise tibial direita e esquerda do animal (Figura A7A, A7B, A7C e A7D). Anestesia local (Cloridrato de Mepivacaína 2% + Adrenalina 1:100.000) também foi aplicada na região, para permitir uma vasoconstrição periférica diminuindo o sangramento local e otimizando o procedimento cirúrgico (Figura A7E). A seguir, com uma lâmina de bisturi nº 15 (Free-Bac, P.R.C., China) foi realizada uma incisão dermoperiosteal de aproximadamente 5 cm de comprimento (Figuras A7F, A7G e A7H). Isto permitiu uma dissecção delicada, de modo que a superfície óssea da metáfise tibial foi exposta (Figura A7I), e os leitos cirúrgicos preparados de acordo com as recomendações dos fabricantes (Neodent® - Curitiba, Brasil), utilizando brocas de metal (Figura A6) sob refrigeração abundante com solução fisiológica estéril (Figuras A7J, A7K, A7L, A7M, A7N e A7O). Foram instalados 2 implantes de cada tipo nas tíbias, sendo que o lado foi selecionado de forma aleatória. Os implantes superiores apresentaram uma distância de 3 centímetros em relação aos inferiores (Figuras A7P, A7Q, A7R, A7S e A7T). Ambos os implantes apresentavam o mesmo tipo de superfície (Jateamento + ataque ácido + imersão em solução isotônica de cloreto de sódio 0,9 %). Os parafusos de cobertura foram instalados (Figura A7U) e em seguida, os tecidos moles foram reposicionados e suturados plano a plano com fio reabsorvível (Vicryl® ETHICON, 4-

0, Johnson & Johnson, Brasil) e não reabsorvível (Nylon 3-0, Shalon®, Brasil) (Figuras A7V e A7W). Após a cirurgia todos os animais receberam uma dose única via intramuscular de antibiótico (Pentabiótico®, Wyeth-Whitehall Ltda, São Paulo, Brasil - 0,1 ml/kg) e analgésico (Cloridrato de Tramadol 50mg/ml, Tramadol®, Medley, São Paulo, Brasil - 5 mg/Kg IM). Após os períodos de 2, 4 e 8 semanas os animais foram eutanasiados através de sobredose anestésica.

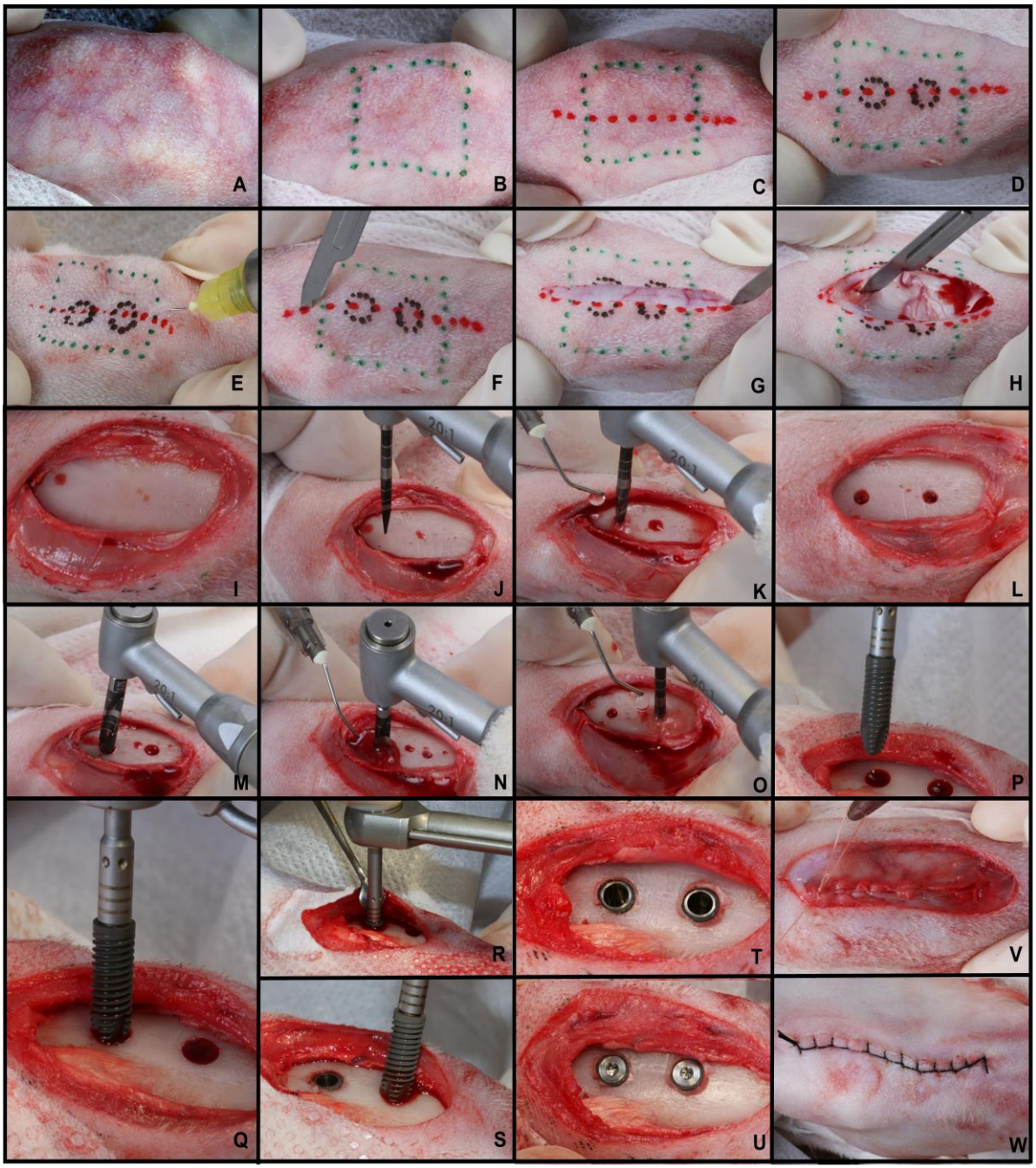
Figura A6 - Sequência de fresagem dos implantes com diferentes macroestruturas utilizados nas **Publicações 2 e 3**

Sequência de Fresagem

CI	HCI
Broca Lança 2.0	Broca Lança 2.0
Broca Helicoloidal 2.0	Broca Helicoloidal 2.0
Broca Piloto 2/3	Broca Cônica 3,5
Broca Helicoloidal 3.0	Broca Cônica 3.5+
Broca Helicoloidal 3.3	Broca Cônica 3.75
Broca Piloto 3/3.75	Broca Cônica 3.75+
Rotação de Perfuração: 800 RPM	Rotação de Perfuração: 800 RPM

Fonte: Elaboração própria.

Figura A7 - Procedimento cirúrgico da **Publicação 1**



*Etapas do procedimento cirúrgico na metáfise tibial dos coelhos: Tricotomia e antissepsia (A); Desenho esquemático para delimitar a metáfise tibial (B e C); Anestesia local (D); Incisão em camadas (G e H); Descolamento e exposição da tíbia (I); Perfuração segundo as recomendações do fabricante sob irrigação salina estéril abundante (J, F, L, M, M e O); Instalação dos implantes (P, Q, R e S); Implantes e parafusos de cobertura instalados (T e U); Sutura interna da fáscia muscular com fio reabsorvível e externa com fio não reabsorvível (V e W)

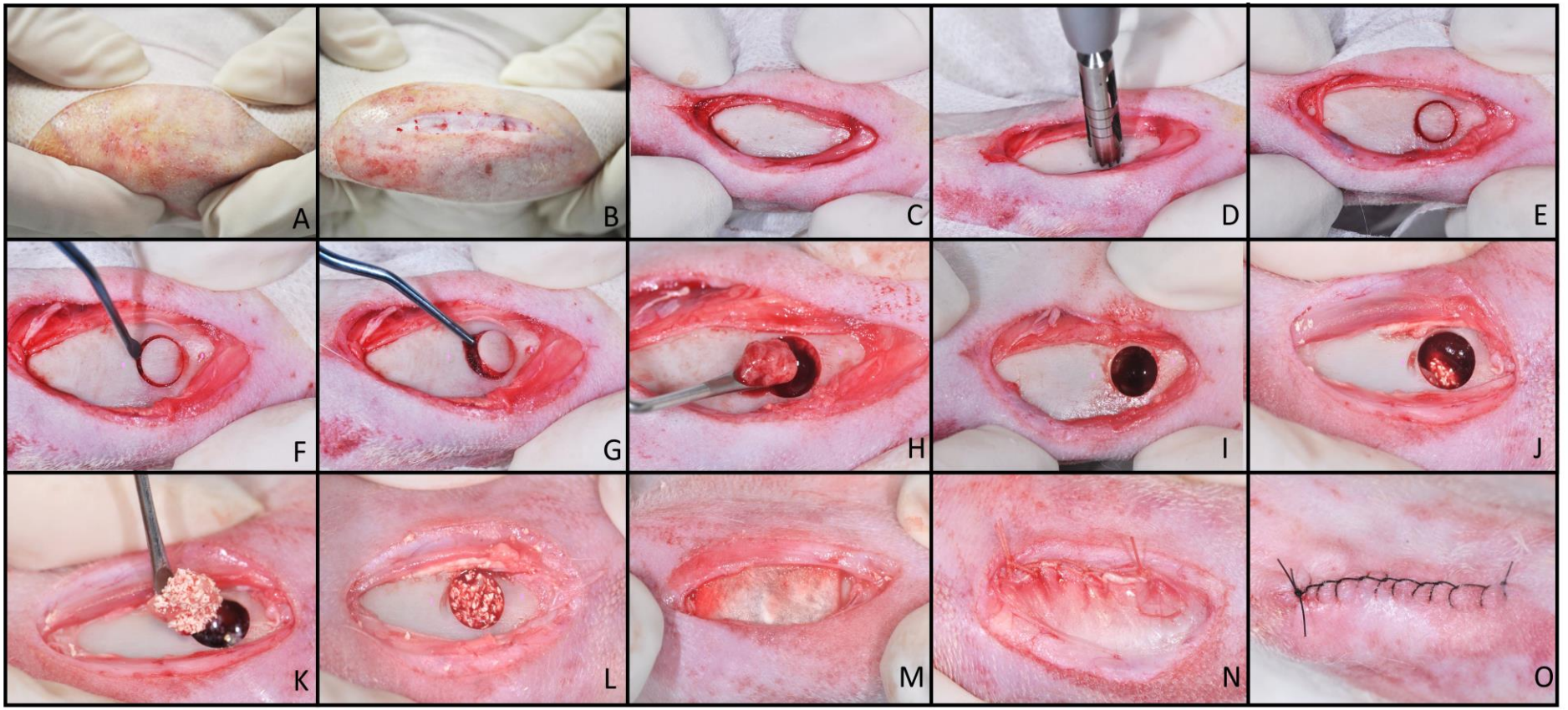
Fonte: Elaboração própria.

Publicações 2 e 3:

Publicação 2 (DIA 0) - Criação bilateral de um defeito de 5mm de diâmetro na metáfise tibial e enxerto

Os procedimentos pré-cirúrgicos foram os mesmos da Publicação 1 (Figuras A8A, A8B e A8C). O preparo para criação do defeito cirúrgico foi realizado utilizando uma broca trefina de 5mm de diâmetro (3i, Neodent, Brasil), acoplada em contra-ângulo (INTRAmatic® 2068 FGBN, Kavo, Brasil) e micromotor (INTRAmatic® 181 DBN, Kavo, Brasil) e sob refrigeração abundante com soro fisiológico estéril 0,9% (Linhamax®, Eurofarma, Brasil) (Figuras A8D e A8E). O tecido ósseo ostectomizado foi removido e a medula óssea coletada com o auxílio da cureta de Lucas nº86 (Figuras A8F, A8G, A8H e A8I). Marcações circulares também foram realizadas a 2mm anterior e a 2 mm posterior às margens do defeito cirúrgico e preenchidas com guta percha. Essas marcações ficaram localizadas sobre uma linha imaginária longitudinal que divide o defeito cirúrgico ao meio. As marcações foram feitas com uma broca carbide tronco-cônica (Broca Carbide Tronco Cônica p/ Alta Rotação n° 16 702, Dentsply, Brasil) acoplada em alta rotação (Turbina EXTRAtorque 605C, Kavo, Brasil) sob irrigação contínua com solução salina estéril 0,9% (Linhamax®, Eurofarma, Brasil). Estas marcações foram úteis para a identificação do centro do defeito cirúrgico original durante a instalação dos implantes e processamento laboratorial, permitindo localizar as margens ósseas originais do defeito durante a análise histológica. Depois de preencher os defeitos com os respectivos materiais (Grupo Controle - DBB e Grupo Experimental - DBB/BM), uma membrana de colágeno foi alocada na superfície do defeito (Jason® membrane, Straumann®, Curitiba, Brasil) (Figuras A8J, A8K, A8L e A8M). Finalmente, os tecidos moles foram reposicionados e suturados da mesma forma descrita na Publicação 1 (Figuras A8N e A8O).

Figura A8 - **Publicação 2** (Dia 0) - Criação bilateral de um defeito de 5mm de diâmetro na metáfise tibial e enxerto



*Etapas do procedimento cirúrgico: Tricotomia e antissepsia (A); Incisão em camadas (B); Descolamento e exposição da metáfise tibial (C); Preparo para criação do defeito cirúrgico realizado com uma broca trefina de 5mm de diâmetro (D e E); Exérese do tecido ósseo ostectomizado (F e G) Coleta da medula óssea fresca com o auxílio da cureta de Lucas (H); Esvaziamento da loja óssea e preenchimento com os diferentes biomateriais de acordo com os diferentes grupos (Grupo Controle - lado direito: OBD e Grupo Experimental - lado esquerdo: OBD/MO)(I, J, K e L); Uma membrana de colágeno (Jason® membrane, Straumann®, Curitiba,Brasil) também foi alocada sobre a janela óssea (M); Sutura interna da fáscia muscular com fio reabsorvível e externa com fio não reabsorvível (N e O).

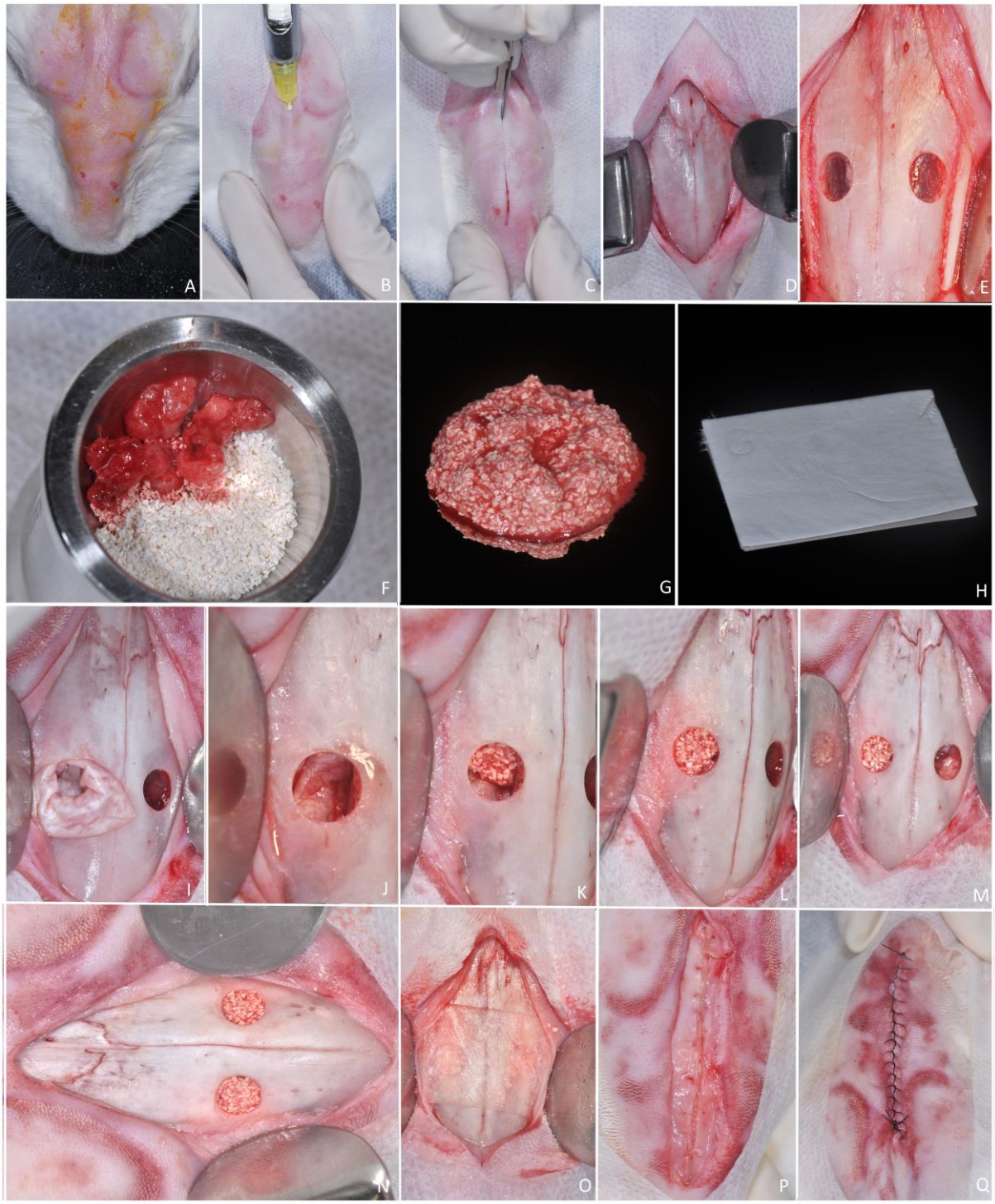
Fonte: Elaboração própria.

Publicação 3 (DIA 0) - Elevação bilateral do assoalho do seio maxilar e enxerto

Os animais foram submetidos à elevação do assoalho do seio maxilar e enxertia com acesso extra-oral. Para isso, uma incisão de 5cm foi realizada seguindo a linha média do dorso nasal após tricotomia, antissepsia e anestesia local na região (Figuras A9A, A9B e A9C). Descolamento de ambos os lados da sutura naso-incisal foi realizado e o osso nasal foi exposto até a sutura naso-frontal (Figuras A9D). Com o auxílio de uma broca trefina de 5mm de diâmetro, com o centro localizado a uma distância de cerca de 5 mm lateralmente à linha média do dorso nasal e cerca de 10mm à frente do ápice da sutura naso-frontal, uma janela óssea em ambos os lados foi obtida (Figuras A9E). Quando a mucosa do seio foi exposta, os instrumentos de elevação do assoalho sinusal foram utilizados (Neodent, Curitiba, Paraná, Brasil). A mucosa sinusal foi descolada 3-4mm nas porções medial, lateral, anterior e inferior da janela óssea obtida. O espaço criado foi forrado com uma membrana de colágeno (Jason® membrane, Straumann®, Curitiba, Brasil) (Figura A9H, A9I e A9J) e posteriormente preenchido com os biomateriais de acordo com os diferentes grupos (Figura A9F, A9G, A9K, A9L, A9M e A9N) e recobertos com uma membrana de colágeno (Jason® membrane, Straumann®, Curitiba, Brasil) (Figura A9O). Os tecidos moles foram reposicionados e suturados por planos por meio de uma sutura continua festonada com fio reabsorvível (Vicryl® ETHICON, 4-0, Johnson & Johnson, Brasil) e não reabsorvível (Nylon 3-0, Shalon®, Brasil) (Figuras A9P e A9Q).

Ao final das cirurgias do dia 0, os animais receberam doses de analgésico e antibiótico conforme descrito na Publicação 1.

Figura A9 - Publicação 3 (Dia 0) - Elevação bilateral do assoalho do seio maxilar e enxerto



*Etapas do procedimento cirúrgico para acesso extra-oral e elevação do assoalho do seio maxilar: Tricotomia e antissepsia (A); Anestesia local (B); Incisão em camadas (C); Descolamento de ambos os lados da sutura naso-incisal e exposição do osso nasal até a sutura naso-frontal (D); Obtenção de uma janela óssea bilateral com o auxílio de uma broca trefina de 5mm de diâmentro (E); Descolamento da membrana sinusal e preenchimento da loja com uma membrana de colágeno (Jason® membrane, Straumann®, Curitiba,Brasil) (H, I e J) e os materiais de enxerto de acordo com os diferentes grupos (F, G, K, L, M e N); Uma membrana de colágeno (Jason® membrane, Straumann®, Curitiba,Brasil) também foi alocada sobre a janela óssea (O); Sutura interna da fáscia muscular com fio reabsorvível e externa com fio não reabsorvível (P e Q)

Fonte: Elaboração própria.

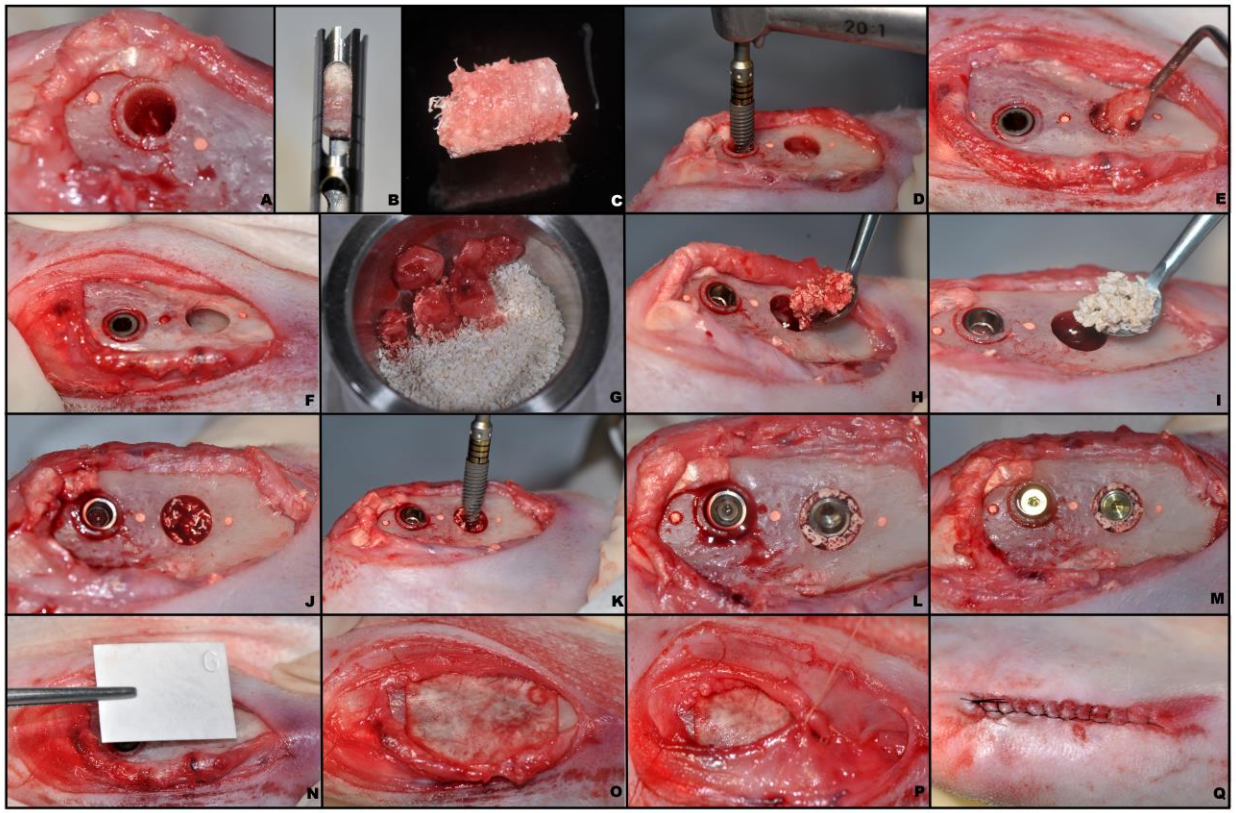
Publicação 2 (DIA 90) – Instalação dos implantes na metáfise tibial previamente enxertada e criação do segundo defeito de 5mm de diâmetro na metáfise tibial, enxerto e instalação imediata de implante

Novamente os animais foram submetidos aos procedimentos pré-cirúrgicos. Incisão dermoperiosteal na região e descolamento permitiram acesso ao defeito ósseo previamente obtido e enxertado. Com o auxílio de uma sonda milimetrada, o centro do defeito cirúrgico foi localizado para instalação dos implantes. Previamente à perfuração para instalação do implante, foi coletada uma biópsia óssea do defeito utilizando uma broca trefina de 3.3mm (Figura A10A, A10B e A10C). O preparo para instalação dos implantes foi realizado do lado direito e esquerdo, de acordo com as recomendações dos fabricantes do sistema de implantes (Neodent®, Curitiba, Paraná, Brasil), de modo que o osso foi fresado com brocas de metal sob refrigeração abundante com soro fisiológico. A perfuração para instalação dos implantes foi realizada com a sequência padronizada de brocas (Figura A6). Os implantes foram inseridos até ao nível ósseo e os parafusos de cobertura foram rosqueados. Foi instalado um implante do lado direito e um no esquerdo de acordo com os diferentes grupos (CI ou HCI) (Figura A10 D).

Ainda neste mesmo ato cirúrgico, os animais foram submetidos à confecção do segundo defeito de 5mm de diâmetro na metáfise tibial e preenchimento com enxerto (DBB ou DBB/BM de acordo com o grupo experimental) (Figura A10E, A10F, A10G, A10H, A10I e A10J). A confecção do defeito e seu preenchimento foram realizados da mesma forma descrita para o primeiro defeito na tíbia, contudo, este defeito foi realizado à 2mm da marcação inferior com guta percha do primeiro defeito e recebeu um implante imediatamente após (Figura A10K, A10L, A10M, A10N e

A10O). Finalmente, os tecidos moles foram reposicionados e suturados da mesma forma descrita na **Publicação 1** (Figura A10P e A10Q).

Figura A10 - Publicação 2 (Dia 90) - Instalação dos implantes na metáfise tibial previamente enxertada e criação do segundo defeito, enxerto e instalação imediata do implante na metáfise tibial



*Coleta da biópsia óssea da região previamente enxertada com uma broca trefina de 3,3 mm de diâmetro (A, B e C); Perfuração e instalação do implante na loja previamente enxertada (D); Coleta da medula óssea fresca com o auxílio da cureta de Lucas da segunda loja óssea e esvaziamento da loja óssea (E e F); Perfuração de acordo com as recomendações do fabricante para cada sistema de implantes e preenchimento da segunda loja óssea com os materiais de acordo com os diferentes grupos (DBB vs. DBB/BM)(G, H, I e J); Imediata instalação do implante na segunda loja óssea (K); Implantes e parafusos de cobertura instalados (L e M); Uma membrana de colágeno (Jason® membrane, Straumann®, Curitiba,Brasil) foi alocada sobre a janela óssea (N e O); Sutura interna da fáscia muscular com fio reabsorvível e externa com fio não reabsorvível (P e Q).

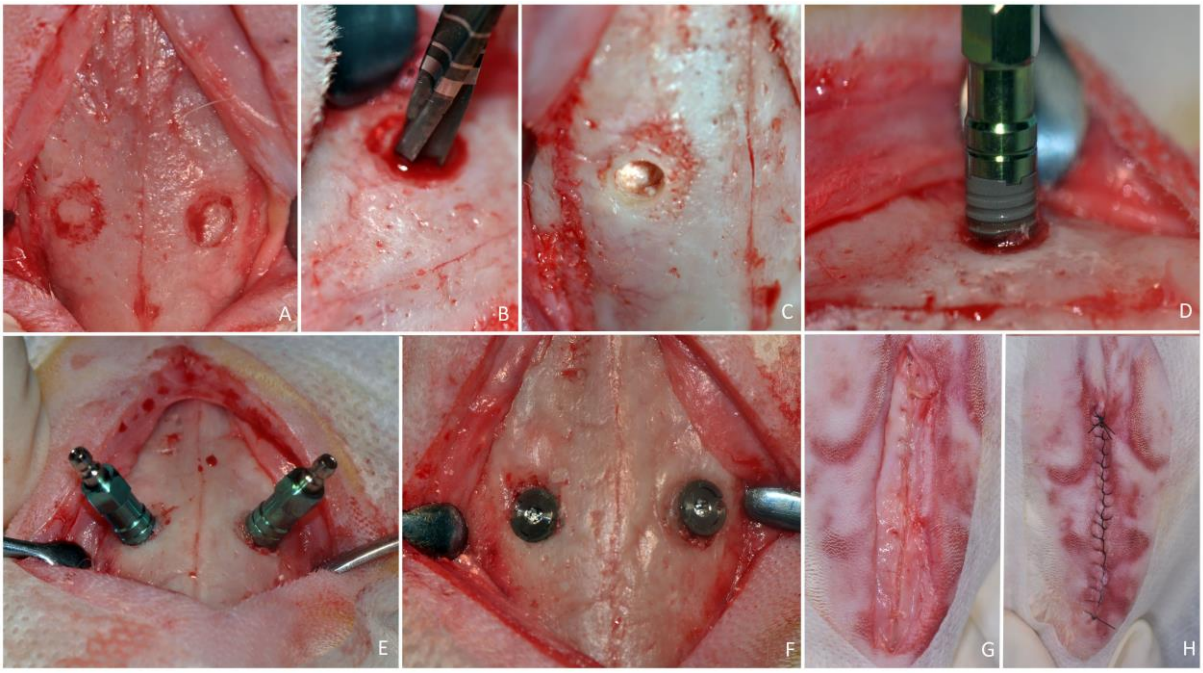
Fonte: Elaboração própria.

Publicação 3 (DIA 90) – Instalação dos implantes na área de elevação do assoalho do seio maxilar com enxertia prévia

Os procedimentos pré-cirúrgicos foram os mesmos descritos no dia 0. Após exposição da área enxertada (Figura A11A), foi executado o preparo para instalação de implantes curtos (Neodent®, Curitiba, Brasil – Kit WS), de modo que o osso foi fresado com brocas de metal sob refrigeração abundante com soro fisiológico estéril (Figura A11B e A11C). A perfuração para instalação dos implantes foi realizada com a sequência padronizada de brocas para implantes de 4mm x 5mm (Sequência de fresagem: Broca lança, broca helicoidal 2.0, broca piloto 2/3, broca helicoidal 3.0, broca helicoidal 3.3, broca piloto 3.3/4). Os implantes foram inseridos até ao nível ósseo e os parafusos de cobertura foram rosqueados (Figura A11D e A11E). Cada animal recebeu de forma randômica um tipo de implante em cada seio maxilar (NP ou AQ). Finalmente, os tecidos foram reposicionados e suturados conforme descrito no dia 0.

Ao final das cirurgias do dia 90, os animais receberam doses de analgésico e antibiótico conforme descrito na **Publicação 1**.

Figura A11 - Publicação 3 (Dia 90) - Instalação dos implantes na área de elevação do assoalho do seio maxilar com enxertia prévia



*Etapas do procedimento cirúrgico para instalação dos implantes: Acesso a área previamente enxertada (A); Perfuração e instalação bilateral dos implantes e dos parafusos de cobertura (B, C, D, E e F); Sutura plano a plano (G e H).
Fonte: Elaboração própria.

Publicações 2 e 3 (DIA 180) - Eutanásia

Os animais foram eutanasiados através de sobredose anestésica 90 dias pós-instalação dos implantes.

Avaliação Biomecânica (Torque de Inserção e Torque de Remoção)

Publicação 1: Todos os implantes instalados foram submetidos à avaliação do torque de inserção, sendo que para isso, foi anotado o torque após a instalação dos implantes estando os mesmos à nível ósseo. Após a eutanásia, em cada período de análise (2, 4 e 8 semanas), as porções mediais das amostras obtidas da tíbia foram reabertas para exposição dos implantes e realização do torque reverso. As amostras foram estabilizadas em uma pequena morsa, e uma chave hexagonal foi conectada tanto no implante como no torquímetro (Tohnichi, modelo ATG24CN-S - com escala graduada de 0.05 N/cm, medindo a força de 3 a 24 N/cm) (Figura A12) e foi realizado um movimento anti-horário para remoção dos implantes aumentando-se o torque até que ocorresse a rotação do implante no interior do tecido ósseo, rompendo-se completamente a interface osso-implante, momento em que o torquímetro registrou o pico máximo de torque necessário para esse rompimento. Este pico máximo necessário para movimentar o implante foi anotado como o valor do torque de remoção. Os remanescentes da tíbia referentes aos implantes superiores que foram previamente removidos para a análise do torque de remoção foram então reduzidos e imersos em formaldeído 10% por 48 horas, lavados em água corrente por 4 horas e posteriormente descalcificados em solução de ácido etilenodiaminotetracético (E.D.T.A. 7%) por dois meses.

Figura A12 - Torquímetro Digital Portátil utilizado na análise biomecânica

Fonte: Elaboração própria.

Análise Microtomográfica (µCT)

Após a eutanásia, as biopsias foram removidas e fixadas em formol a 10% por 48 horas e mantidas em álcool 70º até o momento do escaneamento em µCT scan (Skyscan, Aatselaar, Belgium). **Publicação 1:** biópsias contendo os implantes inferiores da tíbia com diferentes macroestruturas (CI vs. HCI); **Publicação 2:** biópsias ósseas coletadas das tíbias previamente enxertadas (DBB vs. DBB/BM) e biópsias contendo os implantes com diferentes macroestruturas (CI vs. HCI) das tíbias prévia ou imediatamente enxertadas (DBB vs. DBB/BM); **Publicação 3:** biópsias contendo os implantes com diferentes microestruturas (AQ vs. NP) dos seios maxilares previamente enxertados (DBB vs. DBB/BM).

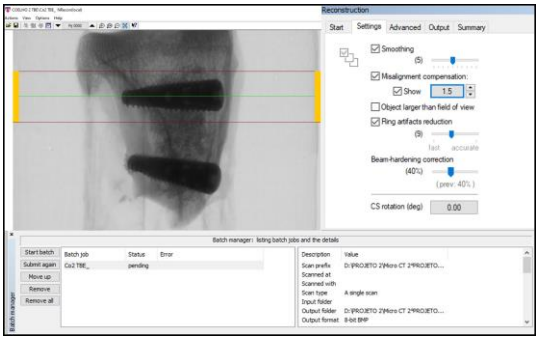
Os seguintes parâmetros foram utilizados para o escaneamento das amostras: Voxel de 18 μm^3, magnificação da imagem de 10x, voltagem do tubo de raios-X de 50 kV, feixe de 496 uA e a corrente elétrica ajustada para 0.1 mA. As imagens tridimensionais foram reconstruídas (NRecon 1.6.1.5 - SkyScan N. V. Belgium) com os seguintes parâmetros: Beam Hardening Correction 40%, Ring Artifact Correction = 9, Smoothing = 5, Postalignment = 1.00 (Figura A13). Posteriormente, as imagens escaneadas foram reorientados para realização da análise volumétrica (Data Viewer SkyScan N. V. Belgium) (Figura A14). As mensurações para a análise Volumétrica (3D) foram realizadas utilizando um software específico (CT Analyser 1.10.1.0 - SkyScan, Belgium), seguindo a seleção de uma área de interesse circular ao redor dos implantes que ultrapassou em 5mm todo o seu diâmetro (ROI - region of interest). O limiar em escala de cinza utilizado foi de 25 a 90 (Figura A15).

A ROI estabelecida para as biópsias coletadas da tíbia previamente enxertada abrangeu todo seu diâmetro e comprimento em um formato cilíndrico (**Publicação 2**). Enquanto que a ROI ao redor dos implantes foi delimitada em todo seu comprimento e ultrapassou em 5mm o seu diâmetro (**Publicações 1, 2 e 3**). Apesar dos implantes terem recebido parafuso de cobertura, houve formação óssea dentro do parafuso de cobertura em alguns casos. Para evitar que essa formação óssea interferisse na análise do volume de tecido mineralizado ao redor do implante, foi estabelecido um segundo ROI para remover o volume de tecidos mineralizados que eventualmente poderiam ter sido formados nesta região. Com os resultados obtidos nas duas ROIs, foi possível definir o volume de tecidos mineralizados como uma porcentagem utilizando a seguinte fórmula:

Volume Total de tecidos mineralizados no ROI principal – Volume de tecidos mineralizados dentro do parafuso de cobertura = Volume de tecidos mineralizados (Pinotti et al., 2018).

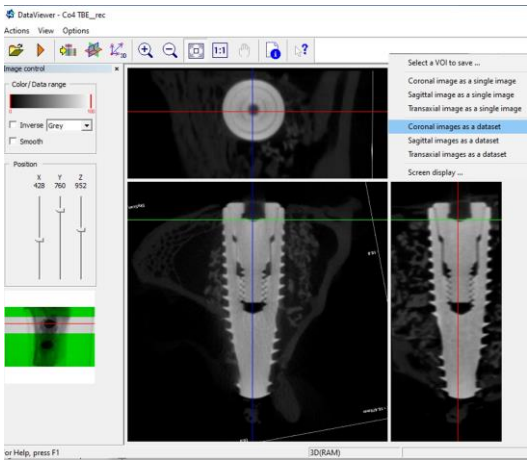
A análise foi realizada por um único examinador, em esquema cego para o tipo de tratamento.

Figura A13 - Reconstrução das peças escaneadas com o software NRecon (**Publicações 1, 2 e 3**)



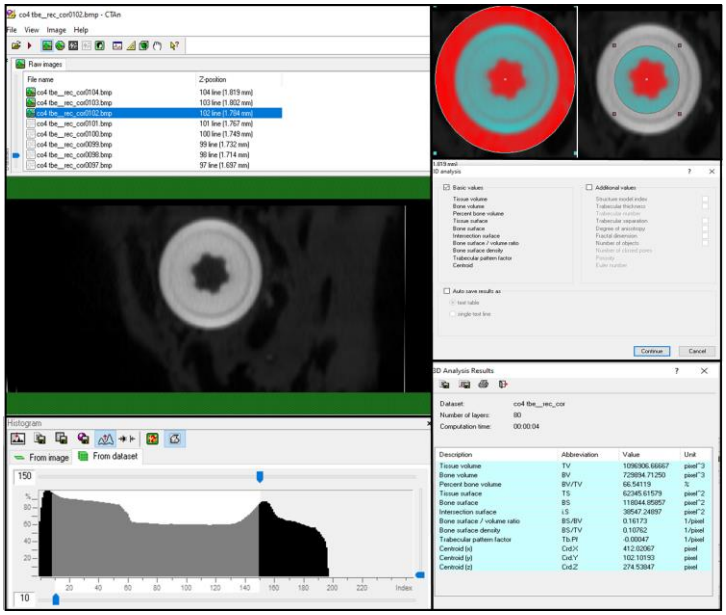
Fonte: Elaboração própria.

Figura A14 - Reorientação dos planos com o software Data Viewer (**Publicações 1, 2 e 3**)



Fonte: Elaboração própria.

Figura A15 - Análise tridimensional com o software CT Analyser (**Publicações 1, 2 e 3**)



Fonte: Elaboração própria.

Histologia Descritiva dos cortes descalcificados

Publicação 1: Após o período de descalcificação, as peças provenientes dos sítios aonde os implantes foram removidos para análise do torque de remoção, foram incluídas em parafina, cortadas em micrótomo (6µm de espessura) e coradas pela técnica de hematoxilina-eosina (HE). Foram obtidas 5 lâminas com 3 cortes em cada na região central do sítio aonde o implante estava inserido. Foram avaliados 3 cortes que estavam distantes 36µm entre si, sendo que o primeiro corte para avaliação foi selecionado de forma randômica. Os cortes foram avaliados por meio de um microscópio óptico DIASTAR (Leica Reichert & Jung products, Alemanha) com aumentos de 5x e 10x sendo avaliado a qualidade do tecido ósseo nas proximidades do leito do implante. Essa análise foi executada por um examinador experiente, treinado e cego para o tipo de implante utilizado.

Análise Histométrica

Biópsias ósseas coletadas das áreas previamente enxertadas - cortes descalcificados (**Publicação 2**): Após o período de descalcificação, as biópsias ósseas coletadas das áreas previamente enxertadas foram incluídas em parafina, cortadas em micrótomo (6µm de espessura) e coradas pela técnica de hematoxilina-eosina (HE). Foram obtidas 5 lâminas da biópsia. Foi avaliado toda extensão do corte da biópsia escolhido aleatoriamente. Os cortes foram avaliados por meio de um microscópio óptico DIASTAR (Leica Reichert & Jung products, Alemanha) com aumento de 10x sendo avaliado a quantidade de tecido ósseo, biomaterial e tecido conjuntivo. A região correspondente à cortical externa da tíbia não foi considerada na análise (Figura A16). Para padronizar a análise os cálculos foram realizados como porcentagem da área total do enxerto (ATE = 100%) e as seguintes fórmulas foram utilizadas:

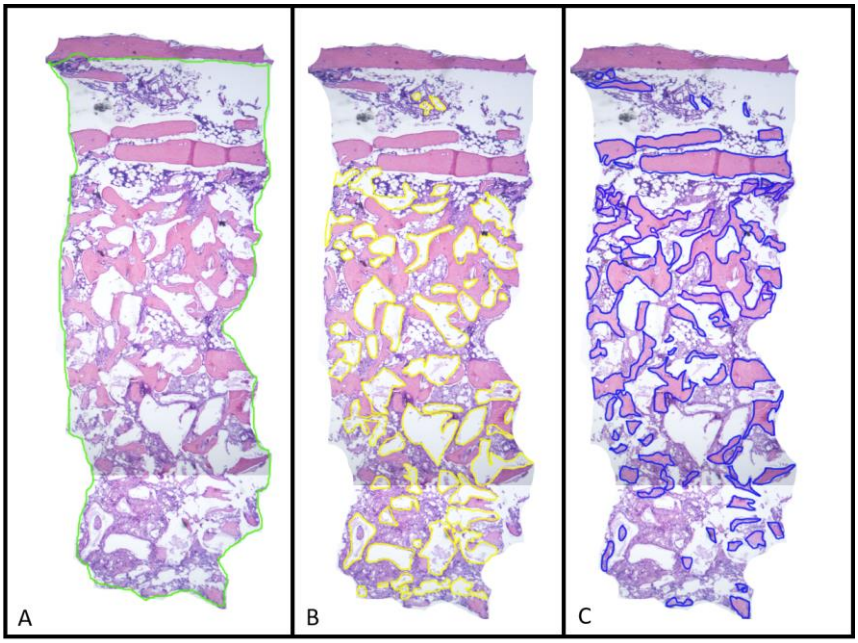
a) $\%\ \text{Tecido ósseo} = \frac{\text{Área total de osso neoformado X 100}}{\text{Área total do enxerto}}$

b) $\%\ \text{Biomaterial} = \frac{\text{Área total de biomaterial X 100}}{\text{Área total do enxerto}}$

c) % Tecido conjuntivo = Área total do enxerto – (% Tecido ósseo + % Biomaterial)

Essa análise foi executada por um examinador experiente, treinado e cego para o tipo de enxerto utilizado.

Figura A16 - Análise das biópsias ósseas coletadas das áreas previamente enxertadas - cortes descalcificados (**Publicação 2**):



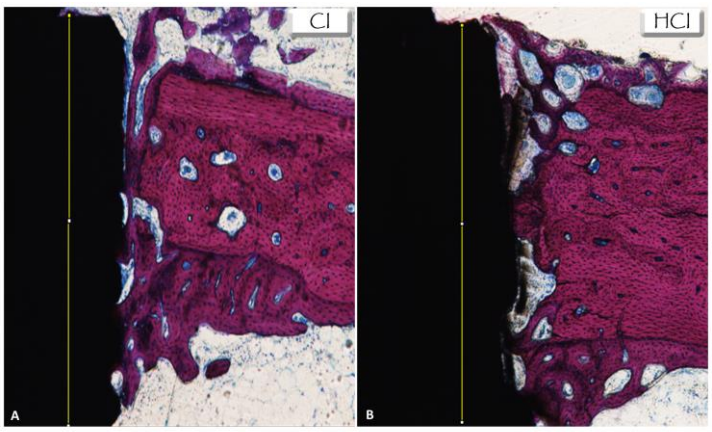
* Linha verde: Área total do enxerto (A); Linha amarela: Área de biomaterial (B); Linha azul: Área de osso neoformado (C).

Biópsias contendo os implantes - cortes não descalcificados (**Publicações 1, 2 e 3**): Após análise microtomográfica, as peças com os implantes foram desidratadas em solução de álcool em séries de concentração crescente. A infiltração plástica foi realizada com misturas de glicolmetacrilato (Technovit 7200 VLC) e álcool etílico, seguindo aumentos gradativas na concentração do glicometacrilato até a infiltração nessa solução pura. Os espécimes foram incluídos em resina, polimerizados e seccionados longitudinalmente ao longo do eixo principal do implante com um disco de diamante de alta precisão. Os blocos foram montados em lâmina acrílica com o auxílio da resina Tecnovit 4000 (Kulzer, Wehrheim, Alemanha), e utilizando um sistema de corte e microdesgaste (Exact-Cutting, System, Apparatebau Gmbh, Hamburgo, Alemanha) as lâminas foram processadas para que tivessem uma secção

de aproximadamente 50 a 70 µm de espessura. As peças foram coradas com Stevenel's Blue e fucsina. A análise histométrica avaliou a quantidade de contado direto do osso com a porção coronal do implante (3,5mm de extensão em linha reta no comprimento do implante - **Publicação 1**) (Figura A18) ou a superfície ao longo de todo implante (BIC %) (**Publicações 2 e 3**). As mensurações foram realizadas por meio de fotomicrografias obtidas por um microscópio óptico (DIASTAR, Leica Reichert & Jung products, Alemanha), com aumento de 10x, com o auxílio de uma câmera de vídeo (Leica Reichert & Jung products, Alemanha). A determinação dos valores foi realizada utilizando um software analisador de imagens (Image J, Jandel Scientific, San Rafael, CA, USA), por um examinador cego, calibrado e treinado para essa análise.

a) $$\text{BIC \%} = \frac{\text{Contato direto do osso com a superfície do implante X 100}}{\text{Comprimento do Implante}}$$

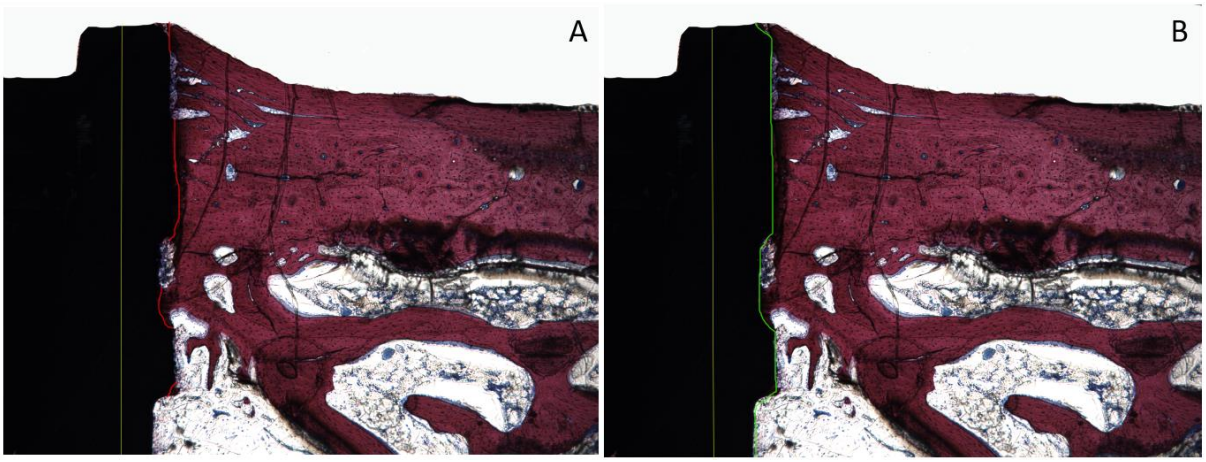
Figura A17- Extensão padronizada na região cervical dos implantes para avaliação do contato osso-implante (**Publicação 1**)



***** Implante Cilíndrico (A) e Implante Cônico Híbrido (B). Linha amarela: extensão padronizada de 3,5mm.

Fonte: Elaboração própria.

Figura A18 - Análise do contato osso-implante (BIC%) (**Publicação 1**)



* Extensão do contato osso-implante (A) e extensão da superfície do implante (B).
Fonte: Elaboração própria.

Análise Estatística

Publicação 1: Os dados das análises biomecânica (torque de inserção e remoção), microtomográfica (BV/TV) e histométrica (%BIC) foram submetidos ao teste de normalidade de Shapiro-Wilk que confirmou que os dados se distribuíram de acordo com o teorema da distribuição central. O teste paramétrico t-pareado foi utilizado para análise inferencial dos dados comparando-se dos diferentes grupos de macroestruturas (CI vs. HCI). O teste de One-way Anova foi aplicado para comparação entre os diferentes períodos de avaliação dentro de cada grupo.

Publicações 2 e 3: Os dados obtidos das análises microtomográfica (BV/TV%) e histométrica (**Publicação 2:** % de osso, % de biomaterial e % de tecido conjuntivo das biópsias ósseas coletadas e **Publicações 2 e 3:** % BIC) também foram submetidos ao teste de normalidade de Shapiro-Wilk confirmando a distribuição central dos dados. O teste t-pareado foi utilizado para análise inferencial dos dados comparando-se dos diferentes grupos de macroestruturas (**Publicação 2:** CI vs. HCI) e microestruturas (**Publicação 3:** NP vs. AQ). O teste t-não pareado para comparação

entre os resultados das áreas enxertadas (**Publicações 2 e 3:** DBB vs. DBB/BM) e na comparação da forma de instalação dos implantes (**Publicação 2:** Imediata vs.Tardia).

O software GraphPad Prism 6 (San Diego, CA, USA) foi utilizado para realização dos testes estatísticos. Todos os testes desse estudo foram aplicados com nível de significância de 95% ($p<0.05$).

O cálculo de amostra foi realizado através do teste de t-pareado com base nos dados histométricos do contato osso-implante do estudo de Faeda et al 2012, o qual avaliou o efeito de diferentes superfícies de implantes sobre a osseointegração em coelhos. Foi verificado que a diferença entre as médias do BIC entre diferentes superfícies de implantes para promover uma diferença estatisticamente significativa foi de 25.95% com desvio padrão de 8.34. Portanto, a utilização de 8 coelhos por grupo em cada período seria suficiente para obtenção de um poder β do estudo maior que 0.9 e α de 0.05.

ANEXO A – Certificado de aprovação nº 11/2016 do Comitê de Ética na Utilização de Animais (CEUA) (**Publicação 1**)

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

CERTIFICADO

Certificamos que a proposta intitulada ***"OSSEOINTEGRAÇÃO DE IMPLANTES DE TITÂNIO COM DIFERENTES MICRO E MACROESTRUTURAS - ESTUDO BIOMECÂNICO, HISTOMORFOMÉTRICO E IMUNO-HISTOQUÍMICO EM OSSO MEDULAR (ILÍACO) E CORTICAL (TÍBIA) DE COELHOS"***, registrada com o nº 11/2016, sob a responsabilidade do(a) **Prof(a). Dr(a). Élcio Marcantonio Júnior** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela **COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA** em reunião de 18/08/2016.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência do Projeto	Dezembro/2017
Espécie/linhagem	Coelho/ Nova Zelândia
Nº de animais	24
Peso/Idade	3-4 Kg – 5 meses
Sexo	Macho
Origem	Biotério da UNESP - Botucatu

Prof. Dr. PAULO SÉRGIO CERRI
Coordenador da CEUA

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ANEXO B – Certificado de aprovação nº 15/2017 do Comitê de Ética na Utilização de Animais (CEUA) (**Publicações 2 e 3**)

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" Câmpus de Araraquara
FACULDADE DE ODONTOLOGIA

CERTIFICADO

Certificamos que a proposta intitulada ***"IMPLANTES COM DIFERENTES MACRO E MICROESTRUTURAS INSTALADOS EM ÁREAS ENXERTADAS – ESTUDO PRÉ-CLÍNICO EM COELHOS"***, registrada com o nº 15/2017, sob a responsabilidade do(a) **Prof(a). Dr(a). Élcio Marcantonio Júnior** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela **COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA** em reunião de 20/06/2017.

Finalidade	( ) Ensino (**X**) Pesquisa Científica
Vigência do Projeto	Outubro/2018
Espécie/linhagem	Coelho/Nova Zelândia
Nº de animais	16
Peso/Idade	3-4 Kg – 5 meses
Sexo	Macho
Origem	Biotério da UNESP – Campus de Botucatu

Profa. Dra. **CARINA APARECIDA FABRÍCIO DE ANDRADE**
Coordenadora da CEUA

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Não autorizo a publicação deste trabalho até março de 2022.

Araraquara, 30 de março de 2020.

Amanda de Carvalho Silva Leocádio