UNIVERSIDADE FEDERAL DE CAMPINA GRANDE

CENTRO DE SAÚDE E TECNOLOGIA RURAL

UNIDADE ACADÊMICA DE MEDICINA VETERINÁRIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E SAÚDE ANIMAL

DANIEL CARDOSO GARCIA

Avaliação da osseointegração do implante cerâmico cilíndrico de β - tricálcio fosfato (β - TCP) de fase pura em defeitos segmentares críticos do osso do rádio de coelhos

Patos - PB
Fevereiro/2022

DANIEL CARDOSO GARCIA

Avaliação da osseointegração do implante cerâmico cilíndrico de β - tricálcio fosfato (β - TCP) de fase pura em defeitos segmentares críticos do osso do rádio de coelhos

Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Doutor em Ciência e Saúde Animal.

Orientador: Professor Doutor Marcelo Jorge Cavalcanti de Sá

Patos - PB
Fevereiro/2022

G216a Garcia, Daniel Cardoso.

Avaliação da osseointegração do implante cerâmico cilíndrico de β - tricálcio fosfato (β - TCP) de fase pura em defeitos segmentares críticos do osso do rádio de coelhos / Daniel Cardoso Garcia. - Patos, 2022.

144 f. : il. Color

Tese (Doutorado em Ciência e Saúde Animal) - Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, 2022.

"Orientação: Prof. Dr. Marcelo Jorge Cavalcanti de Sá".

Referências.

1. Biomateriais - Implante Cerâmico. 2. Implantes Ósseos. 3. Beta - Tricálcio Fosfato. 4. β - TCP. 5. Enxerto ósseo Cortical Alógeno. 6. Osteossíntese. 7. Osso do Rádio de Coelhos. I. Sá, Marcelo Jorge Cavalcanti de. II. Título.

CDU 62:61(043)

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECÁRIA ITAPUANA SOARES DIAS GONÇALVES CRB-15/93

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
PÓS-GRADUAÇÃO EM CIÊNCIA E SAÚDE ANIMAL
Rua Aprigio Veloso, 882, - Bairro Universitário, Campina Grande/PB, CEP 58429-900

FOLHA DE ASSINATURA PARA TESES E DISSERTAÇÕES

DANIEL CARDOSO GARCIA

AVALIAÇÃO DA OSSEOINTEGRAÇÃO DO IMPLANTE CERÂMICO CILÍNDRICO DE B - TRICÁLCIO FOSFATO (B - TCP) DE FASE PURA EM DEFEITOS SEGMENTARES CRÍTICOS DO OSSO DO RÁDIO DE COELHOS

Tese apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Doutor em Ciência e Saúde Animal.

Aprovada em: 21/02/2022

BANCA EXAMINADORA:

Prof. Dr. Marcelo Jorge Cavalcanti de Sá (Orientador - PPGCSA/UFCG)

Prof. Dr. Pedro Isidro da Nóbrega Neto (Examinador Interno - PPGCSA/UFCG)

Prof. Dr. Sérgio Ricardo Araújo de Melo e Silva (Examinador Externo - UFCG)

Prof. Dr. Daniel Curvello de Mendonça Müller (Examinador Externo - UFSM)

Prof. Dr. Marcelo Weinstein Teixeira (Examinador Externo - UFRPE)

Documento assinado eletronicamente por **Daniel Curvello de Mendonça Müller**, **Usuário Externo**, em 21/02/2022, às 09:46, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **SERGIO RICARDO ARAUJO DE MELO E SILVA**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 21/02/2022, às 12:08, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **MARCELO JORGE CAVALCANTI DE SA**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 21/02/2022, às 14:17, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **PEDRO ISIDRO DA NOBREGA NETO**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 22/02/2022, às 11:20, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **ANTONIO FERNANDO DE MELO VAZ**, **COORDENADOR(A)**, em 22/02/2022, às 15:56, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
PÓS-GRADUAÇÃO EM CIÊNCIA E SAÚDE ANIMAL
Rua Aprigio Veloso, 882, - Bairro Universitário, Campina Grande/PB, CEP 58429-900

REGISTRO DE PRESENÇA E ASSINATURAS

ATA DA DEFESA DE TESE

Aos 21 dias do mês de fevereiro do ano de 2022, às 08h00min, sob a presidência do Professor Doutor Marcelo Jorge Cavalcanti de Sá, por meio de videoconferência na plataforma Google Meet, reuniram-se os Professores Doutores Pedro Isidro da Nóbrega Neto, Sérgio Ricardo Araújo de Melo e Silva, Daniel Curvello de Mendonça Müller e Marcelo Weinstein Teixeira, para participarem da banca de Tese do discente Daniel Cardoso Garcia. O resultado da Tese intitulada: "Avaliação da osseointegração do implante cerâmico cilíndrico de β - tricálcio fosfato (β - TCP) de fase pura em defeitos segmentares críticos do osso do rádio de coelhos", foi considerado APROVADO. Do que, para constar, eu, orientador, lavrei a presente Ata, que depois de lida e aprovada, vai assinada por mim e com anuência dos demais membros da banca examinadora.

PROF. DR. MARCELO JORGE CAVALCANTI DE SÁ

Presidente e Orientador

PROF. DR. PEDRO ISIDRO DA NÓBREGA NETO

Examinador Interno

PROF. DR. SÉRGIO RICARDO ARAÚJO DE MELO E SILVA

Examinador Externo

PROF. DR. DANIEL CURVELLO DE MENDONÇA MÜLLER

Examinador Externo

PROF. DR. MARCELO WEINSTEIN TEIXEIRA

Examinador Externo

PROF. DR. ALMIR PEREIRA DE SOUZA

Coordenador do PPGCSA

OBSERVAÇÕES:

1 - Por não possuir cadastro como usuário externo no SEI, o examinador Marcelo Weinstein Teixeira receberá cópia da presente Ata e dará ciência e aprovação dos termos por e-mail.

2 - Os examinadores internos signatários certificam que o examinador externo acima identificado participou da defesa da tese e tomou conhecimento do teor deste documento.

Documento assinado eletronicamente por **Daniel Curvello de Mendonça Müller**, **Usuário Externo**, em 21/02/2022, às 09:46, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **SERGIO RICARDO ARAUJO DE MELO E SILVA**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 21/02/2022, às 12:08, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **PEDRO ISIDRO DA NOBREGA NETO**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 22/02/2022, às 11:20, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **MARCELO JORGE CAVALCANTI DE SA**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 22/02/2022, às 12:54, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

Documento assinado eletronicamente por **ANTONIO FERNANDO DE MELO VAZ**, **COORDENADOR(A)**, em 02/03/2022, às 14:16, conforme horário oficial de Brasília, com fundamento no art. 8º, caput, da Portaria SEI nº 002, de 25 de outubro de 2018.

A autenticidade deste documento pode ser conferida no site https://sei.ufcg.edu.br/autenticidade, informando o código verificador **2128021** e o código CRC **103B4AF9**.

DEDICATÓRIAS E AGRADECIMENTOS

Dedico este trabalho a todos aqueles que estão comprometidos com a pesquisa e tentam de alguma forma, buscar saídas que melhorem a qualidade de vida das pessoas e dos animais, diminuindo seu sofrimento.

Agradeço primeiramente a Deus por tudo que tenho na vida, em especial minha família e meu trabalho, e por tudo o que Ele pôde me proporcionar ao longo deste caminho. Só tenho a agradecer.

Agradeço à minha esposa e colega de trabalho Larissa Eckmann Mingrone Garcia e minha filha Manuela Mingrone Garcia pelo apoio dado ao longo destes anos. Vocês foram muito importantes e deram muita força para que eu pudesse realizar este Projeto. Assumiram muitas responsabilidades, muitas delas não muito fáceis, para que tudo se encaixasse dentro de nossa rotina, enquanto me dedicava à pesquisa. Enfatizo ainda que, a ajuda de Larissa no Projeto e em nossa clínica foi indispensável e essencial durante estes anos. Vocês duas são pessoas sensacionais e maravilhosas, e tenho orgulho de tê-las ao meu lado. Amo vocês.

Agradeço à minha família, e em especial a meus pais, Carlos Alberto Santa Maria Garcia e Bartyra Cardoso Garcia por todo suporte e apoio dados ao longo da vida e durante este período, especificamente. Cada um ajudando como pôde. À minha mãe, que em minha ausência em vários momentos, sempre esteve disponível para os cuidados com minha filha. E a meu pai, pelas longas conversas, dicas e conselhos para o Projeto, mas principalmente para a vida. Leu e corrigiu muitos artigos, e mesmo não sendo da área da Medicina Veterinária, acrescentou

muito ao trabalho e aos artigos dando sua visão e fazendo suas correções. Vocês são pais sensacionais, são presentes, e têm muita luz. São meu espelho como pessoa. Amo vocês.

Agradeço ao meu orientador, Professor Dr. Marcelo Jorge Cavalcanti de Sá, pela oportunidade de realizar o Doutorado sob sua orientação na Universidade Federal de Campina Grande. Hoje mais que um colega de trabalho e orientador, o considero um amigo. Apesar da distância, este Projeto nos permitiu estreitar relações e construir uma amizade que certamente será duradoura. Muito obrigado por tudo.

Agradeço a todas as outras pessoas que de alguma forma cruzaram meu caminho e que puderam contribuir de alguma forma com este Projeto. Muitos deles já são parceiros de trabalho de longa data e que neste momento inclusive se entusiasmaram pelo tema da pesquisa e se prontificaram em ajudar da maneira como podiam. Leonardo Seade pelos procedimentos anestésicos. Rosane Melo pela realização das radiografias. Luana pela realização das micro-tomografias. Suleima Ferreira pela realização dos cortes histológicos e imagens de microscopia óptica. Agradeço também a Felipe Pinotti, pela contribuição intelectual ao Projeto, dando uma outra visão sobre a área de biomateriais e seu uso em odontologia. Contribuiu muito com a metodologia empregada, assim como para a análise dos resultados e discussão sobre o uso da cerâmica de β - TCP. Não se faz a pesquisa com uma pessoa só, mas sim com trabalho em equipe, envolvendo várias pessoas com capacidades diversas em diferentes áreas do conhecimento. Sem vocês todo este Projeto jamais teria sido concluído.

Agradeço também aos graduandos, mestrandos e doutorandos do Laboratório de Ortopedia da UFCG, assim como aos estagiários da clínica em que trabalho pela participação e contribuição aos artigos escritos ao longo deste período. É muito importante que possamos

colocar todo o conteúdo investigado no papel (e agora, online) para divulgar todo o conhecimento adquirido. Para os mais novos que galgam este caminho da pesquisa, certamente é um processo de muito aprendizado com aqueles que já passaram por outras fases, o qual contribuirá de forma indispensável para suas formações.

Universidade Federal de Campina Grande
Centro de Saúde e Tecnologia Rural
Comitê de Ética em Pesquisa
(Comissão de Ética no Uso de Animais)
Av. Santa Cecília, s/n, Bairro Jatobá, Rodovia Patos,
CEP: 58700-970, Cx postal 64, Tel. (83) 3511-3045

Ao: Prof. DR. **Marcelo Jorge Cavalcanti de Sá**

Protocolo CEP/CEUA nº046-2018

CERTIDÃO

Certificamos a V.Sa. que seu projeto INTITULADO **"AVALIAÇÃO DA OSSEOINTEGRAÇÃO DO IMPLANTE CERÂMICO CILÍNDRICO DE β - TRICÁLCIO FOSFATO (β - TCP) DE FASE PURA EM DEFEITOS SEGMENTARES CRÍTICOS DO OSSO DO RÁDIO DE COELHOS"** teve parecer consubstanciado orientado pelo regulamento interno deste comitê e foi **Aprovado**, em **REUNIÃO** NO DIA 27 DE AGOSTO DE 2018, estando à luz das normas e regulamentos vigentes no país atendidas as especificações para a pesquisa científica.

Patos, 29 de Agosto de 2018.

Rosália Severo de Medeiros
Coordenadora do CEP/CEUA/UFCG/Patos

RESUMO

A boa formação e aceleração da cicatrização óssea em casos de fraturas e grandes perdas ósseas ocasionadas por diferentes motivos são temas atuais e de grande relevância, e são estudados por vários grupos em todo mundo, tanto na medicina humana e odontologia, quanto na medicina veterinária. Em muitos casos ortopédicos, o uso de implantes e enxertos, sejam naturais ou sintéticos, tem sido utilizado com o objetivo de reparar os defeitos ósseos causados nessas situações, com diferentes resultados sendo relatados. Especificamente neste estudo, os objetivos principais foram avaliar os resultados clínicos, radiográficos, micro-tomográficos e histológicos relacionados à formação e evolução da cicatrização óssea em ossos do rádio de coelhos ostectomizados quando utilizado o implante cerâmico de β - tricálcio fosfato (β - TCP) ou enxerto ósseo cortical alógeno para preenchimento dos defeitos críticos segmentares ósseos. Para tanto, neste experimento foram utilizados 19 coelhos da raça Nova Zelândia, divididos em 3 Grupos (A, B e C) compostos de 06 animais em cada, sendo que nos animais do Grupo A foi implantado o bloco de β - TCP e nos animais do Grupo B foi utilizado o enxerto ósseo cortical alógeno para preenchimento dos defeitos. Para os animais do Grupo C, a ostectomia foi realizada como nos Grupos A e B, porém foi deixada sem qualquer tipo de preenchimento. Um animal remanescente foi utilizado para a confecção dos enxertos ósseos corticais alógenos. Tanto para o Grupo A, como para o Grupo B, placas e parafusos bloqueados de 1.5 mm confeccionados em titânio foram utilizados para realização das osteossínteses e estabilização dos implantes e enxertos nos sítios ostectomizados. Avaliações clínica, radiográfica, micro-tomografia computadorizada (μCT) e histológica foram realizadas em diferentes momentos para verificar a ocorrência de osseointegração, a função dos membros operados e a possível reabsorção dos implantes. Aos 120 dias de pós-operatório, em todos os animais do Grupo C houve crescimento ósseo nos defeitos criados, porém culminando com ocorrência de não-uniões ósseas visíveis. Já nos animais do Grupo B houve adesão celular e integração óssea sem que ocorressem quaisquer sinais de infecção ou inflamação, resultados que diferem daqueles encontrados no Grupo A, nos quais não foram observados sinais de ocorrência de osseointegração entre o implante de β - TCP e osso hospedeiro, apesar da boa aderência e posicionamento dos mesmos nos defeitos ósseos, e nem os implantes foram reabsorvidos. A biocompatibilidade do biomaterial cerâmico corroborou com os resultados de outros estudos, porém a osseocondutividade apresentou resultado diferente, ou seja, não foi observado bioatividade, fato provavelmente relacionado à característica de baixa porosidade do implante. Conclui-se então, que os implantes β - TCP de fase pura customizados não apresentaram as

características osseocondutoras e osseointegrativas citadas por outros autores e não foram eficazes para a consolidação e integração entre os ossos do hospedeiro e os biomateriais neste estudo. Novos estudos devem ser realizados para fornecer mais informações a respeito desse bloco cerâmico, principalmente quanto à sua porosidade e formato, a fim de considerá-lo como um possível biomaterial para substituição óssea que possa ser utilizado de forma rotineira na cirurgia ortopédica e traumatológica reconstrutiva em medicina veterinária.

Palavras-chave: biomateriais; implantes ósseos; cerâmica; beta - tricálcio fosfato; β - TCP; enxerto ósseo cortical alógeno; osteossíntese

ABSTRACT

Good and fast bone healing in cases of fractures and large bone losses are actual themes of such relevance, which have been widely studied by various groups worldwide, not only in human medicine and dentistry, but also in veterinary medicine. The use of different types of implants and grafts, whether natural or synthetic, has been widely tested for these purposes, also with different results being achieved. Specifically in this study, the main objectives were to evaluate the clinical, radiographic, micro-tomographic and histological results related to the formation and evolution of bone healing in ostectomized rabbit's radius bones when using a ceramic implant of β - tricalcium phosphate (β - TCP) or allogeneic cortical bone graft to fill the critical segmental bone defects created. For this purpose, 19 New Zealand rabbits were used in this experiment, divided into 3 Groups (A, B and C) composed of 06 animals each, in which the bone defects were filled with β - TCP block for animals of Group A, and with allogeneic cortical bone graft for animals of Group B. For the animals in Group C, ostectomy gap were performed as in Groups A and B, but they were left without any type of filling. A remaining animal was used to make the allogeneic cortical bone grafts. For both Group A and Group B, osteosynthesis with 1.5 mm titanium locking plates and crews were used to stabilize the implants and grafts in the ostectomized sites. Clinical, radiographic, micro-computed tomography (μCT) and histological evaluations were performed at different times to verify the occurrence of osseointegration, function of the operated limbs and possible implant resorption. At 120 days after surgery, all animals of Group C had bone ingrowth inside the bone defects but ending with visible bone non-union occurrence. In the animals of Group B, there was cell adhesion and bone-graft integration, without any signs of infection or inflammation, results that differ from those found in Group A, in which there were no signs of osseointegration process between the β - TCP implant and bone host, despite their good adherence and positioning in bone defects, and not even the implants were reabsorbed. The biocompatibility of the ceramic biomaterial corroborated with the results of other studies, but osseoconductivity showed a different result, that is, no bioactivity was observed, a fact probably related to the low porosity characteristic of the implant. It is therefore concluded that this customized pure phase β - TCP implants did not present the osseoconductive and osseointegrative characteristics mentioned by other authors and were not effective for the consolidation and integration between the host bones and the biomaterials for this study. New studies should be carried out to provide more information about this ceramic block, especially regarding its porosity and shape, in order to

consider it as a possible biomaterial for bone replacement that can be used routinely in reconstructive orthopedic and trauma surgery in veterinary medicine.

Keywords: biomaterials; bone implants; ceramics; beta - tricalcium phosphate; β - TCP; allogeneic cortical bone graft; osteosynthesis

LISTA DE FIGURAS

Capítulo I

Capítulo II

Capítulo III

LISTA DE TABELAS E QUADROS

Capítulo I

Capítulo III

LISTA DE ABREVIATURAS E SIGLAS

A lista de abreviaturas e siglas está em ordem criada pelo programa. Algumas abreviaturas e siglas estão em inglês. Assim, quando necessário, a tradução em português está descrita entre parênteses ao lado:

10x: Aumento de 10 vezes

15^{th}: Em inglês significa fifteenth (décimo quinto em português)

1^{st}: Em inglês significa first (primeiro em português)

2,5x: Aumento de 2,5 vezes

20x: Aumento de 20 vezes

40x: Aumento de 40 vezes

5x: Aumento de 5 vezes

A-F implant: Acquous-based silk fibroin implant (implante de fibroína de seda à base de água)

Aprox.: Aproximadamente

ATG: Autogenous tooth graft (enxerto dentário autógeno)

BMC Veterinary Research Journal: BioMed Central Veterinary Research Journal (Periódico BMC Veterinary Research)

BMP`s: Bone Morphogenetics Proteins (proteínas morfogenéticas do osso)

BV: Bone Volume (volume ósseo)

CEP: Comitê de Ética em Pesquisa

CEUA: Comitê de Ética para Uso de Animais

Cr-Ca: Cranio-caudal

CSD: Critical Size Defect (defeito de tamanho crítico)

D: Dias

DCG: Daniel Cardoso Garcia

DFDBA: Demineralized Freeze-Dried Bone Allograft (enxerto ósseo congelado, desidratado e desmineralizado)

EDTA: Ethylenediamine Tetraacetic Acid (ácido etilenodiamino tetra-acético)

et al.: Abreviação de expressão em latim que significa “e outros”

EUA: Estados Unidos da América

Farm.: Farmacêuticos

FDBA: Freeze-dried Bone Allograft (enxerto ósseo congelado e desidratado)

FINGER: É um acrônimo que significa: Factível, Interessante, Nova (inovadora), Good (boa), Ética e Relevante. São características de uma boa questão/pergunta de pesquisa

FP: Felipe Pinotti

GO: Goiás

HA: Hidroxiapatita

HFIP-F implant: Hexafluoro isopropanol-based silk fibroin implant (implante de fibroína de seda à base de hexafluoro isopropanol)

IGF: Insulin-like Growth Factor (fator de crescimento semelhante à insulina)

IJDR = International Journal of Development Research (Periódico Internacional de Pesquisa e Desenvolvimento)

IL: Interleucina

in vivo: Expressão em Latin que significa “dentro do vivo; dentro da vida”. Na ciência a expressão significa a experimentação que ocorre ou tem lugar dentro do tecido vivo de um organismo vivo

ISO: International Organization for Standardization (Organização Internacional para Normatização)

JBI: É uma organização internacional de pesquisa com sede na Faculdade de Saúde e Ciências Médicas da Universidade de Adelaide, Austrália. A JBI desenvolve e fornece informações exclusivas baseadas em evidências, softwares, educação e treinamento projetados para melhorar a prática e resultados de saúde

LEM: Larissa Eckmann Mingrone

LS: Leonardo Seade

Ltda: Limitada

M0: Momento zero

M1: Momento um

M2: Momento dois

M3: Momento três

M4: Momento quatro

MEV: Microscopia eletrônica por varredura

MG: Minas Gerais

MJCS: Marcelo Jorge Cavalcanti de Sá

M-L: Médio-lateral

MsC: Masters in Science (Título de Mestre)

MTE: Membro Torácico Esquerdo

MTF: Musculoskeletal Transplant Foundation (Fundação de Transplantes Músculo-esqueléticos)

MV: Médico Veterinário

N: Nenhum

NDF: No Defect Filling (Sem Preenchimento)

NM: Não mencionado

OCS - B: OsteoConductive Substitute Bovine bone xenograft (enxerto ósseo heterólogo bovino substituto osteocondutor)

OTM: Orthodontic Tooth Movement (movimentação dentaria ortodôntica)

PB: Paraíba

PDGF: Platelet-Derived Growth Factor (fator de crescimento derivado de plaquetas)

PhD: Philosophy Doctor (Título de Doutor)

PICO: É um acrônimo que significa: Paciente, Intervenção, Comparação e "Outcomes" (resultados). Ele orienta a construção da pergunta de pesquisa e da busca bibliográfica e permite que o profissional, da área clínica e de pesquisa, ao ter uma dúvida ou questionamento, localize, de modo acurado e rápido, a melhor informação científica disponível

PMMA: Polimetilmetacrilato

PO: Pós-operatório

PR: Paraná

PRISMA: "Preferred Items for Systematic Reviews and Meta-analysis" (Itens de Eleição para Revisões Sistemáticas e Meta-Análises)

Prod.: Produtos

PRP: Platelet-Rich Plasma (plasma rico em plaquetas)

Quim.: Químicos

Raios X: são ondas eletromagnéticas que possuem a capacidade de atravessar corpos de baixa densidade e são absorvidos por materiais de densidade maior. O raio X é um tipo de radiação eletromagnética

RM: Rosane Melo

ROI: Region Of Interest (região de interesse)

Si - CaOP: Silicon-doped Calcium Alkali Orthophosphate (ortofosfato alcalino de cálcio modificado com silicone)

Si -TCP: Silicon-doped Tricalcium Phosphate (fosfato tricálcico modificado com silicone)

SP: São Paulo

SRA: Stitches Removed by Animal (pontos removidos pelo animal)

TbSp: Trabecular separation (separação trabecular)

TbTh: Bone thickness (espessura óssea)

TbWi: Bone width (espessura ou largura óssea)

TCP: Tricálcio Fosfato

TGF - β: Transforming Growth Factor Beta (fator de transformação do crescimento beta)

TV: Tissue Volume (volume do tecido)

UFCG: Universidade Federal de Campina Grande

UNESP: Universidade Estadual Paulista

US: United States

USA: United States of America (Estados Unidos da América)

USP: Universidade de São Paulo

α - TCP: Alfa - Tricálcio Fosfato

β - TCP: Beta - Tricálcio Fosfato

β: Beta

μCT: Micro-tomografia computadorizada

LISTA DE SÍMBOLOS

A lista de símbolos está em ordem criada pelo programa. Alguns símbolos estão em inglês. Assim, quando necessário, a tradução em português está descrita entre parênteses ao lado:

$: Dólares

%: Porcento

(AL_2O_3): Fórmula da Alumina

($Ca_{10}(PO_4)6(OH)_2$): Fórmula da hidroxiapatita

($Ca_2KNa(PO_4)_2$): Fórmula do Osseolive® (cerâmica de vidro composta de ortofosfato alcalino de cálcio)

($Ca_3(PO_4)_2$): Fórmula do tricálcio fosfato

($Ca_4P_2O_9$): Fórmula do tetracálcio fosfato

(ZrO_2): Fórmula da zircônia

-: Sem dor

+: Dor pós-operatória

++++: Classificação em cruzes (4 cruzes)

≈: Aproximadamente

®: Marca registrada

°C: Graus Celsius

µA: Intensidade dos raios em micro amperes

µm: Micrômetros

Al: Alumínio

Ca: Cálcio

CaP: Calcium Phosphate (fosfato de cálcio)

cm: Centímetros

g: Gramas

hs: Horas

Kg: Quilogramas

KVP: Tensão de Pico em Quilovoltagem

mg: Miligramas

mm: Milímetros

ms: Milissegundos

n: Número de indivíduos

n°: Número

P: Fósforo

p: Nível de significância

Qualis A1 e B1: O Qualis Periódicos é um conjunto de procedimentos utilizados na avaliação de periódicos científicos no Brasil. Esse instrumento é fundamental quando se trata da produção intelectual. A classificação A1 é a classificação mais elevada que um periódico pode receber

R$: Reais

S/A: Sociedade Anônima

µl: Microlitros

SUMÁRIO

1. INTRODUÇÃO

Várias técnicas cirúrgicas avançadas do tecido ósseo normalmente são baseadas em reconstrução e transplante de tecidos. No entanto, várias limitações terapêuticas e de metodologia foram observadas. Como alternativa a este tipo de abordagem, surge a engenharia de tecidos, a qual tem sido utilizada para restaurar o tecido ósseo danificado através da proliferação e diferenciação de células ósseas dentro de arcabouços sintéticos. (Yasemski et al., 1996; Krieger, 2003; Azevedo et al., 2007).

1.1 História dos biomateriais

A aplicação de biomateriais no corpo do ser humano remonta à pré-história, como já indicaram algumas descobertas de crânios com trepanações nas quais foram utilizadas placas de ouro e prata (Laurencin, 2004; Ratner, 2004; Azevedo et al., 2007). Também, há milhares de anos, já foram descritas a aplicação de implantes dentários e a utilização de fios de sutura.

Nos tempos atuais, o uso de biomateriais sofreu um forte impulso com o aparecimento de lentes intraoculares, próteses articulares, implantes mamários, próteses valvulares e vasculares, e implantes dentários (Azevedo, 2007; Pinotti et al., 2018). No entanto, a palavra "biomaterial" como a aplicamos atualmente, só há poucos anos foi introduzida na nomenclatura médica. Na conferência de Chester, em 1991, definiu-se a palavra biomaterial como um "material destinado a contactar com sistemas biológicos para avaliar, tratar, aumentar, ou substituir qualquer tecido, órgão ou função do organismo" (Williams et al., 1991; Williams, 1999; Azevedo, 2007). Recentemente, sua definição se tornou um pouco mais complexa: "um biomaterial é uma substância que foi projetada para tomar uma forma que, sozinha ou como parte de um sistema complexo, seja utilizada para direcionar, através do controle de interações com componentes de sistemas vivos, o curso de qualquer procedimento terapêutico ou diagnóstico em medicina humana ou veterinária" (Williams, 2009). Ao ser aplicado, o biomaterial deve manter as suas propriedades e características estruturais, mas simultaneamente deve substituir a função para a qual foi criado. É também importante que ele permita uma boa adesão celular à sua superfície, tenha uma resistência mecânica adequada, não possua características oncogênicas, seja esterilizável e, por fim, que a sua produção em larga escala seja fácil e com custos aceitáveis. (Gutierres et al., 2006; Azevedo, 2007).

1.2 Importância dos substitutos ósseos

Os biomateriais para uso na ortopedia podem ser definidos como “todo o material de origem humana, animal, vegetal ou sintético, destinado à implantação no homem com as perspectivas de uma reconstituição óssea, de reforço de uma estrutura óssea, ou para o preenchimento de uma perda de substância óssea de origem traumática ou ortopédica” (Tomford, 2000).

A alta capacidade de regeneração óssea, particularmente em indivíduos jovens, significa que, a princípio, a maioria das fraturas poderia cicatrizar sem a necessidade de quaisquer intervenções. Apesar disso, quando há grandes perdas ósseas, como por exemplo, naquelas observadas após ressecção de tumores ósseos e casos severos de não-união óssea, essa cicatrização pode ser dificultosa ou mesmo inexistente, e por isso intervenções cirúrgicas são necessárias para que se possa tentar manipular o processo de regeneração óssea, utilizando-se, para isso, algum tipo de material que substitua o osso perdido (Yasemski, 1996; Carlo et al., 2007; Walsh et al., 2008; Stevens, 2008; Almeida et al., 2020).

De modo geral, os materiais utilizados com o fim de reconstituição dos defeitos ósseos incluem os enxertos ósseos autógenos, enxertos ósseos alógenos, xenoenxertos ósseos, cimento ósseo não degradável, metais e cerâmicas (Crane et al., 1995).

Mas para qualquer que seja o material descrito anteriormente, sem exceção, eventualmente podem ocorrer problemas associados ao seu uso, tais como a dificuldade de esculpir o enxerto no formato desejado, a limitação da quantidade de auto-enxerto que pode ser adquirido do próprio paciente retirado do sítio doador, o aumento da morbidade do paciente e desconforto pela coleta, a possibilidade de infecção transferida através do aloenxerto ósseo (Allegrini Júnior et al., 2018), a fadiga e falha do implante, ou mesmo a ocorrência de stress ao redor ou sobre o osso onde se encontra o enxerto ou implante aplicado. Além disso, também podemos nos deparar com dificuldades associadas às características dos tecidos próximos ao defeito ósseo, assim como as características do próprio defeito ósseo, ou mesmo do tamanho, formato e tipo de osso envolvido, e sobretudo, da escolha do material que será selecionado para reparar/regenerar a perda do tecido ósseo (Stevenson et al., 1996).

Um material ideal utilizado para a reconstrução óssea deveria apresentar características e propriedades tais como a facilidade de manuseio, a possibilidade de um bom encaixe no defeito ósseo não deixando espaços vazios, não ser prejudicial aos tecidos adjacentes, ser biocompatível, e imitar o ambiente fisiológico do tecido ósseo (Stevenson et al., 1996).

A enxertia óssea é o segundo tipo de transplante tecidual mais realizado no mundo, perdendo em números somente para o uso de sangue, ou seja, transfusão sanguínea (Giannoudis et al., 2005; Marino e Ziran, 2010; Zorzi e de Miranda, 2012).

Os substitutos ósseos são atualmente objetos de intensa investigação mundial, tendo como objetivo encontrar alternativas viáveis para se lidar justamente com as limitações decorrentes tanto da colheita de enxerto ósseo autógeno, como da necessidade de se recorrer a bancos de ossos. Além disso, a utilização de aloenxertos ósseos apresenta sempre o risco potencial de transmissão de doenças infecciosas (Khan et al., 2000; Dasgupta et al., 2019). E, de fato, tem sido notável o desenvolvimento destes biomateriais utilizados em cirurgia ortopédica, traumatológica e buco-maxilo-facial, particularmente dos substitutos ósseos (Vaccaro et al., 2002; Carlo et al., 2007; Dorozhkin, 2010; Pinotti et al., 2018; de Paula et al., 2018; de Oliveira et al., 2020).

Para se ter uma idéia do quanto os enxertos ósseos são utilizados, somente nos Estados Unidos foram realizadas em torno de 600.000 enxertias ósseas anualmente na década de 2000 (Marino e Ziran 2010), e no ano de 2002, mais de 986.000 aloenxertos foram utilizados. Somente a Fundação de Transplantes Musculo-Esqueléticos (MTF) distribuiu aproximadamente 250.000 enxertos ósseos (Beebe et al., 2009). Avaliando-se um censo mundial por volta do início dos anos 2.000, foram efetuadas mais de 2 milhões de cirurgias ortopédicas nas quais se recorreu à colheita de autoenxerto (Nishida et al., 2000). Um exemplo disso, foram os procedimentos de fusões vertebrais, os quais representaram um crescimento massivo no uso de enxerto autólogo, dando um salto da 41ª posição entre as cirurgias mais realizadas nos Estados Unidos em 1997, para a 19ª posição em 2003 (Stevens, 2008).

E efetivamente, este tipo de enxerto é o que apresenta os melhores resultados clínicos, além de possuir as melhores características que se espera de um substituto ósseo, tais como a osteogênese (capacidade de células progenitoras ósseas vivendo dentro do enxerto doador, e as quais sobreviveram ao transplante, de formar, proliferar e diferenciar-se em osteoblastos, e eventualmente em osteócitos); a osseoindução (capacidade de estimulação e ativação das células tronco mesenquimais do tecido adjacente em se diferenciarem em células progenitoras de linhagem osteoblástica); a osseocondução (facilitação e orientação do crescimento de vasos sanguíneos e criação de novos sistemas Harvesianos dentro do enxerto ou implante); e a osseointegração (capacidade de ligação ou integração entre a superfície do osso e o material implantado ou enxertado) (Proubasta et al., 1997; Buser et al., 1998; Giannoudis et al., 2005; Stevens, 2008; Beebe et al., 2009; Schmidt et al., 2013; Pinotti et al., 2018). É difícil concentrar estas quatro propriedades em um único material sintético, e por este motivo são evidenciadas

diversas dificuldades para alcançar resultados biológicos semelhantes com o uso de implantes (Allegrini Júnior et al., 2018). Mas é possível adicionar a uma matriz osseocondutora como as cerâmicas (hidroxiapatita ou tricálcio fosfato), agentes bioativos (por exemplo, aspirado de medula e BMP‘s) que lhe forneçam as outras características restantes para substituírem com sucesso o uso tanto do auto, como dos aloenxertos (Davies, 2000; Pinotti et al., 2018; de Oliveira et al., 2020).

A utilização de substâncias com capacidade osteogênica em conjunto com outros materiais exibindo outras características, como a osseoindução ou osseocondução, abre novas perspectivas não só para o tratamento de fraturas, mas também para casos de pseudo-artroses, remoção de tumores ósseos, diversas doenças congênitas, artrodeses, fusões vertebrais, e reconstrução de grandes defeitos ósseos (Yasemski et al., 1996; Giannoudis et al., 2005; Stevens, 2008; Walsh et al., 2008; Pinotti et al., 2018; de Paula et al., 2018; de Oliveira et al., 2020).

Em geral, estes substitutos ósseos consistem das cerâmicas, vidros, polímeros naturais ou sintéticos, e compósitos (associações) destes (Stevens, 2008; Almeida et al., 2020).

1.3 Resposta biológica aos biomateriais

O êxito da aplicação de um material estranho ao organismo depende essencialmente de dois fatores:

A - Sua biofuncionalidade, a qual está diretamente relacionada com a capacidade do biomaterial em desempenhar uma determinada função (ou parte desta) do organismo.

B - Sua biocompatibilidade, que se baseia nas reações ocorridas na superfície do implante, não só no momento de sua implantação, mas também ao longo do tempo, quando este sofre posteriormente um processo de degradação e desgaste (Proubasta et al., 1997).

Assim, em termos de resposta biológica, após a implantação de um biomaterial ocorre a formação de um hematoma, com uma resposta de tipo inflamatória, na qual serão levadas ao local água e glicoproteínas, que revestem e se aderem ao implante. Por quimiotaxia, numerosas células são recrutadas para o local, sendo elas os neutrófilos, eosinófilos, monócitos e macrófagos, as quais iniciam uma resposta de reação ao corpo estranho. Estas últimas, além da sua atividade fagocítica, estimulam a ação dos linfócitos, fibroblastos, osteoclastos e células polimorfonucleares. Seguidamente, inicia-se a angiogênese, com a migração e proliferação de células endoteliais que vão formar uma rede de capilares que constituirá o suporte vascular

daquela área (Davies, 2000; Allegrini Júnior et al., 2018). Por fim, devido à ação de citocinas (IL-1 e IL-2) e de diversos fatores de crescimento (TGF-β, PDGF, IGF, BMP´s) ocorrerá um processo de diferenciação das células mesenquimais pluripotenciais com a formação de matriz óssea e de osso imaturo (Dasgupta et al., 2019). A maturação e remodelação que encerram este processo demonstram toda a semelhança que existe em relação à formação do calo ósseo fisiológico que ocorreria subsequente a uma fratura (Anderson, 2001).

1.4 Classes de biomateriais

De uma forma geral, os biomateriais podem ser classificados sob dois aspectos:

A - Por sua composição química.

Nesta classificação há uma subdivisão dos biomateriais em 4 classes distintas (Bauer e Muschler, 2000; Almeida et al., 2020):

a) Metais e ligas metálicas
b) Cerâmicas
c) Polímeros
d) Compósitos

B - Por seu comportamento biológico.

Esta classificação é baseada na resposta do tecido hospedeiro ao biomaterial (Crane et al, 1995; Bauer e Muschler, 2000):

a) Bioinertes - são aqueles que não provocam reação de corpo estranho ao organismo, encontrando-se em ligação direta ao tecido receptor. Exemplos: titânio, zircônia e alumina.

b) Biotolerados - são moderadamente aceitos pelo tecido receptor, sendo geralmente envolvidos por uma cápsula fibrosa. Exemplos: aço inoxidável, liga de cromo-cobalto, e o polimetilmetacrilato (PMMA).

c) Bioativos - nestes, existe a formação de uma ligação direta aos tecidos vivos, pois geralmente têm na sua composição, íons de cálcio e/ou fósforo (no caso dos substitutos ósseos), que vão

estabelecer uma ponte química com o osso envolvente. Exemplos: hidroxiapatita, tricálcio-fosfato e vidros bioativos.

d) Reabsorvíveis - são aqueles que lentamente vão sendo degradados, e gradualmente vão sendo substituídos por tecido ósseo onde são implantados. Exemplos: tricálcico-fosfato e vidros bioativos.

Para o sucesso clínico do substituto ósseo, é necessário que uma boa osseointegração esteja associada a uma boa resistência mecânica, a qual será necessária para o desempenho de funções de suporte. No sentido de potencializar as suas propriedades mecânicas e físico-químicas, podem ser combinados diferentes tipos de materiais que se complementam entre si. Por esta razão, muitos ortopedistas utilizam materiais compósitos para reconstrução óssea (Fleming et al., 2000), cujas propriedades são superiores às que resultariam da simples adição individual de seus componentes. Mas para tanto, alguns princípios fundamentais da engenharia de tecidos devem também ser respeitados (Vaccaro et al., 2002). Por exemplo, para ocorrer regeneração tecidual (osseointegração) é necessária a presença de células capazes de formar novo tecido ósseo (osteogênese); e que estas consigam aderir, crescer e atravessar todo o material (osseocondução); e que estejam presentes fatores que estimulem a sua diferenciação fenotípica em osteoblastos (osseoindução) (Yasemski et al., 1996; Giannoudis et al., 2005; Calvo-Guirado et al., 2011; Lew et al., 2016).

1.5 Materiais cerâmicos

É possível produzir materiais cerâmicos sintéticos com uma composição semelhante à matriz óssea inorgânica. Estes materiais não têm limitações em termos de quantidade disponível, nem requerem qualquer procedimento cirúrgico adicional para sua aquisição. Como desvantagens à sua utilização, salientam-se a sua inexistente atividade osteogênica ou osseoindutiva, e o seu fraco desempenho mecânico em situações de tração para servirem de estrutura de suporte, dadas a inerente fragilidade e elevada rigidez destes materiais (Hogset e Bredberg, 1988; Peltier e Jones, 2004). No entanto, possuem ótimas características osseocondutoras (Buser et al., 1998; LeGeros e Legeros, 2003; Alegrini Júnior et al., 2018), e quando a cerâmica é anexada ao osso sadio, osteóide é produzido diretamente sobre a superfície desta cerâmica. Consequentemente, o osteóide mineraliza, e o novo osso produzido se remodela (Giannoudis et al., 2005).

As cerâmicas mais comuns do grupo são a alumina (Al2O3), zircônia parcialmente estabilizada, (ZrO_2), fosfato cálcico (ex: HA ($Ca_{10}(PO_4)6(OH)_2$), e tricálcio-fosfato (TCP) ($Ca_3(PO_4)_2$) (Lew et al., 2016), sendo as mais importantes o tricálcio-fosfato (TCP) e hidroxiapatita (HA). Ambas são altamente biocompatíveis. Elas diferem entre si na resposta biológica criada no local hospedeiro: O TCP poroso é reabsorvido do local implantado à medida que ocorre crescimento ósseo em seu arcabolço. Já a HA é um material mais permanente, e leva mais tempo para ser reabsorvido (Kamitakarahara et al., Walsh et al., 2008; Costa et al., 2015).

As camadas superficiais do TCP aumentam a ligação com o osso hospedeiro adjacente, e são materiais com alta capacidade osseocondutora. Isso estimula a reabsorção osteoclástica e formação de novo osso dentro do implante que está sendo reabsorvido. Já a hidroxiapatita passa por uma reabsorção bem mais lenta. Implantes compostos de HA podem se tornar um foco de estresse mecânico (Alegrini Júnior et al., 2018).

O uso do TCP pode também ser combinado com outros materiais (combinação de HA/TCP somente, ou combinados com osso autógeno) para melhorar sua funcionalidade e acelerar sua reabsorção (LeGeros e Legeros, 2003; Costa et al., 2015; Pinotti et al., 2018; de Paula et al., 2018; de Oliveira et al., 2020).

Em um estudo realizado por Ogose et al. (2005), comparando o uso de HA e Beta - TCP (β - TCP, um dos tipos de TCP) como substitutos ósseos após excisão de tumores ósseos, foi demonstrado que o β - TCP foi mais eficiente na incorporação do biomaterial ao tecido ósseo hospedeiro (23 casos incorporados) em relação ao uso da HA (21 casos incorporados). A radioluscência dos implantes observada nas radiografias pós-operatórias levou em média 9 semanas para desaparecer nos casos em que se utilizou o β - TCP, ao passo que nos casos nos quais se utilizou a HA, o desaparecimento das áreas radioluscentes levou em média 17 semanas.

Outro estudo realizado, no qual a implantação de β - TCP foi realizada de forma semelhante ao estudo anterior, foi demonstrado que em 164 de 167 casos operados, as radiografias mostraram nova formação de osso através deste tipo cerâmica (Ozawa et al., 2000).

Em um estudo histopatológico e histomorfométrico conduzido por Jensen et al. (2006) comparando o uso de β - TCP, autoenxerto e xenoenxerto bovino, o autor mostrou em seus dados histomorfométricos que após 8 semanas de implantação da cerâmica e dos enxertos, houve maior crescimento ósseo nos grupos com β - TCP e autoenxerto em relação ao grupo no qual se utilizou o xenoenxerto, não havendo diferença significativa entre este crescimento ósseo em relação aos dois primeiros grupos. Além disso, a reabsorção do implante de β - TCP foi praticamente total após as 8 semanas, enquanto o xenoenxerto ainda se mantinha estável.

Em outro estudo realizado por Costa et al. (2015), os resultados obtidos com o β - TCP demonstraram boa osseointegração e formação óssea, inclusive no interior do grânulo do implante. Demonstrou-se também rápida absorção do implante e a alteração na morfologia granular foi intensa. Aos 90 dias foi observado tecido ósseo de boa qualidade preenchendo praticamente todo o defeito, com presença de pouco biomaterial remanescente. Foi observada também a presença marcante de células de linhagem osteoblástica nas superfícies do tecido ósseo em construção. Por meio da análise visual das avaliações de microscopia eletrônica e miscroscopia óptica, foi observado que este foi o biomaterial implantado mais rapidamente absorvido quando comparado com a hidroxiapatita ou mesmo com a composição bifásica HA/β - TCP. E a qualidade do tecido ósseo formado foi considerada como muito boa.

1.6 Tricálcio fosfato (TCP)

Materiais sintéticos, como os polímeros, cerâmicas, metais ou seus compósitos, foram largamente investigados, e seus usos foram explorados para a aplicação em reparação óssea tanto em estudos in vivo, como também em estudos in vitro (Crane et al., 1995). Devido ao tema abordado nesse estudo, aqui mencionaremos somente questões relacionadas às cerâmicas.

Dentre as cerâmicas, existem várias compostas por tricálcio-fosfato consideradas biocompatíveis. Destas, a maioria é reabsorvível e dissolverá quando exposta a ambientes fisiológicos (Dasgupta eta al., 2019). Em ordem de solubilidade, estes materiais são elencados abaixo (Langer e Vacanti, 1993):

a) Tetracálcio fosfato - ($Ca_4P_2O_9$)
b) Fosfato de cálcio amorfo - ($Ca_3(PO_4)_2$)
c) α - Tricálcio fosfato - α ($Ca_3(PO_4)_2$)
d) β - Tricálcio fosfato - β ($Ca_3(PO_4)_2$)
e) Hidroxiapatita - ($Ca_{10}(PO_4)_6(OH)_2$)

O TCP, uma das formas de cerâmica, existe sob duas formas alotrópicas, alpha e beta (α - TCP e β - TCP). O β - TCP pode ser convertido em α - TCP quando aquecido a 1125°C (Rey et al, 2008; Lew et al. 2016). Atenção tem sido dada mais ao β - TCP que ao α - TCP, devido à maior instabilidade e citotoxicidade deste último. O TCP possui a fórmula química $Ca_3(PO_4)_2$ e relação molar de Ca/P = 1,5 (Lascart et al., 1998; Lew et al., 2016), muito semelhante ao tecido ósseo natural (Dasgupta et al., 2019), contendo cerca de 39% de cálcio e

20% de fósforo (Lascart et al., 1998). Em sua forma pura, o β - TCP, assim como a forma pura de HA, não pode ser obtido diretamente de soluções. Ele é preparado através de uma reação de seu estado sólido ou pela sinterização de uma apatita deficiente em cálcio obtida em soluções (LeGeros e LeGeros, 2003).

É bem aceito que estas biocerâmicas de fosfato de cálcio possuem boas propriedades osseocondutivas, mas pouca capacidade osteogênica e osseoindutiva (LeGeros e LeGeros, 2003). Além disso, também foi demonstrado que elas são biocompatíveis e bioativas (Ogose et al., 2005). Além destas características já mencionadas, o TCP é considerado uma cerâmica de rápida reabsorção (Vaccaro et al., 2002; LeGeros e LeGeros, 2003; Walsh et al., 2008). O seu mecanismo de ação depende da alta concentração de cálcio e fósforo na sua superfície, fator que potencializa sua osseointegração e inicia o processo de biomineralização, estimulando os osteoclastos e influenciando a diferenciação fenotípica das células osteogênicas. Sob a forma de pasta são menos eficazes que as formas sólidas, por falta da porosidade necessária ao crescimento ósseo.

Como previamente demonstrado por Langer e Vacanti (1993), biomateriais porosos aumentam a eficácia terapêutica das células transplantadas, evitando ou diminuindo a ocorrência de morte celular (Crane et al., 1995). Uma cerâmica composta de alta porosidade e baixa densidade, fornece uma melhor área para neovascularização e crescimento ósseo. Este arcabouço osseocondutivo promove um ambiente apropriado para sobrevivência de células ósseas e proteínas ósseas morfogenéticas (BMP's) (LeGeros e LeGeros, 2003).

Associando o β - TCP, no entanto, a produtos como o naftaleno, o peróxido de hidrogênio ou o amido, é possível obter em sua composição poros com 100 a 300 mícrons, permitindo uma osseointegração mais rápida (Gaasbeek et al., 2005). Para se fazer uma comparação, os tamanhos dos poros de um osso cortical normal vão de 1 a 100 µm, e de 200 a 400 µm no osso esponjoso (LeGeros e LeGeros, 2003).

De acordo com Dorozhkin et al. (2010), existem várias formas físicas desta biocerâmica, como pó, partículas, grãos, blocos densos, "scaffolds" porosos, soluções injetáveis, pastas, coberturas de implantes e compósitos. A forma granular seria a mais eficiente segundo Ozawa et al. (2000) e Almeida et al. (2020), porque os espaços entre os grânulos aumentam a porosidade da matriz e sua superfície de contato. Na sua apresentação mais pura, o β - TCP é superior ao HA em relação à osseocondutividade (Ozawa et al., 2000). Uma das principais desvantagens do TCP comparativamente com a hidroxiapatita se deve ao fato de não possuir um suporte estrutural e propriedades mecânicas adequados por conta de sua rápida reabsorção, em função da sua macroporosidade. Devido a esta fragilidade estrutural, quando utilizado, por

exemplo, sob a forma de cunhas de adição interna em osteotomias de valgização tibial, alguns autores sugerem a utilização de uma osteossíntese estável para evitar perdas de correção (Regner et al., 1998; Yamamoto et al., 2000; Koshino et al., 2003; Lew et al., 2016).

Apesar de faltar às cerâmicas implantadas características mecânicas iguais às do osso, após sua incorporação gradualmente elas adquirem força mecânica similar ao do osso esponjoso (Giannoudis et al., 2005).

Em um estudo sobre as características histológicas do β - TCP realizado em cirurgias de fêmur em humanos, após 4 semanas de pós-operatório, foi demonstrado que havia nova formação óssea em abundância e proliferação de células semelhantes a osteoclastos permeados na cerâmica (Ogose et al., 2002).

Atualmente, com os avanços científicos na área dos substitutos ósseos, especificamente aqueles relacionados aos materiais osseocondutores, a evolução deu-se com a introdução de técnicas inovadoras na preparação de compósitos bifásicos HA/TCP com comportamentos mecânicos e biológicos mais próximos aos do osso (Azevedo et al., 2007; Costa et al., 2015; Pinotti et al., 2018; de Oliveira et al; 2020).

As principais aplicações deste tipo de material incluem a reparação de defeitos ósseos, reparos de defeitos periodontais, implantes de ouvido, implantes oculares, reconstrução maxilofacial, fusão vertebral, preenchimentos ósseos, cobertura de implantes metálicos, entre outros (LeGeros e LeGeros, 2003; Almeida et al., 2020).

1.7 Aloenxertos ósseos

Aqui cito os aloenxertos ósseos e algumas de suas características e técnicas de obtenção e armazenamento, pois fazem parte de um dos grupos de comparação de nosso estudo.

Os enxertos ósseos podem ser classificados quanto aos aspectos imunológicos e morfológicos. Quanto ao aspecto imunológico, podem ser subdivididos em autógenos ou autoenxertos (retirados do próprio animal e enxertados nele mesmo); alógenos ou aloenxertos (retirados de um animal e enxertados em outro, entretanto são animais da mesma espécie); e xenógenos ou xenoenxertos (retirados de um animal e enxertados em outro, porém são animais de espécies diferentes). Pelo aspecto morfológico, os enxertos ósseos podem ser esponjosos, corticais, córtico-esponjosos, osteocondrais ou de medula óssea (Alievi et al., 2007; de Freitas et al., 2008).

Contudo, a utilização de enxertos ósseos requer a escolha de meios de conservação que os mantenham viáveis e que preservem suas funções osseocondutoras e osseoindutoras, além de preservar seu bom suporte mecânico (Alievi et al., 2007; de Freitas et al., 2008).

Especificamente o aloenxerto é um dos tipos de enxertos mais utilizados na medicina humana como substituto ósseo, e normalmente costuma ser a segunda opção de uso do cirurgião, ficando atrás somente dos autoenxertos. A sua utilização aumentou consideravelmente por volta do início dos anos 2.000, sendo utilizado em mais de um terço das enxertias ósseas realizadas nos Estados Unidos nesse período. O aumento de sua disponibilidade fez com que fosse possível desenvolver diversos formatos customizados, como cavilhas, tiras, cunhas e outros tipos de fragmentos geométricos (Giannoudis et al., 2005).

Na medicina veterinária, assim como na humana, o uso destes tipos de enxertos ósseos tem se tornado cada vez mais frequente na prática cirúrgica de pequenos animais como método alternativo na osteossíntese de fraturas cominutivas, na substituição do tecido ósseo em casos de neoplasias ósseas, nos casos de não-uniões e más-uniões ósseas, e essa prática tem reduzido significantemente a necessidade de amputações de membros acometidos por diversos fatores e patologias, além de promover uma opção de tratamento apresentando bons resultados. (del Carlo et al., 1999; Alievi et al., 2007; de Freitas et al., 2008).

Para que se obtenha uma conservação adequada dos aloenxertos, algumas técnicas de conservação podem ser utilizadas, tais como conservação em solução de glicerina a 98%, congelamento (“fresh-frozen”), liofilização (“freeze-dried”), uso de óxido de etileno, raios gama, nitrogênio líquido, ou até mesmo o mel de abelhas, entre outras (Cavassani et al., 2001; Vaccaro et al., 2002; Ziliotto et al., 2003; Amendola et al., 2003; Galia et al., 2005; Alievi et al., 2007; Amendola, 2007; Melo Filho et al., 2011).

Quanto mais agressivo o método de processamento do enxerto após sua colheita, menor será a resposta imunogênica. Maiores respostas imunogênicas e retardo na infiltração de capilares no enxerto retardam a taxa de nova formação óssea, assim como a incorporação óssea e remodelamento (Giannoudis et al., 2005).

Atualmente, tanto na medicina humana, quanto na medicina veterinária, os aloenxertos já não são mais utilizados sem processamento ou preparação prévia (uso do aloenxerto a fresco), devido à alta resposta inflamatória e imunogênica que podem desencadear e à possibilidade dos riscos de transmissão de doenças (Hogset e Bredberg, 1988; Filgueiras et al., 2004; Fawzi-Grancher et al., 2009).

Dentre os métodos de conservação de aloenxertos mais utilizados na medicina humana se destacam a técnica por congelamento a -70°C, também conhecida por “fresh-frozen”, na

qual, após uma lavagem com antibiótico, a sua utilização pode se dar desde 1 ano (se for mantido posteriormente a -20°C), até 5 anos (se mantido congelado a -70°C); e a técnica chamada de liofilização ou "freeze-dried", na qual, após lavagem dupla com antibiótico, congelamento a -70°C e secagem até que se obtenha um conteúdo de água de cerca de 5%, possui um período de duração ilimitada para utilização. Estas duas formas de preparo apresentam maior capacidade osseocondutiva quando comparadas à antiga forma de uso a fresco, no entanto as suas capacidades osseoindutivas são inferiores se comparadas à mesma (Vaccaro et al., 2002).

A técnica por congelamento reduz a imunogenicidade e consequente rejeição crônica, mas não elimina o risco de transmissão de doenças (Peterson et al., 2004). Ainda assim, o processamento por congelamento induz a respostas imunes mais exacerbadas que o processamento por liofilização (Giannoudis et al., 2005).

Ao contrário, a liofilização reduz ainda mais a imunogenicidade e o risco de transmissão de doenças, mas também diminui a capacidade de osseoindução e as propriedades mecânicas do enxerto. Além dos problemas imunogênicos e infecciosos, os aloenxertos apresentam ainda outras desvantagens, como a sua inexistente capacidade osteogênica, a variabilidade dos resultados clínicos da sua aplicação, e por fim, o seu elevado custo para o acondicionamento em temperaturas extremamente baixas (Tomford, 2000).

Outro método de conservação de enxertos ósseos muito utilizado principalmente na medicina veterinária, o qual também diminui a resposta imunogênica, possui ação antibacteriana e antifúngica, e ainda possui o baixo custo como grande vantagem em relação aos métodos descritos acima, é a utilização da solução de glicerina a 98% como conservante (Filgueiras et al., 2004). Costa (1996) utilizou com sucesso em seus estudos a glicerina a 98% como meio de preservação de ossos para uso em enxertos. Segundo ele, a glicerina desidrata o tecido ósseo substituindo a maior parte da água intracelular sem que se altere a concentração iônica das células, atuando como eficaz protetor da integridade celular. Além disso, a solução age como antisséptico ante os vários gêneros de microrganismos patogênicos ou não, atuando como bactericida e fungicida, exceto contra formas esporuladas (Filgueiras et al., 2004; Amendola, 2007).

Como metodologia utilizada por eles para a obtenção e conservação dos enxertos em solução de glicerina a 98%, procedeu-se com a colheita dos ossos, sendo que as epífises, os restos musculares, e o periósteo foram removidos com auxílio de serra manual e bisturi. Para a remoção da medula óssea foi utilizado pino metálico com rosca sob água corrente. A seguir, os fragmentos ósseos foram imersos em glicerina a 98% e mantidos à temperatura ambiente, por

30 dias. Antes de realizar a enxertia óssea após este período, os fragmentos ósseos eram reidratados durante dez minutos em solução fisiológica. Como resultado, observaram que os cães receptores apresentaram recuperação do membro afetado em 100% dos casos, concluindo que esse método de preservação é viável para manutenção da função osseoindutora da matriz óssea, e permitiram concluir que a solução de glicerina a 98% é um bom meio para conservação de fragmentos ósseos destinados a enxertia.

Em outro estudo, Ziliotto et al. (2003) coletaram epífises de rádio de animais eutanasiados por indicações não infecciosas ou neoplásicas. Os fragmentos ósseos foram lavados e colocados em frasco com glicerina a 98% por no mínimo 30 dias. Para posterior enxertia, o implante ósseo apresentando o diâmetro mais próximo ao do rádio do animal receptor e previamente conservado em glicerina a 98% com seu canal medular preenchido com poliuretana de mamona era selecionado e deixado em soro fisiológico por aproximadamente 10 minutos. A glicerina a 98% atuou como eficiente meio de conservação para os fragmentos ósseos, mantendo os mesmos livres de contaminação durante o período de estocagem, e agindo como agente redutor de antigenicidade, preservando as funções de osseoindução e osseocondução, além de apresentar baixo custo em todo o processo.

Del Carlo et al. (1999), também em outro estudo, realizaram a manutenção de enxertos ósseos em solução de glicerina a 98% em tempo mínimo também de 30 dias obtendo resultados satisfatórios.

Embora a literatura não estabeleça tempo mínimo ou máximo para a manutenção de enxertos ósseos em solução de glicerina a 98%, Cavassani et al. (2001), de Freitas et al. (2008) e Padilha Filho et al. (2008a) e (2008b), conservaram enxertos em tempo mínimo de 15 dias e verificaram que não houve alterações na radiopacidade e/ou presença de fissuras nas amostras testadas radiograficamente, demonstrando, portanto, que a solução de glicerina 98% apresentou bons resultados em relação à conservação óssea, durante 15 dias, quanto ao aspecto radiológico.

Apesar desses meios de conservação apresentarem boa qualidade no que se refere ao aspecto de conservação óssea, tem-se ampliado muito o interesse quanto às condições microbiológicas e histopatológicas de ossos submetidos a essas condições de conservação (Giovani et al., 2006; Melo Filho et al., 2011).

Tem-se buscado também avaliar a possibilidade de existência de alterações biomecânicas provocadas por esses métodos de conservação óssea, por meio de avaliações físicas de compressão e distração destes fragmentos tratados (Amendola et al., 2008; Melo Filho et al., 2011). Entretanto, referências literárias quanto às densidades ósseas por meio de avaliações radiográficas ficam muitas vezes restritas a acompanhamentos radiológicos pós-

cirúrgicos *in vivo* (Alievi et al., 2007; de Freitas et al., 2008), e pouco tem se estudado sobre a interferência que esses meios poderiam causar sobre a densidade e capacidade de lise óssea por conta do método de conservação utilizado.

Contudo, salienta-se a necessidade de maior conhecimento radiológico a respeito de ossos mantidos em diferentes meios de conservação, a fim de se verificar, se a reabsorção óssea dos enxertos *in vivo* ocorre devido à interferência do meio de conservação sobre os enxertos ou por deficiência óssea do animal doador. Por este motivo, a realização de análises histopatológicas e densitometrias ósseas de tais enxertos precisariam de estudos mais aprofundados em tempos superiores a 15 dias (Freire e Poggiani, 2017).

Como alternativa, mais recentemente, a micro-tomografia computadorizada (µCT) tem sido descrita como um dos métodos de diagnóstico que vem sendo utilizado em pesquisas para a avaliação óssea e osseointegração e vem se consolidando como padrão ouro para estes tipos de estudos. Ela segue os princípios básicos da tomografia computadorizada médica, na qual a amostra é colocada no caminho de um feixe de raios-X formando uma imagem de projeção no cintilador ou outro conjunto de detectores sensíveis aos raios. A amostra é girada e visualizada em um grande número de ângulos, e a sequência de imagens de projeção é "retroprojetada" para reconstruir a absorção de raios-X em cada ponto dentro do volume digitalizado. A µCT é atualmente utilizada para avaliar as características morfométricas tanto ósseas, quanto de enxertias e uso de implantes ósseos, sendo uma alternativa complementar à análise histológica convencional, ao considerar um volume tridimensional da amostra em cortes histológicos bidimensionais. O uso da µCT permite uma análise mais representativa em toda a extensão da amostra, sendo a mais vantajosa em relação à análise histológica, porém, deve-se ressaltar que a histologia continua sendo o método mais indicado para avaliação de células, proteínas e composição (Irie et al., 2018).

E, por fim, a maior quantidade disponível de enxerto alógeno comparativamente ao enxerto autólogo, é sem dúvida uma vantagem; assim como tem a vantagem de não ser necessário um procedimento cirúrgico adicional no paciente para a sua colheita, diminuindo a morbidade e tempo cirúrgico. Também, é possível se ter acesso a aloenxertos ósseos com uma grande variedade de formas físicas, como gel, pó, fibras, pastas, entre outras. Atualmente, está havendo um grande esforço de investigação no sentido de melhorar a integração e remodelação deste tipo de enxerto através de modificações físico-químicas. As modificações físicas passam por criar instrumentos de corte através de laser, por exemplo, com objetivo de obter formatos especiais, com furações precisas, seguidos de desmineralização da superfície para melhorar a revascularização. A modificação química dos aloenxertos pode passar ainda pela adição de

fatores de crescimento, como o BMP-2 e BMP- 7, que irão estimular o crescimento ósseo para o processo de cicatrização (Tomford, 2000).

2. JUSTIFICATIVA E HIPÓTESE

Como já descrito anteriormente, apesar do enxerto autógeno e do enxerto alógeno serem os melhores tipos de enxertos no que se diz respeito à osteogênese, osseoindução, osseocondução e osseointegração quando comparados a outros tipos de implantes, ainda assim nos deparamos com uma série de fatores que são muito importantes no momento da escolha de procedimentos como enxertias ósseas e uso de implantes sintéticos para substituição óssea.

Devemos sempre considerar como desvantagem o tempo cirúrgico mais prolongado e aumento da morbidade dos pacientes nos casos de coleta de enxertos ósseos autógenos, assim como possibilidade de ocorrência de infecção do local de coleta dos enxertos, ou mesmo fissuras ou fraturas do osso no momento de coleta. E se levarmos em conta o uso dos aloenxertos corticais ósseos, dependemos da criação e manutenção de bancos de ossos e procedimentos logísticos que podem levar a um custo mais elevado de coleta, processamento e conservação dos ossos, além de nos depararmos com questões complexas de pós-enxertia como imunogenicidade e riscos de transmissões de doenças e infecções pós-enxertia.

Por estas razões, vários estudos relacionados a implantes sintéticos têm sido realizados mundo afora, na tentativa de se encontrar um substituto ósseo ideal que permita estimular e conduzir a consolidação óssea, levando a uma osseointegração de forma efetiva. Estes tipos de implantes devem ter características e composições que permitem a formação de osso novo, e por isso vêm sendo testados como novas alternativas ao uso dos enxertos ósseos.

O β - tricálcio fosfato (β - TCP) é um tipo de implante sintético que vem sendo testado na medicina humana e na odontologia, tanto em sua fase pura, como na forma de compósitos, e é utilizado em casos de perdas e reabsorções ósseas, sendo principalmente utilizado na forma de grânulos. A possibilidade do uso de blocos cilíndricos maiores abre uma perspectiva para que seja mantido o comprimento ósseo em falhas críticas de ossos longos e em fraturas complexas com perda óssea severa, considerando seu uso principalmente para os animais de pequeno porte.

Na medicina veterinária pouco ou raramente se utiliza este tipo de implante em casos ortopédicos ou traumatológicos de rotina. Apesar de apresentar somente características osseocondutivas na sua fase pura, alguns trabalhos já demonstraram seu uso com eficácia em relação à neoformação óssea.

Por estes motivos, utilizaremos o β - TCP em fase pura e em formato de bloco cilíndrico, mimetizando o diâmetro ósseo do rádio de coelhos. O mesmo será posicionado em uma falha crítica óssea realizada no osso do rádio destes coelhos, e será averiguada a integração óssea que ocorrerá entre ele e o osso, e a possibilidade de formação de osso novo através desse implante ao mesmo tempo que se espera observar alguma reabsorção do mesmo à medida que o osso vai se formando e se entremeando no implante. Como comparação no processo de consolidação óssea e osseointegração utilizaremos o aloenxerto cortical ósseo retirado também do osso do rádio de coelhos.

A hipótese formada é a de que se a osseocondução e reabsorção desse bloco cerâmico forem efetivas, poderemos tentar consolidar seu uso para cães e gatos de forma mais rotineira nos procedimentos cirúrgicos ortopédicos e traumatológicos que exijam grandes quantidades de substitutos do osso, possivelmente sem perder o comprimento original do membro afetado, evitando assim, outros procedimentos ortopédicos futuros, necessários para novas correções do membro. Isso poderá trazer aos animais uma melhora na qualidade de vida pós-operatória, além de diminuir custos com procedimentos cirúrgicos e materiais necessários para estas futuras correções.

3. REFERÊNCIAS BIBLIOGRÁFICAS

1. Allegrini Júnior, S.; Silva, A. C.; Tsujita, M.; Salles, M. B.; Gehrke, S. A.; Braga, F. J. C. Amorphous calcium phosphate (ACP) in tissue repair process. **Microsc Res Tech**, v.13, p.1-11, 2018.

2. Alievi, M. M.; Schossler, J. E. W.; Guimarães, L. D.; de Oliveira, A. N. C.; Traeslel, C. K.; Ferreira, P. A. Implante ósseo cortical alógeno conservado em mel na reconstrução de falha óssea diafisária em fêmur de cães: avaliação clínica e radiográfica. **Cienc Rural**, v.37, n.2, p.450-457, 2007.

3. Almeida, R. S.; Prado da Silva, M. H.; da Rocha, D. N.; Ribeiro, I. I. A.; Júnior, A. A. B.; Miguel, F. B.; Rosa, F. P. Regeneration of a critical bone defect after implantation of biphasic calcium phosphate (β-tricalcium phosphate/calcium pyrophosphate) and phosphate bioactive glass. **Ceramica,** v.66, p.119-125, 2020.

4. Amendola, G. F.; Ilha, M.; Berger, R.; Stedile, R.; Schossler, J. E. Correção de defeito ósseo femoral em cães utilizando implante cortical homólogo conservado em mel. **Acta Cir Bras**, v.18, p.302-307, 2003.

5. Amendola, G. F. Aspectos biomecânicos, bacteriológicos e micológicos de diáfises femorais caninas conservadas em glicerina a 98% ou mel. **Tese de doutorado**. Universidade Federal de Santa Maria, Rio Grande do Sul, 2007, 100p.

6. Amendola, G. F.; Raiser, A. G.; Soares, J. M. D.; Beckmann, D. V. Aspectos biomecânicos compressivos de diáfises femorais caninas conservadas em glicerina a 98% ou em mel. **Cienc Rural**, v.38, n.5, p.1341-1345, 2008.

7. Anderson, J. M. Biological responses to materials. **Annu Ver Mater Res**, v.31, p.81-110, 2001.

8. Azevedo, V. V. C.; Chaves, S. A.; Bezerra, D. C.; Costa, A. C. F. M. **REMAP**, v.2.3, p.35-42, 2007.

9. Bauer, T. W.; Muschler G. F. Bone Graft Materials. **Clin Orthop**, v.37, p.10-27, 2000.

10. Beebe, K. S.; Benevenia, J.; Tuy, B. E., de Paula, C. A.; Harten, R. D.; Enneking, W. F. Effects of a New Allograft Processing Procedure on Graft Healing in a Canine Model: A Preliminary Study. **Clin Orthop Relat Res**, v.467, p.273-280, 2009.

11. Buser, D.; Hoffmann, B.; Bernard, J. P.; Lussi, A.; Mettler, D.; Schenk, R. K. Evaluation of filling materials in membrane-protected bone defects. A comparative histomorphometric study in the mandible of miniature pigs. **Clin Oral Implants Res,** v.9, p.137-150, 1998.

12. Calvo-Guirado, J. L.; Delgado-Ruíz, R. A.; Ramírez-Fernández, M. P.; Maté-Sánchez, J. E.; Ortiz-Ruiz, A.; Marcus, A. Histomorphometric and mineral degradation study of Ossceram®: a novel biphasic β-tricalcium phosphate, in critical size defects in rabbits. **Clin Oral Implants Res**, v.6, n.2, p.667-675, 2011.

13. Carlo, C.; Borges, B.; Pacheco, A.; Rezende, D. F.; Maria, C.; Lopes, C.; César, C.; Pontes, S.; Cristine, K.; Duarte, S. Avaliação do efeito osteoindutor da hidroxiapatita e do biovidro implantados em tecido subcutâneo de cão. **Rev. Ceres**, v.54, n.316, p.492-500, 2007.

14. Cavassani, M. M.; De Moraes, J. R. E.; Padilha Filho, J. G. Função osteoindutora de fragmentos ósseos conservados em glicerina a 98%. Estudo experimental em ratos. **Cienc Rural**, v.31, n.3, p.445-448, 2001.

15. Costa, B. D.; Camargo, N. H.; Oleskovicz, N.; Gava, A.; Dallabrida, A. L.; Regalin, D.; Lima, M. P. A.; Moraes, A. N. Neoformação óssea e osteointegração de biomateriais micro e nanoestruturados em ovinos**. Pesq Vet Bras**, v.35, n.2, p.177-187, 2015.

16. Costa, J. L. O. Reconstrução de grande falha óssea com enxerto cortical alógeno conservado em glicerina, fixado com placa e parafusos de aço inoxidável da série 304. Estudo experimental em cães (*Canis familiaris*). **Dissertação de Mestrado.** Universidade Estadual Paulista - Faculdade de Ciências Agrárias e Veterinárias, São Paulo, 1996.

17. Crane, G. M.; Ishaug S. L.; Mikos A. G. Bone tissue engineering. **Nature Med**, v.1, p.1322-4, 1995.

18. Dasgupta, S.; Maji, M.; Nandi, S. K. Investigating the mechanical, physiochemical and osteogenic properties in gelatin-chitosan-bioactive nanoceramic composite scaffolds for bone tissue regeneration: In vitro and in vivo. **Mater Sci Eng C,** v.94, p.713-728, 2019.

19. Davies, J. E. Histodynamics of endosseous wound healing. In: Davies J. E., editors. **Bone engineering.** v.2, p.1-11, 2000.

20. de Freitas, S. H.; Dória, R. G. S.; Mendonça, S. F.; Neto, J. E.; de Camargo, L. M. Aspecto radiológico de heteroenxerto ósseo cortical fragmentado na reparação de falhas ósseas em coelhos. **Rev Bras Cienc Vet,** v.15, n.3, p.107-110, 2008.

21. de Oliveira, G. J. P. L.; Maroni, M. A. T.; Pinotti, F. E.; Marcantonio Jr., E.; Marcantonio, R. A. C. Low-level laser therapy (LLLT) in sites grafted with osteoconductive bone substitutes improves osseointegration. **Lasers Med Sci**, p.1-11, 2020.

22. de Paula, L. G. F; de Oliveira, G. J. P. L; Pinotti, F. E.; Grecchi, B. B.; de Aquino, S. G.; Marcantonio, R. A. C. Effect of avocado/soybeanunsaponifiables (ASU) on osteointegration in rats with experimental arthritis. **Int J Oral Maxillofac Implants**, v.33, n.3, p.1-10, 2018.

23. del Carlo, R. J.; Galvão, S. R.; Viloria, M. I. V.; Souza, T. D.; Maia Filho, F. Aloenxertos ósseos caninos diferentemente preservados. **Rev Bras Cienc Vet**, v.6, n.3, p.121-126, 1999.

24. Dorozhkin, S. V. Bioceramics of calcium orthophosphates. **Biomaterials**, v.31, n.7, p.1465-1485, 2010.

25. Fawzi-Grancher, S.; Goebbels, R. M.; Bigare, E.; Cornu, O.; Gianello, P.; Delloye, C.; Dufrane, D. Human tissue allograft processing: impact on in vitro and in vivo biocompatibility. **J Mater Sci Mater Med,** v.20, p.1709-1720, 2009.

26. Filgueiras, R. R.; Del Carlo, R. J.; Viloria, M. I. V.; Odenthal, M. E.; De Lavor, M. S. L.; Duarte, T. S. Aloenxerto ósseo, preservado em glicerina, para reconstrução isquiopúbica após acesso ventral à cavidade pélvica de cadelas. **Rev Ceres**, v.51, n.298, p.719-728, 2004.

27. Fleming, J. E. J.; Cornell, C. N.; Muschler, G. F. Bone cells and matrices in Orthopedic Tissue Engineering. **Orthop Clin North Am**, v.31, p. 357-74, 2000.

28. Freire, T. J. F.; Poggiani, F. M. Avaliação radiológica de enxertos ósseos conservados em nitrogênio líquido ou glicerina 98%. **Revista Científica do Curso de Medicina Veterinária - FACIPLAC,** v.4, n.1, p.1-12, 2017.

29. Gaasbeek, R.; Toonen, H.; Heerwaarden, R.; Buma, P. Mechanism of bone incorporation of β-TCP bone substitute in open wedge tibial osteotomy in patients. **Biomaterials,** v.26, n.33, p.6713-9, 2005.

30. Galia, C. R.; Rosito, R.; Mello, T. M. Uso de enxerto ósseo homólogo e heterólogo em diáfise femoral de ratos: comparação entre enxerto ósseo congelado e liofilizado. **Rev Bras de Ortop,** v.40, n.3, p.141-146, 2005.

31. Giannoudis, P. V.; Dinopoulos H.; Tsiridis, E. Bone substitutes: An update. **Injury, Int J Care Injured**, v.36, p.20-27, 2005.

32. Giovani, A. M. M.; Croci, A. T.; Oliveira, C. R. G. C. M.; Filippi, R. Z.; Santos, L. A. U.; Maragni, G. G.; Albhy, T. M. Comparative study of cryopreserved bone tissue and tissue preserved in a 98% glycerol solution. **Clinics,** v.61, n.6, p.565-570, 2006.

33. Gutierres, M.; Lopes, M. A.; Hussain, N. S.; Cabral, A. T.; Almeida, L.; Santos, J. D. Substitutos Ósseos. Conceitos Gerais e Estado Actual. **Arq Med**, v.19, n.4, p.153-162, 2006.

34. Hogset, O.; Bredberg, G. Plaster of Paris and hair cell morphology. A scanning electron microscopic study of an alternative implant materials for ear surgery. **Acta Otolaryngol**, v.106, p.331-8, 1988.

35. Irie, M. S.; Rabelo, G. D.; Spin-Neto, R.; Dechichi, P.; Borges, J. S.; Soares, P. B. F. Use of Micro-Computed Tomography for Bone Evaluation in Dentistry. **Braz Dent J**, v.29, n.3, p.227-238, 2018.

36. Jensen, S. S.; Broggini, N.; HjØrting-Hansen, E.; Schenk, R.; Buser, D. Bone healing and graft resorption of autograft, anorganic bovine bone and β - tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. **Clin Oral Impl Res,** v.17, p.237-243, 2006.

37. Kamitakarahara, M.; Ohtsuki, C.; Miyazaki, T. Review Paper: Behavior of Ceramic Biomaterials Derived from Tricalcium Phosphate in Physiological Condition. **J Biomater Appl**, v.23, p. 197-212, 2008.

38. Khan, S. N.; Tomin, E.; Lane, J. M. Clinical applications of bone graft substitutes. **Orthop Clin North Am,** v.31, p.389-98, 2000.

39. Koshino, T.; Murase, T.; Saito, T. Medial opening-wedge high tibial osteotomy with use of porous hydroxyapatite to treat medial compartment osteoarthritis of the knee. **J Bone Joint Surg Am**, v.85, p.78-85, 2003.

40. Krieger, S. **Biocerâmica**. Universidade de São Paulo, 2003.

41. Langer, R.; Vacanti, J. P. Tissue engineering. **Science**, v.260, p.920-6, 1993.

42. Lascart, T.; Favard, L.; Burdin, P.; Traore, O. Utilisation du phosphate tricalcique dans les osteotomies tibiales de valgisation par addition interne. **Ann Orthop Ouest,** v.30, p.137-41, 1998.

43. Laurencin, C. T. Chapter I- Bone graft and bonegraft substitutes a brief history. In: Laurencin, C. T., editors. **Bone Graft Substitute** - ASTM International, 2004.

44. LeGeros. R. Z.; LeGeros, J. P. Calcium Phosphate Bioceramics: Past, Present and future. **Key Eng Mater**, vols.240-242, p.3-10, 2003.

45. Lew, K. S.; Othman, R.; Ishikawa, K; Yeoh, F. Y. Macroporous bioceramics: A remarkable material for bone regeneration. **J Biomater Appl**, v.27, n.3, p.345-58, 2016.

46. Marino, J. T.; Ziran, B. H. Use of solid and cancellous autologous bone graft for fractures and nonunions. **Orthop Clin North Am**., v.41, p.15-26, 2010.

47. Melo Filho, E. V.; Della Lúcia, R. M.; Salgado, A. E. P.; Miranda, F. B.; Drago, M. A.; Taffarel, M. O.; Vilela L. M.; Mussi, J. M. S.; dos Santos, W. G.; Zanini, M. S.; Freitas, P. M. C. Mecânica e microbiologia de placas produzidas a partir de osso cortical bovino, conservadas em diferentes meios. **Cienc Rural**, v.41, n.4, 2011.

48. Nishida, K.; Gilbertson, L. G.; Evans, C. H.; Kang, J. D. Potential applications of gene therapy to the treatment of spinal disorders. **Spine**, v.25, p.1308-14, 2000.

49. Ogose, A.; Hotta, T.; Hatano, H.; Kawashima, H.; Tokunaga, K.; End, N.; Umezu, H. Histological examination of beta-tricalcium phosphate graft in human femur. **J Biomed Mater Res (Appl Biomater)**, v.63, p.610-604, 2002.

50. Ogose, A.; Hotta, T.; Kawashima, H.; Kondo, N.; Gu, W.; Kamura, T.; Endo, N. Comparison of Hydroxyapatite and Beta Tricalcium Phosphate as Bone Substitutes After Excision of Bone Tumors. **J Biomed Mater Res Part B (Appl Biomater)**, v.72B, p.94-101, 2005.

51. Ozawa, M.; Tanaka, K.; Morikawa, S.; Chazono, M.; Fuji, K. Clinical study of the pure β-tricalcium phosphate: reports of 167 cases. **J East Jpn Orthop Traumatol,** v.12, p.409-413, 2000.

52. Padilha Filho, J. G.; Carvalho Penha, L. H.; de Souza, S. F. Uso do enxerto ósseo cortical bovino conservado em glicerina a 98% na osteotomia femoral em gatos. **Cienc Anim Bras**, v.9, n.4, p.1071-1078, 2008a.

53. Padilha Filho, J. G.; Eimantas, G. C.; de Souza, S. F. Osteossíntese femoral distal em cães e gatos jovens com fíbula de cão conservada em glicerina a 98%. **Vet Not**, v.14, n.1, p.49-55, 2008b.

54. Peltier, L. F.; Jones, R. H. Treatment of unicameral bone cysts by curetage and packing with plaster of Paris pellets. **Clin Orthop**, v.422, p.145-7, 2004.

55. Peterson, B.; Whang, P. G.; Iglesias, R.; Wang, J. C.; Lieberman, J. R. Osteoinductivity of commercially available demineralized bone matrix. **J Bone Joint Surg Am**, v.86, p.2243-50, 2004.

56. Pinotti, F. E.; de Oliveira, G. J. L.; Aroni, M. A. T.; Marcantonio, R. A. C.; Marcantonio Jr., E. Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: a pre-clinical study. **Clin Oral Impl Res**, v.00, p.1-10, 2018.

57. Proubasta, J.; Mur, J.G.; Planell, J.A. Biocompatibilidad, materiales implantables, tipos de implante. In: **Fundamentos de Biomecanica y Biomateriales**, Ediciones Ergon. Madrid., p.271-350, 1997.

58. Ratner, B. D. A history of biomaterials. In: Ratner, B. D., edits. **Biomaterials Science**. 2nd edition. Elsevier Inc., p.10-19, 2004.

59. Regner, L.; Carlsson, L.; Karrholm, J.; Herbert, P. Ceramic coating improves tibial component fixation in total knee arthroplasty. **J Arthroplasty**, v.13, p.882-889, 1998.

60. Rey, C.; Combes, C.; Drouet, C.; Somrani, S. Tricalcium phosphate-based ceramics. In: Kokubo, T. (ed.) Tricalcium phosphate-based ceramics. **Bioceramics and their clinical applications**, 2008; p. 326-355.

61. Schmitt, C. M.; Doering, H.; Schmidt, T.; Lutz, R.; Neukam, F. W.; Schlegel, K. A. Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone. A randomized controlled clinical trial. **Clin Oral Implants Res**, v.24, n.5, p.576-585, 2013.

62. Stevens, M. M. Biomaterials for bone tissue engineering. **Mater Today**, v.11, n.5, p.18-25, 2008.

63. Stevenson, S.; Emery, S. E.; Goldberg, V. M. Factors Affecting Bone Graft Incorporation. **Clin Orthop Relat**, v.324, p.66-74, 1996.

64. Tomford, W. W. Bone allografts: Past, Present and future. **Cell Tissue Bank**, v.1, p. 105-9, 2000.

65. Vaccaro, A. R.; Chiba, K.; Heller J. G. Bone grafting alternatives in spinal surgery. **Spine J,** v.2, p.206-15, 2002.

66. Walsh, W. R.; Vizesi, F.; Michael, D.; Auld, J.; Langdown, A.; Oliver, R.; Yu, Y.; Irie, H.; Bruce, W. β-TCP bone graft substitutes in a bilateral rabbit tibial defect model. **Biomaterials**, v.29, p.266-271, 2008.

67. Williams, D. F.; Doherty, P. J.; Williams, R. L.; Lee, A. J. C. Biomaterial-Tissue Interfaces: **Proceedings of the Ninth European Conference on Biomaterials**, Chester, UK, 1991.

68. Williams, D. F. **The Williams dictionary of biomaterials**, Liverpool Un. Press, Liverpool, 1999.

69. Williams, D. F. On the nature of biomaterials. **Biomaterials,** v.30, p.5897-5909, 2009.

70. Yamamoto, T.; Onga, T.; Marui, T.; Mizuno, K. Use of Hydroxyapatite to fill cavities after excision of begin bone tumors: clinical results. **J Bone Joint Surg**, v.82b, p.1117-20, 2000.

71. Yasemski, M. J.; Payne, R. G.; Hayes, W. C.; Langer, R. S.; Mikos, A. G. The evolution of bone transplantation: molecular, cellular, and tissue strategies to engineer human bone. **Biomaterials**, v. 17, p. 175-85, 1996.

72. Ziliotto, L.; Fantinatti, A. P; Daleck, C. R.; Padilha Filho, J. G.; de Souza, A. P.; Diniz, P. P. V. P. Utilização de implante ósseo cortical alógeno conservado em glicerina para preservação de membro torácico. Estudo experimental em cães. **Acta Cir Bras,** v. 18, n. 2, p. 107-115, 2003.

73. Zorzi, A. R.; de Miranda, J. B. (2012). Introduction, Bone Grafting, Dr Alessandro Zorzi (Ed.), ISBN: 978-953-51-0324-0, **InTech**, Available from: http://www.intechopen.com/books/bone-grafting/introduction

4. CAPÍTULO I

EVALUATION OF OSSEOINTEGRATION AND BONE HEALING USING PURE-PHASE β - TCP CERAMIC IMPLANT IN BONE CRITICAL DEFECTS. A SYSTEMATIC REVIEW.

Artigo submetido à revista **Frontiers in Veterinary Sciences**

Qualis Capes A1 - Medicina Veterinária

Evaluation of osseointegration and bone healing using pure-phase β - TCP ceramic implant in bone critical defects. A systematic review.

Daniel Cardoso Garcia [1,3*], Larissa Eckmann Mingrone [3], Marcelo Jorge Cavalcanti de Sá[2]

[1] Doctoral Student at the Department of Small Animal Surgery, Faculty of Veterinary Medicine, Federal University of Campina Grande (UFCG), Patos, Paraíba, Brazil, [2] Full Professor, Department of Small Animal Surgery, Faculty of Veterinary Medicine, Federal University of Campina Grande (UFCG), Patos, Paraíba, Brazil, [3] Small Animal Surgery Service, Animal Care Barueri Clínica Veterinária, Barueri, São Paulo, Brazil.

[*] Correspondence: Daniel Cardoso Garcia. danielcardosogarcia@gmail.com

ABSTRACT

Background: The gold standard for osseointegration remains the autogenous bone graft, but biomaterials such as β - TCP in its pure-phase showed promising results to be practical bone substitutes. This kind of implants are optimal candidates for bone integration due to their osseoconductive, biocompatibility, bioactivity, and absorptive properties.

Methods: A systematic review was conducted using 5 databases for searching published studies between January 1st 2011 and June 15th 2021. Only clinical and experimental studies, and case reports were included in this research. Human and animal studies published only in Portuguese or English with clinical, radiologic, and histologic evidence of new bone formation, osseoconduction and osseointegration were included. This systematic review was reported according to PRISMA guidelines.

Results: Approximately 14.500 articles were initially found, but after advanced searching using specific including and excluding keywords, matching Boolean operators "AND", "OR" and "NOT", and after excluding duplicates, a total of 11 articles were included for this systematic review, including experimental works, a retrospective study, a randomized controlled clinical study, a randomized prospective study, a prospective observational study, and a case report. All articles showed 100% effectiveness in bone integration after β - TCP implantation by clinical, radiological and/or histologic assessment. Implant shape and porosity seem to have influence in osseointegration process. β - TCP can give predictable, sustainable, and adequate new bone formation with the least infection rates in implant placement cases and patient morbidity, which is the current goals for bone integration, augmentation and replacement.

Conclusion: More clinical studies in the future, demonstrating specific metric measurements in relation to bone consolidation, as well as showing results with other shapes of this implant

are needed to evaluate further in depth osseoconductive and osseointegrative characteristics of this biomaterial, in order to develop new comparisons and quantitative analyses. β - TCP is widely used in dentistry and there is a lack of information about the use of this biomaterial for filling critical segmental defects of long bones in veterinary medicine, but only in small defects and in experimental studies, with no clinical cases performed in animals with a longer observation time.

Key words: Biomaterials; β - TCP; Osseoconduction; Osseointegration; Bone healing; Synthetic implant; Bone defect feeling.

INTRODUCTION

Restoring lost tissue through the application of tissue engineering principles is a complex process that involves interaction between cells, growth factors and scaffolds (1). What still remains a challenge is how to improve the integration of the newly formed bone with the surrounding tissues (2).

Replacement of part of the bone tissue is often necessary in the surgical routine, whether due to fractures, bone neoplasms or orthopedic diseases that lead to bone loss (3). For over 100 years, the gold standard and the most favorable results for bone repair has been the transplantation of autologous bone from the iliac crest, providing the advantages of osseoconductive, osseoinductive and osteogenic actions at the implantation site (4-6). It has been reported that the release of growth factors such as platelet-rich plasma (PRP) and transforming growth factor-β (TGF-β) associated with early vascularization of the donor bone allows for remodeling within 4-6 months after placement of the implant (5). However, this procedure can be hampered by donor site morbidity (7), limited graft availability (4-5), donor site infection (4,8), need for additional surgical intervention (8-9), excessive resorption (4,8) and greater stress for both the surgeon and the patient (5).

As readily available and low-cost alternatives, allografts, xenografts and alloplastics have been used (9-10). Of all these options described, all have been shown to have osseoconductive properties (except lyophilized demineralized bone allograft, which is an osseoinductive material). However, allografts carry the risk of problems integrating with the surrounding bone tissue and, more importantly, they can induce immune responses in the recipient with unpredictable consequences (4). Its preparation requires sterilization and deactivation of proteins normally found in healthy bone, which eliminates the extracellular matrix, as well as bone growth factors, proteins, and other bioactive materials necessary for

osseoinduction (7). They have some disadvantages, including the demineralization process and the need to use frozen tissue. The use of dehydrated human bone is also controversial. Clinically and histologically, allografts present very good results (7,11). On the other hand, such materials present the risk of transmitting infectious diseases. This fact led to greater interest in the research and development of synthetic biomaterials similar to bones (9).

Natural and synthetic bone substitutes are widely used for bone regeneration due to its biocompatibility, osseoinductive and osseoconductive effects, and absence of risk of antigenicity. These materials concurrently serve as a "scaffold" and as osteogenesis stimulators, facilitating bone regeneration, and are often replaced by newly formed bone tissue after graft or implant resorption (8-9,11). These biomaterials are provided mechanical, and inductive support transformers to tissues in reflex, features related to bone substitutes (9).

In this sense, the biomaterial is very capable of promoting bone replacement in order to avoid the use of grafts or bone transplants (3). Synthetic products were formed as replacements for bioinert compounds, several used over decades with the primary purpose of filling and augmenting bone in skeletal facilities. It is known that phosphate compounds have affinity for specific tissues such bones and showed promising results among the various alloplastic materials used for grafting (6).

Alloplastics do not have osteogenic or osseoinductive properties but are osseoconductive materials. On the other hand, these materials are widely available, inexpensive, and do not have the potential to transmit disease or infection (7,10). Furthermore, as they are synthetic, their chemical composition and fabrication can be increasingly manipulated to mimic the characteristics of natural bone (10).

Ideally, as a bioactive calcium phosphate (CaP) ceramic for use in bone material, it exhibits a good behavior in relation to bone bonding, stimulating the formation of bone tissue at the interface between the biomaterial and the features, and to add these features, also must show a high rate of degradation, thus finding a balance between rapid bone formation and rapid biodegradation. (8).

CaP bioceramics in the form of granules or microporous blocks stand out in the researches as biomaterials for defect repair and bone tissue reconstruction, as mentioned by different authors, who observed bone neoformation when these biomaterials were applied in vivo (12).

Microporous CaP bioceramics are manufactured with architecture similar to structure of bone tissue, formed by fine grains, with interconnected microporosity, which helps in the

mechanism of cell adhesion and proliferation through osseoconduction, osseointegration and formation of new bone (12).

It is verified that the performance of these bioceramics in the process of bone neoformation is associated with the characteristics of bioactivity, solubility, wettability, interconnected microporous microstructures, higher superficial areas of grains and micropores.

These characteristics provide conditions for the adhesion of osteoblasts to the surface of the granulated biomaterial, as well as in the interconnected microporosity throughout the granulated biomaterial (3), leading to biomineralization of bone tissue.

Due to their ability to bond to bone and stimulate bone tissue formation, bioactive CaP ceramics are seen as excellent materials for implantation for candidates who need bone augmentation, filling, or replacement (8). Among the biodegradables and osseoconductors biomaterials currently in use and mentioned above, highlights the β - TCP (β - tricalcium phosphate), which is the most popular (2,8). The β - TCP is reabsorbed completely and has no intrinsic osteogenic or osseoinductive properties (7). However, β - TCP has excellent osseoconductivity and biocompatibility. About its microporosity, pores of 50 μm are sufficient to allow osseoconduction (2). Furthermore, it was reported that the β - TCP surface architecture can stimulate monocyte/macrophage differentiation invaders in osteoclasts, and these cells may be essential for ectopic bone formation (2).

Synthetic bone substitutes, such as β-TCP, are clinically very beneficial because they can avoid surgery on a donor site. Thus, β - TCP is considered an ideal grafting material (5). It has the property of being absorbed by the body while being replaced by bone; in addition, its usefulness has been well reported in maxillofacial surgery (5) and it is mainly used in dentistry for filling dental sockets and augmenting of the maxillary sinus floor (10). However, the predictability of the material and the change in absorption of the transplanted material remain largely unknown (10). Significant resorption and integration of β - TCP particles are expected 3-6 months after placement (2,4,6) allowing for a re-arrangement of trabecular bone during this period (6), but little change is observed after 1.5 years (5). Most β - TCP is biodegraded both by osteoclastic activity subsequent to particle breakdown, as well as by chemical dissolution of the molecule into calcium and phosphate components followed by replacement with healthy bone (4,7).

Thus, an important characteristic of synthetic bone substitutes is their bioactivity, which ideally should allow a well-defined resorption of the implant, which is balanced with the speed of new bone formation, resulting in true osseointegration and “restitutio ad integrum”. In this context, it is important to state that the so-called ‘‘resorbable’’ bone substitutes, including

calcium phosphates, were somewhat unsuccessful in this regard, since they could still be detected years after of their implantation due to inadequate resorption (5). In contrast, some studies demonstrated an accelerated resorption of several types of biomaterials, which was not compensated by the increased formation of new bone at the site. Thus, the choice of the applied biomaterial needs to be made according to the patient's comorbidities, in order to provide the best type of treatment combined with excellent results (8).

RATIONALE AND OBJECTIVE

The objective of this study was to carry out a systematic review to evaluate osseointegration and bone consolidation when using the β - TCP ceramic implant in its pure phase in critical bone defects, concomitantly evaluating other pertinent attributes related to the biomaterial studied. The idea is that we would find literature that would serve as a support and basis for the use of this biomaterial in critical bone defects in small animals, especially in more severe cases with great bone loss, where there will be the need to fill or replace the bone gap created after the loss of bone tissue.

MATERIALS AND METHODS

This systematic review adopted and followed the protocols specified by the "Preferred Reporting Items for Systematic Reviews and Meta Analysis" (PRISMA) (13), with the following question elaborated according to the FINGER criteria (14): Is there osseointegration and bone consolidation of long bones critical segmentar defects using β - TCP ceramic implant in its pure phase to fill the created defect?

Criteria for inclusion and exclusion of articles

Using the PICO strategy (P = population/patients; I = intervention/exposure; C = comparison/control; O = "outcome"/outcome/result) (15), articles that met the following criteria were included in this review systematic.

Population: Patients (humans or animals) in which the ceramic implant of β – TCP was used in bone defects.

Intervention: Use of pure β-TCP ceramic phase in critical bone defects in long bones.

Comparison: Other types of grafts and other types of implants for filling, or even defects without filling.

Outcome: Osseoconduction, osseointegration, and bone healing of critical bone defects.

Article selection criteria

• Only full articles between January 2011 and June 2021.

• All human and animal studies published in English or Portuguese with clinical, radiological and/or histological evidence of bone neoformation.

• Experimental work, clinical trials, and case reports.

Exclusion criteria for articles

• Abstracts without the presence of full articles.

• Review articles.

• Articles published prior to 2011.

• Articles in languages other than Portuguese or English.

Study search and selection strategies

A systematic search was carried out exploring the Cochrane Library, PubMed, Scielo, Medline-Bireme and Google Scholar databases for articles that addressed the role of pure phase β - TCP in osseointegration and bone healing between January 1st 2011 and June 15th 2021.

Keywords associated with Boolean operators were used for such searches. For this, the words "β - TCP" OR "Beta TCP" OR "Beta Tricalcium Phosphate" OR "Beta Phosphate Tricalcium" AND "Osseointegration" OR "Osteointegration" were included. Due to the high number of articles in the Google Scholar database, we restricted the search by adding one more keyword filter with the Boolean operator AND and the word "pure phase", in addition to using the Boolean operator NOT to the keywords "composite" OR "biphasic" in order to exclude these last two words. For the other databases mentioned above, advanced search was not an issue as there were few articles. The search query was modified for each database if necessary to reach the most relevant studies. Then, data were collected based on the relevance to the study topic and the main objective, through a tool for evaluating titles and abstracts called Rayyan (16), which was performed by 2 independent researchers in a blind way. From this, any conflicts between authors were resolved by reading the full text to determine whether a particular article was selected for inclusion or exclusion in the systematic review.

Data extraction:

In all, 113 studies were chosen according to title reading and abstract screening, and then the references of these studies were manually searched and checked on Google Scholar. After removing duplicates, we added relevant articles based on title and abstract screening.

From the selected articles, relevant information about the study design, study population, methodology and results were extracted. Other relevant characteristics related to the implant used in the study were also mentioned. In the final step, the inclusion of articles in this systematic review was made by mutual agreement between 2 authors, and at the end 11 studies were chosen for data extraction.

Risk of bias

The methodological quality for the selection of studies was evaluated by two reviewers (DCG and LEM) using the JBI critical appraisal tool (17) for each study individually. The two authors resolved any disagreement regarding the risk of bias of each study by discussion and consensus among reviewers, and if any doubts or disagreements persisted between the first two reviewers, a third reviewer (MJCS) was consulted.

RESULTS

A total of 113 articles published between 2011 and 2021 were identified using the databases through a systematic electronic search. After evaluation by two independent reviewers, the full text of 11 articles was obtained (Figure 1). Below we can identify the number of articles found in each of them:

- Cochrane Library: A total of 5 articles were found by searching the keywords “β - TCP” OR “Beta TCP” OR “Beta Tricalcium Phosphate” OR “Beta Phosphate Tricalcium” AND “Osseointegration” OR “Osteointegration”.
- Pubmed: 23 articles were found using the keywords “β - TCP” OR “Beta TCP” OR “Beta Tricalcium Phosphate” OR “Beta Phosphate Tricalcium” AND “Osseointegration” OR “Osteointegration” in the search.
- Scielo: Only 1 article was found using the keywords “β - TCP” OR “Beta TCP” OR “Beta Tricalcium Phosphate” OR “Beta Phosphate Tricalcium” AND “Osseointegration” OR “Osteointegration”.
- Medline-Bireme: 25 articles were found using the keywords “β - TCP” OR “Beta TCP” OR “Beta Tricalcium Phosphate” OR “Beta Phosphate Tricalcium” AND “Osseointegration” OR “Osteointegration”.
- Google Scholar: Approximately 14,500 articles were found using the keywords “β - TCP” OR “Beta TCP” OR “Beta Tricalcium Phosphate” OR “Beta Phosphate Tricalcium” AND “Osseointegration” OR “Osteointegration” in the searches.

Subsequently, refining the search through the advanced search, in which the Boolean operator AND and the words “osseoinduction” and “pure phase” were added, 239 articles were found. Then, a new advanced search using the Boolean operator NOT to exclude the terms “composite” OR “biphasic” brought us to a total of 59 articles. Further refining the searches, the words “long bones” were included by the Boolean operator OR, associating it with the previously mentioned terms “β - TCP” OR “Beta TCP” OR “Beta Tricalcium Phosphate” OR “Beta Phosphate Tricalcium” AND “Osseointegration” OR Osteointegration” AND “pure phase” NOT “biphasic” OR “composite”, and a total of 4 studies were filtered. However, of these four studies, none of the articles corresponded to the use of β - TCP in its pure phase in long bones as an implant that stimulated osseointegration and bone consolidation in segmental defects, therefore, the words "long bones" were excluded from the search, and after reading the titles and abstracts of the final articles, a total of 8 full articles were included from the Google Scholar database to verify the results of β - TCP associated with osseintegration.

After excluding duplicates of articles across databases, a total of 11 articles were included for this systematic review. The authors of the articles related to each study design, the description of the observation time and the number of patients or animals included in the studies are summarized in Box 1.

Of the eleven articles selected, six were experimental studies, one was a retrospective study, one was a randomized controlled clinical trial, one was a randomized prospective clinical, radiological, and histological study, one was a prospective observational study, and one was a case report.

Of the eleven studies, six articles reported clinical, radiological, and histological evidence of bone neoformation using pure β - TCP phase, two showed radiological and histological analysis, one presented clinical and histological analysis, one revealed only histological study, and one reported only a SEM analysis. More information on the clinical, radiological, and histological findings can be found in Boxes 2 and 3.

Regarding the countries where the experimental works were carried out, one was in Japan (2), two in Brazil (3,12), one in Turkey (9), one in Israel (7) and one in Germany (8). The case report was carried out in the USA (10). And finally, clinical studies took place in Egypt (6), India (4), South Korea (11) and Japan (5), respectively.

All eleven studies included in this systematic review reported a significant increase in the percentage of new bone formation when β - TCP in its pure phase was used, including two randomized controlled trials. Some of the articles described this increase as a percentage of the volume density of the newly formed bone and one article also described it as a bone mineral

content in milligrams. None of the articles reported complications, except (9,11). Only five articles mentioned residual biomaterial at the implanted site (Box 4). When cost analysis was searched, of the eleven original studies that reported scientific evidence of new bone formation using pure-phase β - TCP, two mentioned costs, but the information was very vague (Box 4). They only mentioned the best cost-benefit using the β-TCP implant but did not say the exact value of the costs of implants or procedures related to their use.

For this reason, we found another review article, which is not included in this systematic review, but mentions the price-brand relationship in its data, and for this reason we included here the prices of three brands of β - TCP compared in this article for the knowledge of the reader only. The estimated cost of pure phase β-TCP granules was US $ 63,00 to US $ 73,60. With the articles selected for this systematic review, we were not able to corroborate the prices of all pure-phase beta tricalcium phosphate brands, but only the value of two of them (Box 5). There were no mentions of additional indirect costs associated with using this technology.

Others relevant attributes related to β - TCP implantation were also described. Of the eleven articles included in this review, six emphasized the use of β - TCP as a form of granules. Only one article used it as powder. Four of them did not mention the shape of the implant, but there is a trade name associated with it where readers can search for. Only five of the eleven papers showed particle sizes and only four told us about the porosity of the granules. The size of the bone defects created for implant insertion was explained only in eight of the eleven studies. The mentioned data can be seen in Box 6.

DISCUSSION

The systematic review of articles using β - TCP in its pure phase to assess osseointegration between the years 2011 and 2021 generated few articles (11 articles) after databases searching. In the initial phase of the search, more than 14.500 articles were found in the databases with the selected keywords, but in the end, after the inclusion and exclusion criteria, only 11 articles met the prerequisites for selection. However, we expected a greater number of articles, a fact that may be related to the more frequent use of β - TCP associated with some other type of material, forming composites. Many of these articles were excluded from this systematic review because they did not fit the chosen search terms.

The association of biomaterials could even be noticed in some articles of this review, which despite presenting results with the use of pure β - TCP as a control, also brought us results from other types of biomaterials not mentioned here, or even comparisons between them. However, those who used pure phase ceramic were only included in this review due to the

keywords selected and relation to β - TCP and the results mentioned about it, which could be used independently in the context of the article (2-4 ,6,8,11-12).

It was found that the pure-phase use of this ceramic is currently more used in dentistry (5-7,10-11), especially in cases related to bone augmentation of the floor of the maxillary sinuses for later placement of titanium implants and for alveolar filling, as described by some authors in randomized and prospective clinical studies with human patients (4-6).

Most of the articles included in this study were classified as experimental studies (6 articles), which leads us to believe that even if some of the researches inform us about new forms and associations of materials to promote the growth and differentiation of bone tissue at the with the implant and through its micropores, there are still authors who research the use of pure phase β - TCP ceramic to evaluate the osseoconduction and osseointegration process, in addition to other characteristics that this implant could bring to bone neoformation, even if it already has been extensively studied (2-3,7-9,12).

This could be verified by the different species that were used in the experimental models listed above, such as sheep, rabbits, pigs, and mice, as well as the observational study periods, which ranged from 1 week to 18 months after the implantation of β - TCP. In addition, we could also verify the various types of bones used in the experiments for implantation of bioceramics, such as the bone of the maxilla, tibia, femur, scapula, and frontal bone of the skull. An interesting fact is that in all these experimental animal studies using bone defects for implantation of β - TCP, the bone defects created for implantation were only cylindrical holes, with diameters varying up to 6 mm in a single cortical bone and performed using a drill. It was not identified in any of the articles the use of β - TCP, the occurrence of osseoconduction and bone neoformation, which were performed in bone defects with diameters greater than 6 mm and compromising the 2 cortical bones, or even critical segmental defects in long bones that would create a total loss of contact between the ends of the ostectomized bone.

As for the other 5 articles in which the patients were human, all were included in clinical studies (4-6,11), except for one of them, which was a clinical case report (10), and in all these cases the articles were related to alveolar filling or maxillary sinus floor elevation in dentistry. It is important to mention that no article selected for this search period showed the use of β - TCP in long bones, spine, or other bones, nor was performed in the areas of orthopedics or traumatology, either clinically or experimentally, as in cases of vertebral fusions, filling of bone cysts, bone replacement in cases of neoplasms, bone non-unions, or even for cases of fractures with great bone loss that required an implant for bone replacement and concomitant preservation of bone length.

Despite the good results obtained with the use of pure β - TCP in relation to bone neoformation by the osseoconduction process, in which all authors reported 100% osseointegration with the use of this bioceramic, some of the articles showed that the association of other types of materials with osteogenic characteristics and/or osseoinductive properties to β - TCP, or even other forms of composites, showed superior results compared to the use of pure phase β - TCP, either by a greater local bone production or even for a quicker time in the osseointegration process through cell growth at the implant interface (2-4,6,8,11-12).

In all eleven studies, clinical, radiographic, or histological signs of bone neoformation were evidenced when β - TCP was used in its pure phase, and only two presented complications, being (2,91%) (11) and (11,1 %) (9), respectively, but not due to the use of the implant itself, but due to problems associated with the surgical technique.

Regardless of the design of the studies included in this systematic review, the effectiveness of using this ceramic has been proven. It was observed that the bone tissue invades the environment in which the ceramic granules are implanted, and adheres to them due to their porous characteristics, which are replaced over time by new bone, which proves its bioactive and osseoconductive activity.

The β - TCP implant also did not cause immunological reactions or inflammatory response when in contact with the host tissue, indicating its biocompatible properties (7).

In the histological evaluations, what was described were different degrees of bone maturation depending on the implant used, and different degrees of bone maturation with time, as described by (6). And despite the formation of new bone, there was still an amount of residual implant that remained after a long period of implantation (4,6). The first author shows that there was formation of well-vascularized connective tissue and no presence of inflammatory cells at the site, differing from the study conducted by (4), which showed that despite the formation of new bone around the β - TCP implant, there was less osteoid formation in relation to the other study group. In addition, the connective tissue around the β-TCP implant was poorly vascularized and presented several inflammatory cells, also differing from the other author.

The osseoconduction process takes place through the constant formation of osteoblasts that form around the implant and osteoclasts that destroy the implant over time, opening space for the formation of new bone (9). This process was also described by (2) and (7), who showed in histological sections the presence of osteoclastic cells on the surface of the β - TCP implant, as well as the formation of Havers' canals during the bone healing phase. In another study, (10) showed that bone maturation, bone remodeling and bone formation occurred with

characteristics similar to those of the Haversian system in multiple sites where β - TCP was implanted.

Throughout the process of bone formation and remodeling, it was observed that bone/implant integration occurs over time, being a dynamic process that occurs as new bone forms at the same time as the implant is degraded and reabsorbed. (12). It is known that the faster the biomaterial is absorbed, the more bone is deposited between the implant gaps and faster is the bone maturation process at the site. The same author (12) also showed that there is a different degree of absorption for each implant or even for combined proportions of implants over time. He had demonstrated that the pure phase β - TCP presented a better degree of absorption when compared to the β - TCP associated with HA in the proportions 20/80 and 30/70, respectively. On the other hand, when compared with the proportions 80/20 and 70/30 respectively for each material, the pure phase β - TCP presented worse absorption rate and degree of osseoconduction and osseointegration in relation to the composite.

Another particularly interesting factor observed in this systematic review is that in all the articles included in the study, the ceramic format used by the authors was always that of granules with different granulometries and porosities, except in the article of (2), in which the β - TCP used was in powder. No other papers used β - TCP in a different format such as paste, blocks or compact cylinders to fill in defects and promote osseointegration.

It has been reported that granulation can interfere with the osseoconduction process (2,9). Larger particles would allow for earlier bone regeneration, forming a more stable structure compared to materials that have smaller particles. And materials with smaller micropores have better osseoconductivity. These properties make them more effective in forming new bone (9).

One question we raise in this regard is whether the use of β - TCP cylinders or compact blocks could be used in bone replacement in cases of critical segmental bone defects in long bones, such as extensive fractures with great loss of bone tissue, for example. Cases in which there would be the need for a more compact material to restore the bone defect longitudinally, while maintaining the original length of the bone, and biomechanically being more rigid to withstand forces over time. In this way, what we would see was the union of the two bone segments, proximal and distal, at the interfaces with the ends of the implant. Thus, we imagined that osseoconduction would occur from the proximal and distal ends of the implant and over the surface of the implant, towards the center of the implant. This could open perspectives for further studies, initially using the pure phase β - TCP, but also, later associating the implant in

question with other types of materials, forming composites for analysis of osseointegration in critical segmental defects in veterinary medicine.

However, as observed in the PRISMA flowchart, during the inclusion/exclusion process of the selected articles, when the keyword “long bones” was inserted, there was a considerable decrease in the number of articles, but none of them fit the eligibility criteria after reading the titles, abstracts, and objectives. This factor may indicate that there is a perspective of action in this area using β - TCP to correct large bone losses in fractures, excision of bone tumors and bone non-unions in veterinary medicine.

Comparing different brands of pure phase β - TCP, it was found that even though all of them presented new bone formation, there was a difference in the values presented in relation to osseoconductivity. This was verified by (9), when the author compared 3 different types of pure phase β - TCP, Poresorb M®, Kasios® and Cerasorb®, and showed that the first one presented the best results for bone formation, presenting a higher percentage of osseoconductivity and better values of bone thickness (TbTh) and bone width (TbWi), while Cerasorb® presented the worst indexes for the same evaluated parameters. And regarding the rate of implant resorption over time, Kasios® was the worst among the three.

All the studies that cited the effectiveness of this biomaterial through radiological evaluation showed good bone integration and consolidation, with different values of bone volume and trabeculation, especially for those clinical studies in humans in which maxillary sinus floor elevation and filling of dental sockets were performed. However, an important information given by one of the authors (5) was in relation to the mean bone volume and the thickness (height) of the maxillary sinus floor after 2 years of β - TCP implantation in 30 patients. The author found that in several of these patients (70%), radiographically there was a decrease in the evaluated parameters mentioned above when compared to the radiographic study of the 6-month postoperative period, which may be related to bone resorption that occurred over time. This means that, due to this bone resorption, there was exposure of the tip of the titanium implants inserted in the maxillary bone associated with β - TCP, but without complications. This study, unlike most of the others mentioned, is one of those that evaluated for the longest time patients who received β - TCP ceramic implants to increase bone volume. Therefore, we cannot know whether in the other selected articles, specifically referring to experimental studies in which the observation time was less than six months, whether such a process of bone volume reduction would occur after osseointegration. These findings may be associated with the natural process of bone remodeling that occurs in a physiological way.

On the other hand, another study, a case report of 1 patient who was evaluated for 4 years after a maxillary sinus floor augmentation procedure using β - TCP, did not evidence such bone absorption due to time (5). Despite presenting the results with a much longer time than in the previous article, we must consider that the report presents only one patient. Perhaps if there were more patients in this study group, the results could be different.

We did not find in the evaluated articles the prices related to the β - TCP implants used. As different brands were used, a comparison between them would be necessary to stipulate the cost-benefit. As none presented numbers, but only a vague mention of "lower costs", we turned to another article that was initially excluded from the initial research, because during its reading it was the only one in which we found a price list with some of the implant brands used.

We believe that we had some limitations in our systematic review, most of them related to the heterogeneity of the studies. Different animal species, as well as the bones used as experimental models, including long bones, calvaria bone and maxillofacial bones were used in combination with various implant application techniques in different species, as well as the size of the bone defects created. Regarding the β - TCP implant, there is heterogeneity regarding its sizes, porosity, brands, amount of biomaterial implanted in the defect sites. Regarding the chosen method of observation time, there is heterogeneity in terms of follow-up periods. Times ranged from 1 week to 4 years.

In addition, in relation to the osseointegration process and bone healing, which are the main objectives of this systematic review, they were approached in different ways by the studies, with some evaluated by radiographic images, others by histological analysis, others by clinical evaluation, and others evaluated through combinations of these different methods.

Even knowing that all of them reported positive changes in the implanted area with good results in bone formation and integration, this limits our study when we try to make a more adequate comparison between the parameters evaluated, which may cause a bias in the study.

Due to the lack of quantitative evidence in some of the studies, as well as the heterogeneity observed regarding the brands of biomaterials, granule sizes, different locations, and sizes in the realization of the bone defect for implant placement, patient species, follow-up periods and lack of information of some comparable items, it was not possible to perform a meta-analysis on the eleven articles included in this systematic review.

CONCLUSION

β - TCP in its pure phase is a biomaterial that has been tested and used for cases of bone augmentation and replacement for decades. In general, after autogenous grafts, we can say that

β - TCP is the most reliable and widely used synthetic biomaterial category of materials for grafting in dentistry and maxillofacial surgery. It can produce predictable, sustainable, and adequate bone formation, with minimal infection rates and low patient morbidity, essential characteristics to search for when considering bone filling and bone replacement. More future clinical studies showing the exact and standardized values of measurements that evidence bone healing, as well as studies showing other formats (such as cylinders and blocks), and clinical uses of β - TCP alone or in association with other biomaterials, are needed to evaluate their osseoinductive, osseoconductive and osseointegrative properties, as well as to determine possible new comparisons and quantitative analyzes for further evaluations on the subject.

Conflict of interests

The authors report that there are no conflicts of interest and are solely responsible for the writing and content of this work.

Authors' contribution:

DCG and MJCS conceived and designed this systematic review. DCG and LEM performed the searches in the databases. DCG and MJCS coordinated the research and selected the articles. DCG and LEM read and selected the articles for inclusion/exclusion of the same and the relevant data of each article. DCG and LEM wrote the article. All authors critically reviewed the manuscript and approved the final version.

Financial support and sponsorship

There was no financial support for this research. There will only be a financial contribution from the UFCG postgraduation program if the article is approved and published in a Qualis Capes A1 Journal. In this way, the financial aid will only be used to pay the journal's publication fee.

REFERENCES

1. Javaid MA, Esfandiari SA. Health technology assessment report on role of platelet derived growth factor in intra-osseous periodontal regeneration (Report no: S2013.01). Montreal, Canada: Faculty of Dentistry, McGill University (2013). Retrieved from: https://www.mcgill.ca/dentistryohs/courses-and-events/dent-655/hta-report

2. Bhawal UK, Ryoichirob U, Noboruc K, Tetsuod A, Koichia H, Norihirobet N. Effect of the surface morphology of silk fibroin scaffolds for bone regeneration. *Bio Med Mater Eng.* (2016) 27: 413-424. doi: 10.3233/BME-161595

3. Costa BD, Camargo NH, Oleskovicz N, Gava A, Dallabrida AL, Regalin D, et al. Neoformação óssea e osteointegração de biomateriais micro e nanoestruturados em ovinos. *Pesq Vet Bras.* (2015) 35(2): 177-187. doi: 10.1590/S0100-736X2015000200015

4. Joshi CP, Dani NH, Khedkar, SU. Alveolar ridge preservation using autogenous tooth graft versus betha-tricalcium phosphate alloplast: A randomized, controlled, prospective, clinical, pilot study. *J Indian Soc Periodontol.* (2016) 20(4): 429-434. doi: 10.4103/0972-124X.188335

5. Okada T, Kanai T, Tachikawa N, Munakata M, Kasugai S. Long-term radiographic assessment of maxillary sinus floor augmentation using beta-tricalcium phosphate: analysis by cone-beam computed tomography. *Int J Implant Dent.* (2016) 2(8): 1-9. doi: 10.1186/s40729-016-0042-6

6. Abdullah AAB, Edrees MF, Bakry AM. Clinical, radiographic, and histological assessment of socket preservation using melatonin with beta-tri-calcium phosphate for receiving dental implant. *Biomed Sci.* (2021) 1: 10-16. doi: 10.11648/j.bs.20210701.12

7. Klein Y, Kunthawong N, Fleissig O, Casap N, Polak D, Chaushu S. The impact of alloplast and allograft on bone homeostasis: orthodontic tooth movement into regenerated bone. *J Periodontol.* (2020) 91(8): 1067-1075. doi: 10.1002/JPER.19-0145

8. Knabe C, del-Khattab A, Hübner WD, Peters F, Knauf T, Peleska B, et al. Effect of silicon-doped calcium phosphate bone grafting materials on bone regeneration and osteogenic marker expression after implantation in the ovine scapula. *J Biomed Mater Res. Part B: Appl. Biomater.* (2018) 107(3): 594-614. doi: 10.1002/jbm.b.34153

9. Damlar I, Erdoğan O, Tatli U, Arpağ OF, Görmez U, Üstün Y. Comparison of osteoconductive properties of three different b-tricalcium phosphate graft materials: A pilot histomorphometric study in a pig model. *J Craniomaxillofac Surg.* (2015) 43(1): 175-180. doi: 10.1016/j.jcms.2014.11.006

10. Daher S, Leary J, Ewers R, Coelho PG, Bonfante EA. Histological analysis of an implant retrieved from a β tricalcium phosphate graft after 4 years: a case study. *J Long Term Eff Med Implants.* (2019) 29(2): 135-140. doi: 10.1615/JLongTermEffMedImplants.2019031828.

11. Kim J, Sohn DS, Heo JU, Moon JW, Lee JH, Park IS. Benefit of the replaceable bony window in lateral maxillary sinus augmentation: clinical and histologic study. *Implant Dent.* (2014) 23(3): 277-282. doi: 10.1097/ID.0000000000000070

12. Dallabrida AL, Camargo NHA, Moraes AN, Gava A, Dalmônico GML, Costa BD, et al. Caracterização de biocerâmica de fosfatos de cálcio microestruturada em diferentes composições em ovinos. *Pesq Vet Bras.* (2018) 38(7): 1327-1336. doi: 10.1590/1678-5150-PVB-4930

13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372(71): 1-9. doi: 10.1136/bmj.n71

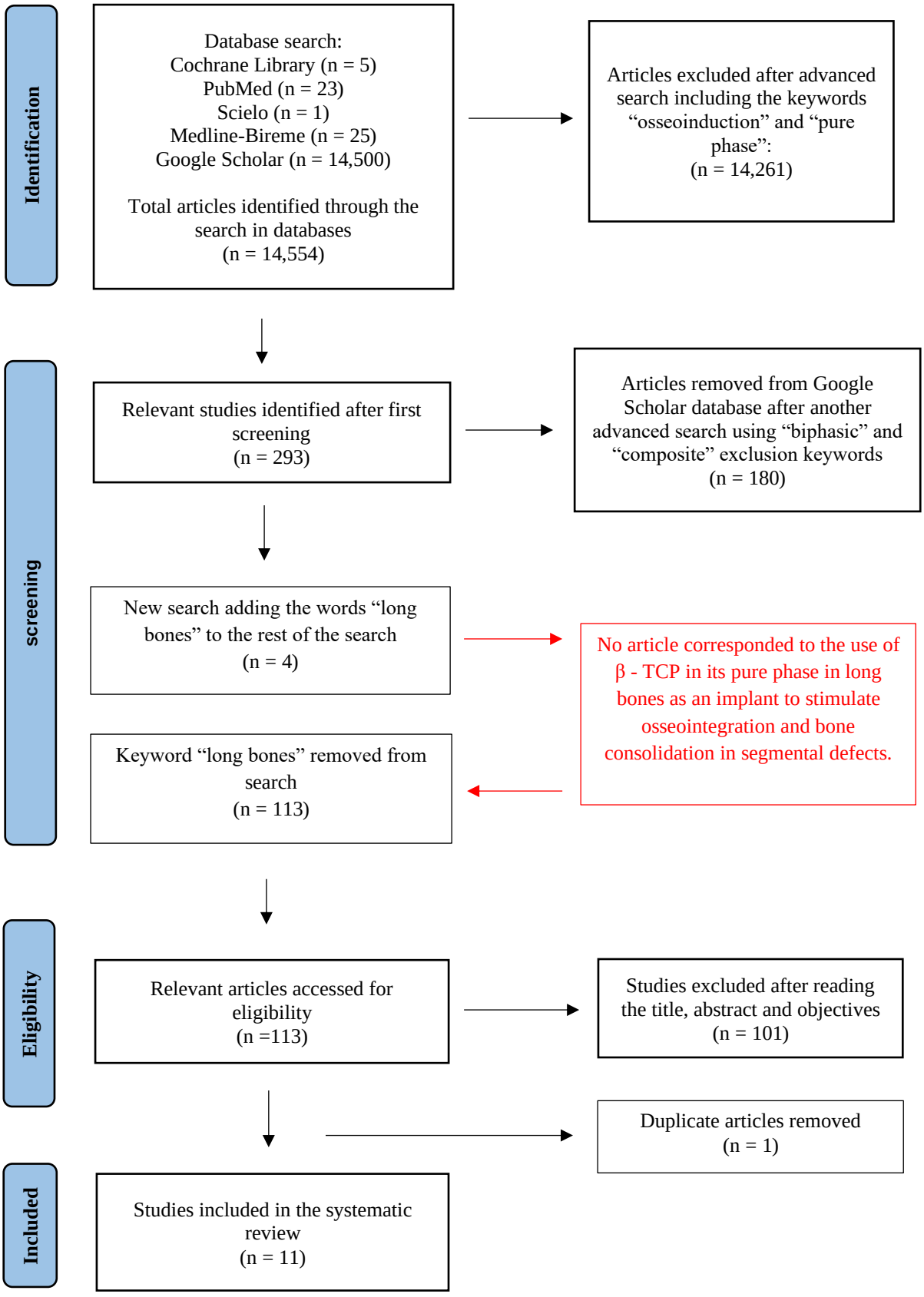
14. Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB. Designing clinical research. 4th ed. Philadelphia (PA): Lippincott Williams and Wilkins; 2013.

15. Haynes R. Forming research questions In: Haynes R, Sackett D, Guyatt GH, Tugwell P, editors. Clinical Epidemiology: How to do Clinical Practice Research. Philadelphia, PA: Lippincott Williams & Wilkins; 2006. p. 3-14.

16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan - a web and mobile app for systematic reviews. *Systematic Reviews* (2016) 5:210, doi: 10.1186/s13643-016-0384-4.

17. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Chapter 7: Systematic reviews of etiology and risk. In: Aromataris E, Munn Z (Editors). JBI Manual for Evidence Synthesis. JBI, 2020. Available from https://synthesismanual.jbi.global

Figure 1 - PRISMA flowchart of the search strategy for the data collected for inclusion and exclusion of articles in the systematic review.



Box 1 - Articles included in the systematic review showing authors, type of study, observation time, and number of patients or animals used in the study.

Authors / Year	Study Design	Time of Study	Number of Patients / Animals
Abdullah et al., 2021	Randomized controlled clinical trial	6 months	24 humans
Bhawal et al., 2016	Experimental study	1 and 2 weeks	24 rabbits
Costa et al., 2015	Experimental study	3 months	8 sheeps
Daher et al., 2019	Case report	4 years	1 human
Dallabrida et al., 2018	Experimental study	3 months	8 sheeps
Damlar et al., 2015	Experimental study	8 weeks	8 pigs
Joshi et al., 2016	Prospective randomized clinical, radiographic, and histological study	4 months	15 humans
Kim et al., 2014	Retrospective study	6-8 months	81 humans (103 maxillary sinuses)
Klein et al., 2020	Experimental study	2-6 weeks	84 mice
Knabe et al., 2019	Experimental study	2 weeks to 18 months	36 sheeps
Okada et al., 2016	Prospective observational study	6 months and 2 years	30 humans

Box 2 - Articles in alphabetical order of authors included in the systematic review showing the methodology and biomaterials used, as well as the effectiveness of the studies demonstrated by clinical, radiological and/or histological evidence of newformed bone after placement of the implant.

Authors / Year	**Methods / Biomaterials**	**Clinical/Radiological/Histological Evidence of New Bone Formation / Residual Implant**
Abdullah et al., 2021	Preservation of the socket after tooth extraction. Group A - defect filled with 1% melatonin + β-TCP Group B - defect filled with pure β-TCP phase	All had neoformed bone, residual biomaterial, and well-vascularized non-inflamed connective tissue. Group (A) showed the highest mean values of bone density, height, and bone width, followed by Group (B). The histological study reported more bone maturation in group A than in group B. Complete bone maturation occurred later in group B than in group A.
Bhawal et al., 2016	Implantation of biomaterial in defects created in the knee joints (lateral epicondyle of the femur). Group 1 - 6 animals without implant Group 2 - 6 animals with HFIP-F implant [a] Group 3 - 6 animals with implant A-F [b] Group 4 - 6 animals with β-TCP implant [c]	The A-F scaffold, as well as the β-TCP, have a more evident osseoinductive capacity than the HFIP-F scaffold. Osseointegration was observed between native tissue and new tissue within the bone defects. In histological sections, osteoclast-like cells were observed on the surface of β-TCP. New bone formation was observed from residual bone towards β-TCP more frequently after 2 weeks compared to the 1-week post-surgery group. Areas rich in connective tissue/bone marrow cells were observed between the bone substitute particles and the residual bones. However, there was new bone among the β-TCP granules.
Costa et al., 2015	Creation of 3 bone defects in each tibia of each animal and filled with: 2 proximal defects - HA[d]; HA/β-TCP 60/40 [e]; 2 medial defects - autogenous bone graft only; 2 distal defects - β-TCP; HA/Al_2O_3 5% [f]	HA, β-TCP and HA/β-TCP showed great osseoregenerative capacity. HA/β-TCP appears to be better for a long-term outcome. 5% HA/Al_2O_3 is not a good answer. The β-TCP showed intermediate results regarding the osseoregenerative capacity.
Daher et al., 2019	β-TCP only for maxillary sinus floor elevation	They exhibited bone maturation, remodeling, and development similar to the Havesian bone morphology. Presence of multiple lamellar bone remodeling sites with a configuration similar to the Haversian cortical.

Dallabrida et al., 2018	Creation of 3 bone defects in each tibia of each animal. Filling: 2 with bone autograft (both proximal defects of each tibia); 1 with AH (right middle tibia); 1 with β-TCP (left middle tibia); 1 with 80/20 HA/β-TCP or 70/30 HA/β-TCP (distal right tibia); 1 with HA/β-TCP 20/80 or HA/β-TCP 30/70 (left distal tibia).	All synthetic bioceramics showed good capacity for osseoinduction, osseoconduction and osseointegration, promoting good ability to stimulate bone formation. In all materials, the graft granules and implants were well integrated with varying degrees of active bone remodeling occurring within 3 months. All biomaterials showed varying degrees of absorption over the evaluation period, the most promising being the proportions of HA/β-TCP 20/80 and HA/β-TCP 30/70, followed by β-TCP, and then by the proportions of HA/β-TCP 80/20, HA/β-TCP 70/30 and HA, respectively.
Damlar et al., 2015	Five bone defects in the frontal bone of the skull of each animal: 3 defects tested with three types of β-TCP; 1 defect tested with allograft as a positive control; and 1 defect tested with blood clot as a negative control.	All groups showed new bone formation. Among the β-TCP groups, Poresorb M® had better bone formation, through better % of osseoconductivity, TbTh and TbWi; while Cerasorb® had the worst result for these parameters. Cerasorb® had the highest TbSp; and Kasios® had the worst % residual graft. Regarding the percentage of bone conduction, Poresorb® presented the best values, while Cerasorb® presented the worst.
Joshi et al., 2016	Preservation of the alveolar edges after 3 extracted teeth: 1 well filled with β-TCP 1 alveolus filled with ATG 1 alveolus not grafted	All alveoli had new bone formation. The ATG-grafted sites showed harder consistency than the β-TCP-grafted sites and less reduction in the height and width of the vertical bone crest. Histologically, β-TCP showed less osteoid formation and poor integration with newly formed bone. The connective tissue around the β-TCP was poorly vascularized and there was infiltration of inflammatory cells.
Kim et al., 2014	Allografts were implanted in 40 sinuses, xenografts in 26 sinuses, and a mixture of allografts and xenografts in 35 sinuses. A mixture of allografts and alloplastics was implanted in 2 sinuses. The allografts used were lyophilized bone allograft, Orthoblast [g], Tutoplast Spongiosa Microchip [h], Allotis [i] or Grafton [j]. The xenografts used were inorganic bovine bone matrix [k], Biocera [l] coated with calcium phosphate nanocrystal or OCS-B [m]. The alloplastic used was pure phase β-TCP [n].	Clinically 97.09% (100 of 103) of all maxillary sinuses showed complete bone healing at the bone windows. Three cases had infection (2,91%). Radiographically, all sinus walls (97,09%) were reconstructed. Histologically, all samples evaluated (97,09%) showed bone formation without formation and invagination of fibrous connective tissue.
Klein et al., 2020	Part 1 - Alveolar bone regeneration model. Bone defects created. 1 filled with allograft, 1 filled with β-TCP alone, 1 left unfilled.	Bone volume and bone trabeculation were shown to be reduced in the β-TCP group compared to the allograft group and the non-grafted group at 2 and 4 weeks after graft use but were similar at 6 weeks. Graft particles could be detected 2 weeks after grafting for the β-TCP group, and 2 and 4 weeks for the allograft. The presence of a higher number of osteoclasts was observed in the β-TCP group at 2 and 4

	Part 2 - Orthodontic tooth movement in a local restoration model. Bone defects. 1 filled with β-TCP, 1 filled with allograft, only 1 filled without filling.	weeks compared to allograft and control. OTM [o] behaved similarly in the two grafted groups but was worse compared to the non-grafted control.
Knabe et al., 2019	4 critical defects were created in each animal's scapula: 1 filled with Si-CAOP [p]; 1 filled with Si-TCP [q]; 1 filled with β-TCP [r]; 1 defect left unfilled.	After 2 weeks and after 1 month, the defects grafted with Si-CAOP exhibited significantly greater bone area, bone-particle contact, osteogenic marker expression, and significantly smaller particle area than the defects grafted with Si-TCP and β-TCP. At 3 and 6 months, all materials showed excellent defect regeneration, with additional bone remodeling at 12 and 18 months.
Okada et al., 2016	Maxillary sinus floor elevation. Use of pure phase β-TCP in all patients.	Implant osseointegration achieved in all patients. Different degrees of newly formed bone replacing β-TCP in the second surgery. Radiographically, the mean bone volume and implant height decreased with time.

[a] HFIP-based Silk Fibroin (HFIP-F); [b] water-based silk fibroin scaffold (A-F); [c] β-TCP - (Taihei Chemical Industrial Co., Nara, Japan); [d] Hydroxyapatite; [e] hydroxyapatite/β-TCP 60/40 composite; [f] 5% hydroxyapatite/alumina nanocomposite; Poresorb M® (Curasan AG, Kleinostheim, Germany); Cerasorb® (Lasak, Prague, Czech Republic); Kasios® (Kasios, L'Union, France); TbTh - trabecular thickness; TbWi - trabecular width; TbSp - trabecular separation; [g] Orthoblast - (IsoTis OrthoBiologics Inc., Irvine, CA); [h] Tutoplast Spongiosa Microchip - (Pures; Zimmer Dental, Carlsbad, CA); [i] Allotis - (Biotis, Seoul, Korea); [j] Grafton - (Osteotech, Eatontown, NJ); [k] inorganic bovine bone matrix (Bio-Oss; Geistlich Pharma AG, Wolhusen, Switzerland); [l] Biocera - (Oscotec, Cheonan, Korea); [m] OCS-B - (Nibec, Seoul, Korea); [n] β-TCP - (Cerasorb M; Curasan, Kleinosthein, Germany); [o] OTM - orthodontic tooth movement. [p] Osseolive - glassy crystalline material with main crystalline phase $Ca_2KNa(PO_4)_2$ with a mixture of 4% sodium and magnesium silicate; [q] Ceracell - pure β-TCP phase with a 4% sodium and magnesium silicate mixture; [r] Cerasorb M - pure phase of β-tricalcium phosphate - β-TCP ($Ca_3(PO_4)_2$); β-TCP - beta tricalcium phosphate; ATG - autogenous tooth graft.

Box 3 - Efficacy of the β - TCP implant shown through newformed bone, volumetric density, bone mass content and residual graft demonstrated in some articles. Implant costs, complications and other intercurrences were also mentioned when present.

Author / Year	Effectivity [a]	BV/TV [b]	Residual Implant	Cost	Complications
Abdullah et al., 2021	100%	NM	NM	NM	N
Bhawal et al., 2016	100%	Approx. 20% in 2 weeks	NM	NM	N
Costa et al., 2015	100%	NM (Show classification of ++++ / 0 to 4+)	NM	NM	N
Daher et al., 2019	100%	NM	NM	NM	N
Dallabrida et al., 2018	100%	NM	NM	NM	N
Damlar et al., 2015	100%	Cerasorb [c] - 41,28 ± 4,02%; Kasios [d] - 43,85 ± 3,07%; Poresorb [e] - 48,71 ± 2,56% (all after 8 weeks)	[*] Cerasorb - 29,42 ± 6,29%; [*] Kasios - 38.85 ± 4.87%; [*] Poresorb - 37.14 ± 3.38%	NM	No postoperative complications, but 1 was excluded due to frontal sinus perforation during defect preparation.
Joshi et al., 2016	100%	NM	NM. Only that a few particles were left.	Material with better cost-benefit but does not show numbers.	N

Kim et al., 2014	97,09%	NM	NM	NM	Infection – 2,91% (3 patients). Cracked bone in 2 other cases. 12 sinus perforations during osteotomies.
Klein et al., 2020	100%	Approx. 60% in 4 and 6 weeks.	All absorbed in 4 weeks.	Reduced costs but does not show numbers.	NM
Knabe et al., 2019	100%	0,75% in 2 weeks; Approx. 43% in 1 month; Approx. 62% in 3 months; Approx. 68% in 6, 12 e 18 months.	Approx. 64% in 2 weeks. Approx. 32% in 1 month. Approx. 21% in 3 months. Approx. 16% in 6 months. Approx. 12% in 12 months. Approx. 8% in 18 months.	NM	N
Okada et al., 2016	100%	NM	24,4% in 6 months and 45,1% in 2.5 years.	NM	The implant tip protruded into the maxillary sinus by approx. 70% of cases (41/58 implants) in 2 years.

[a] Efficacy through new bone formation; [b] Volumetric density (VB / VT) in percentage; VB - bone volume (VB); TV - tissue volume. * $p < 0.0001$ (statistically significant); [c] Cerasorb® (Lasak, Prague, Czech Republic); [d] Kasios® (L'Union, France); and Poresorb M® (Curasan AG, Kleinostheim, Germany). NM = not mentioned; N = none.

Box 4 - Price ratio of β - TCP implants with brand names and granule sizes. References about their prices and links to companies that market them are also provided.

Chemical Composition	Comercial Name	Size [#]	Price U.S. $	References
β-TCP	Cerasorb [a]	0.5	73.6	**Bluesky Bio** http://blueskybio.com/store/pure-phase-b-tcp
β-TCP	Premier TCP [b*]	0.5	70.0	**Osseous Technologies of America** http://www.osseoustech.com/promotions/2012-ao/
β-TCP	Synthograft [c]	0.5	63.0	**eBiologics Dental** http://www.ebiologicsdental.com/SearchResults.asp?searching=Y &scrt=13&search=synthograft&show=1&page=6

[a] Cerasorb (Lasak, Prague, Czech Republic); [b] Premier Biomaterials (Riverside Business Park, Nenagh, Co. Tipperar, Ireland); [c] SynthoGraft (Arborway, Boston, MA, USA); [#] in grams or cubic centimeters; * Implant was not used in any of the articles selected in the systematic review. Source: Javaid and Esfandiari, 2013.

Box 5 - Brand, shape, porosity and particle size of the implants used by the authors. The sizes of bone defects created for implantation of β - TCP are also shown in this chart.

Authors / Year	Name of Implant (Brand)	Shape of Implant	Particle Size	Particle Porosity	Critical Size of Bone Defect
Abdullah et al., 2021	IngeniOs β-TCP [a]	Granules	NM	NM	22,03 ± 2,96 mm (alveolus depth) x 8,50 ± 2,60 mm (oral-lingual width)
Bhawal et al., 2016	β-TCP-100 [b]	powder, ρ = 3.14 g/cm3	20 - 200 µm	NM	3,25 mm (diameter) x 4,95 mm (depth)
Costa et al., 2015	NM	Granules	NM	46,07 - 54,44%	6 mm (diameter)
Daher et al., 2019	SynthoGraft [c]	Granules	50 - 500 µm	NM	Not mentioned size in mm (osteotomy in the position of the right maxillary first molar)
Dallabrida et al., 2018	Tricalcium-phosphate-β [d]	Granules	200 - 500 µm	73,57 ± 0,82%	6 mm (diameter)
Damlar et al., 2015	Cerasorb M [e] Kasios [f] Poresorb [g]	All 3 are granules	Cerasorb M micro-pores smaller than 50 µm, and macro-pores from 50 to 500 µm. Kasios - micro-pores of 1 - 5 µm and macro-pores of 200 - 500 µm. Poresorb - 1 - 5 µm micro-pores and 100 µm macro-pores.	Cerasorb - 62% Kasios - 60-80% Poresorb - 30-40%	1 cm (diameter) x 4 mm (depth)
Joshi et al., 2016	SyboGraft [h]	NM	NM	NM	Does not mention size in mm (implants were inserted in alveolar sockets after tooth extractions in the maxilla and mandible)

Kim et al., 2014	Cerasorb M	NM	NM	NM	Vertical anterior osteotomy - 2 to 3 mm distal to the vertical anterior wall of the maxillary sinus. Distal osteotomy - 15 mm away from the vertical anterior osteotomy. Vertical osteotomy height was approx. 10 mm.
Klein et al., 2020	β-TCP. But it doesn't mention the brand used.	NM	NM	NM	Extended dental socket with a cutter and a drill, resulting in a 4-sided defect ≈ 15µl.
Knabe et al., 2019	Cerasorb M	Grânulo	1000-2000 µm	65% (pores size 0.1-500 µm)	8 mm (diameter) e 8 mm (depth)
Okada et al., 2016	OSferion [i]	NM	NM	NM	NM (only that it was an elevation of the maxillary sinus floor in the premolar and molar region).

[a] Bioactive Synthetic Bone Particles, Zimmer Biomet Dental, USA; [b] Taihei Chemical Industrial Co., Nara, Japan; [c] Bicon, LLC; Boston, MA; [d] Department of Mechanical Engineering, CCT, UDESC / Joinville / SC Brazil; [e] Curasan AG, Kleinostheim, Germany; [f] Kasios, L'Union, France; [g] Lasak, Prague, Czech Republic; [h] T Plug, Eucare Pharmaceuticals Unip. Ltd., Chennai, Tamil Nadu, India; [I] Olympus Terumo Biomaterials Corp., Japan; NM = not mentioned.

5. CAPÍTULO II

A NEW CRITICAL SEGMENTAR RADIAL BONE DEFECT MODEL IN RABBITS.

Artigo publicado na revista **International Journal of Development Research (IJDR)**

Qualis Capes A2 - Multidisciplinar

B1 - Medicina Veterinária

A new critical segmentar radial bone defect model in rabbits.

Daniel Cardoso Garcia[1,3*]; Larissa Eckmann Mingrone[3]; Marcelo Jorge Cavalcanti de Sá[2]

[1] DVM, MsC, PhD student at Campina Grande Federal University (UFCG)

[2] DVM, MsC, PhD, Full Professor at Campina Grande Federal University (UFCG)

[3] DVM, MsC Veterinary Surgeon at Animal Care Barueri Clínica Veterinária

Abstract

The loss of large bone segments remains one of the most challenging problems in orthopedic surgery and considerable research continues with different results being reported. We proposed an experimental model of critical bone segmental defect. Thirteen New Zealand rabbits were used. The animals were divided into 2 groups composed of 06 animals each and a 7 mm ostectomy was performed in the radial bone of each animal. In Group A the bone defect was kept without any filling. In Group B the bone defect was filled by allogenous cortical bone graft. The remaining animal was used for grafts` acquisition. Stabilization with plates and screws was done in animals of Group B. Clinical and radiographic evaluations were performed in the postoperative period at five specific moments. All animals in Group B showed bone union through radiographic studies, while all animals in group A showed bone neoformation within the bone gap, but none of them showed bone union at 120 days after surgery. The segmental bone defects were easy to make, with quick surgical time, without complications, and due to the non-union observed during the bone healing process in Group A, we can state that the ostectomy length used in this study can be considered as a critical bone segmental defect and be used as experimental model for this specie.

Keywords: Segmental bone defect.; critical bone defect.; radius; experimental model; rabbits.

1 Small Animal Surgery Department UFCG, Patos - PB - Brazil.

2 Small Animal Surgery Department UFCG, Patos - PB - Brazil.

3 Small Animal Surgery Department Animal Care Barueri Clínica Veterinária, Barueri, SP - Brazil.

* Correspondence to: Daniel Cardoso Garcia. - Rua Duque de Caxias, 226 - Centro - Barueri - SP - Brazil. Zip code: 06401-010. Phone number: (55 11) 99618-3304.

Introduction

The loss of large bone segments remains one of the most challenging problems in orthopedic surgery (Clements et al., 2008; Azi et al., 2012; Pobloth et al., 2017; Ruan et al., 2018; Huang et al., 2020) due to their weak capacity to self-regenerate (Huang et al., 2020). In some cases, the treatment of bone defects can lead to serious complications, such as nonunion, bone atrophy, and bone deformity (Leng et al., 2020). Prior to the advent of complex reconstructive procedures this kind of problems culminated in limb amputation or permanent functional deficits (Gugala et al., 2007; Reichert et al., 2009).

Segmental bone defects may result in limb length inequity and may complicate a patient's postoperative course (Clements et al., 2008). Although there are several ways to treat segmental bone loss, few methods allow treatment with primary fusion and maintenance of limb length using a 1-stage procedure (Clements et al., 2008). Traditionally, bone grafts, either autogenous or allogenous, have been used in attempt to restore or maintain segmental length (Clements et al., 2008; Cipitria et al., 2013, Pobloth et al., 2017).

Bone allografts are bone tissues harvested from another animal of the same specie after processing it to reduce antigenicity. However, this treatment results in a decrease of incorporation capacity of the graft with host tissue and includes the risk of immune rejection and pathogen transmission to recipient (Yassine et al., 2017).

The vast majority of bone defects can heal spontaneously under suitable physiological environmental conditions due to the regeneration ability of bone (Reichert et al., 2009), unless the segmental size defect is extensive (Zhao et al., 2016). However, the healing process of bone defect is time consuming, and new bone generation takes place slowly because of decreased blood supply to the fracture site and insufficiency of calcium and phosphorus to strengthen and harden new bone. In addition, large defects, also known as critical bone defects, may not heal spontaneously and lead to nonunion prognosis due to the size of defects or unstable biomechanical properties, unfavorable wound environment, suboptimal surgical technique, metabolic factors, hormones, nutrition, and applied stress (Li et al., 2015).

For these reasons, segmental bone defect repair remains a clinical and experimental challenge in tissue engineering (Cao et al., 2012), and considerable research continues toward developing novel devices to restore and accelerate bone integrity (Wancket, 2015).

Recently, with the advances made in the realm of bone tissue engineering, the development and use of artificial bone has been successfully utilized to fill limited and focal bone defects (Cao et al., 2012). Synthetic bone graft substitutes are expected to be of major importance in the treatment of these large bone defects. However, the osseoregenerative

potential of these bone substitutes still needs to be improved to obtain materials equivalent to autologous bone (Bodde et al., 2008).

Guidelines are provided for the dimensions of implants for in vivo studies, based on the size of animal and bone chosen and on the implant design, in order to avoid pathological fracture of the test site. For example, cylindrical implants placed into rabbit tibial and femoral diaphyseal bone should be no larger than 2 mm in diameter and 6 mm in length (International Standard ISO 10993-6, 1994; Pearce et al., 2007).

Experimental models are useful for assessing the efficacy of new treatments, and should be reproducible, well controlled and afford the application of standardized methods of analysis (Azi et al., 2012).

An adequate animal model involves the creation of a standardized bone defect that resembles bone loss encountered in clinical situations. To eliminate the influence of instability, adequate fixation of the bone defect is mandatory (Boer et al., 1999). Also, must permit angiogenesis, provide all types of cells needed for bone repair in situ, and minimize the suffering of the animal (Horner et al., 2010).

For the testing of tissue destined for long bones, the use of nonhealing segmental bone defect models is well established and includes fracture and osteotomy models, based upon those Bonnarens and Einhorn et al. described in 1984, it means, there are two main methods of creating a critical defect in the long bone, using either an osteotomy approach or a traumatic approach. Osteotomy utilizes a drill or saw to surgically remove the required length of bone from a predetermined site and producing a consistent defect in all subjects. The edges of the defect are usually cut straight (not jagged) with less trauma. To reflect the conditions after traumatic injury, the defect can be created via trauma, which will produce a jagged cut edge and traumatize both the bone and surrounding soft tissue (Horner et al., 2010).

Rabbit is one of the most commonly used animal models, and it ranks first among all the animals used for musculoskeletal research (Castañeda et al., 2006; Li et al., 2015), being used in approximately 35% of studies (Pearce et al., 2007). They offer advantages over large animal models by reaching skeletal maturity at a relatively early age (approximately 6 months) and advantages over other rodents by undergoing more secondary osteonal remodeling (Pearce et al., 2007; Wancket, 2015).

They also show significant intracortical remodeling, have faster bone turnover than other rodents (Li et al., 2015), even than primates (Castañeda et al., 2006). It was reported that there were similarities in bone mineral density and the fracture toughness of mid diaphyseal bone between rabbits and humans (Li et al., 2015). Other advantages are that they are easily

available, and easy to house and handle (Pearce et al., 2007). These characteristics make rabbits the first choice when researchers develop animal model for the in vivo test of a new bone substitute biomaterials (Li et al., 2015).

In the evaluation of artificial bone regenerative materials, the so-called ‘‘critical-size defect’’ (CSD) is an essential model. A CSD is defined as the smallest size of a defect, that does not heal spontaneously when left untreated for a certain period or that shows less than 10% bone regeneration during the lifetime of an animal (Bodde et al., 2008; Reichert et al., 2009; Li et al., 2015; Zhao et al., 2016).

Nevertheless, a critical defect in long bone cannot simply be defined by its size but may also be dependent on the species phylogenetic scale, anatomic defect location, associated soft tissue and biomechanical conditions in the affected limb as well as age, metabolic and systemic conditions, and related morbidities affecting defect healing (Reichert et al., 2009).

Disregarding study dissimilarities, CSD varies between 10 and 20 mm in general (Nielsen et al., 1992; Bodde et al., 2008; Xia et al., 2019; Huang et al., 2020). Earlier studies investigated scaffolds for degradation and bone formation in 15 mm segmental radial rabbit defects (Walsh et al., 2008; Bodde et al., 2008; Zhou et al., 2010; Ruan et al., 2018; Leng et al., 2020). Empty defects did not confirm a critical size of 15 mm for a period of 12 weeks, because some of the defects had closed (Bodde et al., 2008; Cao et al., 2012). Gauthier et al. (2005) used a cylindrical, 7 e 10 mm critical-size bone defect rabbit model to investigate the efficiency of an injectable calcium phosphate bone substitute for bone regeneration. Yassine et al., 2017 has created a segmental defect of 5 mm in the mid-shaft of radius as a critical size defect bone model and observed for 90 days (Yassine et al., 2017).

However, a review of the literature shows that the exact critical size of radial defects in rabbits is not clear (Bodde et al., 2007; Zhao et al., 2016). For practical purposes, if there is no mineralised area of 30% after 52 weeks, there would never be complete bony regeneration. Although the minimum size that renders a defect “critical” is not well understood, it has been defined as a segmental bone deficiency of a length exceeding 2 to 2.5 times the diameter of the affected bone (Oryan, 2014; Li et al., 2015; Wancket et al., 2015).

One of the fundamental factors for studying a nonhealing bone defect model is whether the size of the defect created remains critical. A problem with comparing different models is that there is often a lack of clear evidence that the model used was actually a critical defect. In 1934, Key hypothesized that to create a critical size defect in canine ulna, the defect must be over 1.5 times the diameter of the bone. It has also been repeatedly shown that the periosteum

is very important for bone regeneration and that unless this is removed the defect will often spontaneously heal (Horner et al., 2010).

The method of fixation is another important variable to be studied in bone fracture defect models. Fixation is more important for defects within weight-bearing bones than the nonweight-bearing bones (Horner et al., 2010). At present, most long bone defect models use either bone plates or intramedullary rods to fix the defect, thus reflecting the situation within the clinic (Horner et al., 2010).

A good site for studying the regeneration of segmental long bone defects is the mid-diaphyseal radius. It is claimed that this model does not need internal fixation or external splinting in small animals as the adjacent intact ulna provides stability to the created radial defect. (Bodde et al., 2008; Zhao et al., 2016).

The time scale of an in vivo study will vary depending on the choice of model and species as well as the parameters to be monitored. Studies using rabbits, for example, normally takes 8 to 20 weeks observation (Horner et al., 2010).

Materials and Methods

This study was previously submitted to the Bioethics Committee of the Federal University of Campina Grande (CEUA - UFCG) to assess the use of animals and was approved under protocol number CEP 046/2018.

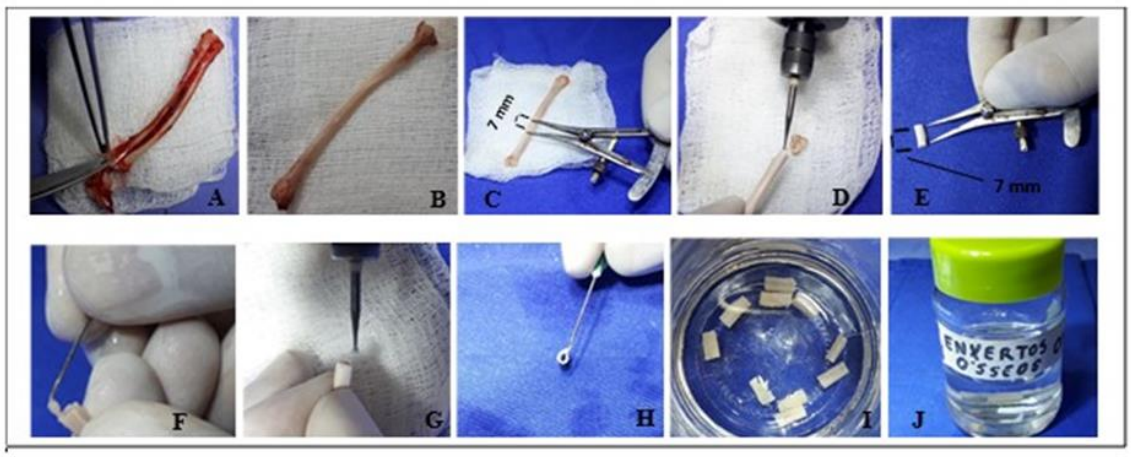
Allogeneous cortical bone graft collection and storage procedures, as well as surgical and postoperative procedures were performed at Animal Care Barueri Clínica Veterinária, in Barueri, São Paulo.

For this experiment 13 New Zealand rabbits, male and female, weighing between 3 and 4 kg were used. The animals were subdivided into 2 groups consisting of 06 animals each. Group A was considered the group in which the radial diaphysis ostectomy was performed and it was kept without any filling of the bone defect by a graft. Group B was considered the control group of the study, in which radial diaphysis ostectomy was performed and cortical bone allograft was used to fill the bone gap. The other spare animal was used for aseptic collection of the two bones of the radius for manufacturing of all the allogenous bone cortical grafts that were part of the bone bank, and which were later implanted in the radial bone of the animals of Group B.

Grafts` acquisition

After obtaining the bone fragments for grafting, they were cleaned and washed through irrigation with 0.9% saline solution[4], and any tissue adhering to the bone cortex, including the periosteum, was removed. The medullary canal was also cleaned with the aid of a hypodermic needle, and any structures within this same canal were removed, such as blood vessels and bone marrow. Afterwards, the grafts already completely free of any non-bone tissue were again abundantly washed with 0.9% saline solution before being stored in a bottle containing 98% glycerin solution[5] (Padilha Filho et al., 2008a and 2008b), product chosen to serve as a preservative for the grafts (Figure 1). In this bottle, the bone allografts remained preserved for a minimum period of 30 days before being used.

Figure 1 - Photographic images demonstrating the procedures for collecting, confection and storing of allogeneic cortical bone grafts.



Removal of soft tissue adhered to the bone after dismemberment (A). Radius appearance after cleaning the soft tissue and removing the ulna (B). Graft length measurement (7 mm) with a Castroviejo caliper (C). Cutting the grafts with a high-speed drill and spherical dental drill (D). Graft cut to desired size and measured for exact verification of 7 mm in length (E). Removal of all tissue within the medullary canal (F). Trimming any irregularities on the edge of the graft (G). Image after irrigation of the graft and cleaning of the medullary canal (H). Grafts are placed in 98% glycerin for conservation (I). Pot identified with the grafts to be used after 30 days of rest in 98% glycerin (J).

4 Cloreto de sódio 0,9% - Fresenius Kabi Brasil Ltda - Barueri - SP - Brazil.

5 Glycerin 98% - Biohervas Farmácia de Manipulação - Barueri - SP - Brazil.

Anesthetic protocol

As pre-anesthetic protocol, Acepromazine[6] was used at a dose of 1 mg/kg via the intramuscular route, associated with Ketamine[7] at a dose of 40 mg/kg, also via the intramuscular route. After a 15 minute interval, the animals were catheterized in the venous blood vessel of the ear and maintained on fluid therapy with Ringer Lactate[8]. Then, isoflurane[9] was used for anesthetic induction by vaporization in the mask. For anesthetic maintenance, the same isoflurane was maintained in an open anesthetic circuit with the use of the mask mentioned above. The animals were monitored by electrocardiogram, heart rate, respiratory rate, blood pressure, oxygen saturation and temperature.

Surgical protocol

Prior to surgery, the hairs on the left thoracic limb of each animal were shaved and then antisepsis was performed by cleaning the skin with alcoholic chlorhexidine[10]. A longitudinal cranial incision of approximately 6 centimeters was made in the skin over the topography of the left radius of each animal and the tissues adjacent to the diaphysis of the bone were dissected. In the middle third of the radial shaft, an ostectomy was performed with the aim of removing a bone fragment of approximately 7 mm in length. The measurement was demarcated over the bone with the aid of a Castroviejo compass and electrocautery (Figure 2A). Then, the ostectomy procedure was performed with the aid of a mini electric high-speed drill[11] and a dental burr[12] (Figure 2B). Small holes were made transversely in the bone until it was possible to fracture and completely remove the bone fragment. Trimmings were performed on the remaining bone through burr wear, in order to make the bone defect as homogeneous as possible, with the osteotomies well parallel. Therefore, irrigation of the ostectomized sites was performed at the time of cuts in order to prevent the occurrence of thermal necrosis.

---

6 Acepran 0,2% - Univet S/A - São Paulo - SP - Brazil.

7 Dopalen - Ceva Santé Animale - Paulínia - SP - Brazil.

8 Ringer Lactato - JP Indústria Farmacêutica S. A. - Ribeirão Preto - SP - Brazil.

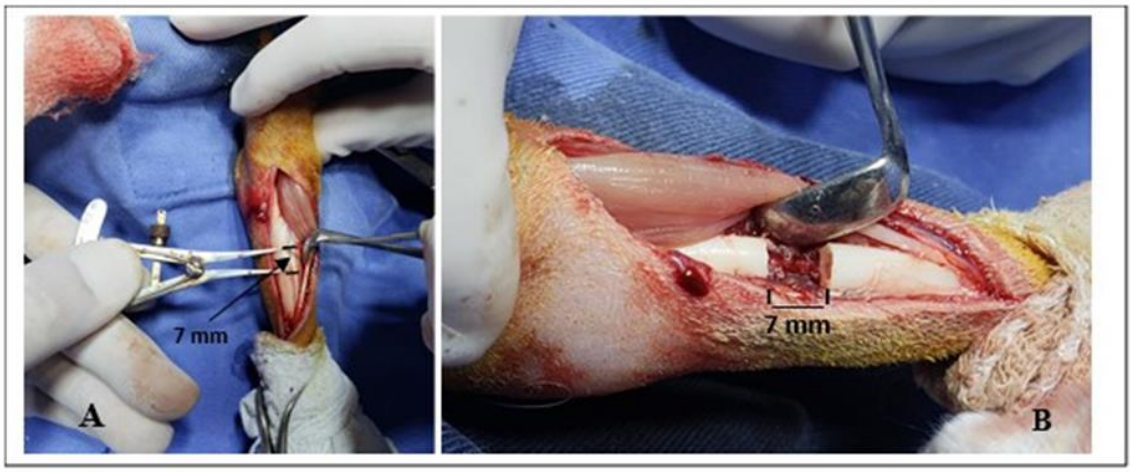
9 Isoforine - Cristália Prod. Quím. Farm. Ltda. - Itapira - SP - Brazil

10 Riohex 2% - Indústria Farmacêutica Rioquímica Ltda. - São José do Rio Preto - SP - Brazil.

11 Dremel Bosch - BSH Store - Curitiba - Paraná - Brazil.

12 Broca Carbide esférica FG n° 3 - Angelus Prima Dental Ltda - Londrina - PR - Brazil.

Figure 2 - Photographic images demonstrating the demarcation site in the middle third of the radius bone and the bone defect created as a critical segmental bone defect after the ostectomy procedure.



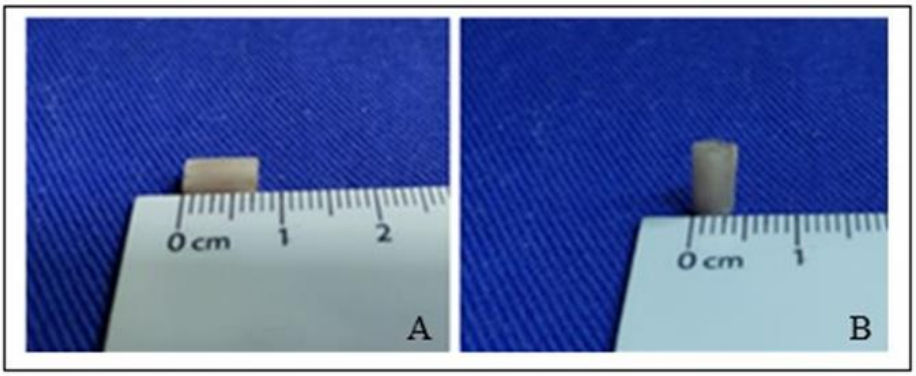
Demarcation of the osteotomy site in the middle third of the left radius diaphysis with the aid of a Castroviejo compass (7 mm in length) (A). Post-ostectomy image showing a 7 mm segmental defect in the left radius bone of one of the rabbits (B).

For the animals in Group A, the ostectomy site was left without any type of filling material and the anatomical planes were sutured as described below.

For Group B animals, cortical bone allografts removed from the bone bank were positioned in the bone defect created by the ostectomy (Figure 3). The grafts were inserted through pressure at the ostectomy site so that their proximal and distal ends were in intimate contact and compressed with those of the animal's bone.

At the moment that allogenous cortical bone graft were supposed to be used, they were removed from the 98% glycerin pot and were subjected to an abundant prior washing with 0.9% saline solution for a period of 10 minutes before being placed in bone defect, in order to remove the glycerin and rehydrate the fragment to be used.

Figure 3 - Photographic images showing the shape and measurements of the allogeneic cortical bone graft.



Allogeneic cortical bone graft: length = 7 mm (A). Diameter = 3 mm (B).

For osteosynthesis, stabilization of the operated limb, and protection and support of the graft at the ostectomy site, six holes 1.5 mm titanium locking plates[13] and 1.5 mm titanium locking screws[14] were used. Both the plates and screws used in this research were donated.

Osteosynthesis was performed by bridging the grafted defect, that is, although the plate had 6 holes along its length, only 4 screws were used for locking, 2 of them being placed in the two most proximal holes of the plate, and another 2 placed in the two most distal holes, leaving the central part of the plate free of screws.

After performing the ostectomy and osteosynthesis procedures, suturing of the muscle planes, subcutaneous tissue and skin with nylon 3.0[15] using simple and separate pattern were performed in a conventional manner. Dressings and care of the surgical wound stitches throughout the postoperative period were performed twice a day. The stitches were removed after 2 weeks post-operatively.

For infection control, Enrofloxacin[16] was administered at a dose of 5 mg/kg, subcutaneously, once a day, for 7 days. To control pain and inflammation, Morphine[17] was used at a dose of 2.5 mg/kg, subcutaneously in the immediate postoperative period, followed by

13 Lincevet - Rio Claro - SP - Brazil.

14 Lincevet - Rio Claro - SP - Brazil.

15 Nylon - Bioline fios cirúrgicos Ltda. - Anápolis - GO - Brazil.

16 Chemitril 2,5% - Chemitec Agro-Veterinária Ltda - São Paulo - SP - Brazil.

17 Dimorf - Cristália Prod. Quím. Farm. Ltda. - Itapira - São Paulo - Brazil.

Tramadol Hydrochloride[18] at a dose of 5 mg/kg, subcutaneously, twice a day, for 7 days, in addition to Meloxicam[19], at a dose of 0.2 mg/kg, subcutaneously, once a day, for 7 days.

The animals were clinically and radiographically[20] evaluated postoperatively (PO) for a period of 120 days. The evaluated moments were considered: M0 (immediate postoperative period); M1 (30 days of PO); M2 (60 days of PO); M3 (90 days of PO); M4 (120 days of PO).

Results

Clinically, all animals in Group A and Group B evolved satisfactorily. Even after the procedures, they rested their paws on the ground and walked without limping. The alignment of the operated limb remained adequate throughout the entire period. They presented mild discomfort on palpation of the site, but there were no complications in the operated region, such as infection, drainage of purulent content, limb edema, or opening of the surgical wound. Two animals tore off some of the stitches, but without any complications. Radiographically, for both Group A and Group B, it could be observed that the osteotomies were performed properly and with good positioning in the middle third of the diaphysis of each radius (Figures 4A1 and 4A2). The animals in Group A showed an onset of bone mineralization on the radiographs of M1 (Figures 4B1 and 4B2), but in a slower process compared to other moments. In M2 (Figures 4C1 and 4C2), the bone healing process was more advanced, and a good part of the bone gap was already filled with new bone. However, in M3 and M4, the healing process seemed to stop, since in the images of 90 and 120 days PO were very similar in terms of bone growth within the defect, not reaching the proximal and distal ends of the ostectomized bone (Figures 4D1 and 4D2, and 4E1 and 4E2, respectively).

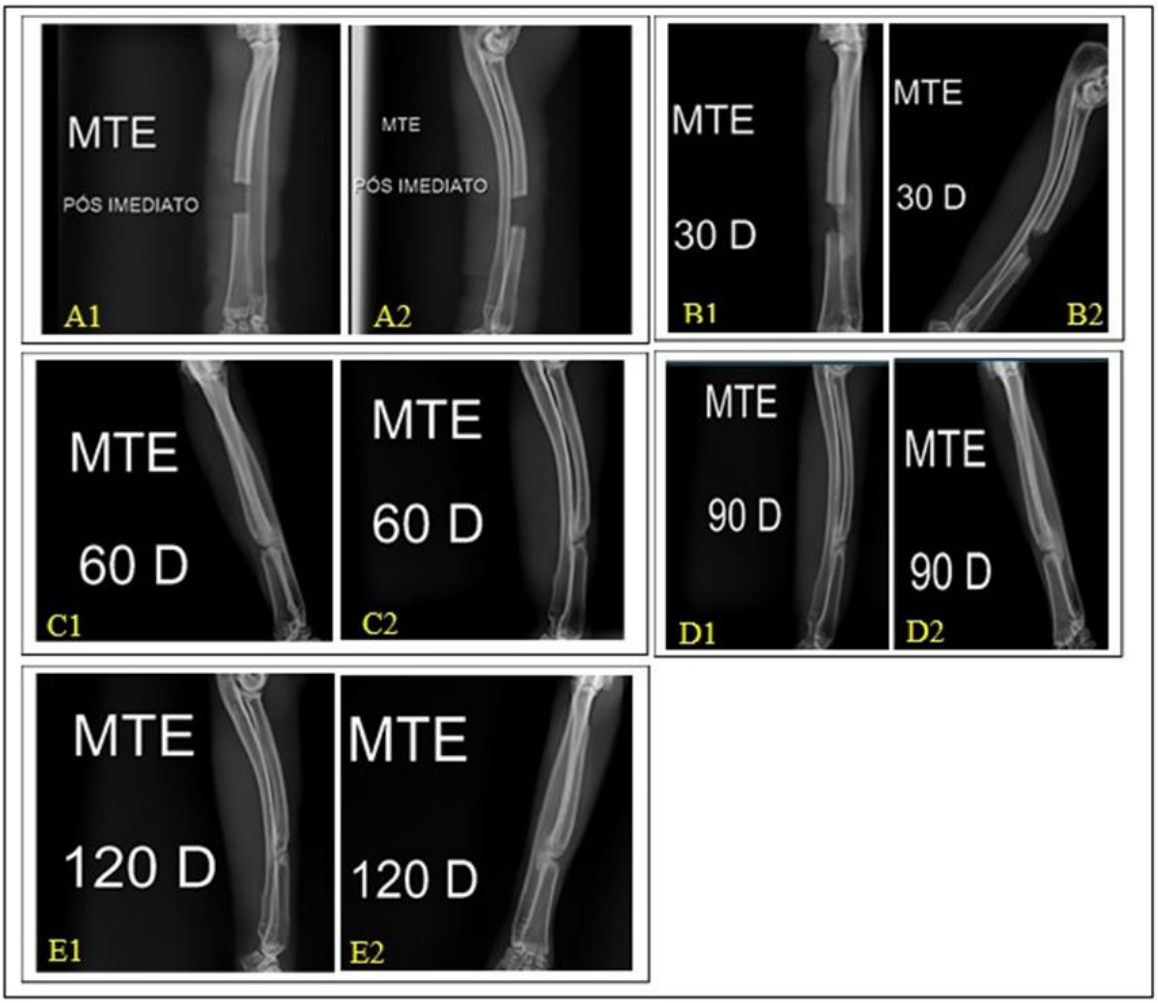
---

18 Tramadol - União Química Farmacêutica Nacional S/A - Pouso Alegre - MG - Brazil.

19 Maxicam 0,2% - Ourofino Saúde Animal Ltda - Cravinhos - SP - Brazil.

20 System DR Wireless, Model Mars 1417V - TSI - iRay Technology - Shanghai - China and Portable X Ray Model Orange 1060HF - Digicare - Oxson Technology - São Paulo - SP - Brazil.

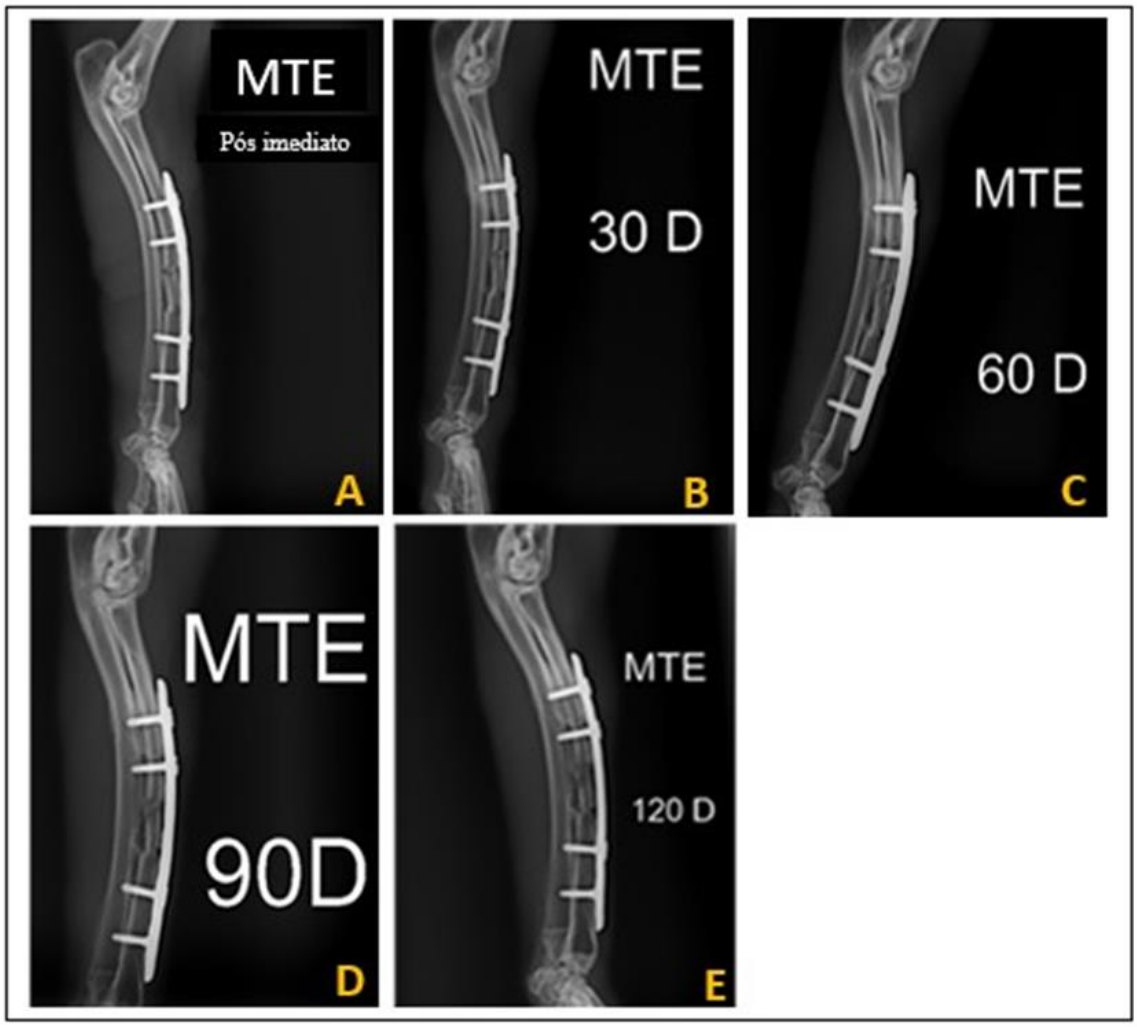
Figure 4 - Postoperative radiographic images from moments M0 to M4 showing the critical segmental bone defect performed in the middle third of the left radius of one of the animals of Group A and the body's attempt to carry out the bone healing process over time.



MTE = left thoracic limb; Pós imediato = immediate post-operative moment (M0); 30D = 30 days after surgery (M1); 60D = 60 days after surgery (M2); 90D = 90 days after surgery (M3); 120D = 120 days after surgery (M4).

In Group B, it was possible to notice the good positioning of the graft within the bone gap in all animals (Figures 5A to 5E). In all six animals there was bone integration of the graft and the host bone. In M1 (Figure 5B) a radiolucent line between the graft and the bone could still be observed, but in M2 (Figure 5C) the beginning of the bone consolidation process that extended to M3 and M4 (Figures 5D and 5E, respectively).

Figure 5 - Radiographic images of one of the animals of Group B at moments M0 to M4 after filling the bone defect with an allogeneic cortical bone graft.



MTE = left thoracic limb; Pós imediato = immediate post-operative moment (M0); 30D = 30 days after surgery (M1); 60D = 60 days after surgery (M2); 90D = 90 days after surgery (M3); 120D = 120 days after surgery (M4).

Discussion

In order to choose a critical bone segmental defect model, several factors must be taken into account, such as species used, animal age, bone, observation time for bone growth, speed of bone turnover and similarity of bone mineral density of the species in question (Castañeda et al., 2012; Li et al., 2015). Despite countless works already carried out with rabbits, we chose to use this species for several reasons, such as high availability, small size, easy handling, reduced shelter space (Pearce et al., 2007), and mainly because its turnover is faster than from other rodents and species, and have significant intracortical remodeling, as mentioned by Li et

al., 2015, being this species the first choice in cases of in vivo experimental models for bone substitutes.

Although several authors use other bones to produce the bone defect in rabbits, radius can be considered an adequate bone for simulating critical bone defects (Yassine et al., 2017). In our study, we used this same bone and it proved to be adequate for making the bone defect, being easy to perform, without complications in relation to the surgical and postoperative procedures, and the surgical time was considered reduced to perform the procedure. Many authors choose this bone as an experimental model because they report that it is unnecessary to stabilize the bone failure or even protect the implant or graft used in the failure, due to the integrity of the ulna that serves as support and alignment of the limb (Bodde et al., 2008; Horner et al., 2010). However, we chose to stabilize the radius as indicated by Boer et al., 1999 in Group B (use of the graft), as this would mimic exactly what would happen in a clinical/surgical situation. We allow cranial support to the bone graft, reducing mechanical stress on it, which would be adequate if porous implants were to be used. On the other hand, we chose not to stabilize the animals in Group A (failure without filling), as this would reproduce extensive bone loss without treatment, simply letting the bone try to spontaneously consolidate without any help (Reichert et al., 2009) whether this occurred or not (Huang et al., 2020).

The big question that arises for this type of experimental model, and there are still divergences between the studies, is in relation to the length of the critical bone defect. In the literature used as reference, the size ranged from 5 mm to 2 cm of bone defect produced in the radius of rabbits (Nielsen et al., 1992; Gauthier et al., 2005; Bodde et al., 2008; Walsh, 2008; Yassine et al., 2017; Ruan et al., 2018; Xia et al., 2019; Huang et al., 2020; Leng et al., 2020). Despite the controversy, we chose to perform the radial ostectomy length based on the definition cited by Oryan, 2014; Li et al, 2015 and Wancket et al., 2015 in which the critical bone defect is 2 to 2.5 times the diameter of the bone in question. As the radius of the animals used in this experiment was 3 mm, which was even the diameter of the allogenous grafts used, we chose to perform the critical bone defect with a length of 7 mm. Despite being a shorter length than most defects created by various authors, it is longer than the defects created by Yassine et al., 2017 and equal to that performed by Gauthier et al., 2015.

The critical defect used in this experiment did not consider the guidelines of the International Standard ISO 10993-6, 1994 regarding the size of implants, which advocate the use of cylindrical implants in the tibia and femur of rabbits in experimental models of segmental bone defect with maximum diameter and length of, respectively, based on the possibility that larger sizes could generate greater local stress and occurrence of pathological fracture in the

test area. In our study, the allogeneic bone cortical graft was 3 mm in diameter and 7 mm in length, and contrary to what was previously described, there was no fracture at the site, either in the graft or even in the host bone.

Regarding the time of study, Horner et a., 2010 reports temporal observation between 8 and 20 weeks. Our study used a time limit of 4 months (16 weeks), which is longer than many studies, but a few weeks less than the longest time reported above. What we were able to assess during this period was the occurrence of graft osseointegration (Group B), which could already be observed from 60 days after the operation, and the beginning of bone growth in the critical failure of animals in group A (without filling of failure). In Group A, this process occurred slowly in the first 30 days, but then, between 30 and 60 days, bone growth in the gap was fast and with good filling, but it did not reach the bone union until 120 days. And in fact, from the radiographs we can see that the filling in M4 is practically the same as in M2, which may mean that from M2 onwards, with no union of the bone ends, the consolidation process generated by the bone may have stopped from 60 days after surgery. We cannot say for sure, if we could observe any longer, whether there would still be enough bone activity to culminate in bone union. This could even determine whether the size of the segmental bone defect created is really critical or not. Other studies using more observational time and different lengths of bone gap could be carried out to resolve this question.

Conclusion

The segmental bone defects performed in this study were easy to make, with a quick surgical time, and without complications. Although there was bone growth through both bone ends of the defects, none of the defects showed complete bone union at the end of 4 months, unlike the graft group, showing that the length of the bone defect performed in the radius of rabbits during this period can be considered as a critical defect and can be considered as an experimental model for this specie.

References

Azi, M. L.; Kfuri Jr., M.; Martinez, R.; Salata, L. A.; Paccola, C. A. J. (2012). Desenvolvimento de um modelo experimental de falha óssea infectada na ulna de coelhos. Acta Ortop. Bras. 20(3):136-138.

Bodde, E. W. H.; Spauwen, P. H. M.; Mikos, A. G.; Jansen, J. A. (2008). Closing capacity of segmental radius defects in rabbits. Journal of Biomedical Materials Research Part A. 85(1):206–217.

Boer, F. C.; Patka, P.; Bakker, F. C.; et al. (1999). New segmental long bone defect. Model in sheep: quantitative analysis of healing with dual energy x-ray absorptiometry. Journal of Orthopaedic Research. 17(5):654-660.

Bonnarens, F.; Einhorn, T. A. Production of a standard closed fracture in laboratory animal bone. J Orthop Res. 2(1):97-101.

Cao, L., Liu, X., Liu, S., Jiang, Y., Zhang, X., Zhang, C.; Zeng, B. (2012). Experimental repair of segmental bone defects in rabbits by angiopoietin-1 gene transfected MSCs seeded on porous β-TCP scaffolds. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 100(5):1229-1236.

Castañeda, S.; Largo, E.; Calvo, E.; Rodríguez-Salvanés, F.; Marcos, M. E.; Díaz-Curiel, M.; Herrero-Beaumont, G. (2006). Bone mineral measurements of subchondral and trabecular bone in healthy and ostoporotic rabbits. Skelet Radiol. 35:34-41.

Cipitria, A.; Reichert, J. C.; Epari, D. R.; Saifzadeh, S.; Berner, A.; Schell, H.; Mehta, M.; Schuetz, M. A.; Duda, G. N.; Hutmacher, D. W. (2013). Polycaprolactone scaffold and reduced rhBMP-7 dose for the regeneration of critical-sized defects in sheep tibiae. Biomaterials. 34:9960-9968.

Clements J. R., Carpenter, B. B.; Pourciau, J. K. (2008). Treating segmental bone defects: a new technique. J. Mater Sci Mater Med. 19:2367-2376.

Gauthier, O.; Müller, R.; von Stechow, D.; Lamy, B.; Weiss, P.; Bouler, J. M.; Aguado, E.; Daculsi G. (2005). In vivo bone regeneration with injectable calcium phosphate biomaterial: a three-dimensional microcomputed tomographic, biomechanical and SEM study. Biomaterials, 26(27):5444-5453.

Gugala, Z.; Lindsey, R. W.; Gogolewski, S. (2007). New Approaches in the treatment of critical-size segmental defects in long bones. Macromol Symp. 253:147-161.

Horner, E. A.; Kirkham, J.; Wood, D.; Curran, S.; Smith, M.; Thomson, B; Yang, X. B. (2010). Long bone defect. Models for tissue engineering applications: a criteria for choice. Tissue Engineering. 16(2):263-271.

Huang, Q.; Liu, Y.; Ouyang, Z.; Feng, Q. (2020). Comparing the regeneration potential between PLLA/Aragonite and PLLA/Vaterite pearl composite scaffolds in rabbit radius segmental bone defects. Bioactive Materials. 5(4):980-989.

International Standard ISO 10993-6 (1994). Biological evaluation of medical devices - Part 6. pp.1-11.

Key, J. A. (1934). The effect of a local calcium depot on osteogensis and healing of fractures. J Bone Joint Surg Am. 16(1):176-184.

Leng, Y.; Ren, G.; Cui, Y.; Peng, C.; Wang, J.; Wu, D.; Liu, H. (2020). Platelet-rich plasma-enhanced osseointegration of decellularized bone matrix in critical-size radial defects in rabbits. Annals of Translational Medicine. 8(5):198-198.

Li, Y.; Chen, S. K.; Li, L.; Li, L.; Qin, L.; Wang, X. L.; Lai, Y. X. (2015). Bone defect animal models for testing efficacy of bone substitute biomaterials. Journal of Orthopaedic Translation. 3(3):95-104.

Nielsen, F. F.; Karring, T.; Gogolewski, S. (1992). Biodegradable guide for bone regeneration. Acta Orthopaedica Scandinavica. 63(1):66-69.

Oryan, A.; Bigham-Sadegh, A.; Abbasi-Teshnizi, F. (2014). Effects of osteogenic medium on healing of the experimental critical bone defect in a rabbit model. Bone. 63:53-60.

Pearce, A. I.; Richards, R. G.; Milz, S.; Schneider, E.; Pearce, S. G. (2007). Animal models for implant biomaterial research in bone: a review. European Cells and Materials. 13:1-10.

Padilha Filho, J. G.; Carvalho Penha, L. H.; de Souza, S. F. (2008a). Uso do enxerto ósseo cortical bovino conservado em glicerina a 98% na osteotomia femoral em gatos. Ciência Animal Brasileira. 9(4):1071-1078.

Padilha Filho, J. G.; Eimantas, G. C.; de Souza, S. F. (2008b). Osteossíntese femoral distal em cães e gatos jovens com fíbula de cão conservada em glicerina a 98%. Vet. Not. 14(1):49-55.

Pobloth, A. M.; Schell, H.; Petersen, A.; Beierlein, K.; Kleber, C.; Schmidt-Bleek, K.; Duda, G. N. (2017). Tubular open-porous β-tricalcium phosphate polycaprolactone scaffolds as guiding structure for segmental bone defect regeneration in a novel sheep model. Journal of Tissue Engineering and Regenerative Medicine. 12(4):897-911.

Reichert, J. C.; Saifzadeh, S.; Wullschleger, M. E.; Epari, D. R.; Schütz, M. A.; Duda, G. N.; Schell. H.; Van Griensven, M.; Redl, H.; Hutmacher, D. W. (2009). The challenge of establishing preclinical models for segmental bone defect research. Biomaterials. 30:2149-1263.

Ruan, S.; Deng, J.; Yan, L.; Huang, W. (2018). Composite scaffolds loaded with bone mesenchymal stem cells promote the repair of radial bone defects in rabbit model. Biomedicine & Pharmacotherapy. 97:600-606.

Xia, P.; Wang, S.; Qi, Z.; Zhang, W.; Sun, Y. (2019). BMP-2-releasing gelatin microspheres/PLGA scaffolds for bone repairment of X-ray-radiated rabbit radius defects. Artificial Cells, Nanomedicine, and Biotechnology. 47(1):1662-1673.

Walsh, W. R.; Vizesi, F.; Michael D.; Auld J.; Langdown A.; Oliver R.; Yua, Y.; Irieb, H.; Brucea, W. (2008). Beta-TCP bone graft substitutes in a bilateral rabbit tibial defect model. Biomaterials. 29(3):266-271.

Wancket L. M. (2015). Animal models for evaluation of bone implants and devices: comparative bone structure and common model uses. Veterinary Pathology. 52(5):842-850.

Yassine, K. A.; Mokhtar, B.; Houari, H.; Karim, A.; Mohamed, M. (2017). Repair of segmental radial defect with autologous bone marrow aspirate and hydroxyapatite in rabbit radius: A clinical and radiographic evaluation, Veterinary World. 10(7):752-757.

Zhou, J.; Lin, H.; Fang, T.; Li, X.; Dai, W.; Uemura, T.; Dong, J. (2010). The repair of large segmental bone defects in the rabbit with vascularized tissue engineered bone. Biomaterials. 31:1171-1179.

Zhao, M. D.; Huang, J. S.; Zhang, X. C.; Gui, K. K.; Xiong, M.; Yin, W. P.; Yuan, F.; Cai, G. P. (2016). Construction of Radial Defect Models in Rabbits to Determine the Critical Size Defects. PLOS ONE. 11(1):1-15.

6. CAPÍTULO III

EVALUATION OF PURE-PHASE β - TRICALCIUM PHOSPHATE (β - TCP) CYLINDER CERAMIC IMPLANT OSSEOINTEGRATION IN RABBITS CRITICAL SEGMENTAR RADIAL BONE DEFECTS.

Artigo encaminhado à revista **BMC Veterinary Research**

Qualis Capes - A1

Evaluation of pure-phase β - tricalcium phosphate (β - TCP) cylinder ceramic implant osseointegration in rabbits critical segmentar radial bone defects.

Daniel Cardoso Garcia[1,2*]; Larissa Eckmann Mingrone[2]; Felipe Eduardo Pinotti[3]; Leonardo Seade[2]; Rosane de Melo[2]; Marcelo Jorge Cavalcanti de Sá[1]

[1] Department of Surgery - Faculty of Veterinary Medicine - Federal University of Campina Grande (UFCG) - Patos - Paraíba - Brazil - Zip code 58708-110.

[2] Surgery Department - Animal Care Barueri Veterinary Clinic - Barueri - São Paulo - Brazil - Zip code 06401-010.

[3] Department of Surgery - School of Dentistry - State University of São Paulo (UNESP) - Araraquara - São Paulo - Brazil - Zip code 14801-130.

[*] Correspondence: Daniel Cardoso Garcia. Animal Care Barueri Veterinary Clinic. Rua Duque de Caxias, 226 - Centro - Barueri - São Paulo - Brazil - Zip code 06401-010. Phone: +55(11) 99618-3304.

ABSTRACT

Background: Autograft, allograft, and bone substitute synthetic materials play an important role in reconstructive orthopedic surgery and understanding of the biological effects of these materials is necessary for their best use. Although autografts and allografts show excellent skeletal incorporation, the host's morbidity and graft acquisition limit their availability. Several synthetic bone substitute materials may present osseoconductive characteristics similar to grafts, such as beta tricalcium phosphate (β - TCP), a biomaterial of the ceramic class. In the present study, 19 New Zealand rabbits were used, divided into 3 groups (A, B and C), in which animals of Group A were implanted a block of β - TCP, and in animals of Group B allogenous cortical bone grafts were used, both inserted in critical segmentar defects performed by ostectomy on the radius bone of rabbits. In Group C, ostectomies were done as in Groups A and B, but no defect fillings were used. Clinical, radiographic, micro-computed tomography (μCT) and histological evaluations were performed at different times to verify the occurrence of osseointegration between bone and β - TCP implant and between bone and graft, as to assess possible implant resorption.

Results: At 120 days of PO, in all animals of Group B there were cell adhesion and bone integration, without signs of infection or inflammation, differing from Group A, where there were not any osseointegration process between β - TCP implant and host bone, nor have the implants reabsorbed. The biocompatibility of such material corroborated with the results of

other studies, but osseoconductivity showed different results, it means, no bioactivity signs, probably because of implant low porosity characteristics.

Conclusion: It could be concluded that the customized pure-phase β - TCP implants used for this study did not show such osseoconductive and osseointegrative characteristics mentioned by other authors and they were not effective for the consolidation and integration between the hosts bones and biomaterials. Further studies must be carried out to provide more information about this ceramic block, specially concerning to its porosity and shape in order to classify it as a proper material for use in the routine of orthopedic reconstructive surgery in veterinary medicine.

Keywords: Biomaterials, bone critical defect, bone segmentar defect, bone implant, beta - phosphate tricalcium, β - TCP, ceramics, orthopedics, ostectomy, rabbits.

INTRODUCTION

Currently, tissue engineering has been widely used to try to manipulate the bone regeneration process and restore damaged bone tissue through the proliferation and differentiation of bone cells within synthetic structures, using, for this, some type of material that replaces the bone tissue lost or the damaged bone [1-6]. In general, the most commonly used materials for bone defects reconstitution include bone grafts (autogenous, allogeneic or xenografts) and biomaterials, such as non-degradable bone cement, metals and ceramics [7, 8].

The word biomaterial as we currently apply it is defined as any material that is intended to come into contact with biological systems to assess, treat, augment or replace any tissue, organ or organism function [4, 9]. When applied, the biomaterial must maintain its properties and structural characteristics, but at the same time it must replace the function for which it was created. It is also important that it allows good cell adhesion to its surface [6], has adequate mechanical strength, does not have oncogenic characteristics, is sterilizable, is easy to handle, allows good fit in the bone defect [10, 11], is not harmful to tissues adjacent, be biocompatible, mimic the physiological environment of bone tissue and, finally, that its large-scale production is easy and cost-effective [4, 12]. Still, the best expected features of bone substitutes are the promotion of osteogenesis, osseoinduction, osseoconduction and osseointegration [5, 13-16].

In this context, bone autograft presents the best clinical results. However, it is difficult to concentrate these four properties in a single synthetic material, but it would be possible to add to an osseoconductive matrix, such as ceramics, bioactive agents that will provide the remaining

characteristics to replace the use of autografts and allografts, such as bone marrow aspirate or bone morphogenetic proteins (BMP's) [5, 17, 18].

Although autogenous and allogeneic grafts are the best types of grafts in terms of osteogenesis, osseoinduction, osseoconduction and osseointegration when compared to other types of implants, we still face several very important factors when choosing procedures with the use of autografts, such as prolonged surgical time, increased morbidity for removal of autogenous grafts and the possibility of infection or fracture during graft collection [19, 20].

Likewise, for the use of allografts, we depend on bone tissue banks, logistical procedures and the high cost associated with processing and preservation methods [21], in addition to facing complex issues such as immunogenicity and risks of disease transmission when these are in use and contact with the recipient bone tissue [15, 22-24].

For these reasons, several studies related to synthetic implants have been carried out in an attempt to find a bone substitute that allows effective stimulation, conduction and integration of bone cells with the objective of bone healing. These types of implants have characteristics and compositions that allow the formation of new bone, so they have been tested as new alternatives to the use of bone grafts [25].

Regarding synthetic ceramic biomaterials, it is possible to produce them with a composition similar to that of inorganic bone matrix. These materials do not have limitations of availability, nor do they require any additional surgical procedure for their acquisition. The disadvantages of its use include a non-existent osteogenic or osseoinductive activity and its poor mechanical performance in tensile situations to serve as a supporting structure, given the inherent fragility and high rigidity of these materials [26, 27]. However, they have excellent osseoconductive characteristics [14, 28]. When ceramic is fixed to healthy bone, the osteoid is produced directly on the ceramic surface. Consequently, the osteoid mineralizes, and the new bone produced remodels [15].

One of the most important ceramics in this group is tricalcium phosphate (TCP). There are two allotropic forms, alpha and beta (α - TCP and β - TCP). It has the chemical formula Ca3(PO4)2 and molar ratio Ca/P = 1.5, containing about 39% calcium and 20% phosphorus [29]. It is highly biocompatible and bioactive [30, 31]. In the biological response created in the host bone defect, the porous TCP is reabsorbed from the implanted site as bone growth occurs within its structure [2, 28, 32]. Porous biomaterials increase the therapeutic efficacy of transplanted cells, preventing or decreasing the occurrence of cell death [7, 33]. A high-porosity, low-density composite ceramic provides a better area for neovascularization and bone growth [6, 28].

The surface layers of TCP increase its attachment to the adjacent host bone [28] and this stimulates osteoclastic resorption and new bone formation concomitantly with implant resorption. The use of TCP can also be combined with other materials (HA/TCP combination, or combined with autogenous bone, or even BMP's) to improve its functionality and accelerate its resorption [18, 28, 31, 34-36].

In its purest presentation, β - TCP is superior to α - TCP or hydroxyapatite in terms of osseoconductivity [34]. One of the main disadvantages of β - TCP in relation to hydroxyapatite is due to the fact that it does not have adequate structural support due to its rapid reabsorption, due to its macroporosity [37-39]. Although implanted ceramics do not have mechanical characteristics equal to those of bone, after their incorporation into bone they gradually acquire mechanical strength similar to that of cancellous bone [15, 28, 40].

Beta - tricalcium phosphate (β - TCP) is a type of synthetic implant widely tested in human medicine and dentistry, used in cases of bone loss, and used mainly in the form of granules [10].

In view of the above, we used β - TCP in a cylindrical block format and in its pure phase to clinically, radiographically, micro-tomographically (μCT) and histologically evaluate the occurrence of osseoconduction and osseointegration processes involving the ceramic implant and the bone of the host animal, and also to assess whether there will be resorption of this biomaterial as a new bone is formed and incorporated into the implant. And for that, we used the allogeneic cortical bone graft for comparison.

The possibility of using larger cylindrical blocks opens a perspective to maintain bone length in critical segmental bone defects, and in complex fractures with severe bone loss, considering its use mainly for small animals for having an affordable and cheap price. In veterinary medicine, this type of implant is not or is rarely used in routine orthopedic trauma cases.

The hypothesis that was formulated is that there would be osseoconduction and osseointegration caused by the implant, as well as its partial or total resorption as mentioned by other authors, and in this way, its use could be consolidated as a bone substitute material that would be used in a routine in traumatological cases of dogs and cats that would require large amounts of bone to fill gaps, necessary for healing and bone consolidation.

RESULTS

All animals in the three groups were clinically and radiographically evaluated for a period of 120 days, except for one animal in Group B that died 76 days after the experiment

due to an unknown cause. From a clinical point of view, an initial assessment was performed daily in the first 15 postoperative days to assess some pre-established parameters (Table 1) that were subsequently continued weekly. None of them presented anatomical deviation of the bone axis, demonstrating good alignment of the limbs. The support of the limbs occurred in the first days of the postoperative period (PO), with no signs of lameness in the operated limbs, and the gait was considered normal. Some animals had mild pain on palpation on the ostectomy site and on the plaque, a condition that was remedied over the days with the use of analgesics and anti-inflammatory drugs.

None of them showed signs of swelling, hematoma, seroma, or edema of the operated limb. Some animals insistently removed the bandages, sometimes trying to eat them, so it was decided to keep the animals without bandages and only routine cleaning procedures were performed. However, some animals bit and removed some of the stitches from the surgical wound, but without damage or opening in the skin. One animal in Group B developed a skin wound more distal to the stitches due to self-trauma, which healed after a few days.

After removal of the stitches and complete healing of the skin, no animal presented any skin or subcutaneous tissue problem related to the presence of the plate, screw, implant or graft. They also did not show signs of infection or foreign body reaction such as erythema, nodules, presence of secretion, fistula, or pus due to the presence of the synthetic implant or allogeneic cortical bone graft. The animals without any filled defects also recovered well, with no signs of infection.

A curious fact that was noticed in some of the rabbits was the persistence of non-growth or failure of hair growth on the operated limbs after shaving. Not only did the radius region remain alopecic after a few months of the surgical procedure, but also the shoulder of the same limb, contralateral thoracic limb, and ears, that is, areas of the body that were shaved for procedures during the surgical approach, but that were not operated on.

Table 1 - Clinical signs evaluated after radial ostectomy and placement of a synthetic β – TCP ceramic implant or allogeneic cortical bone graft in the segmental bone defect (Groups A and B, respectively), and after radial ostectomy without defect filling (Group C).

	Group	Material	Limb alignment	Limb support	Lameness	Surgical wound	Pain	Edema
Animal 1	A	β-TCP	great	great	no	SRA	+	no
Animal 2	A	β-TCP	great	great	no	normal	-	no
Animal 3	C	NF	great	great	no	normal	+	no
Animal 4	A	β-TCP	great	great	no	normal	+	no
Animal 5	A	β-TCP	great	great	no	SRA	-	no
Animal 6	C	NDF	great	great	no	SRA	-	no
Animal 7	C	NDF	great	great	no	normal	-	no
Animal 8†	B	Graft	great	great	no	normal	-	no
Animal 9	B	Graft	great	great	no	normal	+	no
Animal 10	A	β-TCP	great	great	no	normal	-	no
Animal 11	B	Graft	great	great	no	SRA	-	no
Animal 12	C	NDF	great	great	no	SRA	+	no
Animal 13	A	β-TCP	great	great	no	normal	-	no
Animal 14	B	Graft	great	great	no	normal	-	no
Animal 15	C	NDF	great	great	no	normal	-	no
Animal 16	B	Graft	great	great	no	SRA	-	no
Animal 17	B	Graft	great	great	no	SRA	+	no
Animal 18	C	NDF	great	great	no	normal	+	no

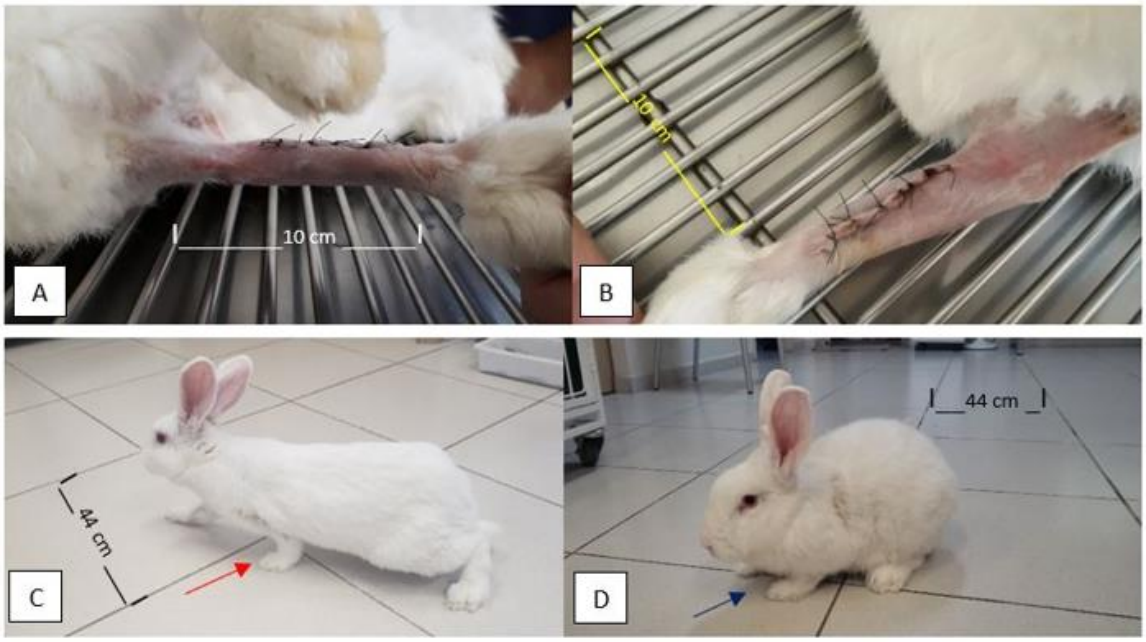
β - TCP = Beta-tricalcium-phosphate; NDF = no defect filling; SRA = stiches removed by animal; + = mild post-operatory pain; - = no pain; † Animal died after 76 days of PO, between M3 and M4, of unknown cause.

Over the months, from the time the stitches were removed until the 120 day postoperative evaluation, the function, alignment, and support of the limbs remained satisfactory, with no clinical signs indicative of problems that could be associated with the surgical procedures or the presence of the ceramic implant or allogeneic cortical bone graft used to fill the bone defects (Figure 1).

From the radiographic point of view, all animals in Group B, in which allogeneic cortical bone grafts were used, had good evolution and bone consolidation in 120 days, including the animal in Group B that died before the stipulated period for evaluation at 76 days of PO, which presented bone neoformation and osseointegration between the graft and the host bone already

in the radiographs of M2 (60 days PO). Normally, in the radiographs in the immediate PO (M0) and 30 days PO (M1), a proximal and distal radiolucent line was still observed between the edges of the bone and the graft, but at all other moments M2, M3 and M4 (60, 90, and 120 days PO, respectively) were no longer observed due to osseointegration between bone and graft. In some cases, bone remodeling had already occurred in M4.

Figure 1 - Photographic images of the rabbits' limbs a few days after the immediate postoperative period and, later, at 120 days after the surgical procedure.



Note the alignment of operated limbs and absence of edema, swelling, redness, signs of inflammation/infection or foreign body reaction (A and B) at five days PO. In C and D, note the good support of the thoracic limbs and load placement on the left thoracic limb at 120 days PO (red and purple arrows in C and D, respectively).

For Group A, there are images that suggest possible biological activity between the implant and the host bone, and there were no signs of bone or implant resorption present in M4. There were still radiolucent lines between the implant and the bone edges, showing that there was possibly not complete osseointegration between them. In Group C, at 120 days postoperatively, there was no complete bone growth and integration between the proximal and distal bone edges, culminating in bone non-union in all animals in the group (Figure 2).

In none of the cases in which osteosynthesis was performed, was there a fracture, loosening of bone plates or screws, or even any sign of osteomyelitis. On the contrary, good alignment of the limb, as well as of the plates, grafts, and implants, was always observed. In one of the animals in Group B, there were only 3 screws inserted into the plate instead of the 4

recommended for the experiment, due to the iatrogenic fracture of the bone during the passage of the drill to make the third hole (from proximal to distal), making it impossible to drill and insert a fourth screw.

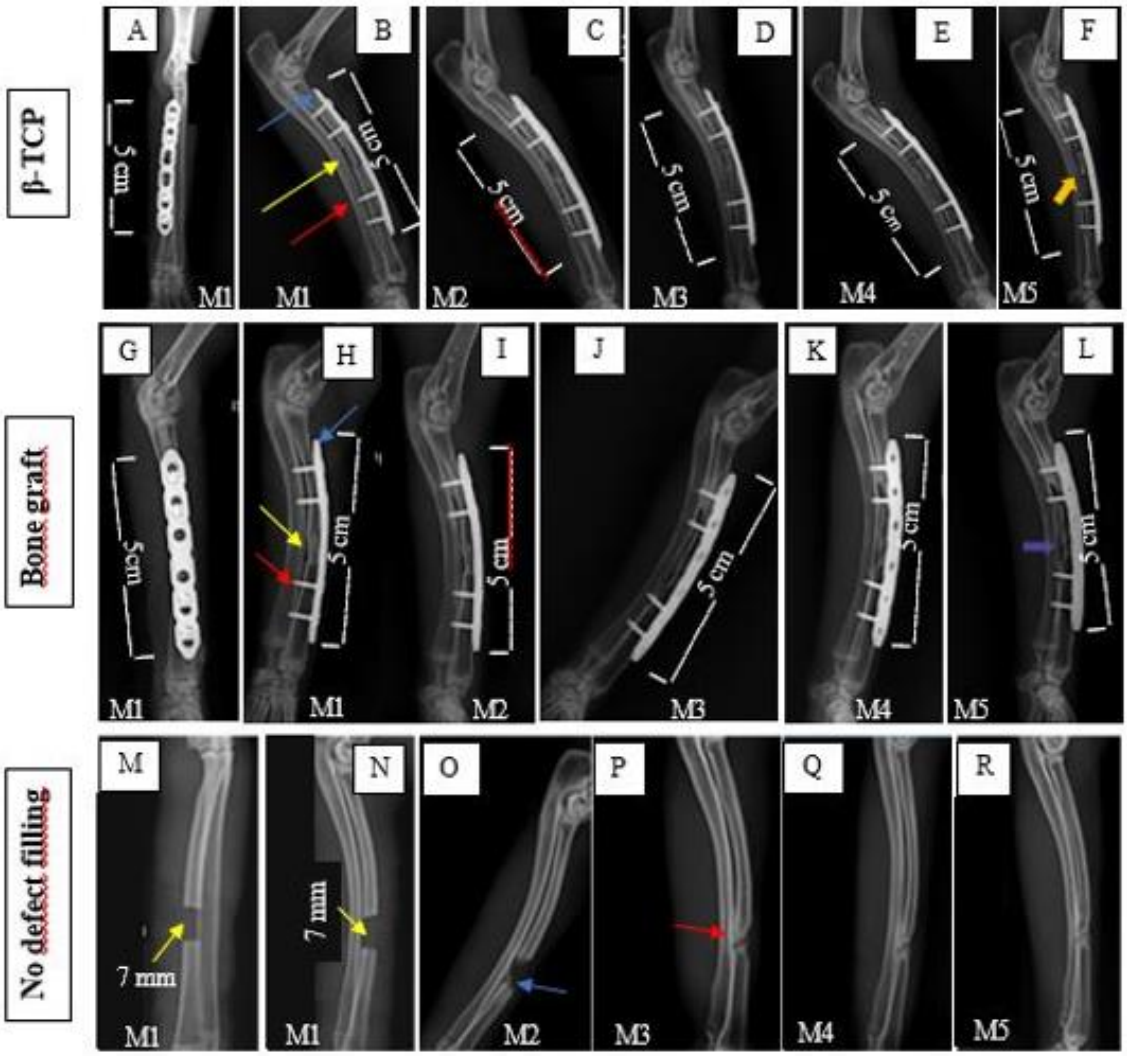
From the surgical point of view, as observed 120 days after surgery at the time of euthanasia (and after the death of the animal in group B at 60 days PO), all twelve animals in Groups A and B had the plaque and the screws well positioned and aligned in the bone, without any change after the mentioned period. There was growth of connective tissue on the superficial bone lamina and plate, as well as exuberant growth of mineralized tissue around and on it in some points, both in animals in Group A and in Group B (Figure 3).

There were no signs of infection at the operated sites. When removing the plates and screws, it was possible to notice for all rabbits in Group A and Group B, that there was good insertion and aligned positioning of the implants and grafts at the ostectomy site, and possible integration observed macroscopically between the recipient bone and the materials inserted into the bone defect, as there was no movement of them that would justify the contrary, even with pressure with the fingers on them. They were firm in their positions.

No signs of bone resorption or any other signs that demonstrate a loss of biological activity occurred at the ostectomy site were observed, either by the ostectomy itself during cutting with a dental drill, or by the presence of synthetic biomaterial or bone graft stored in 98% glycerin. At that moment, the tests of cultures and antibiograms were collected and all had their negative results.

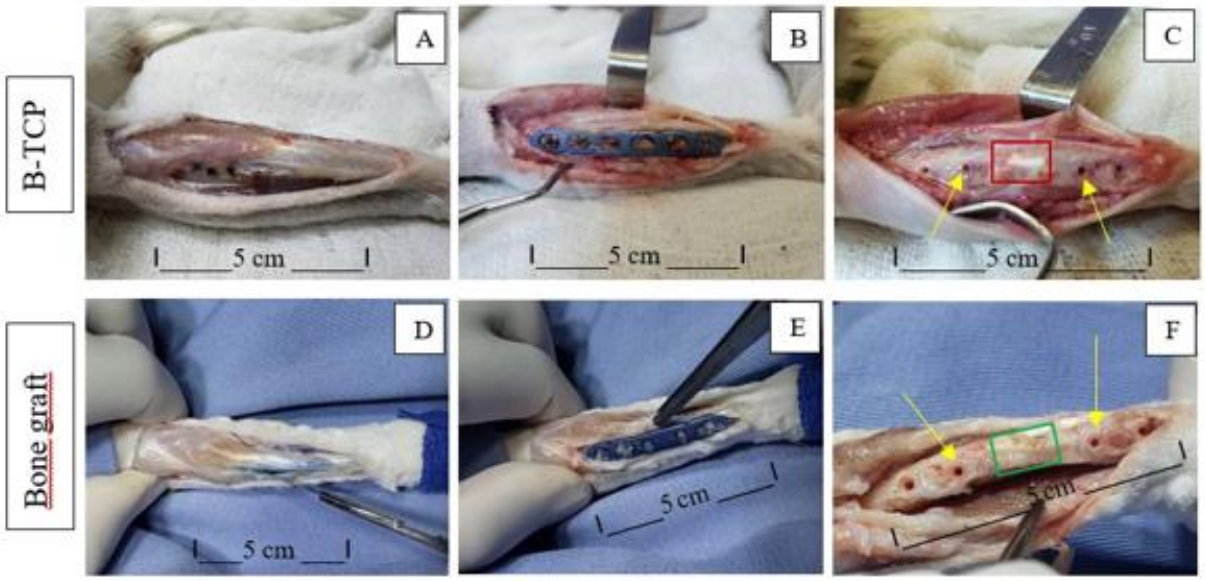
After disarticulation of the limb and removal of all soft tissue adhered to the bone and implant, it could be noted that there was a large amount of connective tissue around the implant and graft, as well as around the interface between the implant or graft with the edges proximal and distal to the ostectomy site.

Figure 2 - Postoperative radiographic images of the left forelimbs of animals in Groups A, B and C, respectively, at moments M0 to M4.



Craniocaudal images of immediate PO (M1) (A, G and M) and mid-lateral images of immediate PO (M1), 30 (M2), 60 (M3), 90 (M4) and 120 days (M5) of PO (B-F, H-L, N-R, respectively) of animals from Groups A (β - TCP), Group B (bone graft) and Group C (no bone defect filling). In both groups with defect filling, note the ceramic implant and bone graft inserted in the ostectomy site (yellow arrow on B and H), 1.5 mm titanium plate (blue arrow in B and H) and 1.5 mm titanium screws (red arrow in B and H). In figure F, in M5, the integration of the implant and bone can not be confirmed (solid orange arrow) because radiotransparent lines between bone and implant, although its perfect positioning. In L, in M5, it is possible to notice the integration between bone and graft, with the beginning of bone remodeling at the bone critical defect site (solid purple arrow). In M and N, in M1, we can see the ostectomy site with 7 mm length (yellow arrows). In O, in M2, there is initial growing of bone inside the defect (blue arrow) that increses until P, in M3 (red arrow). From that moment, it seems that bone growing process stops, since the image inside the defect is the same for the next 60 days (P, Q and R - M3 to M5).

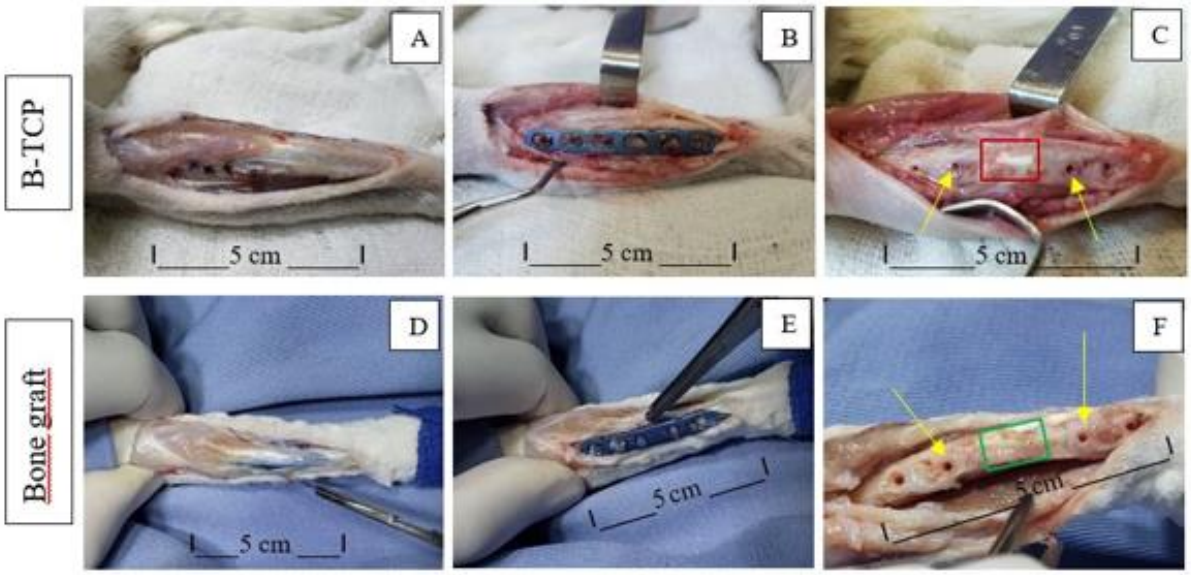
Figure 3 - Photographic images of the left forelimb of an animal from Group A (β - TCP) and another from Group B (allogenic cortical bone graft) shortly after euthanasia and exposure of the operated site for removal of bone fragments for µCT and histological study.



Photographic images showing good limb alignment, no signs of inflammation, infection, or foreign body reactions (A to F). After removing the plate and the screw, it was possible to notice the good alignment of the β - TCP implant (C) and allogenous cortical bone graft (F) in relation to the bone and a possible osseointegration of the implant with the surrounding tissues inside the bone defect and the bone edges. Macroscopically, it seems that there was no implant resorption. The yellow arrows in C and F show the positions of the second and third screws (left to right direction), corresponding to the proximal-distal direction of the forelimb. The red and green squares indicate the positions where β - TCP implant and allogenous graft were inserted in the bone defect. In Group B osteointegration seems to be more effective and macroscopically visible than Group A. For both Groups is visible that there are not any kind of foreign bone reaction or infection in the bones or other tissues surfaces below the mettalic implantes as seen after plates and screws removal.

After the careful removal of this tissue, it was possible to observe the interaction of the biomaterial and the bone graft with the host bone without mobility or loosening between them. The implants appeared intact, with no resorption appearance, while the allogeneic cortical bone grafts appeared to be part of the host bone, indistinguishable from the recipient bone of rabbits after these 120 days. Even after cutting the bone piece to obtain the 2 cm fragment for making the histological slides, both the β - TCP implant and the allogeneic cortical bone graft did not destabilize with each manipulation of the fragment (Figure 4).

Figure 4 - Photographic images of the left forelimb of an animal from Group A (β - TCP) and one from Group B (allogenic cortical bone graft) after limb disarticulation and specimen preparation for μCT and histological study.

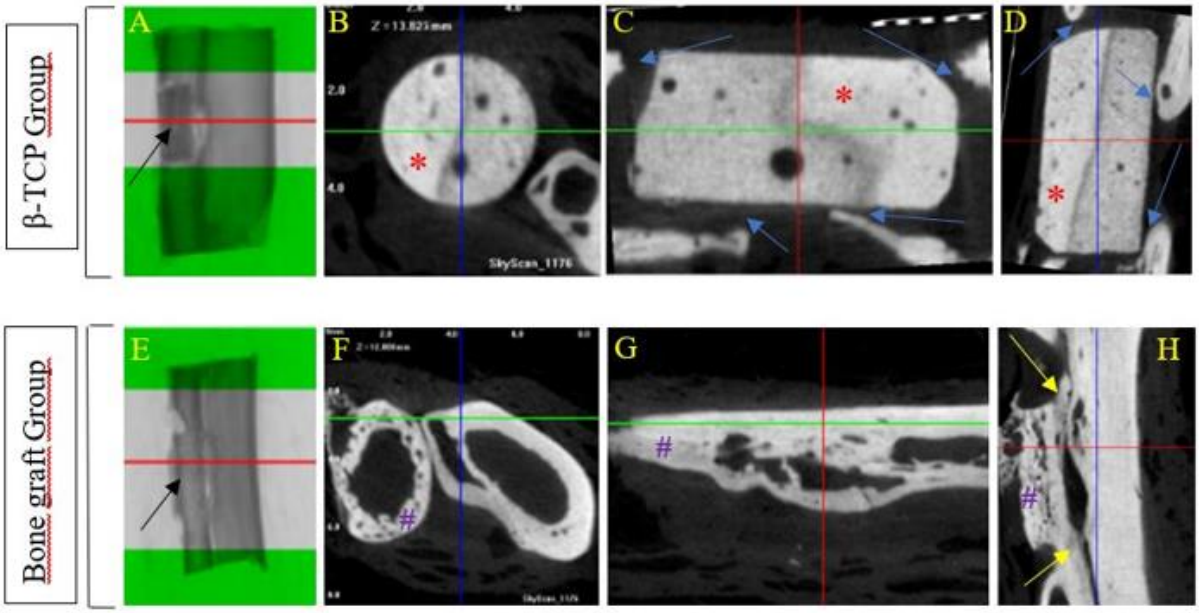


Photographic images showing good limb alignment, no signs of inflammation, infection, or foreign body reactions (A to F). After removing the plate and the screw, it was possible to notice the good alignment of the β - TCP implant (C) and allogenous cortical bone graft (F) in relation to the bone and a possible osseointegration of the implant with the surrounding tissues inside the bone defect and the bone edges. Macroscopically, it seems that there was no implant resorption. The yellow arrows in C and F show the positions of the second and third screws (left to right direction), corresponding to the proximal-distal direction of the forelimb. The red and green squares indicate the positions where β - TCP implant and allogenous graft were inserted in the bone defect. In Group B osteointegration seems to be more effective and macroscopically visible than Group A. For both Groups is visible that there are not any kind of foreign bone reaction or infection in the bones or other tissues surfaces below the mettalic implantes as seen after plates and screws removal.

In the μCT images, what we can see is that there was no osseointegration between the β - TCP implant and bone in the animals of Group A, while there was visible osseointegration of the allogeneic cortical bone graft and bone in the animals of Group B. In Group A good positioning of the implants was observed, with no mobility in the places where they were initially inserted. The edges of the recipient bone grew towards the implant but were not able to integrate with the ceramic to form a single continuous piece seen in the image. The cylindrical blocks of the implants kept their original sizes, and we did not clearly see a resorption process in them. There were some small radiolucent circular dots inside the implant contrasting with the rest of the piece, which could be interpreted as a possible process of initial absorption of the implant or an area of greater porosity dots inside the implant (Figure 5).

In Group B, the bone grafts showed an osseointegration process and also an initial bone remodeling process. The edges of the recipient bone grew towards the graft and touched each other forming a single piece seen in the image. The grafts were well positioned and without mobility. It seemed that there was also integration with the other bone below the radius, the ulna, where we can see the two touching each other (Figure 5).

Figure 5 - Tomographic images of selected fragments of animals from Group A (β - TCP) and Group B (cortical allogeneic bone graft).



Tomographic images of 2 cm bone with β - TCP implant (group A) and bone graft (Group B). A and E - Middle part of the implant and graft as region of interest (ROI) for μCT analysis and acquisition of the cross-sectional images. Note the implant and bone graft inserted in the receptor bones (black arrows in A and E, respectively). B and F - Trans-axial cut. C and G - Coronal cut. D and H - Sagital cut. * β -TCP implant; # Allogenous cortical bone graft. Note that there is no implant-bone osteointegration by μCT images on Group A (purple arrows in C and D), but on contrary, there is positive osteointegration between bone graft and bone as shown in H (yellow arrows).

After acquiring the μCT images, the 2 cm pieces from Group A were prepared for histological study. Five of the six pieces of calcified bone were randomly assigned to perform the Exakt protocol. Unexpectedly, the β - TCP implants reacted with resin or alcohol baths during the fixation protocol and dissolved for unknown reasons, except for some parts, which remained intact after the protocol was performed. There is no newly formed bone within the area corresponding to the implant. Despite this, the bone edges did not resorb, and connective tissue coating of the implant could be observed, except in some areas of the most cranial part

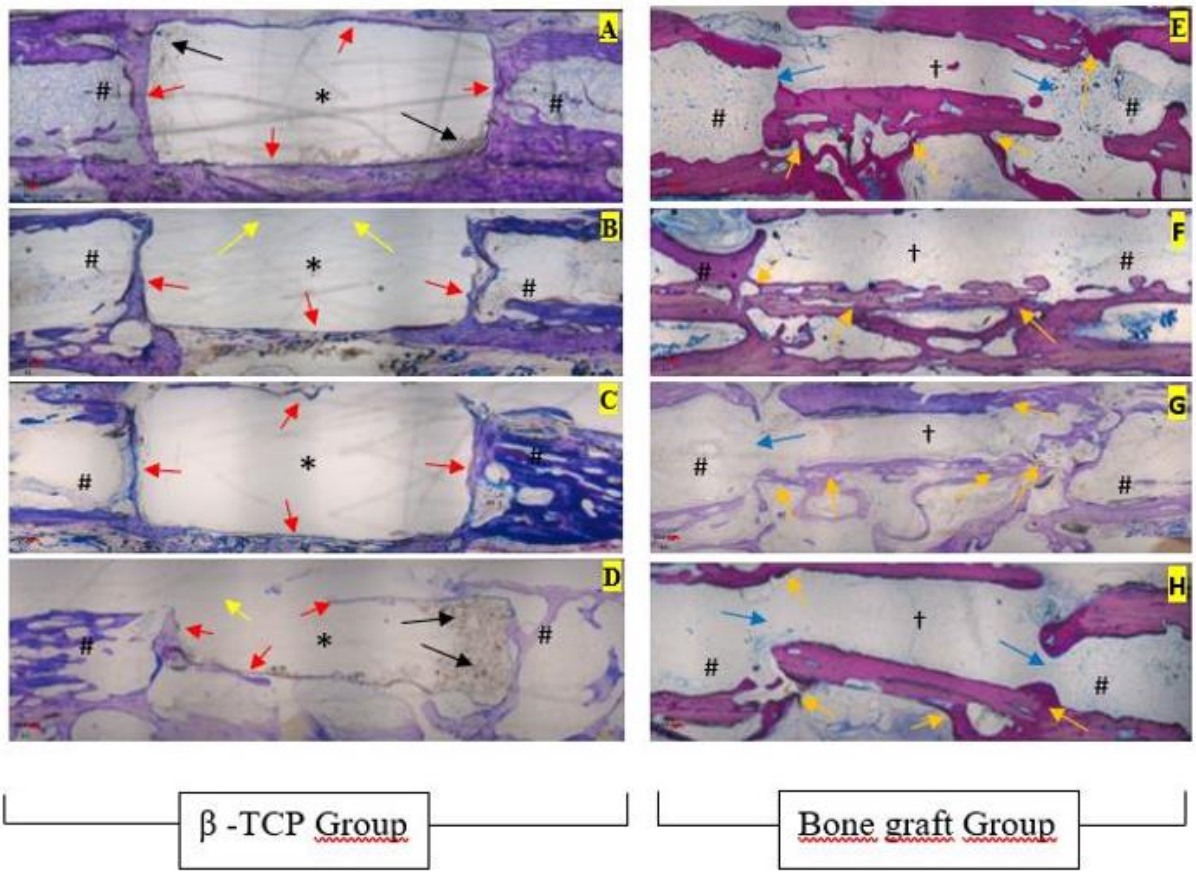
of the implant, where there is no surrounding tissue. The edges of the bone created a kind of wall between the implant and itself. There were no signs of a foreign body reaction, demonstrating that there are signs of biocompatibility. The ceramic implant appears to have the same shape as the original when implanted 120 days ago, with no resorption process. (Figure 6).

The allogeneic cortical bone graft formed connections with the recipient bones and remodeled in most cases, presenting integration between the edges and surfaces of the graft. The graft and bone communicate through a spinal canal in most cases, as if they were a single piece. There was a more efficient osseointegration process compared to Group A. In addition, there was greater activity of bone neoformation compared to the other group. As in Group A, in Group B there was no evidence of foreign body reaction (Figure 6).

The other two pieces (one with β - TCP and the other with bone graft) that were randomly selected for histological study by the staining process with hematoxylin and eosin underwent a bone decalcification process to perform the slides. Regarding the synthetic ceramic implant from Group A, its location within the bone defect was much clearer to observe than the graft from Group B, as its color was much whiter than the animal's bone and, therefore more noticeable macroscopically. In addition, it was possible to notice the rounded aspect of its surface in relation to the original bone of the rabbit, unlike the graft, which presented a flatter aspect in relation to the synthetic implant. The decalcification process took about 10 months and for the β - TCP pieces (Group A) it was necessary to remove the ceramics that were inserted into the bone, as they did not decalcify like the adjacent host bone, maintaining their initial hardness characteristic.

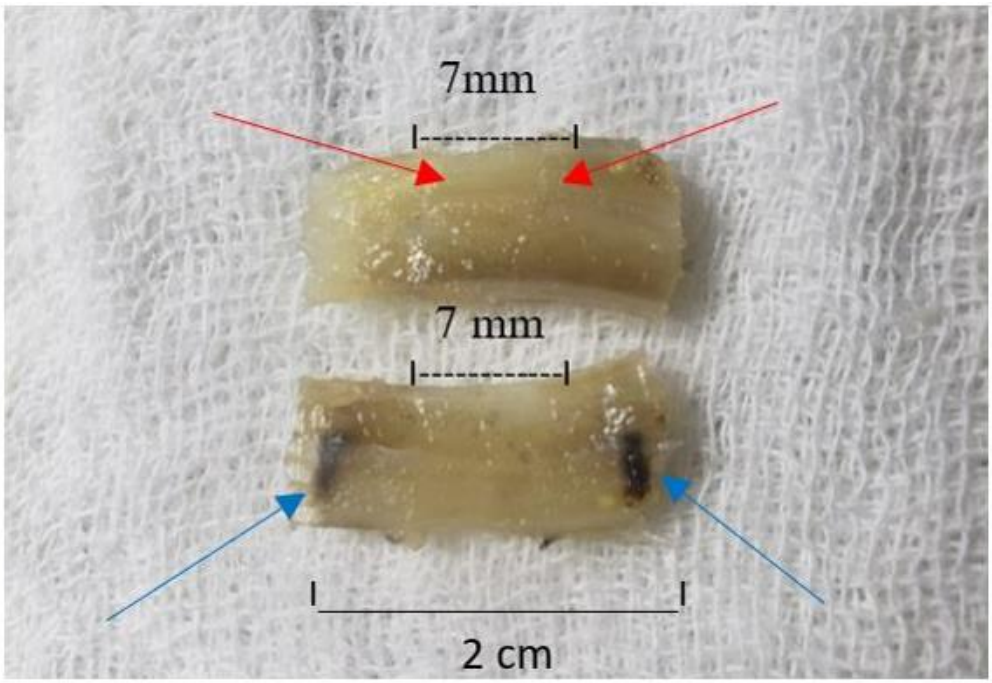
For Group B, the decalcification process was performed without intercurrences, and after the procedure, a longitudinal cut was made in the piece, and it was practically impossible to see in which region the bone graft was implanted. The only colors that we could macroscopically distinguish on the specimen were a darker delimited area referring to the insertion site of the screws and the slightly lighter color of the graft in relation to the bone (Figure 7).

Figure 6 - Histological images of animals in Group A (β - TCP) and Group B (bone graft). Exakt protocol performed with calcified parts.



Photographic images of Exakt protocol to visualize β - TCP implant (A - D) and bone graft (E - H) positions and their interfaces with receptor bone. β-TCP implant reacted with the resin during the fixation protocol and dissolved (A - D), except for some parts that still remained intact (black arrows in A and D). Note that there is not newly formed bone within the implant area. Receptor bone edges did not reabsorb and encompassed the implant (red arrows in A and C), except in some areas in B and D (yellow arrows). There is not signs of foreign body reactions showing its biocompatibility. Implant seems tohave the same shape as its original form when implanted 120 days before, showing no reabsorbation process. Allogenous bone graft formed connections to receptor bones and remodeled, showing integration between graft´s edges and surfaces (orange arrows). Graft and bone comunicates through a medullary channel in most cases (blue arrows). * Marks the center of the implant. # Marks rabbits receptor proximal and distal portions of the bone. † Marks the center of the allogenous cortical bone graft. Magnification 40x. Histological slides with 40 µm thickness.

Figure 7 - Photographic image of the longitudinal section of the radius and ulna bone of a specimen from Group B (allogenic graft) after the decalcification process.



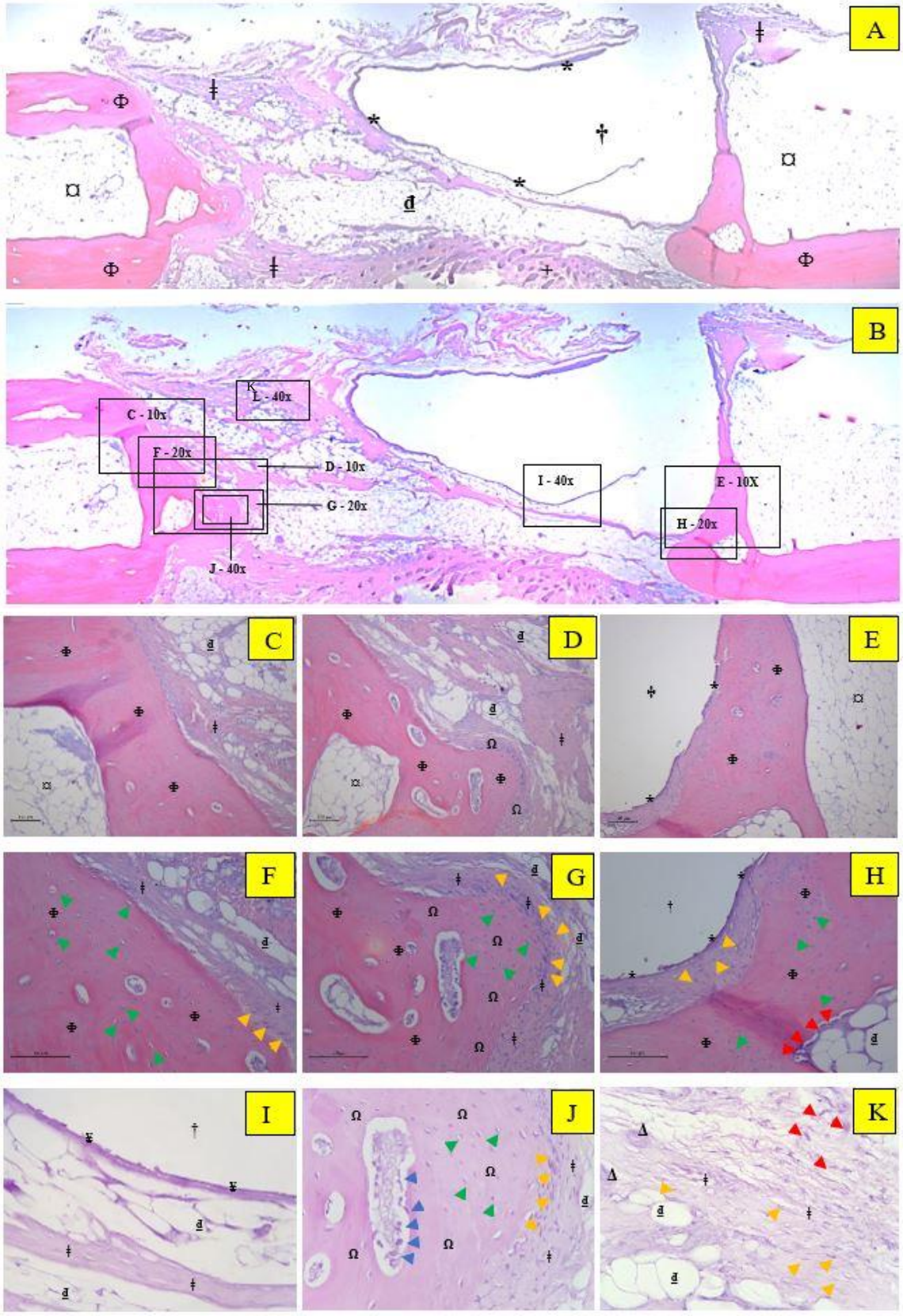
Internal view of the medullary channel of Group B radius and ulna bone (allogenous bone graft) after decalcification process and longitudinal section to prepare the slide for histological study. Note the darker areas corresponding to the screw's insertion sites (blue arrows) and a slightly lighter area in the central portion of the pice corresponding to the bone graft that was inserted within the radius bone defect (red arrows).

In the histological section of decalcified bone from the animal in Group A, what we could observe was that there was no invasion of bone cells into the area of the implant, as well as no formation of neovascularization. The edges that are in contact with the implant showed only fibrous tissue and connective tissue growth, with no osseointegration of the β - TCP implant to the host bone. Fibrocytes and fibroblasts are present at the implant margins, but osteoblasts and osteocytes are present only in the lamellar bone area corresponding to the host bone, and not as bone neoformation expected by implant bioactivity. We observed that the medullary canal closed with a bone "wall", but there is no invasion of this bone to connect to the implant. However, there was no resorption of the bone ends. In a deeper cut area, we could observe some remnants of the ceramic material (Figure 8).

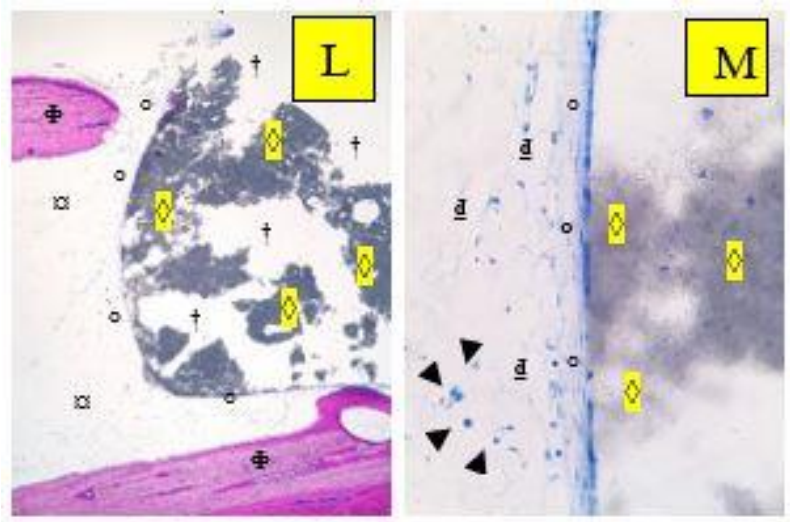
In the decalcified bone section of the animal in Group B, what we could observe was the osseointegration between the host bone and the allogeneic cortical bone graft. It is practically impossible to differentiate what would be the bone graft and the bone, as the two are

confused and there are few areas where graft remnants can be found. It is possible to distinguish them only by the types of bone present in the region of interest. The bone neoformation that occurred integrates with the lamellar bone of the host and joins the graft region, forming a single bone piece. Osteoblasts and osteocytes can be seen in various regions of the slide. Blood vessel neoformations are also observed in some regions of the graft (Figure 9).

Figure 8 - Photographic images of histological section of bone fragment/implant from an animal in Group A (β - TCP). Protocol using Hematoxylin and Eosin in a decalcified bone fragment.

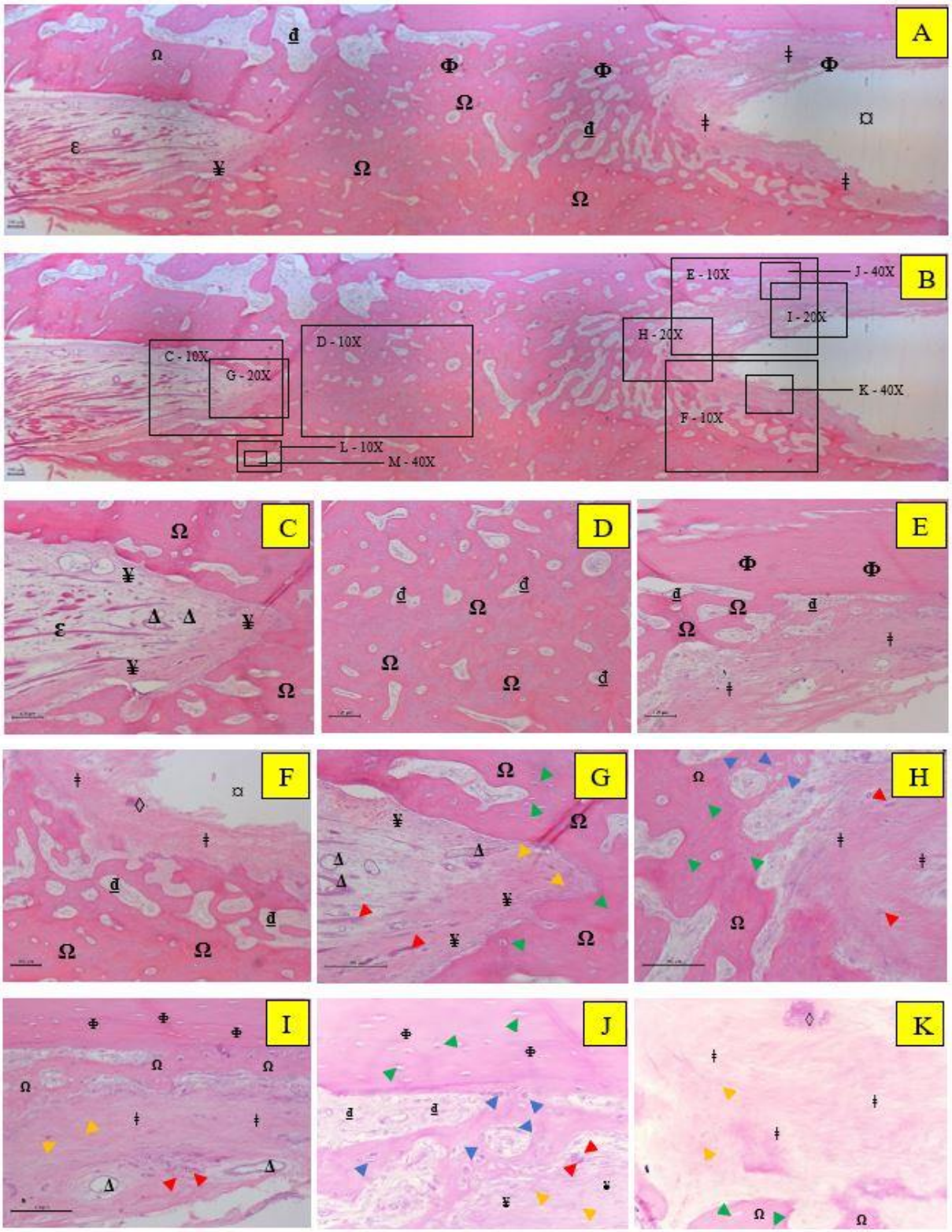


Continuation of Figure 8.

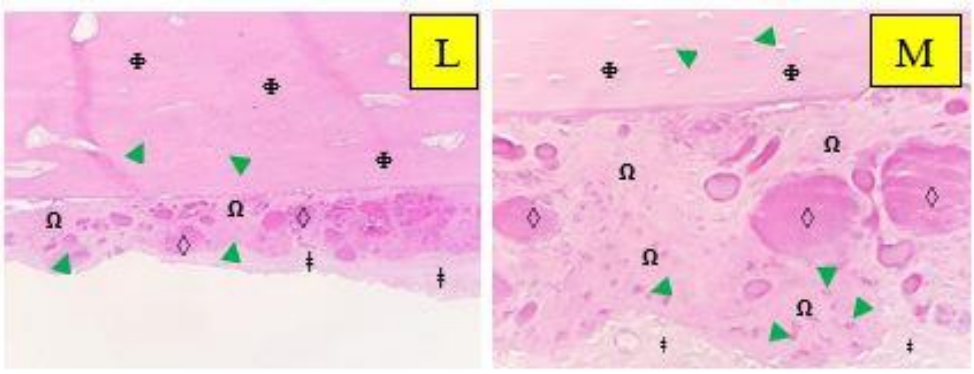


A - Optical microscopy photographic image with HE staining and 2.5x magnification. B - Same image as figure A with 2.5x magnification showing the areas of interest (ROI) delimited by black rectangular boxes, which indicate the letters of the following images and corresponding magnification of the lens used. C, D and E - Optical microscopy photographic images with 10x magnification of a segment delimited in B. Note that in E, there is only a fibrous tissue capsule in contact with the implant. F, G and H - Optical microscopy photographic images with 20x magnification of a segment delimited in B. I, J and K - Optical microscopy photographic images with 40x magnification of a segment delimited in B. Note that in H and I, there is only fibrous tissue capsule, fibrocytes and connective tissue in contact with the implant. L and M - Optical microscopy photographic images with magnification of 5x and 40x, respectively, of deeper segments of the slide, from which fragments of the β - TCP ceramic remained after its removal for microtomy process after decalcification. Note how there are no newly formed bone cells in contact with the implant, but only connective tissue. **+** Muscle tissue; ***** Fibrous tissue capsule; Δ Blood Vessels; ° Connective tissue capsule; Ω Bone neoformation; ¥ Connective tissue; ǂ Fibrous tissue; ◊ Ceramic remnant of β - TCP; ¤ Adipose tissue in the spinal canal; Φ Lamellar bone; ⫝̸ Extramedullary adipose tissue; † Cleft created with the negative image of the β - TCP implant; Osteocytes - (green arrowhead); Osteoblasts - (blue arrowhead); Fibroblasts - (red arrowhead); Fibrocytes - (orange arrowhead); Myeloid cells - (black arrowhead).

Figure 9 - Photographic images of histological section of bone fragment/graft from Group B animal (allogenous cortical bone graft). Protocol using Hematoxylin and Eosin in a decalcified bone fragment.



Continuation of Figure 9.



A - Optical microscopy photographic image with HE staining and 2.5x magnification. B - Same image as figure A with 2.5x magnification showing the areas of interest (ROI) delimited by black rectangular boxes, which indicate the letters of the following images and corresponding magnification of the lens used. C, D, E, F and L - Optical microscopy photographic images with 10x magnification of a segment delimited in B.G, H and I - Optical microscopy photographic images with 20x magnification of a segment delimited in B.J, K and M - Optical microscopy photographic images with 40x magnification of a segment delimited in B. Note how, unlike Figure 8, the use of allogeneic cortical graft promoted osseoconduction and osseointegration with bone neoformation, as seen in Figure 9, letters A , B, C, D, E, F, G, H, I, K, L and M at different magnifications and locations. Δ Blood Vessels; Ω Bone neoformation; ¥ Connective tissue; ǂ Fibrous tissue; ¤ Adipose tissue in the spinal canal; Φ Lamellar bone; ◊ Bone graft waste; ⸸ Extramedullary adipose tissue; Osteocytes - (green arrowhead); Osteoblasts - (blue arrowhead); Fibroblasts - (red arrowhead); Fibrocytes - (orange arrowhead).

DISCUSSION

According to the results obtained, although there was an osseointegration process between the bone graft and the host bone in the animals of Group B, we did not observe the same process occurring between the β - TCP implant and the host bone in any of the animals of Group A. The allogeneic cortical bone graft is a material with osseoconductive and osseoinductive properties [19, 20], unlike the ceramic implant in its pure phase, which has only osseoconductive characteristics [6, 41]. Due to their ability to bind to bone and stimulate bone tissue formation, bioactive CaP ceramics are seen as excellent candidates for grafting materials for bone augmentation or replacement [42] and among them, β - TCP is considered the ideal material [43]. Despite this, the β - TCP in the form of a cylindrical block and in pure phase, made exclusively for this study, proved to be ineffective in relation to the osseoconductive characteristics, as mentioned by the authors above and by [28].

Many studies have shown osseoconduction and osseointegration properties using β - TCP in granules [42, 44-48]. But the granulated implant shape has been used only for alveolar filling and maxillary sinus lift in dentistry [41, 43, 48-51], and in orthopedics [1, 2, 5,

10, 36, 44, 52, 53], stronger, rigid and stable biomechanical characteristics are not required of them to be used in critical defects of long bones.

Thinking not only of filling purposes, but also in cases of bone replacements and maintenance of bone length combined with single-stage surgeries, that is, correction by acute osteosynthesis, we thought of using a compacted ceramic implant with osseoconduction properties in the expectation that it could lead us to a bone healing process through bone/implant integration and remodeling. Furthermore, it was reported that the surface architecture of β - TCP could stimulate the differentiation of monocytes/macrophages into osteoclasts, these cells being essential for ectopic bone formation [46]. But we inferred that the lack of enough space between the implant particles could have been the reason that prevented and prevented the formation and penetration of blood clots, cells, and growth factors inside them for neovascularization and formation of new bone cells to occur. This would certainly allow the adhesion of these new cells to the implant with greater firmness, thus achieving complete integration of the implant with the host bone and bone neoformation within the ostectomy defect.

Even being a compacted implant, the β - TCP block has the porosity like any other ceramic implant [44, 47]. The size of the pores described by other authors differs a lot, which makes the implant to be classified as micro or macroporous [34, 36, 45, 47]. Pores of 50 μm are described as sufficient to promote osseoconduction [46]. However, one of the authors who used different β - TCP implants with micropores smaller than 50 μm, around 1 to 5 μm, also reported osseointegration, but with implants in granular formats [45]. In this case, we classified our β - TCP implant as having micropores, with porosity around 1.16 μm reported by the manufacturer after the sintering process. As it is a customized implant, we sought to gain more rigid and firmer mechanical characteristics for the implant during the sintering process, which consequently reduced its porosity. Our hypothesis was that by reducing porosity and gaining rigidity, we could have an alternative to maintain a good mechanical characteristic, without forcing and breaking the implant and at the same time, allowing blood vessels, cells and growth factors permeate the pores of the implant and form new bone throughout. But this feature was not enough to promote the conduction of bone cells nor the integration between them in our implant. Certainly, the porosity that we gained from the sintering process was too low to allow osseoconduction, which differed from the findings of [45]. Perhaps here, the granular shape of the author's implant can be taken into account, which despite the low porosity, the granular shape could allow better passage of cells between the granules, a fact that would not be possible when using the cylindrical block.

This suggests that this specific biomaterial, despite being a biocompatible material, proved to be inert in this study, not promoting or stimulating bone growth within it. And, in this case, it could not be considered a bone substitute for this period of 120 days of study, and it could not be used in cases of severe bone loss or critical bone failures and defects, as it did not physiologically allow bone healing or did not serve as a a biomaterial that stimulated the formation, induction or conduction of cells to restore bone column and limb function as described by [36]. Probably, if we increase the porosity, even if we decrease its rigidity, we could obtain an implant that is more suitable for the purposes described here.

As expected, there was a difference in the time of osseoconduction and bone integration observed between the two materials used in the study. Contrary to what was shown by [35], through characteristics that could be observed in radiographs and macroscopic longitudinal sections, we saw that for β - TCP, 120 days were not enough for integration and remodeling of the ceramic piece as occurred with the graft. allogeneic cortical bone, but this could possibly have occurred if there had been a longer evaluation time, even considering the low porosity characteristics described above.

Some animals in Group B already showed evidence of bone consolidation on radiographs at 60 days after surgery, corroborating the studies by [54, 55]. Another fact that confirms this was the surgical specimen obtained from one of the animals in Group B, which died at 76 days. In relation to this animal, it was observed both in the radiography and in the macroscopic aspects of the piece that there was already bone consolidation between the graft and the host bone.

The evolution time between the implantation of the biomaterial and the evaluated period leads us to question some aspects that we must consider: firstly, during the entire period recommended in this study, there was no absorption of the studied biomaterial, contrary to what has been reported by other colleagues [2, 28], who state that resorption is an inherent characteristic of this type of implant, and it was expected that it would occur in this study, at least in part, at the end of the 120-day evaluation period and as described by [18, 36]. Perhaps with longer periods of study, we could have observed greater absorption of the implant and changes in the original characteristics of the structural surface of the implant, from a macroscopic point of view, and mainly through radiographs and CT scans.

Second, we must consider the biomechanical aspects related to the biomaterial. As already reported by some colleagues [26, 27], the β - TCP is a porous implant and has poor biomechanical characteristics and can fracture as the load is exerted on it. Even being a cylindrical and compacted block, it probably could not withstand excessive and repetitive loads

without suffering fracture [7]. Therefore, a longer evaluation time, without resorption of the implant and subsequent filling of it with new bone, could lead to the stress of this implant and its consequent fatigue, which would possibly culminate in its fracture [11].

Thinking not only about the evaluation of osseointegration, but also considering the risk of breaking the biomaterial and mechanical interference in the implant, we tried to isolate the implant within the bone defect, performing a bridge osteosynthesis with plate and screws and making a "by-pass" over ceramic, which certainly reduced the load and mechanical stress on it [2, 38]. This procedure, in a clinical-surgical context actually simulates what would be done in real situations for which we are testing the cylindrical block β - TCP. In situations where there is a fracture with enough bone loss to create a critical bone defect, the objective would be to perform osteosynthesis, while keeping the length of the limb as close to what it would be originally, but for this, some materials with osseoconductive properties would have to be grafted or implanted in place [38].

One should always consider the use of some internal or external fixation device that transposes the loads through the implant and prevents the action of forces and movements of traction, compression, torsion, shear and bowing of the bone in relation to the implant [23]. This is extremely important, because in such situations, initially minimal or no movement is desired in the fractured focus. As we have a material that requires cell adhesion to integrate at the defect site, this must be mandatory so that osteoclasts can act through the implant and allow osteoblasts and blood vessels to grow and permeate between the micro-tunnels formed by osteoclasts through the pores of the biomaterial and osteoid is produced through and on the surface of the implant so that it subsequently mineralizes [15].

As cited by [6, 28], a ceramic composed of high porosity and low density offers a better area for neovascularization and bone growth. Thus, we can also say that β - TCP, in this case, did not present one of the important characteristics of implants that are normally used as bone substitutes and that is conferred by its biological behavior [30], that of being bioactive. We can say that for an osseointegration process to occur, there must be a direct connection between the implant and the living bone tissue, as the former has calcium and phosphorus ions in its composition, which would establish a chemical bridge with the surrounding bone [30], and in this case, we do not observe this happening.

However, the β - TCP implant showed good behavior in relation to biocompatibility, as described by [11]. Radiographic, tomographic, and histological studies, in addition to clinical and macroscopic evaluations of post-euthanasia surgical specimens, as well as culture of the surgical sites proved this. Corroborating with previous radiographic studies [40, 56], we could

observe that there were no tissue reactions that suggested osteomyelitis, bone resorption or bone sequestration. We also did not see any type of fistula or drainage of pus on or under the skin, or around the tissues, when the bone was collected for the histological examinations. This behavior was similar to that observed in the Group with bone graft (Group B).

In fact, especially for Group B after 120 days, we could even observe bone remodeling between the host bone and the allogeneic cortical bone graft. This shows that the graft preservation method in 98% glycerin was effective in controlling and preventing the growth of bacteria and fungi on the graft and in preventing contamination of the ostectomized area. This is also supported by some studies [20, 54, 55, 57] that used this same method to preserve the grafts that would be used in bone replacements. This is extremely important, as it greatly reduces the cost of performing the bone bank, drastically reducing the amounts spent on obtaining, preserving, and processing bones, proving to be a feasible, replicable, and adequate technique for the storage and preservation of grafts, differing from methods applied by [23, 24]. At least if we think of a bone bank for veterinary medicine, unlike human medicine, which has clear and strict health protocols for the creation of bone banks, this methodology could be a solution for cases in which there is no available some kind of biomaterial for implantation and bone replacement.

For animals in Group A, bacterial or fungal contamination related to the implant itself would not be expected, since the implant is synthetic and is already sterilized by gamma irradiation. In fact, 120 days after the operation, fungal and bacterial culture tests with antibiogram were performed for all specimens to rule out the possibility of contamination, with negative results for all of them. What could perhaps be observed in this case would be a foreign body reaction caused by the implant at the ostectomy site. However, in none of the cases such a reaction was observed, reaffirming what had already been reported by other colleagues [32] regarding the biocompatible characteristic of synthetic β - TCP ceramic.

This was also observed by the clinical evaluation of the animals. In none of the cases were edema reactions, local redness, swelling, pain on palpation, fistula or local abscess formation observed. Even after euthanasia and opening of the surgical focus and exposure of the implanted area, there was no evidence of contamination of the ceramic implant, bone graft, titanium plate or screws, or of the bone itself or adjacent soft tissues. This could be proven even by the negative culture tests cited above that were performed on the pieces, as well as in studies reported by [23, 58]. This demonstrates that from the point of view of biocompatibility, β - TCP is a material that could be used in the veterinary clinical-hospital routine. Even if it has to remain

for long periods in the body, it would probably not produce adverse reactions that would justify its withdrawal.

Considering the resorbable properties of β - TCP, significant resorption of β - TCP particles and concomitant bone neoformation were expected 3-6 months after its placement [46, 48, 50] allowing for new trabecular bone rearrangement during this period [48]. For this study, after 4 months of evaluation, what we would expect at some point is an implant that would be replaced by new bone, while the same implant would be reabsorbed by the body over time, partially or completely. In this way, we would have more blood vessels interspersed by the implant and, consequently, more cells of the immune system being taken to the site, which would allow the formation of a defense system in that environment that was previously synthetic, but which, over time, would transforming into a more biological environment, gradually. This resorption mechanism would suggest that the entire implanted environment would be favorable to bone growth. But in this study, we could see this happening only in Group B, between the bone and graft connections, but not in Group A with the β - TCP. In the latter case, all biological activity took place around the implant and not inside it.

Finally, in this context of infection, osseointegration and implant resorption, we know that bone consolidation that occurs in fractures with or without bone graft could occur even in infected environments, but for this to happen, absolute stabilization of the fracture with devices is necessary to prevent micro-movements at the fracture focus. We could not say whether even using a non-absolute stabilization such as the bridge used here in this study, through osteosynthesis with plates and screws, there would be cell adhesion in the ceramic implant and osseointegration in the presence of infection or contamination of the surgical site, since as a material synthetic, initially it is not vascularized until capillaries grow through it. Probably, in this case, there would be a biofilm surrounding the implant and maintaining a contaminated environment. Anyway, the objective here was to mimic in the most real way what would be applied in reconstructive surgery from the point of view of asepsis and uncontaminated surgery, and this objective was achieved because we were able to observe a four-month follow-up with no evidence of infection, and with the occurrence of a consolidation process in one of the groups.

CONCLUSION

It can be concluded that there was no osseoconduction process by the biomaterial β - TCP that would lead to osseointegration between the implant and the bone. There was no resorption of the ceramic implant, nor did the implant cause any inflammatory, infectious, or

foreign body reactions during the 120 days it remained within the critical segmental bone defect. However, it did not show bioactive features with this design and porosity that were used. More studies combining its porosity and its shape must be done to achieve the objectives we aim for in this experiment, so that we can have an implant that can be routinely used as a bone substitute in cases of considerable bone loss and the need for biomaterials to stimulate the cell adhesion between host cells and the implant.

MATERIALS AND METHODS

This study was previously submitted to the Bioethics Committee of the Federal University of Campina Grande (CEUA - UFCG) for evaluation of the use of animals and was approved under protocol number CEP 046/2018. The procedures for the collection and storage of allogeneic cortical bone grafts, as well as the surgical and postoperative procedures were performed at Animal Care Barueri Clínica Veterinária, in Barueri, São Paulo. For this study, 19 New Zealand rabbits, male and female, weighing between 3 and 4 kg were used. The animals were divided into 3 groups consisting of 06 animals in each. Group A was considered the group in which the radial diaphysis ostectomy was performed and the synthetic implant of β – TCP was used to fill the critical bone defect. Group B was considered the one in which the radial diaphysis ostectomy was performed and the cortical bone allograft was used to fill the bone gap. Group C was considered the control group of the study, and it was the one in which the ostectomy of the middle third of the radial diaphysis was performed, but the bone defects were not filled with any materials. The other spare animal was used for the aseptic collection of the two bones of the radius to make all the allogeneic cortical bone grafts that were part of the bone bank, which were later implanted in the segmental defects of the radius bones of the animals in Group B.

Implantation of β – TCP:

The implants used in the experiment were made with a high degree of purity (99.9%) of ceramic composed of β - tricalcium phosphate (β - TCP) in its pure phase, without association with any other type of material. They were donated by the company Procell (Rio Claro - São Paulo - Brazil) and customized for this study, unlike its commercial line already available. The shape of the implants was a porous cylindrical block, custom-made with 7 mm in length and 3 mm in diameter. The pore diameter of the implant was 1.1640 µm. These implants were already sterilized by gamma irradiation from the factory, packaged and identified in ready-to-use surgical grade paper packaging.

Acquisition of allogeneic cortical grafts:

Euthanasia was performed on one of the animals as described below (euthanasia section) for aseptic collection of the forearm of both forelimbs. After obtaining the bone fragments for grafting, they were cleaned and washed through irrigation with 0.9% saline solution (0.9% Sodium Chloride - Fresenius Kabi Brasil Ltda. - Barueri - SP - Brazil), and all tissue adhered to the cortical bone, including the periosteum was removed. The medullary canals were also cleaned with the aid of a hypodermic needle, and any structures such as blood vessels and bone marrow were removed. Subsequently, the grafts, already completely free of any non-bone tissue, were washed again abundantly with 0.9% saline solution before being stored in a flask containing 98% glycerin solution (Biohervas Farmácia de Manipulação - Barueri - SP - Brazil), product chosen to serve as a preservative for the grafts [54-58]. In this bottle, the bone allografts were preserved for a minimum period of 30 days before being used.

Anesthetic protocol:

As a pre-anesthetic protocol, Acepromazine (Acepran 0.2% - Univet S/A - São Paulo - SP - Brazil) at a dose of 1 mg/kg associated with Ketamine (Dopalen - Ceva Santé Animale - Paulínia - SP - Brazil) was used at a dose of 40 mg/kg, both intramuscularly. After an interval of 15 minutes, the animals were catheterized in the marginal venous vessel of the ear and maintained in fluid therapy with Ringer Lactate (Ringer Lactato - JP Indústria Farmacêutica S. A. - Ribeirão Preto - SP - Brazil). Then, isoflurane (Isoforine - Cristália Prod. Quím. Farm. Ltd. - Itapira - SP - Brazil) was used for anesthetic induction by mask vaporization. For anesthetic maintenance, the same isoflurane was kept in an open anesthetic circuit using the mask mentioned above. Animals were monitored by electrocardiogram, heart rate, respiratory rate, blood pressure, oxygen saturation and temperature [61, 62].

Surgical protocol:

Before surgery, the hairs of the left forelimb of each animal were shaved and then antisepsis was performed with skin cleansing with alcoholic chlorhexidine (Riohex 2% - Indústria Farmacêutica Rioquímica Ltda. - São José do Rio Preto - SP - Brazil). A longitudinal cranial incision of approximately 6 cm was made in the skin over the topography of the left radius of each animal and the tissues adjacent to the bone diaphysis were dissected. In the middle third of the radial diaphysis, an ostectomy was performed with the aim of removing a bone fragment of approximately 7 mm in length and creating a critical segmental bone defect, as shown by [59]. The measurement was marked on the bone with the aid of a Castroviejo

compass and electrocautery. Then, the ostectomy procedure was performed with the aid of a mini high-speed electric drill (Dremel Bosch - BSH Store - Curitiba - PR - Brazil) and a spherical dental drill (Broca Carbide FG n°3 - Angelus Prima Dental Ltda. - Londrina - PR - Brazil). Small holes were made transversely in the bone until it was possible to fracture it and completely remove the bone fragment.

Trimmings were performed in the remaining bone in order to make the bone defect as homogeneous as possible, with the osteotomies well parallel to each other. Therefore, irrigation of the ostectomized sites was performed at the time of the cuts, in order to avoid the occurrence of bone thermal necrosis. For the animals in Group A, the implants, which were already ready for use, were removed from the sterilized casings and were placed in the bone defect created by the ostectomy. For the animals in Group B, cortical bone allografts were removed from the bone bank and placed in the bone defect created by the ostectomy. Both materials were inserted by means of pressure at the ostectomy site, so that their proximal and distal ends were in intimate contact and compressed with those of the animal's bone.

At the time of using the allogeneic cortical bone graft, they were removed from the 98% glycerin pot and subjected to an abundant prewash with 0.9% saline solution for a period of 10 minutes before being placed in the bone defect, in order to to remove the glycerin and rehydrate the fragment to be used.

For the animals in Group C, the ostectomy site was left without any type of filling material and the anatomical planes were sutured as described below.

Then, osteosynthesis of Groups A and B were performed in order to promote stabilization of the operated limb and provide protection and mechanical support to implants and grafts at the ostectomy site. For this purpose, 1.5 mm locked plates and screws made of titanium were used. Both the plates and screws used in this research were donated by the company Lincevet (Lincevet - Rio Claro - SP - Brazil). Osteosynthesis were always performed in the form of a bridge over the implanted/grafted defect, that is, although the plate had 6 holes along its length, only 4 screws were used for blocking, 2 of which were placed in the two most proximal holes of the plate. and another 2 placed in the two most distal holes, leaving the central part of the plate free of screws. After performing the ostectomy and osteosynthesis procedures, sutures were performed in the muscle planes, subcutaneous tissue, and skin with nylon 3.0 (Nylon - Bioline surgical wires Ltd. - Anápolis - GO - Brazil) in a simple and conventionally separated pattern.

Dressings and care of the surgical wound stitches throughout the postoperative period were performed twice a day. Stitches were removed after 2 weeks postoperatively. To control

the infection, Enrofloxacin (Chemitril 2.5% - Chemitec Agro-Veterinária Ltda - São Paulo - SP - Brazil) was administered at a dose of 5 mg/kg, subcutaneously, once a day for 7 days. To control pain and inflammation, morphine (Dimorf - Cristália Prod. Quím. Farm. Ltd. - Itapira - São Paulo - Brazil) was used at a dose of 2.5 mg/kg, subcutaneously in the immediate postoperative period, followed by of Tramadol Hydrochloride (Tramadol - União Química Farmacêutica Nacional S/A - Pouso Alegre - MG - Brazil) at a dose of 5 mg/kg, subcutaneously, twice a day, for 7 days, associated with Meloxicam (Maxicam 0, 2% - Ourofino Saúde Animal Ltda - Cravinhos - SP - Brazil) at a dose of 0.2 mg/kg, subcutaneously, once a day for 7 days [61].

Postoperative evaluation:

The animals from Groups A, B and C were clinically and radiographically evaluated in the postoperative period (PO) for a period of 120 days. The exact timing of each assessment was specifically described below for each study. Then, the animals were euthanized and the μCT and histological studies were performed only for Group A and B, for each bone segment containing the implant or bone graft, respectively.

1. Postoperative clinical assessment:

Daily inspections of the surgical wound and monitoring of the healing process were performed in the three groups. Cleaning of the skin and stitches was performed twice a day with 0.9% saline solution and 2% non-alcoholic chlorhexidine solution. It was also checked daily for swelling, redness, and edema at the operated site, as well as limb alignment, limb support on the ground and gait status. This evaluation was purely macroscopic, subjective, and qualitative, through visualization and palpation of the operated site during the 120 days after surgery.

2. Postoperative radiographic evaluation:

All animals in the three groups were radiographically evaluated with digital radiographs postoperatively (System DR Wireless, Model Mars 1417V - TSI - iRay Technology - Shanghai - China and Portable X Ray Model Orange 1060HF - Digicare - Oxson Technology - São Paulo - SP - Brazil). Two radiographic views were performed on each animal, one craniocaudal (Cr-Ca) with the animal in the prone position and another mediolateral (M-L) with the animal in the left lateral position. Five moments were recommended for the radiographs, called M0, M1, M2, M3 and M4. The referred moment M0 was considered the immediate postoperative period,

M1 was the period of 30 days after the surgery, M2 was the period of 60 days after the surgery, M3 was considered the period of 90 days after the surgical procedure, and finally, M4 was the period of 120 days after the surgical procedure.

Euthanasia:

After a period of 120 days, each of the 18 animals used in the experiment was euthanized. For this, Acepromazine was initially administered at a dose of 1 mg/kg intramuscularly, associated with Ketamine at a dose of 40 mg/kg, also intramuscularly. Then, the marginal venous blood vessel of the rabbit's ear was catheterized and sodium thiopentax (1.0 g Thiopentax - Cristália Prod. Quim. Farm. Ltd. - Itapira - SP - Brazil) was administered at a dose of 30 mg/kg per intravenous route. After reaching an ideal anesthetic plane, potassium chloride was administered at a dose of 1 mg/kg intravenously. Vital signs were evaluated by the multiparametric monitor and then checked by the veterinarian, attesting to death due to cardiorespiratory arrest and absence of pulse [63].

Collection of bone fragments with β - TCP implant or bone graft for µCT and histology studies:

After euthanasia, and before the left radius of each operated animal from Groups A and B were removed for µCT and histology, the regions where the β - TCP implants or allogeneic bone graft were inserted were tested for possible contamination, being performed culture tests for fungi, bacteria and antibiogram of the surgical site. The plates and screws were also removed, and two screws (second and third - most proximal and most distal, respectively) were also sent for culture testing along with the previous swab.

With the aid of a dremell and a dental drill, the bone was transversely sectioned in two places in order to obtain a smaller bone fragment (approximately 2 cm in length) containing the implant or graft. For this, only the implant or graft was left in the most central area and about 0.65 cm of the original bone of the host was left in both the proximal and distal ends. As a final piece for µCT examination and for making slides for histological study, longitudinally, the new fragment was approximately 2 cm long and 3 mm in diameter. Again, the fragment was washed in 0.9% saline solution, packaged, and identified in a glass jar containing 10% formalin.

3. Evaluation by µCT:

Computed tomography micro-tomography was performed and evaluated in the micro-tomography laboratory of the Faculty of Dentistry, Universidade Estadual Paulista (UNESP - Araraquara).

All samples from Groups A and B were processed and tomographically analyzed after the animals were euthanized. The collections of implanted bones and grafted bones were performed as reported by [60]. Bone fragments from the left radius of rabbits containing the β-TCP implant or allogeneic cortical bone graft were removed from the flask with 10% formalin, where they were fixed for at least 48 hours, and subsequently stored in 70-degree alcohol.

Then the samples were removed from alcohol at 70 degrees and supported on paper towels. They were wrapped in this paper towel and moistened with water using a syringe and needle. Then, the samples were stored in circular styrofoam so that there was no interference in the generation of images and were inserted into the micro-tomography device.

The samples were scanned by micro-tomography (Skyscan, Aatselaar, Belgium) with the following parameters: camera pixel: 12.45; X-ray tube power: 65 kVP, X-ray intensity: 385 µA, integration time: 300 ms, filter: Al-1 mm and voxel size: 18 µm3. The images were reconstructed, spatially repositioned, and analyzed by specific software (NRecon, Data Viewer, CTAnalyser, Aatselaar, Belgium).

On average, it took 17 minutes of scanning to generate the images of each part. All micro-tomographic procedures were performed by the same laboratory technician and interpreted by the same radiologist, in order to avoid any bias in the technique and interpretation of the images.

4. Histological evaluation:

Histological evaluations were performed in the histological processing laboratory of the Faculty of Dentistry of the Paulista State University (Universidade Estadual Paulista - UNESP - Araraquara).

All samples from Groups A and B were evaluated after euthanasia of the animals and after the acquisition of µCT images.

Randomly, for five samples from Group A and five samples from B, slides for optical microscopy analysis were acquired through a grinding system (Exakt Apparatebeau, Hamburg, Germany), according to the protocol reported by [36]. This methodology was used to analyze calcified bone samples.

For the other specimens of each Group A and B, slides were performed for histological study by optical microscopy using the Hematoxylin and Eosin staining methodology. In this case, the methodology was used for analysis of decalcified bone samples. The decalcification process took place through immersion and maintenance of bone pieces in EDTA and took around 10 months to have a bone suitable for cutting with a conventional microtome. For the ceramic piece, the biomaterial had to be removed to perform the cut, because even after this period, it had not decalcified as bone. As for the piece with graft, this was not necessary, as the graft decalcified exactly like the host bone, not preventing the cut by the microtome.

Slide images were obtained and evaluated using optical microscopy (DIASTAR, Leica Reichert & Jung Products) at 2.5x magnification for calcified samples and 2.5x, 5x, 10x, 20x and 40x magnification for decalcified samples.

All images were captured with a camera (Leica Application Suite V3.8; Leica Reichert & Jung Products). For the 2.5x magnification images, three consecutive linear regions of interest (ROI) were identified. Photos of each ROI segment were taken (left side, center, and right side) and uploaded to a computer. With the aid of a computer program (Autostitch - UBC Industry Liason Office, British Columbia, USA) the 3 parts of the photographs were reassembled to form 1 new complete photograph of the entire segment, which served for the histological analysis of the calcified bones.

As for the images of the decalcified bones, the above process was only necessary for the 2.5x magnification, not being necessary for the highest magnifications, just taking the photographs of the slides directly with the magnifications used at 5x, 10x, 20x and 40x. For magnifications greater than 2.5x, ROI were identified in these same slides with a magnification of 2.5x, from which the images were made in the other aforementioned magnifications of the locations corresponding to the contact zone of the host bone with the materials that were used for filling and adjacent areas.

List of abbreviations

CaP - calcium phosphate; β - TCP - Beta - tricalcium phosphate; µCT - micro-computed tomography; PO - postoperative period; BMP's - bone morphogenetic proteins; TCP - tricalcium phosphate; HA - hydroxyapatite; NDF - without filling the defect; SRA - animal removed the stitches; M0 - zero moment; M1 - moment one; M2 - moment two; M3 - moment three; M4 - moment four; mm - millimeters; cm - centimeters; 2.5x, 5x, 10x, 20x, and 40x - 2.5, 5, 10, 20, and 40x magnifications, respectively; CEUA – UFCG - Bioethics Committee of the Federal University of Campina Grande; CEP - Research Ethics Committee; µm -

micrometer; Ltd.- Limited; SP - Sao Paulo; S/A - Corporation; mg - milligrams; Kg - kilograms; Product - Products; Kim. - Chemicals; Farm. - Pharmacists; PR - Paraná; GO - Goiás; MG - Minas Gerais; Cr-Ca - craniocaudal; M-L - mid-lateral; UNESP - State University of São Paulo; KVP – kilovoltage; µA - micro-amperage; ms - milliseconds; Al - aluminum; ROI - region of interest (area of interest); EDTA - ethylenediamine tetraacetic acid.

STATEMENTS

Ethics approval section

This study was previously submitted to the Bioethics Committee of the Federal University of Campina Grande (CEUA / UFCG – Committee on Ethics for the Use of Animals / Federal University of Campina Grande) for evaluation of the use of animals and was approved under protocol number CEP 046/2018. All experimental protocols were approved by the previously mentioned committee. All methods were carried out in accordance with relevant guidelines and regulations**.**

All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Conflict of interests

The authors declare no conflicts of interest.

Financing

There was no financial support for this research. There will only be a financial contribution if the article is approved and published in a Qualis Capes A1 journal. In this way, the financial aid will only be used to pay the journal's publication fee.

Authors' contribution

DCG and MJCS conceived and designed this research. DCG and LEM performed the bibliographic reference searches. DCG and MJCS coordinated the research. DCG and LEM operated on the animals and conducted the experiments and postoperative evaluations. LS performed the anesthesia. MRI performed the radiographs. DCG and LEM wrote the article. FP contributed the results and discussion of CT scans and histology. MJCS reviewed the article. All authors critically reviewed the manuscript and approved the final version.

Acknowledgements

Suleima Ferreira - for carrying out all the preparation of the slides for histological study.

Procell® - for the donation of β - TCP implants.

Marcelo José Prado from Lincevet® Soluções em Medicina Veterinária Ltda. - for the donation of titanium plates, screws, and orthopedic materials.

Leonardo Seade - for performing the anesthesia.

Rosane Melo - for performing the radiographs.

Prof. Dr. Luciana Corrêa from the Department of Pathology, Faculty of Dentistry, USP - for her expertise, help and guidance in carrying out the decalcification process.

REFERENCES

1. Yasemski MJ, Payne RG, Hayes WC, Langer RS, Mikos AG. The evolution of bone transplantation: molecular, cellular, and tissue strategies to engineer human bone. Biomaterials. 1996;17:175-185. https://doi.org/10.1016/0142-9612(96)85762-0

2. Vaccaro AR, Chiba K, Heller JG. Bone grafting alternatives in spinal surgery. Spine J. 2002;2:206-215. https://doi.org/10.1016/S1529-9430(02)00180-8

3. Krieger S. 2003. Biocerâmica. Geosciences Institute. São Paulo, University of São Paulo (USP).

4. Azevedo VVC, Chaves SA; Bezerra DC, Costa ACFM. Materiais cerâmicos utilizados para implantes. Revista Eletrônica de Materiais e Processos (REMAP). 2007;2(3):35-42. www.dema.ufcg.edu.br/revista

5. Stevens MM. Biomaterials for bone tissue engineering. Mater Today. 2008;11(5):18-25. https://doi.org/10.1016/S1369-7021(08)70086-5

6. Dasgupta S, Maji M, Nandi SK. Investigating the mechanical, physiochemical and osteogenic properties in gelatin-chitosan-bioactive nanoceramic composite scaffolds for bone tissue regeneration: In vitro and in vivo. Sci Eng C Mater Biol Appl. 2019;94:713-728. https://doi.org/10.1016/j.msec.2018.10.022

7. Crane GM, Ishaug SL, Mikos AG. Bone tissue engineering. Nature Med. 1995;1:1322-1324.

8. Gokturk E, Turgut A, Baygu C, Gunal I, Seber S, Gulbas Z. Oxygen-free radicals impair fracture healing in rats. Acta Orthop Scand. 1995;66(5):473-475. https://doi.org/10.3109/17453679508995590

9. Williams DF. The Williams dictionary of biomaterials. In: Williams DF., Liverpool Un. Press, Liverpool (UK); 1999.

10. Carlo C, Borges B, Pacheco A, Rezende DF, Maria C, Lopes C, César C, Pontes S, Cristine K, Duarte S. Avaliação do efeito osteoindutor da hidroxiapatita e do biovidro implantados em tecido subcutâneo de cão. Rev Ceres. 2007;54(316):492-500. https://locus.ufv.br//handle/123456789/20486

11. Allegrini Júnior S, Silva AC, Tsujita M, Salles MB, Gehrke SA, Braga FJC. Amorphous calcium phosphate (ACP) in tissue repair process. Microsc Res Tech. 2018;13:1-11. https://doi.org/10.1002/jemt.23013

12. Gutierres M, Lopes MA, Hussain NS, Cabral AT, Almeida L, Santos JD. Substitutos Ósseos. Conceitos Gerais e Estado Atual. Arq Med. 2006;19(4):153-162.

13. Proubasta J, Mur JG, Planell JA. 1997. Biocompatibilidad, materiales implantables, tipos de implante. In: Fundamentos de Biomecanica y Biomateriales. Ediciones Ergon, Madrid, 1980. p. 271-350.

14. Buser D, Hoffmann B, Bernard JP., Lussi A, Mettler D, Schenk RK. Evaluation of filling materials in membrane-protected bone defects. A comparative histomorphometric study in the mandible of miniature pigs. Clin Oral Impl Res. 1998;9(3):137-150. https://doi.org/10.1034/j.1600-0501.1998.090301.x

15. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: An update. Injury, Int. J. Care Injured. 2005;36:20-27. https://doi.org/10.1016/j.injury.2005.07.029

16. Beebe KS, Benevenia J, Tuy BE, de Paula CA, Harten RD, Enneking WF. Effects of a New Allograft Processing Procedure on Graft Healing in a Canine Model: A Preliminary Study. Clin Orthop Relat Res. 2009;467:273-280. https://doi.org/10.1007/s11999-008-0444-8

17. Davies JE. Histodynamics of endosseous wound healing. In: Daves, J. E. Bone engineering. 2 ed. Published by: em squared inc Toronto, Canada, 2000. p. 1-11.

18. Williams DF. On the nature of biomaterials. Biomaterials. 2009;30:5897-5909. https://doi.org/10.1016/j.biomaterials.2009.07.027

19. Alievi MM, Schossler JEW, Guimarães LD, de Oliveira ANC, Traeslel CK, Ferreira PA. Implante ósseo cortical alógeno conservado em mel na reconstrução de falha óssea diafisária em fêmur de cães: avaliação clínica e radiográfica. Ciênc Rural. 2007;37(2):450-457. https://doi.org/10.1590/S0103-84782007000200024

20. de Freitas SH, Dória RGS, Mendonça SF, Neto JE, de Camargo LM. Aspecto radiológico de heteroenxerto ósseo cortical fragmentado na reparação de falhas ósseas em coelhos. Rev Bras Ciênc Vet. 2008;15(3):107-110. http://dx.doi.org/10.4322/rbcv.2014.209

21. Tomford WW. Bone allografts: Past, Present and future. Cell Tissue Bank. 2000;1:105-109. https://doi.org/10.1023/A:1010158731885

22. Amendola GF, Raiser AG, Soares JMD, Beckmann DV. Aspectos biomecânicos compressivos de diáfises femorais caninas conservadas em glicerina a 98% ou em mel. Ciênc Rural. 2008;38(5):1341-1345. https://doi.org/10.1590/S0103-84782008000500022

23. Melo Filho EV, Della Lúcia RM, Salgado AEP, Miranda FB, Drago MA, Taffarel MO, Vilela LM, Mussi JMS, dos Santos WG, Zanini MS, Freitas PMC. Mecânica e microbiologia de placas produzidas a partir de osso cortical bovino, conservadas em diferentes meios. Ciênc Rural. 2011;41(4):660-666. https://doi.org/10.1590/S0103-84782011000400018

24. Zhang W, Yao D, Zhang Q, Zhou JG, Lelkes PI. Fabrication of interconnected microporous biomaterials with high hydroxyapatite nanoparticle loading. Biofabrication. 2010;2(3):1-10. https://doi.org/10.1088/1758-5082/2/3/035006

25. Khan SN, Tomin E, Lane JM. Clinical applications of bone graft substitutes. Orthop Clin North Am. 2000;31:389-98. https://doi.org/10.1016/S0030-5898(05)70158-9

26. Hogset O, Bredberg G. Plaster of Paris and hair cell morphology. A scanning electron microscopic study of an alternative implant materials for ear surgery. Acta Otolaryngol. 1988;106:331-8. https://doi.org/10.3109/00016488809122254

27. Peltier LF, Jones RH. Treatment of unicameral bone cysts by curetage and packing with plaster of Paris pellets. Clin Orthop. 2004;422:145-147. https://doi.org/10.1097/01.blo.0000131645.68869.6e

28. LeGeros RZ, LeGeros JP. Calcium Phosphate Bioceramics: Past, Present and future. Key Eng Mater. 2003;240(242):3-10. https://doi.org/10.4028/www.scientific.net/KEM.240-242.3

29. Lascart T, Favard L, Burdin P, Traore O. Utilisation du phosphate tricalcique dans les osteotomies tibiales de valgisation par addition interne. Ann Orth Ouest. 1998;30:137-41. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=1585867

30. Bauer TW, Muschler GF. Bone Graft Materials. Clin Orthop. 2000;371:10-27.

31. Ogose A, Hotta T, Hatano H, Kawashima H, Tokunaga K, Endo N, Umezu H. Histological examination of beta-tricalcium phosphate graft in human femur. J Biomed Mater Res (Appl Biomater). 2002;63:610-604. https://doi.org/10.1002/jbm.10380

32. Kamitakarahara M, Ohtsuki C, Miyazaki T. Review Paper: Behavior of Ceramic Biomaterials Derived from Tricalcium Phosphate in Physiological Condition. J Biomater Appl. 2008;23:197-212. https://doi.org/10.1177/0885328208096798

33. Langer R, Vacanti JP. Tissue engineering. Science. 1993;260(5110):920-926. http://doi.org/10.1126/science.8493529

34. Ozawa M, Tanaka K, Morikawa S, Chazono M, Fuji K. Clinical study of the pure β-tricalcium phosphate: reports of 167 cases. J East Jpn Orthop Traumatol. 2000;12:409-413.

35. Jensen SS, Broggini N, HjØrting-Hansen E, Schenk R, Buser D. Bone healing and graft resorption of autograft, anorganic bovine bone and b-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. Clin Oral Impl Res. 2006;17:237-243. https://doi.org/10.1111/j.1600-0501.2005.01257.x

36. Almeida RS, Prado da Silva MH, da Rocha DN, Ribeiro IIA, Júnior AAB, Miguel FB, Rosa FP. Regeneration of a critical bone defect after implantation of biphasic calcium phosphate - β-tricalcium phosphate/calcium pyrophosphate - and phosphate bioactive glass. Cerâmica. 2020;66:119-125. https://doi.org/10.1590/0366-69132020663782707

37. Regner L, Carlsson L, Karrholm J, Herbert P. Ceramic coating improves tibial component fixation in total knee arthroplasty. J Arthroplasty. 1998;13:882-889. https://doi.org/10.1016/S0883-5403(98)90194-2

38. Yamamoto T, Onga T, Marui T, Mizuno K. Use of Hydroxyapatite to fill cavities after excision of begin bone tumors: clinical results. J Bone Joint Surg. 2000;82B:1117-1120. https://doi.org/10.1302/0301-620X.82B8.0821117

39. Koshino T, Murase T, Saito T. Medial opening-wedge high tibial osteotomy with use of porous hydroxyapatite to treat medial compartment osteoarthritis of the knee. J Bone Joint Surg Am. 2003;85:78-85.

40. Ogose A, Hotta T, Kawashima H, Kondo N, Gu W, Kamura T, Endo N. Comparison of Hydroxyapatite and Beta Tricalcium Phosphate as Bone Substitutes After Excision of Bone

Tumors. J Biomed Mater Res Part B: Appl Biomater. 2005;72B:94-101. https://doi.org/10.1002/jbm.b.30136

41. Klein Y, Kunthawong N, Fleissig O, Casap N, Polak D, Chaushu S. The Impact of Alloplast and Allograft on Bone Homeostasis: Orthodontic Tooth Movement into Regenerated Bone. J Periodontol. 2020;91(8):1067-1075. http://dx.doi.org/10.1002/JPER.19-0145

42. Knabe C, Adel-Khattab D, Hübner W, Peters F, Knauf T, Peleska B, Barnewitz D, Genzel A, Kusserow R, Sterzik F, Stiller M, Müller-Mai C. Effect of silicon-doped calcium phosphate bone grafting materials on bone regeneration and osteogenic marker expression after implantation in the ovine scapula. J Biomed Mater Res Part B: Appl Biomater. 2019;107(3):594-614. epub 2018. http://dx.doi.org/10.1002/jbm.b.34153

43. Costa BD, Camargo NH, Oleskovicz N, Gava A, Dallabrida AL, Regalin D, Lima MPA, Moraes AN. Neoformação óssea e osteointegração de biomateriais micro e nanoestruturados em ovinos. Pesq Vet Bras. 2015;35(2):177-187. http://dx.doi.org/10.1590/S0100-736X2015000200015

44. Okada T, Kanai T, Tachikawa N, Munakata M, Kasugai S. Long-term radiographic assessment of maxillary sinus floor augmentation using beta-tricalcium phosphate: analysis by cone-beam computed tomography. Int J Impl Dent. 2016;2(8):1-9. http://dx.doi.org/10.1186/s40729-016-0042-6

45. Damlar I, Erdoğanb Ö, Tatlib U, Arpağc OF, Görmezd U, Üstüne Y. Comparison of osteoconductive properties of three different b-tricalcium phosphate graft materials: A pilot histomorphometric study in a pig model. J Craniomaxillofac Surg. 2015;43(1):175-180. http://dx.doi.org/ 10.1016/j.jcms.2014.11.006

46. Bhawal UK, Ryoichirob U, Noboruc K, Tetsuod A, Koichia H, Norihirobet N. Effect of the surface morphology of silk fibroin scaffolds for bone regeneration. Biomed Mater Eng. 2016;27:413-424. http://dx.doi.org/10.3233/BME-161595

47. Dallabrida AL, Camargo NHA, Moraes NA, Gisele GA, Dalmônico GML, Costa BD, Oleskovicz N. Caracterização de biocerâmica de fosfatos de cálcio microestruturada em

diferentes composições em ovinos. Pesq Vet Bras. 2018;38(7):1327-1336. http://dx.doi.org/10.1590/1678-5150-PVB-4930

48. Abdullah AAB, Edrees MF, Bakry AM. Clinical, Radiographic, and Histological Assessment of Socket Preservation Using Melatonin with Beta-Tri-Calcium Phosphate for Receiving Dental Implant. Biomed Sci. 2021;7(1):10-16. http://dx.doi.org/10.11648/j.bs.20210701.12

49. Kim J, Sohn D, Heo J, Moon J, Lee J, Park I. Benefit of the Replaceable Bony Window in Lateral Maxillary Sinus Augmentation: Clinical and Histologic Study. Implant Dent. 2014;23(3):277-282. http://dx.doi.org/10.1097/ID.0000000000000070

50. Joshi CP, Dani NH, Khedkar SU. Alveolar ridge preservation using autogenous tooth graft versus betha-tricalcium phosphate alloplast: A randomized, controlled, prospective, clinical, pilot study. J Indian Soc Periodontol. 2016;20(4):429-434. http://dx.doi.org/10.4103/0972-124X.188335

51. Daher S, Leary J, Ewers R, Coelho PG, Bonfante EA. Histological analysis of an implant retrieved from a β tricalcium phosphate graft after 4 years: a case study. J Long-Term Eff Med Implants. 2019;29(2):135-140. http://dx.doi.org/10.1615/JLongTermEffMedImplants.2019031828

52. Walsh WR, Vizesi F, Michael D, Auld J, Langdown A, Oliver R, Yua Y, Irieb H, Brucea W. Beta - TCP bone graft substitutes in a bilateral rabbit tibial defect model. Biomaterials. 2008;29(3):266-271. https://doi.org/10.1016/j.biomaterials.2007.09.035

53. Dorozhkin SV. Bioceramics of calcium orthophosphates. Biomaterials. 2009;31:1465-1485. https://doi.org/10.1016/j.biomaterials.2009.11.050

54. Padilha Filho JG, Carvalho Penha LH, de Souza SF. Uso do enxerto ósseo cortical bovino conservado em glicerina a 98% na osteotomia femoral em gatos. Ciênc Anim Bras. 2008a;9(4):1071-1078. https://www.revistas.ufg.br/vet/article/view/1173

55. Padilha Filho JG, Eimantas GC, de Souza SF. Osteossíntese femoral distal em cães e gatos jovens com fíbula de cão conservada em glicerina a 98%. Vet. Not. 2008b;4(1):49-55. http://www.seer.ufu.br/index.php/vetnot/article/view/18881

56. Freire TJF, Poggiani FM. Avaliação radiológica de enxertos ósseos conservados em nitrogênio líquido ou glicerina 98%. Revista Científica do Curso de Medicina Veterinária - FACIPLAC. 2017;4(1):1-12.

57. Ziliotto L, Fantinatti AP, Daleck CR, Padilha Filho JG, de Souza AP, Diniz PPVP. Utilização de implante ósseo cortical alógeno conservado em glicerina para preservação de membro torácico. Estudo experimental em cães. Acta Cir Bras. 2003;18(2):107-115. https://doi.org/10.1590/S0102-86502003000200007

58. Giovani AMM, Croci AT, Oliveira CRGCM, Filippi RZ, Santos LAU, Maragni GG, Albhy TM. Comparative study of cryopreserved bone tissue and tissue preserved in a 98% glycerol solution. Clinics. 2006;61(6):565-570. https://doi.org/10.1590/S1807-59322006000600013

59. Garcia1 DC, Mingrone LE, de Sá MJC. A new critical segmentar radial bone defect model in rabbits. Int J Dev Res. 2021;11(08):49279-49283. https://doi.org/10.37118/ijdr.22549.08.2021

60. Irie MS, Rabelo GD, Spin-Neto R, Dechichi P, Borges JS, Soares PBF. Use of Micro-Computed Tomography for Bone Evaluation in Dentistry. Braz Dent J. 2018;29(3):227-238.

61. Comissão de ética no Uso de Animais - CEUA. Guia de anestesia e analgesia em animais de laboratório. Universidade Federal de São Paulo (UNIFESP). Vigência 2020-2021. 48 pg.

62. Bedin RAC, Schultz M. Anestesia para coelhos submetidos a cirurgias de experimentação: Relato de série de oito anestesias. Revista Científica Multidisciplinar Núcleo do Conhecimento. 2020; 3(6):151-158. https://doi.org/ 10.32749/nucleodoconhecimento.com.br/saude/anestesia-para-coelhos

63. Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, et al. Recommendations for euthanasia of experimental animals: Part 2. Laboratory Animals. 1997; 31(1):1-32. doi:10.1258/002367797780600297

7. CONCLUSÕES FINAIS

Podemos concluir com este estudo que o implante cerâmico cilíndrico de β - TCP em sua fase pura não foi capaz de produzir osseointegração e produção de osso novo que estimulasse a cicatrização óssea. Dessa maneira, não observamos a ocorrência do processo de osseocondução e reabsorção da cerâmica, características inerentes a este tipo de implante. Com isso, não podemos recomendar este biomaterial, com as características utilizadas nesta pesquisa, para uso rotineiro como substituto ósseo nos casos de defeitos ósseos críticos segmentares. Tampouco podemos extrapolar seu uso para cães e gatos no momento, com os resultados obtidos. Como apresenta uma característica relevante de biocompatibilidade e vários estudos atestando sua capacidade osseointegrativa, há perspectiva real de seu uso num futuro, desde que se realizem outros estudos relacionados à sua microporosidade e formato que possam atestar posteriormente sua eficácia como material osseocondutor, osseointegrativo e com características absortivas.

8. CONSIDERAÇÕES FINAIS

Aspectos relevantes

Este projeto foi realizado no período de agosto de 2018 a maio 2021. Todos os animais foram adquiridos de um criador de coelhos na cidade de Araçariguama, São Paulo. Os coelhos eram trazidos do criatório para a cidade de Barueri, onde ficavam por período de quarentena de 20 dias na Clínica Veterinária Animal Care Barueri. Lá eles recebiam tratamento antiparasitário, água e comida ao longo do período de espera para as cirurgias experimentais. Ficavam em sala com temperatura controlada e em gaiolas de 1 metro largura por 1 metro de comprimento.

Todos os coelhos já eram adultos, e isso era importante pois era necessário que as placas de crescimento do rádio e da ulna já estivessem fechadas, para que não atrapalhassem ao longo

do estudo com algum problema relacionado ao fechamento precoce das mesmas e possível ocorrência de defeito anatômico do eixo ósseo, ou mesmo fratura fisária nos animais que não iriam receber a placa e parafusos para osteossíntese como os outros animais dos Grupos do enxerto e Grupo do implante.

Foram realizados procedimentos pilotos para que se pudesse estabelecer uma metodologia em vários aspectos do projeto, e durante esta fase inicial, encontramos diversos problemas e dificuldades que fomos solucionando ao longo do período de estudo. Os mesmos estão descritos no item a seguir "Dificuldades encontradas no desenvolvimento do projeto", e são citados justamente para que outros pesquisadores interessados em dar continuidade a esta linha de pesquisa, possam através da leitura deste projeto, iniciarem seus estudos já evitando alguns erros que cometemos no início, e que poderiam contribuir de forma positiva para o andamento da pesquisa, principalmente no que se refere à celeridade dos processos e diminuição dos custos com aquisição de coelhos, anestésicos, implantes, e metodologia de avaliação com imagens e histopatológico.

Todos os procedimentos cirúrgicos e pós-operatórios ao longo dos 120 dias após a cirurgia foram realizados na Clínica Veterinária Animal Care Barueri, meu local de trabalho diário, onde estou presente de segunda à sábado das 10 hs às 19 hs. Durante este período estava sempre avaliando os coelhos em horários vagos e que me permitiam o contato diário com os animais do experimento. O único dia em que outra pessoa estava cuidando dos animais era aos domingos.

As radiografias e as anestesias foram realizadas por grandes colegas de trabalho e amigos de longa data, Rosane Melo e Leonardo Seade, aos quais devo imensa gratidão pela contribuição neste projeto, pois estavam sempre presentes e prontos para ajudar nas diversas fases do estudo, e colaborando para que obtivéssemos as melhores imagens e anestesias possíveis para nossos animais do experimento.

As tomografias e os exames histológicos das peças calcificadas e descalcificadas foram realizadas no Laboratório de Odontologia da UNESP de Araraquara, São Paulo, e nesta fase obtive grande ajuda de algumas pessoas que foram essenciais para o projeto, como a técnica Suleima Ferreira, que preparou todas as lâminas e do doutorando em Odontologia do Laboratório, Felipe Pinotti, que fazia as imagens das peças calcificadas e me enviava por e-mail, para que pudéssemos debatê-las e interpretá-las, e ao final incluí-las no projeto e nos artigos a serem enviados às revistas científicas.

Já a parte da leitura das lâminas com material descalcificado e avaliação celular, as mesmas foram realizadas no Laboratório HISTOPET, em São Paulo, com a ajuda do Veterinário Emerson, o qual laudou e fez todas as imagens desta parte do projeto.

Dificuldades encontradas no desenvolvimento do projeto

Inicialmente, nosso primeiro problema encontrado foi o osso que faríamos a ostectomia para uso do implante e enxerto. Quando tivemos a ideia do projeto, o primeiro local que pensamos em fazer a ostectomia foi a tíbia, pois gostaríamos de utilizar um osso mais plano e que tivesse um outro osso adjacente como suporte. Porém realizando as cirurgias-piloto, vimos que a fíbula tinha um contato muito íntimo com a tíbia, e isso dificultava a osteotomia, pois sempre fraturávamos os dois ossos durante o processo de corte.

E quando entramos em contato com o fabricante do implante cerâmico, à princípio ele nos enviou implantes convencionais que tinham a mesma largura do fêmur (2 cm de comprimento x 1 cm de diâmetro). E por este motivo, resolvemos então iniciar as ostectomias deste osso, pensando que osso e implantes mais largos facilitariam nosso experimento. Porém, após 3 casos operados, vimos que devido à biomecânica dos membros pélvicos desta espécie, a qual é muito diferente daquela dos cães e gatos, ocorreram, soltura de parafusos, fratura de placa e mesmo fratura e falha dos implantes cerâmicos. Os implantes utilizados no fêmur eram muito mais porosos e possuíam orifícios em seu eixo longitudinal e transversal, onde inclusive colocávamos parafusos prendendo o implante na placa, mas isso gerava estresse no mesmo e culminava com sua fratura.

Por isso, neste momento, tivemos que mudar tanto o local de ostectomia, quanto o formato e porosidade do implante. A partir dessas dificuldades que encontramos com o osso de escolha, passamos a fazer a ostectomia no rádio dos coelhos. E ao invés de utilizar a serra oscilatória, a qual destruía a cortical fina do osso do rádio destes animais, passamos a utilizar o dremel com broca dentária esférica pequena, pois dessa maneira controlávamos mais a linha de corte sem danificar as corticais ósseas. Inclusive a ulna é mais separada do rádio, ao contrário da fíbula e tíbia, o que permitiu uma ostectomia de um osso mantendo um suporte adicional do osso adjacente. Com isso conseguíamos manter os implantes e enxertos mais estáveis em seu local, diminuindo o estresse sobre eles. E assim, pudemos isolar este fator mecânico e avaliar somente o processo de condução, integração e cicatrização óssea.

Por sua vez, o implante teve que ser readaptado à largura e diâmetro do rádio, e em comprimento menor, sendo produzido de forma personalizada para este experimento. Ao invés

de 2 cm de comprimento por 1 cm de largura / diâmetro (implante inicial para o fêmur), passou a ser sinterizado em 7 mm de comprimento por 3 mm de largura / diâmetro para adequação no osso do rádio. Isso fez com que a porosidade diminuísse e mecanicamente ficasse mais firme. Porém este processo fez com que se perdesse os orifícios originais do implante, e isto impedia maior permeabilidade de coágulos e células penetrarem no interior do implante, pois o bloco ficou mais compacto.

Um outro fator que nos gerou vários problemas ao longo do projeto, foi estabelecer uma anestesia adequada. Inicialmente tentamos entubar os coelhos para manter uma anestesia inalatória. Quisemos fazer uma anestesia melhor do que encontrado em alguns artigos, que utilizavam anestesia dissociativa somente e muitas vezes intramuscular. Porém é extremamente difícil de entubar coelhos, o que atrasava muito o início das cirurgias. Muitos coelhos inclusive tinham sangramento da região da laringe, pois durante o processo de intubação, se forçava muito a sonda para tentar encontrar a traqueia, mas sem sucesso. Daí optou-se em não entubar, mas em dois casos, os coelhos tiveram apneia, e infelizmente vieram a óbito, pois não tínhamos controle sobre a ventilação.

Desta maneira, chegamos a um consenso final, onde iríamos induzir estes animais com a medicação intravenosa, porém manteríamos a máscara com o anestésico inalatório. Isso permitiu que não houvesse outros episódios de apneia, e a partir deste momento, não tivemos mais óbitos durante o experimento.

Uma das maiores dificuldades encontradas, foi no momento que tínhamos que iniciar a fase da coleta dos ossos para realização das micro-tomografias e lâminas para os estudos histológicos. Esta fase se iniciou em janeiro de 2020, quando eu estava em contato com o pessoal da odontologia da USP, já fazendo os testes iniciais de descalcificação. Devido à pandemia, todos os laboratórios fecharam, e não pude dar continuidade com esta fase neste período. Além disso, víamos que a cerâmica que foi produzida de forma customizada, não descalcificava como esperávamos. E na USP não havia um micrótomo para realizar cortes de peças calcificadas. A partir desse momento, houve um grande lapso de tempo até que eu pudesse encontrar um local que realizasse as lâminas com as peças calcificadas e também realizasse o processo de descalcificação para o estudo celular. Entrei em contato com vários laboratórios particulares (humanos e veterinários), assim como vários laboratórios de universidades. Ou não estavam funcionando devido à pandemia, ou não tinham o micrótomo adequado, ou o valor para se produzir as peças era astronômico.

E foi somente em novembro de 2020 (10 meses depois) que após entrar em contato com alguns professores de odontologia, acabei encontrando o Laboratório de Odontologia da

UNESP de Araraquara. Lá, com a ajuda da técnica de laboratório, Suleima Ferreira, conseguimos realizar as lâminas das peças calcificadas através do protocolo Exakt, além das lâminas com as peças descalcificadas. Para a confecção das lâminas com o protocolo Exakt, as mesmas levaram em torno de 4 meses para ficarem prontas. Já as lâminas de HE levaram 10 meses para sua conclusão, devido ao fato do processo de descalcificação em EDTA ser muito lento.

E foi neste momento que encontrei o Laboratório de odontologia da UNESP, que também encontrei o micro-tomógrafo na própria UNESP Araraquara para fazer as imagens de µCT antes dos materiais irem para a histologia. Inicialmente Essas imagens de tomografia seriam realizadas na Escola Politécnica da USP, mas devido à pandemia estava fechada e o valor a ser pago seria algo em torno de R$ 25.000,00. Já no Laboratório da odontologia da UNESP, o valor das micro-tomografias e histologia saíram por R$ 1.500,00. Como não possuía bolsa de estudos nem financiamento para o projeto, a redução deste custo possibilitou que concluíssemos todas as etapas do projeto a um custo muito mais baixo.

Limitações do estudo

Acredito que a maior limitação do estudo foi não haver o processo de osseointegração do implante com o osso hospedeiro, pois dessa maneira não conseguimos realizar a histomorfometria, que era também um dos objetivos iniciais do projeto, mas que teve que ser excluída do estudo devido à não formação de osso novo e à não reabsorção do implante. Dessa maneira, além de não haver algo qualitativo para análise, também não conseguimos estudos quantitativos para comparação, o que prejudicou nossa análise, inclusive para a realização de uma comparação estatística.

Um outro fator limitante foi não conseguir realizar a descalcificação das peças com os implantes sem ter que removê-los para fazer as lâminas. Seria o ideal, pois conseguiríamos ter mais lâminas para microscopia óptica com aumentos superiores ao que foi avaliado pelo protocolo Exakt. Dessa maneira teríamos um "n" maior mostrando os tipos celulares envolvidos no processo de integração. E não precisaríamos lidar somente com a impressão negativa do implante, já que teve que ser removido para realizar o corte com o micrótomo convencional e confecção do bloco de parafina e lâmina com método HE. Talvez isso nos mostrasse detalhes mais ricos em termos celulares. E a remoção do implante pode ter gerado alguma perda celular ou processo interessante de se avaliar por conta disso.

Perspectivas

Apesar de ser um biomaterial bastante estudado, quando avaliamos seu uso na medicina humana, o mesmo é associado a algum material osteogênico para preenchimento ósseo.

Raramente ou nunca é utilizado sozinho em sua fase pura. Diferentemente na odontologia, existem várias marcas que produzem o β - TCP em fase pura, mas todos são em formato de grânulos ou pó, e isso é compreensível, já que o tamanho do local que será implantado é pequeno.

Porém, na medicina veterinária, eu vejo seu uso como algo promissor. Obviamente que há necessidade de se melhorar a estrutura do implante para que ele possa conduzir mais células ósseas e integrar de forma melhor aos ossos longos dos animais, principalmente os de pequeno porte. Mas isso e factível. Todos os estudos encontrados nas revisões para este projeto indicam produção de osso novo e reabsorção do implante. Então é possível que se consiga, através da tecnologia que se tem hoje, de se produzir o mesmo implante utilizado neste projeto com características um pouco diferenciadas a ponto de aumentar a sua porosidade, sem perder as características biomecânicas. Há como intervir e controlar seu processo de produção, e por isso pode-se chegar a um implante mais adequado. Desta maneira, poderíamos realmente solucionar alguns problemas encontrados durante as cirurgias ortopédicas nos pequenos animais, principalmente no que se refere ao tempo cirúrgico e coleta de enxerto para preenchimento de grandes defeitos ósseos, como vemos normalmente em nossa rotina em animais com fraturas com severa perda óssea. E combinar isso com procedimentos cirúrgicos de um único estágio, diminuindo muito a morbidade dos pacientes e custos associados a vários procedimentos.

Do ponto de vista acadêmico, este projeto abre as portas para outros alunos de mestrado e doutorado que porventura venham a continuar com esta linha de pesquisa do laboratório de ortopedia, e que venham a estudar e trabalhar com a cerâmica de beta - tricálcio fosfato em seus experimentos.