UNESP - Universidade Estadual Paulista
"Júlio de Mesquita Filho"
Faculdade de Odontologia de Araraquara

Pedro Henrique de Azambuja Carvalho

Avaliação clínica, tomográfica, microtomográfica e histológica da reconstrução alveolar horizontal com uso de enxerto heterógeno em bloco.

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Orientador: Valfrido Antonio Pereira Filho

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DADOS CURRICULARES

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Dedico este trabalho aos meus antepassados, cirurgiões-dentistas há mais de quatro gerações familiares, exemplos de ética e dedicação à profissão.

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À minha mãe **Flávia Braga de Azambuja.**

Aos meus pais **Delmar Broglio Carvalho** e **Gustavo Mata Zechlinski**

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"[...] nunca aceitar como verdadeira qualquer coisa sem a conhecer evidentemente como tal; isto é, evitar cuidadosamente a precipitação e a prevenção; não incluir nos meus juízos nada que se não apresentasse tão clara e tão distintamente ao meu espírito, que não tivesse nenhuma ocasião para o pôr em dúvida."

René Descartes*

*DESCARTES, René. Discurso do método; Meditações; Objeções e respostas; As paixões da alma; Cartas. São Paulo: Abril, 1979

Carvalho PHA. Avaliação clínica, tomográfica, microtomográfica e histológica da reconstrução alveolar horizontal com uso de enxerto heterógeno em bloco. [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2020.

RESUMO

Este trabalho teve por objetivo avaliar o comportamento do osso bovino desproteinizado em bloco (DBBM), na reconstrução horizontal de maxila atrófica, em comparação com bloco de osso autógeno do ramo mandibular (AB). As etapas e resultados estão aqui apresentadas na forma de quatro artigos científicos, o primeiro uma revisão sistemática acerca do uso de enxertos ósseos heterógenos para aumento horizontal de rebordo, o segundo e o terceiro com resultados do ensaio clínico randomizado em humanos, e o quarto a avaliação morfológica e laboratorial do material testado. Foram selecionados 12 pacientes com edentulismo total da maxila, sem comprometimento sistêmico, maiores de 18 anos e rebordo remanescente com espessura mínima de 2mm e altura mínima de 10 mm, excluídos os fumantes, irradiados ou em tratamento com medicações que alteram o metabolismo ósseo. As reconstruções ósseas horizontais foram realizadas com DBBM ou AB, aleatoriamente distribuídos em modelo de boca dividida. Tomografias de feixe cônico e medidas transoperatórias da espessura do rebordo foram realizadas em três momentos: inicial (T0), imediatamente após a enxertia óssea (T1) e prévio a instalação de implantes (T2). Após nove meses, os pacientes foram submetidos a reabertura dos sítios enxertados para a instalação de implantes, nos quais foram aferidos os valores do torque de inserção e coeficiente de estabilidade inicial (ISQ), biópsias foram obtidas das áreas enxertadas para avaliação histológica e microtomográfica. Para análise laboratorial as amostras foram submetidas a avaliação *ex-vivo* e de potencial pro-inflamatório em cultura celular de osteoblastos humanos. A instalação de implantes foi possível em todos os sítios enxertados, 5 pacientes apresentaram uma ou mais complicações no leito receptor (AB:3; DBBM:2), sendo as principais: deiscência da ferida e exposição de membrana e/ou enxerto. O ganho de volume não foi diferente entre os grupos, mas a reabsorção média em porcentagem foi menor no grupo AB: (10.83% ± 8.23 vs. 16.73% ± 8,01). O torque de instalação e ISQ não apresentaram diferença estatística entre os grupos. Nos parâmetros microtomográficos, a superfície óssea foi menor no grupo AB: (12,01 ± 2,16 vs. 14.69 ± 2,66) enquanto a espessura de trabécula foi maior (0.5 ± 0.33mm vs. 0.28 ± 0.04mm), A quantidade de tecido mineralizado foi maior no grupo AB, mas não houve diferença para a área de tecido mole e de osso vital nos cortes histológicos. Na análise *ex-vivo* foram identificados remanescentes orgânicos e celulares, no entanto o DBBM testado não alterou a expressão de citocinas pró infamatórias dos osteoblastos *in-vitro*. Como conclusão, no presente estudo os enxertos de osso bovino em bloco apresentaram comportamento clínico, tomográfico e histológico semelhante ao osso autógeno para os parâmetros avaliados, além de não estimular a expressão de citocinas pró-inflamatórias em osteoblastos.

Palavras chave: Aumento do rebordo alveolar. Xenoenxertos. Substitutos ósseos. Ensaio clínico controlado aleatório.

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ABSTRACT

The aim of this work was to evaluate the features involving the use of deproteinized bovine bone matrix DBBM in maxillary horizontal ridge augmentation. The phases and results of this work are presented in four full papers. The first one is a systematic review about the use of xenogenous grafts in horizontal ridge augmentation. The second and third presented the results of a randomized clinical trial comparing DBBM block with block of autogenous bone from mandibular ramus (AB). And the fourth evaluates the morphologic and *in vitro* behavior of the tested material Twelve adult patients with edentulous atrophic maxillary ridges and without systemic health diseases were random selected in a list of patients for oral rehabilitation with implants. Irradiated patients, patients with systemically diseases and post menopause women were excluded. The patients were submitted to reconstructive surgery under general anesthesia. Each side of anterior maxilla received one type of graft, according to randomization, DBBM or AB. Cone bean Computerized Tomography (CBCT) scans and trans-operatory thickness assessment were performed at three times: initial (T0), immediate post-operative (T1) and nine months after surgery (T2). Nine months later an all-on-four protocol was installed, and it was measured implant torque and implant stability quotient (ISQ). Also, biopsies were obtained from grafted areas for microtomographic and histological evaluation. DBBM block and granules were submitted to *ex-vivo* morphologic analysis and *in-vitro* inflammatory induction in primary human osteoblasts(pOB). All the 24 grafted areas were able to implant placement, 5 patients presented one or more complications, (AB: 3, DBBM: 2). The main complications were wound dehiscence and graft exposure. The volumetric changes were not statically different between groups, but the mean resorption was lower in AB group (10.83% ± 8.23 vs 16.73% ± 8,01). Installation torque and ISQ presented no statistical difference. In the microtomographic parameters the specific bone surface was lower in the AB: (12,01 ± 2,16 vs. 14.69 ± 2,66), while the trabecula was thicker in AB (0.5 ± 0.33mm vs. 0.28 ± 0.04mm). The mineralized tissue area was grater in AB, but no differences were observed between soft tissue and vital bone at the evaluated histologic slides. In the *ex-vivo* analysis cellular and organic remnants were found in DBBM blocks, but the tested material did not upregulate the pro-inflammatory cytokines in pOB. In the current study the low temperature sintered OBDB presented as an alternative for maxillary horizontal augmentation, with clinical, tomographic and histological behavior similar to AB, and have not induced pro-inflammatory response in vitro.

Keywords: Alveolar ridge augmentation. Xenografts. Bone substitute materials.

Randomized controlled trial.

LISTA DE ABREVIATURAS E SIGLAS

AB	Osso autógeno em bloco do ramo mandibular
BS	Superfície óssea (microtomografia)
BV	Volume ósseo (microtomografia)
BS/BV	Densidade da superfície óssea específica (microtomografia)
BS/TV	Densidade da superfície óssea (microtomografia)
BV/TV	Percentual de volume ósseo (microtomografia)
BT/ST	Razão entre tecido mineralizado e tecido mole (histometria)
CBCT	Tomografia Computadorizada de Feixe Cônico
Conn.	Conectividade (microtomografia)
DBBM	Matriz de osso bovino desproteinizado
DICOM	Comunicação de imagens digitais em medicina
ELISA	Ensaio de imunoabsorção enzimática
IL-1β	Interleucina 1 beta
IL-6	Interleucina 6
IL-8	Interleucina 8
IL-17	Interleucina 17
ISQ	Quociente de estabilidade do implante
kg	Quilograma
mg	miligrama
Micro-CT	Microtomografia computadorizada
ml	mililitro
mm	milímetros
M	Molar
N	Newton
pOB	Cultura primária de osteoblastos humanos
Por.	Porosidade (microtomografia)
PRISMA	Itens recomendados para reportar revisões sistemáticas e meta-análises

PTFe	Politetrafluoretileno
OPN	Osteopontina
OPG	Oteoprotegerina
RPM	Rotações por minuto
Tb.Pf	Padrão trabecular (microtomografia)
Tb.Sp	Separação das trabéculas (microtomografia)
Tb.Th	Espessura de trabéculas (microtomografia)
TGF-β	Fator de crescimento tecidual beta
TNF-α	Fator de necrose tecidual alfa
TV	Volume de tecido (microtomografia)
TS	Superfície de tecido (microtomografia)
º	Graus
µ	micro
ANOVA	Análise de variância

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1 INTRODUÇÃO

Os procedimentos de aumento do rebordo alveolar com enxertia óssea são um recurso importante para o tratamento reabilitador com implantes dentários, principalmente quando o remanescente alveolar após a extração foi severamente reabsorvido vertical ou horizontalmente[1–3]. A perda dentária precoce, os traumas severos, e/ou doenças periodontais avançadas estão entre as principais etiologias da reabsorção do rebordo alveolar[2,4–6].

Os implantes dentários possuem um importante papel na reabilitação de pacientes com perda parcial ou total de elementos dentários[2,7,8]. Entretanto, o sucesso desses está diretamente relacionado à qualidade do tecido ósseo, sendo assim, a compreensão da biologia óssea e dos problemas resultantes de sua atrofia são essenciais para a o planejamento do tratamento[2,9–11]. A adequada morfologia do leito receptor, a qualidade e a quantidade óssea são primordiais para o sucesso dos implantes dentários[12,13].

As reabsorções do rebordo alveolar, incluindo análise dos tecidos duros e moles, foram inicialmente classificadas por Seibert[14], em horizontais, verticais ou combinadas, entretanto este classificação não considerava a magnitude das deficiências e foi posteriormente modificada por Allen[15] que nomeou as deficiências horizontal, vertical e combinada de tipo A, B e C respectivamente e incluiu as subclassificações de média (< 3mm), moderada (3-6mm) e severa 9 (>6mm). Em atualização constante, os tipos A, B e C foram renomeados por Wang & Al-Shamari (2002)[16], para H (horizontal), V (vertical) e C (combinada), e as classificações quantitativas para s (*Small,* <3mm), m (*medium*, 3-7mm) e l(*large*, >7mm). Estas classificações ajudam na tomada de decisão clínica e indicação correta de procedimentos de aumento do rebordo, por exemplo casos com classificação C-l devem, preferencialmente, serem resolvidos com enxertos ósseos em blocos, autógenos ou de biomaterial[17].

A partir da ampliação da implantodontia como forma de tratamento reabilitador e das dificuldades para a reabilitação destes rebordos atróficos, foram desenvolvidas diversas técnicas de aumento do rebordo alveolar, principalmente com uso de enxertia óssea. Dentre as técnicas mais utilizadas podemos citar a regeneração óssea guiada (ROG), os enxertos interposicionais (*In-lay)* e aposicionais (*On-lay)* e as osteotomias para aumento do rebordo alveolar com ou sem distração óssea[2,3,11].

Dentre as alternativas de reconstrução alveolar, os procedimentos de enxertia apresentam alta taxa de sucesso e baixo índice de complicações, a ROG com uso de osso autógeno, osso liofilizado de origem animal ou partículas de hidroxiapatita, recobertos por tela de titânio ou por membranas biocompatíveis, é uma alternativa eficaz para casos de preenchimento alveolar[18–20], assim como as osteotomias com expansão de corticais. No entanto, em revisão sistemática de Milinkovic e Cordaro[11], foi observado que os enxertos em bloco apresentam maior taxa de sucesso e menores índices de complicações em relação ao uso de enxerto particulado isolado para aumento horizontal do rebordo alveolar. Ainda, as osteotomias com expansão de corticais apresentam o risco de fratura do rebordo, podendo levar a impossibilidade de instalação imediata do implante ou à necessidade de enxertos ósseos[2,4,11].

Entre as alternativas para reconstrução alveolar, os enxertos por meio de osso autógeno são considerados o “padrão ouro”, devido as suas propriedades osteogênica, osteocondutora e osteoindutiva[9,21–23], entretanto, estes enxertos apresentam dificuldades de técnica e complicações associadas a cirurgia do leito doador[22,24]. Para a escolha da área doadora de enxerto autógeno, a morbidade associada, quantidade e qualidade de osso disponível e a reabsorção esperada devem ser avaliados[11,24,25].

Os enxertos autógenos em bloco ou particulados podem ser obtidos de diversas áreas doadoras, como a crista ilíaca, calvária, costelas e áreas intrabucais, como ramo mandibular, mento e tuberosidade maxilar. Enxertos de crista ilíaca são os enxertos de área extraoral mais frequentes, devido a quantidade disponível e características morfológicas. Entretanto, apresentam elevada morbidade (até 49%) e complicações associadas, a saber: dor pós-operatória persistente, alteração de função, hematomas, parestesia e fratura de crista ilíaca[26,27]. Os enxertos removidos de áreas intrabucais apresentam menores índices de complicações, e complicações de menor grau. Estas complicações incluem parestesia, defeitos estéticos na região de mento e dor-pós-operatória persistente[24,25,28].

Além destas desvantagens, o enxerto autógeno também apresenta a limitação da quantidade possível de ser obtida, e estas questões tem estimulado a pesquisa por alternativas que possam substituir o enxerto de osso autógeno quando este está contraindicado[18,26,28,29].

Esposito et al. (2009)[7], avaliaram, em uma revisão sistemática da literatura, a eficiência de procedimentos de aumento do rebordo alveolar, horizontal e vertical,

entretanto eles não encontraram evidência suficiente acerca do aumento horizontal do rebordo com apenas um ensaio clínico randomizado incluído na revisão.

Os enxertos de origem animal podem ser uma alternativa ao tratamento com enxertos autógenos[10,12,30], pois apresentam boa biocompatibilidade e propriedades osteocondutivas[31–33]. Ainda, estes enxertos mostram resultados equivalentes aos autógenos, com taxa de reabsorção e de sucesso semelhantes após a instalação de implantes dentários[12]. Os substitutos ósseos de origem animal já estão bem estabelecidos como alternativa para procedimentos de levantamento de seio maxilar, enxerto após extração dentária e para ROG em pequenos defeitos[2,11,34]. Esta modalidade de enxerto vem sendo amplamente estudada[35,36], e apresenta boa capacidade osteocondutiva in vitro e em modelos animais[36,37]. Os bons resultados ocorrem principalmente devido ao arranjo da micro e nano estrutura porosa, na qual são arranjadas partículas minerais que permitem a embebição do material com fatores de crescimento ósseo[31,36,38,39].

Schmitt et al.[36], em estudo realizado em calvária suína, demostraram o potencial de incorporação e neoformação óssea de um bloco de osso bovino comercialmente disponível. O osso bovino em bloco apresenta microestrutura que favorece sua incorporação ao leito receptor e a neoformação óssea na interface osso enxerto. Ainda Schmitt et al.[36], demostraram que a adição de fatores de crescimento como BMP (proteína óssea morfogênica) e VEFG (fator de crescimento endotelial) não contribuíram para maior neoformação óssea no enxerto de osso bovino em bloco. Outros estudos prévios em animais demostraram neoformação óssea e a propriedade osteocondutiva do OBDB em grânulos[31,35] ou em bloco[37].

Pistilli et al.[10], em estudo clínico randomizado, compararam enxertos autógenos de ramo mandibular ou de crista ilíaca com enxertos de origem equina, para aumento vertical e/ou horizontal de maxila e mandíbula. A partir dos resultados, verificaram que o enxerto ósseo autógeno se mostrou superior, e que os enxertos heterógenos em bloco de origem equina apresentam 50% de falhas[10,30].

Estudos analisando osso de origem bovina evidenciam bons resultados em aumentos horizontais. Block et al.[32], mostraram em seu estudo a estabilidade de enxertos de origem bovina particulados para aumento horizontal, apresentando taxa de reabsorção inferior a 25% para aumentos de até 4 mm em um período de 500 dias. Felice et al.[30], em estudo clínico randomizado de boca dividida, encontraram resultados semelhantes nas taxas de sucesso de enxertos de crista ilíaca e de origem

bovina em enxertos "*In-lay*" para aumento vertical mandibular. Neste mesmo estudo, a análise histomorfométrica demonstrou que os enxertos apresentaram neoformação óssea semelhante, contudo, a reabsorção no grupo osso bovino foi significativamente menor em um período de quatro meses.

Mordenfeld et al.[39], em estudo de boca dividida, realizaram análise radiográfica e histomorfométrica da combinação do enxerto bovino desproteinizado com enxerto autógeno, misturados em diferentes proporções no sítio cirúrgico, e evidenciaram aumento médio de 82% do volume ósseo e taxa de reabsorção entre 27% e 49%.

Enquanto os enxertos particulados podem promover um aumento médio de espessura de até 3,7 mm, os enxertos em bloco podem alcançar em média 4,5 mm[40–43]. Ainda, os enxertos particulados apresentam baixa taxa de neoformação óssea, mesmo em estudos de longo prazo[40,41,44,45], e estão mais frequentemente associados a deiscência de sutura e exposição do material[42].

Portanto, considerando a morbidade relacionada aos enxertos autógenos, a possibilidade de reconstrução óssea por meio de enxertos heterógenos e os melhores resultados associados aos enxertos em bloco de origem bovina em relação à outras espécies, estudos clínicos se fazem necessários para comparar estes enxertos, verificando taxas de reabsorção, viabilidade celular e previsibilidade para instalação de implantes dentários.

Os procedimentos para aumentos ósseos do rebordo alveolar são uma alternativa segura e eficaz para permitir a reabilitação com implantes dentários em pacientes com atrofia alveolar. Embora o osso autógeno seja considerado o padrão ouro para aumentos alveolares, existem complicações e limitações inerentes à técnica e morbidade pós-operatória. Assim, os enxertos de origem animal têm se mostrando uma alternativa ao osso autógeno. Entretanto, seu uso na forma particulada tem resultados menos previsíveis e, apesar de suas baixas taxas de reabsorção, apresentam pouca quantidade de novo osso formado em longo prazo. Ainda, não existem estudos clínicos controlados, com adequado controle de variáveis, avaliando os enxertos de origem bovina em bloco para aumento ósseo horizontal. Desta forma, foi identificada a necessidade da comparação de enxertos ósseos heterógeno e autógeno, em bloco, para a reconstrução óssea horizontal e instalação de implantes dentários.

2 PROPOSIÇÃO

O presente estudo propôs-se a realizar uma avaliação do uso de enxertos ósseos heterógenos em bloco, em procedimentos de aumento horizontal do rebordo alveolar. O projeto original (Apêndice A) foi submetido e aprovado pelo Comitê de ética em Pesquisa em seres Humanos (CEP) da Faculdade de Odontologia de Araraquara sob o nº 2.07.842 (Anexo A) Quatro publicações distintas foram obtidas a partir da proposição original, a saber:

- Publicação 1: Realizar uma revisão crítica da literatura a respeito do uso de enxertos ósseos heterógenos para o aumento horizontal do rebordo alveolar, avaliando o ganho ósseo, a reabsorção do enxerto, as taxas de complicações e a taxa global de sucesso.
- Publicação 2: Avaliar o comportamento clínico e tomográfico, por meio de um estudo prospectivo, clínico, randomizado, de um enxerto ósseo heterógeno de origem bovina, em bloco, produzido com tecnologia nacional, e submetido a um processo de purificação exclusivamente químico.
- Publicação 3: Avaliar a incorporação, a microarquitetura e as características histológicas, por meio de estudo prospectivo, clínico, randomizado, de áreas enxertadas com osso heterógeno de origem bovina em bloco.
- Publicação 4: Analisar as características morfológicas *ex-vivo*, o grau de pureza e a resposta inflamatória *in-vitro*, de enxertos heterógenos de origem bovina em diferentes apresentações, particulado ou em bloco,

3 PUBLICAÇÕES

O desenvolvimento desse projeto resultou na produção de quatro artigos científicos originais.

1) “Horizontal ridge augmentation using xenogenous bone graft – Systematic review”, publicado no periódico Oral and maxillofacial Surgery – Springer-Verlag, Alemanha, autorizada a reutilização netes trabalho (Anexo B)

2) “Deproteinized bovine bone block for horizontal ridge augmentation: a clinical split-mouth prospective study – part I, clinical and radiographic evaluation”, a ser submetido ao periódico Clinical Oral Implants Research – Wiley Online, Publicação official da Associação Européia de Osseointegração.

3) Deproteinized bovine bone block for horizontal ridge augmentation: a clinical split-mouth prospective study – part II, histologic and microtomographic evaluation”, a ser submetido ao periódico Clinical Oral Implants Research – Wiley Online, Publicação official da Associação Européia de Osseointegração.

4) “Cellular immunologic response of human osteoblasts to different presentations of DBBM”, a ser submetido ao periódico Journal of Biomedical Materials Research – Wiley Online, Publicação oficial da sociedade americana de biomateriais, sociedade japonesa de biomateriais, sociedade australiana de biomateriais e sociedade sul coreana de biomateriais.

3.1 Publicação 1[1]

HORIZONTAL RIDGE AUGMENTATION USING XENOGENOUS BONE GRAFT – SYSTEMATIC REVIEW

Short title: Horizontal ridge augmentation with xenograft

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Key words: Alveolar Ridge Augmentation; Alveolar Bone Loss; Bone Substitutes; Systematic Review.

[1] *Artigo publicado no periódico Oral and Maxillofaical Surgery – Qualis CAPES B2 – Fator de Impacto 1.050 Journal Impact Metrics, Prediction and Ranking: 2019 (Dentistry, Oral Surgery & Medicine)*

ABSTRACT

The objective of this study was to do a systematic review about the use of xenogenous bone graft in horizontal ridge augmentation to answer the following question: *In implant patients, treated with xenografts for horizontal ridge augmentation, what would be the outcomes in terms of bone gain, bone resorption, implant survival, and complication rates?* The main search was performed at PubMed, Cochrane, and Scopus databases, and found 2610 articles. After selection and duplicates removal, 29 studies were included in the final review. The collected data were sample size, number and type of graft, site, horizontal gain, resorption rate, and complications. A total of 610 patients were submitted to 853 bone grafts, both in maxilla and mandible. Most studies (n=26) used particulate grafts, isolated or associated with autogenous bone, and covered by collagen membrane or titanium mesh. The mean of horizontal bone gain was 4.44mm. In addition, the augmented ridges allowed placement of 1325 successful dental implants. The complication rate was 7.85%, being membrane exposure the most common. In conclusion, although the autogenous bone graft remains as the gold standard for alveolar reconstruction, this review suggests that xenogenous bone graft is a feasible alternative for horizontal bone augmentation.

Key words: Alveolar Ridge Augmentation; Alveolar Bone Loss; Bone Substitutes; Systematic Review.

INTRODUCTION

The alveolar ridge resorption can restrict dental implant placement[1]. Usually, the bone resorption occurs as consequence of tooth loss, trauma and pathologies[2]. Therefore, augmentation procedures are performed to provide adequate bone volume for dental implant placement[3]. Residual alveolar ridges according to the main resorbed region are classified as horizontal, vertical, or combined defects. This classification guides the surgeon to the adequate diagnosis and support the treatment decision[4]. Different techniques are available to reconstruct and/or regenerate atrophic alveolar ridges[5-7], including ridge split crest, bone block graft, biomaterials, distraction osteogenesis, and guided bone regeneration[6, 8-11].

The autogenous bone is the gold standard for graft procedures due to osteogenesis, osteoinduction, and osteoconduction features. It is used as block and/or particulate graft[6, 12, 13]. However, the autogenous grafts has some disadvantages including: requirement of a donor site, high morbidity, potential graft resorption and difficulty to adaptation. Therefore, alternative bone materials from different origins are available, represented by allogenic bone graft (derived from human cadavers), xenogenous bone graft (derived from other animal species), and bone graft substitutes (completely synthetic)[14-16].

The xenogenous bone is used for alveolar ridge augmentation with reliable results, low morbidity and decreased complication rate[14, 17, 18]. Also, they show a good long-term stability due to the slow resorption characteristic[19]. It is important to highlight that none bone substitute material has osteoinductive feature similar to autogenous bone. Actually, they support the bone healing process by their osteoconduction characteristic[16, 18-20]. Nevertheless, the efficiency of bone substitute materials in augmentation procedures is proved in many studies[17, 19, 21].

The aim of this study was to perform a systematic review of literature on horizontal ridge augmentation using xenogenous bone graft for dental implant placement, in order to evaluate the bone gain, graft resorption, complication rate, and success.

MATERIALS AND METHODS

This systematic review was directed in accordance for the PRISMA statement (Preferred Reporting Items for Systematic Review and Meta-Analysis)[22], and aimed to answer the following question:: *In implant patients, treated with xenografts for horizontal ridge augmentation, what would be the outcomes in terms of bone gain, bone resorption, implant survival, and complication rates?*

Search Strategy and Selection Criteria

The search strategy was performed in MEDLINE (Medical Literature Analysis and Retrieval System Online, via PubMed), ELSEVIER (via Scopus), and Cochrane Library databases. It was searched all possible combinations of following descriptors: “xenograft”, “Xenogenous”, “bone augmentation”, “bone reconstruction”, “bone particulate”, “bone block”, “bone augmentation”, “bone reconstruction”, “bone particulate”, “bone block”, “lateral augmentation”, “ridge augmentation” and “horizontal augmentation”.

Three independent reviewers (GC, GST, LBM) analyzed titles and/or abstracts according to the following inclusion criteria: specific studies that evaluated horizontal ridge augmentation using Xenogenous bone grafts; studies on humans; reported in English language; no time restriction regarding to publication date; and study types: case series, retrospective or prospective clinical trials. The inclusion criterial were broad, aiming to bring general results without specifying the type of technique, use of

membranes, or type of prosthetic rehabilitation. Furthermore, bone grafts used to sinus lift procedure or vertical augmentation were not included on this study.

After initial selection, the researchers evaluated the full-text of the selected articles according to the same inclusion criteria to define the final included studies. Any disagreements between the reviewers were settled by additional discussion.

Data extraction

Data from the included studies was extracted by the reviewers, including the following variables: type of study; augmentation procedure (bone block and/or particulate graft, Xenogenous or Xenogenous-autogenous mixture); number of patients, age and gender; number of bone grafts; anatomic region of augmentation; horizontal bone gain, resorption rate, complications, implant viability and success rate. Again, disagreements between reviewers were solved by further discussion. Data were analyzed by descriptive statistics and horizontal bone gain was evaluated by the confidence interval (95%) from the data.

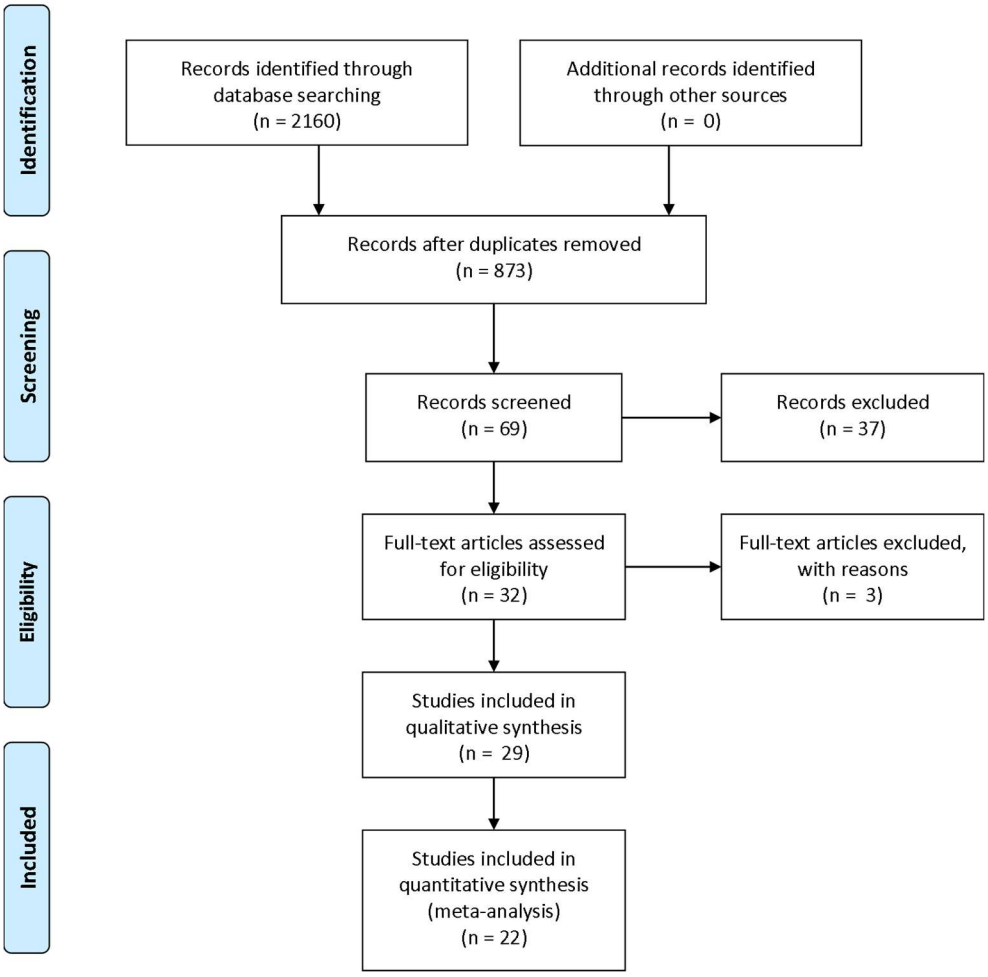
Quality evaluation

All included studies were evaluated using the PRISMA statement[22] criteria to define the scientific evidence for the clinical decision-making process. This evaluation classifies the potential risk of bias of each study, analyzing the following criteria: random sample selection; definition of inclusion and/or exclusion criteria; report of losses to follow-up; validated measurements obtained; and statistical analysis. Studies meeting all criteria were classified as low risk of bias; those that did not meet one of the criteria were classified as moderate risk of bias; and those that did not meet two or more criteria were classified as high risk of bias.

RESULTS

The electronic search was performed by two authors (GC and GST) in March 04, 2017 resulting in 2160 articles. After duplicate removal and the reading of titles and/or abstracts, 69 articles were selected. The full-text of the all selected articles was reviewed for the inclusion criteria. Thus, 37 articles did not meet one or more inclusion criteria in title and/or abstract, and three articles were excluded after full reading. Therefore, 29 articles were included in the final selection. A flowchart of the selection and inclusion process is present in Figure 1.

Figure 1. Flowchart of systematic review process, according to PRISMA statement**.** de Azambuja Carvalho et al. 2019, retrieved from Oral and Maxillofacial Surgery - Springer



Source: de Azambuja Carvalho et al, 2019. Oral and Maxillofacial Surgery, Springer.

All the included articles ranged between 2001 and 2017. Among them 18 studies were prospective, 10 were retrospective, one was case-control, and one was case series. Table 1 shows the quality assessment and bias risk of selected papers.

Table 2 presents the extracted data for each reviewed article. The mean of horizontal bone gain was 4.44mm, ranging from 0.11mm to 7.72mm (Figure 2). In contrast, 18 studies reported resorption data, in mm and/or percentage. The means of resorption rate were 1.29±1.11mm and 24.4±11.04%. The complication rate was 7.95%, and membrane exposure was the most frequent reported one. Furthermore, the achieved horizontal volume allowed implant placement with a success in 96.93% of the cases.

Table 1. PRISMA Quality assessment of selected papers**.** de Azambuja Carvalho et al. 2019, retrieved from Oral and Maxillofacial Surgery - Springer.

Year	Author	Randomization	Include/Exclude criteria	Loss of folLow-up	Valid measurements	Statistical analysis	Risk of bias
2016	Amoian et al.	Yes	yes	yes	yes	yes	Low
2016	Gultekin et al.	No	yes	yes	yes	yes	Moderate
2016	Meloni et al.	No	yes	yes	yes	yes	Moderate
2016	Pelegrine et al.	Yes	yes	yes	yes	yes	Low
2016	Schwarz et al.	No	yes	yes	yes	no	High
2016	Urban et al.	No	yes	yes	yes	no	High
2016	Wessing et al.	No	yes	yes	no	no	High
2015	Merli et al.	Yes	yes	yes	yes	yes	Low
2015	Monje et al.	No	yes	yes	yes	yes	Moderate
2014	Kolerman et al.	No	yes	yes	yes	yes	Moderate
2014	Mordenfeld et al.	Yes	yes	yes	yes	yes	Low
2014	Pistilli et al.	Yes	yes	yes	yes	yes	Low
2013	de Stavola et al.	Yes	yes	yes	yes	no	Moderate
2013	Poulias et al.	Yes	yes	yes	yes	yes	Low
2013	Shalash et al.	No	yes	yes	yes	yes	Moderate
2013	Urban et al.	No	yes	yes	yes	yes	Moderate
2012	Block et al.	No	yes	yes	yes	yes	Moderate
2012	Khammees et al.	No	yes	yes	yes	yes	Moderate
2012	Pagliani et al.	No	yes	yes	yes	yes	Moderate
2011	Calvo-Guirado et al.	No	no	no	no	yes	High
2011	Cordaro et al.	Yes	yes	yes	yes	yes	Low
2011	Urban et al.	No	yes	yes	yes	yes	Moderate
2009	Di Stefano et al.	No	yes	yes	yes	yes	Moderate
2008	Pieri et al.	No	yes	yes	yes	yes	Moderate
2007	Hammerle et al.	No	no	yes	yes	yes	High
2006	Steigman	No	no	yes	yes	no	High
2006	Von Arx et al.	No	yes	yes	yes	no	High
2003	Hellem et al.	No	yes	yes	yes	no	High
2001	Hising	No	yes	yes	yes	yes	Moderate

Table 2. Data extraction of included papers after full reading screening. de Azambuja Carvalho et al. 2019, retrieved from Oral and Maxillofacial Surgery - Springer

Year	Author	Study Design	Sample	Grafts	Graft sort	Garfted area	Horizontal gain (mm)	Resorption (mm or %)	Implants (Sucess rate)	Complications	Impossibility of Implant / New graft needed	Age (Mean ± SD; range)
2016	Amoian et al.	Prospective	10	13	Particulate xenogenous (CenoBone and Bio-Oss) + collagen membrane	Mandible	2.93 (Cenobone)/ 3.37 (Bio-Oss)	*	*	*	*	30 a 50
2016	Gultekin et al.	Retrospective	24	28	Particulate autogenous or xenogenous + coollagen membrane	Maxilla	5.42 ± 0.76	12.48 ± 2.67%	23 (100%)	-	1	48.82±10.17 (28-67)
2016	Meloni et al.	Prospective	18	22	Particulate autogenous or xenogenous (1:1) + collagen membrane	Posterior maxilla and mandible	5.03 ± 2.15	*	55 (100%)	Membrane exposure without graft loss (2)	-	56.8 (24 - 78)
2016	Pelegrine et al.	Prospective	8	8	Particulate xenogenous (control) or Particulate xenogenous + aspired bone marrow (test)	Anterior maxilla	4.34 ± 1.58 (control) 4.09 ± 1.33 mm (test)	*	>16 (100%)	-	-	52.4 ± 2.2
2016	Schwarz et al.	Retrospective	10	10	Xenogenous block and particulate + collagen membrane	Maxilla and mandible	3.00 ± 2.20 (n=10)/3.88 ± 1.75 (n=8)	*	8 (80%)	Dehiscence (7); Block resorption (4); Screw exposure (1).	2	47.4 (34-70)
2016	Urban et al.	Retrospective	16	19	Particulate autogenous and xenogenous (1:1) + d-PTFe or e-PTFe or collagen membrane	Maxilla e mandible	7.0 ± 1.5	1.4 ± 1.0 mm	122 (97,6%)	-	-	64.6 ± 14.6 (48-80)

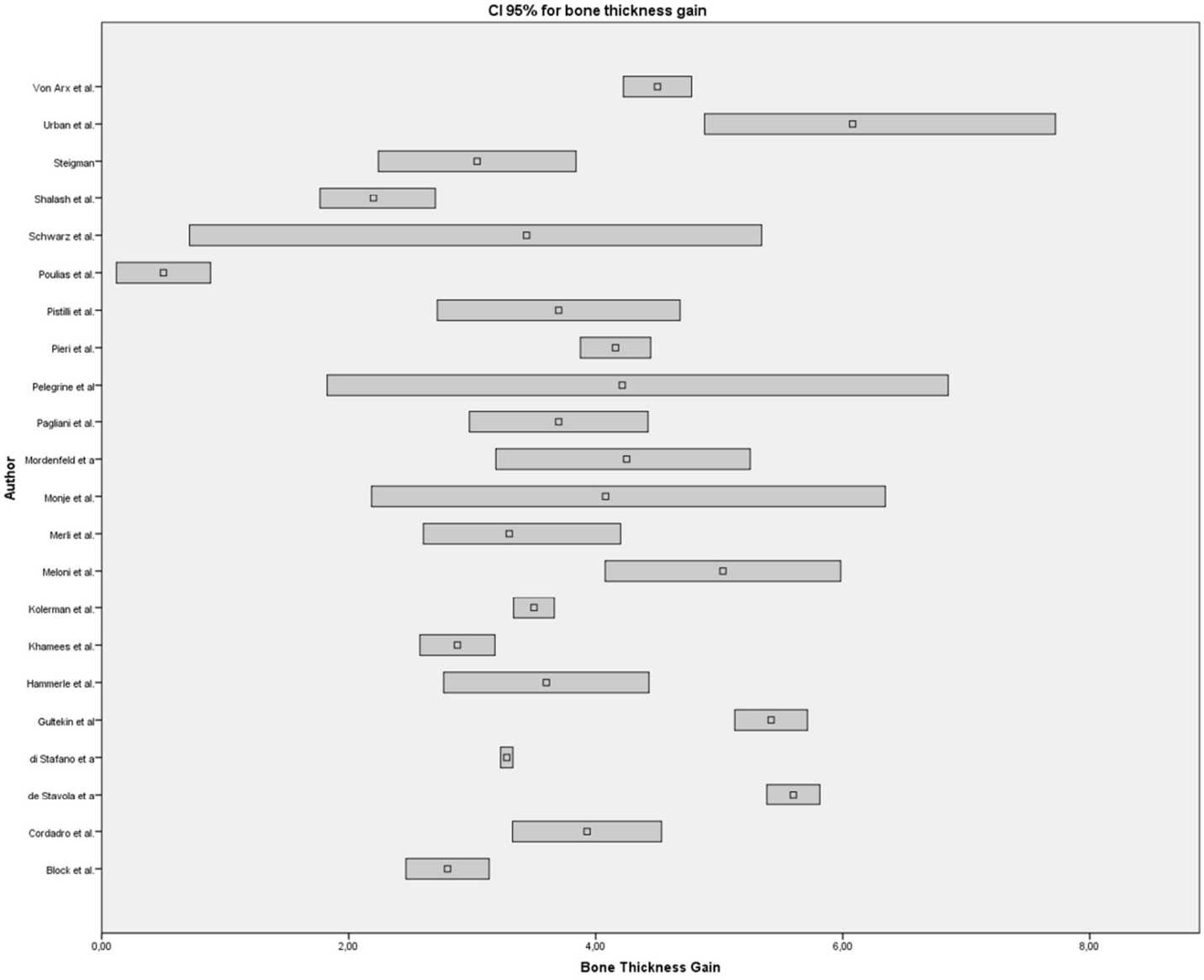
2016	Wessing et al.	Retrospective	36	49	Particulate xenogenous or xenogenous + autogenous chips (1:1) + collagen membrane	Maxilla e mandible	*	*	103 (100%)	Dehiscence (6); Graft loss (2)	1	57.7 ± 12 (32-76)
2015	Merli et al.	Prospective	50	50	Particulate xenogenous + collagen membrane or β-TCP + collagen membrane	Maxilla e mandible	3.1 ± 1.2 (Bio-Oss)/3.5 ± 1.7 (β-TCP)	0.77 ± 0.36mm (Bio-Oss) / 0.54 ± 0.45mm (β-TCP)	61 (100%)	Bio-Oss: dehiscence (1), infection (1), paresthesia(1); β-TCP: deiscence (1), infection (2)	-	Bio-Oss: 56 ± 13 (31-76); β-TCP: 53,4 ± 12,4 (30-76)
2015	Monje et al.	Retrospective	14	19	Autogenous block (mandibular ramus) and particulate xenogenous + collagen membrane; Autogenoud block (Iliac Crest) and particulate xenogenous + collagen membrane	Anterior maxilla	3.23 ± 1.46 (RM); 4.93 ± 1.84 (CI)	*	*	-	-	18 - 85
2014	Kolerman et al.	Retrospective	41	122	Split crest + Particulate xenogenous + collagen membrane	Maxilla and mandible	3.5 ± 0.93	*	122 (95,80%)	Deiscence (15); cover screw exposure (18)	6	(19-77)
2014	Mordenfeld et al.	Prospective	13	28	Particulate autogenous and xenogenous	Maxilla and mandible	4.0±1.4 (G90:10) / 4.5±1.3 (G60:40)	2.3±1.7mm [34.7±23.5%] (G90:10) / 1.8±1.4mm [27.2±18.7%] (G60:40)	71 (97,18%)	Dehiscence (7)	1	59.6 (29-75)

2014	Pistilli et al.	Prospective	20	20	Autogenous block or xenogenous block + collagen membrane	Maxilla and mandible	3.7±2.1	*(Graft loss in 50% of cases)	53 (64%)	Dehiscence with graft loss (9), Severe or total graft resorption (1), Graft mobility (1), Bone sequestration (1), Graft recontourning needed (1).	10	46.8 (21 a 60)
2013	de Stavola et al.	Prospective	10	10	Particulate xenogenous + resorbable membrane	Posterior mandible	5.6 ± 0.3	0.25 ± 0.29mm	*	-	-	46.6 (20 - 63)
2013	Poulias et al.	Prospective	12	12	Particulate xenogenous + resorbable membrane	Maxilla and mandible	0.50 ± 0.60	0.30 ± 0.9mm	23 (100%)	-	-	G1: 52 ± 16 (26 - 77); G2: 58 ± 11 (38 a 71)
2013	Shalash et al.	Prospective	18	18	β-TCP (G1) or β-TCP and particulate xenogenous (G2) + non resorbable membrane	Maxilla and mandible	1.96 ± 0.25 (G1) / 2.44 ± 0.34 (G2)	*	19 (89,47%)	Membrane exposure (2)	2	31.5 (18-45)
2013	Urban et al.	Prospective	25	76	Particulate autogenous and xenogenous (1:1) + collagen membranae	Maxilla and mandible	5.68 ± 1.42	*	76 (100%)	Infection (1)	1	52.7 (30-72)
2012	Block et al.	Retrospective	12	12	Particulate xenogenous + resorbable membrane	Anterior maxilla	2.8 ± 0.53	<1mm	12(100%)	Dehiscence with partial graft loss (1)	-	42.5 (19-65)
2012	Khamees et al.	Prospective	13	16	Autogenous block + particulate xenogenous + Titanium mesh	Maxilla	2.88 ± 0.57	1.67±1.00mm	23 (100%)	Mesh exposure (4)	-	28.19 ± 11.39 (13-55)
2012	Pagliani et al.	Prospective	19	19	Particulate xenogenous + resorbable membrane	Maxilla and mandible	3.7±1.5	1.0 ± 1.1	34 (97.1%)	-	1	46.3
2011	Calvo-Guirado	Case-control	20	20	Particulate xenogenous + Titanium mesh	Posterior mandible	*	*	*	-	-	*

2011	Cordaro et al.	Prospective	17	22	Autogenous block + Particulate xenogenous + bi-layer collagen membrane	Maxilla and mandible	3.93±1.36	0.25±0.23	55 (100%)	Dehiscence (1); Membrane esposure wiht partial graft loss (2).	-	42 (19-66)
2011	Urban et al.	Prospective	18	20	Particulate autogenous and xenogenous + resobable membrane (glycolide and trimethylene carbonate)	Maxilla and mandible	5.56 ± 1.45†	*	43 (100%)	-	-	49.91 (30-60)
2009	Di Stefano et al.	Retrospective	5	5	Particulate xenogenous + Ti-PTFe	Posterior mandible	3.28±0.04	*	15 (100%)	-	-	45.5 (32-59)
2008	Pieri et al.	Prospective	16	19	Particulate autogenous and xenogenous (70:30) + titanium mesh	Maxilla and mandible	4.16 ± 0.59	1.37 ± 0.32mm	44 (93,18%)	Mesh exposure (1); Resorption around implants (3)	-	49.63 ± 10.56 (29-64)
2007	Hammerle et al.	Prospective	12	15	Xenogenous particulate or Xenogenous block + resorbable membrane	Maxilla	3.6 ± 1.5	*	15 (100%)	-	-	44 (20-82)
2006	Steigman	Retrospective	8	19	Xenogenous particulate + collagen membrane	Maxilla and mandible	3.04 ± 1.66	*	19 (100%)	Implants threads exposure (1)	1	(35-68)
2006	Von Arx et al.	Prospective	42	58	Autogenous block and Xenogenous particulate + collagen membrane	Maxilla and mandible	4.6 ± 1.05	0.36 ± 0.52mm	*	Hematoma e dehiscence (1). Membrane exposure (3).	2	34 (17-75)
2003	Hellem et al.	Prospective	30	29	autogenous and xenogenous particulate + fibrinogen	Maxilla and mandible	*	*	82 (95.9%)	Embolism (1) [related to donor site]	-	41.6
2001	Hising	Retrospective	71	92	autogenous and xenogenous particulate + Trombin	Maxilla and mandible	*	<1mm	231 (80.5%)	Infection (2)	2	60 ± 11(24-84)
Total			**608**	**850**								

* Absent information; † Authors presents autogenous bone grafts and autogenous + xenogenous grafts, the mean gain represents the gain for both groups.

Figure 2. Horizontal bone gain (in millimeters), 95%CI according to available data**.** de Azambuja Carvalho et al. 2019, retrieved from Oral and Maxillofacial Surgery - Springer



DISCUSSION

This study aimed to aggregate qualified scientific information about horizontal ridge augmentation using xenogenous bone grafts in order to clarify and discuss its advantages, indications, and complications. In total, 610 patients were submitted to 853 augmentation procedures, involving both maxilla e mandible. The xenogenous bone grafts were used in different forms, 73.0% of studies used

xenografts as particulate graft, alone or associated with autogenous bone. Furthermore, usually the grafts were covered by a membrane. Most of the studies used absorbable membrane[2, 14, 23-40], and few studies used titanium mesh[41-43]. Moreover, two studies applied a fibrin sealant – containing fibrinogen, aprotinin, and thrombin – to the grafted area[44, 45]. The application of barriers probably decreases the resorption rates, however the type is not relevant for bone gain[3, 7, 10, 23, 24, 29].

This systematic review was not limited to clinical trials in order to achieve more data about the use of xenografts. Thus, it was observed that particulate xenograft was the most frequently used, followed by the mixture between autogenous and xenogenous particulate grafts.

Some disadvantages of autogenous bone such as high rates of resorption, harvesting surgery morbidity, and limited amount of volume, stimulated researchers to investigate about bone material substitutes as feasible alternatives[46-48]. Furthermore, the overwhelming majority of the studies is from the last ten years revealing that this subject is recent and there is a lack of absolute information. The autogenous graft seems to have a significant higher resorption rates when compared with xenografts. In our review, the average resorption for xenografts was 24.4%, while the literature report average resorption rates varying from 10% to 49% for autogenous bone grafts[14, 49-52].

Regarding to complications, 13 studies did not report any type[1,2,26,28,30,31,33,36,37,40,42,53]. On the other hand, the remaining studies demonstrates the dehiscence as the most common complications, however not leading to major problems. Another common complication was membrane exposure with no need of surgical interventions. However, seven studies reported graft infection, failure and need re-operation.

Horizontal augmentation procedures using xenografts are feasible presenting significant bone gain and low rates of complications. Esposito et al.[18] published a systematic review evaluating the efficacy of both horizontal and vertical augmentation procedures. However, they found few evidence about horizontal augmentation, with only one clinical trial. In our review, 18 studies were prospective and seven of them presented low risk of bias.

Wessing et al. (2018)[54] published a similar review, however they group considered any type of grafts, as fresh frozen bone grafts, autogenous grafts or xenografts. Beyond our analysis considered only graft procedures with presence of annorganic bone materials we found a similar treatment success rate, 99.13% (CI, 97.23 – 99.96) in Wessing et al. study and 96.43% (CI, 95.43 – 97.43) in our study.

According to the reviewed studies, Xenogenous graft provides proper amount of bone augmentation in thickness (mean 4.44mm), and high rates of success for implant placement. Just one study[14] presented lower success for implant placement (64%). However, this study was the only one that used bone blocks from equines and showed 50% of graft loss, which is not reported in any other study[14].

The highest thickness gain was shown by Urban et al.[37] and Gultekin et al.[30], both using a combination of autogenous and Xenogenous particulate grafts. These findings agree with the hypothesis that annorganic Xenogenous graft could slow the resorption of autogenous bone[7, 25, 30] increasing the volume to grafted area[1, 2, 27, 52].

The study with greatest sample size was Kolerman et al.[38] and achieved a mean gain of 3.5mm (SD: 0.93mm) using a combined technique of split crest and interpositional particulate graft.

The limitation of this systematic review was the impossibility to perform meta-analysis due the variability and lack of standardization of data. Moreover, despite the number of studies included, only one of them was a randomized clinical trial. Therefore, future studies should explore this lack of clinical trials about the use of bone substitutes in augmentation procedures, especially for horizontal augmentation.

The Xenogenous bone grafts, regardless of form of use, presented high success rate without major complications. Those procedures allowed implant placement in 96.63% of the cases. Autogenous block grafts show success rates from 92% to 100 [55]. However, there are few data about implant installation in grafted areas. Therefore, it is possible to conclude that xenografts are a feasible alternative to autogenous bone grafts in horizontal augmentation. Additionally, we encourage researchers to perform controlled randomized clinical trial in this area due to the lack of strong evidence about implant insertion torque, initial stability and osseointegration failures in grafted areas.

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3.2 Publicação 2[2]

DEPROTEINIZED BOVINE BONE BLOCK FOR HORIZONTAL RIDGE AUGMENTATION: A CLINICAL SPLIT-MOUTH PROSPECTIVE STUDY – PART I, CLINICAL AND RADIOGRAPHIC EVALUATION.

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Key-words: Alveolar ridge augmentation; Heterografts; Deproteinized Bovine Bone Block; Randomized Clinical Trial

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ABSTRACT

Objectives This study evaluated the clinical and radiographic characteristics of maxillary ridge reconstruction with deproteinized bovine bone block (DBBM) compared to autogenous bone graft from the mandibular ramus (AB).

Materials and methods Twelve patients with edentulous atrophic maxillary ridges were included. They were submitted to reconstruction surgery under general anesthesia. Each side of the anterior maxilla received a type of graft, according to randomization: AB or DBBM. CBCT volumes of the alveolar ridges were acquired at three moments: pre-augmentation (T0), immediate post-augmentation (T1), and pre-implants (T2), and the alveolar ridge volume at T0 was compared with the volumes at T1 and T2. Also, thickness of alveolar ridges was measured trans-surgically with a surgical caliper, in three standardized positions (medial, lateral, and above graft's fixation screw), in three moments: T0, T1 and T2. Nine months after grafting procedure (T2), implants were installed following an “all-on-four” protocol and the trans-surgical thickness was assessed again. Also, implant torque and implant stability quotient (ISQ) were measured. The clinical follow-up was performed during the whole study period, and complications were recorded in the patient’s chart.

Results: Five patients (3 from AB, 2 from DBBM) presented one or more complications. The main complications were wound dehiscence and graft exposure, with a mean time of 74.2 days after surgery (range 20 – 120). Three patients presented complications of the donor site. The volumetric changes were not statically different between AB and DBBM. Resorption percentages in the CBCT volumes, were 10.83% ± 8.23 (AB) and 16.73% ± 8.01 (DBBM). The trans-surgical bone thickness was increased by 3.7 mm ± 9.7 in AB and 4.5 mm ± 8.3 in DBBM. Torque (AB: 40.83N ± 15.49; DBBM: 30.41N ± 14.43) and ISQ (AB: 62 ± 7.45; DBBM: 53.37 ± 13.04) were equal in the anterior placed implants of both groups.

Conclusion: DBBM presented as a viable alternative for maxillary horizontal augmentation, with clinical and radiographic behavior comparable to AB.

Keywords Alveolar ridge augmentation; Heterografts; Deproteinized Bovine Bone Block; Randomized Clinical Trial

INTRODUCTION

Bone grafting is a valuable procedure for alveolar ridge augmentation when the final aim is to restore function in atrophic jaws with dental implants. The main indication for ridge augmentation is severe resorbed ridges with the horizontal and vertical dimensions compromised (Tolstunov, 2019; Chiapasco, 2009; Buser et al., 2002). Early teeth loss, severe trauma or advanced periodontitis are the main etiologies for ridge resorption (Lim et al., 2018; Aghaloo et al., 2016; Monje et al., 2015; Chiapasco, 2009). Dental implants play a crucial role on rehabilitation of patients with partial or total teeth loss (Chiapasco et al., 2015; Chiapasco, 2009; Esposito et al., 2009). However, the success of dental implants, in this cases, hinge on the quality and quantity of remaining bone tissue (Motamedian et al., 2016; Manzano-Moreno et al., 2015).

Several techniques have been described to rehabilitate atrophic ridges, including guided bone regeneration (GBR), alveolar ridge osteotomy, osteogenic distraction, and bone grafting (Tolstunov, 2019; Milinkovic & Cordaro, 2014; Chiapasco, 2009). Regarding the precise indications, all grafting procedures present high rates of success and few complications. It can be performed with autologous bone (AB), homologous, xenogeneic bone, or synthetic material, in combination with collagen or other biocompatible membranes, all these alternatives are supported with enough literature data. (Pelegrine et al., 2016; Barone et al., 2008; Brugnami et al., 1999). Previous studies showed that in large augmentations, block bone grafts have higher success rates and lower complication rates when compared to bone graft in granules (Milinkovic & Cordaro, 2014).

AB presents good results due to its osteoconductive, osteoinductive, and osteogenic properties (Spin-Neto et al., 2014; C. Sbordone et al., 2012; Clementini et al., 2011; L. Sbordone et al., 2009). However, it demands technical ability, a

second surgical site, and it is related to higher operative costs and donor-site complications (Pereira et al., 2019; Spin-Neto et al., 2014). To minimize the burden of a donor site creation, bone substitute materials have been extensively studied (Barone et al., 2008, 2017; Nkenke & Neukam, 2014; Carlsen et al., 2013). A viable alternative is the xenogeneic bone graft, due its biocompatibility and osteoconductive properties (Schwarz et al., 2017; Motamedian et al., 2016; Veis et al., 2015; Pistilli, Felice, Piatelli, Nisii, Barausse, 2014; Block et al., 2012; Felice et al., 2009). Xenogeneic bone grafts have similar results to AB regarding resorption rates and implant success (Motamedian et al., 2016). Deproteinized bovine bone matrix (DBBM) is a consolidated xenogeneic material for maxillary sinus augmentation, post extraction socket preservation, and small-size GBR (Milinkovic & Cordaro, 2014; Chiapasco, 2009; Artzi et al., 2000).

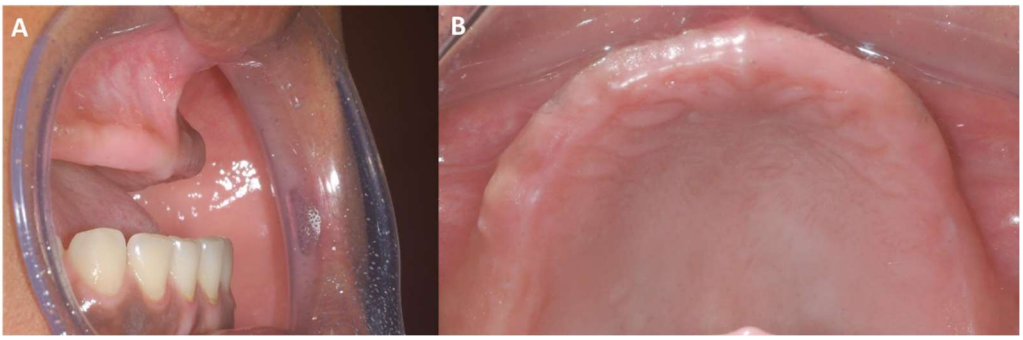
Commercially available blocks of DBBM have been associated to low mechanical resistance, making impossible to use screws for fixing the block to the recipient site without cracking the material (Gehrke et al., 2019; Fontana et al., 2008). Therefore, most previous studies used DBBM blocks as inlay graft. However, with the development of new technologies for low temperature sintering and purification of the material, it became possible to manufacture DBBM blocks with appropriate mechanical resistance (Gehrke et al., 2019; Cingolani et al., 2018; Kacarevic et al., 2018). Thus, this study evaluated the clinical and radiographic characteristics of maxillary ridge reconstructions with DBBM block compared to AB graft from mandibular ramus.

MATERIAL AND METHODS

Sample selection

This study was conducted in accordance with the Helsinki Declaration and was approved by the local and national ethical committee in research under the number 673443017.0.0000.5416. The patients included in the sample voluntarily presented themselves for oral rehabilitation with dental implants at the outpatient clinic of the Oral Surgery and Diagnosis Division from São Paulo State University, Araraquara Dental School of Dentistry (FOAr-UNESP). Patients with totally edentulous maxilla, and with horizontal atrophy of the maxillary ridge were further examined (Figure 1).

Figure 1. Clinical evaluation of horizontal deficiency in patients with complete maxillary edentulism. (A) Lateral view of anterior alveolar ridge. (B) Occlusal view from atrophic maxillary ridge.



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In order to be selected for the study, the patients should accomplish the following inclusion criteria: adult (over 18 years old), of any sex, with totally edentulous maxillary ridge, remaining alveolar bone width between 2 and 10mm, and remaining alveolar height of at least 10mm, and accept to participate in the study by signing the Free Consent Term. Patients with chronic unstable systemic illnesses, pregnant or nursing women, under chemotherapy or radiotherapy, with active sites of infection, who take medications which affects the bone metabolism,

smokers, with recent extraction socket in the area of interest, or patients with bad oral habits that could affect the bone repair, were excluded.

Study design

The present study followed the CONSORT 2010 statement, to draw and report a clinical trial (Moher et al., 2010). The study was delineated as a clinical split-mouth prospective study, and all the patients received both treatments, to know: one side of maxilla was augmented with DBBM block (Bonefill Porous Block, Bionnovation, Brazil) and the other with autogenous bone block (AB) from mandibular ramus, thereby setting the study groups. The groups' assignment was randomized previously to the surgical procedure, by a dichotomous method for graft (AB or DBBB) and side (Right or Left), in the software Microsoft Excel 365 (Office365, Microsoft, USA), and this information was taken to the surgery room.

A baseline (T0) CBCT acquisition (i-CAT Classic, i-Cat, Imaging Sciences International, Hartfield, PA, EUA; FOV of 16 x 13 cm, voxel size 0.25 mm voxel, 120 kVp, 36 mAs) was performed for each patient at the moment they agreed to participate in the study.

Sample size

Sample size was calculated based on previous similar studies, considering an alfa of 5% and study power of 80%. Sample size was estimated in 10 samples for group. Considering an error margin of 20% a total 'n' of 12 patients, and 24 augmentation sites, were included.

Surgical protocol for bone grafting

One hour pre-operatively, all patients received Cefazolin 1g (Kefazol, Lilly S.A., Brazil) or Clindamycin 600 mg (Dalacin, Pharmacia S.A., Brazil) in case of penicillin allergy, Dexamethasone 10 mg (Decadron, Aché S.A., Brazil) and monohydrated Sodic Dipyrone 1g (Dipyrone, Medley S.A., Brazil), or Acetaminophen 750 mg (Paracetamol, Medley S.A., Brazil), in case of Dipyrone allergy or intolerance.

Surgical procedures were performed under hospitalization and general anesthesia. After antisepsis with chlorhexidine 0.5% (Riohex, Rioquímica, Brazil), a local anesthetic infiltration with Articaine 4% with epinephrine 1:100,000 (Articaine, DFL, Brazil) was performed for bleeding control and post-operative comfort.

All surgeries were performed by the same experienced surgeon (VAP). A biangular incision was performed extending bilaterally between the areas were the first premolars were supposed to be. After the incision and the cautious periosteal detachment the recipient site in maxilla was decorticated with stainless-steel pear shape burs (H251E Maxicut, Komet Brazil, Brazil) and perforated with 16mm conic burs (H33L 701, Komet Brazil, Brazil), under copious irrigation with 0.9% saline solution (Equiplex, Brazil), aiming to maximize the blood supply and enhance the vascularization toward the graft base (Figure 2A).

To prepare the recipient areas, the mandibular ramus was accessed with a linear incision over the mandibular oblique line, with sufficient extension to allow the graft removal. The autogenous bone block was removed with piezoelectric motor (Piezosonic, Driller, Brazil) and piezoelectric saw (ES007A-Driller, Driller, Brazil), aiming to reduce complications and to improve the cellularity in the grafted block (Altimpark et al., 2015) and three cortectomies were performed, the first one over the external oblique line, then two vertical osteotomies determining the graft length, finally disc saw (Härte instruments, Brazil) was used to the cortectomy of

the graft base, final removal was performed with straight and curve surgical chisels (Quinelato, Brazil) (Figure 2B). The DBBM was removed from sterile package and moistened in saline solution according to the manufacturer orientations.

Both AB and DBBM were reshaped with piezoelectric drill and surgical burs (Komet Brazil, Brazil). The sharp angles were removed, and they were adapted to the receiving site. Titanium screws of 1.4x12mm, with extended heads (Bionnovation, Brazil) were used to fix the biomaterial to the alveolar bone with a leg-screw like technique. (Figure 2C).

Trans-surgical measurements of alveolar ridges' thickness were obtained before the graft positioning (T0), with a surgical caliper (Quinelato, Brazil, QD.308.10), in three standardized positions (medial, lateral, and above graft's fixation screw), between the canine fossa and anterior nasal spine, in both sides. Immediately after the grafts fixation, the thickness was again recorded with the surgical caliper (T1) positioned 90º with the maxillary occlusal plane and in the exact same locations. During the implant installation surgery, the ridge thickness was assessed once more (T2) in the same positions. Measurements were recorded in millimeters and were taken in triplicate by the same examiner.

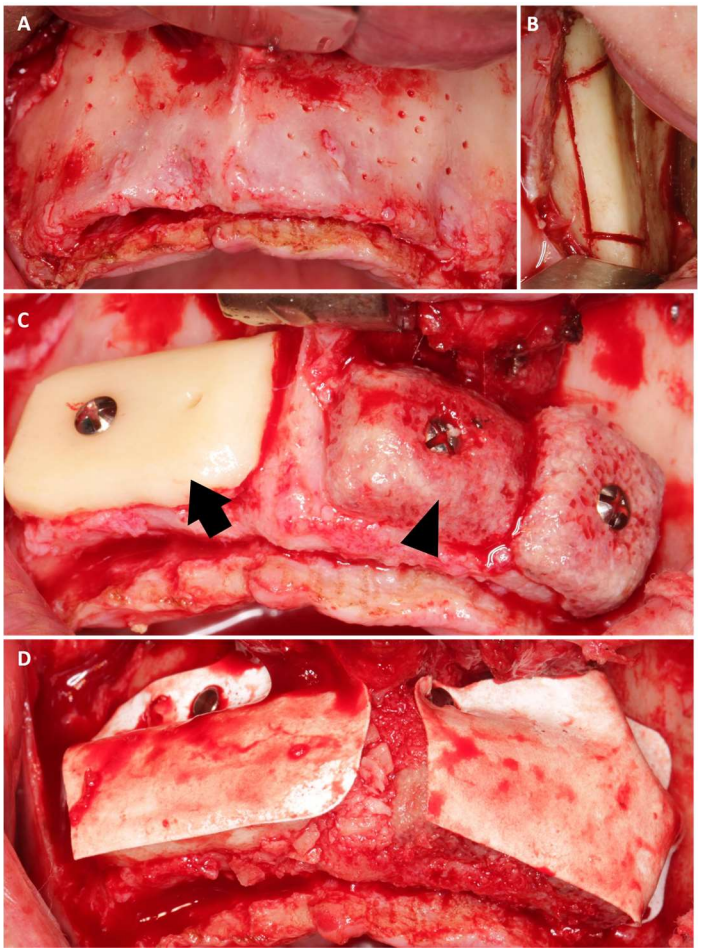
Both augmented sites were covered with polytetrafluorethylene (PTFE) non-resorbable membranes (Surgitime PTFE 0.25 mm, Bionnovation, Brazil). The membranes were fixed in the residual bone with titanium tacks (AutoTac®, BioHorizons Implant System, EUA). Finally, the surgical wounds were closed by first intention with polyglactin 910 (Ethicon, Johnson & Johnson, Brazil).

After the surgical procedures, the patients were prescribed with Amoxicillin 500 mg or Clindamycin 300 mg, in case of penicillin allergy, three times daily for 7 days. Nimesulid 100 mg twice daily for 4 days and monohydrated Sodic Dipyrone 1g, or Acetaminophen 750 mg, in case of Dipyrone allergy, 4x/day, in case of pain.

The sutures were partially removed between days 7 and 10, and completely removed in all cases up to 15 days.

A new CBCT acquisition with the same configurations as the baseline was performed immediately after the surgical procedure (T1), as well as the clinical evaluation of post-operative complications (e.g. suture dehiscence, graft exposure, infection, hemorrhage, paresthesia, bone fracture).

***Figure 2**. Horizontal ridge augmentation procedure with mandibular ramus bone blocks (AB) or Deproteinized Bovine Bone (DBBM). (A) bone marrow perforation and decortication of receiving site. (B) Donor site osteotomy. (C) Graft fixation according to randomization, arrow indicate de OA group and arrowhead the DBBM group. (D) Augmented sites covered with non-resorbable PTFe membrane.*



Source: From author.

Surgical protocol for augmented sites reopening and placement of dental implants
Nine months following the maxillary ridge augmentation patients were submitted to a final CBCT scan (T2), with the same parameters used in T0 and T1, and the grafted areas were reopened for implant placement and trans-surgical bone thickness measurements. The drug prophylaxis protocols for preventing excessive pain and infection were the same cited for the first surgery.

Surgical procedures for implant placement and clinical measurements of grafts were undertaken in outpatient conditions. Initially, chlorhexidine 0.12% and 2% (Rioquímica, Brazil) was used for intra-buccal and facial antisepsis. Local anesthesia was performed with Articaine 4%, with epinephrine 1:100,000.

A complete mucoperiosteal flap was detached to remove the membranes and membrane's tacks, and the clinical measurements were performed with the surgical caliper in the same places of the initial measurements, taking the graft medial screw as reference (T2). The screws were only removed in cases which the graft biopsies (unpublished data) or implant placement were impaired, otherwise they have been kept in position, as shown in figure 3.

Socket preparation in augmented bone was performed according to the manufacturer recommendations for 3.5 external hexagon implants placement (BioDirect, Bionnovation, Brazil). Briefly, using a surgical handpiece (Neodent/NSK, Brazil) in a electric motor (NeoSurgC XT plus, Neodent/NSK, Brazil) the bone was drilled at 800 rpm and 20N with specific burs, increasing in width until reach the 3.5/3.75 mm at the desired length, always under constant refrigeration with sterile saline.

All patients received two dental implants (BioDirectSWE, Binnovation, Brazil) by augmented side. In the anterior area the implants length was of 10mm and in

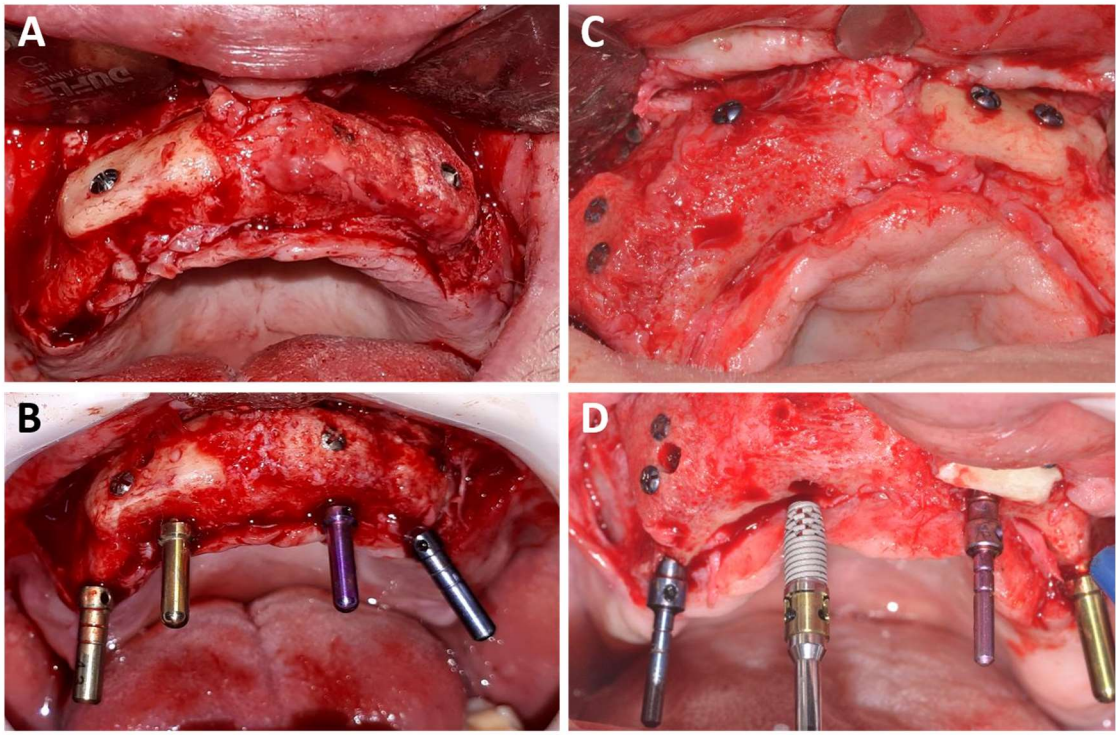
the posterior area the implants length was 13mm, and the posterior implants were placed with a 30º backwards inclination according to the all-on-four concept.

In all cases the implants were placed with manual ratchet with torque wrench, provide by the manufacturer. The final insertion torque was assessed in triplicate by a torque meter attached to the ratchet.

The implant stability quotient was assessed at the moment of the implant placement, using the resonance frequency index by Osstell device (Osstell ISQ, Sweden), using the smart-peg A1 (Osstell ISQ, Sweden) for universal external hexagon connection, then three measurements were taken with the Osstell probe in the buccal-palatal way of the smart-peg.

Following implant placement, the soft tissues were repositioned, implants were protected with cover screw, and sutures were performed using polyglactin 910 wire, which was removed after 7 to 10 days. The post-operative therapeutic protocol was the same as first described to the graft surgery.

Figure 3. Surgical procedure for implant placement in augmented maxillary ridges with autogenous bone (AB) or deproteinized bovine bone blocks (DBBM). (A-D) Examples of augmented areas after nine months, and implant placement.



Source: From author.

Clinical assessment

The clinical assessment of the patients took place at the first post-operative day, third post-operative day and 7th post-operative day, followed by weekly returns in the first month, and then increasingly spaced until the moment of implant surgery.

In each appointment the patients were evaluated for: general health conditions as fever, swollen lymph nodes (local or at distance), weakness, discoloring, and others; also was evaluated the local condition of wounds noting the main signs and symptoms of complications as: bleeding, hematomas, swallow, paresthesia, bruising, wound dehiscence, infection, seroma, granulomatosis, pain, or others.

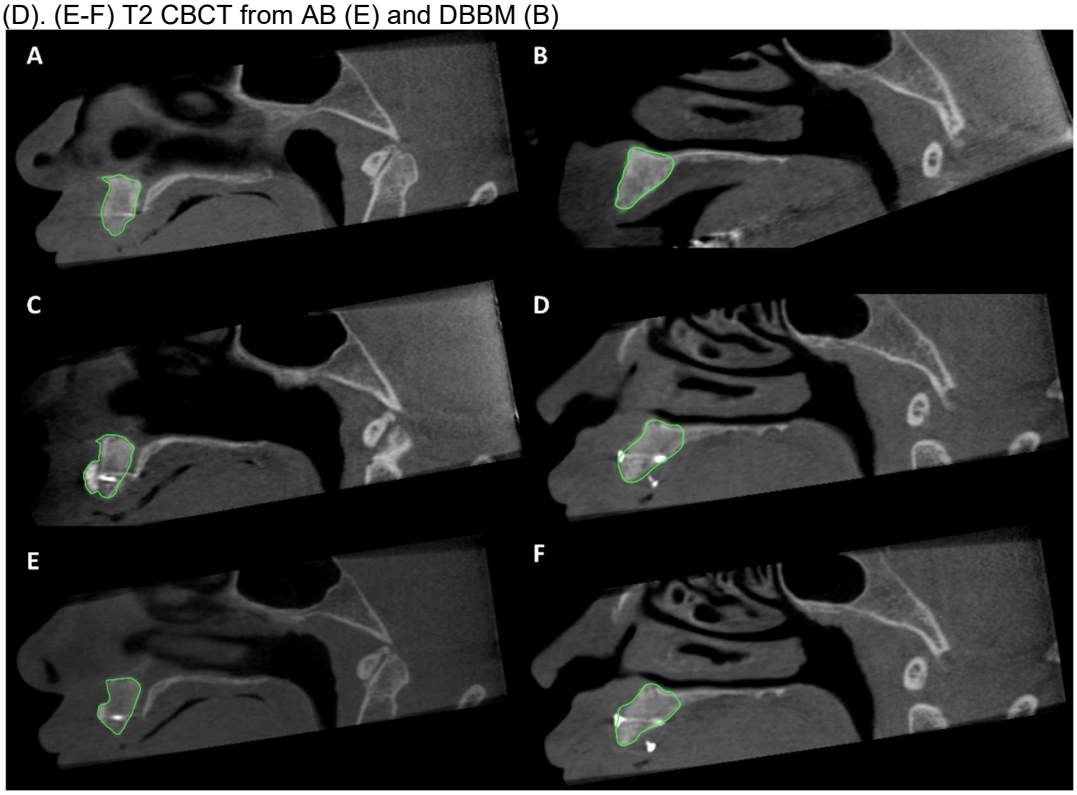
Tomographic evaluation

The DICOM datasets from the CBCT acquisitions at T0, T1, and T2 were anonymized and stored in a hard drive for posterior tridimensional reconstruction in the software OsiriX (GNU LGPL, Geneva, Switzerland). The reconstructed images were standardized regarding the spatial orientation, the Frankfurt plane was settled parallel to ground, the coronal plane crossing the pterygoid plates settled perpendicular to Frankfurt, and the sagittal plane fixed according to the midline crossing the coronal plane in 90 degrees. After head re-orientation, 40 sections (0.25 mm and 1 mm interval) from each side of maxilla were selected in sagittal view. The region of interest (ROI) for volume measurement was determined based on these sections. Measurements were performed in these sagittal 0.25 mm CBCT sections, spaced by 1mm.

Briefly, in each section, the ROI was hand-delimited with digital drawing tablet Intuos Graphics (Wacom Co. Ltd, Saitama, Japan), by two experienced examiners. To enhance visualization of important structures the threshold was

standardized with level (L) and width (W) fixed according to the findings of Spin-Neto et al. (2011)(Spin-Neto et al., 2011), W=3086 e L=667. For each section, the area (A) of residual bone or augmented bone was calculated by the ROI tool in the OsiriX software (Figure 4). The sum of all areas was used to determine the volume (V), based on Cavalieri's principle.

Figure 4. Tomographic evaluation of bone volume and ROI delimitation in the different timepoints. (A-B) T0 CBCT from AB (A) and DBBM (B) grafted sides.(C-D) T1 CBCT from AB (C) and DBBM (D). (E-F) T2 CBCT from AB (E) and DBBM (B)



Source: From author.

Statistical analysis

The clinical and tomographic measurements were tabulated and codified to allow blind statistical analysis.

Volume in CBCT scans were assessed by two independent examiners (ICC 0.86) and the average was used for statistical analysis. To evaluate the initial bone augmentation in CBCT, the area (A) of residual bone or augmented bone was calculated for each section and the sum of all areas was used to determine the volume (V), then the initial volume gain was determined by the difference betweenT1 and T0 , the resorption rate by the difference between T2 and T1, and the effective bone gain by the difference between T2 and T0, as a % and in mm^3. Differences were calculated individually for each patient.

The mean of the triplicate measurements of trans-surgical ridge thickness was used for analysis. The initial bone augmentation was represented by the difference betweenT1 and T0, and the bone thickness resorption by difference between T2 and T1 as a % and in mm. The torque (N) and the ISQ values were computed for each implant, and statistical comparisons were performed between the implant position (anterior or posterior) and graft type (AB or DBBM).

After confirming normal distribution of quantitative variables (Shapiro-Wilk's test) and assessed homoscedasticity (Levene's test), those were compared by ANOVA with Tukey post-hoc test for multiple comparisons. Qualitative variables, as complication rates and implant survival were submitted to Fisher's exact test.

RESULTS

The population of this study were 80% women and 20% men with the mean age of 51.25 ± 11.1 years-old, ranging between 29 and 65 years-old.

A total complication rate of 20.83% (n=5) was recorded, without statistical difference between the AB group (16.67%) and the DBBM (25%) (Table 1). The suture dehiscence was the main recorded primary complication, this complication leads to membrane exposure (AB: 25%; DBBM 16.67%) or even graft exposure (16.7% in both groups). One membrane exposure was late reported without initial suture dehiscence. The median time, in days, until membrane exposure was 90 days (30-120) in the AB group and 57.5 (20-95) days in DBBM grafts. Despite of occurrence of dehiscence, membrane exposure, or graft exposure, in all cases it was possible to keep the graft in position, and all reconstructed sites were able to receive dental implants at the end of healing period.

Complications were observed in 25% of donor sites, but in some cases the same site presented two or more complications. Often post-operative complications were suture dehiscence (2), seroma formation (2) and one trans-operatory hemorrhage. There was no cases of graft loss or intercurrences during implant surgery.

Table 1. Frequency and distribution of post-operative complications in horizontal ridge augmentation procedures, according to the graft type, and donor or receiving areas. Carvalho P.H.A., Araraquara, 2020

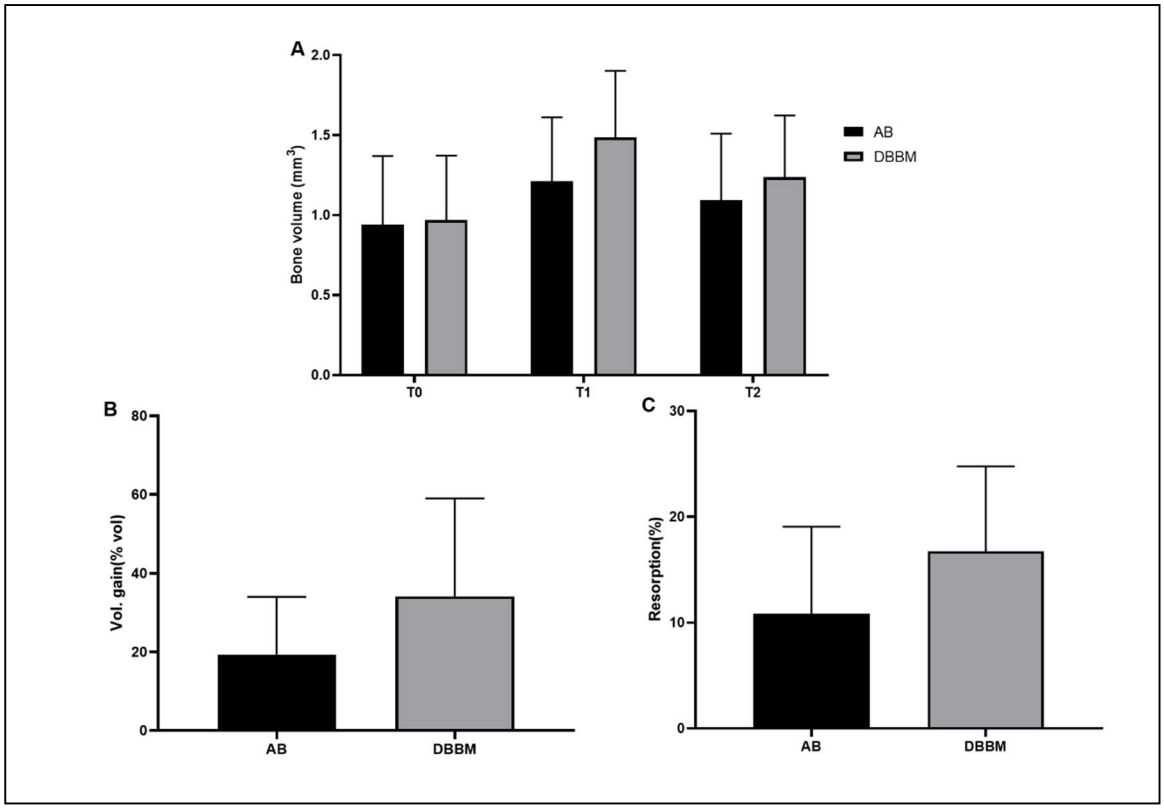
Sites presenting clinical intercurrences and complications		
	n	
AB	3 (25.00%)	
DBBM	2 (16.67%)	
Total complications at receptor site		
AB	7 (41.18%)	
DBBM	10 (58.82%)	
Total complications at donor site		Patients with complications at donor sites
AB	7 (-)	4 (33.33%)
DBBM	-	-
Complications at receptor site by type		
	AB	DBBM
Infraorbital nerve paresthesia	2 (16.67%)	1 (8.33%)
Hematoma	-	-
Swelling	1(8.33%)	1 (8.33%)
Hemorrhage	-	-
Seroma		1 (8.33%)
Suture dehiscence	2 (16.67%)	3 (25.00%)
Membrane exposure	3 (25.00%)	2 (16.67%)
Graft exposure	2 (16.67%)	2 (16.67%)
Infection	-	-
Necrosis	-	-
Hemorrhage	1	16.67%
Seroma	3	50.00%
Suture dehiscence	3	50.00%
Infection	-	-

Tomographic evaluation

The T0 (baseline) tomographic volume was equal between groups (p>0.05), the average volume was 0.94 mm³ ± 0.43 (AB) versus 0.97 ± 0.4 mm (DBBM), showing an efficient randomization routine. (Figure 5 A).

DBBM provided a final volume gain of 34.1% ± 24.94 in average, in contrast with 19.36% ± 14.65 of AB (p<0.05, *One-Way* ANOVA), in mm³ this values correspond to 15.01 mm^3 ± 11.49 and 26.79 mm^3 ± 13.67, to AB and DBBM respectively (Figure 5 B). The volume resorption in 9 months, in comparison to the volume obtained in T1 was of 10.83% ± 8.23 for AB and 16.73% ± 8.01 for DBBM grafts, without statistical difference,(p>0.05, *One-Way* ANOVA), or 15.29 mm^3 ± 11.48 and 26.79 mm^3 ± 13.66 in absolute measurements (Figure 5 C).

Figure 5. CT volumetric evaluation of DBBM and AB grafts for maxillary reconstruction. (A) Total bone volume (mean and upper SD) for AB and DBBM in three different timepoints. (B) Volume gain (mean and upper SD) in percentage for AB and DBBM. (C) Resorption rate (mean and upper SD) after 9 months, for both AB and DBBM.

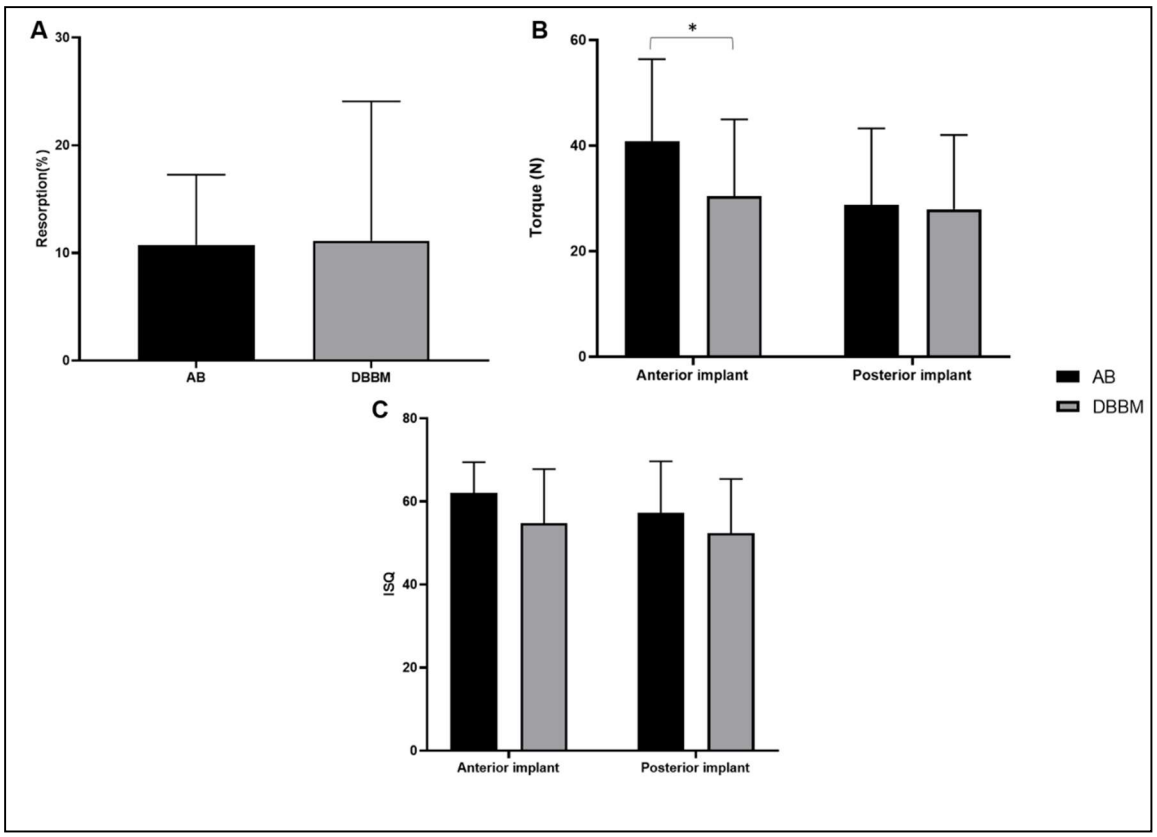


Source: From author.

Trans-operatory measurements

The trans-surgical assessed bone thickness was increased by 3.7 mm ± 9.7 for AB and 4.5 mm ± 8.3 for DBBM, and after the healing period there was a resorption of 10.74% ± 6.53% in AB and 11.13% ± 12.96 in DBBM (Figure 6A). The insertion torque of the straight anterior implants was in average 40.83 ± 15.5 in AB and 30.42 ± 14.53 in DBBM ($p<0.05$) (Figure 5 B). The posterior angulated implants in AB grafted areas showed an average torque of 28.75 ±14.48 and the angulated implants in the DBBM grafts an average torque of 27.92 ± 14.05, without statistical difference (Figure 6 B). The mean ISQ in the anterior implants was 62,03 ± 7,45 in AB and e 54.78 ± 13.04 in DBBM, while for the posterior implants it was in average 57.33 ± 12.34 in AB group and 52.39 ± 13.04 in DBBM group (Figure 6 C).

Figure 6. Thickness resorption, implant installation torque and stability quotient (ISQ), of AB and DBBM grafts used in maxillary reconstruction (A) Thickness resorption (mean and upper SD) in AB and DBBM grafts, expressed as resorption rate. (B) Insertion torque (mean and upper SD) of anterior straight implants and posterior inclined implants in AB and DBBM. (C) ISQ (mean and upper SD) of anterior straight implants and posterior inclined implants in AB and DBBM.



Source: From author.

DISCUSSION

Horizontal ridge augmentation procedures with bone blocks are a safety and efficient technique to the rehabilitation of atrophic jaws. Therefore, this clinical trial was designed in a split-mouth research design, which evaluates the clinical and tomographic behavior of DBBM blocks, used for alveolar ridge augmentation.

AB represents the standard for augmentation procedures in the oral and maxillofacial area, however several studies showed that the morbidity associated to the donor site can range between 17% to 51.85% (Pereira et al., 2019; Nkenke & Neukam, 2014; Scheerlinck et al., 2013). Also, for large reconstructions, it is necessary to retrieve bone from extra-oral donor areas, which most times demands a second surgical team and increase in procedures costs. Some studies showed that graft resorption of autogenous iliac grafts can achieve up to 49%, due to its marrow characteristic (Nkenke & Neukam, 2014; C. Sbordone et al., 2012; L. Sbordone et al., 2009). Bone harvesting from intra-oral sites is not completely free of complications, as paresthesia, hemorrhage and infection still occur (Pereira et al., 2019; Milinkovic & Cordaro, 2014; Scheerlinck et al., 2013). The bone from mandibular ramus presents lower resorption rates (Pereira et al., 2019; Gultekin et al., 2016), and this information was corroborated in our study (10.83% volume loss of AB).

Horizontal ridge augmentation procedures are well documented in the literature, but for most of them there is a lack of randomized clinical studies, and most data are provided by case series or retrospective analysis (Elnayef et al., 2018; Troeltzsch et al., 2016). DBBM alone or in combination with autogenous bone can promote new bone formation up to 40% of graft volume, which can be explained by the

properties of bovine bone on inducing osteoclast activity, and promote the cytokine releasing in osteoblasts (Shi et al., 2018; Amerio et al., 2010).

Several studies evaluating DBBM achieved good results in horizontal ridge augmentation (de Azambuja Carvalho et al., 2019). Block et al. (2012), showed that bone graft procedures using DBBM were time standing and present a resorption rate lower than 25% of initial volume. The bone block used in the present study presented in average a resorption rate of 16.73%, in accordance with the findings of Block et al. (2012), even their study was performed with particulate bovine bone grafts, and with a maximum of 4mm of augmentation, and our mean thickness augmentation was 8.48 mm .

A systematic review by Esposito et al. (2009) evaluated the effectiveness of horizontal and vertical alveolar ridge augmentation procedures, however they have not found enough evidence regarding ridge augmentation procedures with xenogenous bone blocks. They found only one randomized trial, and after this review, some advances were developed in the sintering and manufacturing of heterogenous bone blocks, but few studies evaluate it effectiveness and only one more trial was included in the review updates.

Recently, Lima et al. (2018), performed a similar split-mouth clinical trial, comparing a deproteinized bovine bone block, from a different commercial brand than ours, with autologous block from mandibular ramus. In the study of Lima et al. (2018) the resorption rates for both autologous and bovine blocks were 2.6% and7.3% respectively, lower than resorption rates found in our study. However, the study of Lima et al. (2018) reopened the augmented sites after 6 months against 9 months in our study, and the implants insertion torque in DBBM block areas in their study was also lower than implants placed in AB augmented areas.

Using DBBM, Ortiz-Vigón et al (2017) achieved a thickness increment of 4.12 mm, compatible to the one reported in the present study. However, the resorption rates were not assessed by them. In the present study, graft resorption rate for DBBM was 11.43%, without statistical difference to AB. Also in the study of Ortiz-Vigón (2017), the wound dehiscence and graft exposure were observed in 33% of the sample, higher than in our sample (16.78%) but in the range of what is expected for block grafts in the literature (Checchi et al., 2019; Ortiz-Vigón et al., 2017; Jensen et al., 2016). The soft tissue is a challenge in these augmentation procedures, because the increment in bone tissue is not immediately followed by soft tissue growth, and in most cases the patients included are elder, with low tissue elasticity and poor repair. Despite the complications, the success rate for the grafting procedures in our study was 100%, all areas were able to receive implants, which is corroborated by the previous findings of Ortiz-Vigón et al. (2017).

Mordenfeld et al. (2014), also performed a split-mouth study to evaluate horizontal ridge augmentation with DBBM, but using a combination of particulate DBBM with particulate autogenous bone in different proportions, 60:40 and 90:10, achieving a horizontal gain of 82% of initial volume and resorption rate between 27% e 49%. The combination of both grafts in the same site seems to enhance properties of xenogenous graft, and minimize the limitations of autogenous bone alone, however this type of study design impairs the evaluation of the properties of bovine bone as bone substitute alone. In our study the resorption rates, even in clinical or tomographic evaluation were lower, which goes along with a supposed superior stability over time of block grafts (Laass et al., 2020; Benic et al., 2019).

Pistilli et al. (2014), performed a clinical trial with equine xenogenous bone, and compared the equine derived xenogeneic bone block with autogenous bone of

mandibular ramus or iliac crest. They found high failure rate and graft loss in the equine bone group, up to 50% of failure. In autogenous group all procedures performed in maxilla were successful. However, the authors include augmentation sites in both maxilla and mandible. The bone in the posterior region of mandible is considered a poor-quality bone, and provide low vascularization, which could lead to increase in graft loss. Also equine bone did not present good clinical results in human studies (Felice et al., 2009).

One of major concerns about DBBM is the risk of the graft to carry the Bovine Spongiform Encephalopathy (BSE) Prion protein, or RNA fragments of the Prion, which could lead to an increased risk of Creutzfeld-Jakob Disease (CJD) (Kim et al., 2013, 2016). This risk can be controlled using tracked cattle of countries BSE-free. Submitting the material to several alkaline baths and high temperatures over 1000ºC would improve its safety (Kacarevic et al., 2018). A disadvantage of treating the material in high temperatures is the loss of the micro and nano- porosity architecture, naturally provided in the bovine bone (Kacarevic et al., 2018). This nano porosity up to 100μm could be one of the reasons for the superior clinical behavior of DBBM in relation to other grafts, due to a higher bone surface in the same volume of material (Turco et al., 2018). The material tested in this study was not submitted to high temperatures, and the purification consists of ”a sequence of baths that solubilize the organic structures such as cells remaining from organic matrix, fibers and proteins, remaining only the mineral portion […] and sterilized through Gamma Radiation (25kGy)”, according to the manufacturer.

The architecture of block grafts fits perfectly to the function of a scaffold, which allow cell adhesion and migration through the porous, also the chemical properties of DBBM allow a superior moistening in comparison to other bone

substitutes (Gehrke et al., 2019; Weibrich et al., 2000). This property allows the DBBM to be soaked with patient's blood and growth factors in the moment of its placement, enhancing the proliferation of blood cells, endothelial cells, and osteoblasts (Amerio et al., 2010).

CONCLUSION

DBBM presented as a viable alternative for maxillary horizontal augmentation, with clinical and radiographic behavior comparable to AB.

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3.3 Publicação 3[3]

DEPROTEINIZED BOVINE BONE BLOCK FOR HORIZONTAL RIDGE AUGMENTATION: A CLINICAL SPLIT-MOUTH PROSPECTIVE STUDY – PART II, HISTOLOGIC AND MICROTOMOGRAPHIC EVALUATION

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Key-words: Alveolar ridge augmentation; Deproteinized Bovine Bone Block; Randomized Clinical Trial; Histology; Microarchitecture

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ABSTRACT

Objectives To evaluate the micro architectural and histological patterns of new bone formation in areas grafted with deproteinized bovine bone block (DBBM) compared to autogenous graft from mandibular ramus (AB).

Materials and methods Twelve patients with edentulous atrophic maxillary ridges were submitted to maxillary ridge reconstruction surgery under general anesthesia. Each side of the anterior maxilla received a type of graft, according to randomization: AB or DBBM. Nine months after ridge augmentation, cylindrical bone biopsies of 2.0 x 6.0 mm were obtained of augmented sites using a 2.5 mm diameter trephine bur, including graft, interface, and native bone. Biopsies were submitted to microtomography (micro CT) to evaluate the micro architectural features of new bone formation (tissue volume [TV], and surface [TS],Bone volume [BV], an surface [BS] bone volume percent [BV%],, bone surface density [BS/TV], specific bone density [BS/BV], trabecular number [Tb.N], separation [Tb.Sp], thickness [Tb.Th] and pattern [Tb.Pf], porosity, and connectivity). From each group, six samples were randomly selected for histological analysis. Histological images were qualitatively evaluated for tissue pattern and osteocytes infiltration. Further the areas of new bone formation, vital bone, soft tissue, residual biomaterial, and necrotic bone were defined by Hue - Saturation - Value thresholding, with dedicated software Image J.

Results: TV, TS, BV, BV/TV, BS/TV, Tb.N, Tb.Sp, Tb.Pf, porosity, were equal for both groups. BV/BS was larger for DBBM (14.69 ± 2.66) than for AB (12.01 ± 2.16), but Tb.Th was larger in AB (0.55 ± 0.33mm) than in DBBM (0.28 ± 0.04mm). At the histologic analysis, area of mineralized tissue was larger in the AB than in DBBM (55.29% ± 11.24 vs 37.04% ± 9.04, $p<0.05$), vital bone and soft tissue areas did not differ between groups.

Conclusion DBBM presented suitable incorporation to the grafted site, allied to new bone formation, bone volume, bone density, and soft tissue areas similar to AB.

Keywords Alveolar ridge augmentation; Deproteinized Bovine Bone Block; Randomized Clinical Trial; Histology; Microarchitecture

INTRODUCTION

The ideal bone substitute should present attributes as osteoconductivity and biocompatibility, to avoid antigenicity and be gradually resorbed, as new bone formation takes place (Schwarz et al., 2017; Chiapasco, 2009; Jensen et al., 2006). The autogenous bone (AB) graft presents the best known osteogenic, osteoconductive, and osteoinductive properties, however the need of a donor site, limited amount and short-term resorption are some limitations of its use (Troeltzsch et al., 2016; Nkenke & Neukam, 2014).

Bone substitute materials have been under copious development in the past years, in attempt to achieve regenerative properties compatible with those of AB (Stumbras et al., 2019; Ebrahimi et al., 2017). Among the possible bone substitute materials, xenogeneic bovine derived bone, also known as deproteinized bovine bone matrix (DBBM), has been studied for 30 years, and is one of the best documented bone substitutes of animal origin (de Azambuja Carvalho et al., 2019; Kacarevic et al., 2018; Troeltzsch et al., 2016; Esposito et al., 2009). The incorporation of DBBM to the grafted site has been reported in trials evaluating grafts after up to 10 years, and due to its low resorption pattern the granules of DBBM remain in the grafted site all the time long, being surrounded by new host bone (Danesh-Sani et al., 2017; Degidi et al., 2013). Bone augmentation procedures in humans, using DBBM demonstrated a thickness increase up to 7.72mm, with resorption rates ranging from 10 to 49% (de Azambuja Carvalho et al., 2019). Also, DBBM grafts have a success rate of 98%, considering the implant placement and graft survive.

DBBM is produced in diverse presentations and by innumerous commercial brands. The two most common presentations are granules and blocks. DBBM granules presented the most reliable results in procedures as sinus lift, socket preservation, and

cavity defects, when the remaining anatomy provides stability and support to the grafted material (Kolerman et al., 2019; Mendoza-Azpur et al., 2019; Troeltzsch et al., 2016; Block et al., 2012).

DBBM in blocks have an intrinsic structural design, which promotes stability and facilitate cell migration (Benic et al., 2017; Veis et al., 2015). However, DBBM blocks need to be processed for cleaning and removal of organic and antigenic remnants, so they become suitable for human's health-related application.(Barbeck et al., 2014). The purification process negatively affects the mechanical properties of DBBM blocks, making it hard to fixate with screws in the host bone (Gehrke et al., 2019; Kacarevic et al., 2018; do Desterro et al., 2014; Weibrich et al., 2000). Therefore, the exclusive use of chemical treatment could improve the biomechanichal and surface characteristics of DBBM blocks. However, little is known regarding the clinical behavior and host bone interaction of chemically treated DBBM.

The aim of this randomized clinical trial was to evaluate the incorporation of DBBM block grafts in comparison to AB block grafts, by histological and microtomographic analysis.

MATERIAL AND METHODS

This study was conducted in accordance with the Helsinki Declaration and was approved by the local and national ethical committee in research under the number 673443017.0.0000.5416.

This study is a "follow-on" from a prospective randomized clinical trial (Carvalho et al., 2020 - unpublished data), which assessed new bone formation and success rate of DBBM block (Bonefill® Porous block, Bionnovation, Brazil), used as graft for horizontal ridge augmentation.

Sample selection and surgical procedures

Sample selection, surgical procedures and follow up were previous described by Carvalho et al. (2020, unpublished data). Briefly, 12 healthy adults were randomly selected from a list of patients searching for oral rehabilitation with implants. The patient needs to fit the following inclusion criteria: adult (over 18 years old), of any sex, with totally edentulous maxillary ridge, remaining alveolar bone width between 2 and 10 mm, and remaining alveolar height of at least 10mm, and accept to participate in the study by signing the Informed Consent Form.

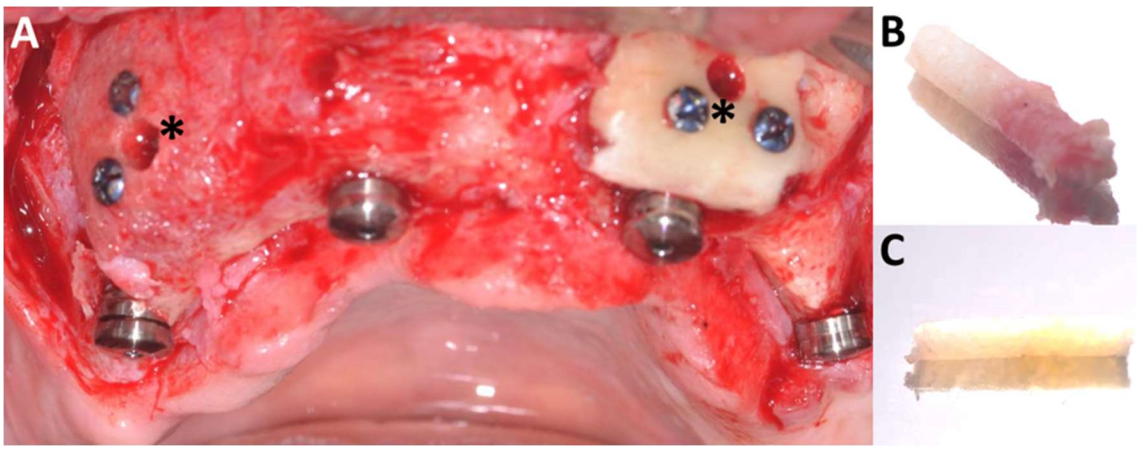
The anterior maxillary ridge was accessed with an crestal incision and two relaxing incisions, followed by cautious periosteal detachment, thus the recipient site in maxilla was decorticated with stainless-steel pear shape burs (H251E Maxicut, Komet Brazil, Brazil) and perforated with 16mm conic burs (H33L 701, Komet Brazil, Brazil). After, the mandibular ramus was accessed and the autogenous bone block (AB) was removed with piezoelectric motor (Piezosonic, Driller, Brazil) and piezoelectric saw (ES007A-Driller, Driller, Brazil), disc saw (Härte instruments, Brazil) and surgical chisels (Quinelato, Brazil). The DBBM was removed from sterile package and moistened in saline solution according to the manufacturer orientations.

AB and DBBM blocks were reshaped to remove sharp angles and then were adapted and fixed to the receiving site by titanium screws of 1.4x12mm, with extended heads (Bionnovation, Brazil), then covered with polytetrafluorethylene (PTFE) non resorbable membranes (Surgitime PTFE 0,25mm, Bionnovation, Brazil), fixed in the residual bone with titanium tacks (AutoTac®, BioHorizons Implant System, EUA). Finally, the surgical wounds were closed by first intention with polyglactin 910 (Ethicon, Johnson & Johnson, Brazil).

After 9 months surgical procedures for implant placement and clinical measurements of grafts were undertaken in outpatient conditions. A complete mucoperiosteal flap was detached to remove the membranes, membrane's tacks, and the screws in the cases which the graft biopsies or implant placement were impaired by them, otherwise those screws have been kept in position.

Two cylindric bone biopsies of 2.0 x 6.0mm were obtained with a 2.5mm diameter trephine bur (Härte instruments, Brazil), retrieved at 1200rpm in a perpendicular way to the augmented area (Figure 1). Bone biopsies were obtained from both, AB and DBBM, and immediately placed in sterile saline and fixed in formaldehyde 4% (Synth, Brazil) buffered with sodium phosphate buffer 0.1M (pH 7.2) for 48h. After, the samples were washed in flowing water during 6h and stored in a 70% ethanol solution.

Figure 1. Surgical approach to the augmented sites to implant placement, 9 months after grafting procedure, (A)Clinical aspect of both augmented sides, DBBM in the left side and AB in the right. (*) indicates the sites where the biopsies were performed.(B) Bone fragment of the area augmented with DBBM block. (C) Bone fragment of the area augmented with autogenous block of mandibular ramus. Carvalho, P.H.A. Araraquara, 2020



Source: From author.

Microtomographic evaluation

First, specimens were scanned in a computed digital microtomography system, SkyScan (SkyScan 1176 Bruker Micro CT, Aatselaar, Belgium), with voxel resolution

of 9 µm (50Kv and 500µ), Cu-Al filtered and rotation settled to 0.3mm. The obtained images were stored and reconstituted by determining the Region of Interest (ROI) in the software NRecon (SkyScan, Version 1.6.6.0). The software Data Viewer (SkyScan, Version 1.4.4 64-bit) was used to correct the spatial orientation of images and to set the aspect view of analysis, in three plans: coronal, sagittal, and longitudinal view.

After reorientation, the ROI was created in software CTAnalyser – CTAn (Bruker MicroCT Version 1.12.4.0), and the image measurements were performed according to a gray scale threshold. The range of threshold was settled between 25-90 shades of gray, which defined the newly formed bone volume and its characterization. For micro architectural analysis, the parameters were: tissue volume [TV], tissue surface [TS],bone volume [BV], bone surface [BS] bone volume percent [BV/TV],, bone surface density [BS/TV], specific bone density [BS/BV], trabecular number [Tb.N], trabecular separation [Tb.Sp], trabecular thickness [Tb.Th] and trabecular pattern [Tb.Pf], porosity percentage (por.), and connectivity (conn.).

Histologic evaluation

The same bone fragments used for microtomography were again washed in flowing water for 6h and decalcified in ethylenediaminetetraacetic acid (EDTA) at 14% (Synth, São Paulo, Brazil). EDTA was changed each two days, for two weeks or until the specimens were susceptible to a needle perforation. The decalcification was immediately followed by dehydration of samples in progressive ethanol concentrations, from 50ºGL to absolute ethanol, and finally included in paraffin blocks.

Histologic slides preparation was performed with an microtome (Micron HM 325, Thermo Scientific, UK), and cuts of 4 µm thickness were obtained in the longitudinal aspect of the sample, for each sample three histologic slides were obtained, the first

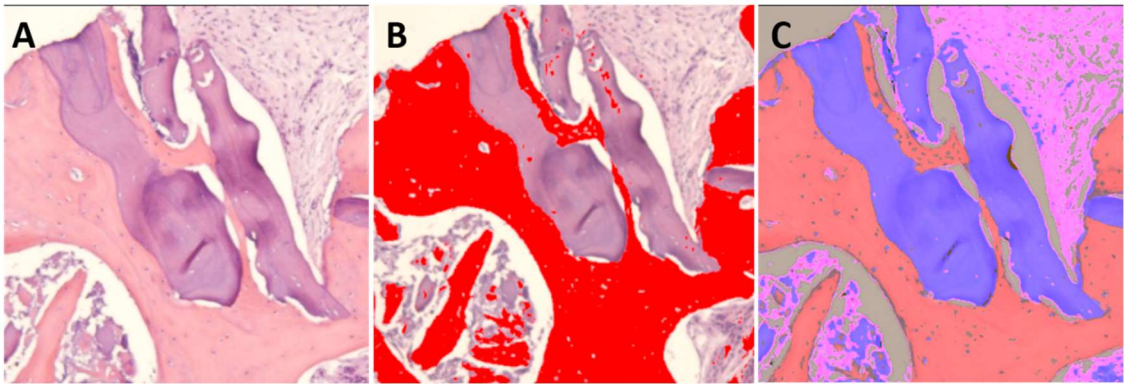
one once the sample was completely displayed in slide, then two subsequent cuts with 28μm interlude. Histologic slides were stained in Hematoxylin & Eosin (HE), for qualitative and quantitative histomorphometry.

An experienced examiner performed the histomorphometry analysis in triplicate, the quantitative analysis was limited to the graft and interface area. In attempt to standardize the technique, for all specimens it was considered a 6mm distance, from the buccal margin of the graft to the native bone.

Samples were first scanned and digitized to RAW files in computer by an optical microscopic (Diastar – Leica Reichert Jung Products, Germany) with attached digital camera (Olimpus, CAMEDIA C50/60 Wide Zoom, Japan), using an objective lens with 4.0/100x magnification and 10x optical zoom.. The acquired images were processed in the software ImageJ (Image J, NIH, Bethesda, US). Total sample areas was delimited and settled as 100%, new formed bone, remnant biomaterial and soft tissue were selected using the color threshold tool, in the HSV/HSB configuration (Figure 2), empty spaces and identifiable structures were subtracted of calculation when present. The final percentage of each component was obtained by the mean of triplicates. The parameters evaluated at the histomorphometry were mineralized tissue (%), soft tissue (%), proportion of bone/soft tissue, the vital and non-vital bone, and the residual biomaterial.

Figure 2. Method for determining the area of new bone, residual biomaterial and soft tissue in the software Image J, H&E (40x). (A) Original scan of light microscopy sections stained with hematoxylin and eosin. (B) Example of threshold limitation in HSV/HSB method for bone tissue, (C) Segmentation

of different surfaces after threshold selection, Magenta: new bone, Blue: residual biomaterial, and Pink: soft tissue. Carvalho, P.H.A. Araraquara, 2020.



Source: From author

RESULTS

All patients presented successful graft incorporation and were able to receive dental implants. Whenever any complications occurred, they were treated with local therapy, and do not affect the graft incorporation in any patients of this study. Bone biopsies were taken for all the patients: 12 samples of each graft were used for microtomographic assessment and 6 samples of each graft were randomly selected to histologic analysis.

Microtomographic evaluation

No statistical difference was observed between AB and DBBM bone biopsies in regarding TV, TS, BV, BS, BV/TV, BS/TV, Tb.N, Tb.Sp, Tb.Pf, connectivity, and porosity percentage ($p>0.05$, ANOVA One Way) (Table 1).

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Table 1. Microtomography parameters evaluated in biopsies obtained from augmented sites in maxilla. A comparison between the autogenous bone from mandibular ramus (AB*)* and deproteinized bovine bone block (DBBM). Carvalho P. H A., Araraquara, 2020

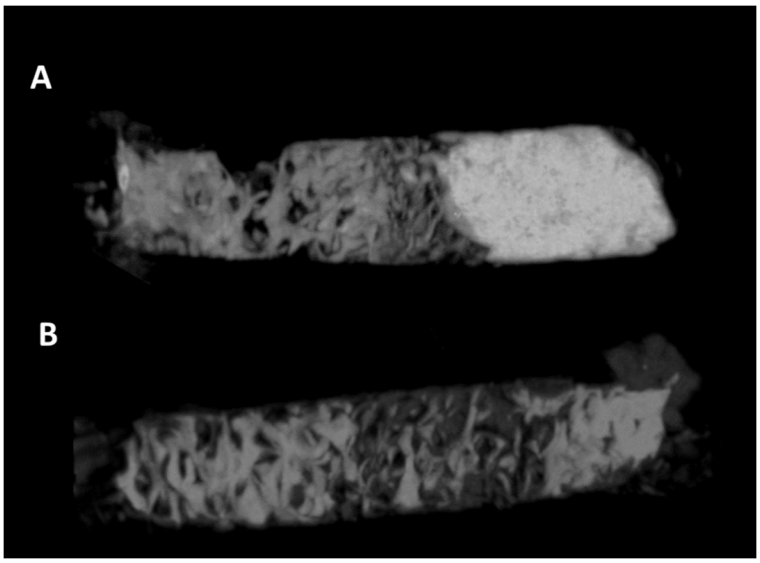
	MicroCT parameters [mean (SD)]						
Material	**TV (mm^3)**	**TS (mm^2)**	**BV (mm^3)**	**BV/TV (%)**	**BS (mm^2)**	**BS/TV (mm^{-1})**	**BS/BV (mm^{-1})**
AB	32.47 ± 12.87	64.02 ± 17.91	8.54 ± 3,39	29.47 ± 12.39	100.57 ± 35.31	3.39 ± 1.19	12.01 ± 2.16*
DBBM	37.9 ± 14.81	70.49 ± 15.31	8.34 ± 2.85	24.32 ± 9.05	117.48 ± 35.38	3.34 ± 0.81	14.69 ± 2.66*
Material	**Tb.N (mm^{-1})**	**Tb.Sp (mm)**	**Tb.Th (mm)**	**Tb.Pf**	**Por. (%)**	**Conn.**	
AB	0.64 ± 0.35	0.58 ± 0.25	0.55 ± 0.33†	-1.62 ± 8.84	70.53 ± 12.39	1679.79 ± 2133.84	
DBBM	0.84 ± 0.25	0.58 ± 0.18	0.28 ± 0.04†	-2.34 ± 4.31	75.68 ± 9.05	1147.71 ± 849.56	

*Indicate statistical difference between rows (ANVOA One way, p<.05). † Indicate statistical difference between rows (U-Mann Whitney, p<.05). Source: From author

BV/BS in AB group were 12.01 ± 2.16 versus 14.69 ± 2.66 in DBBM ($p<0.05$ ANOVA One Way). Tb.Th of AB was 0.55 ± 0.33mm, thicker than in DBBM, which presents Tb.Th mean values of 0.28 ± 0.04mm ($p<0.05$,U-Mann Whitney) (Table 1). In the tridimensional reconstruction of the bone biopsies, both grafts presented a good incorporation and a threshold similar to basal bone, the threshold of remnant autogenous graft was higher due to its more cortical aspect. (Figure 3)

Figure 3. Tridimensional reconstruction of the microtomography data from biopsies of grafted areas in maxilla. (A) Bone fragmented biopsied from a site augmented with autogenous block from mandibular

ramus. (B) Bone fragmented biopsied from a site augmented with deproteinized bovine block (DBBM). Carvalho, PHA. Araraquara, 2020



Source: From author.

Histologic evaluation and histomorphometry

The histologic evaluation and the histomorphometry were performed in 6 random samples of each group, AB and DBBM. All samples were carefully handled during the process to keep the buccal area identified. Native bone was present in the samples of both groups, organized in a complete lamellar structure, of thick interconnected trabecula with normal marrow space in between. Biomaterial remnants were evident in the samples of DBBM group, but surrounded by woven bone and some thin lamellar trabeculae, with detectable osteoblasts, also some osteoid was visible and new bone seems to be deposited on the surface of biomaterial (Figure 4 A). The separation line between the graft and native bone was clearly visible in the AB group samples, also the cortical pattern of mandibular bone was present, a thicker trabecular structure was present, and some empty osteocytes lacunae, surrounded by low saturation eosin staining areas, were visible indicating areas of necrotic bone (Figure 4 B).

Table 2. Parameters of histological quantitative analysis of AB and DBBM graft biopsies. Carvalho, P.H.A., Araraquara, 2020

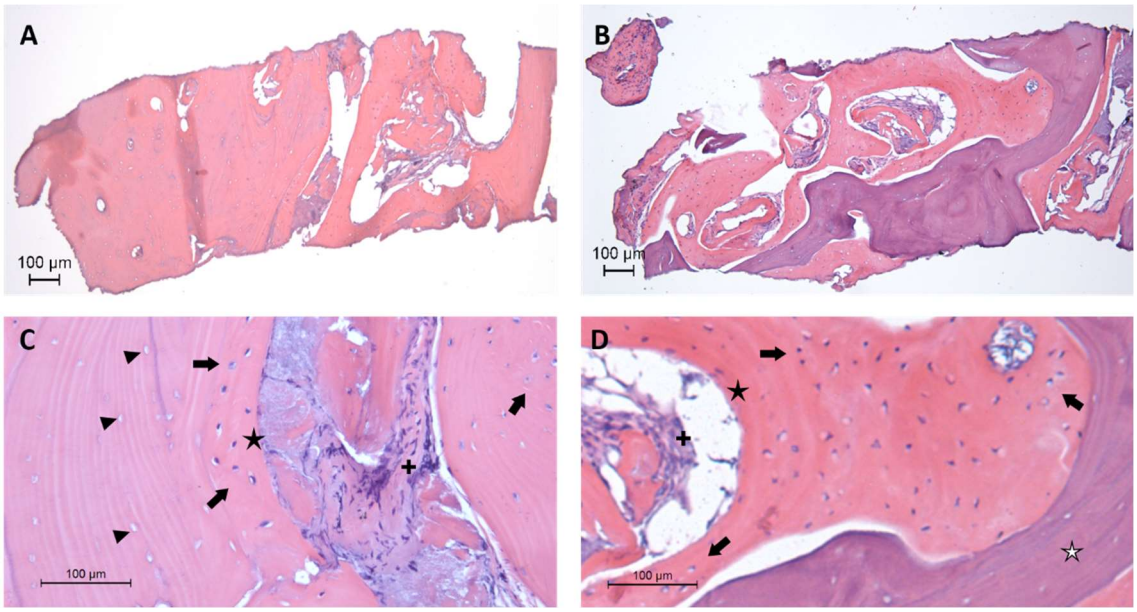
	Bone tissue	Soft tissue	BT/ST ratio	Vital bone	Non-vital bone	Biomaterial
AB	55.29 ± 11.24*	25.82 ± 6.12	2.22 ± 0.56	46.52 ± 7.01	15.09 ± 6.86	-
DBBM	37.04 ± 9.04	22.89 ± 2.29	1.64 ± 0.48	37.04 ± 9.04	-	20.84 ± 6.45

*Statistically significant difference (Unpaired t-test, $p<0.05$). Source: From author.

The area of mineralized tissue was larger in AB than in DBBM (55.29% ± 11.24 vs 37.04% ± 9.04, $p<0.05$), however in AB group the remnants of graft were undifferentiable of new bone, therefore the areas with non-vital bone, characterized by bone lacunae without cells were marked as non-vital bone. Then, considering only the vital bone areas there was no difference between the percentage of vital bone in AB and DBBM (46.52% ± 7.01 vs 37.04% ± 9.04).(Table 2)

The soft tissue area, and the ratio between bone and soft tissue areas did not differ between groups. The remnant biomaterial was considered only in the DBBM samples and represented 20.84% ±6.45 of total area.

Figure 4. Histological light microscopy of biopsies taken from augmented areas after 9 months healing.(A) Overview biopsy off augmented area with autogenous bone from mandibular ramus [AB] H&E 40x. (B) Overview biopsy of augmented area with deproteinized bone block [DBBM], H&E, 40x. (C) High magnification (100x) of a representative area of AB augmented area,. (D) High magnification of a representative area of DBBM augmented site. Black arrows indicate regions with plenty of cells, black arrows heads indicate the empty osteocytes lacunae, black stars indicate new formed bone, white star indicate the residual biomaterial, and plus sign the osteoid matrix. Carvalho, P.H.A., Araraquara, 2020.



Source: From author

DISCUSSION

The aim of this study was to evaluate the incorporation and the microarchitecture of augmented ridges with a new commercially available deproteinized bone block. The deproteinized bone block used was chemically purified in alkaline baths and not submitted to any kind of low/high temperatures. In this split mouth randomized trial, we performed histological and microtomographic analysis, using autogenous bone from mandibular ramus as control.

DBBM has been used as bone substitute material for the past 30 years, and presented good results in *in vitro* and *in vivo studies,* most of them in its particulate form (de Azambuja Carvalho et al., 2019; Stumbras et al., 2019; Broggini et al., 2015). For example, Block et al.(2012) found a good stability in the volume of alveolar ridges

augmented with DBBM in granules, with a resorption rate lower than 25% after 500 days.

In the past, the block form of DBBM was not expected to promote enough new bone formation, and its use was recommended with the addition of morphogenetic proteins or growth factors (Schwarz et al., 2010). However, changes in the processing and purification treatment improved the physic and chemical characteristics and allowed a higher hydrophilicity (Cingolani et al., 2018), interconnected micro porosity (do Desterro et al., 2014), without change the biocompatibility (Melchior et al., 2018).

AB is still the gold standard material for ridge reconstruction and augmentation procedures in oral and maxillofacial surgery (Sakkas et al., 2017; Troeltzsch et al., 2016). However, some limitations as the need of a donor site, the post-operative morbidity, and a resorption rate, which can achieve up to 49%, are some of the reasons to research for feasible bone substitutes, to use in alveolar ridge augmentation and bone regenerative procedures (Pereira et al., 2019; Nkenke & Neukam, 2014; Sbordone et al., 2009).

Most studies performed with bone substitute materials took place in sinus lift procedures, a model of cavity defect, surrounded by health bone, which implicates in high quality bone augmentation procedures regardless the tested materials (Kolerman et al., 2019). However even in the sinus lift procedures the incorporation of the bone substitute and the new bone formation remains controversial and some authors showed that deproteinized bovine bone could remain in the grafted area for uncertain time (Mordenfeld et al., 2010; Traini et al., 2007). In our histological samples it is possible to identify the bovine bone remnants surrounded by new bone, this maintenance for longer period could represents an advantage, due to the mechanical properties and the slowest resorption rate.

Nevertheless, the loss of connectivity between the new formed trabecula and the native bone can be a disadvantage of DBBM granules, it occurs due to the absence of a preformed scaffold and stable structure, which leads to the graft presents as a necrotic bone as most distant from implantation bed (Kolerman et al., 2019; de Lange et al., 2014). It is supposed that block grafts could provide this structural stability to enhance bone formation in all grafted area (Benic et al., 2017; Veis et al., 2015), however our histological samples showed an immature bone, soft tissue, and residual biomaterial in the most buccal aspect, confirming the groundbreaking findings of Araujo et al. (2002) whose demonstrated, in dogs, the dynamics of new bone formation at the interface of DBBM in comparison with AB.

De Lange et al. (2014) performed a prospective study evaluating ridge augmentation in human's maxillary sinus. Their study compared the microtomographic and histological results from deproteinized bovine bone and biphasic calcium phosphate, and they showed a mineralized surface up to 20% of total area for both grafts, but with different trabecular thickness between biomaterial and native bone. These results agree with the lower trabecular thickness (Tb.Th) observed in DBBM, in our study. According to some authors minor trabecular thickness can indicate new bone formation (Kivovics et al., 2017; de Lange et al., 2014).

The microtomographic parameters evaluated in this study were similar in the control and tested groups, without statistical difference for bone volume, percentage of bone volume, bone surface and bone surface density, trabecular number and separation, or porosity. Trabecular thickness (Tb;Th) and specific bone surface (BS/BV) were the only parameters with statistical difference, Tb.Th was higher in the AB, and BS/BV in an inverse proportion, which can be explained by the most cortical nature of the AB from mandibular ramus, also observed in the histological analysis.

Also, a smaller trabecular thickness corroborates the expected behavior of a porous micro architecture of DBBM (de Lange et al., 2014).

The trabecular number (Tb.N) dod not presented statistical difference, however the values seemed to be slightly higher in DBBM, this increase in trabecular number could act as a structural compensation for the reduced trabecular thickness in DBBM group.

New bone formation from DBBM samples were not statistically different from the AB, reaching up to 37% of the biopsied area. In comparison, particulate grafts in long-term studies presented low rates of new bone formation (Mendoza-Azpur et al., 2019; Degidi et al., 2013; Hämmerle et al., 2008; Steigmann, 2006).

Slotte et al.(2017) in a comparable study, evaluated horizontal augmentation with cancellous bovine bone blocks in nine patients, and identified a percentage of new bone formation ranging from 7.7 to 34.5%, 21.4% in average, and a mean percentage of residual biomaterial of 20.43%, ranging between 2.9 and 48.2%.. The results of our trial are agreed with the actual literature for different DBBM, supporting the previous findings of case reports and retrospective studies.

The graft incorporation occurs in a series of coordinated events, starting with a cellular reaction at the host bone, followed by growth factors, cytokines and blood impregnation at the block graft (Barbeck et al., 2016; Ghanaati et al., 2014; Spin-Neto et al., 2014). These early events lead to macrophages, monocytes, and osteoblasts migration, to the new bone formation and graft incorporation (Shi et al., 2018). These mechanisms differ in quantity and quality for different graft types (Ghanaati et al., 2012).

Schmitt et al. (2013), demonstrated the potential of incorporation and new bone formation of a DBBM block, using an animal model in pigs calvaria. Bovine bone

has a micro architectural pattern which enhance its incorporation in the bone-graft interface, due to some features as wettability, chemical and physical stability, and a micro porosity like the human marrow. Also, Schmitt et al. (2013), showed that adding growth factors as BMP and VEGF to the bovine bone grafts did not contribute significantly to a greater new bone formation. Other animal studies evidenced the osteoconductive and new bone formation for different presentations of DBBM (Moest et al., 2015; Veis et al., 2015; Thorwarth et al., 2006).

In this study, there were no immunohistochemical or molecular analysis to enhance the results of new bone formation and vital bone evaluation, which is a limitation of the adopted method. Also, the limitation of a specific threshold for new formed bone, graft material and residual bone was also not applicable to our samples in the microtomography. However, the samples were processed according to the standard histological methods for immunohistochemistry, and microtomographic data are stored in a large institutional database, therefore future studies can be performed using the same sample to segment the mineralized tissue and correlate the histological and microtomographic findings.

AB still presents the most reliable results, but DBBM poses as a predictable and safe alternative to alveolar ridge reconstruction. DBBM blocks provides a scaffold to new bone formation, with good microarchitectural arrange, promoting an augmentation procedure with similar results to autogenous bone.

CONCLUSION

DBBM presented suitable incorporation to the grafted site, new bone formation, bone volume, bone density and soft tissue similar to AB. It is an adequate bone substitute to be used in horizontal ridge augmentation procedures.

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3.4 Publicação 4[4]

CELLULAR IMMUNOLOGIC RESPONSE OF HUMAN OSTEOBLASTS TO DIFFERENT PRESENTATIONS OF DBBM.

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Key-words: Deproteinized bovine bone (DBBM), Guided bone regeneration, Purification, Cellular inflammatory response

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ABSTRACT

Objectives This study evaluated the cellular immunologic response of osteoblasts exposed to different presentations of a low temperature non-sintered deproteinized bovine bone matrix (DBBM) granules (G) and block(B), and the purity degree of both materials. *Materials and methods* Six different baths of a commercially available DBBM block (Bonefill® Porous Block) and one bath of DBBM granule (Bonefill® Porous) were embedded in paraffin, cut in a microtome and stained to identify the mineral structure and organic or cellular remnants. Samples of the same baths were processed in TRIZOL for RNA extraction and quantification by Nanodrop. For the immunologic cell reaction assay primary humans osteoblasts (pOB) of three different donors were cultured in 48-well plates for 24h, after initial growth they were exposed to DBBM block (pOB+B) or granules (pOB+G), or none (control) for 1, 3 or 7 days. Pro-inflammatory cytokines expression by pOB was evaluated in triplicate from the supernatant by cross-linked ELISA assay, in the three timepoints. Osteopontin immunofluorescence (OPN) and histological staining were performed at day 1 and 7 to evaluate the cell growth in the DBBM surfaces, also the total DNA amount and LDH were assessed for the quantification of cell viability. *Results:* A lamellar structure was present in demineralized sample slides. Organic remnants were present in DBBM blocks, and 45.55% (±7.12) of osteocytes lacunae presented cellular remnants in blocks against 17.31% (±1.31) in granules. In 3 of 5 batches of blocks it was possible to find and isolate bovine RNA , any trace of RNA was found in granules. Expression of TGF-ß1 by pOB+G at day 7 (218.85[234.62] pg/ml) was higher than both pOB+B (24.34[15.59] pg.ml) and control (62.6[39.55] pg/ml) ($p<0.05$), pOB+B presented the lowest amount of TGF-ß1 secretion at the end of evaluation (30.22[14.94] pg/ml, $p<0.05$),. For IL-6 and OPG, there was no statistical difference between groups, IL-8 secretion was higher in the pOB+G at day1 (6.49[2.57] ng/ml), IL-8 release increase in all groups, The pOB+G induced more IL-8 secretion than control (3.03[3.38] ng/ml, $p<0.05$). Considering the kinetics of cytokines release during the study period all groups presented a similar pattern of cytokines increment for Interleukin-6 (IL-¨6),Interleukin-8 (IL-8) and osteoprotegerin (OPG) . lactate dehydrogenase (LDH) expression corrected by DNA amount did not present statistical difference. Cellularity was observed in both materials surface at day 7, in H&E and OPN staining. *Conclusion:* Despite the difference in the purity degree both materials have not upregulated the expression of pro-inflammatory cytokines by osteoblasts.

Key-words: Deproteinized bovine bone (DBBM), Guided bone regeneration, Purification, Cellular inflammatory response

INTRODUCTION

Bone regeneration is a common procedure in regenerative medicine and dentistry and there are innumerous commercially available materials which are indicated for bone regeneration procedures.[1,2] Those materials differ from each other in chemical composition, physicochemical structure, mechanical properties and producing or purification processes.[3,4] Regarding their origin the bone materials can be autologous (derived from the same individual), allogeneic (from different individuals of same specie), xenogeneic (from different species) and synthetic or alloplastic. [5,6]

Each type of biomaterials demands a particular processing method, and for the allografts and xenografts the purification is crucial to remove the organic remnants of donor, which potentially carry pathogens, proteins or foreign genetic material which can lead to disease transmission or exacerbates inflammatory reaction.[7,8] Bone substitutes materials should accomplish a series of requirements to be suitable for clinical use. Besides the main requirements are the osteoconductive, osteoinductive and osteogenic properties the biocompatibility is one of the most important characteristic of a biomaterial.[9–12]

The osteogenesis and graft incorporation are mediated by several cellular and molecular pathways that promote and regulate the activity of osteoblasts and osteoclasts, due to this the bone substitutes also must not be cytotoxic, be compatible with human cells, not induce foreign body reaction, serve as scaffold for cellular migration and vessel formation, be hydrophilic and must have mechanical resistance similar to the host bone. [13]

Regarding the cytotoxicity and inflammatory reaction, the purity has been considered to be a good parameter to evaluate the suitability of biomaterials. Non-autologous bone materials should ideally contain a pure mineral structure, without

organic remnants or antigenic properties. [7,8] However, some of commercially available purification methods for allografts or xenogeneic bone materials present some degree of organic remnants These purity has been classified by Ghanaati et al. [8] in 5 levels ranging from 0 (no organic remnants and no lamellar bone structure) to 4 (material containing donor cellular remnants in the trabecula).

The current purification methods described implicate in complex physical and chemical steps to free the material from immunogenic components and attend to safety requirements, but despite the efforts for a standard purification process some naturally derived bone materials still containing cellular or organic remnants in their composition, by other side materials that have been proved to be free of organic components lose their lamellar structure as the purification process changes its physicochemical properties. [8,14,15]

Bone substitutes have been applied for several medical indications for example to treat fractures, to maintain bone structure and mechanical properties, and to support oral and facial bone regeneration. [6,11,13] However, as most studies in bone regeneration and bone substitute materials focused on new bone formation or bone maintenance, less is known regarding the biology of the interaction between bone substitutes and the receptor site, which could be affected by the biological properties of each biomaterial. As well as there is lack of information about how the manufacturing, purification process and final purity degree affects the cellular response to bone substitutes.

The aim of this study is to evaluate the cellular reaction *in vitro* from primary human osteoblasts (pOB) exposed to different forms of deproteinized bovine bone matrix (DBBM) (Bonefill® Porous Block/Granules, Bionnovation, Bauru, Brazil),

submitted to exclusively chemical purification process, and to evaluate the histological structure and biochemical composition of each tested material.

MATERIAL AND METHODS

Material origin

Bonefill® Porous (Bionnovation, Bauru, Brazil) is a commercially available DBBM in chips and blocks, derived from bone of bovine femur. According to the information obtained by the manufacturer's manual inside the product pack, Bonefill® is obtained by "crushed fresh bone submitted to a sequence of baths that solubilize the organic structures such as cells remaining from organic matrix, fibers and proteins, remaining only the mineral portion […] and sterilized through Gamma Radiation (25kGy)", according to the manufacturer. The biomaterial originates from tracked Brazilian herd, declared free of Bovine Spongiform Encephalopathy (BSE) according to the International Zoosanitary Code and Scientific Seeing Committee of the European Union (SSCEC of August 2005). Also, according to the manufacturer, the purification process does not submit the bovine bone to high temperature treatment.

Ex-vivo evaluation

Sample preparation

Five different batches of Bonefill® Porous Block (Bionnovation, Bauru, Brazil) and one of chips were randomly obtained directly from manufacturer in two different time points. The samples were partitioned, under sterile conditions, in two parts: one piece for histological analysis and another for RNA extraction.

For Histological analysis samples were treated as previously described. [7,8] In brief, the samples were decalcified in 10% Tris-buffered EDTA solution at 37 °C for 7 days. After, samples were dehydrated in a series of increasing alcohol concentrations and xylene in a preprogrammed tissue processor (TP1020, Leica Biossytems Nussloch Gmb) and embedded in paraffin blocks. Using a rotatory microtome (Leica M2255, Wetzlar, Germany) seven slides of 3-5 µm thick were obtained from the most central part of material and prepared for Hematoxylin and eosin (H&E) and AZAN trichrome histological staining, also Tartrate-resistant acid phosphatase (TRAP) was performed to assess the presence of multinucleated cells or osteoclasts.

Histological analysis

The histological analysis was performed with a Nikon Eclipse 80i light microscope to evaluate the macro and microstructure of the biomaterial, as well as the arrangement and presence of its organic and inorganic components. A microscope-connect DS-Fi1 Digital camera and a DS-L2 digital sigh control unit (Nikon, Tokyo, Japan) were used to scan and digitalize slides.

From each sample the H&E stains were used to identify osteocytes lacunae and cellular remnants presence. The total bone lacunae and the lacunae with cellular remnants were counted in the NIS-Elements software (Nikon Instruments Inc., Melville, NY) at a 100x magnification. Azan trichrome was used to qualitatively assess bone matrix and the presence of collagen remnants, and TRAP staining was used for TRAP+ cells searching.

RNA extraction

Samples for total RNA extraction were laid inside 2.0 ml cryogenic tubes and immersed for 5 minutes at liquid nitrogen. After, samples were removed from cryogenic tubes

with aid of a sterile forceps, placed in a sterile plastic covering and smashed with a hammer, all under sterile and RNAase free conditions.

The powder obtained from this process was placed in a new sterile 1.5ml tube and the total RNA extraction and purification was performed using 1ml of TRIZOL reagent (Sigma-Aldrich, Brøndby, Denmark) added to each sample and incubated for 15 minutes at room temperature and for 2h at 4 °C. Purification was performed according to manufacturer instructions. Briefly, 200 µl of Chloroform was added to each tube following by 10s vortex and incubated at room temperature for 15 minutes. Tubes were centrifuged at 12000 g for 15 minutes at 4 °C, then the aqueous phase (transparent phase), containing RNA, was transferred to a new 1.5 ml tube before addition of 500 µl of isopropanol.

These tubes were incubated at room temperature for 10 minutes for RNA precipitation and then submitted to a new centrifugation step (12 000 g, 4 °C, 10 minutes), after which supernatant was removed and a pellet containing RNA was formed. The pellet was submitted to a DNase digestion step with 2. µl of DNase I stock solution (Qiagen RNase-free DNase set, Quiagen, Hilden, Germany), 10 µl of RDD Buffer (Quiagen, Hilden, Germany) and 87.5 µl of RNase-Free Water (RFW, Quiagen, Hilden, Germany), after 10 minutes incubation at 37 °C DNase digestion was stopped adding 50 µl of TRIZOL reagent and 50 µl of chloroform, mixed through pipetting. Solution were centrifuged (7200g, 10 min, 4 °C) and transparent phase was added to 250 µl of absolute ethanol and 10 µl 3M-sodium-acetate in a new tube incubated a -20 °C for 90 minutes. After, tubes were centrifuged (max speed, 10 min, 4 °C), supernatant was removed and 75% ethanol added to pellet and centrifuged again (max speed, 5 min, 4 °C). Supernatant was removed and pellets were left to dry on air inside

sterile hood. Died pellets were suspended with 11 µl of RFW, and RNA concentration measured with nanodrop spectrophotometer (NanoDrop, Wilmington, USA).

Human Osteobalsts (pOB) response *in-vitro*

Primary Cell Culture

Primary human osteoblasts (pOB) were cultured according to previous described protocol.[16,17] Informed consent was obtained from all donors. Briefly, excess tissues from surgery room were obtained from different donors whose do not present any health condition that affects bone metabolism, For the present study cells from three different donors were used up to passage 3, one bone sample from a male 6 month-old baby calvaria, other from a 42 years-old woman mandible and the third one from the calvaria of a 61 years-old man

After bone osteotomy, bone fragments which would otherwise be discarded were transferred to saline solution and taken to cell culture lab. Then bone fragments were minced and placed in 25 cm^2-cell culture flasks with Dulbecco`s Modified Essential Medium Nutrient Mixture F-12 (DMEM/F-12. Sigma Aldrich, St. Louis, MO, USA), with 10% Fetal Calf Serum (FCS, Gibco, Carlsbad, CA, USA) and 1% Penicillin/Streptomycin (P/S) (Invitrogen, Carlsbad, CA, USA) at 37°C and 5% CO2. Medium was changed twice a week and after a monolayer confluence cells were checked for phenotype and preserved at cryogen.

Cell viability and Immunologic response of pOB to Biomaterial

Human Osteoblasts from cryogen were first reactivated in 25cm^2 cell flasks during seven days or until confluence was obtained, after confluence cells were detached with

Trypsin and suspended in DMEM/F-12 with 10% FCS and 1% P/S, osteoblasts were counted with a Neubauer counting chamber and set up to a concentration of 1.5 x 10^4cells/ml.

Bovine bone block (B) samples were standardized into cylinders of 2mm radius and 2 mm height and bovine bone granules (G) samples volume was standardized to 0.25cc. Samples were prepared and placed in a 48-wells culture plate under sterile conditions.

One milliliter of cell suspension (1.5 x 10^4 cells/ml) was seeded onto blocks (pOB+B), and onto granules (pOB+G), in duplicate. Also, 1ml of cell suspension without biomaterial (pOB) and biomaterial with 1ml culture medium without cells (B and G) were used as negative control groups. After 24h of culture at 37°C and CO2, biomaterials were transferred to new wells with culture medium to evaluate just the attached cells. Assay plates were cultured at 37°C and 5%CO2 for seven days. The culture medium was changed, and supernatant of culture was collected after 24h, 72h and 7 days. Lactate-dehydrogenase assay (LDH) and Enzyme-linked immunosorbent assay (ELISA), were performed in duplicate for supernatants from the three different time points at days 1, 3 and 7. Histology and immunofluorescence were performed for samples after 24h and 7 days.

Pierce LDH cytotoxicity assay kit (Thermo Fisher Scientific, Rockford, IL, USA) used and the assay was performed according to manufacturer`s instructions and absorbance was measured with a microplate reader (Infinite M200, TECAN, Grödig, Austria) set to 490nm wavelength and 680nm correction reference reading.

Supernatants from test and control groups were collected and replaced a 1, 3 and 7 days of cultivation. The concentrations of osteoblasts inflammatory markers and growth factors as: TGF-ß1, TNF-α, IL-1ß, IL-6 and IL-8, were assessed using DuoSet®

ELISA Development Systems (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. All samples were measured in duplicate with a microplate reader (Infinite M200, TECAN, Grödig, Austria) set to 450nm wavelength and 570nm correction reference reading. Outputs were plotted o concentration estimative by interpolating a parameter logistic curve against standard in Graph-Pad Prism version 8.0 (Graph-Pad Software).

Immunofluorescence

Samples of tests and controls were stained for Osteopontin (OPN) to evaluate active osteoblasts at the material surface. Rabbit anti-human OPN (rabbit MA5-29580; Invitrogen Molecular Probes, USA), 1:200 in 1% bovine serum albumin/PBS, was used as primary antibody. Samples previous fixed in paraformaldehyde 4% were washed three times with PBS before permeabilization with 0.5% Triton-X/PBS. Permeabilized samples were washed again 3 times in PBS and incubated with first antibody for 1h at room temperature. The washing step was repeated after incubation and samples were treated with secondary anti-rabbit Alexa Fluor 546 antibody (goat A-11010; Invitrogen, Molecular probes, USA) diluted 1:200 in 1% BSA/PBS for 60 min in the dark at room temperature. The cell nuclei were DAPI counterstained.

Statistics

All statistical analysis was performed considering a 95% confidence level. The average results in each time point were compared by ANOVA Two-Way with Tukey's post hoc test for multiple comparisons.

RESULTS

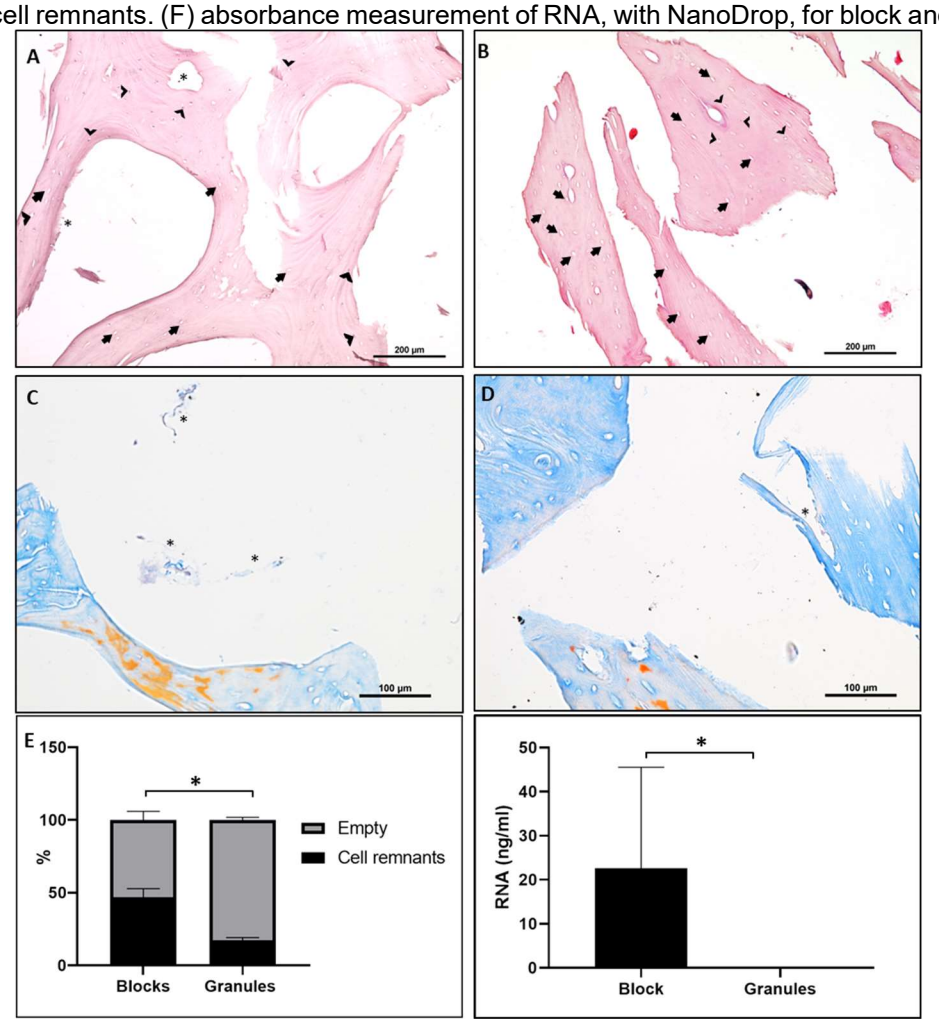
Ex-vivo evaluation

A lamellar structure was present in demineralized sample slides, with thin trabeculae and interconnected porous, as stated by manufacturer the bone blocks macro morphology is similar to the human bone, but with increased porosity. In H&E staining organic remnants were present, inside the Haversian canals and in the margin of trabecula, indicating possible fragments of periosteum not removed by the chemical baths. Also, regarding all tested lots of bone blocks (B), in average 45.55% (±7.12) osteocytes lacunae presented cellular remnants (Figure 1A). For the tested lot of granules 17.31% (±1.31) of lacunae presented some trace of organic remnants (Figure 1B)

For both materials Azan staining evidenced a mature mineralized bone, with presence of possible collagen remnants and connective like-tissue, there were Haversian canals without any organic remnants although others presented connective-like tissue fragments (Figure 1C-D). No TRAP positive cells were found in any of examined batches.

In 3 of 5 batches of Bonefill® Porous Block it was possible to find and isolate RNA. RNA amount ranged from 14.4 to 47.7 ng/µl, with a 260/280 rate of purity of 1.76 to 1.94 (Figure 1 E-G). In the samples of Bonefill® Porous Granules it was not found any trace of RNA (Figure 1 H-I).

Figure 1. Ex-vivo H&E staining and histometry of DBBM block and granules. (A) and granules (B) at 100x magnification, (C and D) Ex-vivo Azan trichrome staining of bovine bone blocks (C) and granules (D) 400x, arrows indicate empty osteocyte lacunae and arrows` heads indicate examples of osteocyte lacunae with cell or organic remnants of bovine tissue inside, Asterisks mark organic or connective tissue-like remnants outside the lacunae. (E) Ratio between empty osteocytes lacunae and lacunae presenting cell remnants. (F) absorbance measurement of RNA, with NanoDrop, for block and granules.



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Cell viability and immunological response

The release of TGF-ß1 in the group pOB+G increased at the time point 2 (day 3), and was higher than release in group pOB+B, at time point 3 (day 7) the TGF-ß1 secretion by pOB+G (218.85[234.62] pg/ml) was higher than both pOB+B (24.34[15.59] pg.ml) and control (62.6[39.55] pg/ml) ($p<0.05$) (Figure 2A). For IL-6 and OPG, there was no statistical difference between groups, for this both cytokines there was an increasing in OPG production from time point 1 to 7, in the test groups pOB+B and pOB+G (Figure 2B-C). The IL-8 secretion was higher in the pOB+G at the time

point 1 (6.49[2.57] ng/ml), and in the other time points, IL-8 release increase in all groups, but was slightly less in the control group (2.64[3.04] ng/ml).

Considering the kinetics of cytokines release during the study period all groups presented a similar pattern of cytokines increment for IL-6, IL-8 and OPG (Figure 3). Regarding TGF-ß1 release the pOB+B presented the lowest amount of TGF-ß1 secretion at the end of evaluation (30.22[14.94] pg/ml, $p<0.05$), and pOB+G presented the highest increment of TGF-ß1 from day 3 to 7 (85.68[62.89] pg/ml to 304.53[295.63] pg/ml, $p<.05$) (Figure 3A). IL-6 release in pOB+G was higher than pOB+B in days 3 (7.5 [1.32] ng/ml vs 2.6 [1.78] ng/ml, $p<0.05$) and 7 (11.31 [1.67] vs 4.91 [2.91] ng/ml, $p<.05$), but similar to control (9.01 [1.18] ng/ml, $p>.05$) (Figure 3B).

The pOB+G IL-8 cumulative secretion (8.12[1.74] ng/ml) was higher than both control (0.39[0.34] ng/ml) and pOB+B (1.72[1.80] ng/ml), for the entire evaluated period pOB+G (14.21[4.74] ng/ml) induced more IL-8 secretion than control (3.03[3.38] ng/ml, $p<0.05$), but similar to pOB+B (6.38[7.13] ng/ml, $p>0.05$) (Figure 3D).

After 24h OPG in control group (1.78[0.45] ng/ml) was higher than both pOB+B (0.75[0.26] ng/ml, $p<.05$) and pOB+G (0.31[0.14] ng/ml, $p<.05$). After 7 days OPG secretion in pOB+G (1.35[1.24] ng/ml) remains lower than control (5.87[3.46] ng/ml, $p<.05$), but OPG in pOB+B (4.29[2.85] ng/ml) was similar to control ($p>.05$).

LDH expression corrected by DNA amount did not present statistical difference.

Figure 2**.** Mean and SD of Inflammatory cytokines' concentrations in the supernatant, at different culture timepoints. (*) indicates statistical difference (p<.05; Two-way ANOVA and Tukey's post test)**.**

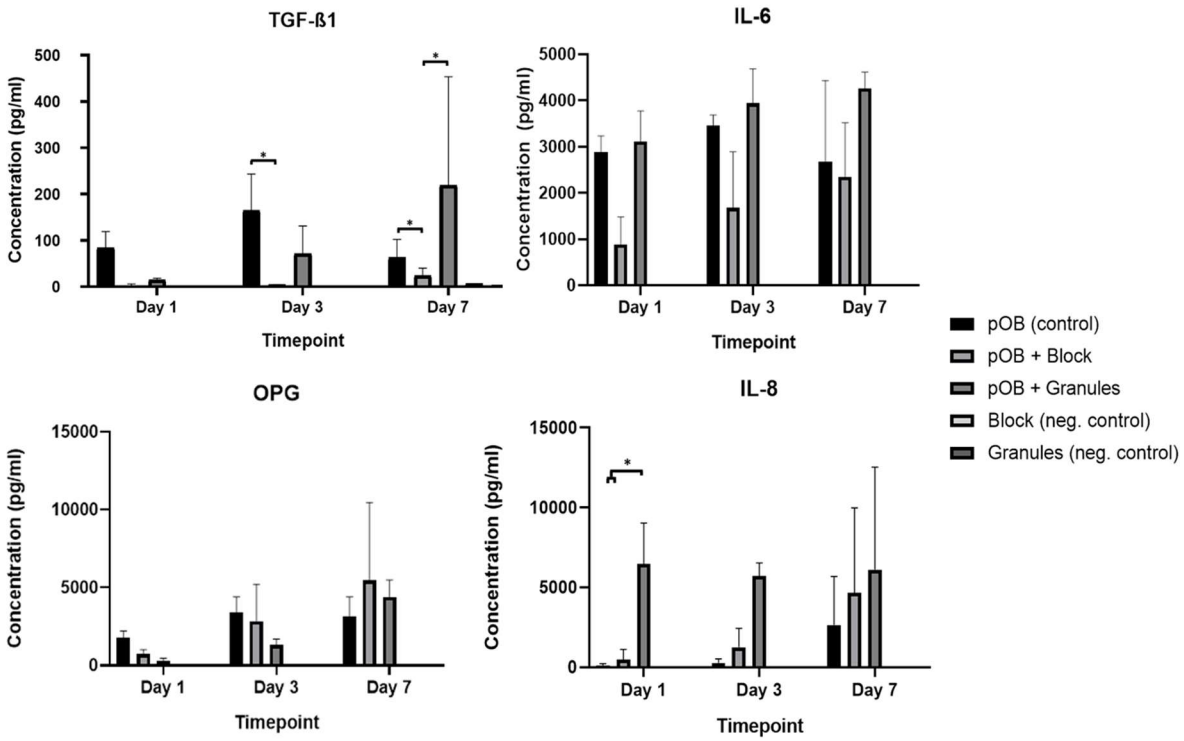

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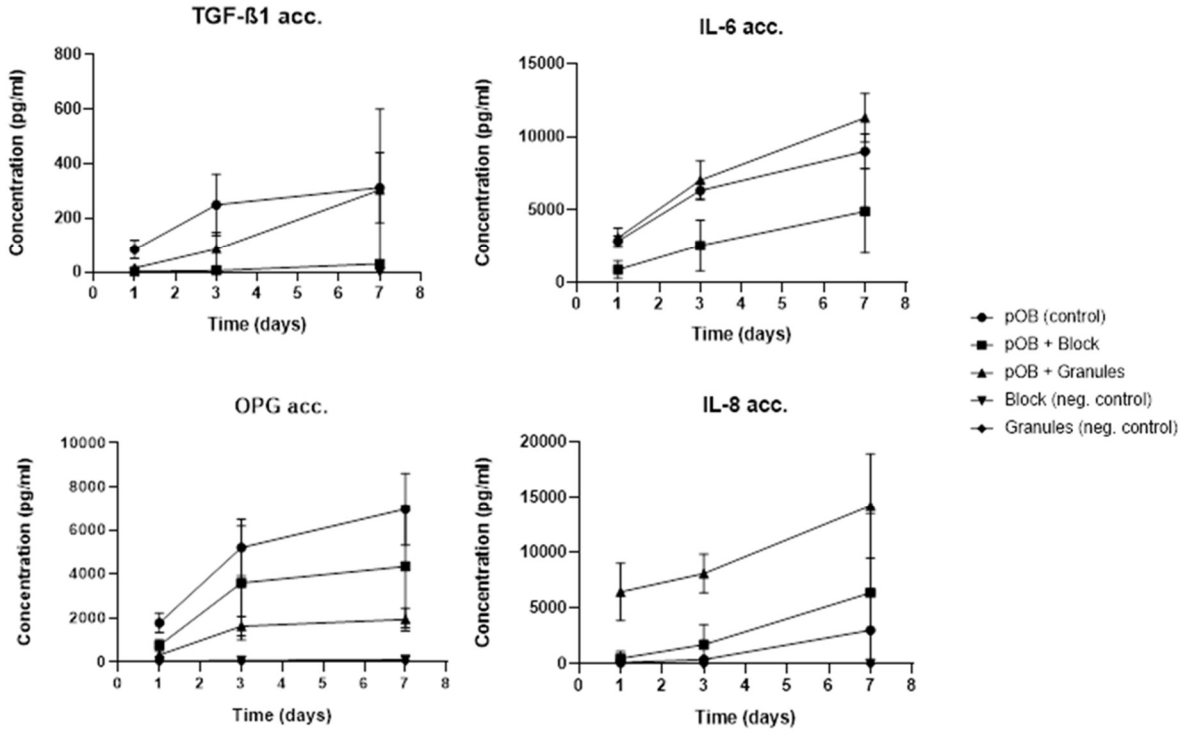
Figure 3. . Accumulated values for Inflammatory cytokines' concentrations kinetics in the supernatant**..**



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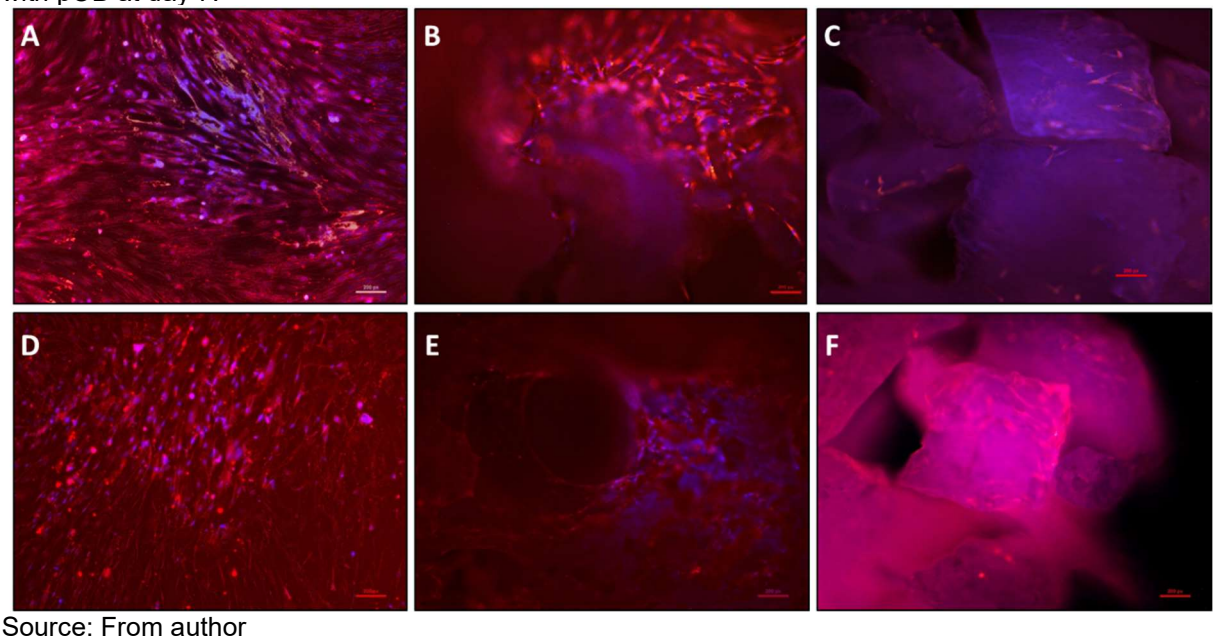
Histology and immunofluorescence

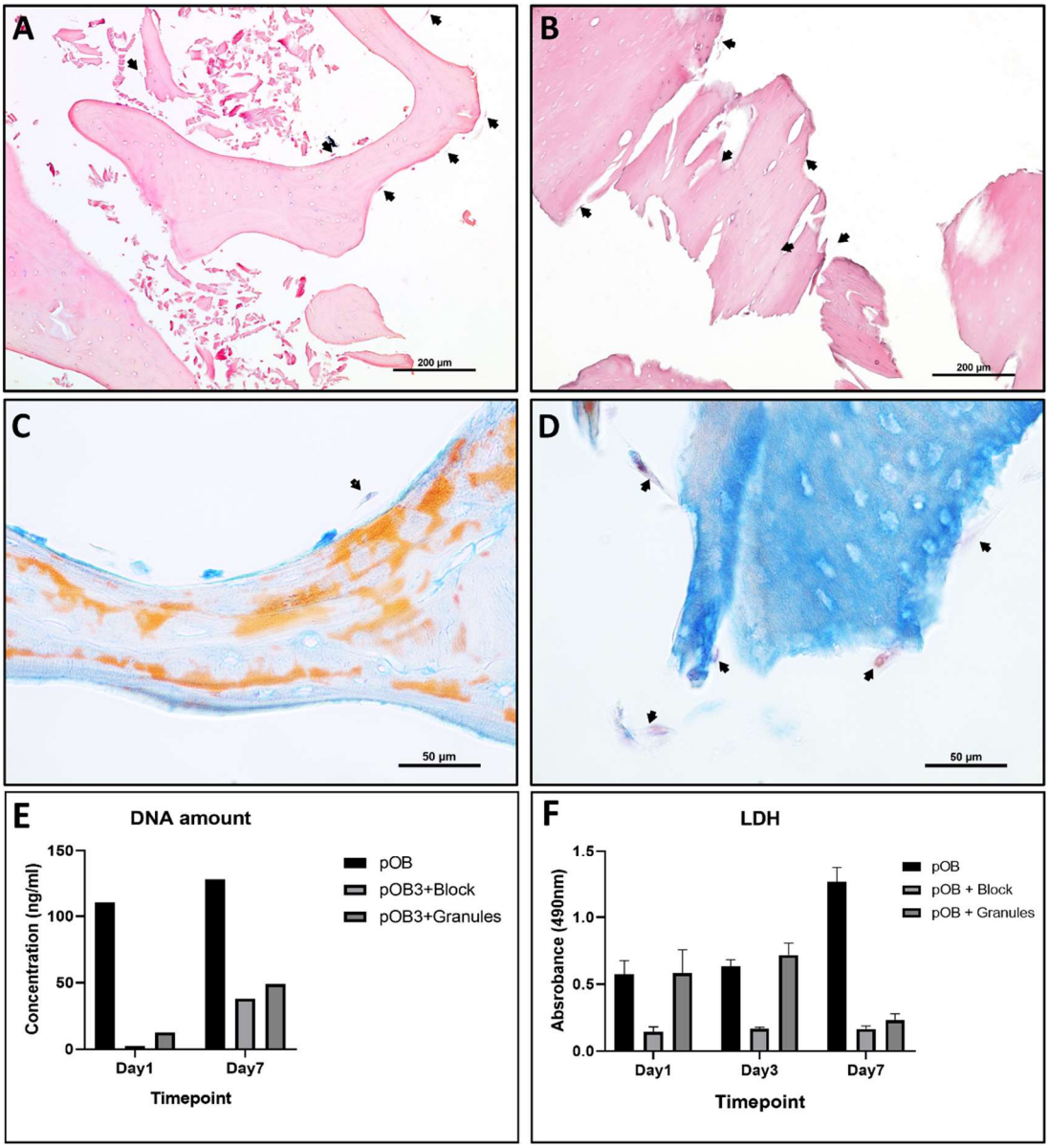
Samples were fixed and prepared for histological analysis after one and seven days of culture. H&E staining show large cellularity at day 1 in blocks and granules (Figure 4A-B), indicating adherence of cells to the tested materials surface. Large cellularity can be observed in both materials at day 7 (Figure 4C-D), with cells penetrating in material lacunae. Azan staining at day one evidences cells well distributed in material surface of granules but less cells attached to block`s surface (Figure 4E-F), while in 7 days it is possible to observe cells filling the lacunae of both tested materials (Figure 4G-H). Samples were also stained with Osteopontin (OPN), for immunofluorescence, and osteoblasts were positive marked in both materials' surfaces at day 1 and 7 (Figure 5), in bone blocks it was also possible to observe he migration of osteoblasts from surface to the lamellar structure (Figure 5E).

Figure 4**.** Osteopontin immunofluorescence staining and DAPI nuclei staining for osteoblasts at 200x magnification. (A) Primary Human osteoblasts (control) at day 1. (B) surface of Bovine bone block cultured with pOB at day 1. (C) Bovine bone granules cultured with pOB at day 1. (D) pOB at day 7 (control). (E) surface of Bovine bone block cultured with pOB at day 7. (F) Bovine bone granules cultured with pOB at day 7.



Source: From author

Figure 5. Histologic samples of DBBM in block and granules after 7 days in primary humans ostoeblasts (pOB) cell culture. . (A) pOB + Block after 7 days, H&E 100x. (B) pOB + Granules after 7 days, H&E 100x. (C and D) Azan trichrome staining of bovine bone blocks (C) and granules (D) 400x, arrows indicate examples of pOB on material surface. (E) DNA amount in samples after 1 and 7 days of culture, for estimative of cell growth. (F) LDH absorbance for cell death in the three different timepoints.



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DISCUSSION

Purity has been an issue on the subject of grafting materials since its conception [18]. Non-autologous bone substitutes from different origins, either from same species donors (allografts) or different species (xenografts) carry the potential of disease transmission or even immunological response inducing [9,19–21]. These materials can

contain bacteria, virus, antibodies, immunological molecules and proteins that ideally should be eliminated or inactivated by standardized procedures before clinical application.[8,14,20,22]

Several techniques have ben purposed to eliminate organic remnants from non-autologous grafts, and standardized method have been published and validated in the past 20 years. [19,20] Specifically, for the bovine derived materials there is a strong worry about Creutzfeldt-Jakob disease (CJD), in humans, and Bovine Spongiform Encephalitis (BSE). In Europe, a publication of German Ministry of Health, dated from 1994 and reviewed in 1996 (*Bundesgesundheitsamt,* 1996),[23] state the requirements of bovine, caprine and sheep products must attend to minimize the risk of BSE transmission to humans. According to this, the material should be evaluated by 6 parameters: (1) origin and feeding of the animals; (2) type of tissue used for production; (3)processing steps for inactivation of prions; (4) amount of raw material needed for the production of one daily dose; (5) number of daily doses; and (6) method of application. Each parameter is classified according to a logarithmic scale. Higher numbers indicate lower risk of infection. The origin and feed of animals are a strong parameter, and the manufacturer of materials analyzed in this study uses bone derived from tracked Brazilian cattle, free of BSE, which minimizes the risk of prion containing, also bone tissue is classified, as above, as the lowest risk tissue for Prion containing. According to these parameters the tested biomaterial had achieved a score of 22 points, over the 20 points sum necessary to be considered safe for CJD transmission.

For parameter 3 (processing steps for inactivation) several methods of purification have been described in literature, but is know that heating above 1000 °C is the most effective way of protein denaturation. [15,20] However, special attention have been dispensed for cleaning procedures that does not affect the ultrastructure,

mechanical and osteocondutive properties of materials.[19] High temperature heating reduces the material porosity and melt the lamellar structure, impairing material wettability and osteogenic cells attachment. By the other side, the manufacturer of tested material state that Bonefill® is submitted to a “sequence of baths that solubilize the organic structures such as cells remaining from organic matrix, fibers and proteins”, and it is processed at low temperature.

Some previous evaluated bovine-derived bone material, as Bio-OSS®, are protein free considered,[8] and its purification procedure consists of an initial bath for fat and gross organic residues removal, followed by heating up to 300 °C and finalizing with high alkalinity solution bath (pH >13), and previous studies proved that Bio-OSS® is free of organic remnants. [8,20,24] However, the process results in loss of resistance and mechanical properties, also usually this material when presented in blocks cannot be fixed at bone with screws.

The DBBM block evaluated in his study preserves the macro lamellar structure similar to human bone, with mature mineralized interconnected trabecula and porosity, which has been reported to promote a good mechanical resistance. Clinical studies make successful use of screws to fix the Bonefill® blocks in the remaining bone. However, the exclusive chemical processing shows to no be efficient in remove all organic material from bovine blocks, while in granules it appears to be more effective. These differences between granules and blocks can be related to the tridimensional structure of block which could avoid the complete soaking of the material in the chemical baths.

Orlowska et al.[7] in a similar ex-vivo study identified the presence of lamellar structure and cellular remnants in a xeno-synthetic DBBM, with intentional collagen addition. According to the authors, collagen containing bone substitutes could provide

stability to bone formation and improve the biomaterial results. However more studies are necessary to evaluate the biocompatibility of bone substitutes materials containing organic remnants

Most of available bone substitute materials contain some degree of impurity, and it is no clear how these features interfere in the biocompatibility and bone formation. Inflammatory foreign body reaction is a current concern for the use of biomaterials for bone augmentation, and the pattern of macrophages reaction seems to be an important marker to predict if a biomaterial will be successful incorporated to host bone, and allow new bone formation, or if it will be completely encapsulated and resorbed. [9,13,25,26]

In our study the DBBM block, containing organic remnants, do not induce a pronounced inflammatory pattern in human osteoblasts, comparing to the DBBM granules without traces of RNA or cellular residuals. However, the secretion of TGF-ß1-at the group pOB+B was significative lower than pOB+G in all timepoints, which could be explained by a lower cellularity in the pOB+B group. TGF-ß1 is commonly secreted by osteogenic cells, and is considered a pleiotropic interleukin in bone, which usually induces to bone formation by stimulating OPG and inhibiting RANK pathway, inducing the osteoid formation. [27,28]

IL-6 is a pro-inflammatory cytokine naturally expressed by osteoblasts, it's functions in bone homeostasis are related with the osteoclast's activation and osteoblast's maturation, also it is higher expressed by immature osteoblasts then mature ones. Amerio et al [29] previously evaluated the secretion of IL-6 by osteoblasts exposed to DBBM, and found a down regulation in the group exposed to DBBM in relation to the control. In our study we did not observed significant difference in the IL-

6 expression between groups, however any of the tested materials act to upregulate this cytokine.

IL-8 was also higher for pOB+G then control and pOB+B, this cytokine is particularly related, *in vivo,* to a particle induced chemotaxis, and with the recruitment of neutrophils in the early stage of inflammatory response.[30] It was previously demonstrated the high temperature sintered biomaterials induces more IL-8 production and polymorphonucleated cells (PMG) reaction.[30–32] Osteoblasts are a potent inflammatory mesenchymal cell, and beyond its native osteogenic function it is also accumulate the cytokine releasing when stresses and modulate the bone immune reaction [28,33].

For both types of DBBM tested it was possible to identify the colonization by osteoblasts at the material surface, in the immunofluorescence images at day 1 the cells appears to be at the top of material, when in day 7 the colonization could be observed towards the porous structure, which was also observed at histologic staining. Furthermore, a larger surface area of pOB+G compared to pOB+B could explain the increased cellularity and consequently the higher levels of cytokines expression in pOB+G group.

Our results suggest that both tested materials induce a similar inflammatory response by osteoblasts, despite the differences in the presentation (block or granules) and purity degree. The results of monoculture cells limit the extrapolation of results, and *in vivo* inflammatory reaction studies should be performed to evaluate biomaterials with different purification degrees

At this relationship between purity, biocompatibility, and mechanical properties an ideal balance should be searched, while collagen remnants can be desirable and

enhance bone formation, cellular remnants with immunogenic properties should be avoided.

CONCLUSION

The DBBM tested presented different purity degree according to its presentation, granules were free of cell remnants and did not contain RNA residuals. Despite the difference in the purity degree both materials have not upregulated the expression of pro-inflammatory cytokines by osteoblasts.

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4 CONCLUSÕES

A partir dos resultados obtidos por meio das metodologias aplicadas neste estudo, foi possível concluir que:

1- O uso de osso bovino desproteinizado é uma alternativa viável para o aumento horizontal do rebordo alveolar, e apresenta altas taxas de sucesso, permitindo a instalação de implantes dentários, independente da forma de apresentação.

2- O osso bovino desproteinizado em bloco, utilizado neste estudo, apresenta-se como alternativa viável ao enxerto de osso autógeno do ramo mandibular, no aumento horizontal do rebordo alveolar, com bons resultados clínicos e tomográficos

3- A microarquitetura e a incorporação do biomaterial testado, em áreas de aumento horizontal do rebordo alveolar suportam sua indicação para este tipo de procedimento.

4- O osso bovino desproteinizado, em ambas as apresentações, não exacerba a expressão de citocinas pró-inflamatórias em cultura primária de osteoblastos humanos, mesmo contendo remanescentes orgânicos.

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APÊNDICE A – METODOLOGIA DO ESTUDO CLÍNICO RANDOMIZADO

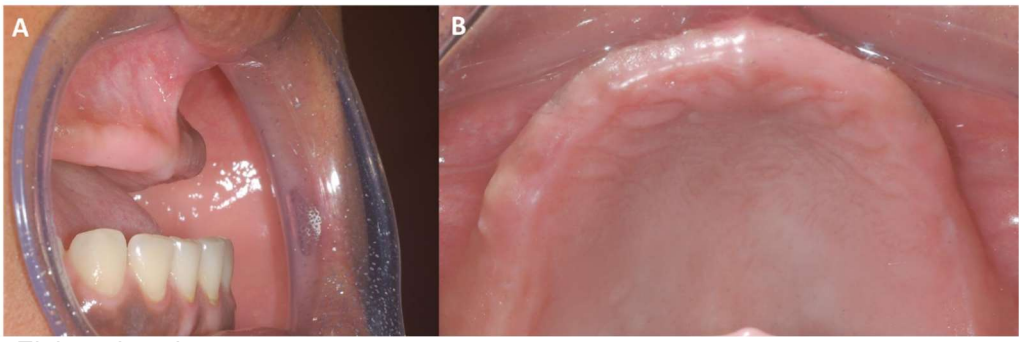
Submissão ao Comitê de Ética em Pesquisa

Este estudo seguiu as normas previstas pela Resolução 466/12 do Conselho Nacional de Saúde – MS e foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia de Araraquara da Universidade Estadual Paulista “Júlio de Mesquita Filho”, sob o número do CAEE: 67443017.0.0000.5416.

Seleção da amostra

Foram selecionados pacientes desdentados totais de maxila, atendidos na Faculdade de Odontologia de Araraquara, candidatos a receberam tratamento reabilitador com prótese total implanto-suportada apresentando atrofia horizontal do rebordo alveolar (Figura 1).

Figura 1- Avaliação clínica da deficiência horizontal do rebordo alveolar superior de pacientes desdentados totais de maxila. (A) vista lateral da porção anterior do rebordo alveolar, (B) vista oclusal do rebordo maxilar atrófico.



, Fonte: Elaborado pelo autor.

Foram selecionados os pacientes de acordo com os seguintes critérios de inclusão: pacientes saudáveis, com ausência de doenças graves ou descompensadas as quis influenciassem os processos fisiológicos de regeneração óssea; de ambos os

sexos, com idade superior a 18 anos e que concordassem em participar do estudo, assinado Termo de Compromisso Livre e Esclarecido.

Não foram incluídos na amostra: pacientes fumantes, pacientes com foco de infecção bucal no momento da avaliação, indivíduos com histórico de extração na região há menos de seis meses, pacientes com doenças sistêmicas não controladas que contraindicariam os procedimentos, pacientes com histórico de radioterapia na região de cabeça e pescoço e/ou em uso de quimioterápicos, pacientes usuários drogas que interferem com a remodelação (*turnover*) óssea e/ou corticóides de forma crônica e gestantes.

Desenho do estudo

Este estudo seguiu o desenho experimental de Ensaio Clínico Randomizado (ECR) de boca dividida, com os pacientes recebendo os dois tipos de enxerto ósseo, alocados aleatoriamente cada um em um lado da região de pré-maxila. Um sítio com enxerto autógeno de ramo mandibular e outro com enxerto em bloco heterógeno de origem bovina desproteinizado (Bonefill Porous Block, Bionnovation, Brasil), determinando os dois grupos de análise.

A metodologia deste estudo foi dividida em:

1) Avaliação clínica e tomográfica pré-operatória da quantidade e qualidade do remanescente ósseo.
2) Cirurgia: reconstrução de maxila, aumento horizontal do rebordo alveolar com enxerto ósseo em bloco, autógeno e heterógeno, cada um alocado de acordo com sorteio prévio, com aleatorização binomial no software Microsoft Excel 2016 (Office365, Microsoft, Redmond, WA, USA)
3) Análise: avaliação clínica e tomográfica pós-operatória imediata, avaliação clínica por um período de 9 meses, reavaliação tomográfica e reabertura das áreas

enxertadas para instalação de 4 implantes dentários seguindo o protocolo *"all-on-four".*

4) Análise microtomográfica e histomorfométrica de biópsias obtidas no momento da instalação dos implantes. O tecido mineralizado biopsiado envolvia tanto o enxerto como o osso nativo de cada área aumentada.

Protocolo cirúrgico para realização de enxertia óssea

Uma hora antes do início do procedimento os pacientes foram medicados com 2g de Amoxicilina (Amoxicilina, Medley Farmacêuticos S.A., Brasil) ou 600g de Clindamicina (Clindamicina, Medley Farmacêuticos S.A., Guarulhos, Brasil) para pacientes alérgicos à penicilina, 10mg de Dexametasona (Decadron, Aché Laboratórios Farmacêuticos S.A., Brasil) e 1g de Dipirona Sódica Monoidratada (Dipirona, Medley Farmacêuticos S.A., Guarulhos, Brasil), ou 750mg de Paracetamol (Paracetamol, Medley Farmacêuticos S.A., Brasil), em caso de alergia à Dipirona.

Os procedimentos cirúrgicos foram realizados em regime hospitalar, sob anestesia geral. Após antissepsia com solução de Clorexidina 0,5% (Riohex, Rioquímica, Brasil), foi realizada anestesia local com Articaina 4% com epinefrina 1:100.000 (Articaine, DFL, Brasil).

Inicialmente foi realizado retalho trapezoidal, para exposição dos sítios receptores na maxila, estendendo-se entre as regiões de segundo pré-molar de cada lado . Após a incisão o remanescente ósseo da maxila foi decorticado com fresas de aço cirúrgico (H251E Maxicut, Komet Brasil, Brasil) e perfurado com broca tronco-cônica 701L de 16mm de diâmetro (H33L 701, Komet Brasil, Brasil) em motor elétrico (LB100, Beltec, Brasil), para expor a matriz orgânica do osso e favorecer a incorporação do enxerto (Figura 2A).

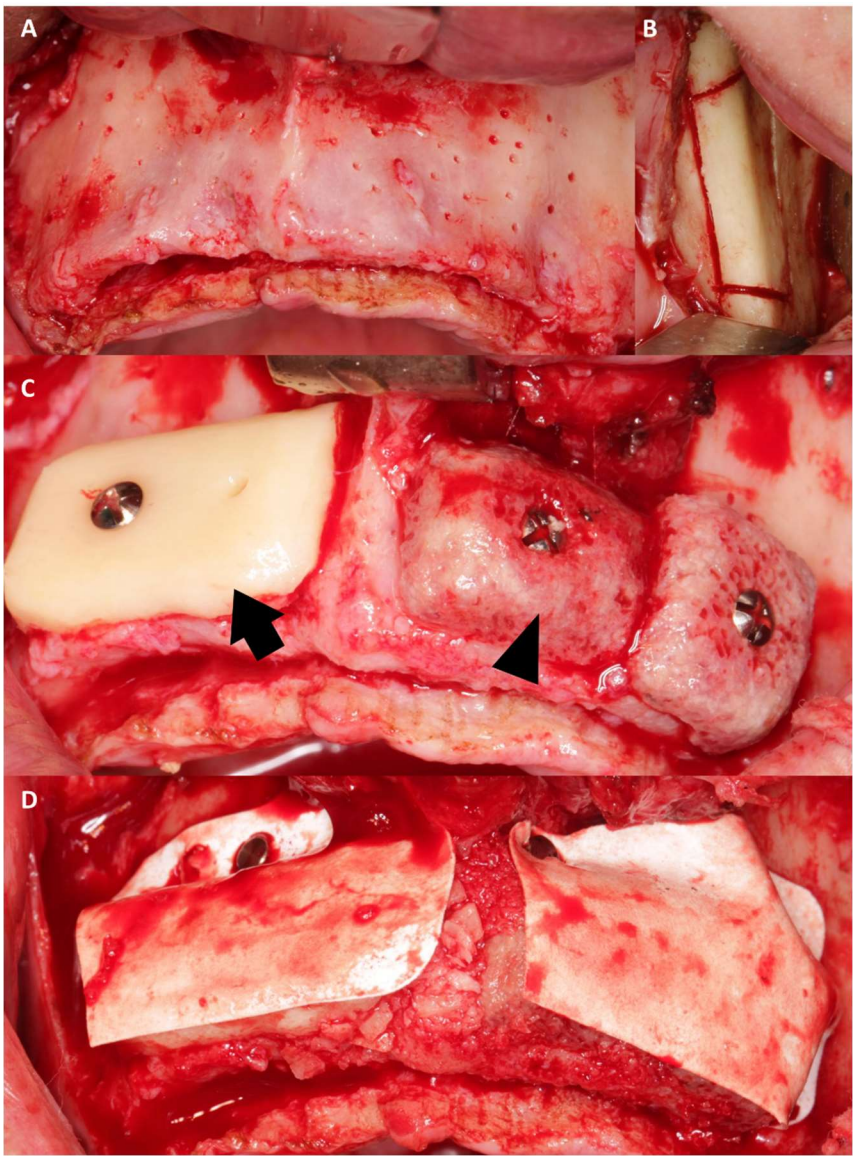
Após o preparo dos leitos receptores, o ramo mandibular foi acessado por meio de incisão linear sobre a linha obliqua externa com extensão suficiente para a remoção do enxerto, o retalho mucoperiosteal foi descolado expondo o ramo mandibular e a área retro molar, na sequência foram realizadas três osteotomias corticais completas com motor piezoelétrico (Piezosonic, Driller, Brasil) e serra para motor piezoelétrico (ES007A-Driller, Driller, Brasil), uma sobre a linha oblíqua externa e duas verticais, anterior e posterior, conforme o tamanho necessário para o enxerto; finalmente uma quarta osteotomia, localizada inferiormente, unindo as osteotomias verticais foi realizada para facilitar a remoção do enxerto utilizando disco serrilhado para osso (Disco serrilhado 8mm, Härte instrumentos, Ribeirão Preto, Brasil) acoplado em ponta reta (Kavo ind. e comércio, Joiville, Brasil). Concluídas as osteotomias, o enxerto foi removido com auxílio de cinzeis ósseos reto e curvo (Quinelato, Brasil, QD.120.02, QD. 121.04) (Figura 2B). Já o enxerto heterógeno em bloco, já estéril de fábrica, foi utilizado após hidratação em solução salina estéril durante um minuto, conforme recomendações do fabricante.

Ambos os enxertos foram modelados com o auxílio de motor piezoelétrico e fresas de aço cirúrgico (Komet Brasil, Brasil) para remoção de ângulos vivos e para adaptação ao leito receptor de acordo com sorteio prévio. Os enxertos foram fixados com dois parafusos de titânio de 1,4mm de diâmetro por 12mm de comprimento (Bionnovation, Brasil), com cabeça expandida e sem torque no enxerto, para estabilização destes (Figura2C). Após a fixação dos enxertos, as regiões imediatamente adjacentes a perfurações dos parafusos foram mensuradas com espessímetro cirúrgico (Quinelato, Brasil, QD.308.10) posicionado perpendicularmente ao plano oclusal da maxila para verificar a espessura obtida, em milímetros, com cada medida tomada em triplicata.

Realizadas as mensurações, os enxertos foram recobertos por membranas de Politetrafluoretileno (PTFE) (Surgitime PTFE 0,25mm, Bionnovation, Brasil, fixadas com tachinhas de titânio (AutoTac®, BioHorizons Implant System, EUA). Por fim, os leitos cirúrgicos foram suturados por primeira intenção com Poligalactina 910 (Ethicon, Johnson & Johnson, Brasil).

O protocolo medicamentoso pós-operatório utilizado foi: 500mg de Amoxicilina a cada 8 horas, por sete dias; ou 300mg de Clindamicina a cada 8 horas por 7 dias, em caso de alergia à penicilina; 100 mg de Nimesulida a cada 12 horas por 4 dias; 500mg de Dipirona Sódica Monoidratada a cada 6 horas, enquanto dor, por no máximo 4 dias, ou 750mg de Paracetamol a cada 6 horas, enquanto dor, por no máximo 4 dias, caso alergia à Dipirona. Em todos os casos as suturas foram parcialmente removidas entre 7 e 10 dias pós-operatórios, e completamente removidas em 15 dias. Uma tomografia computadorizada de feixe cônico pós-operatória imediata foi realizada, assim como avaliação clínica em relação a presença de complicações relacionadas com o procedimento cirúrgico, a saber: deiscência de sutura, exposição do enxerto, infecção, hemorragia, parestesia e fratura óssea. As repercussões clínicas foram avaliadas em ambos os sítios cirúrgicos, doador e receptor.

Figura 2- Procedimento cirúrgico de aumento horizontal do rebordo alveolar com enxertos em bloco autógeno do ramo mandíbula (OA) ou heterógeno de origem bovina desproteinizado em bloco (OBDB). (A) Preparo, exposição e decorticação do leito receptor. (B) Osteotomia no leito doador para obtenção do OA. (C) Fixação dos enxertos seguindo critério de aleatorização binomial, seta indicando grupo OA e cabeça de seta indicando grupo OBDB. (D) cobertura dos enxertos ósseos com membrana não reabsorvível de PTFe.



. Fonte: Elaborado pelo autor.

Protocolo cirúrgico para reabertura dos enxertos e instalação dos implantes dentários

Nove meses após o procedimento de enxertia óssea foi realizada cirurgia de acesso a área enxertada para instalação dos implantes. O protocolo medicamentoso foi idêntico ao protocolo para cirurgia de enxertia óssea.

Os procedimentos cirúrgicos de reabertura e instalação dos implantes dentários foram realizados em regime ambulatorial. Foram realizados antissepsia extrabucal com Clorexidina 2% (Riohex, Rioquímica, Brasil) e bochecho com 10 ml de Clorexidina 0,12% (Periogard, Colgate-Palmolive Industrial Ltda, Brasil). Após, foi realizada anestesia local por meio de infiltração de articaina 4% com epinefrina 1:100.000 (Articaine, DFL, Brasil).

Um novo retalho trapezoidal foi realizado na mesma posição da incisão anterior, para exposição dos enxertos e realização de mensurações de espessura nas mesmas regiões utilizadas previamente. Foram removidos os fixadores de membrana e as membranas, os parafusos de enxerto foram removido apenas nos casos em que atrapalhavam a realização das biópsias ou a trajetória de inserção dos implantes, caso contrário esses foram mantidos (Figura 3). Antes da perfuração para instalação dos implantes, foram removidos fragmentos ósseos de 2,0mm de largura por 6mm de altura por meio de broca trefina de 2,5 x 6mm, a 1200 RPM (Härte instrumentos, Ribeirão Preto, Brasil), das duas áreas enxertadas (Figura 3D-F). O fragmento ósseo após a remoção foi imediatamente lavado com soro fisiológico estéril (Equiplex, São Paulo, Brasil) e fixado em formaldeído 4% (Synth, São Paulo, Brasil) tamponado em tampão de fosfato de sódio 0,1M (pH 7,2). Após a biópsia a fresagem para instalação dos implantes dentários foi realizada conforme instruções do fabricante para instalação de implantes hexágono externo (BioDirect, Bionnovation, Brasil), sob

irrigação externa com solução estéril de Cloreto de Sódio 0,9% (Equiplex, Brasil), com a utilização de um contra ângulo cirúrgico 20:1 (Neodent/NSK, Curitiba, Brasil) acoplado em motor elétrico (NeoSurg® XT plus, Neodent/NSK, Cuririba, Brasil) com rotação entre 800 e 1200 rpm. Foram instalados 2 implantes de 3,5mm x 10mm na região anterior e dois implantes de dimensões 3,5x13mm na região posterior com inclinação de cerca de 30 graus, conforme protocolo *all-on-four*. Em todos os casos a inserção do implante foi finalizada com catraca manual com torquímetro, fornecidos no kit de instalação do fabricante. O torque de instalação de cada implante foi registrado por meio de torquímetro acoplado a catraca manual.

O coeficiente de estabilidade dos implantes foi mensurado, no momento da instalação, por meio do índice de frequência de ressonância (ISQ) pelo dispositivo Osstell (Osstell ISQ, Suécia), Para isto, foi utilizado o smart-peg A1 (Osstell ISQ, Suécia) específico para a conexão dos implantes HE, e com a sonda do aparelho foram realizadas três medições no sentido vestíbulo-palatino do smart-peg.

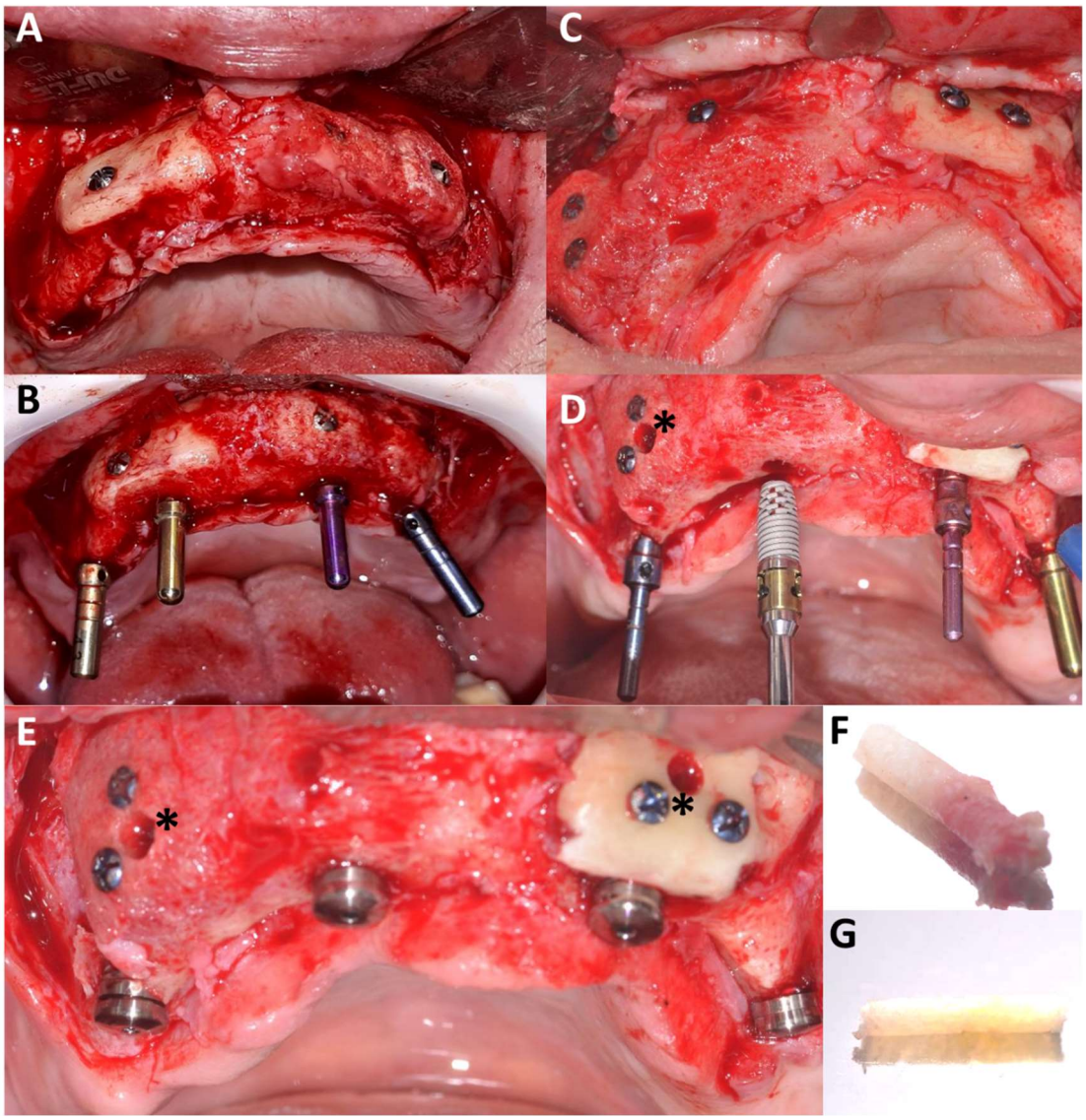
Após a instalação dos implantes, biópsia e mensurações clínicas os tecidos foram reposicionados e suturados com Poligalactina 910 (Ethicon, Johnson & Johnson, Brasil), e a sutura removida entre 7 a 10 dias. O protocolo medicamentoso pós-operatório utilizado foi igual ao realizado após a cirurgia de enxertia óssea.

Avaliações clínicas

Foram realizas avaliações clínicas semanalmente no período pós-operatório imediato, sendo estas espaçadas após o primeiro mês. Na avaliação clínica foi avaliada a situação sistêmica do paciente, como: febre, linfadenoaptia regional, debilidade, entre outros; além da avaliação clínica do local da intervenção, anotando os principais sinais e sintomas, como: hematoma, edema, equimose, parestesia, e

situação da ferida operatória quanto a ocorrência de deiscência, infecção, seroma, formação granulomatosa, dor local, entre outros.

Figura 3- Procedimento cirúrgico de aumento horizontal do rebordo alveolar com enxertos em bloco autógeno do ramo mandíbula (OA) ou heterógeno de origem bovina desproteinizado em bloco (OBDB). (A-D) exemplos de reabertura e remoção das membranas decorridos 9 meses do procedimento de enxertia e disposição dos implantes all-on-four. (B) Osteotomia no leito doador para obtenção do OA. (E) *Indicação da área de obtenção das amostras biopsiadas nos grupos OA e OBDB. (F-G) Fragmentos de biópsias Core incluindo leito receptor, interface osso enxerto e área enxertada.



Fonte: Elaborado pelo autor.

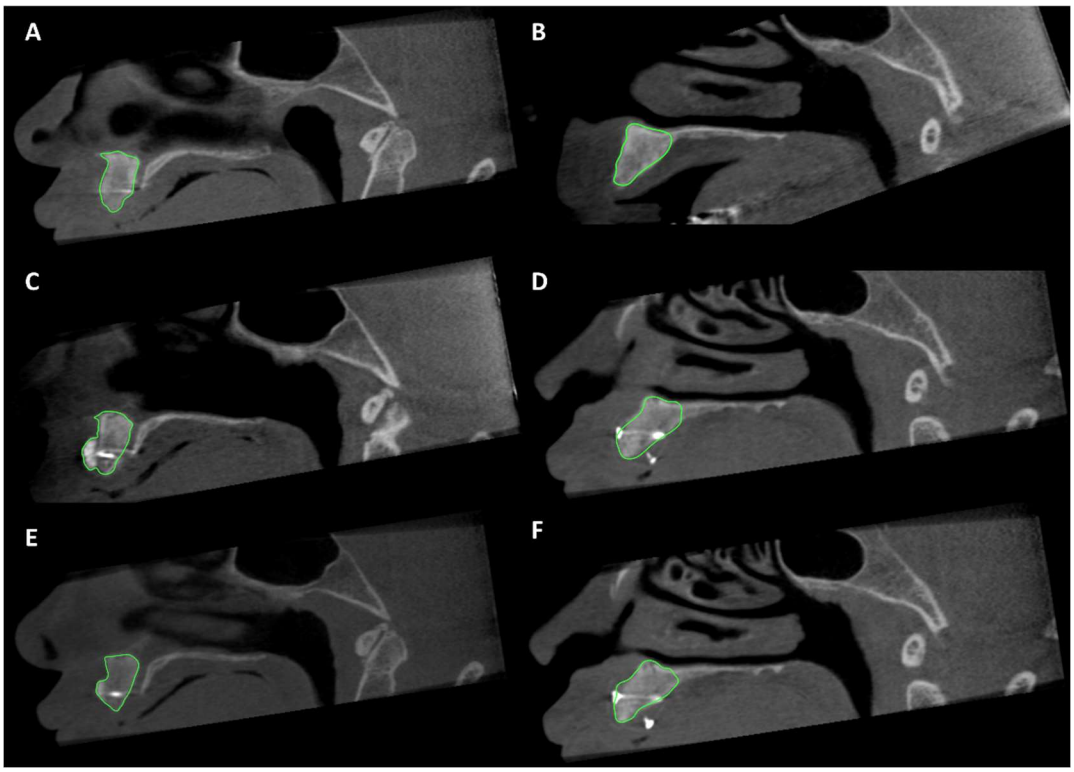
Avaliação tomográfica

Os conjuntos de dados DICOM das tomografias obtidas nos tempos pré-operatório (T0), pós-operatório imediato (T1) e com 9 meses de pós operatório (T2), foram salvos em disco rígido e reconstruídos por meio de software específico OsiriX (software-livre, Genebra, Suíca). As reconstruções foram orientadas quanto a posição espacial tridimensional, tendo como referência posição do plano de Frankfurt paralelo ao solo, o plano coronal cruzando as placas pterigoides e o plano sagital fixado na linha média perpendicular ao plano coronal. Após a orientação da cabeça, no plano sagital 40 cortes para cada lado foram padronizados a partir da linha média, sobre os quais foi definida a região de interesse e calculado a área de cada secção (Figura 4).

Para determinar o volume (V) das regiões enxertadas de maneira padronizada, as áreas (A) de cada tipo de enxerto, foram medidas em 40 cortes seccionados para cada lado da linha média. Todas as mensurações foram realizadas em secções sagitais da TC com espessura de 0,25 mm e com distância entre fatias de 1mm. O contorno das regiões de interesse (ROI) em cada secção foi traçado manualmente por meio de mesa digitalizadora Intuos (Wacom, Brasil). Para facilitar a delimitação das estruturas, o contraste de exposição das imagens foi padronizado, e o nível de centro (L) e a largura de banda (W) definidos de acordo com as sugestões de Spin-Neto et al. (2011)[46], W=3086 e L=667. A área (A) de cada corte foi calculada automaticamente pela ferramenta de ROI do software OsiriX. O volume da região óssea vestibular, medido em cada fatia da tomografia computadorizada foi calculado pela multiplicação da área (A) e a altura (H), que equivale a distância entre as fatias sagitais. O volume (V) de toda a região, resulta da soma de todos os volumes medidos em cada fatia (Princípio de Cavalieri).

Para avaliar o aumento ósseo enxertado incialmente, foram comparados os volumes obtidos nos períodos T1 e T2, para avaliar a taxa de reabsorção dos enxertos, o volume obtido em T3 foi subtraído de T2, e para avaliar o aumento ósseo final o volume de T3 subtraído de T1.

Figura 4- Avaliação tomográfica do volume ósseo e delimitação da região de interesse (ROI) realizada nos diferentes períodos do estudo:



Fonte: Elaborado pelo autor.

Avaliação por meio de microCT e histomorfometria

As biópsias ósseas obtidas durante a instalação dos implantes foram utilizadas para análise de microCT e histomorfometria, para tanto, as amostras ósseas foram mantidas em formaldeído 4% tamponado em tampão de fosfato de sódio 0,1M (pH 7,2) por 48h, sendo posteriormente lavadas em água corrente por 6 horas e armazenadas em álcool 70%.

Os espécimes foram submetidos à análise por varredura de feixe de raios-X em um sistema de microtomografia digital computadorizada, escaneados pelo microtomógrafo SkyScan (SkyScan 1176 Bruker MicroCT, Aatselaar, Bélgica, 2003) utilizando cortes de 9µm de espessura (50Kv e 500µ), com filtro de cobre e alumínio e passo de rotação de 0,3mm. As imagens obtidas pela projeção dos raios-x nas amostras foram armazenadas e reconstituídas determinando a área de interesse pelo software NRecon (SkyScan, 2011; Versão 1.6.6.0). No software Data Viewer (SkyScan, Versão 1.4.4 64-bit) as imagens foram reconstruídas para adequação do posicionamento padrão para todas as amostras, podendo ser observada em três planos (transversal, longitudinal e sagital). Após a reorientação do posicionamento, a área de interesse (ROI) foi configurada no software CTAnalyser – CTAn (2003-11SkyScan, 2012 Bruker MicroCT Versão 1.12.4.0) , na sequência foi realizada a análise e mensuração da imagem de acordo com a escala de cinza (*threshold*). O *threshold* utilizado na análise foi de 25-90 tons de cinza, possibilitando a obtenção do volume de osso formado e sua caracterização.

Após o escaneamento os mesmos fragmentos ósseos utilizados na análise de microCT foram brevemente lavados em água corrente e descalcificados em solução de ácido etilenodiaminotetracético (EDTA) a 7,5% (Synth, São Paulo, Brasil). Após a

descalcificação as peças foram desidratadas em concentrações crescentes de etanol a partir de 50o GL até álcool absoluto e incluídas em parafina. Foram realizados cortes seriados no sentido longitudinal da peça com 4 µm de espessura com auxílio de micrótomo (Micron HM 325, Thermo Scientific, Reino Unido). Os cortes foram corados com hematoxilina e eosina (HE) (Synth, São Paulo, Brasil) e submetidos a histomorfometria e contagem de osteócitos.

Para histomorfometria, três lâminas de cada fragmento ósseo foram selecionadas pela técnica de estereometria. A quantificação do tecido ósseo foi realizada por um examinador experiente. A análise quantitativa foi limitada a área de enxertia óssea e a interface osso-enxerto. As amostras foram digitalizadas para computador por meio de uma câmera fotográfica (Olimpus, CAMEDIA C50/60 Wide Zoom, Japão) acoplada ao Microscópio Óptico (Diastar – Leica Reichert Jung Products, Alemanha) com objetiva de aumento 4.0/100X e oculares de aumento de 10X. Com auxílio de um software livre para análise de imagens (Image J, NIH, Bethesda, Estados Unidos), a área total do enxerto foi delimitada e quantificada como 100% e em seguida subtraídas das áreas mineralizadas outras estruturas, como espaços vazios, células e vasos sanguíneos. A porcentagem final de cada região foi obtida pelo cálculo da média de área óssea das três lâminas quantificadas. Em seguida, a contagem de osteócitos foi realizada em duplicata, em toda a extensão das lâminas.

Análise estatística

As mensurações realizadas foram tabuladas e codificadas por grupo para avaliação estatística. As medidas volumétricas nos exames de imagem foram

analisadas por dois examinadores calibrados (CI 0,86), e as médias das mensurações realizadas por cada examinador foram consideradas para análise estatística.

Para os dados quantitativos, após a confirmação do modelo de distribuição dos dados por meio dos testes de normalidade de Shapiro-Wilk e homocedasticidade de Levene, foi utilizada a análise de variância (ANOVA *One-way* e *Two-way*) com pós teste de Sidak em casos de comparações múltiplas. Para dados que não obtiveram distribuição normal foi utilizado teste U de Willcoxon e Mann-Whitney. No caso de dados de caráter qualitativo nominal as comparações foram realizadas com teste exato de Fisher.

Cálculo amostral

Para realização deste estudo foi utilizada amostra de conveniência. Baseando-se em estudos semelhantes e considerando erro α de 5% e o poder de estudo desejado em 80%, o tamanho amostral (n) foi estimado em 10 amostras por grupo, com margem de 20% de perdas foram incluídos 12 pacientes, com o total de 24 sítios, divididos em dois grupos de n=12.

ANEXO A – APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA COM SERES HUMANOS

UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Enxertos xenógenos
Pesquisador: Valfrido Antonio Pereira Filho
Área Temática:
Versão: 2
CAAE: 67443017.0.0000.5416
Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.070.842

Apresentação do Projeto:
O estudo tem a duração prevista para 3 anos, e buscará comparar o sucesso de enxerto xenógeno de origem bovina (disponível comercialmente) com o enxerto autógeno de ramo mandibular, na reabilitação de maxilas com atrofia de rebordo alveolar para ganho ósseo e posterior implante dentário.
Serão selecionados 14 pacientes desdentados totais com atrofia horizontal da região anterior da maxila. Cada lado da região anterior de maxila será reconstruído com um tipo de enxerto em bloco, conforme randomização.
Serão realizadas comparações clínicas pré cirúrgicas, trans cirúrgicas imediatas e trans cirúrgicas e clinicas tardias. Bem como comparações laboratoriais em biópsias obtidas das regiões enxertadas submetidas a histomorfometria, micro tomografia, análise de reação em cadeia de polimerase (PCR), além de comparação volumétrica tomográfica dos enxertos através de TCs obtidas no pré operatório, pós imediato e pós operatório de 9 meses. Por fim será realizada a instalação de quatro implantes dentários na região enxertada seguindo a técnica de protocolo all-in-four, e realizada mensurações de estabilidade do implante com auxílio de mensurador específico.

Objetivo da Pesquisa:
Comparar a qualidade da osseointegração de enxertos ósseos em bloco de origem animal com o

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padrão ouro (osso autógeno).

Avaliação dos Riscos e Benefícios:

Riscos: Os riscos do presente trabalho incluem algumas complicações pós-operatórias que podem ocorrer na área doadora do enxerto ósseo como dor, inchaço, desconforto, parestesia (dormência) e sangramentos; ou na área de instalação dos enxertos e implantes como: inflamação, dor, inchaço, desconforto, sangramento, comunicação buco sinual ou contaminação do seio maxilar. Existe ainda o risco de perda de um ou mais implantes dentários ou enxertos.

Benefícios: Os pacientes receberão sem custo o tratamento dentário com implantes osseointegráveis e próteses do tipo protocolo superiores, além de todas as consultas prévias a cirúrgia bem como sus consultas de retorno.

Comentários e Considerações sobre a Pesquisa:

A metodologia no projeto está bem descrita em 4 etapas: 1) Avalição pré-operatória: Anamnese, exame clínico, avaliação de exame tomográfico pré-operatório e planejamento cirúrgico; 2) Procedimento cirúrgico de enxerto ósseo: Realização de enxerto ósseo conforme seleção por randomização, obtenção de exame tomográfico pós-operatório imediato; 3) Procedimento cirúrgico para instalação de implantes dentários: avaliação do exame tomográfico para planejamento, remoção de biópsia óssea para avaliação histomorfométrica, e instalação de implantes; 4) Análise: avaliação volumétrica das tomografias e avaliação por microCT, histomorfometria e PCR das biópsias.

Considerações sobre os Termos de apresentação obrigatória:

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Conclusões ou Pendências e Lista de Inadequações:

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Considerações Finais a critério do CEP:

Protocolo APROVADO em reunião de 18 de Maio de 2017.

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Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

Continuação do Parecer: 2.070.842

Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_867509.pdf	16/05/2017 09:18:36		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_xenoenxertos_em_bloco_Corrigdo_CEP_08052017.pdf	16/05/2017 09:17:24	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito
Orçamento	Termo_de_Ciencia_Orcamentaria.pdf	11/04/2017 19:36:35	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito
Declaração de Instituição e Infraestrutura	Termo_de_autorizacaoo_clinica_laboratorial.pdf	11/04/2017 19:36:18	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito
Projeto Detalhado / Brochura Investigador	Avaliacao_Clinica_e_Histologica_de_Xenoenxertos_em_bloco.pdf	15/03/2017 11:18:30	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito
Folha de Rosto	folha_de_rosto_assinada.pdf	06/03/2017 10:10:39	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito
Declaração de Pesquisadores	termo_de_ressarcimento_de_gastos_assinado.pdf	06/03/2017 10:10:20	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito
Declaração de Pesquisadores	Termo_de_cumprimento_das_normas_do_CEP_assinado.pdf	06/03/2017 10:09:48	PEDRO HENRIQUE DE AZAMBUJA CARVALHO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARARAQUARA, 18 de Maio de 2017

Assinado por:
Lígia Antunes Pereira Pinelli
(Coordenador)

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